

DIGESTIVE PHYSIOLOGY

OF

Pigs



Edited by
J.E. Lindberg and B. Ogle



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The Digestive Physiology of Pigs

Proceedings of the 8th Symposium

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J.E. Lindberg and B. Ogle

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Preface

This book contains a compilation of papers presented at the 8th Symposium on Digestive Physiology in Pigs, held at the Swedish University of Agricultural Sciences, Uppsala, 20–22 June 2000. In this book the most recent findings relating to the effect of nutrition on the gut physiology in pigs are highlighted.

The 8th Symposium on Digestive Physiology in Pigs was a continuation of a series of earlier meetings held on this subject, with the first meeting being held in Great Britain in 1979. This was followed by symposia in France, Denmark, Poland, The Netherlands and Germany. The 7th Symposium was held in St Malo, France, in 1997. The present symposium, as well as previous symposia, was under the auspices of the European Association of Animal Production (EAAP).

The gastrointestinal tract is a complex system that has a number of different functions. It is the site for interaction between feed and the body, and while the main function is digestion and absorption of nutrients the gastrointestinal tract is also a sophisticated defence organ and the body's largest endocrine organ. The interplay between the different functions is carefully modulated by a variety of direct and indirect mechanisms determining the ability of the gastrointestinal tract to withstand damage that potentially may be aimed against it. Luminal factors are now recognized as having direct or indirect effects on the gastrointestinal tract.

The 8th Symposium on Digestive Physiology in Pigs had the health and wellbeing of the pig in focus and during this symposium questions relating to the development and function of the gastrointestinal tract, and the possible interactions between nutrition and gut function, were addressed. The sessions dealt with factors influencing gut maturation and function in prenatal and postnatal pigs, as well as the gastrointestinal immune system in pigs and its implications for health maintenance. Further, nutrient fluxes at the intestinal level and nutrient utilization and metabolism by the gastrointestinal tract were discussed, in addition to dietary and animal-related factors affecting digestion and digestive secretions. Finally, the influence of the gut microflora on the digestive processes, the influence of nutrition on the gut microflora and the role of the gut microflora in the prevention of disease were discussed.

The symposium attracted delegates from countries worldwide and resulted in fruitful discussions and many interactions between the participants. It is our hope that these proceedings will contribute to the development of science within this area and create a platform for future research that will increase our knowledge on how to optimize the nutrition of the pig and how to prevent diet-related gastrointestinal disturbances.

Acknowledgements

The organizers are grateful to the speakers and to the poster presenters both for their excellent presentations and for provision of written versions which form the basis of these proceedings. Thanks are also due to the chairs of the sessions. Finally, thanks are due to all the delegates not just because of their presence, which is an essential feature of any meeting, but for their contribution to formal discussions.

Sincere thanks are also due to the following for their financial contributions, without which the symposium and workshops could not have taken place.

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Part I

Gut Development and Function

1 Transitions in the Life of the Gut at Birth

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The demands placed on the gastrointestinal tract (GIT) change dramatically at birth. Before birth, the GIT is exposed to only small amounts of complex nutrients via swallowed amniotic fluid. Immediately after birth, the GIT must digest and absorb nutrients from a large intake of energy- and protein-rich colostrum. Which factors influence GIT adaptation to enteral nutrition *ex utero*? In the pig, a series of GIT maturational events do not take place until immediately before and after birth. Endocrine signals and enteral food intake itself play key roles in this rapid adaptation. This review shows how some basic characteristics of the GIT (growth, digestion, absorption) change in the perinatal period of the pig, and how hormones (e.g. glucocorticoids) and food (e.g. colostrum and milk) influence the transition of the GIT from its 'silent being *in utero*' to its highly active life just after birth.

The Timing of Gastrointestinal Maturation in Different Species

In humans, the functional maturation of the digestive system (e.g. digestive enzymes) progresses relatively slowly, but maturation also starts early, i.e. long before birth. At birth, the infant gastrointestinal tract (GIT) is sufficiently mature to digest significant amounts of non-milk carbohydrates and proteins. In contrast to humans, rodents (e.g. rats, mice) and carnivores (e.g. mink) have a very immature GIT at birth, and adult diets are poorly tolerated until shortly before weaning. Adult-type GIT functions develop rapidly, but not until quite late, e.g. postnatally. In the pig (and the other large domestic species), both the timing and the rate of GIT maturation are intermediate to those in precocious species (primates, guinea pigs) and altricial species (rodents, carnivores), respectively. Hence, the major developmental events occur in the immediate perinatal period, with more

gradual changes postnatally. These species-dependent patterns of GIT maturation are illustrated in Fig. 1.1.

At the time of birth, the pig becomes dependent on its own uptake of nutrients for the first time, and the functional characteristics of the GIT must either be sufficiently mature before birth or change rapidly after birth to meet this challenge. As already indicated in Fig. 1.1, the maturation of some key GIT functions in the pig occurs quite rapidly shortly before and after birth, in association with the transition from placental (parenteral) nutrition to enteral (oral) nutrition. Rapid maturation at this time makes pigs (and the other large farm animals) particularly vulnerable to disease, when GIT development in the neonate is immature. It is therefore important to know the factors that influence GIT function in the neonatal pig. This review will focus on the developmental changes that occur in growth, digestive function (enzyme activities) and absorptive

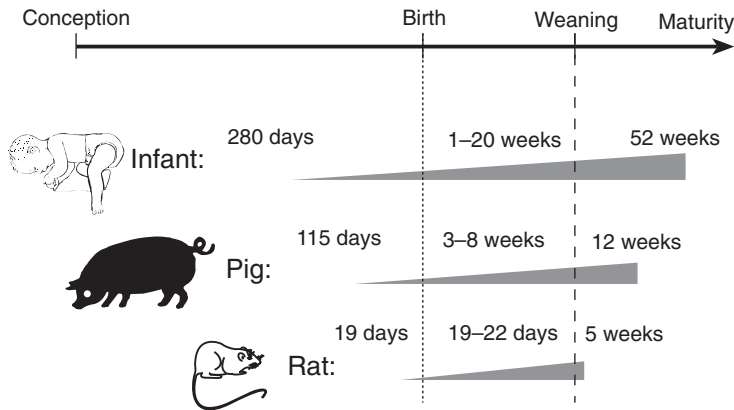


Fig. 1.1. Progression of gastrointestinal maturation in three different species. In farm animals (e.g. pigs), GIT maturational changes occur mainly from shortly before birth to just after weaning. In humans, maturation is slower and starts relatively early (fetal life), while in the rat most changes occur relatively fast and late (postnatally).

capacity (nutrients and immunoglobulins) of the GIT in the immediate perinatal period.

Physiological Transitions at the Time of Birth

It is well documented that the rate of metabolism and maturation of the pig GIT is extremely rapid during the neonatal period (Reeds *et al.*, 1993), when the animal for the first time becomes fully dependent on enteral nutrient intake, via the GIT. Less information is available on the growth and metabolism of the GIT before birth. Nevertheless, it is clear that the rate of cell turnover, and probably the oxygen-demanding cell metabolism, is much slower before birth than after birth (Trahair and Sangild, 1997). Teleologically, it would also be a 'waste of resources' to let the GIT in the fetal pig metabolize a large proportion of the amino acid intake, like in the neonate (Reeds *et al.*, 1993). What prevents the fetal gut from achieving a rate of metabolism and cell turnover similar to that in neonates? Besides the lack of luminal substrates, which may play a separate role (see later), the oxygen supply may be important. The fetus is well adapted to live in a low-oxygen environment for its general

growth and metabolism. At birth, blood arterial oxygen levels increase dramatically (e.g. from 50 to 100% oxygen saturation) as a result of the circulatory changes and the onset of independent respiratory function (Sangild *et al.*, 1996b). This change in blood oxygenation is probably a trigger to the start of a 'new era' of gut function. If the rise in arterial blood oxygenation does not occur immediately after birth, due to immature lung function or birth difficulties (asphyxia), then the developing GIT is severely affected. Thus, neonatal hypoxia is associated with severe malfunction of the intestine and an increased incidence of enterocolitis (Cohen *et al.*, 1991; Powell *et al.*, 1999). In addition to the changes in nutrition and oxygen delivery, a number of other physiological and physical changes occur at birth. Any of these changes may act as a signal for GIT development in the immediate neonatal period. The physiological transitions at birth are illustrated in Fig. 1.2.

Birth-related Changes in Cortisol Secretion

Many hormones and growth factors are potential developmental regulators of GIT structure and function. In this context, we

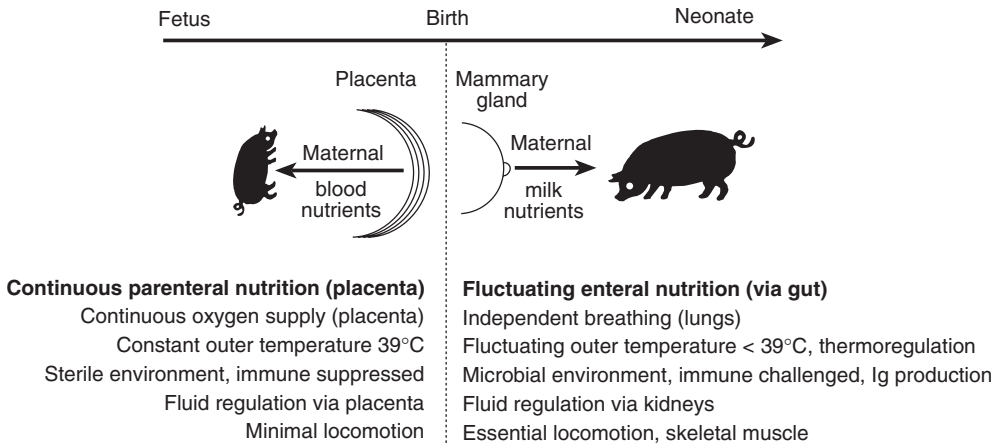


Fig. 1.2. The major physiological and physical changes that occur at birth. These changes may act as signals for maturation of many organs, including the GIT. The GIT assumes a new role by the transition from parenteral to enteral nutrition at birth.

will focus mainly on cortisol from the adrenal cortex because this steroid hormone has been shown to have the most general maturational role for organs in the late fetal and early neonatal periods. The elevated cortisol levels at this time stimulate the functional maturation of GIT (reviewed by Trahair and Sangild, 1997) and other essential organs (e.g. lungs, liver, kidneys) (Liggins, 1976; Silver, 1990). In the pig (and other large domestic species), the major developmental increase in adrenocortical function occurs in late gestation, such that glucocorticoid levels are at their highest at term (Silver and Fowden, 1989; Sangild *et al.*, 1994a). This increase in function is driven by the corresponding high levels of ACTH (adrenocorticotrophic hormone) from the pituitary, which stimulate not only cortisol secretion but also adrenal gland growth. Postnatally, glucocorticoid levels normally decrease and show only minimal changes around the time of weaning in pigs (Sangild *et al.*, 1991). The developmental change in cortisol secretion and its relation to adrenal gland weight are shown in Fig. 1.3 (left panel).

In relation to the acute changes taking place at birth in the pig GIT, it is of interest to know whether the final increase in cortisol levels is driven by parturition itself, or whether it is more dependent on fetal age.

Since the prepartum increase has already started 2 weeks before birth, it can be concluded that at least part of the change is time dependent. However, the fact that newborn pigs delivered by vaginal birth have higher plasma cortisol levels than newborn pigs delivered by elective caesarean section (no parturition) indicates that the final rise in cortisol is related to the labour process (Fig. 1.3, right panel). This is substantiated by the fact that the difference between delivery types is present irrespective of gestational age in the interval 100–115 days (Sangild *et al.*, 1995a). Thus, the mode of delivery may influence GIT development via changes in the circulating cortisol levels around birth.

Birth-related Changes in the Mode of Nutrient Administration

Before birth the fetal pig receives its nutrients for growth and development via the placental circulation (Fig. 1.2). These elemental maternal nutrients (amino acids, glucose, minerals, vitamins) pass into the pig via the hepatic portal vein. In contrast to the neonatal GIT, the fetal GIT is therefore exposed to nutrients mainly from the circulation (parenteral nutrition) and not from the GIT lumen (enteral nutrition).

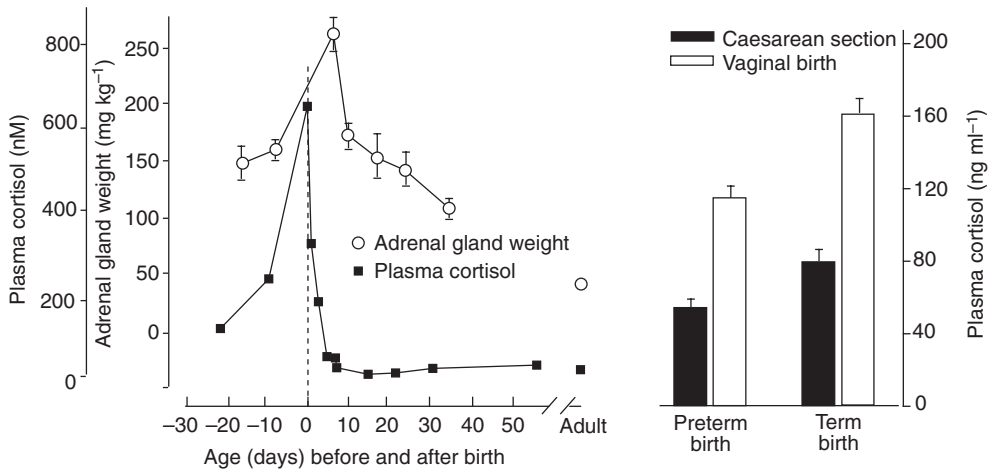


Fig. 1.3. Plasma cortisol and relative adrenal gland weights in pigs before and after birth (left panel). Plasma cortisol in newborn premature and term pigs born either by elective caesarean section or after vaginal delivery (right panel). Cortisol level in newborn vaginally delivered pigs is higher than at any other time throughout life. Cortisol stimulates the pre- and neonatal functional maturation of the GIT.

Although the fetal GIT seems to have little to do *in utero*, it is not completely devoid of material passing through it. The fetus swallows large amounts of amniotic fluid each day (about 20% of body weight). Although the nutrient content is relatively low (< 1% protein), swallowed amniotic fluid protein has been estimated to contribute up to 20% of fetal energy demands (Mulvihill *et al.*, 1985). Does it matter for GIT epithelial cells whether they are exposed to nutrients from the apical side or from the basolateral side of the cell membrane? A series of studies in both fetuses and newborns has documented that not only the quality and quantity of nutrients, but also the mode of nutrient administration, parenteral versus enteral nutrition, plays a role in determining the growth and development of the GIT in the period around birth. Some examples of these influences will be given later.

Growth of the Gastrointestinal Tissues

In the case of the gastrointestinal tract, the size of the organ has a profound influence on its functional capacity. Not only the

presence and tissue concentration of mucosal enzymes and nutrient transporters are important, but also the total hydrolytic or absorptive capacity as reflected in the total mucosal surface area. Hence, a function that normally decreases in concentration with age on a specific tissue basis (e.g. the postnatal decline in lactase activity per gram intestine) may show a developmental increase in total hydrolytic capacity for the entire intestine because of the concomitant increase in tissue weight (Zhang *et al.*, 1997; Park *et al.*, 1998). It is therefore important to describe the functional maturation of the GIT both on a tissue-specific basis and on a total organ basis to get a true picture of physiological development.

In the weeks before birth, the ratio of stomach to body weight is constant. Within the first week after birth it increases by about 25% to reach its maximal relative weight at 1 week (Fig. 1.4). Neither before nor after birth is the relative stomach weight stimulated by cortisol (Sangild *et al.*, 1991, 1994b; Sangild, 1995). At birth, new demands are also placed upon the endocrine and exocrine pancreas. It is therefore surprising that relative pancreas weight decreases by about 25% during the

last weeks before term (Fig. 1.4). On the other hand, it grows very rapidly after birth, and within the first postnatal week its relative weight is 60–80% higher than at birth. After 1 week of age there is a more gradual weight increase, although some accelerated growth occurs at the time of weaning. In contrast with the stomach and pancreas, the small intestine grows more rapidly than the body as a whole, both before and after birth. During the last 3–4 weeks of gestation the increase in relative weight is 70–80% (Fig. 1.4). The increase immediately after birth is extremely rapid and results in an intestine that has a 50% higher relative weight at 24 h than at birth.

GIT Growth as Affected by Oral Nutrients Before and After Birth

Studies on fetal lambs show that if the fetus is prevented from swallowing amniotic fluid during a substantial part of gestation then the small intestine undergoes some degree of hypotrophy (Trahair and Harding, 1995; Trahair and Sangild, 1999). If amniotic fluid ingestion plays a similar role for pig fetuses, it seems that prevention of the swallowing of amniotic fluid by oesophageal ligation would have the greatest effect on pigs in late gestation, when the intestine grows more rapidly than the body as a whole (Fig. 1.4). Nevertheless,

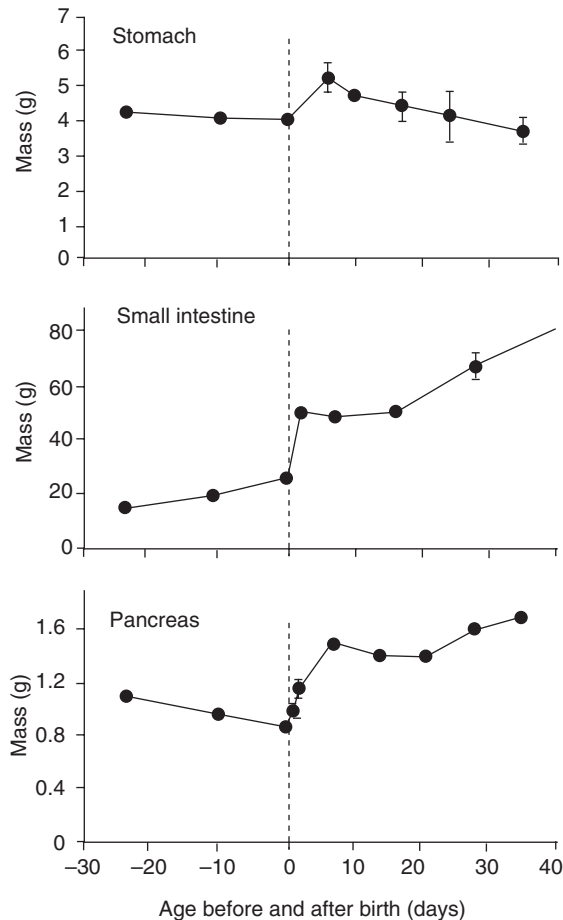


Fig. 1.4. Relative weight (g kg^{-1} body weight) of the different parts of the gastrointestinal tract in pigs. Note the developmental changes taking place around birth.

we have been unable to demonstrate any effect on intestinal growth by ligating the fetal oesophagus at 90 days gestation and removing the pigs at 105 days gestation. Despite the fact that body weight was reduced in the ligated fetuses ($\sim 20\%$), the GIT was apparently less sensitive and needed to be denied access to swallowed amniotic fluid for a longer time before intestinal growth was affected. This indicates that luminal factors may be less important in mediating intestinal growth in the fetal pig than they are in the neonatal pig.

The pioneering studies of Widdowson *et al.* (1976) made it clear that colostrum intake has a very selective effect on the growth and maturation of the gut. In these studies, fasted versus fed newborn pigs were compared and the rapid growth of the pancreas and small intestine in the fed animals was taken to reflect an effect of oral feeding with sow's colostrum. It can be argued, however, that the fasted pigs in these studies were not in a normal physiological state and therefore were not ideal to

show the effect of oral feeding on organ growth. A more appropriate study design is to compare organ growth in pigs receiving the same amount of nutrients either enterally (via the gut) or parenterally (via the circulation). Figure 1.5 shows how the stomach, pancreas and small intestine in pigs respond to 6 days of either enteral or parenteral feeding (Sangild *et al.*, 2000b). From the results it can be concluded that, in the immediate postnatal period, stomach growth is independent of enteral food administration, while pancreas growth is in part dependent on enteral food. Intestinal growth is almost entirely dependent on enteral food, because relative intestinal weight decreased after birth when pigs were fed parenterally (Fig. 1.5). This effect of luminal nutrition is not restricted to feeding of colostrum or natural milk, since enteral feeding of milk formulas or even elemental diets have similar effects on intestinal growth compared with total parenteral nutrition (Park *et al.*, 1998; Burrin *et al.*, 2000). Also, the above effects are not restricted to the postnatal period,

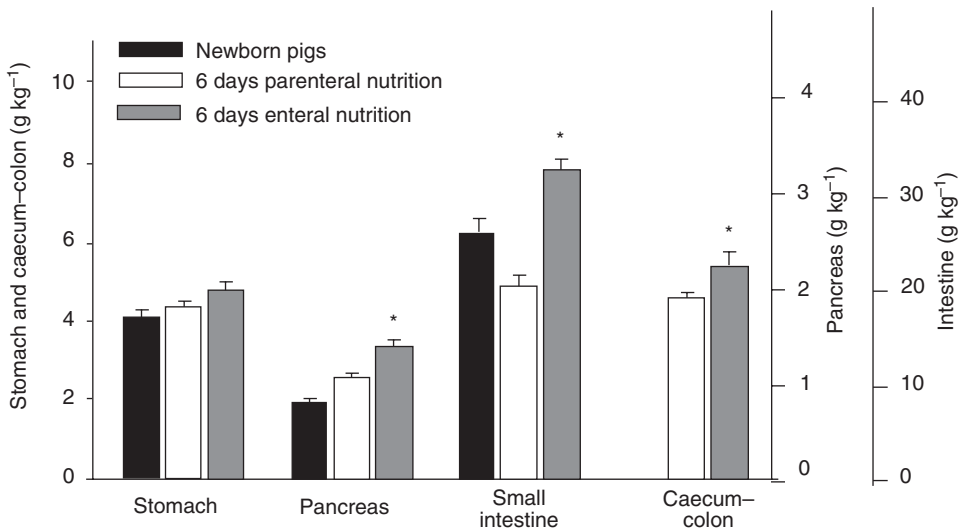


Fig. 1.5. The effect of total enteral nutrition (oral intake of sow's milk) and total parenteral nutrition (TPN, systemic infusion of an elemental diet) on the relative weight of the stomach, pancreas, small intestine and caecum-colon in 6-day-old pigs. Despite similar nutrient intake and body weight in the two fed groups, organ weights were 10–60% higher for pigs fed enteral food. *, $P < 0.05$ relative to the TPN group.

though the lack of enteral nutrients may play a more critical role in the neonate because of the rapid growth and development of the intestine at this time. Thus, the rate of intestinal protein synthesis and metabolism of amino acids have been shown to be extremely high during the first weeks postnatally (Reeds *et al.*, 1993).

Milk and, particularly, colostrum are rich sources of protein and fat but also contain a number of hormones and growth factors that may have a stimulating effect on gut growth. Although a number of studies using single growth factors or hormones have shown that these regulatory components play a role, it remains unclear whether the stimulating effects of colostrum on the growth of the pancreas and small intestine are mediated mainly by nutrient-dependent or nutrient-independent factors. Among the nutrients, proteins are probably most important, due to the high dependence of GIT cell metabolism on amino acids (Reeds *et al.*, 1993). IGF-I and IGF-II are the regulatory factors that have been studied most as effectors on gut growth and differentiation in pigs (Xu *et al.*, 1994; Burrin *et al.*, 1996; Park *et al.*, 1998, 1999), but considerable attention has also been given to the epidermal growth factor (EGF) family of peptide growth factors (James *et al.*, 1987; Shen and Xu, 1998). These peptides are found in large quantities in colostrum and smaller peptide concentrations are also present in the amniotic fluid swallowed by the fetus. It remains, however, that neither of these peptides alone has a pronounced effect on the growth of the developing pig GIT in the complete absence of nutrients. In addition, these growth factors are not specific for the GIT, but have a variety of effects in epithelia and organs throughout the body.

Although IGFs and EGFs may explain in part the high growth-promoting effects of certain fluids (amniotic fluid, colostrum) on the developing pig GIT, they do not explain why the provision of luminal nutrients themselves (without the growth factors) have a direct effect on the growth of the mucosa (Burrin *et al.*, 2000). After birth, the developing GIT may be specifi-

cally dependent on luminal nutrients as an energy and protein source for the enterocytes. On the other hand, it cannot be excluded that the effect of luminal nutrients is, at least in part, mediated via a humoral factor. In this context, a lot of research has recently been devoted to a novel growth factor peptide, glucagon-like peptide 2 (GLP-2), as a mediator of the GIT growth effect of luminal nutrition. GLP-2 is produced in the ileal L-cells and is released in response to a meal high in fat and carbohydrate.

If GLP-2 is a key regulator of the response to luminal nutrients, one would expect this regulatory system to be absent or at least work differently in the fetus. The fetus swallows amniotic fluid protein (which does not stimulate GLP-2 release) and luminal nutrition plays a limited role. In agreement with this, circulating GLP-2 levels are much lower in late-gestation fetuses (5 ± 2 pM), compared with newborn pigs (17 ± 3 pM) or pigs that have received luminal nutrients for a week (60 ± 10 pM) (Sangild, Petersen and Holst, unpublished observations). To support the view that GLP-2 is a potent growth factor for the intestinal mucosa only after birth, when pigs normally receive enteral and not parenteral nutrition, we have compared the effects of exogenous infusion into parenterally fed fetal, premature neonatal and term neonatal pigs using the same dose and treatment regimen. The results for the relative weight of the small intestinal mucosa are shown in Fig. 1.6, which shows that exogenous GLP-2 has a significant effect on mucosal growth after birth (irrespective of age at delivery) but has little, if any, effect on intestinal mucosal growth *in utero*.

Perinatal Development of Stomach Function

In the fetal pig, stomach fluid pH is close to neutral and it is not until the last couple of weeks before birth that gastric acidity starts to increase as a result of HCl production. Gastric fluid pH is 2–4 in newborn unsuckled pigs (Sangild *et al.*, 1994b, 1995a) and

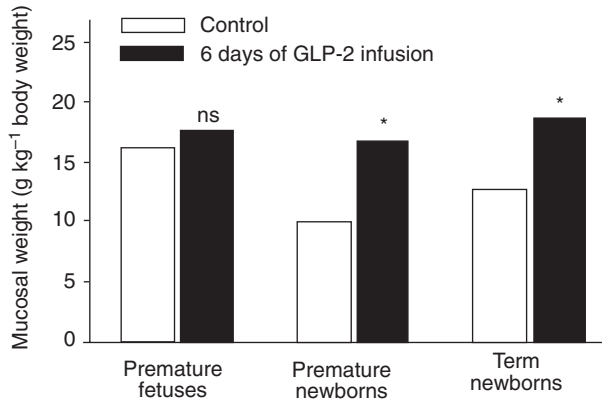


Fig. 1.6. Intestinal mucosal weight relative to body weight in GLP-2 treated fetal, premature neonatal and term neonatal pigs. All groups were infused intravenously with GLP-2 ($96 \mu\text{g kg}^{-1} \text{ day}^{-1}$) or vehicle (control) for 6 days. Newborn pigs were kept on total parenteral nutrition (Petersen *et al.*, 2000a,b). *, $P < 0.05$ relative to controls.

acid-secretory function continues to develop in the immediate postnatal period. As measured by the secretagogue-stimulated acid secretion, mature levels are attained at 5 days of age (Sangild *et al.*, 1992). This profile of development (from 2 weeks before birth to 1 week after birth) is compatible with a role of the prepartum cortisol surge in the development of acid secretion. In addition, the levels of gastrin (the main hormonal stimulator of acid secretion) also develop in parallel with the circulating glucocorticoid levels. Accordingly, treatment of fetal pigs with glucocorticoids induces precocious secretion of acid and gastrin (Sangild *et al.*, 1994a) (Fig. 1.7). However, elevated glucocorticoid levels have little effect on gastrin-stimulated acid secretion after birth (Sangild *et al.*, 1992), which suggests that porcine parietal cells may become unresponsive to cortisol once acid secretion has been initiated.

In fetal pigs, the increases in circulating gastrin levels observed in response to changes in enteral stimuli (Sangild *et al.*, 1996a) are relatively small compared with the response to exogenous cortisol administration (Sangild *et al.*, 1994b) or to the normal rise in circulating gastrin that occurs before birth (Sangild *et al.*, 1994a). Circulating glucocorticoids are therefore

more important in regulating gastrin secretion in the pig during the perinatal period than the presence in the gastric lumen of amniotic fluid before birth or colostrum after birth.

In the pig, gastric protease activity develops either shortly before or just after birth (Sangild *et al.*, 1991). The activity of the proteases depends on an acidic environment (pH 2 to 4) and a shift in the nature of protease zymogens occurs during development. Hence, the major milk-clotting enzymes (chymosins) are predominant during early life; in the pig, stomach chymosin production is maximal at birth (Fig. 1.7). With increasing age, these enzymes are gradually replaced by components exhibiting more general proteolytic activity (pepsins). Immediately before and after birth, glucocorticoids stimulate protease development (Sangild *et al.*, 1994b; Sangild, 1995) (Fig. 1.7). Elevations in glucocorticoid levels during the first 5 weeks of postnatal life continue to induce small increases in some protease activities (Sangild *et al.*, 1991). However, such effects may be of little physiological relevance, because glucocorticoid levels normally decrease during this period (Fig. 1.3). In the fetal and neonatal pig, we have not observed any effect of enteral food on protease activity.

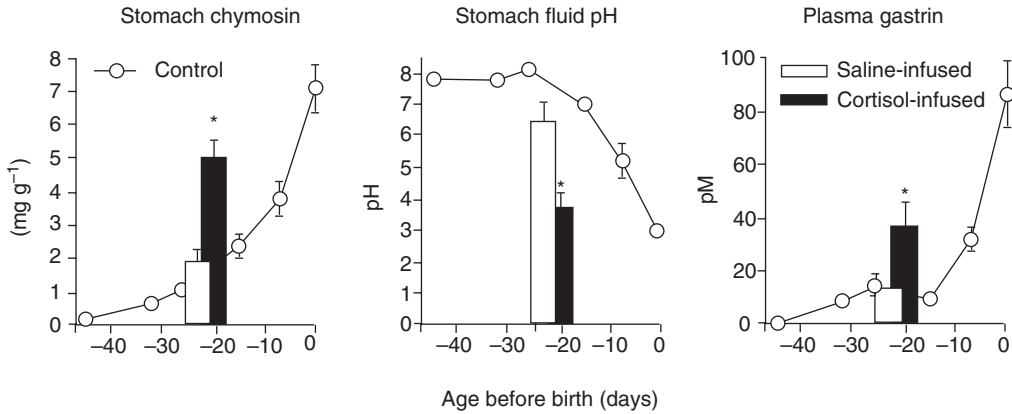


Fig. 1.7. Stomach function before birth, and effects of cortisol infusion on chymosin, acid and gastrin secretion in the fetus. *, $P < 0.05$ relative to saline-infused fetuses.

Perinatal Development of Intestinal Digestive Enzymes

In the fetus, hydrolysis of substrates in the intestinal lumen is minimal (Britton and Koldovsky, 1989) and in immature (vacuolated) enterocytes most of the digestive processes are intracellular, not luminal. However, the prenatal development of brush-border hydrolases is an important component in the preparation for life and nutrition *ex utero*. Among the major pig brush-border enzymes, only lactase and peptidase activities reach notable levels in the fetus, and they reach peak tissue-specific activities at birth or shortly after birth (Sangild *et al.*, 1995b). Glucosidase (sucrase, maltase) activities do not rise until postnatally, paralleling a fall in lactase activity.

The developmental rise in lactase and aminopeptidase activities in the prenatal pig is regulated, at least in part, by cortisol, because infusion of cortisol into immature fetuses stimulates the activity of these enzymes (Sangild *et al.*, 1995b). The lower cortisol level observed in piglets after caesarean delivery (as opposed to spontaneous vaginal birth) is also associated with altered GIT enzymic development in the newborn pig (Sangild *et al.*, 1996b). However, the effects of glucocorticoids on the enzymic development are variable and depend on

the intestinal region and the specific enzyme. These variable effects may be due to differences in the number and activity of glucocorticoid receptors on epithelial cells and/or to differences in the molecular mechanisms by which the hormones alter enzyme synthesis in these cells.

Before birth, amniotic fluid ingestion has a documented role in the structural development of the fetal lamb small intestine (see earlier), but its effects on intestinal function is somewhat equivocal (Trahair and Sangild, 1997). In fetal pigs prevented from swallowing amniotic fluid for 1–2 weeks in late gestation, we have not detected notable differences in the activity of gastrointestinal enzymes, including intestinal lactase and aminopeptidase A (Fig. 1.8). Although the lack of amniotic fluid may produce significant effects for selected intestinal enzymes and regions (Sangild *et al.*, 1997a), these effects are quantitatively small and are unlikely to result in major effects on the intestinal digestive capacity. Enterocyte turnover is relatively slow in the fetal intestine and the fetal GIT may require a relatively long exposure to luminal nutrients before this results in altered GIT maturation.

In contrast to the effects of amniotic fluid ingestion, studies in both fetuses and neonates have shown that colostrum intake is associated with changes in the

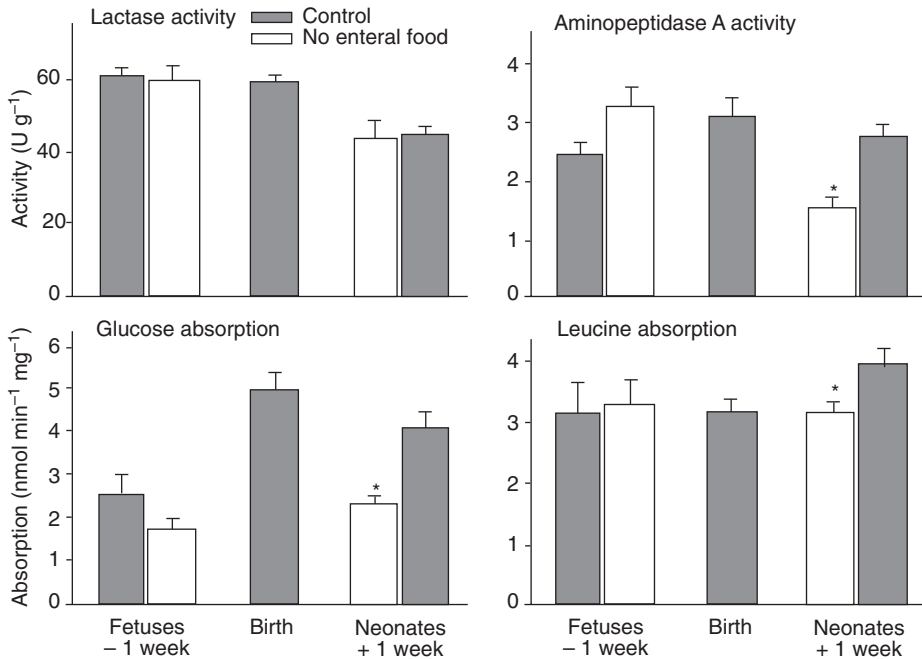


Fig. 1.8. Digestive enzyme activity and nutrient absorption (expressed per tissue weight in the middle small intestine) in fetal pigs (–1 week), newborn pigs and postnatal pigs (+1 week). ‘No enteral food’ groups of pigs represent oesophageal-ligated fetuses and parenterally fed postnatal pigs, respectively. Fetal amniotic fluid ingestion has no effects, while milk intake after birth increases aminopeptidase A activity and glucose/leucine absorptive capacity. *, $P < 0.05$ relative to controls.

tissue-specific activity of brush-border enzymes. Colostrum ingestion has been reported to either increase or decrease the specific activity of lactase (expressed per intestinal weight, protein or DNA) while consistently increasing the specific activities of enzymes such as maltase and aminopeptidases (Sangild *et al.*, 1996b; Wang and Xu, 1996; Zhang *et al.*, 1997). Exposure of the neonatal intestine to colostrum is associated with profound changes both in mucosal structure and in the biochemical events which lead to the insertion of functional enzymes into the brush-border membrane (Dudley *et al.*, 1996). The nature of the nutrient components or regulatory factors in colostrum that causes these structural and functional changes remains unknown and neither the IGFs nor EGFs present in colostrum and milk can adequately account for the changes in enzyme expression and

activity at this time (James *et al.*, 1987; Xu *et al.*, 1994; Park *et al.*, 1998). Likewise, a potent trophic factor released in response to enteral food, GLP-2, does not induce changes in enzyme expression and activity that are identical to those induced by the first intake of colostrum and milk (Petersen *et al.*, 2000a,b).

More gradual changes in intestinal enzyme activities occur after the colostrum period. Hence, at 1 week of age the specific activity of many brush-border enzymes is not notably dependent on enteral milk supply, at least not in the short term. Lactase (Fig. 1.8), maltase, sucrase and aminopeptidase N specific activities were similar in parenterally fed and enterally fed 6-day-old pigs. However, the activity of aminopeptidase A (Fig. 1.8) and dipeptidyl peptidase IV were highest in the pigs fed enterally (Sangild *et al.*, 2000b).

Perinatal Development of Nutrient Absorption

Absorption of nutrients as measured by the uptake of monosaccharides and amino acids by the intestinal mucosa is low during the first half of gestation, but increases rapidly thereafter (Buddington and Malo, 1996). During the final weeks of gestation, there is a particularly rapid increase in glucose uptake capacity (Sangild *et al.*, 1993, 2000a), while the ability of the mucosa to take up most amino acids remains unchanged (Fig. 1.8). The possibility that the prenatal cortisol rise stimulates the increase in glucose uptake capacity, and possibly the expression of its sodium–glucose coupled transporter protein (SGLT-1), has not been investigated in pigs or in other large domestic species. However, our recent studies on fetal pigs (unpublished) have shown no significant correlation between circulating cortisol levels and glucose uptake capacity. Most likely, glucose uptake function is less sensitive to glucocorticoid induction in the fetus than the enzyme responsible for the release of glucose from milk lactose, lactase-phloridzin hydrolase. Likewise, the lack of swallowed amniotic fluid has no apparent effect on the normal prepartum development of absorptive capacity for glucose and amino acids (Fig. 1.8).

Just after birth when the newborns begin to suckle, there is a dramatic decrease in the tissue-specific uptake of both glucose and amino acids which may be associated with the colostrum-induced changes of the microvillus membrane (Zhang *et al.*, 1997). Nevertheless, total transport capacities along the entire length of the small intestine remain constant or even increase due to the large increase in intestinal mass (Buddington *et al.*, 2000). In the days after the colostrum period, the tissue-specific nutrient uptakes increase again, probably due to the insertion of new nutrient transporters into the microvillus membrane. Interestingly, this process depends on enteral nutrition, since parenterally fed 1-week-old pigs show significantly lower uptakes of glucose and leucine than corre-

sponding enterally fed pigs (Fig. 1.8). In conclusion, the transition from mainly par-enteral nutrition in the fetus to enteral milk nutrition in the neonate stimulates not only the amount of intestinal tissue (Fig. 1.5) but also the concentration of certain intestinal nutrient transporters (Fig. 1.8).

Perinatal Development of Intestinal Immunoglobulin Absorption

One of the most striking features of the small intestine in most newborn animals is its ability to take up macromolecules, including immunoglobulins, and to transport them intact across the epithelium into the circulation. The ability to take up bulk amounts of macromolecules by (un)specific pinocytosis provides the neonate with the passive immunity present in the mother's colostrum. Only in species that have extensive transplacental transfer of immunoglobulins to the fetus before birth (e.g. primates) is the protein-absorptive capacity of the neonatal intestine negligible. In the pig, this ability ceases within the first day or two after birth, by a process known as 'intestinal closure' (Weström *et al.*, 1984). Intestinal macromolecule uptake is present *in utero* during the last 2 weeks of gestation but it is markedly less in the fetus than in the neonate (Sangild *et al.*, 1999). These observations suggest that the ability to take up and transfer intact proteins from the epithelium into the circulation is a very specific process that develops close to term. This hypothesis is supported by the finding that the capacity for protein absorption is lower in newborn piglets delivered prematurely than in those born at full term (Sangild *et al.*, 1997b). Hence, the uptake of macromolecules by the newborn farm animal is not merely a result of 'an immature or leaky epithelium', but it reflects a specific maturational process that is timed to maximize immunoglobulin uptake just after birth. The perinatal development in the ability to absorb macromolecules is illustrated in Fig. 1.9.

Studies in rats and mice have shown that glucocorticoids stimulate the normal

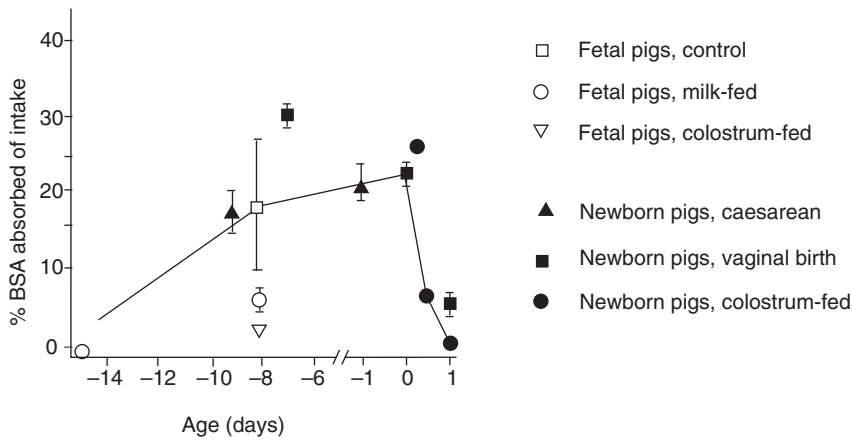


Fig. 1.9. Perinatal development of the intestinal capacity to absorb macromolecules. The results show proportion of bovine serum albumin (BSA) absorbed within 6 h of infusion of sow's colostrum + BSA. Results are compiled from a number of different studies (Weström *et al.*, 1984, 1985; Sangild *et al.*, 1999).

advancement of intestinal closure, which occurs at 2–3 weeks in these species. In the pig, the effects of glucocorticoids are opposite, in that the low cortisol levels associated with preterm birth by elective caesarean section (Sangild *et al.*, 1997b) or after metyrapone treatment of newborn pigs (Sangild *et al.*, 1993) are associated with severely reduced IgG uptake capacity. Nevertheless, it seems that the time of intestinal closure to macromolecule transport in the pig and other farm animals is influenced more by luminal factors than by endocrine factors such as glucocorticoids. Studies in fetal and neonatal pigs (Weström *et al.*, 1985; Sangild *et al.*, 1999) indicate that colostrum itself plays the most important role in the induction of intestinal closure. When colostrum whey was infused into the fetal pig intestine at 101–107 days gestation, the uptake of macromolecules declined from 104 days onwards. In contrast, fetuses infused with amniotic fluid or milk whey remained fully capable of taking up macromolecules during the entire period of infusion (Fig. 1.9). In these studies, some fetuses had elevated levels of plasma cortisol (probably in response to surgery), but this did not alter the time available for macromolecule absorption in these fetuses. Hence, the ability of the

developing small intestine to absorb macromolecules is an excellent example of how the pattern of GIT development varies among species (rodents, farm animals) and how the effects of regulatory factors (e.g. cortisol) differ.

Conclusions and Perspectives

The main focus of this review has been on the development of the pig gastrointestinal tract (GIT) in the immediate perinatal period (1–2 weeks before and after birth). During this period, and particularly during the process of birth, a series of profound changes occurs in the physical and physiological environment of the pig. Some of these changes affect the maturation of the GIT and thus the ability of the pig to digest and absorb the large amounts of nutrients provided immediately after birth. Glucocorticoid hormones and the first colostrum intake both play central roles as modifiers of the genetic 'biological clock' of gastrointestinal maturation, but they interact with a number of other factors.

Both the GIT ontogenetic pattern and its regulatory factors differ among species although there are many similarities within the large farm animals (pig, cow, horse,

sheep, goat). All these species show a relatively high perinatal mortality (e.g. 10–20%). A large part of this mortality may arise because the newborns in these species are born at a time in gestation when they are only just able to survive. Thus, the final prepartum maturation of the fetal tissues and organs, including the gastrointestinal tract, plays a critical role in determining neonatal viability. Improving neonatal viability therefore depends on identifying the precise nature of the systemic and luminal factors that influence GIT development during the critical period just before and after birth. Such studies will also help to optimize the clinical and

nutritional care of farm animals born both at term and prematurely.

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References

- Britton, J.R. and Koldovsky, O. (1989) Development of luminal digestion: implications for biologically active dietary polypeptides. *Journal of Pediatric Gastroenterology and Nutrition* 9, 144–162.
- Buddington, R.K. and Malo, C. (1996) Intestinal brush-border membrane enzyme activities and transport functions during the prenatal development of pigs. *Journal of Pediatric Gastroenterology and Nutrition* 23, 51–64.
- Buddington, R.K., Elnif, J., Puchal, A.A. and Sangild, P.T. (2001) Intestinal apical amino acid absorption during development of the pig. *American Journal of Physiology* 280, R241–R247.
- Burrin, D.G., Wester, T.J., Davis, T.A., Amick, S. and Heath, J.P. (1996) Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. *American Journal of Physiology* 270, R1085–R1091.
- Burrin, D.G., Stoll, B., Jiang, R., Chang, X., Hartmann, B., Holst, J.J., Greeley, G.H. and Reeds, P.J. (2000) Minimal enteral nutrient requirements for neonatal intestinal growth in piglets: how much is enough? *American Journal of Clinical Nutrition* 71, 1603–1610.
- Cohen, I.T., Nelson, S.D., Moxley, R.A., Hirsh, M.P., Counihan, T.C. and Martin, R.F. (1991) Necrotizing enterocolitis in a neonatal piglet model. *Journal of Pediatric Surgery* 26, 598–601.
- Dudley, M.A., Burrin, D.G., Quaroni, A., Rosenberger, J., Cook, G., Nichols, B.L. and Reeds, P.J. (1996) Lactase phlorhizin hydrolase turnover *in vivo* in water-fed and colostrum-fed newborn pigs. *Biochemical Journal* 15, 735–743.
- James, P.S., Smith, M.W., Tivey, D.R. and Wilson, T.J.G. (1987) Epidermal growth factor selectively increases maltase and sucrase activities in neonatal piglet intestine. *Journal of Physiology* 393, 583–594.
- Liggins, G.C. (1976) Adrenocortical-related maturational events in the fetus. *American Journal of Obstetrics and Gynecology* 126, 931–941.
- Mulvihill, S.J., Stone, M.M., Debas, H.T. and Fonkalsrud, E.W. (1985) The role of amniotic fluid in fetal nutrition. *Journal of Pediatric Surgery* 20, 668–672.
- Park, Y.K., Monaco, M.M. and Donovan, S.M. (1998) Delivery of total parenteral nutrition (TPN) via umbilical catheterization: development of a piglet model to investigate therapies to improve gastrointestinal structure and enzyme activity during TPN. *Biology of the Neonate* 73, 295–305.
- Park, Y.K., Monaco, M.H. and Donovan, S.M. (1999) Enteral insulin-like growth factor-I augments intestinal disaccharidase activity in piglets receiving total parenteral nutrition. *Journal of Pediatric Gastroenterology and Nutrition* 29, 198–206.
- Petersen, Y.M., Elnif, J., Schmidt, M., Burrin, D.G., Hartmann, B., Holst, J.J. and Sangild, P.T. (2000a) Brush-border enzymes are differentially regulated by glucagon-like peptide 2 in the newborn pig maintained on total parenteral nutrition. *Gastroenterology* 118, A76.

- Petersen, Y.M., Hartmann, B., Schmidt, M., Holst, J.J. and Sangild, P.T. (2000b) Exogenous glucagon-like peptide 2 has a limited effect on mucosal growth and enzyme activity in fetuses when compared to neonates. *Gastroenterology* 119, A561.
- Powell, R.W., Dyess, D.L., Collins, J.N., Roberts, W.S., Tacchi, E.J., Swafford, A.N. Jr, Ferrara, J.J. and Ardell, J.L. (1999) Regional blood flow response to hypothermia in premature, newborn, and neonatal piglets. *Journal of Pediatric Surgery* 34, 193–198.
- Reeds, P.J., Burrin, D.G., Davis, T.A. and Fiorotto, M.L. (1993) Postnatal growth of gut and muscle: competitors or collaborators. *Proceedings of the Nutrition Society* 52, 57–67.
- Sangild, P.T. (1995) Stimulation of gastric proteases in the neonatal pig by a rise in adrenocortical secretion at parturition. *Reproduction, Fertility and Development* 7, 1293–1298.
- Sangild, P.T., Foltmann, B. and Cranwell, P.D. (1991) Development of gastric proteases in fetal pigs and pigs from birth to thirty six days of age. The effect of adrenocorticotropin (ACTH). *Journal of Developmental Physiology* 16, 229–238.
- Sangild, P.T., Cranwell, P.D. and Hilsted, L. (1992) Ontogeny of gastric function in the pig: acid secretion and the synthesis and secretion of gastrin. *Biology of the Neonate* 62, 363–372.
- Sangild, P.T., Diernæs, L., Christiansen, I.J. and Skadhauge, E. (1993) Intestinal transport of sodium, glucose and immunoglobulin in neonatal pigs. Effect of glucocorticoids. *Experimental Physiology* 78, 485–497.
- Sangild, P.T., Hilsted, L., Nexø, E., Fowden, A.L. and Silver, M. (1994a) Secretion of acid, gastrin and cobalamin-binding proteins by the fetal pig stomach: developmental regulation by cortisol. *Experimental Physiology* 79, 135–146.
- Sangild, P.T., Silver, M., Fowden, A.L., Turvey, A. and Foltman, B. (1994b) Adrenocortical stimulation of stomach development in the prenatal pig. *Biology of the Neonate* 65, 378–389.
- Sangild, P.T., Hilsted, L., Nexø, E., Fowden, A.L. and Silver, M. (1995a) Vaginal birth versus elective caesarean section: effects on gastric function in the neonate. *Experimental Physiology* 80, 147–157.
- Sangild, P.T., Sjöström, H., Norén, O., Fowden, A.L. and Silver, M. (1995b) The prenatal development and glucocorticoid control of brush-border hydrolases in the pig small intestine. *Pediatric Research* 37, 207–212.
- Sangild, P.T., Hilsted, L., Silver, M. and Fowden, A.L. (1996a) Gastrin secretion in response to amniotic fluid or milk in the fetal pig GI tract. *Regulatory Peptides* 64, 167.
- Sangild, P.T., Silver, M., Schmidt, M. and Fowden, A.L. (1996b) The perinatal pig in pediatric gastroenterology. In: Tumbleson, M.E. and Schnook, L. (eds) *Advances in Swine in Biomedical Research*. Plenum Press, New York, pp. 745–756.
- Sangild, P.T., Holtug, K., Diernæs, L., Schmidt, M. and Skadhauge, E. (1997a) Birth and prematurity influence intestinal function in the newborn pig. *Comparative Biochemistry and Physiology* 118A, 359–362.
- Sangild, P.T., Trahair, J.F., Silver, M. and Fowden, A.L. (1997b) Luminal fluids affect intestinal enzyme activities in prenatal pigs. *European Association of Animal Production* 88, 194–197.
- Sangild, P.T., Trahair, J.F., Loftager, M.K. and Fowden, A.L. (1999) Intestinal macromolecule absorption in the fetal pig after infusion of colostrum *in utero*. *Pediatric Research* 45, 595–602.
- Sangild, P.T., Fowden, A.L. and Trahair, J.F. (2000a) How does the fetal gastrointestinal tract develop in preparation for enteral nutrition after birth? *Livestock Production Science* 66, 141–150.
- Sangild, P.T., Petersen, Y.M., Elnif, J., Schmidt, M., Buddington, R.K. and Burrin, D.G. (2000b) Premature and term newborn pigs differ in their intestinal response to parenteral and enteral nutrition. *Gastroenterology* 119, A76.
- Shen, W.H. and Xu, R.J. (1998) Stability and distribution of orally administered epidermal growth factor in neonatal pigs. *Life Sciences* 63, 809–820.
- Silver, M. (1990) Prenatal maturation, the timing of birth and how it may be regulated in domestic animals. *Experimental Physiology* 75, 285–307.
- Silver, M. and Fowden, A.L. (1989) Pituitary–adrenocortical activity in the fetal pig in the last third of gestation. *Experimental Physiology* 74, 197–206.
- Trahair, J.F. and Harding, R. (1995) Restitution of fetal swallowing restores intestinal growth after mid-gestation oesophageal obstruction. *Journal of Pediatric Gastroenterology and Nutrition* 20, 156–161.
- Trahair, J.F. and Sangild, P.T. (1997) Systemic and luminal influences on the perinatal development of the gut. *Equine Veterinary Journal* 24 (Suppl.), 40–50.

- Trahair, J.F. and Sangild, P.T. (1999) Enteral diet composition alters development of the GIT and other fetal organs. *Gastroenterology* 116, A582.
- Wang, T. and Xu, R.J. (1996) Effects of colostrum feeding on intestinal development in newborn pigs. *Biology of the Neonate* 70, 339–348.
- Weström, B.R., Svendsen, J., Ohlsson, B.G., Tagesson, C. and Karlsson, B.W. (1984) Intestinal transmission of macromolecules (BSA and FITC-labelled dextrans) in the neonatal pig. *Biology of the Neonate* 46, 20–26.
- Weström, B.R., Ohlsson, B.G., Svendsen, J., Tagesson, C. and Karlsson, B.W. (1985) Intestinal transmission of macromolecules (BSA and FITC-dextran) in the neonatal pig: enhancing effect of colostrum, proteins and proteinase inhibitors. *Biology of the Neonate* 47, 359–366.
- Widdowson, E.M., Colombo, V.E. and Artavanis, C.A. (1976) Changes in the organs of pigs in response to feeding for the first 24 hours after birth. II. The digestive tract. *Biology of the Neonate* 28, 272–81.
- Xu, R.J., Mellor, D.J., Birtles, M.J., Breier, B.H. and Gluckman, P.D. (1994) Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. *Biology of the Neonate* 66, 280–287.
- Zhang, H., Malo, C. and Buddington, R.K. (1997) Suckling induces rapid intestinal growth and changes in brush border digestive functions of newborn pigs. *Journal of Nutrition* 127, 418–426.

2 Effects of Dietary Fermentable Carbohydrates on the Empty Weights of the Gastrointestinal Tract in Growing Pigs

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In two experiments, different types and levels of dietary fermentable carbohydrates were fed to group-housed, growing pigs. The experiments showed that increasing the amounts of dietary fermentable carbohydrates mainly increased the empty weight of the stomach and colon. Addition of straw to the diet increased the empty weight of most parts of the gastrointestinal tract, but in particular that of the stomach. Fermentation in the gastrointestinal tract mainly increased the empty weight of the colon. Type of dietary fermentable carbohydrates did not affect the empty weight of the gastrointestinal tract.

Introduction

The interest of pig nutritionists in the role of dietary fermentable carbohydrates is

increasing. There is evidence that the amount of net energy from dietary fermentable carbohydrates in growing pigs equals that of dietary starch (Schrama *et*

al., 1998). However, dietary fibre may increase the slaughtering losses due to an increased weight of the digestive tract (e.g. Jorgensen *et al.*, 1996). In this chapter, two studies on the effects of dietary fermentable carbohydrates on the empty weights of different parts of the gastrointestinal tract (GIT) are discussed.

Materials and Methods

In experiment 1, eight groups of 14 pigs each were used. The groups were fed one of four experimental diets. Two experimental diets contained gelatinized maize starch as carbohydrate source (easily digestible) and two contained raw potato starch (stimulation of fermentation). The difference between diets with the same carbohydrate source was the addition of 15% milled wheat straw (no fermentation) to one of those diets. Thus, the effects of fermentation and diet volume (i.e. straw addition) on the empty weights of the GIT were studied. At dissection of the GIT (12 pigs per treatment), pigs had been fed the experimental diet for 7 weeks and had an average live body weight (BW) of 55.3 kg. In experiment 2, 20 groups of 14 pigs each were used. The groups were fed one of ten experimental diets. Five experimental diets contained solvent-extracted coconut meal and the other five soybean hulls as fermentable carbohydrate source. The diets with the same fermentable carbohydrate

source differed in the amount added (4%, 15%, 26%, 37%, and 48%) of either solvent-extracted coconut meal or soybean hulls, which were exchanged with gelatinized maize starch as appropriate. Thus, the effects of type and added amount of dietary fermentable carbohydrates on the empty weights of the GIT were examined. At dissection of the GIT (six pigs per treatment), pigs had been fed the experimental diet for 6 weeks and had an average live BW of 63.0 kg. In both experiments the GIT of the dissected pigs was removed and weighed. The GIT was emptied and separated into the major components – stomach, small intestine, colon and caecum – and weighed. All groups were fed 2.5 times the maintenance requirement for net energy (293 kJ kg^{-0.75} day⁻¹).

Results

Experiment 1 showed that both carbohydrate source and addition of straw affected the empty weight of the GIT. Inclusion of raw potato starch in the diet compared with gelatinized maize starch increased the empty weight of the total GIT by 8.2% (43.2 and 39.7 g kg⁻¹ BW, respectively, *P* = 0.010; Table 2.1). This effect was due to the increased empty weight of the colon (18.5 and 14.3 g kg⁻¹ BW, respectively, *P* < 0.001; Table 2.1). The addition of wheat straw to the diets increased the empty weight of the total GIT by 6.7% (42.9 and

Table 2.1. Effects of carbohydrate source (C) and addition of straw (S) on empty weights (g kg⁻¹ BW) of the gastrointestinal tract (GIT) in growing pigs (Experiment 1).

	Carbohydrate source				SEM	Effects
	Raw potato starch		Gelatinized maize starch			
	No straw	Straw	No straw	Straw		
GIT	42.3	44.2	37.7	41.6	1.3	C**, S*
Stomach	6.0	6.8	6.2	6.7	0.2	S**
Small intestine	16.5	16.6	16.1	18.2	0.7	
Caecum	1.7	1.8	1.7	1.7	0.1	
Colon	18.0	19.0	13.7	14.9	0.7	C***

* *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001.

40.0 g kg⁻¹ BW, respectively, $P = 0.033$; Table 2.1). It seems that the effect of straw addition to the diet is due to an increased empty weight of the stomach, caecum and colon, but only the increase in the empty weight of the stomach was significant (6.1 and 6.7 g kg⁻¹ BW, respectively, $P = 0.004$; Table 2.1). No interactions were found between carbohydrate source and the addition of wheat straw.

Experiment 2 showed that the source of non-starch polysaccharides (NSP) in the diet did not affect the empty weights of the GIT (Table 2.2). There was, however, an effect of the added amount of the feed ingredients (rich in NSP) on the empty weight of the GIT (Table 2.2). The empty weight of the GIT increased with an increasing added amount of dietary fermentable carbohydrates ($P < 0.01$; Table 2.2). This was caused in particular by an

increased empty weight of both the stomach ($P < 0.001$; Table 2.2) and colon ($P < 0.001$; Table 2.2). No interactions between source and added amount of NSP were found.

Conclusions

Addition of straw to the diet increased the empty weight of the stomach of growing pigs. Fermentation in the gastrointestinal tract of growing pigs mainly increased the empty weight of the colon. The type of non-starch polysaccharides in the diet did not affect the empty weight of different parts of the gastrointestinal tract of growing pigs. An increased intake of dietary fermentable carbohydrates increased the empty weight of the stomach and the colon of growing pigs.

Table 2.2. Effects of source^a and added amount of dietary non-starch polysaccharides (NSP) (g kg^{-0.75} day⁻¹) on empty weights (g kg⁻¹ BW) of the gastrointestinal tract (GIT) in growing pigs (Experiment 2).

	Dose of diet ingredient (%) ^a					SEM	Effects ^b
	4	15	26	37	48		
NSP intake (g kg ^{-0.75} day ⁻¹)							
Coconut	8.76	12.45	17.28	22.02	26.23		
Soya	8.54	13.12	18.20	21.03	26.77		
Empty weights							
<i>GIT</i>							
Coconut	42.5	45.9	46.1	46.8	52.7	1.7	D**
Soya	44.7	41.1	45.9	49.0	47.5		
<i>Stomach</i>							
Coconut	6.4	6.7	6.9	7.1	8.2	0.3	D***
Soya	6.6	6.7	7.4	7.9	7.6		
<i>Small intestine</i>							
Coconut	21.9	21.8	22.2	21.4	22.1	1.1	
Soya	23.5	19.3	21.4	23.5	21.5		
<i>Caecum</i>							
Coconut	1.8	1.9	2.1	2.1	2.4	0.3	
Soya	2.0	1.8	2.7	1.8	2.0		
<i>Colon</i>							
Coconut	12.4	15.5	14.9	16.2	20.0	0.8	D***
Soya	12.6	13.4	14.4	15.7	16.3		

^a Dose of either solvent-extracted coconut meal (Coconut) or soybean hulls (Soya) in the diet.

^b D, effect of the added amount of dietary fermentable carbohydrates. ** $P < 0.01$; *** $P < 0.001$.

References

- Jorgensen, H., Zhao, X.Q. and Eggum, B.O. (1996) The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *British Journal of Nutrition* 75, 365–378.
- Schrama, J.W., Bosch, M.W., Verstegen, M.W.A., Vorselaar, A.H.P.M., Haaksma, J. and Heetkamp, M.J.W. (1998) The energetic value of NSP in relation to physical activity in group-housed, growing pigs. *Journal of Animal Science* 76, 3016–3023.
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3 Are the Activities of Intestinal Peptidases Age- and Diet-dependent in Piglets?

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Forty-two piglets were randomly assigned to seven groups differing in their age at sampling (0, 13, 21 and 56 days) and in the composition of their diet, consisting of either sow's milk or dry milk substitute or dry starter feed offered at different ages. After a classical drop between birth and 2–3 weeks of age, the activity of peptidases in the jejunal mucosa of weaned piglets tended to recover the initial level observed at birth. Four weeks after weaning, the presence of plant protein in the diet did not modify the activity of intestinal peptidases, except that of γ -glutamyl transferase.

Introduction

The products released from intraluminal protein digestion by pancreatic enzymes are hydrolysed by peptidases located in the brush-border membrane and in the cytosol of enterocytes of the small intestine. Oligopeptides and dipeptides are hydrolysed by aminopeptidases and by dipeptidases, respectively. The functional development of the small intestine has been examined in a number of studies. However, little information was available on the ontogenesis of the development of intestinal peptidases after weaning in

piglets and on the influence of the composition of the diet.

Material and Methods

Experiments and treatments were conducted according to the European Union regulation concerning the protection of experimental animals. Forty-two Pietrain $\times$ Large White piglets born in the same week in a batch of farrowing sows were randomly assigned to seven groups (G1 to G7) of six animals each, according to age, weight, litter origin and dietary treatments.

Group 1 consisted of newborn unsuckled piglets. Piglets of G2 and G3 were suckled by the sow until slaughter at days 13 and 21, respectively, whereas those of G4 were sow-suckled until day 21 and weaned on a dry starter diet until day 56. The piglets of G5 and G6 were suckled by the sows up to day 13 and fed *ad libitum* a pelleted milk substitute until slaughter on days 21 and 56, respectively. The piglets of G7 were first suckled by the sows up to 13 days and fed pelleted milk substitute between 14 and 21 days, then finally given starter feeds until slaughter at day 56. The specific activities were measured in duodenal, jejunal and ileal parts of the small intestine and expressed as international units (IU) mg^{-1} of protein for aminopeptidase N, dipeptidyl peptidase IV, γ -glutamyl transferase, lactase, maltase, sucrase and alkaline phosphatase.

Results and Discussion

The effect of age (at birth and days 13, 21 and 56 for G1, G2, G3 and G4, respectively) and that of dietary treatments (at day 21, G3 vs. G5 and at day 56, G4 vs. G6 and G7) on the specific activities of seven digestive enzymes in the small intestinal mucosa are reported.

The specific activities of aminopeptidase N and dipeptidyl peptidase IV in the duodenum, the jejunum and the ileum were generally high at birth (G1) but decreased gradually during suckling (Fig. 3.1), in agreement with data of Sangild *et al.* (1991). A tendency for a postweaning recovery in the activity of both enzymes has been observed only in the jejunum at day 56. A similar but significant increase ($P < 0.01$) has also been observed for the activity of γ -glutamyl transferase. The protein content in the small intestinal mucosa doubled ($P < 0.01$) from birth to day 21, but did not change thereafter. Therefore, the initial values of the activity of peptidases at birth expressed as per gram of intestinal mucosa, i.e. 6000 IU g^{-1} for dipeptidyl peptidase IV and aminopeptidase N were fully recovered at day 56 (G4) when that of γ -glutamyl transferase was multiplied by 2.7.

Changes observed after birth in the distribution of aminopeptidase N and dipeptidyl peptidase IV activities in the three segments resulted in the setting of gradients from duodenum to ileum for both enzymes with maximum activities in the distal region, in agreement with previous data (Tarvid *et al.*, 1994; Lizardo *et al.*, 1995).

The specific activities of maltase and sucrase increased at the expense of that of lactase up to day 56 (data not shown), as classically demonstrated. The 1.7-fold increase in alkaline phosphatase activity between day 21 and day 56 demonstrated a further digestive adaptation of the small intestine with age.

At day 21, the specific activity of dipeptidyl peptidase IV in the jejunum was higher in suckling piglets (G3) compared with those fed milk substitute (G5) (Table 3.1). At day 56, the specific activities of γ -glutamyl transferase and alkaline phosphatase were significantly higher for animals weaned at day 21 and fed a dry starter diet (G6 and G7 vs. G4). The activity of aminopeptidase N, despite being non-significant, varied in the same extent. These data seemed to indicate that the presence of plant protein in the diet stimulating the activity of pancreatic proteases (Lizardo *et al.*, 1995) could modulate the

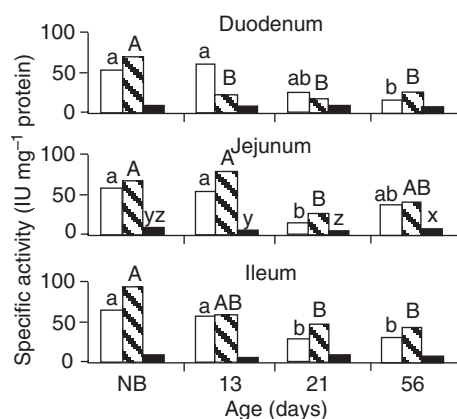


Fig. 3.1. Effect of age and intestinal site on the specific activity of intestinal peptidases (values with a different character differed significantly at $P < 0.05$). □ Aminopeptidase N, ▨ dipeptidylpeptidase IV, ■ glutamyl-transferase.

activity of intestinal peptidases, but to a lesser extent.

Therefore, in contrast to the behaviour of maltase and sucrase activities in the small intestine, after a classical drop between 2 and 3 weeks of age, the activity of peptidases in the small intestinal mucosa of weaned piglets recovered in

general the initial level observed at birth. Four weeks after weaning, and whatever the composition of the diet, it could be hypothesized that peptidases in the brush border of the small intestine were deeply involved in the digestion of dietary protein and in the absorption of amino acids.

Table 3.1. Effect of dietary treatments on the specific activities of intestinal peptidases (IU mg⁻¹ protein).

		Age (days)					Statistical analysis			
		21		56			21 days		56 days	
Site	Peptidase	Group		Group						
		G3	G5	G4	G6	G7	P	RSD	P	RSD
Duodenum	AP N	28.1	20.9	18.2	11.1	19.0	NS	14.0	NS	6.8
	DP IV	20.3	22.0	28.9	22.9	29.9	NS	9.8	NS	8.4
	γ-GT	7.6	8.4	9.2	9.3	8.9	NS	1.9	NS	1.9
Jejunum	AP N	17.5	25.5	40.1	31.1	26.0	NS	10.1	NS	19.2
	DP IV	27.4 ^a	20.1 ^b	41.6	41.1	41.5	*	4.5	NS	20.5
	γ-GT	6.2	7.1	10.7 ^a	9.0 ^b	8.8 ^b	NS	1.1	*	1.1
Ileum	AP N	30.9	31.2	33.2	31.7	26.1	NS	31.9	NS	11.4
	DP IV	48.8	27.9	44.9	41.6	44.9	NS	18.1	NS	13.6
	γ-GT	11.9	8.2	10.1	8.6	8.3	NS	3.7	NS	1.8

AP N, aminopeptidase N; DP IV, dipeptidyl peptidase IV; γ-GT, γ-glutamyl transferase; RSD, residual standard deviation; ^{a,b} values with a different character in the same row differed significantly at $P < 0.01$ (*); NS, not significant.

References

Lizardo, R., Peiniau, J. and Aumaître, A. (1995) Effect of sorghum on performance, digestibility of dietary components and activities of pancreatic and intestinal enzymes in the weaned piglet. *Animal Feed Science and Technology* 56, 67–82.

Sangild, P.T., Cranwell, P.D., Sorensen, H., Mortensen, K., Noren, O., Wetteberg, L. and Sjöström, H. (1991) Development of intestinal disaccharidases, intestinal peptidases and pancreatic proteases in suckling pigs. The effects of age and ACTH treatment. In: Verstegen, M.W.A., Huisman, J. and Den Hartog, L.A. (eds) *Digestive Physiology in Pigs*. Pudoc, Wageningen, pp. 73–78.

Tarvid, I., Cranwell, P.D., Ma, L. and Vavala, R. (1994) The early postnatal development of protein digestion in pigs. II. Small intestinal enzymes. In: Souffrant, W.B. and Hagemester, H. (eds) *Proceedings of the VIth International Symposium on Digestive Physiology in Pigs*. EAAP – Publication No. 80, Dummerstorf, Vol. 1, pp. 181–184.

4 Glucagon-like Peptide 2 Stimulates Small-intestine Growth and Maturation in the Pig Fetus and Neonate

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The goals of the study were to determine whether glucagon-like peptide 2 (GLP-2) stimulates intestinal maturation in the period around birth. GLP-2 was administered to late-gestation fetuses and neonates for 6 days. GLP-2 stimulated intestinal growth in neonates but not in the fetuses. Gut enzyme activity was variably affected by GLP-2 in the fetuses and the neonates.

Introduction

The small intestine of the pig grows significantly, with a concomitant increase in certain disaccharidase and peptidase activities, in the period around birth (Trahair and Sangild, 1997). Glucagon-like peptide 2 (GLP-2) is a 33-peptide, released from the L-cell of the ileum in response to food intake (Jeppesen *et al.*, 1999). GLP-2 significantly increases small-intestinal weight through the induction of intestinal epithelial proliferation (Drucker *et al.*, 1996) and stimulates brush-border (BB) enzyme activities (Brubaker *et al.*, 1997).

The goal of this study was to test whether exogenous infusion of GLP-2 into parenterally fed pigs (absence of enteral food stimulation) could influence small-intestine growth and enzyme activities around the time of birth.

Materials and Methods

Animals

Thirteen newborn pigs (Large White × Danish Landrace) from two litters, delivered by caesarean section at 114–115 days of gestation (term = 115 ± 2 days), were used for postnatal GLP-2 studies. The piglets were fitted with a vascular catheter in the hepatic-portal vein and received either parenteral nutrition alone (TPN, $n = 6$) or parenteral nutrition plus GLP-2 (GLP-2, $n = 7$) for 6 days. The infusions were given intravenously, twice daily, and consisted of either human GLP-2 (12.5 nmol kg^{-1} per 2 h) or the vehicle (0.1% porcine serum albumin in 0.9% saline). After the infusion period, the pigs were killed for tissue collection. Small intestinal length and weight were recorded. The intestine was

divided into three segments of equal length, designated the proximal, middle and distal. Intestinal samples were frozen in liquid nitrogen and kept at -80°C for later determination of enzyme mRNA levels and activity. Blood was collected daily for determination of basal GLP-2 levels by radioimmunoassay (Jeppesen *et al.*, 1999).

Six pregnant sows were used to study the effects of GLP-2 *in utero*. Under anaesthesia, three fetuses per sow were prepared with a carotid artery catheter at 100 days of gestation (term = 115 days). For 6 days, the fetuses were infused intra-arterially, with either GLP-2 ($n = 7$) or saline ($n = 6$) as above. The fetuses were removed by caesarean section at 106 days of gestation and killed for tissue collection.

Enzyme mRNA measurements

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to assess maltase-glucoamylase (MGA) (EC 3.2.1.20), sucrase-isomaltase (SI) (EC 3.2.1.48-10), aminopeptidase N (ApN) (EC 3.4.11.2), aminopeptidase A (ApA) (EC 3.4.11.7) and dipeptidyl peptidase IV (DPP IV) (EC 3.4.14.5) mRNA expression in the small intestine. Total RNA was extracted by the guanidium phenol chloroform method (Chomczynski and Sacchi, 1987) and reverse transcribed. Specific primers were designed for all the genes tested using the cDNA sequences in the Blast database with accession numbers NM_004668, NM_001041, AF176122, U66371 and X73278, respectively. Optimal conditions were established for all genes and a cycle number in the linear range of amplification was chosen for the fetal and neonatal guts. Lactase-phloridzin hydrolase (LPH) (EC 2.1.23-62) was determined by Northern blot techniques. The results were analysed by densitometry and presented as the ratio of the gene to 18S rRNA (arbitrary units, AU).

Enzyme activity measurements

Enzymes were extracted from the frozen samples and activity was determined with specific enzyme substrates (Sangild *et al.*, 1995).

Results

Basal GLP-2 concentrations in the GLP-2-treated fetuses were 252 ± 98 pM vs. 14 ± 2 pM in the controls. Intestinal weight and length were unchanged with GLP-2. Fetal MGA, ApA mRNA and DPP IV mRNA levels were significantly decreased by GLP-2 (0.17 ± 0.04 vs. 1.5 ± 0.1 AU, 0.08 ± 0.02 vs. 0.9 ± 0.1 AU and 0.8 ± 0.1 vs. 1.6 ± 0.1 AU, respectively). MGA and ApA activities were unchanged but DPP IV activity was significantly increased (2.27 ± 0.15 vs. 1.67 ± 0.1 U g $^{-1}$ tissue). ApN mRNA was unchanged but ApN activity (5.35 ± 0.54 vs. 8.55 ± 0.57 U g $^{-1}$ tissue) was increased with GLP-2 treatment.

Basal GLP-2 concentrations in GLP-2-treated neonates were 309 ± 120 pM vs. 23 ± 5 pM in the controls. Small-intestinal weight was significantly increased by GLP-2 treatment (43.7 ± 1.6 g vs. 32.24 ± 0.6 g; $P < 0.05$). Tissue-specific MGA mRNA levels (1.8 ± 0.1 vs. 1.14 ± 0.2 AU) and activity were significantly increased by GLP-2 treatment. LPH, SI, ApN, ApA and DPP IV mRNA levels and activities were unchanged but the values for the total intestine were generally elevated because of the increase in intestinal mass.

Conclusions

Exogenous GLP-2 stimulates gut growth in neonatal pigs but not in fetuses. GLP-2 appears to exert its effects at the transcriptional and post-transcriptional level by perhaps affecting BB enzyme mRNA stability, enzyme processing efficiency or proteolysis in pig fetuses and neonates.

References

- Brubaker, P.L., Izzo, A., Hill, M. and Drucker, D.J. (1997) Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. *American Journal Physiology* 272, E1050–1058.
- Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* 162, 156–159.
- Drucker, D.J., Erlich, P., Asa, S.L. and Brubaker, P.L. (1996) Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proceedings of the National Academy of Sciences USA* 93, 7911–7916.
- Jeppesen, P.B., Hartmann, B., Hansen, B.S., Thulesen, J., Holst, J.J. and Mortensen, P.B. (1999) Impaired meal stimulated glucagon-like peptide 2 response in ileal resected short bowel patients with intestinal failure. *Gut* 45, 559–563.
- Sangild, P.T., Fowden, A.L., Sjöström, H., Norén, O. and Silver, M. (1995) The prenatal development and glucocorticoid control of brush-border hydrolases in the pig small intestine. *Pediatric Research* 37, 207–212.
- Trahair, J.T. and Sangild, P.T. (1997) Systemic and luminal influences on the perinatal development of the gut. *Equine Veterinary Journal Supplement* 24, 40–50.

5 Induced Functional Maturation of the Gut Mucosa due to Red Kidney Bean Lectin in Suckling Pigs

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The possibility of inducing gastrointestinal tract maturation in suckling piglets by the administration of red kidney bean lectin was studied. Piglets, 10 days of age, were gavaged either a crude preparation of lectin or saline on each of 3 days. Decreased absorption/permeation of different-sized markers and changes in small intestinal disaccharidase activities were found after the lectin treatment. These results indicated that lectin exposure had induced functional maturation of the gut in young pigs before weaning.

Introduction

During the neonatal period the mammalian gut undergoes major development, which leads to structural as well as functional changes (Henning, 1987). Fetal-type enterocytes with high endocytotic activity are gradually replaced, in a proximal–distal direction by new adult-type cells devoid of such activity, being completed in the distal intestine when the pigs are 3–4 weeks old (Baintner, 1986). The expression of brush-border enzymes, e.g. disaccharidases, in the small intestinal mucosa is also linked to the change in enterocyte generations. Lactose activity is high in the newborn and declines with age until it becomes minimal after weaning, while sucrase and maltase activities are low at birth but increase after weaning (Pluske *et al.*, 1997). Lectins isolated from the red kidney bean (*Phaseolus vulgaris*) have been shown to bind to complex carbohydrate structures on the surface of enterocytes and induce cell proliferation and growth of the gut mucosa (Pusztai *et al.*, 1999) and may also influence intestinal maturation (Weström *et al.*, 1999). The aim of the present study was to explore the effect of gavage-feeding red kidney bean lectin on the small intestinal function in suckling piglets.

Methods

The study included 27 crossbred pigs, which were divided into control pigs (CP; $n = 16$) and lectin-treated pigs (LP; $n = 11$). Between 10 and 12 days of age, the CP group was given vehicle alone (4 ml 0.9% NaCl kg⁻¹ body weight) while the LP group was given a crude red kidney bean lectin preparation (400 mg kg⁻¹ body weight, dissolved in 0.9% NaCl). The lectin preparation contained about 25% pure lectin and was performed according to Pusztai and Watt (1974). All piglets were kept with the sow during the study and were not creep fed. At 13 days of age, a test of the intestinal absorptive capacity *in vivo* was performed. The pigs were gavage-fed a marker molecule cocktail (4 ml kg⁻¹ body weight) containing bovine serum albumin (BSA,

67,000 Da, 500 mg kg⁻¹), 1-deamino-8-D-arginine vasopressin (dDAVP, 1067 Da, 0.01 mg kg⁻¹), and Na-fluorescein (NaF, 376 Da, 9.4 mg kg⁻¹) dissolved in 0.9% NaCl. Blood samples were taken before (0 h) and during the following 24 h. The next day, at 14 days of age, a test of intestinal tissue permeability *in vitro* was performed. After anaesthesia, 20 cm segments from the proximal and distal jejunum were excised and mounted as tissue sheets in Ussing chambers (Pantzar *et al.*, 1993). The buffer on the mucosal side was replaced with a buffer containing the marker molecules: ovalbumin (OVA, 45 kDa, 25 mg ml⁻¹), FITC-dextran 4400 (FD4, 4400 Da, 1 mg ml⁻¹) and ¹⁴C-mannitol (182 Da, 1.38 μM) and samples were taken from the serosal receiver side during 2 h. All markers were analysed according to Pantzar *et al.* (1993). For disaccharidase analysis, intestinal segments were quickly collected and frozen in liquid nitrogen (–80°C) and then analysed according to Dahlqvist (1984).

Results

There was no significant difference in initial or sacrifice (CP 5.0 ± 0.9 kg; LP 4.5 ± 0.6 kg) weights between CP and LP following treatment. After gavage feeding, the absorption of the different-sized markers into plasma was lower for LP (Table 5.1). In addition, the mucosal to serosal passage of the markers ¹⁴C-mannitol, FD4 and OVA *in vitro* was lower for LP when compared with CP (results not shown). In the proximal small intestine, lectin treatment caused a tendency for disaccharidase activities to decrease, while increased maltase and sucrase activities (not shown) were found in the middle and distal parts after the lectin treatment (Table 5.1).

Discussion

The study showed that exposure of young suckling 10–14-day-old piglets to red kidney bean lectin induced functional maturation of the gastrointestinal tract. A

Table 5.1. Plasma peak levels *in vivo* for the marker molecules NaF (0.5 h), dDAVP (2 h), and BSA (4 h). Mucosal disaccharidase activities in the proximal (prox), middle (mid) and distal (dist) small intestine. Results from control pigs (CP) and lectin-treated pigs (LP) were compared statistically using Student's *t*-test.

Group	Marker absorption			Disaccharidase activity (units g ⁻¹ protein)					
	NaF (ng ml ⁻¹)	dDAVP (pg ml ⁻¹)	BSA (μg ml ⁻¹)	Lactase			Maltase		
				prox	mid	dist	prox	mid	dist
CP	1189 ± 433	10.2 ± 9.5	1.4 ± 0.8	186 ± 37	129 ± 79	0 ± 0	142 ± 115	105 ± 10	38 ± 53
LP	548 ± 187***	2.7 ± 2.8**	0.2 ± 0.2***	96 ± 13	103 ± 22	24 ± 3	94 ± 24	210 ± 76*	228 ± 72***

P* < 0.05; *P* < 0.01; ****P* < 0.001.

decreased marker molecule absorption/permeability was found both *in vivo* and *in vitro* after lectin treatment. The reduced absorption of the inert and passively transported molecules ¹⁴C-mannitol and NaF may be explained by a reduction in the absorptive surface area (reduced villi size) due to the lectin treatment. The decreased absorption of FD4 and dDAVP might be explained by a combination of decreased surface area, decreased rate of endocytosis and presumably a tighter epithelium. With respect to the large protein molecules OVA and BSA, the reduction may be explained by decreased endocytosis after lectin administration due to a faster replacement of fetal-type to adult-type enterocytes with fewer lysosomal vacuoles (Baintner, 1986). Since we found a tendency to decreased lactase activity and significant increases in sucrase and maltase activities following lectin exposure, our results demonstrate a

maturation effect also on the brush-border enzymes in the mucosa.

Postweaning diarrhoea in pigs is a serious and complex health problem. At weaning, the gut is suddenly exposed to food with a markedly different composition than milk. This results in villus atrophy and crypt hyperplasia that leads to a decreased digestive and absorptive capacity of the intestine (Pluske *et al.*, 1997). The present study shows the possibility of inducing precocious gut maturation in suckling piglets by the administration of kidney bean lectin. The induced functional maturation is confirmed by structural changes (Biernat *et al.*, 2001). Besides the value of increased basic knowledge about gut maturation, the results from the present study suggest a novel instrument to increase gut growth and maturation that could be of potential use for reducing weaning problems in pig production.

References

- Baintner, K. (1986) *Intestinal Absorption of Macromolecules and Immune Transmission from Mother to Young*. CRC Press, Boca Raton, Florida, pp. 1–216.
- Biernat, M., Gacsalyi, U., Rådberg, K., Zabielski, R., Weström, B. and Pierzynowski, S.G. (2000) Effect of kidney bean lectin on gut morphology. A way to accelerate mucosa development. In: Lindberg, J.E. and Ogle, B. (eds) *Digestive Physiology of Pigs*. CAB International, Wallingford, UK, pp. 46–48.
- Dahlqvist, A. (1984) Assay of intestinal disaccharidases. *Scandinavian Journal of Clinical Laboratory Investigation* 44, 169–172.
- Henning, S.J. (1987) Functional development of the gastrointestinal tract. In: Johnson, L.R. (ed.) *Physiology of the Gastrointestinal Tract*. Raven Press, New York, pp. 285–300.
- Pantzar, N., Weström, B., Luts, A. and Lundin, S. (1993) Regional small-intestinal permeability *in vitro* to different-sized dextran and proteins in the rat. *Scandinavian Journal of Gastroenterology* 28, 205–211.
- Pluske, J.R., Hampson, D.J. and Williams, I.H. (1997) Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* 51, 215–236.

- Pusztai, A., Ewen, S.W.B., Grant, G. and Bardocz, S. (1999) Effect of lectins on digestion of food and body metabolism. *COST-98, Effects of antinutrients on the nutritional value of legume diets* 1, 22–28.
- Pusztai, A. and Watt, W.B. (1974) Isolectins of *Phaseolus vulgaris*. A comprehensive study of fractionation. *Biochimica et Biophysica Acta* 365, 57–71.
- Weström, B.R., Linderöth, A., Dandriofosse, A., Pusztai, A. and Pierzynowski, S.G. (1999) Decreased intestinal macromolecular absorption after enteral exposure to spermine and lectin (PHA) in young rats. *Cost-98, Effects of Antinutrients on the Nutritional Value of Legume Diets* 8, 59–64.

6 The Activity of Lipolytic Enzymes is Low around Weaning – Measurements in Pancreatic Tissue and Small Intestinal Contents

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The influence of weaning on the activity of trypsin, lipase and carboxyl ester hydrolase (CEH) in pancreatic tissue and small intestinal contents was investigated in 64 piglets. The activity of all enzymes in the pancreatic tissue declined following weaning and reached a minimum on day 5 postweaning. The activity of trypsin in small intestinal contents increased during days 1–9 postweaning, whereas the activity of CEH remained constant and the activity of lipase declined. These results suggest that the digestion of fat is of special concern during the immediate postweaning period in piglets due to low lipolytic activity.

Introduction

Weaning influences physiological responses in pigs, particularly intestinal function and secretions, and a poor digestibility of nutrients has been observed in weanling pigs. The digestibility of fat is of special concern because it has been demonstrated that piglets have a high demand for energy in order to reach an optimal growth rate. Due to the low feed intake right after weaning,

this high demand for energy is difficult to achieve without compromising the health of the gastrointestinal tract. The secretions of the exocrine pancreas are responsible for digesting most dietary fat and several studies have been performed to investigate the effect of weaning on the activity of lipolytic enzymes as well as the digestibility of fat in the postweaning period (e.g. Cera *et al.*, 1988, 1990; Jensen *et al.*, 1997). Most attention has been paid to pancreatic lipase

but carboxyl ester hydrolase (CEH) may also be important for fat digestion during the immediate postweaning period.

The purpose of the present experiment was to investigate the changes in pancreatic enzyme activity in pancreatic tissue and intestinal contents during the period immediately after weaning.

Materials and Methods

An experiment was performed with 64 pigs (Danish Landrace $\times$ Yorkshire). The pigs were divided into eight groups and from each group a pig was slaughtered 3 days prior to weaning (day -3), at the day of weaning (day 0) and 1, 2, 3, 5, 7 and 9 days postweaning. The pigs were weaned at 28 days of age without having access to creep feed prior to weaning. Postweaning, the pigs were fed a standard weaner diet *ad libitum*. At slaughter the entire gastrointestinal tract was removed and the pancreas was carefully dissected free. The total contents of the small intestine were collected and weighed. The activity of trypsin, lipase and CEH was determined in the pancreatic tissue and small intestinal contents using methods described by Jensen *et al.* (1997).

Results and Discussion

The activity of trypsin, lipase and CEH in pancreatic tissue was maximal at the day of weaning or day 1 postweaning. During the following days a rapid decline in enzymatic activity was observed and the activity reached a minimum on day 5 postweaning (Table 6.1). The activity of trypsin increased during the following 4 days whereas the activity of lipase and CEH remained at the same (low) level during this period. The trypsin activity in small intestinal contents increased during the postweaning period (days 1–9). A constant activity of CEH was observed during this period whereas the activity of lipase was decreasing (Table 6.1).

A decrease in lipase levels in the pancreas at day 3 and day 7 postweaning was also observed by Cera *et al.* (1990). In small intestinal contents the lipase activity was lower in weaned piglets when compared with suckling littermates (Cera *et al.*, 1990). It has been shown that the activity of CEH in pancreatic tissue is low 4 weeks after weaning (Jensen *et al.*, 1997) but the activity in small intestinal contents has, to our knowledge, not been determined in previous experiments. CEH is a relatively non-specific lipase that cleaves ester linkages; it most likely makes a substantial

Table 6.1. The concentration (U g⁻¹ tissue) of trypsin, lipase and CEH in pancreatic tissue and the total activity (U) of trypsin, lipase and CEH in small intestinal contents in piglets. The results are least square means.

Day	Pancreatic tissue			Digesta		
	Trypsin	Lipase	CEH	Trypsin	Lipase	CEH
-3	4.48 ^{ac}	4670 ^{ab}	26.8 ^{ab}	ND	–	–
0	5.28 ^a	5478 ^{ab}	51.1 ^a	8.4 ^b	2501 ^{ab}	25.9
1	5.83 ^a	5738 ^a	42.7 ^a	14.5 ^b	3091 ^a	12.4
2	4.98 ^{ac}	5113 ^{ab}	23.4 ^{ab}	32.1 ^{ab}	1316 ^{ab}	29.6
3	2.49 ^{abc}	2514 ^{bc}	10.5 ^{bc}	34.1 ^{ab}	1939 ^{ab}	17.2
5	2.10 ^b	1345 ^c	5.8 ^c	44.6 ^{ab}	1666 ^{ab}	24.1
7	4.34 ^a	2599 ^{abc}	6.2 ^c	90.7 ^a	1080 ^b	20.3
9	3.07 ^{ab}	1979 ^c	4.7 ^c	58.6 ^a	1775 ^{ab}	28.6

ND, not determined.

^{a,b,c} Least square means in the same column with different superscript are significantly different ($P < 0.05$).

contribution to fat digestion but its quantitative contribution to fat digestion remains to be elucidated (Gabert and Hedemann, 1999).

Pierzynowski *et al.* (1993) showed that the exocrine pancreatic secretion of lipase and CEH increases following weaning. These measurements were made 1–2 weeks after weaning and this may have masked a decline or a constant secretion during the immediate postweaning period, as observed in the present experiment. However, it should be pointed out that there are immense principal differences between the methods used in these experiments (chronically catheterized animals vs. slaughter experiments), which complicates comparisons.

In our previous experiment no postweaning decline in trypsin activity in pancreatic tissue was observed (Jensen *et al.*, 1997). However, the first observation was made 1 week after weaning and this may have masked a temporary postweaning

decline in trypsin activity. Rantzer *et al.* (1997) observed an increasing output of trypsin during the first 5 days following weaning in chronically catheterized animals, which is in agreement with the results obtained in small intestinal contents in the present experiment.

Measurements of enzyme activity in digesta are confounded by the fact that the enzymes are degraded/inactivated during transit from the duodenum to the distal ileum (Gabert and Hedemann, 1999). Lipase activity decreases more than that of other enzymes; more than 95% of its activity disappears during small intestinal transit. In contrast, 65% of trypsin activity disappears during small intestinal transit (Layer *et al.*, 1990).

The results presented here suggest that the digestion of fat may be of special concern after weaning, as the activity of lipolytic enzymes in small intestinal contents is low whereas the activity of trypsin is increasing.

References

- Cera, K.R., Mahan, D.C. and Reinhart, G.A. (1988) Weekly digestibilities of diets supplemented with corn oil, lard or tallow by weanling swine. *Journal of Animal Science* 66, 1430–1437.
- Cera, K.R., Mahan, D.C. and Reinhart, G.A. (1990) Effect of weaning, week postweaning and diet composition on pancreatic and small intestinal luminal lipase response in young swine. *Journal of Animal Science* 68, 384–391.
- Gabert, W.M. and Hedemann, M.S. (1999) The contribution of exocrine pancreatic secretions to fat digestion. In: Pierzynowski, S.G. and Zabielski, R. (eds) *Biology of the Pancreas in Growing Animals*. Elsevier Sciences BV, The Netherlands, pp. 339–360.
- Jensen, M.S., Jensen, S.K. and Jakobsen, K. (1997) Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. *Journal of Animal Science* 75, 437–445.
- Layer, P., Jansen, J.B.M.J., Cherian, L., Lamers, C.B.H.W. and Goebell, H. (1990) Feedback regulation of human pancreatic secretion. Effects of protease inhibition on duodenal delivery and small intestinal transit of pancreatic enzymes. *Gastroenterology* 98, 1311–1319.
- Pierzynowski, S.G., Weström, B.R., Erlanson-Albertsson, C., Ahrén, B., Svendsen, J. and Karlsson, B.W. (1993) Induction of exocrine pancreas maturation at weaning in young developing pigs. *Journal of Pediatric Gastroenterology and Nutrition* 16, 287–293.
- Rantzer, D., Kiela, P., Thaela, M.-J., Svendsen, J., Ahrén, B., Karlsson, B. and Pierzynowski, S.G. (1997) Pancreatic exocrine secretion during the first days after weaning in pigs. *Journal of Animal Science* 75, 1324–1331.

7 Sphingomyelinase Activity in Gastrointestinal Content and Mucosa from Pigs of Different Ages

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The potential capacity to hydrolyse sphingomyelin to ceramide in bile, small intestinal mucosa and gastrointestinal content from piglets was studied. No sphingomyelinase (SMase) activity was detected in bile, while there was activity in the mucosa, gut content and faeces. A higher ($P < 0.05$) activity of all three SMases was found in intestinal mucosa homogenates from 4-week-old piglets than in newborn piglets. The activity of the alkaline SMase was higher in the ileum than in the duodenum and jejunum. SMase activity of all three isoforms was present in the ileal and faecal content at 16 weeks of age.

Introduction

The phospholipid sphingomyelin (SM) is a major component of mammalian cell plasma membranes and of serum lipoproteins. SM metabolites have been shown to be important lipid mediators affecting cell regulation and are suggested to be involved in regulation of the inflammation and immune response (Zhang and Kolesnick, 1995). The major route of SM degradation is catalysed by three different sphingomyelinases (SMase): one acid, one neutral and one alkaline isoform (Nilsson, 1969).

The SMase may be responsible for the hydrolysis of the dietary SM found in milk, eggs, meat and fish. Also, the SMase may regulate cholesterol absorption, as the hydrolysis of endogenous membrane SM of Caco2 cells by exogenous SMase was found to decrease cholesterol absorption. Further, the hydrolysis products of SM may have an

inhibitory effect on intestinal carcinogenesis (Nyberg, 1998).

The aim with the present study was to obtain data on the potential of bile, mucosa and digesta from piglets of different ages to hydrolyse SM to ceramide.

Material and Methods

Animals and sampling

Crossbred (Yorkshire $\times$ Landrace) piglets of three different age groups, i.e. newborn (0 days old, had not received any colostrum; body weight (BW) 1.5 ± 0.1 kg), suckling (2–4 weeks old; BW 4.1 ± 0.8 kg) and weaned piglets (9 weeks old; BW 22.8 ± 1.3 kg), were used. They were anaesthetized with pentobarbital; and bile, gut content and intestinal segments were collected. In addition, digesta and faeces were collected from ileo-cannulated pigs 16 weeks old.

SMase analysis

The mucosa layer was separated from the underlying tissue using a spatula; then it was weighed and homogenized on ice in a buffer containing protease inhibitors (Nyberg, 1998). SMase activity was determined using ¹⁴C-SM suspended in 0.15 M NaCl containing 3 mM bile salt mixture (Nyberg, 1998). The samples were incubated at 37°C for 30 min and the reaction was terminated by addition of 2 ml chloroform/methanol (2:1). After centrifugation the radioactivity was determined by liquid scintillation and normalized as nmol h⁻¹ mg⁻¹ sample protein for mucosa sample, and expressed per gram for faeces and per millilitre for intestinal content.

Statistical analysis

The data on intestinal mucosa and faecal activity of SMase were analysed according to a factorial design and one-way analysis of variance, respectively. For the remaining data, mean values with their standard deviations were calculated.

Results

No SMase activity was detected in the bile samples. As Table 7.1 shows, higher activity of all three SMases was found in intestinal mucosa homogenates from 4-week-old than in newborn piglets. The activity of acid and neutral SMase was similar in 4- and 9-week-old piglets. In contrast, the activity of alkaline SMase was lower in 9-week-old than in 4-week-old and newborn piglets.

Only the activity of alkaline SMase differed with the site of sampling along the small intestine: it was higher in the ileum than in the duodenum and jejunum (Table 7.1). In contrast, no differences were observed in the activity of acid and neutral SMase with the site of sampling along the small intestine (Table 7.1). The activity of neutral SMase in digesta from growing pigs (4–9 weeks old) tended to be higher in jejunum than in duodenum (2301 ± 486 and 676 ± 48 nmol h⁻¹ ml⁻¹ intestine juice, respectively). The same pattern but with higher values was observed for alkaline SMase (5931 ± 2834 and 857 ± 144 nmol h⁻¹ ml⁻¹, respectively). The activity of acid SMase, on the other hand, was similar in

Table 7.1. Sphingomyelinase (SMase) activity in tissue homogenates from the small intestine of growing pigs.

	SMase activity (nmol h ⁻¹ mg ⁻¹ protein)		
	Acid	Neutral	Alkaline
Age, weeks			
0	13.0 ^a	12.7 ^a	16.1 ^b
4	17.0 ^b	18.3 ^b	20.9 ^c
9	20.2 ^{bc}	18.8 ^b	8.4 ^a
SEM	1.2	1.4	1.4
Level of significance	0.01	0.01	0.001
Part of the gut			
Duodenum	16.7	18.4	12.3 ^a
Jejunum	17.0	14.4	11.3 ^a
Ileum	16.5	16.9	21.8 ^b
SEM	1.2	1.4	1.4
Level of significance	NS	NS	0.001

^{a,b,c} Means within columns with different superscripts are significantly different; NS, not significant.

all three segments investigated, with an average value of $493 \pm 49 \text{ nmol h}^{-1} \text{ ml}^{-1}$.

The activity of acid, neutral and alkaline SMase in ileal digesta from 16-week-old pigs was 17 ± 5 , 292 ± 124 and $1643 \pm 575 \text{ nmol h}^{-1} \text{ ml}^{-1}$, respectively. No differences in activity were found for any of the three SMases in faecal samples from newborn, 4-, 9- and 16-week-old pigs, and the average activities were 70, 89 and $158 \text{ nmol h}^{-1} \text{ g}^{-1}$ for the acid, neutral and alkaline SMase, respectively.

Discussion

The high activity of all three SMases in 4-week-old piglets could be explained by the suckling of these animals and thus ingestion of milk fat, which is rich in SM (Nyberg, 1998). The present data showed that the alkaline SMase activity, but not the acid and neutral SMases, had declined in 9-week-old piglets. The alkaline SMase is involved in the transformation of dietary SM and in absorption of cholesterol. Thus, the enzyme activity should be expected to

decline with decreasing ingestion of dietary SM. In contrast, the acid SMase is involved in the bulk turnover of membrane or lipoprotein-associated SM and also in digestion of LDL-associated SM, and the neutral SMases are involved in the catabolism of cell-surface SM and in mobilization of cholesterol. Therefore, it seems reasonable that the acid and neutral SMases preserve their activities independent of the dietary SM concentration. The absence of alkaline SMase activity in pig pancreas (Nilsson, 1969) and bile juice, and the highest activity in the middle and distal parts of the small intestine, implies that the enzyme might have other specific functions besides the digestion of dietary SM.

The results from this study show that the activity of the alkaline SMase in mucosa and in ileal and faecal content tended to be highest during the suckling period. The activity decreased when the pigs were weaned from milk to a commercial dry feed, but did not disappear. Further studies seem warranted in order to elucidate fully the biological role of SMase in pigs.

References

- Nilsson, Å. (1969) The presence of sphingomyelin- and ceramide-cleaving enzymes in the small intestinal tract. *Biochimica et Biophysica Acta* 176, 339–347.
- Nyberg, L. (1998) Digestion and absorption of sphingomyelin from milk. Doctoral thesis, University of Lund, Sweden.
- Zhang, Y. and Kolesnick, R. (1995) Signaling through the sphingomyelin pathway. *Endocrinology* 136, 4157–4160.

8 Weaning of Supernumerary Piglets at 7 Days of Age: Effects on Digestive Function – Preliminary Results

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The present trial was designed to evaluate the effect of weaning at 7 days of age on the digestive capacity of piglets. After weaning, both pancreas and small intestine weight decreased transiently. Pancreatic lipase and chymotrypsin activities were lowered, whereas intestinal maltase and aminopeptidase N were enhanced. It is assumed that pancreatic and small intestinal functions are able to respond and adapt to weaning changes as early as 7 days of age.

Introduction

During recent years, sow prolificacy has been considerably improved. As a result, piglets in excess to the rearing capacity of the batch are more and more frequent. Fostering at 24–36 h after birth on a nurse sow and weaning 1 week later has proved to be efficient in improving survival of these supernumerary piglets (Orgeur *et al.*, 2000). Although incidence of weaning in 14–28-day-old piglets on digestive function has been well described (Lindemann *et al.*, 1986; Kelly *et al.*, 1991; Pluske *et al.*, 1997), little information is available on the effects of early weaning at 7 days on the digestive function. Therefore, the present trial was designed to determine the capacity of pancreatic and small intestinal (SI) function to adapt to very early weaning.

Materials and Methods

At 7 days of age, 30 sow-reared piglets were allotted to five groups of six animals

each, on the basis of body weight (BW) and litter origin. Piglets of the first group were killed at day 7 (S7) whereas those of the second group were suckled by the sow up to killing at day 21 (S21). Piglets of the other groups were weaned and killed at day 10 (W10), day 14 (W14) and day 21 (W21). Weaned piglets were tube-fed commercial piglet formula providing 220 g crude protein, 120 g fat, 170 g lactose and 18.5 g lysine kg⁻¹. Piglets were fed four times daily and given increasing amounts of feed (3, 10.5, 18, 25.5, 33, 40.5 and 48 g feed kg⁻¹ BW day⁻¹ at days 1, 2, 3, 4, 5, 6 and days 7–14 post weaning, respectively) mimicking typical intake of piglets given dry feed in practical conditions (Orgeur *et al.*, 2000). The powdered diet was reconstituted with water to a final concentration of 200 g l⁻¹. At killing, both pancreas and small intestine (proximate, intermediate and distal sections) were weighed and sampled for digestive enzyme analysis. The specific activities of pancreatic (trypsin, chymotrypsin and lipase) and intestinal (lactase, maltase and aminopeptidase N)

enzymes as well as tissue protein contents were determined. Differences between treatment means were assessed by the least significant difference test, with $P < 0.05$ considered as significant.

Results and Discussion

A growth check was observed during the first 2 postweaning days, the initial body weight being recovered 4 days after weaning. Pancreas and SI relative weight decreased during the first 3 postweaning days (Table 8.1). However, at 21 days of age, differences between sucking and weaned piglets were no longer significant. These findings are in agreement with previous studies on piglets weaned at 14 days and 21 days (Lindemann *et al.*, 1986; Kelly *et al.*, 1991; Makkink *et al.*, 1994). A transient decrease in the relative weight of small intestinal mucosa was observed during the first postweaning days, suggesting

transient changes in SI morphometry. Preliminary results indicated a dramatic reduction of villus size in W10 and W14 vs. S7 piglets.

Weaning resulted in a significant decrease in pancreatic protein content as well as in pancreatic enzyme activities (U mg⁻¹ protein and U kg⁻¹ BW) being one- to eightfold reduced in W14 compared with S7 piglets. At 21 days of age, activities of chymotrypsin and lipase, but not trypsin, remained lower in weaned piglets. As a result of the low feed intake during the first postweaning days and the change in diet composition, a reduced stimulation of the exocrine pancreatic function by luminal substrates might be involved in the decrease of these activities (Makkink *et al.*, 1994). Similarly, the sharp decrease in lipase activity could be related to the threefold lower level of fat in formula compared with sow milk. The development of intestinal disaccharidase and peptidase activities, but not their distribution (data not shown)

Table 8.1. Effects of age and weaning on tissue weight, mucosa/small intestine (SI) weight ratio and pancreatic and SI enzyme activities.

Units		Treatment					RSD
		S7	W10	W14	W21	S21	
Pancreas weight	(g kg ⁻¹ BW)	1.67 ^a	1.34 ^{bc}	1.34 ^{bc}	1.53 ^{ab}	1.19 ^c	0.21
SI weight	(g kg ⁻¹ BW)	40.5 ^{ab}	32.3 ^c	38.2 ^{ab}	42.8 ^a	36.3 ^{bc}	3.7
Mucosa/SI weight	(g g ⁻¹)	0.71 ^a	0.56 ^d	0.61 ^{cd}	0.66 ^{bc}	0.67 ^{ab}	0.03
Pancreatic enzyme activities							
Trypsin	(U mg ⁻¹ protein)	68 ^a	43 ^b	37 ^b	62 ^a	55 ^{ab}	15
	(kU kg ⁻¹ BW)	16.9 ^a	8.3 ^{bc}	4.9 ^c	9.7 ^{bc}	10.2 ^b	3.9
Chymotrypsin	(kU mg ⁻¹ protein)	4.2 ^{bc}	6.9 ^a	2.7 ^c	3.0 ^c	4.8 ^b	1.3
	(kU kg ⁻¹ BW)	1006 ^a	1387 ^a	378 ^b	509 ^b	876 ^{ab}	442
Lipase	(U mg ⁻¹ protein)	30.6 ^a	12.2 ^c	6.7 ^d	6.2 ^d	21.1 ^b	4.2
	(kU kg ⁻¹ BW)	7.6 ^a	2.4 ^b	0.9 ^c	1.0 ^c	3.5 ^b	1.1
SI enzyme activities							
Lactase	(U mg ⁻¹ protein)	334 ^a	230 ^b	208 ^b	237 ^b	203 ^b	59
	(kU kg ⁻¹ BW)	864 ^a	321 ^c	432 ^{bc}	559 ^b	454 ^{bc}	156
Maltase	(U mg ⁻¹ protein)	38 ^e	86 ^d	122 ^b	215 ^a	134 ^c	33
	(kU kg ⁻¹ BW)	95 ^d	134 ^d	432 ^b	689 ^a	299 ^c	89
Ap N	(U mg ⁻¹ protein)	43.0 ^b	56.4 ^a	57.4 ^a	53.4 ^a	43.3 ^b	6.0
	(kU kg ⁻¹ BW)	95 ^{abc}	78 ^c	102 ^{ab}	112 ^a	88 ^{bc}	17

Days post weaning: 3 in W10; 7 in W14, 14 in W21.

RSD, residual standard deviation; BW, body weight; Ap N, aminopeptidase N; U = 1 nmol substrate hydrolysed min⁻¹.

along the small intestine, was affected by weaning. Compared with 7-day-old suckling piglets, activity of lactase was lowered in weaned piglets at days 10, 14 and 21. However, similar activity was found in 21-day-old weaned and suckling piglets. The presence of lactose in formula likely explains this result whereas the decline in lactase activity commonly observed after weaning is associated with deprivation of lactose (Kelly *et al.*, 1991). As usually demonstrated, weaning sharply enhanced maltase activity in relation to the presence of complex carbohydrates in diet. The increase in aminopeptidase N activity agrees well with Tarvid *et al.* (1995). Thus, the different behaviours of lactase activity

and those of maltase and aminopeptidase N could be related to the more apical distribution of lactase on the villus (Pluske *et al.*, 1997).

The present data point out an overall decline in the growth of piglets as well as in pancreas and SI digestive function during the first postweaning days. Therefore, digestive capacity is affected in the period immediately following weaning by the change in both the amount and composition of food intake, but adaptations occur rapidly. As early as 7 days of age, pancreas and SI function are able to be enhanced and a very early weaning may stimulate maturation of both pancreas and SI digestive enzymes.

References

- Kelly, D., Smyth, J.A. and McCracken, K.J. (1991) Digestive development of the early-weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. *British Journal of Nutrition* 65, 169–180.
- Lindemann, M.D., Cornelius, S.G., El Kandelgy, S.M., Moser, R.L. and Pettigrew, J.E. (1986) Effect of age, weaning and diet on digestive enzyme levels in the piglet. *Journal of Animal Science* 62, 1298–1307.
- Makkink, C.A., Negulescu, G.P., Guixin, Q. and Verstegen, M.W.A. (1994) Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. *British Journal of Nutrition* 72, 353–368.
- Orgeur, P., Salaün, C., Le Roux, T., Venturi, E. and Le Dividich, J. (2000) L'adoption et le sevrage ultra-précoce: une stratégie pour élever les porcelets en surnombre. *Proceedings of the 32èmes Journées de la Recherche Porcine en France*, ITP, Paris, pp. 143–149.
- Pluske, J.R., Hampson, D.J. and Williams, I.H. (1997) Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* 51, 215–236.
- Tarvid, I., Cranwell, P.D., Ma, L., Harrison, D.T. and Campbell, R.G. (1995) Small intestinal peptidases in light and heavy pigs at 4 and 6 weeks of age. In: Hennessy, D.P. and Cranwell, P.D. (eds) *Proceedings of the Australasian Pig Science Association. Manipulating Pig Production V*, Australasian Pig Science Association, Canberra, 178pp.

9 Intestinal Nutrient Absorption in Newborn Pigs in Response to Enteral Nutrition or Treatment with Glucagon-like Peptide 2

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The first intake of enteral feed is known to stimulate development of the gastrointestinal tract (GIT). The objective of this study was to evaluate the importance of enteral feeding compared with total parenteral nutrition (TPN) and the effect of glucagon-like peptide 2 (GLP-2) on small intestine dimensions and mucosal uptake of glucose, leucine, lysine and proline. Newborn pigs were raised for 6 days on TPN alone, or on TPN with two daily doses of GLP-2, or fed enterally with colostrum and milk. Intestinal wet weight and total uptake capacities for glucose and the three amino acids were significantly higher for both the GLP-2 and the enteral groups relative to those in the TPN group.

Introduction

A well-functioning digestive tract is of vital importance for the newborn piglet and the first intake of enteral feed (colostrum) is known to stimulate development of the gastrointestinal tract (GIT). This effect may be mediated via both enteral nutrients and certain growth factors, e.g. glucagon-like peptide 2 (GLP-2) (Drucker, 1999; Xiao *et al.*, 1999). The mucosal uptake of water-soluble nutrients such as sugars and amino acids is dependent on a carrier-mediated transport with specific carriers for the different nutrients. Glucose is absorbed via the SGLT-1 transporter and the three amino acids studied in this experiment represent substrates for different carriers handling neutral (leucine), basic (lysine) and imino

(proline) amino acids. The densities of carriers is dependent on both substrate available and stage of ontogenetic development (Buddington and Diamond, 1989). The objective of this study was to evaluate the importance of enteral feeding compared with total parenteral nutrition (TPN) and the effect of GLP-2 on small-intestine dimensions and mucosal uptake of glucose, leucine, lysine and proline.

Material and Methods

Twenty-two piglets (Landrace White × Danish Landrace) from two litters were delivered by caesarean section at 114–115 days of gestation (term = 115 ± 2 days). The piglets from the two litters were ran-

domly assigned to receive either total parenteral nutrition (TPN, $n = 7$), total parenteral nutrition plus two daily infusions of GLP-2 (GLP-2, $n = 8$) or enteral sow's milk (ENT, $n = 7$). The experiment lasted for 6 days, after which the piglets were killed, the length and weight of the small intestine were recorded, and rates of nutrient transport measured.

Pooled sow's milk was mixed with skimmed cow's milk to match the energy and protein content of the TPN fluid (3350 kJ l⁻¹ and 44 g protein l⁻¹). The milk was administered through an orogastric tube and the TPN solution via the hepatic portal vein using infusion pumps. The infusion rate reached 230 ml kg⁻¹ day⁻¹ (730 kJ and 10.3 g amino acid kg⁻¹ day⁻¹). The GLP-2 (12.5 nmol kg⁻¹) was infused intravenously twice a day over a period of 2 h.

The small intestine was divided into three pieces of equal length (proximal, mid and distal regions) and nutrient uptake was measured by incubating 1 cm of everted sleeves for 2 min at 37°C in mammalian Ringers with 50 mmol l⁻¹ for glucose and each of the amino acids. Accumulation of glucose was quantified by adding trace levels of ¹⁴C-D-glucose. ³H-labelled L-isomers of the amino acids were used to measure

uptake. After weighing the tissue, solubilizer and scintillant were added and radioactivity was measured by liquid scintillation counting. Values for glucose represent carrier-mediated uptake, and those for the amino acids represent the sum of carrier-mediated and carrier-independent absorption. Rates of transport were calculated following Karasov and Diamond (1983).

Results

Intestinal wet weight was comparable for the GLP-2 and enteral groups (27 ± 1 and 33 ± 1 g kg⁻¹, respectively) and lowest for TPN pigs (20 ± 1 g kg⁻¹). Total uptake capacities of the small intestine for glucose and the three amino acids (products of total intestinal mass and specific rates of absorption relative to body weight^{0.75}) were significantly higher for both the GLP-2 and the enteral groups relative to those in the TPN group (Fig. 9.1).

Tissue-specific rates of glucose transport averaged over the entire length of small intestine were significantly lower for TPN pigs (2.7 nmol mg⁻¹ min⁻¹) than for the GLP-2 and enteral groups (3.7 and 3.8 nmol

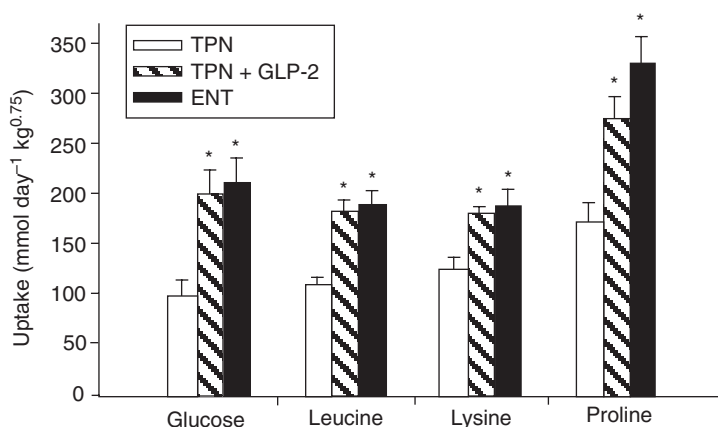


Fig. 9.1. Intestinal nutrient uptake of glucose, leucine, lysine and proline measured *in vitro* and expressed as total capacities. TPN, total parenteral nutrition ($n = 7$); TPN + GLP-2, TPN plus two daily infusions of GLP-2; ENT, enteral feeding with sow's milk. Values marked with stars are different from those in the TPN group ($P < 0.05$).

$\text{mg}^{-1} \text{ min}^{-1}$, respectively). However, the effect of GLP-2 was limited to the proximal and middle regions with no effect in the distal region, whereas the presence of luminal nutrients (ENT) stimulated distal-specific uptake of glucose (Fig. 9.2). GLP-2 stimulated the specific uptake of leucine (+12%) relative to that in the TPN group and the effect was most pronounced in the proximal and middle regions. Specific uptake of proline was stimulated by enteral feeding relative to the TPN group (+22%) with maximal effect in the middle region. Neither enteral feeding nor GLP-2 treatment had any effect on specific uptake of lysine.

Conclusions

Our findings indicate that enteral feeding stimulates the development of the small intestine and its capacities to absorb nutrients. GLP-2 can reduce the intestinal atrophy associated with TPN feeding and elicits absorptive capacities that are comparable to those measured in enterally fed piglets. The effects of GLP-2 on specific nutrient uptake are predominantly in the proximal and middle regions of the small intestine. It is concluded that GLP-2 may be useful for maintaining structure and function in piglets with digestive disorders.

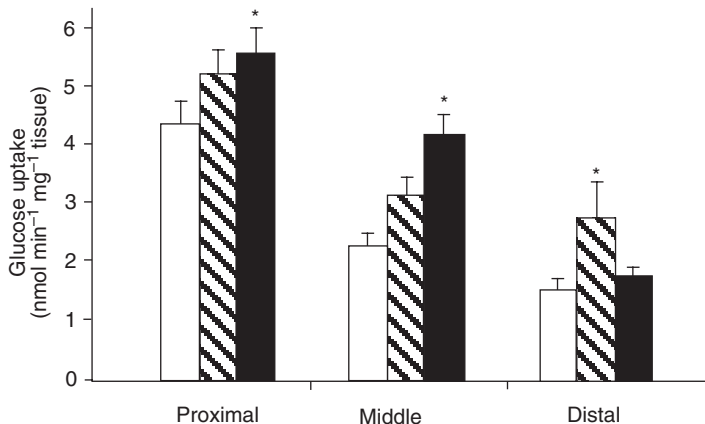


Fig. 9.2. Specific rates of absorption for glucose ($\text{nmol min}^{-1} \text{ mg}^{-1}$ tissue wet weight) measured in three regions of the small intestine (proximal, middle and distal). For detailed description of groups, see Fig. 9.1. Values marked with stars are different from those in the TPN group ($P < 0.05$).

References

- Buddington, R.K. and Diamond, J.M. (1989) Ontogenetic development of intestinal nutrient transporters. *Annual Review of Physiology* 51, 601–619.
- Drucker, D.J. (1999) Glucagon-like peptide 2. *Trends in Endocrinology and Metabolism* 10, 153–156.
- Karasov, W.H. and Diamond, J.M. (1983) A simple method for measuring solute uptake by intestine *in vitro*. *Journal of Comparative Physiology* 152, 1937–1943.
- Xiao, Q., Boushey, R.P. and Drucker, D.J. (1999) Secretion of the intestinotropic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. *Gastroenterology* 117, 99–105.

10 Glucagon-like Peptide 2 Stimulates Intestinal Growth by Decreasing Proteolysis and Apoptosis in Parenterally Fed Premature Piglets

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Glucagon-like peptide 2 (GLP-2) secretion and intestinal growth are substantially decreased in parenterally versus enterally fed neonatal pigs. Thus, our objective was to determine whether GLP-2 stimulates intestinal growth in total parenterally fed (TPN) pigs. Preterm piglets were given TPN, TPN plus GLP-2, or sow's milk enterally and the kinetics of intestinal cell turnover and protein metabolism were assessed after 6 days. We conclude that GLP-2 treatment restored normal intestinal growth in TPN piglets when compared with enterally fed piglets. However, GLP-2 increased intestinal growth by decreasing proteolysis and apoptosis, whereas enteral nutrition acts via increased protein synthesis and cell proliferation and decreased apoptosis.

Introduction

Glucagon-like peptide 2 (GLP-2) is produced in the enteroendocrine L-cells in the distal intestine in response to enteral nutrient intake (Holst, 1997; Drucker, 1999). A number of recent studies have shown that GLP-2 is an intestinal trophic peptide that can augment or restore growth under conditions of compromised intestinal function. Given that enteral nutrition is a critical stimulus of early neonatal intestinal growth, it is conceivable that the secretion and trophic actions of GLP-2 are involved. Consistent

with this, our recent studies in neonatal pigs showed that GLP-2 secretion is directly correlated with the level of enteral nutrient intake and mucosal growth (Burrin *et al.*, 2000). In the present study, we investigated whether the premature neonatal intestine is responsive to GLP-2 under conditions of total parenteral nutrition (TPN) in which gut growth is suppressed. We compared the intestinal response to GLP-2 infusion in TPN-fed pigs with that in pigs fed sow's milk enterally to examine whether GLP-2 may mediate the trophic effects of enteral nutrients on the neonatal intestine.

Materials and Methods

A total of 29 crossbred (Large White $\times$ Danish Landrace) pigs from eight sows were used. Twenty-one pigs from three sows were obtained by caesarean section at 106–108 days of gestation and randomly assigned to receive a parenteral infusion of glucose, amino acids and lipid (TPN, $n = 7$), TPN plus human GLP-2 (25 nmol kg⁻¹ day⁻¹, GLP-2, $n = 8$), or sow's milk enterally (ENT, $n = 6$) for 6 days. Another eight pigs from five sows were obtained by caesarean section at 107 days gestation and killed in order to estimate intestinal weight, protein and DNA content. The TPN and GLP-2 pigs received a continuous umbilical venous infusion of an elemental nutrient solution containing free amino acids, glucose, lipid, electrolytes and vitamins. The TPN solution provided 550 kJ kg⁻¹ day⁻¹, 8 g amino acid kg⁻¹ day⁻¹ and a fluid intake of 170 ml kg⁻¹ day⁻¹. The ENT group received a continuous orogastric infusion of sow's colostrum (1 day) or milk (days 2–6) that provided approximately 700 kJ kg⁻¹ day⁻¹, 12 g protein kg⁻¹ day⁻¹.

After 6 days, the fractional protein synthesis rate (FSR) was measured with a bolus of 1-¹³C-phenylalanine and the fractional protein degradation rate (FDR) was calculated as described by Burrin *et al.* (1999). To measure protein synthesis, pigs received an intravenous injection of a bolus dose of L-phenylalanine (1.5 mmol kg⁻¹ body weight) containing approximately 0.60 mmol kg⁻¹ body weight of L-¹³C-phenylalanine (98% [1-¹³C]-phenylalanine; Cambridge Isotopes, Andover, Massachusetts). Thirty minutes after isotope injection, pigs were killed and intestinal samples were fixed for histology or frozen in liquid nitrogen. The isotopic enrichment of 1-¹³C-phenylalanine in intestinal tissue was determined by gas chromatography–mass spectrometry; protein and DNA were also determined. Crypt cell proliferation index was measured by giving an intravenous injection of 5-bromodeoxyuridine (BrdU, 50 mg kg⁻¹) 4 h before killing the animals and then visualizing the BrdU-labelled nuclei using immunohistochemistry (expressed as % BrdU positive

cells/total crypt cells) as described by Burrin *et al.* (2000). The rate of apoptosis in mucosal enterocytes was determined using the terminal dUTP nick-end labelling (TUNEL) using a commercially available kit (Trevigen, Inc., Gaithersburg, Maryland) (expressed as % TUNEL positive cells/total cells). Villus morphology was determined using light microscopy and image analysis. Plasma GLP-2 was determined by radioimmunoassay using antisera which recognized the N-terminal (1–33), biologically active form of GLP-2 (Wøjdemann *et al.*, 1998).

Results

The initial and final body weights among the three groups were not different and averaged ($\pm$ SD) 1330 $\pm$ 181 g and 1487 $\pm$ 238 g, respectively. The rates of weight gain were not different among the three groups and the mean value for the three groups was 26 $\pm$ 5 g kg⁻¹ day⁻¹. At the end of the experiment, the basal plasma GLP-2 (pM, mean $\pm$ SD) concentrations in the GLP-2 (67 $\pm$ 45) and ENT (56 $\pm$ 21) piglets were higher ($P < 0.05$) than in the TPN piglets (28 $\pm$ 19).

Conclusions

The present study demonstrates that exogenous GLP-2 treatment largely prevents the reduction in intestinal growth associated with TPN in neonates. A novel finding from this study is that the trophic actions of GLP-2 on the neonatal intestine appear to be mediated largely by a suppression of proteolysis and apoptosis rather than a stimulation of protein synthesis and cell proliferation. The nature of the protein anabolic response to GLP-2 was different mechanistically than that of enteral nutrition, which stimulated protein accretion mainly via increased protein synthesis. Enteral nutrition and GLP-2 both increased crypt depth, villus height and DNA content; however, enteral nutrition was associated with both a suppression of apoptosis and stimulation of cell proliferation, whereas

GLP-2 only suppressed apoptosis. We conclude that GLP-2 treatment normalized intestinal growth in TPN-fed preterm piglets when compared with enterally fed piglets. Despite this, the mechanisms whereby GLP-2 and enteral nutrition

increased growth were different: GLP-2 appears to suppress protein catabolism and apoptosis, whereas enteral nutrition not only inhibits apoptosis, but also stimulates proliferation and protein synthesis.

Table 10.1. Endpoints of small intestinal growth, cell kinetics and protein metabolism.

	TPN	TPN + GLP-2	Enteral	Pooled SD
Intestinal weight (g kg ⁻¹ BW)	9.4	12.6*	14.3*	1.9
Intestinal surface area (cm ² kg ⁻¹ BW)	97	130*	151*	18
Villus height (μm)	478	695*	642*	99
Crypt depth (μm)	79.8	89.3*	96.4*	7.5
DNA content (mg kg ⁻¹ BW)	42.1	58.0*	65.7*	8.5
DNA accretion (mg day ⁻¹)	2.5	6.10*	8.08*†	1.7
BrdU positive cells (% crypt cells)	31.1	29.8	43.8*	9.4
TUNEL positive cells (% total cells)	5.56	2.90	2.01	2.13
Protein content (mg kg ⁻¹ BW)	883	1263*	1502*	217
Protein accretion (mg day ⁻¹)	44‡	133*	191*†	49
Protein synthesis rate (% day ⁻¹)	50.1	45.2	63.5*	9.2
Protein degradation rate (% day ⁻¹)	47.6	36.9*	52.5	9.1

*Significantly different from TPN; $P < 0.05$.

†Significantly different from TPN + GLP-2; $P < 0.05$.

‡Not significantly different from zero.

References

- Burrin, D.G., Wester, T.J., Davis, T.A., Fiorotto, M.L. and Chang, X. (1999) Dexamethasone inhibits small intestinal growth via increased protein catabolism in neonatal pigs. *American Journal of Physiology* 276, E269–E277.
- Burrin, D.G., Stoll, B., Jiang, R., Hartmann, B., Holst, J.J., Greeley, G.H. and Reeds, P.J. (2000) Minimal enteral nutrient requirements for neonatal intestinal growth in piglets: how much is enough? *American Journal of Clinical Nutrition* 71, 1603–1610.
- Drucker, D.J. (1999) Glucagon-like peptide 2. *Trends in Endocrinology and Metabolism* 10, 153–156.
- Holst, J.J. (1997) Enteroglucagon. *Annual Review in Physiology* 59, 257–271.
- Wøjdemann, M., Wettergren, A., Hartmann, B. and Holst, J.J. (1998) Glucagon-like peptide-2 inhibits centrally induced antral motility in pigs. *Scandinavian Journal of Gastroenterology* 33, 828–832.

11 Effect of Formula vs. Sow's Milk Feeding on the Gut Morphology in Neonatal Piglets

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The aim of the present study was to assess both the short- and mid-term effects of feeding sow's colostrum followed by sow's milk or formula deprived of growth factors on small intestinal morphometry and mucosa maturation in neonatal piglets. Jejunum samples were collected for light microscopy from piglets killed at birth, 20 h or day 7. At 7 days, feeding sow's milk or formula has no effect on tissue morphometry. However, data on crypt cell mitotic index and enterocyte vacuolation suggest that feeding formula results in a marked delay in the maturation of intestinal mucosa.

Introduction

Milk formulae are being introduced into the pig industry to feed underprivileged or supernumerary piglets. However, little information is available on the mid-term effects of formula compared with maternal feeding on the small intestinal structure and function (Houle *et al.*, 1997). In piglets, the rapid postnatal intestinal growth elicited by colostrum ingestion is usually related to endocytosis of ingested immunoglobulins, mucosa hyperplasia and protein synthesis (Kelly, 1994; Burrin *et al.*, 1997) and is associated with dramatic changes in intestinal morphology (Xu, 1996). Mammary secretions contain a variety of growth factors. The developing intestine is considered to be a target organ for these milk-borne growth factors that may contribute to the specific growth of gastrointestinal tissues in neonatal pigs. The

aim of the present study was to assess both the short- and mid-term effects of feeding formula deprived of growth factors on intestinal morphometry. In addition, effects on mucosa maturation including crypt cell mitotic index and enterocyte vacuolation (Baintner, 1986) were determined.

Materials and Methods

Twenty-four unsuckled newborn piglets were allotted to five groups depending on dietary treatments and age at slaughter: NB, slaughtered at birth; SC1, bottle-fed sow colostrum up to slaughter at 20 h; SCM7, bottle-fed sow's colostrum during the first 36 h followed by sow's milk until slaughter at day 7; and F1 and F7, bottle-fed formula up to slaughter at 20 h and day 7, respectively. Formula was supplemented with purified porcine immunoglobulins during

the first day. Total feed intake in SCM7 and F7 piglets was 3232 g and 3399 g whereas total body weight gain was 950 g and 930 g, respectively. Whole-thickness segments of the mid-jejunum were fixed in formaldehyde, paraffin embedded and stained for light microscopy. Morphometry measurements were performed with a video image analysis system (MultiScan v8.08, Poland). Thickness of tunica mucosa, depth of crypts and length and width of villi were measured. Percentage of vacuolated enterocytes and lysosomal vacuoles (LV) cross-section area (μm^2) were determined in villi, and mitotic index was calculated in crypts. Variance analysis followed by a Tukey test, and Student's *t*-test were used to indicate statistical differences (Statistica PL v5.1, StatSoft, USA).

Results and Discussion

Rapid growth of small intestine weight observed during the first day was mainly caused by an 80% increase in mucosa weight. However, the relative mass of intestinal mucosa was similar at both 20 h

and 7 days in colostrum- and formula-fed piglets (Table 11.1). Houle *et al.* (1997) also reported that at day 7 the relative weight of the small intestine was similar in sow-reared and formula-fed piglets.

At 20 h, crypt depth and villi length tended to be specifically enhanced in SC1 piglets, resulting in an increased thickness of tunica mucosa. These tendencies were not observed in F1, suggesting a colostrum-dependent development. At day 7, there were no more differences in villi and crypt sizes between SCM7 and F7 piglets, in close agreement with data of Houle *et al.* (1997). Colostrum-dependent changes on intestinal morphometry seemed to be transient. However, crypt mitotic index, which mirrored the rate of cell renewal, tended to be higher in piglets fed on sow's colostrum and milk than in formula-fed piglets. In addition, the decrease in the number of vacuolated enterocytes and the size of LV in the mid-jejunum between 20 h and day 7 was less marked in formula-fed than in milk-fed piglets. Abundance of vacuolated enterocytes in 7-day-old piglets may suggest some inhibition in cell turnover and cell maturation (Baintner, 1986). In suckling piglets,

Table 11.1. Effects of age and dietary treatments on the mean small intestine (SI) growth parameters, jejunum mucosa morphometry, crypt cell mitotic index and vacuolated enterocyte indexes.

	Group (n)					P-value
	NB (6)	SC1 (4)	F1 (4)	SCM7 (6)	F7 (6)	
Age at slaughter	0	20h	20h	7d	7d	
SI weight (g kg ⁻¹ BW)	30.5 ^{aA}	46.8 ^b	44.5 ^B	39.4 ^c	40.5 ^C	0.005
SI length (m kg ⁻¹ BW)	2.90	3.10	2.92	2.46	2.45	0.29
Mucosa weight (g kg ⁻¹ BW)	19.3 ^{aA}	35.5 ^b	33.7 ^B	23.2 ^a	24.9 ^A	0.0002
Jejunum						
Tunica mucosa (μm)	800	1060	787	814	817	0.16
Crypt depth (μm)	88	121	109	103	110	0.62
Crypt mitotic index (%)	2.4 ^{aA}	3.2 ^b	2.8 ^A	3.3 ^b	2.7 ^{A*}	0.007
Villus length (μm)	706	875	666	689	701	0.27
Villus width (μm)	80 ^{aA}	121 ^b	109 ^B	90 ^a	93 ^C	0.0001
Enterocytes with LV (%)	46 ^{aA}	45 ^a	89 ^{B*}	13 ^b	71 ^{B*}	0.0001
LV area (μm^2)	53 ^{aA}	149 ^b	171 ^B	46 ^a	60 ^A	0.0001

P-value represents the result of variance analysis.
Values with a different letter (^{abc} for milk-fed and ^{ABC} for formula-fed piglets) in the same row differ significantly (*P* < 0.05, Tukey test).
*Indicates the difference between milk-fed and formula-fed at particular time point (*P* < 0.05, Student's *t*-test).

large LV are characteristically located in the apical and central part of the enterocyte, and disappear between day 4 and day 11. These vacuoles are observed as long as the enterocytes can nonselectively take up the nutrients from the intestinal lumen (Clarke and Hardy, 1971; Baintner, 1986). Our results suggest that formula feeding leads to a marked delay in intestinal mucosa maturation. The concentrations of insulin and IGF-I, respectively, were: in colostrum, 825 $\mu\text{U ml}^{-1}$ and 856 ng ml^{-1} in sow's milk, 194 $\mu\text{U ml}^{-1}$ and 19 ng ml^{-1} ; and in formula, 33 $\mu\text{U ml}^{-1}$ and 11 ng ml^{-1} . However, to

what extent our findings could be related to the low content of insulin and IGF-I and other bioactive compounds in formula is not known. It follows that feeding formula may prolong the period of penetration of noxious agents such as food antigens.

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References

- Baintner, K. (1986) *Intestinal Absorption of Macromolecules and Immune Transmission from Mother to Young*. CRC Press, Boca Raton, Florida.
- Burrin, D.G., Davis, T.A., Fiorotto, M.L. and Reeds, P.J. (1997) Role of milk-borne vs. endogenous insulin-like growth factor I in neonatal growth. *Journal of Animal Science* 75, 2739–2743.
- Clarke, R.M. and Hardy, R.N. (1971) Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. *Journal of Anatomy* 108, 63–77.
- Houle, V.M., Schroeder, E.A., Odle, J. and Donovan, S.M. (1997) Small intestinal disaccharidase activity and ileal villus height are increased in piglets consuming formula containing recombinant human insulin-like growth factor-I. *Pediatric Research* 42, 78–86.
- Kelly, D. (1994) Colostrum, growth factors and intestinal development in pigs. In: Souffrant, W.B. and Hagemester, H. (eds) *Proceedings of the VIth International Symposium on Digestive Physiology in Pigs*. EAAP Publication No. 80, Dummerstorf, pp. 151–166.
- Xu, R.J. (1996) Development of the newborn GI tract and its relation to colostrum/milk intake: a review. *Reproduction, Development and Fertility* 8, 35–48.

12 Effect of Kidney Bean Lectin on Gut Morphology: a Way to Accelerate Mucosa Development

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The effect of a red kidney bean lectin preparation on the microstructure of the small intestine was studied in suckling piglets. Animals received lectin via stomach tube on 3 subsequent days and were then sacrificed and small-intestine samples were collected for light microscopy analysis. The differences in the crypt and villi size between vehicle- and lectin-treated piglets were minor. However, analysis of enterocyte vacuolation in lectin-treated animals (reduced size of lysosomal vacuoles and fewer vacuolated enterocytes/villus) revealed that kidney bean lectin may induce maturation of the enterocytes in suckling piglets.

Introduction

Oral administration of a red kidney bean (*Phaseolus vulgaris*) lectin results in reduction of total body weight, atrophy of skeletal muscle and many other effects (de Oliveira *et al.*, 1988). *In vitro*, it stimulates mitosis of blood cells and intestinal crypt cells (Macgregor, 1993; Bardocz *et al.*, 1995). In the small intestine, lectin causes reversible, dose-dependent hyperplastic growth by lengthening the tissue and thickening the wall in the small intestine (de Oliveira *et al.*, 1988; Bardocz *et al.*, 1990, 1995). Little is known about the influence of lectins on small intestine structure in suckling piglets and around weaning. In this study we have compared mucosa morphometry parameters as well as the index of mucosa maturation: vacuolation in the

enterocytes (Baintner, 1986; Biernat *et al.*, 1999) in suckling piglets.

Materials and Methods

Treatment and experiments were conducted in compliance with European Community regulations concerning the protection of experimental animals. The experiment was performed on suckling piglets (eight control and eight treated with 400 mg kg⁻¹ body weight of crude red kidney bean lectin preparation containing 25% pure lectin). The piglets were kept with the sow, except for the moment of lectin administration. The lectin preparation was administered via stomach gavage on days 12, 13 and 14 of life. On day 16 the piglets were sacrificed and small-intestinal

segments were taken, fixed in Bouin's solution, embedded in paraffin and haematoxyline/eosin-stained for light microscopy using a video image analysis system (MultiScan v8.08, Poland). The depth of crypts, length and width of villi and thickness of tunica mucosa of the small intestine (duodenum, jejunum and ileum) were measured. The enterocytes were examined for the presence of supranuclear lysosomal vacuoles (LV). The cross-section area and the percentage of vacuolated enterocytes were measured. A non-parametric Mann-Whitney test was used to compare the control and PHA-treated piglets (InStat for Macintosh v. 2.03, Graph Pad Software, USA).

Results and Discussion

Lectin treatment did not significantly change the crypt depth and villi width in the small intestine. In rats, lectin treatment resulted in increased length and weight of the small intestine (de Oliveira *et al.*, 1988; Bardocz *et al.*, 1990, 1995), decreased length of villi, reduced thickness of the tunica mucosa (Weinman *et al.*, 1989) and reduced number and size of the giant supranuclear LV in the enterocytes in the distal small intestine (Clarke and Hardy, 1969; Baintner, 1986). In our experiment the villi length significantly decreased in the jejunum, whereas in the ileum the villi had a tendency to shorten their length ($P =$

0.07). The thickness of the tunica mucosa was significantly reduced in the duodenum ($P = 0.03$) and had a tendency to thin in the jejunum ($P = 0.07$) and in the ileum ($P = 0.059$) in lectin-treated piglets. Numerous enterocytes containing lysosomal vacuoles were observed in the slides from the jejunum and ileum of control pigs. The vacuolated enterocytes were observed both in the top and in the bottom parts of the villi. These large vacuoles were located supranuclearly in the middle and upper part of the enterocyte, and did not contain mucus (as evidenced by negative results of staining with PAS and alcian blue). In contrast to slides from control pig intestines, in lectin-treated pigs the vacuolated enterocytes were located only in the upper half of the length of villi. Accordingly, the number of vacuolated enterocytes and the size of the lysosomal vacuoles were reduced as compared with control pigs. Changes in the small intestinal villi and tunica mucosa structure induced by lectin are similar to those usually observed around weaning (Baintner, 1986). The presence and disappearance of the LV in the enterocytes seems to be a good morphological marker of maturation of the small intestine. The LV are present only in the early populations of enterocytes and reflect nonselective absorption by the enterocytes (Clarke and Hardy, 1969). The subsequent enterocyte populations possess more complex absorption mechanisms, and do not contain the LV (Baintner, 1986). Lack of LV in the

Table 12.1. Morphometry (μm) and vacuolated enterocyte index of the small intestine of control and lectin-treated pigs. Mean $\pm$ SEM, $n = 8$.

	Duodenum		Jejunum		Ileum	
	Control	Lectin	Control	Lectin	Control	Lectin
Crypt depth	186 $\pm$ 8	189 $\pm$ 8	155 $\pm$ 7	157 $\pm$ 8	130 $\pm$ 5	137 $\pm$ 5
Villus length	585 $\pm$ 55	494 $\pm$ 28	773 $\pm$ 69	627 $\pm$ 31 ^a	474 $\pm$ 51	375 $\pm$ 26 ^a
Villus width	121 $\pm$ 5	123 $\pm$ 4	107 $\pm$ 5	103 $\pm$ 3	108 $\pm$ 3	109 $\pm$ 3
Tunica mucosa	817 $\pm$ 58	711 $\pm$ 27 ^a	986 $\pm$ 74	815 $\pm$ 45	646 $\pm$ 50	508 $\pm$ 29
Enterocytes with LV (%)	0	0	97	25	98	54
LV area (μm^2)	0	0	103 $\pm$ 2	49 $\pm$ 2 ^a	107 $\pm$ 3	87 $\pm$ 2 ^a

Different letters in a row show the statistical difference ($P < 0.05$, Tukey–Kramer multiple comparison test).

enterocytes of lectin-treated pigs would suggest increased turnover of enterocytes. On the other hand, there is no clear evidence for increased crypt cell proliferation, and simultaneously the length of the villi is reduced.

In conclusion, the present results suggest that administration of red kidney bean lectin preparations may induce numerous modifications resembling the maturation of

the upper gut mucosa structure in suckling piglets.

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References

- Baintner, K. (1986) *Intestinal Absorption of Macromolecules and Immune Transmission from Mother to Young*. CRC Press, Boca Raton, Florida.
- Bardocz, S., Brown, D.S., Grant, G. and Pusztai, A. (1990) Luminal and basolateral polyamine uptake by rat small intestine stimulated to grow by *Phaseolus vulgaris* lectin phytohaemagglutinin *in vivo*. *Biochimica et Biophysica Acta* 1034, 46–52.
- Bardocz, S., Grant, G., Ewen, S.W.B., Duguid, T.J., Brown, D.S., Englyst, K. and Pusztai, A. (1995) Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. *Gut* 37, 353–360.
- Biernat, M., Zabielski, R., Sysa, P., Sosakświderska, B., Le Huërou-Luron, I. and Guilloteau, P. (1999) Small intestinal and pancreatic microstructures are modified by an intraduodenal CCK-A receptor antagonist administration in neonatal calves. *Regulatory Peptides* 85, 77–85.
- Clarke, R.M. and Hardy, R.N. (1969) An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat. *Journal of Physiology* 204, 127–134.
- de Oliveira, J.T.A., Pusztai, A. and Grant, G. (1988) Changes in organs and tissues induced by feeding of purified kidney bean (*Phaseolus vulgaris*) lectins. *Nutritional Research* 8, 943–947.
- Macgregor, H.C. (1993) *An Introduction to Animal Cytogenetics*. Alden Press, Oxford.
- Weinman, M.D., Allan, C.H., Trier, J.S. and Haggen, S.J. (1989) Repair of microvilli in the rat small intestine after damage with lectins contained in the red kidney bean. *Gastroenterology* 97, 1193–1204.

13 Topography of Digestive Enzymes in the Pig Small Intestine at the Early Stages of Ontogeny in the Norm and Pathology

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The distribution of sucrase, maltase, lactase, alkaline phosphatase, amino- and dipeptidases along the small intestine of 1-, 7-, 14- and 30-day-old piglets (normal and hypotrophic) has been investigated. With increasing age, a repression of lactase was observed, as well as induction of sucrase and maltase; the activities of alkaline phosphatase and amino- and dipeptidases decreased and were redistributed along the intestine. In hypotrophic piglets with dyspepsia the significant decrease in activities of lactase, alkaline phosphatase and amino- and dipeptidases and their redistribution along the small intestine were shown. It may be concluded that the dyspepsia resulted in a reduction of the body mass, depending, to a considerable extent, on the enzymatic defects of the small intestine.

Introduction

Pigs may be regarded as adequate models for the study of physiological processes, including those that take place in the human digestive system. Only a few studies have been devoted to the investigation of maturation of digestive enzymes in the pig small intestine during early ontogeny (Zhang *et al.*, 1997), especially under pathology. Thus, in this study, the distribution of some digestive enzyme activities along the small intestine of 1-, 7-, 14- and 30-day-old piglets has been investigated. Activities of sucrase, maltase, lactase, alkaline phosphatase, aminopeptidase M and dipeptidases with different substrate specificity were measured in the duodenum, jejunum 1 and 2 and ileum of normal piglets and hypotrophic piglets with dyspepsia.

Material and Methods

The experiments were carried out on 1-, 7-, 14- and 30-day-old piglets divided into healthy (control group, $n = 5$ for each age) and hypotrophic ($n = 5$ for each experimental group). In the mucosal homogenates of the duodenum, jejunum 1, jejunum 2 and ileum, the activities of sucrase (S), maltase (M), lactase (L), alkaline phosphatase (AP), amino peptidase M (AP M) and dipeptidases (DP, substrates: glycyl-L-leucine, glycyl-L-valine, glycyl-L-phenylalanine) were determined. The carbohydrase activities were measured by the glucose oxidase method (Dahlqvist, 1968); DP by the glycine method (Ugolev and Timofeeva, 1969); AP M by the method of Farr *et al.* (1968); AP with sodium *p*-nitrophenyl phosphate as a substrate; and protein by the method of Lowry *et al.* (1951). The

activities were expressed in $\mu\text{mol products min}^{-1} \text{ g}^{-1} \text{ protein}$. The data were statistically treated using Student's *t*-test.

Results and Discussion

The maximal L activity was found in the jejunum of 1- and 7-day-old pigs (Table 13.1); it declined in the intestine of 14- and 30-day-old pigs. By contrast, S and M activities were increased in older piglets, their maximum being also found in jejunum 1 or 2. The activity of AP was also maximal in jejunum 1 of 1-day-old piglets; with the age it decreased and its distribution along the small intestine became more even. In 1-day-old piglets the activity of AP M was maximal in the ileum, whereas in older piglets its maximum was observed in jejunums 1 and 2. The activities of different DP were maximal in jejunum 2 of 1-day-old piglets. In 7- and 14-day-old piglets the maximum of these activities was observed in jejunum 1 or 2, but in 30-day-old piglets it was displaced to the ileum. They decreased with the age, particularly in the duodenum.

In the hypotrophic piglets with dyspepsia we found a reduction of the body mass during the first month of life. The most significant decrease of carbohydrase activities was detected in the middle part of the small intestine. The AP activity was mostly decreased in the duodenum and in jejunum 1. On the contrary, activity in the ileum of 7-, 14- and 30-day-old piglets increased, i.e. its redistribution along the intestine was found. The activity of AP M was reduced in the duodenum and in the jejunum of piglets of all ages, as well as in the ileum of 1-day-old piglets, and increased in the ileum of older piglets. The DP activities were decreased along the whole small intestine of hypotrophic piglets, and their maximum was displaced to the ileum of older piglets. The morphological study revealed atrophy, shortening and deformation of the villi.

It may be concluded that the dyspepsia resulted in a reduction of the body mass and depends to a great extent on the enzymatic defects of the small intestine. Probably, the displacement of the maximal enzyme activity to the distal part of the small intestine in pathological pigs should

Table 13.1. The activities of enzymes in the small intestine of normal piglets.

Enzyme	Days	Duodenum	Jejunum 1	Jejunum 2	Ileum
Lactase	1	41.8 $\pm$ 6.1	42.3 $\pm$ 9.3	27.3 $\pm$ 4.7	13.1 $\pm$ 2.3
	7	33.8 $\pm$ 6.0	41.4 $\pm$ 7.5	57.5 $\pm$ 8.6	4.9 $\pm$ 1.3
	14	12.9 $\pm$ 0.8	20.5 $\pm$ 2.7	16.4 $\pm$ 3.4	1.7 $\pm$ 0.3
	30	1.2 $\pm$ 0.1	4.6 $\pm$ 0.7	6.0 $\pm$ 0.5	0.5 $\pm$ 0.1
Maltase	1	8.8 $\pm$ 1.6	14.4 $\pm$ 1.8	17.8 $\pm$ 2.9	10.5 $\pm$ 0.9
	7	22.4 $\pm$ 4.6	52.6 $\pm$ 8.1	67.8 $\pm$ 8.9	22.6 $\pm$ 4.6
	14	20.1 $\pm$ 2.6	56.7 $\pm$ 1.0	70.8 $\pm$ 7.8	25.5 $\pm$ 4.8
	30	54.1 $\pm$ 9.8	62.7 $\pm$ 9.4	41.5 $\pm$ 6.8	29.9 $\pm$ 5.4
AP	1	41.8 $\pm$ 4.5	64.7 $\pm$ 9.3	52.3 $\pm$ 8.2	19.1 $\pm$ 2.9
	7	17.5 $\pm$ 3.3	23.4 $\pm$ 3.7	29.6 $\pm$ 5.3	12.4 $\pm$ 2.5
	14	18.6 $\pm$ 1.7	16.1 $\pm$ 2.2	16.3 $\pm$ 2.6	14.0 $\pm$ 1.9
	30	8.4 $\pm$ 1.5	8.9 $\pm$ 1.2	8.7 $\pm$ 1.1	11.7 $\pm$ 1.5
AP M	1	77.3 $\pm$ 9.9	90.4 $\pm$ 8.5	91.3 $\pm$ 8.4	105.1 $\pm$ 1.5
	7	82.9 $\pm$ 7.3	111 $\pm$ 10	128 $\pm$ 10	63.0 $\pm$ 4.8
	14	78.3 $\pm$ 9.6	84.3 $\pm$ 11.3	72.5 $\pm$ 4.0	42.0 $\pm$ 3.2
	30	33.4 $\pm$ 4.8	85.6 $\pm$ 9.2	75.4 $\pm$ 9.5	56.4 $\pm$ 6.7
Glycyl-valine DP	1	1190 $\pm$ 187	1703 $\pm$ 152	2042 $\pm$ 219	1700 $\pm$ 174
	7	763 $\pm$ 107	1104 $\pm$ 131	1224 $\pm$ 157	754 $\pm$ 73
	14	1012 $\pm$ 59	1280 $\pm$ 149	1261 $\pm$ 130	960 $\pm$ 171
	30	636 $\pm$ 116	1067 $\pm$ 101	784 $\pm$ 33	1187 $\pm$ 135

be considered as an adaptive sign, promoting the animal's survival, because it is known that the ileum is a reserved zone of the gut (Membrane Digestion, 1989).

References

- Dahlqvist, A. (1968) Assay of intestinal disaccharidases. *Analytical Biochemistry* 7, 18–25.
- Farr, W., Rehfeld, N. and Haschen, R.I. (1968) Vergleichende Untersuchungen zur Bestimmung der Aminosäurearylamidase. *Zeitschrift Medicinische Labortechnik* 9, 78.
- Lowry, O.H., Rosenbrough, N.Y., Farr, A.L. and Randall, R.Y. (1951) Proteins measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193, 265–275.
- Membrane Digestion (1989) MIR Publisher, Moscow, 288 pp.
- Ugolev, A.M. and Timofeeva, N.M. (1969) Assay of peptidase activity. In: *Studies of the Digestive Apparatus in Man*. Nauka, Leningrad, pp. 178–181. (In Russian.)
- Zhang, H., Malo, C. and Buddington, R. (1997) Suckling induces rapid intestinal growth and changes in brush border digestive functions of newborn pigs. *Journal of Nutrition* 127, 418–426.

14 The Message Underlying Pig Pancreas Regeneration After Partial Pancreatectomy

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We performed a time-course evaluation of growth-related intracellular reactions in the remnant pancreas after partial pancreatectomy (Px) in pigs. Px Piglets were sacrificed 1 h to 30 days after surgery. Pancreatitis-associated protein (PAP), an inflammation marker, was increased 8 h after surgery to a maximum at day 3. Apoptosis was maximal on day 3 with maximal caspase activity. Regeneration was evident on day 5 with increases in MAP kinase activities peaking on day 30. Remnant hypertrophy and hyperplasia were observed on day 30. Pig pancreatic regeneration processes include inflammation, apoptosis and growth.

Introduction

Pancreas regeneration occurred in rats after induced pancreatitis (Jurkowska *et al.*, 1992) and partial pancreatectomy (Px) (Morisset *et*

al., 1999). The pig pancreas also exhibited regeneration after Px (Fiorucci *et al.*, 1998). The human pancreas failed to show regeneration after partial resection (Tsiotos *et al.*, 1999). This study established time-course

intracellular reactions related to pig pancreas regeneration after partial Px.

Materials and Methods

Eight female Yorkshire/Landrace/Duroc crosses, with a mean body weight of 16.2 kg at surgery, had 23.4 ± 5.3 g of their pancreas removed (49.3%) according to Koyama *et al.* (1986). The control pig had laparotomy and pancreas manipulation without resection. The resected sections taken at surgery and the remnants were excised and weighed after Px. Biopsies of the remnants (0.5 g) were taken at the dorsal and ventral parts of the corpus surrounding the mesenteric vein plus one at the duodenal site near the exit of the pancreatic duct. Frozen samples were homogenized in appropriate buffers and evaluated for pancreatitis-associated protein (PAP) (Morisset *et al.*, 1997), for caspase 3 activity (Zhang *et al.*, 1999) and for the pancreatic digestive enzymes, p42/p44 MAP kinases, RNA and DNA (Jurkowska *et al.*, 1992; Lainé *et al.*, 1993; Morisset *et al.*, 1999).

Results

All pigs gained weight after Px, up to 16.9 kg 30 days after the operation; the unresected control animal gained 17.5 kg in 30 days. Weights of the pancreatic remnants were increased by 4.4% and 22.3%, 28 and 30 days after initial surgery, when compared with the remnant weight of the control animal excised at 30 days.

As a stress protein, PAP expression was barely detectable in all resected fragments; however, PAP remained undetectable 1 to 4 h after Px but was significantly induced 4 h later (8 h) in the remnants. Maximal expression was observed 3 days after surgery.

Caspase activity is a good marker of programmed cell death (Nunez *et al.*, 1998). In our experiments, caspase 3 remained at a low level of activity at all times in the resected segments of the pancreas; how-

ever, maximal caspase 3 activation occurred 3 days after Px in pancreatic remnants. Caspase 1, although detected, remained unaffected throughout.

Activity of the MAP kinases p42/p44 (cell cycle markers) remained at low levels in all resected pancreas and in remnants up to 3 days post-surgery. Active phosphorylated forms of both enzymes appeared from day 5 post-Px in the remnants, with maximal activation observed at day 30 post surgery.

Px caused major atrophy of the remaining pancreatic gland 3 days after surgery, as evidenced by major decreases in concentrations of all pancreatic components. Signs of recovery were evident at day 28 and more so at day 30, with increases in all components' concentrations, and signs of remnant hypertrophy. Increases of 9.7% and 44.4% in total DNA were observed 28 and 30 days, respectively, after initial surgery. These data are positive signs of pancreas hyperplasia and regeneration.

Discussion

Three major glandular and cellular different responses to Px highlight this study. The initial inflammatory response started a few hours after resection and was characterized by PAP induction as soon as 8 h after the operation. This induction could be mediated by inflammatory factors such as tumor necrosis factor alpha, interferon gamma and interleukin 6, known to increase PAP mRNA level (Morisset *et al.*, 1997). The second major response probably deals with remodelling of the pancreatic remnant after surgical injury and is characterized by active apoptosis peaking 3 days after surgery. This process also coincides with maximal decreases in pancreatic enzyme contents, a sign of reduced enzyme synthesis and secretion. Comparable reductions in pancreatic enzyme mRNA expression and protein synthesis were observed in rat pancreas after induced pancreatitis (Morisset *et al.*, 1997). Signs

of pancreatic regeneration were already evident a week after surgery, with the increases in MAP kinase activity peaking at day 30 – the time point with maximal pancreatic cellular hypertrophy and hyperplasia. These data strongly suggest that the pancreatic gland initially responded to the stress of surgery, put itself at rest and then initiated the regeneration process.

Conclusion

If similar reactions occur in human pancreas after major surgery, it would suggest that patient management should seek to minimize pancreatic demand in order to favour accelerated recovery and regrowth, since Friess *et al.* (1998) demonstrated that human pancreas can grow in response to inhibition of luminal proteolytic activity.

References

- Fiorucci, S., Bufalari, A., Distrutti, F., Bufalari, A., Lanfranccone, L., Servoli, A., Sarpi, L., Federici, B., Bartoli, A., Morelli, A. and Moggi, L. (1998) Bombesin-induced pancreatic regeneration in pigs is mediated by p46^{shc}/p52^{shc} and p42/p44 mitogen-activated protein kinase upregulation. *Scandinavian Journal of Gastroenterology* 33, 1310–1320.
- Friess, H., Kleeff, J., Isenmann, R., Malfertheiner, P. and Buchler, M.W. (1998) Adaptation of the human pancreas to inhibition of luminal proteolytic activity. *Gastroenterology* 115, 388–396.
- Jurkowska, G., Grondin, G., Massé, S. and Morisset, J. (1992) Soybean trypsin inhibitor and caerulein accelerate recovery of caerulein induced pancreatitis in rat: a biochemical and morphological study. *Gastroenterology* 102, 550–562.
- Koyama, I., Pennington, L.R., Swindle, M.M. and Williams, G.M. (1986) Pancreatic allo transplantation with Roux-en-y jejunal diversion in swine: its technical aspects. In: Tumbleson, M.E. (ed.) *Swine in Biomedical Research*. Plenum Press, New York, pp. 385–389.
- Lainé, J., Beattie, M. and LeBel, D. (1993) Simultaneous kinetic determinations of lipase, chymotrypsin, trypsin, elastase, and amylase on the same microliter plate. *Pancreas* 8, 383–386.
- Morisset, J., Iovanna, J. and Grondin, G. (1997) Localization of rat pancreatitis associated protein during bile salt-induced pancreatitis. *Gastroenterology* 112, 543–550.
- Morisset, J., Aliaga, J.C., Calvo, E.L., Bourassa, J. and Rivard, N. (1999) Expression and modulation of p42/p44 MAP kinases and cell cycle regulatory proteins in rat pancreas regeneration. *American Journal of Physiology* 277, G953–G959.
- Nunez, G., Benedict, M.A., Hu, Y. and Inohara, N. (1998) Caspases: the proteases of the apoptotic pathway. *Oncogenes* 17, 3227–3245.
- Tsiotos, G.G., Barry, M.K., Johnson, C.D. and Sarr, M.G. (1999) Pancreas regeneration after resection: does it occur in humans? *Pancreas* 19, 310–313.
- Zhang, Y., Fujita, N. and Tsuruo, T. (1999) Caspase-mediated cleavage of p21^{Waf1/Cip1} converts cancer cell from growth arrest to undergoing apoptosis. *Oncogene* 18, 1131–1138.

15 The Effect of Weaning Diet on the Intestinal Morphology of Young Piglets

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Sixteen pigs were selected from four related sows at 20 days of age. Treatments were: (A) commercial diet containing uncooked cereals; (B) commercial diet containing cooked cereals; (C) simple diet containing uncooked cereals; and (D) suckling pigs given access to creep feed. At 27 days of age pigs were slaughtered. Villus height in the duodenum (Id), jejunum (Ij) and ileum (Ii) was not affected by treatment ($P < 0.05$). Crypt depth in the Id was greater for treatments A and C than for either B or D ($P < 0.05$). Ii crypt depths were lower for treatment D than for the other treatments ($P < 0.05$). Goblet cell number per mm² of villus or crypt epithelium was unchanged by treatment ($P < 0.05$). Argentaffin cell numbers per mm² of crypt epithelium were higher for treatments B and D than for treatments A and C. In conclusion, including cooked cereals in starter diets helped to maintain the structure of the small intestine.

Introduction

Weaning is associated with changes in the pig's intestinal morphology: principally, a reduction in villus height, a change in villus shape and an increase in crypt depth (Hampson, 1986; Kelly *et al.*, 1991). To help to lessen these changes, diets high in milk products and cooked cereals and with low levels of antigenicity are fed. The objective here was to examine the effect of steam-flaking cereals on the intestinal integrity of weaned pigs.

days of age, one piglet from each litter was assigned randomly to each of four treatments. Treatments A, B and C pigs were weaned on to diet 1 (193 g milk products, 230 g wheat and 200 g maize kg⁻¹), diet 2 (as for diet 1 but with the cereal component steam-flaked) and diet 3 (200 g milk products, 346 g wheat and 160 g barley kg⁻¹), respectively. These pigs were individually penned. Treatment D pigs were unweaned and received creep feed (diet 2). Steam-flaking increased the level of gelatinized starch as a proportion of total starch in both maize (838 cf. 265 g kg⁻¹) and wheat (745 cf. 203 g kg⁻¹). Analysis of diets is shown in Table 15.1.

Materials and Methods

Animals and experimental diets

Sixteen Landrace × Large White pigs from four closely related sows were used. At 20

Sampling and tissue processing

Pigs were slaughtered at 27 days of age by lethal injection with Euthatal (pentobarbi-

Table 15.1. Analysis of the experimental diets (g kg⁻¹).

	Diet 1	Diet 2 ^a	Diet 3
Dry matter	885	884	898
Crude protein	229	226	197
Fat	86	85	60
Crude fibre	25	28	29
Ash	59	61	60

^aDiet 2 contained steam-flaked wheat and maize.

tone sodium BP). On removal of the digestive tract, sections of the duodenum (Id) (10 cm from stomach), the jejunum (Ij) (60 cm from stomach) and the ileum (Ii) (10 cm from caecum) were excised and fixed in 10% phosphate-buffered formalin. Following processing, sections were prepared and then stained using: (i) haematoxylin and eosin (H + E); (ii) periodic acid/Schiff's reagent (PAS); and (iii) Masson Fontana method, according to the methods of Bancroft and Stevens (1990).

The stained sections were viewed using a Letzt Ortholux microscope. Villus height and crypt depth were measured using the H + E stained sections. Sections stained by the PAS method were used to count numbers of goblet cells present in villus and crypt epithelium. Argentaffin cells in the crypt epithelium were quantified from sections stained using the Masson Fontana method and a $\times 63$ Planapo oil immersion objective.

Results and Discussion

Villus height in the Id, Ij and Ii were not affected by treatment ($P > 0.05$). Crypt

hyperplasia was observed in the Id of the weaned pigs in treatment groups A and C, while for treatments B and D shallow crypts were observed ($P < 0.05$). Ii crypt depths were greater for all weaned pigs than for suckled pigs ($P < 0.05$). Crypt depths are summarized in Table 15.2.

Finger-shaped villi with smooth surfaces and rounded tips were seen in all portions of the small intestine (SI) of the unweaned pigs, while complex villus shapes were found in the weaned pigs from treatments A and C. Villus shape for treatment B was similar to that of suckling pigs.

One function of goblet cells is to lubricate ingested food (Junqueira *et al.*, 1998) and it was expected that diet consistency (milk cf. pellets) might influence their number in the small intestine (SI). Dunsford *et al.* (1991) found that weaning *per se* and not diet was the primary cause of decreases in goblet cell populations. In the present study goblet cell numbers were similar for all treatments.

Argentaffin cells have the function of increasing the motility of the SI by releasing 5-hydroxytryptamine. This stimulates contraction of the SI smooth muscle (Junqueira *et al.*, 1998) and greater numbers

Table 15.2. Mean crypt depth (μm) $\pm$ SD of weaned and unweaned pigs.

	Treatment				<i>F</i> test
	A	B	C	D	
Duodenum (Id)	350 $\pm$ 77 ^a	278 $\pm$ 66 ^b	342 $\pm$ 89 ^a	285 $\pm$ 70 ^b	$P < 0.05$
Jejunum (Ij)	281 $\pm$ 49	281 $\pm$ 57	270 $\pm$ 43	253 $\pm$ 55	NS
Ileum (Ii)	249 $\pm$ 59 ^a	270 $\pm$ 70 ^a	254 $\pm$ 68 ^a	211 $\pm$ 73 ^b	$P < 0.05$

Table 15.3. Mean argentaffin cell numbers per mm² of crypt epithelium $\pm$ SD.

	Treatment				F test
	A	B	C	D	
Duodenum (Id)	5.1 $\pm$ 0.5 ^a	10.0 $\pm$ 2.6 ^b	5.9 $\pm$ 2.0 ^a	8.4 $\pm$ 0.8 ^b	$P < 0.05$
Jejunum (Ij)	3.8 $\pm$ 1.6	4.0 $\pm$ 0.9	2.5 $\pm$ 1.2	3.1 $\pm$ 1.0	NS
Ileum (Ii)	1.1 $\pm$ 1.7	1.31 $\pm$ 1.1	1.2 $\pm$ 1.1	0.7 $\pm$ 0.7	NS

of these cells may increase passage rates of ingesta. Higher numbers of argentaffin cells ($P < 0.05$) were found in the Id of treatment groups B and D compared with treatment groups A and C (Table 15.3). Since both cooked cereal and sow's milk are digested with greater efficiency (relative to the raw cereal-based diets), nutrients may be absorbed faster, thus allowing increased passage rates through the SI.

In conclusion, postweaning diet does influence intestinal morphology. The finger-shaped villi, the shallow crypt depths and the high numbers of argentaffin cells in the SI of pigs fed cooked cereals may be expected to promote improved postweaning growth. Morphological changes to the SI of these pigs were similar to those found in unweaned pigs, suggesting a diet of high digestibility.

References

- Bancroft, J.D. and Stevens, A. (1990) *Theory and Practice of Histological Techniques*, 3rd edn. Churchill Livingstone, Edinburgh, 709 pp.
- Dunsford, B.R., Haensley, W.E. and Knabe, D.A. (1991) Effects of diet on acidic and neutral goblet cell populations in the small intestine of early-weaned pigs. *American Journal of Veterinary Research* 52, 1743–1746.
- Hampson, D.J. (1986) Alterations in piglet small intestinal structure at weaning. *Research in Veterinary Science* 40, 32–40.
- Junqueira, L.C., Carneiro, J. and Kelley, R.O. (1998) *Basic Histology*, 9th edn. Appleton and Lange, Stamford, Connecticut, 494 pp.
- Kelly, D., Smyth, J.A. and McCracken, K.J. (1991) Digestive development in the early weaned pig. 11. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. *British Journal of Nutrition* 65, 181–188.

Part II

The Gastrointestinal Immune System

16 Development and Function of the Pig Gastrointestinal Immune System

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The porcine gastrointestinal immune system has evolved for the purpose of maintaining the integrity of the pig. In order to achieve this function it must fulfil two important roles. Firstly, it must be able to recognize and eliminate the effects of microorganisms (and their products) that have the potential to cause infectious diseases. Secondly, it must be able to recognize harmless (mostly dietary) antigens and prevent wasteful and potentially harmful dietary responses to them. In this review we attempt to identify those immunological mechanisms that regulate these responses in adult animals. We then examine how these responses develop in the young piglet. The overall conclusion to be drawn from this work is that the intestinal immune system of the young pig is very immature and that its slow development may underlie the increased susceptibility to disease.

Mucosal Immune Responses

In order to control infections within the gut, the local mucosal immune system has adopted a remarkably different strategy to that which operates systemically. Antigens that gain entry into tissue via cuts or the mouthparts of biting insect have to be actively eliminated, a process that involves inflammation and some degree of tissue damage. In contrast, microorganisms that remain within the gastrointestinal tract are unlikely to cause harm. Only once the organism (or its toxin) has adhered to the epithelial cells and gained entry across the epithelium will it cause disease. In this way, maintaining the integrity of the epithelial surface is a critical process in preventing infections. It is, then, not surprising that mucosal immune defence mechanisms tend not to be inflammatory and are primarily directed toward keeping potentially harmful antigens within the

lumen of the intestine, where peristalsis and the constant flow of digesta will effectively remove them. IgA antibodies, which are unable to activate complement, are clearly well able to fulfil this role. In health, the physical integrity of the mucosal barrier (epithelial cells and mucus) together with IgA form an effective barrier to the entry of potentially harmful antigens. Only once the barrier is breached do other defensive processes play a role. In particular, other immunoglobulin isotypes (IgG), cytotoxic lymphocytes (CTL) and delayed type hypersensitivity reactions have been implicated in the recovery from mucosal infections in pigs.

In pigs as in other species, significant quantities of fed protein are absorbed immunologically intact across the intestinal mucosa (Wilson *et al.*, 1989; Telemo *et al.*, 1991). Immune responses to harmless dietary components must be regulated to prevent tissue damage and impaired

absorption of macromolecules, and systemic tolerance to fed proteins ('oral tolerance') has been demonstrated in the pig (Newby *et al.*, 1979; Bailey *et al.*, 1993). Thus, the immunological structures associated with the gastrointestinal tract have evolved to generate different types of response to pathogens and to harmless environmental antigens. The sites at which such 'value judgements' are made are unclear in any species, but models of the function of mucosal-associated lymphoid tissues must account for both of these outcomes.

Functional Organization

The mucosal-associated lymphoid tissue can be divided into two major compartments: that consisting of the organized lymphoid structures (Peyer's patches, mesenteric lymph nodes, etc.) and that occurring in tissues specialized for other functions (the intestinal lamina propria). The lymphoid tissue comprising the mucosal immune system of the pig has received a significant amount of attention. In the conventional model, the organized tissues are 'inductive' sites, populated by naive cells: following priming they recirculate as memory cells through the diffuse 'effector' sites such as the intestinal lamina propria. However, recent evidence suggests that the conventional model of recirculation of naive and memory T-cells may be less well understood than has been thought (Westermann *et al.*, 1996; Meeusen *et al.*, 1996). The unusual migration pathway of T-cells within the lymph nodes of the pig has made interpretation of experiments in this species difficult (Binns *et al.*, 1985). Both T- and B-cells do appear in afferent lymph draining from the intestine and there is evidence for local proliferation, particularly of T-cells (Rothkotter *et al.*, 1995). Re-transfused cells from afferent lymph return selectively to the intestine, although whether these are from Peyer's patch or lamina propria has not been determined, nor whether this is the sole route of exit (Rothkotter *et al.*, 1993).

Peyer's patches

Within the pig jejunum, 11–26 discrete Peyer's patches have been reported. Each patch contains multiple B-cell follicles separated by interfollicular areas dominated by T-cells. Plasma cells containing IgM, IgG and IgA are present in the dome and between the bases of the follicles (Brown and Bourne, 1976a). Microfold cells have been described in the overlying lymphoepithelium, demonstrable by staining for cytokeratin-18 (Gebert *et al.*, 1994): between the epithelium and the follicle is a dome region containing discrete cells expressing high levels of MHC class II antigens and with morphology characteristic of dendritic cells (Wilders *et al.*, 1983).

Lamina propria

The intestinal lamina propria is heavily populated with leucocytes in mature animals. In the pig, plasma cells and B-cells predominate around the crypts and T-cells in the villi. Plasma cells are predominantly IgA⁺ and IgM⁺ but some IgG⁺ cells are also present (Brown and Bourne, 1976a). In the T-cell-dominated villi there is clear spatial separation between CD8⁺ cells in and under the epithelium and CD4⁺ cells in the lamina propria deep to the capillary plexus (Vega-Lopez *et al.*, 1993). There is extensive expression of MHC class II antigens in the villi and crypts, though a proportion of this is associated with capillary endothelium (Wilson *et al.*, 1996).

Epithelium

The majority of lymphocytes in the epithelium (IEL) express CD2. In mature animals a high proportion also express CD8⁺. Available antibodies have not allowed identification of CD8⁺ $\alpha\alpha$ homodimer IEL as has been described in humans. Intraepithelial cells from mature pigs can produce IL-2 and interferon following activation *in vitro* and also engage in limited cytotoxicity (Wilson *et al.*, 1986a).

Induction and Regulation of Mucosal Responses

There is a substantial body of evidence to document the uptake of antigen via M-cells into the Peyer's patches in the induction of mucosal responses. Whilst few would question the pivotal role of the Peyer's patches in the induction of primary responses, the immunological role of the lamina propria is less clear. The pig lamina propria is densely populated with cells capable of fulfilling an immunological role, and we have hypothesized that it may be of critical importance in orchestrating the responses to dietary antigens.

Using methods developed in this laboratory we have isolated from the intestinal lamina propria populations of low buoyant density cells and T-cells. Three-colour immunofluorescence has revealed that the jejunal lamina propria contains large numbers of MHC class II-bearing cells. These cells could be subdivided into at least three subsets based upon their coexpression of other markers. The majority of MHC class II was coexpressed on CD45⁺ cells; however, capillary endothelium as well as a network of MHC class II-bearing stromal cells lying deep to the capillary plexus could be identified. Flow cytometry of the low-buoyancy lamina propria cells (isolated on 30%/50% Percoll gradients) confirmed the presence of a population of cells which only express MHC class II, and a second population of cells expressing CD16, SwC3 and CD45. By sorting the gated populations of cells we showed that the CD45⁻ and CD45⁺ cells were morphologically very different. The CD45⁺ cells were large and strongly adherent and frequently had bilobed nuclei. In contrast, the CD45⁻ were smaller and elongated, with dense oval nuclei. The two sorted populations were tested in a mixed lymphocyte reaction: whereas the MHC II⁺ CD45⁺ population were found to be potent stimulators of primary responses, the CD45⁻ stromal population were unable to generate any proliferative response (Haverson *et al.*, 1999).

Analysis of the T-cells that are resident in the lamina propria and cytokines that

they produce would further suggest that this is heavily biased in favour of the maintenance of homeostasis by preventing over-reaction to otherwise harmless luminal antigens. The profile of cytokines within the intestinal lamina propria would appear to be independent of other non-mucosal sites and to be highly sensitive to a range of factors. In the pig, activation of isolated lamina propria T-cells with concanavalin A resulted in a highly polarized situation in which the interleukins IL-4 and IL-10 were transcribed but not IL-2 (Bailey *et al.*, 1994). This would be consistent with an environment dominated by Th2 cytokines that supported the synthesis and secretion of IgA. The response of isolated human cells has been shown to be highly dependent upon the nature of the activation signal. For example, it has been shown that ligation of the CD2 surface molecule resulted in secretion of much higher levels of IL-2, IL-4, γ IFN and TNF- α by lamina propria cells than by systemic cells (Targan *et al.*, 1995), whilst co-ligation of CD3 plus CD28 triggered less IL-2 secretion. Other groups have reported that CD3/CD28 triggering resulted in higher levels of IL-4 secretion by intestinal than by systemic cells (Boirivant *et al.*, 1996), further indicating that lamina propria T-cells have the potential to produce high levels of IL-2 or IL-4, depending on the signal delivered during activation. Clearly there are two possible functions for these antigen-driven T-cells: (i) they could be involved in surveillance and expression of active responses to potential pathogens (James and Zeitz, 1994); or (ii) they may be involved in the regulation and maintenance of mucosal tolerance (Bailey *et al.*, 1998). In our laboratory we have shown that activation of isolated pig lamina propria cells results in high levels of cell death when compared with similarly isolated splenic lymphocytes. This suggests that the primary function of this environment may be to prevent the expression of active T-cell responses to antigens normally present in the intestinal lumen.

If, as we have postulated, the lamina propria is so heavily biased in favour of the

maintenance of tolerance, then it is of importance to determine the mechanisms by which this microenvironment is maintained and how it may be manipulated in order to control infectious agents when the mucosal barrier is 'breached'. Two recent studies begin to shed light upon these questions. Firstly, we have shown that whereas the vast majority of both lamina propria and Peyer's patch CD4⁺ T-cells are CD45RC⁻ (Haverson *et al.*, 1999) (i.e. memory or recently activated cells), the majority of CD4⁺ cells in the lymph which drains the intestine are CD45RC⁺ (M. Bailey and H.J. Rothkotter, unpublished observation). There are a number of possible hypotheses as to why the dominant lamina propria population does not emigrate but, no matter what the mechanism, this result would strongly suggest that the maintenance of the lamina propria microenvironment is highly selective. The second piece of work addressed the question of what factors may be able to rescue activated lamina propria lymphocytes (LPLs) from increased cell death by apoptosis. In these studies we showed that addition of recombinant pig IL-2 into the culture results in full rescue of CD8⁺ T-cells but has no effect on the survival of CD4⁺ T-cells. In contrast, addition of TGF- β rescued LPL CD4⁺ T-cells whilst having no effect on CD8⁺ T-cells (F. Plunkett and M. Bailey, unpublished observation). Such a change in response to a infectious agent might allow the expression of a protective IgA response and the generation of cytotoxic cells capable of killing virus-infected cells.

To summarize, immunological features of the intestinal lamina propria that may contribute to a role in the 'downregulation' of responses to dietary antigens in 'adult pigs' include the following:

- compartmentalized organization;
- predominance of CD4⁺CD45RC⁻ T-cells;
- CD2⁺ T-cells coexpress CD3⁺;
- upon activation, T-cells express IL-2R;
- activated cells secrete IL-4 and IL-10, but little IL-2;
- activation leads to apoptosis;
- cells can be rescued from apoptosis by IL-2 (CD8⁺) or TGF- β (CD4⁺);
- high proportion of MHC class II expression on 'non-professional immune cells'.

Development of the Gastrointestinal Immune System

Many features of the young pig's gastrointestinal immune system would suggest that it is relatively immature. Whilst this may in part reflect a lack of exposure to antigen, there is a growing body of evidence to suggest that this immaturity may be of functional significance.

Peyer's patches

Peyer's patches are not fully formed at birth: accumulations of leucocytes are visible but extend and organize rapidly in the first few days of life. Increases in size and organization are at least partially dependent on antigen (Rothkotter and Pabst, 1989).

Epithelium

In young pigs, IEL are mostly CD2⁻CD4⁻CD8⁻. During the first few weeks CD2⁺CD4⁻CD8⁻ appear but CD8⁺ IEL do not appear until 7 weeks onwards (Whary *et al.*, 1995). The ability of IEL from young piglets to respond to mitogens is poor and develops with time (Wilson *et al.*, 1986b), though this can be delayed by early weaning. Failure to proliferate may reflect their inability to produce IL-2 at this age since they are capable of responding to IL-2 *in vitro* (Whary *et al.*, 1995).

Lamina propria

Virtually no T-cells or plasma cells populate the lamina propria of the piglet at birth. Development appears to be antigen-driven and occurs in phases. Plasma cells accumulate in the first 4 weeks of life: the majority of cells are IgM⁺ initially but IgA⁺ cells

rapidly predominate (Brown and Bourne, 1976b). Gamma-delta T-cells are present in the fetus (Trebichavsky *et al.*, 1995), but in low numbers. During the first week, CD2⁺4⁻8⁻ cells enter the intestine; CD2⁺4⁺ cells appear at around 3 weeks old, CD2⁺8⁺ at around 7 weeks (Rothkotter *et al.*, 1991; Bianchi *et al.*, 1992; Vega-Lopez *et al.*, 1995). More recently we have shown that whereas in adult pigs the majority of lamina propria CD2⁺ T-cells coexpress CD3⁺, in pigs younger than 3 weeks of age less than 50% of CD2⁺ cells are also positive for CD3. The number of cells expressing myeloid-associated antigen SwWC1 also increases with age, probably reflecting an increase in granulocyte (eosinophil, mast cell) numbers.

Evidence for a poorly developed intestinal lamina propria in young pigs which may contribute to their failure to 'down-regulate' responses to dietary antigens in the postweaning period includes: paucity of cells; differential development of CD4⁺ and CD8⁺ cells; increased proportions of CD2⁺ CD3⁻ cells; and reduced ability to respond to mitogens.

Although the organized lymphoid tissues of the newborn piglet rapidly develop and express immunological function, the immunological architecture of diffuse effector sites such as the intestinal lamina propria can take up to 7–9 weeks to develop, depending on antigenic challenge. This is consistent with a need to produce effector cells but the time course is delayed, despite continuous environmental challenge. The young piglet is capable of active immune responses to live virus and to dietary components at 3 weeks of age, and these are of comparable magnitude to that to systemic antigen (Welch *et al.*, 1988; Bailey *et al.*, 1994). However, tolerance to continuously fed proteins is not fully achieved until after 8 weeks old; similarly, the magnitude of primary responses to novel dietary components is reduced with age (Wilson *et al.*, 1989; Miller *et al.*, 1994). It has been proposed that this early failure to regulate responses to harmless dietary proteins or commensal bacteria contributes to postweaning diarrhoea in early-weaned piglets (Miller *et al.*, 1984; Li *et al.*, 1991).

Immunological Responses at Weaning

The young pig is especially susceptible to enteritis caused by enterotoxigenic *Escherichia coli* during the period immediately following weaning. Early weaning exacerbates this problem and throughout Europe it is normal commercial practice to wean pigs at an age much younger than would occur in the wild. This results in an increased susceptibility to bacterial infection, the effects of which are controlled by the very wide use of antibiotics.

Weaning is a highly complex event and represents several challenges to a pig's homeostasis, many of which under farm conditions are highly variable in incidence, duration and severity. These include: challenge with potential pathogens (ETEC, Salmonella, Rotavirus); environment (housing and temperature); dietary change; inappetance; stress (mixing with other pigs and removal from the dam); altered microenvironment of the gut (pH, microflora, nutrient supply); and loss of maternal milk (immune protection, non-specific antibacterial protection and bioactive peptides).

Several hypotheses have been put forward why these factors may predispose to postweaning diarrhoea. For example, it has been suggested that dietary change may be harmful as a result of altered nutrient supply (McCracken *et al.*, 1999), hypersensitivity reactions against dietary antigens and harmful responses to ANFs associated with some feedstuffs, particularly soybean. The weaning period in pigs is accompanied by changes in intestinal morphology, including reduced villus height, increased villus width and increased crypt depth. Similar morphological changes are induced in mice as a result of the transient allergic reactions that are induced following the introduction of novel dietary antigens. Based largely upon these results, we hypothesized that a similar mechanism may be involved during the postweaning period in pigs (Miller *et al.*, 1984a,b). The results of studies summarized in the previous section clearly show that the intestinal immune system of the young pig is immature and so poses the question: at what age

is the porcine intestine capable of making an appropriate immune response to both dietary and microbial antigens? During the early postnatal period the young animal has to adapt to increasing amounts of both dietary and microbial antigens and it has been postulated that a transient aberrant immune response to antigens in the post-weaning diet may predispose to bacterial infection and disease.

There is a growing body of evidence of aberrant immunological responses following early weaning. For example, we have shown that weaning is associated with a transient reduction in the ability of intraepithelial lymphocytes to respond to mitogens. Similarly, the ability of other splenic T-cells to secrete IL-2 is reduced. We have also shown that weaning triggers an accumulation of CD2⁺ cells into the intestinal lamina propria and this led us to postulate that this may be due to the relocalization of systemic T-cells to the gut. We reasoned that this might lead to the local secretion of Th1-type cytokines and that this could provide a mechanism for the observed changes in gut morphology at that time. Recent results

have not been entirely consistent with this hypothesis, because rather than showing an increased ability of lamina propria lymphocytes (isolated 4 or 7 days postweaning) to produce IL-2, the secretion of this and other growth factors was reduced. Interestingly, the results did serve to emphasize the immaturity of the pig gut lamina propria at this time: whereas the total number of lamina propria CD2⁺ cells was increased, the proportion that coexpressed CD3⁺ (both CD45RC⁺ and CD45RC⁻) was reduced. Our studies in adult pigs have strongly implicated the importance of these cells in the regulation of responses to dietary antigens. Given the paucity of these cells in the post-weaning pig intestine, it is possible that further study of these cells may start to explain the increased susceptibility of the post-weaning pig to enteric infection.

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References

- Bailey, M., Miller, B.G., Telemo, E., Stokes, C.R. and Bourne, F.J. (1993) Specific immunological unresponsiveness following active primary to proteins in the weaning diets of piglets. *International Archives of Allergy and Immunology* 101, 266–271.
- Bailey, M., Hall, L., Bland, P.W. and Stokes, C.R. (1994a) Production of cytokines by lymphocytes from spleen, mesenteric lymph node and intestinal lamina propria of pigs. *Immunology* 82, 577–583.
- Bailey, M., Miller, B.G., Telemo, E., Stokes, C.R. and Bourne, F.J. (1994b) Altered immune response to fed proteins following neonatal exposure of piglets to the antigen. *International Archives of Allergy and Immunology* 103, 183–187.
- Bailey, M., Plunkett, F., Clarke, A., Sturgess, D., Haverson, K. and Stokes, C. (1998) Activation of T cells from the intestinal lamina propria. *Scandinavian Journal of Immunology* 48, 177–182.
- Bianchi, A.T., Zwart, R.J., Jeurissen, S.H. and Moonen-Leusen, H.W. (1992) Development of the B- and T-cell compartments in porcine lymphoid organs from birth to adult life: an immunohistological approach. *Veterinary Immunology and Immunopathology* 33, 201–221.
- Binns, R.M., Pabst, R. and Licence, S.T. (1985) Lymphocyte emigration from lymph nodes by blood in the pig and efferent lymph in the sheep. *Immunology* 54, 105–111.
- Boirivant, M., Fuss, I., Fiocchi, C., Kleins, S., Strong, S.A. and Strober, W. (1996) Hypoproliferative human lamina propria T-cells retain their capacity to secrete lymphokines when stimulated via CD2/CD28 pathways. *Proceedings of the Association of American Physicians* 108, 55–67.
- Brown, P.J. and Bourne, F.J. (1976a) Distribution of immunoglobulin staining cells in alimentary tract, spleen and mesenteric lymph node of the pig. *American Journal of Veterinary Research* 37, 9–13.

- Brown, P.J. and Bourne, F.J. (1976b) Development of immunoglobulin-containing cell populations in intestine, spleen and mesenteric lymph node of the young pig as demonstrated by peroxidase-conjugated antiserums. *American Journal of Veterinary Research* 37, 1309–1314.
- Gebert, A., Rothkotter, H.J. and Pabst, R. (1994) Cytokeratin-18 is an M-cell marker in porcine Peyer's patches. *Cell and Tissue Research* 276, 213–221.
- Haverson, K., Bailey, M. and Stokes, C.R. (1999) T-cell populations in the pig intestinal lamina propria: memory cells with unusual phenotypic characteristics. *Immunology* 96, 66–73.
- James, S.P. and Zeitz, M. (1994) Human gastrointestinal mucosal T cells. In: Ogra, P., Lamm, M.E., McGhee, J.R. et al. (eds) *Handbook of Mucosal Immunology*. Academic Press, San Diego, California, pp. 275–283.
- Li, D.F., Nelssen, J.L., Reddy, P.G., Blecha, F., Klemm, R. and Goodband, R.D. (1991) Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. *Journal of Animal Science* 69, 4062–4069.
- McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A. and Gaskins, H.R. (1999) Weaning anorexia may contribute to local inflammation in the piglet small intestine. *Journal of Nutrition* 129, 613–619.
- Meeusen, E.N.T., Premier, R. and Brandon, M.R. (1996) Tissue-specific migration of lymphocytes: a key role for Th1 and Th2 cells? *Immunology Today* 17, 421–424.
- Miller, B.G., Newby, T.J., Stokes, C.R. and Bourne, F.J. (1984a) Influence of diet on postweaning malabsorption and diarrhoea in the pig. *Research in Veterinary Science* 36, 187–193.
- Miller, B.G., Newby, T.J., Stokes, C.R., Hampson, D.J., Brown, P.J. and Bourne, P.J. (1984b) The importance of dietary antigen in the cause of postweaning diarrhoea in pigs. *American Journal of Veterinary Research* 45, 1730–1733.
- Miller, B.G., Whittemore, C.T., Stokes, C.R. and Telemo, E. (1994) The effect of delayed weaning on the development of oral tolerance to soya-bean protein in pigs. *British Journal of Nutrition* 71, 615–625.
- Newby, T.J., Stokes, C.R., Huntley, J., Evans, P.A. and Bourne, F.J. (1979) The immune response of the pig following oral immunization with soluble protein. *Veterinary Immunology and Immunopathology* 1, 37–47.
- Rothkotter, H.J. and Pabst, R. (1989) Lymphocyte subsets in jejunal and ileal Peyer's patches of normal and gnotobiotic minipigs. *Immunology* 67, 103–108.
- Rothkotter, H.J., Ulbrich, H. and Pabst, R. (1991) The postnatal development of gut lamina propria lymphocytes: number, proliferation and T and B cell subsets in conventional and germ-free pigs. *Pediatric Research* 29, 237–242.
- Rothkotter, H.J., Huber, T., Barman, N.N. and Pabst, R. (1993) Lymphoid cells in afferent and efferent intestinal lymph: lymphocyte subpopulations and cell migration. *Clinical and Experimental Immunology* 92, 317–322.
- Rothkotter, H.J., Hriesik, C. and Pabst, R. (1995) More newly formed T-lymphocyte than B-lymphocyte leave the intestinal-mucosa via lymphatics. *European Journal of Immunology* 25, 866–869.
- Targan, S.R., Deem, R.L., Liu, M., Wang, S. and Nel, A. (1995) Definition of a lamina propria T-cell responsive state: enhanced cytokine responsiveness of T-cells stimulated through CD2 pathway. *Journal of Immunology* 154, 664–675.
- Telemo, E., Bailey, M., Miller, B.G., Stokes, C.R. and Bourne, F.J. (1991) Dietary antigen handling by mother and offspring. *Scandinavian Journal of Immunology* 34, 689–696.
- Trebichavsky, I., Sinkora, J., Rehakova, Z., Splichal, I., Whyte, A., Binns, R., Pospisil, R. and Tuckova, L. (1995) Distribution of gamma-delta T cells in the pig fetus. *Folia Biologica* 41, 227–237.
- Vega-Lopez, M.A., Telemo, E., Bailey, M., Stevens, K. and Stokes, C.R. (1993) Immune cell distribution in the small intestine of the pig: immunohistological evidence for an organised compartmentalisation in the lamina propria. *Veterinary Immunology and Immunopathology* 37, 49–60.
- Vega-Lopez, M.A., Bailey, M., Telemo, E. and Stokes, C.R. (1995) Effect of early weaning on the development of immune cells in the pig small intestine. *Veterinary Immunology and Immunopathology* 44, 319–327.
- Welch, S.K., Saif, L.J. and Ram, S. (1988) Cell-mediated immune responses of suckling pigs inoculated with attenuated or virulent transmissible gastroenteritis virus. *American Journal of Veterinary Research* 49, 1228–1234.

- Westermann, J. and Pabst, R. (1996) How organ-specific is the migration of 'naive' and 'memory' T cells? *Immunology Today* 17, 278–282.
- Whary, M.T., Zarkower, A., Confer, F.L. and Ferguson, F.G. (1995) Age-related differences in subset composition and activation responses of intestinal intraepithelial and mesenteric lymph-node lymphocytes from neonatal swine. *Cellular Immunology* 163, 215–221.
- Wilders, M.M., Drexhage, H.A., Weltevreden, E.F., Mullink, H., Duijvestijn, A. and Meuwissen, S.G.M. (1983) Large mononuclear Ia-positive veiled cells in peyers patches. 1. Isolation and characterisation in rat, guinea-pig and pig. *Immunology* 48, 453–460.
- Wilson, A.D., Stokes, C.R. and Bourne, F.J. (1986a) Intraepithelial lymphocytes and T-cell mitogens: a comparison of murine and porcine responses. *Immunology* 58, 621–625.
- Wilson, A.D., Stokes, C.R. and Bourne, F.J. (1986b) Morphology and functional characteristics of isolated porcine intraepithelial lymphocytes. *Immunology* 59, 109–112.
- Wilson, A.D., Stokes, C.R. and Bourne, F.J. (1989) Effect of age of absorption and immune responses to weaning or introduction of novel dietary antigens in pigs. *Research in Veterinary Science* 46, 180–186.
- Wilson, A.D., Haverson, K., Bland, P.W., Stokes, C.R. and Bailey, M. (1996) Expression of class II MHC antigens on normal pig intestinal endothelium. *Immunology* 88, 98–103.

17 Effects of Nucleotides on the Immune Function of Early-weaned Piglets

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Feeding nucleotides to early-weaned piglets can enhance T-cell functions in delayed-type hypersensitivity (DTH) to antigenic challenge and *in vitro* proliferative responses, although the length of supplementation may be critical. These immuno-enhancing effects were obvious when nucleotides were supplied for at least 2 weeks at weaning.

Introduction

Early-weaned piglets have lower access to milk nutrients and growth factors than conventionally raised piglets, and thus their immune competence could be compromised. We suggest that the immune status of early-weaned piglets would benefit from

diets containing immuno-enhancing ingredients that do not incur unwanted metabolic costs.

Nucleotides are considered to be semi-essential nutrients for normal metabolism, and are formed in the healthy individual via *de novo* synthesis. However, during times of high growth, gut development or

the activation of lymphoid and other immune cells following infection, they are required in higher amounts and at this time are usually obtained from the diet (Carver 1994; Youping *et al.*, 1994). In the present study, we tested the hypothesis that feeding nucleotides to early-weaned piglets can enhance their immune responses.

Materials and Methods

Large White piglets of both sexes, weaned at 15–19 days of age and housed at the Pig Production Unit, Australasian Pig Institute, Queensland, were used in this study. Weaned piglets were given water and a standard commercial diet *ad libitum*. The nucleotide source used was yeast RNA (Sigma, St Louis, Missouri, USA); it was dissolved in warm isotonic rehydration solution and was provided *ad libitum*. Control piglets received rehydration solution only. Keyhole limpet hemocyanin (KLH) (2 mg in 0.5 ml alum) was injected intramuscularly on postweaning day 7.

In Trial 1, the long-term effect of nucleotide supplement on immune function was studied using three groups of nine piglets receiving a daily dose of 0, 500 or 1000 mg yeast RNA between postweaning days 7 and 35 (for 4 weeks). In Trial 2, the effect of timing of yeast RNA supplementation at various preweaning and postweaning stages (for either 1 or 2 weeks) was examined. The timing of the treatments included group A (non-supplemented control), group B (1-week-long preweaning supplementation), group C (postweaning days 1–7 prior to KLH immunization), group D (1 week preweaning and 1 week postweaning, before KLH immunization), group E (postweaning days 8–15, during the early stage of immunization) and group F (postweaning days 16–23, during the later stage of immunization).

Weekly body weight and postweaning group feed intakes were recorded and feed conversion rate was calculated for growth performance assessment. In Trial 1, DTH reactions to an intradermal challenge with KLH (80 µg in 0.025 ml saline), revealed by

the size of the papule formed at 24 h after challenge, were performed on the back of the piglet at postweaning day 30. Weekly serum samples before and after KLH immunization were used to assess levels of specific antibody against KLH, using an established ELISA (Nguyen *et al.*, 1998). In Trial 2, weekly EDTA-venous blood and serum samples were taken via the anterior vena cava. Total and differential blood leucocyte numbers were counted using an automated haematology analyser (Cell-Dyn 3500, Abbott Diagnostics, Illinois, USA). The numbers of blood lymphocyte phenotypes including CD3⁺, CD4⁺, CD8⁺ and CD4⁺CD8⁺ were identified by flow cytometry as previously described by Nguyen *et al.* (1998). Blood mononuclear cells were also isolated using Ficoll-Paque medium and cultured *in vitro* for the proliferative response to Concanavalin A (Con A, 80 µg in 0.025 ml saline) stimulation as previously described by Nguyen *et al.* (1998).

Repeated measures analysis of variance and Student–Newman–Keuls methods were used. Results are expressed as mean ± SEM.

Results

No differences in growth rate, feed intake and feed conversion rates were observed among groups in the two trials. In Trial 1, the DTH reaction to KLH was significantly ($P < 0.05$) higher in the two nucleotide-fed groups than for the control piglets (Fig. 17.1a). However, there was no obvious difference in serum antibody levels between groups (data not shown). In Trial 2, the piglets that received nucleotides for a 2-week period (group D) also showed a significantly increased *in vitro* lymphocyte proliferative response to Con A stimulation ($P < 0.05$) compared with other groups (Fig. 17.1b). No significant differences among groups were found for lymphocyte phenotypes (CD3⁺, CD4⁺, CD8⁺ and CD4⁺CD8⁺ cells), or for leucocyte counts, DTH reactions to KLH and serum KLH-specific antibody responses (data not shown).

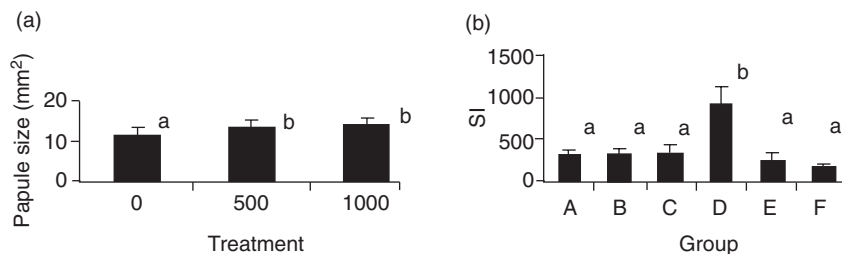


Fig. 17.1. (a) Effect of nucleotide treatment on DTH response to KLH challenge. 'Treatment', nucleotide level mg per pig day⁻¹. (b) Effect of nucleotide treatment on Con A-induced lymphocyte proliferative responses at postweaning day 8. 'SI', stimulation index (stimulated cell count per minute/unstimulated count per minute); 'Group', timing of supplementation with nucleotides. In each graph, different letters indicate significant differences ($P < 0.05$).

Discussion

Piglets fed nucleotides for a longer term (2–4 weeks) appeared to have improved lymphocyte functions at the end of supplementation, as evidenced by their increased T-cell-mediated DTH responses to KLH and *in vitro* proliferative responses to a non-specific T-cell mitogen (Con A). However, we were unable to determine the duration of this impact from this study. As there were no changes in the weekly counts of blood leucocytes or various T-lymphocyte phenotypes, the beneficial effects of nucleotide supplements on T-lymphocytes and their mediated immune responses were likely to be associated with an improved efficiency of T-lymphocyte responsiveness during the challenge. Interestingly, antibody responses to KLH were not affected by nucleotide treatments, but it is worth noting that the absence of a significant impact of nucleotide supplements on growth performance suggests that the effect on T-cells or immune responses is likely independent of metabolizable energy content of the nucleotides. Our findings are similar to

those reported in laboratory species by others (Carver, 1994; Youping *et al.*, 1994) but the range of effects on immune cells are different, which may be due to differences between species. The present findings support the hypothesis that feeding nucleotides to early-weaned piglets can enhance their immune responses, although length of supplementation may be critical. As T-cell-mediated immunity plays an important role in mucosal defence mechanisms, research is currently being undertaken in our laboratory to determine whether feeding nucleotides to pigs can improve gut immunity.

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References

- Carver, J.D. (1994) Dietary nucleotides: cellular immune, intestinal and hepatic system effects. *Journal of Nutrition* 124, 144s–148s.
- Nguyen, V.P., Wong, C.W., Hinch, G.N., Singh, D. and Colditz, I.G. (1998) Variation in the immune status of two Australian pig breeds. *Australian Veterinary Journal* 76, 613–617.
- Youping, H.E., Sanderson, I.R. and Walker, W.R. (1994) Uptake, transport and metabolism of exogenous nucleosides in intestinal epithelial cell cultures. *Journal of Nutrition* 124, 1942–1949.

18 Effect of Antisecretory Factor-derived Peptides on Induced Secretion in the Porcine Small Intestine *in vivo* and *in vitro*

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We assessed whether a small antisecretory factor (AF)-derived peptide (A3), partly including the active site reported from rat studies, could inhibit porcine intestinal secretory responses to different secretagogues. *In vivo*, fluid accumulated in ligated loops (jejunum and ileum) with cholera toxin (CT), *Escherichia coli* heat-labile enterotoxin (LT) or 5-hydroxytryptamine (5-HT) was measured. *In vitro*, electrical parameters in mucosal sheets from jejunum mounted in Ussing chambers, stimulated with vasoactive intestinal peptide, 5-HT and theophylline, was recorded. A3 reduced the response to the enterotoxins but failed to inhibit the response to 5-HT. These findings support the notion that A3 includes an active site of AF and suggest that the mode of action does not involve inhibition of secretory reflexes induced by 5-HT. The lack of effect of A3 on the secretory responses *in vitro* suggests that the antisecretory action of AF is mediated via structures or mechanisms beyond the epithelium and submucosa and involves neuronal structures in submucosa.

Introduction

Diarrhoeal diseases are still major global health problems and thousands of children die every day due to fluid loss, despite the introduction of oral rehydration therapy. The antisecretory factor (AF) may represent a new class of antisecretory drugs (Lundgren, 1997). AF is synthesized and stored in endocrine and epithelial cells in the pituitary gland, and in epithelial and lymphoid cells in gastrointestinal, respiratory and urinary tracts (Lange *et al.*, 1999). The pituitary content of AF increases in response to luminally introduced enterotoxins such as cholera toxin (CT), *Escherichia coli*

heat-labile enterotoxin (LT) and toxin A (Lönnroth *et al.*, 1988) and AF inhibits enterotoxin-induced intestinal inflammation (rat) and hypersecretion (rat and pig) (Lange *et al.*, 1987a; Johansson *et al.*, 1997a). The pathway for the antisecretory effect of AF is not known but it has been speculated to be an attenuation of the secretory reflexes in the ENS (Skadhauge *et al.*, 1986; Lange *et al.*, 1987b; Johansson *et al.*, 1997a). We assessed whether small AF-derived peptide (A3), partly including the active site reported from rat studies (Johansson *et al.*, 1997b), could inhibit porcine intestinal secretory responses to different secretagogues *in vivo* and *in vitro*.

Material and Methods

Experimental protocols

A3 (mol. wt 1158.3), corresponding to the 40–49 amino acids in the human AF (Johansson *et al.*, 1997b), was synthesized by solid-phase peptide synthesis.

Eight 8-week-old fully weaned Danish Landrace pigs (13–15 kg) on a standard diet were used. Twelve hours before anaesthesia and surgery the animals were deprived of food, but had free access to sterile drinking water, containing D-glucose (55 g l⁻¹). The pigs were anaesthetized with isoflurane. Through a midline incision, loops of the small intestine were prepared by ligating between the mesenteric arcades, ensuring a proper blood supply. A series of 20 single loops of 10 cm in length, separated by 3 cm, was prepared; the first loop was located 30 cm from the ligament of Treitz. Subsequently 20 loops were prepared in the distal small intestine, the last loop being 100 cm from the ileocaecal valve. Total length of the small intestine was 12 ± 1 m. Loops were instilled with test solution, or test solution plus 50 mg 5-hydroxytryptamine (5-HT), or test solution plus CT in a dose range, or test solution plus LT in a dose range, or left empty. After incubation of 60 min (5-HT) or 240 min (CT, LT), the loops were carefully removed and weighed with and without content, and then dried in an oven at 60°C for 24 h, to achieve a constant weight. The fluid accumulation was determined by simple subtraction and expressed as mg mg⁻¹ dry loop per 60/240 min. A3 (30 µg in 10 ml saline) or saline (control) was given as an intravenous bolus 1 h before instillation.

From 17 pigs, mucosal sheets from proximal small intestine were mounted in an Ussing chamber bath in a bicarbonate-buffered Ringer's solution at 39°C aerated with 95% O₂ and 5% CO₂. The tissues were short-circuited during the entire experiment. The short-circuit current (I_{sc}) was recorded continuously. Fifteen minutes after mounting, A3 (10 nM) or saline was added bilaterally to the chamber; 30 min later vasoactive intestinal peptide

(VIP) (3.2 × 10⁻⁷ M, serosa), 5-HT (10⁻⁴ M, serosa) and theophylline (2.2 × 10⁻³ M, bilaterally), respectively, were added.

Statistics

All results are expressed as mean ± the standard error of the mean (SEM). The *in vivo* data were analysed using one-way ANOVA. Pairwise comparison was performed using Student's *t*-test with Bonferroni correction, when there were more than two comparisons. Probability values (*P*) of 0.05 or less were considered significant.

Results

There was no differences in the clinical, cardiovascular and blood gas parameters between saline-treated and peptide-treated pigs. CT and LT induced a dose-dependent fluid accumulation in all treatments. CT-induced fluid accumulation was significantly reduced by A3 (about 60%) in the proximal small intestine, whereas it failed to reduce the response in the distal part (Fig. 18.1). LT-induced fluid accumulation was significantly reduced by A3 in both the proximal and distal small intestine (Fig. 18.2). *In vitro*, A3 decreased the TTX-sensitive secretory effect of substance P.

Discussion

The results show that the sequence between amino acids 40 and 49 of the human AF has antisecretory activity in the porcine small intestine. This antisecretory effect of A3 on CT- and LT-induced secretion in the proximal small intestine corresponds to the effect reported from studies using porcine AF (Lange *et al.*, 1987a). A3 inhibited CT-induced fluid accumulation in the proximal small intestine, while no effect was found in the distal part, in agreement with the observation that regional differences exist in the secretory pathway for CT (Hansen *et al.*, 2000).

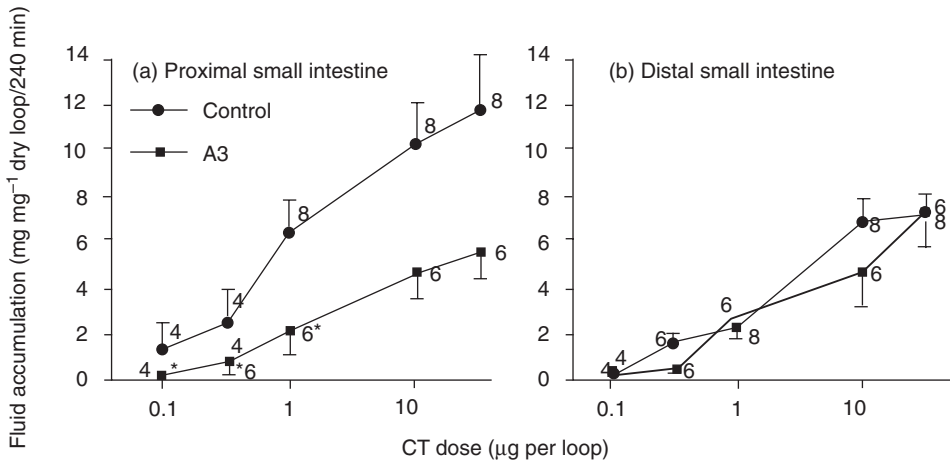


Fig. 18.1. Effect of A3 on cholera toxin-induced fluid accumulation in ligated loops in (a) proximal and (b) distal porcine small intestine. Values are means $\pm$ SEM. Figures indicate number of observations in four pigs. One-way ANOVA and Student's *t*-test: **P* < 0.05 compared with control.

LT- but not CT-induced fluid accumulation was reduced by A3, which indicate that, despite their similarity, CT and LT induce secretion by different mechanisms. This is in agreement with recent findings by Turvill *et al.* (1998), who reported that LT – in contrast to CT – induces secretion without recruiting 5-HT. McEwan *et al.* (1991)

proposed that the mode of action of AF was to inhibit 5-HT receptors, but this seems unlikely, since in the present study A3 failed to reduce the 5-HT-induced fluid accumulation and the increase in short-circuit current. The short incubation time for 5-HT (120 min after A3 infusion) cannot explain the lack of effect, since Johansson

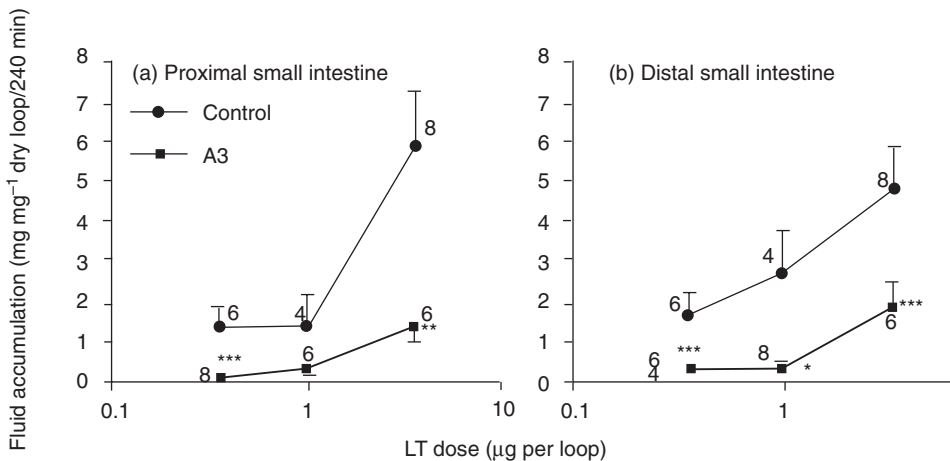


Fig. 18.2. Effect of A3 on *E. coli* heat-labile enterotoxin-induced fluid accumulation in ligated loops in (a) proximal and (b) distal porcine small intestine. Values are means $\pm$ SEM. Figures indicate number of observations in four pigs. One-way ANOVA and Student's *t*-test: **P* < 0.05; ***P* < 0.01; ****P* < 0.001 compared with control.

et al. (1997a) reported antisecretory effect 90 min after AF administration.

In conclusion, A3 reduces the response to the exogenous secretagogues, CT and LT, but fails to inhibit the response to 5-HT. These findings support the notion that A3 includes an active site of AF and suggest that the mode of action does not involve

inhibition of secretory reflexes induced by 5-HT. Additionally, the results give further evidence that there are differences in the secretory pathway between CT and LT. The *in vitro* results suggest that the antisecretory effect of A3 lies beyond the epithelium and involves neuronal structures in submucosa.

References

- Hansen, M.B., Bergreen, P., Grøndahl, M.L., Maltbæk, J.S., Ersbøll, A.K. and Skadhauge, E. (2000) Segmental differences in neurogenic signal transduction pathways for cholera toxin in the porcine jejunum. *Pharmacology and Toxicology* (in press).
- Johansson, E., Jennische, E., Lange, S. and Lönnroth, I. (1997a) Identification of an active site in the antisecretory factor protein. *Gut* 41, 642–645.
- Johansson, E., Lange, S. and Lönnroth, I. (1997b) Identification of an active site in the antisecretory factor protein. *Biochimica et Biophysica Acta* 1362, 177–182.
- Lange, S., Lönnroth, I. and Skadhauge, E. (1987a) Effects of the antisecretory factor in pigs. *European Journal of Physiology* 409, 328–332.
- Lange, S., Lönnroth, I., Palm, A. and Hyden, H. (1987b) The effect of antisecretory factor on the permeability of nerve cell membrane to chloride ion. *Pflügers Archives* 410, 648–651.
- Lange, S., Jennische, E., Johansson, E. and Lönnroth, I. (1999) The antisecretory factor: synthesis and intracellular localisation in porcine tissues. *Cell Tissue Research* 296, 607–617.
- Lönnroth, I., Lange, S. and Skadhauge, E. (1988) The antisecretory factors: inducible proteins which modulate secretion in the small intestine. *Journal of Comparative Biochemistry and Physiology* 90A, 611–617.
- Lundgren, O. (1997) Treating diarrhoea: what might the pituitary offer? *Gut* 41, 719–720.
- McEwan, G.T.A., Schousboe, B. and Skadhauge, E. (1991) Influence of age on antisecretory factor inhibition of enterotoxin action in the pig small intestine. *Journal of Veterinary Medicine A* 38, 222–228.
- Skadhauge, E., Lange, S. and Lönnroth, I. (1986) Effects of secretagogues and of pituitary antisecretory factor (ASF) on Na⁺ and Cl⁻ fluxes across the pig jejunum *in vitro*. *Proceedings of the International Union of Physiological Science* 16, 372A (abstract).
- Turvill, J.L., Mourad, F.H. and Farthing, M.J.G. (1998) Crucial role for 5-HT in cholera toxin but not *Escherichia coli* heat-labile enterotoxin-intestinal secretion in rats. *Gastroenterology* 115, 883–890.

Part III

Nutrient Absorption and Utilization by the Gut

19 Nutrient Requirements for Intestinal Growth and Metabolism in the Developing Pig

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The notion that the gut tissues have their own particular nutrient requirements for growth and function has not been explored in great detail, either quantitatively or qualitatively. We have quantified the nutrient needs of gut tissues in infant pigs by measuring the metabolism of stable isotopically labelled substrates, namely amino acids and glucose. These studies have established that the gastrointestinal tissues: (i) utilize nearly 50% of dietary amino acid intake; (ii) metabolize non-essential amino acids (glutamate, aspartate, glutamine) to a greater extent than essential amino acids; (iii) utilize as much as 30% to 50% of the dietary lysine, leucine and phenylalanine and 85% of threonine; (iv) derive a substantial proportion of their essential amino acid needs from the arterial circulation; and (v) derive a majority of their glucose needs from the arterial circulation (85%) rather than from the diet. The physiological and metabolic bases of these findings and their implications regarding whole-body amino acid requirements in pigs are discussed.

Introduction

The concept that gastrointestinal tissues have an important impact on whole-animal metabolism is well established. The portal-drained viscera (PDV) contribute between 3% and 6% of body weight, but they account for 20–35% of whole-body protein turnover and energy expenditure (Burrin *et al.*, 1989; Yen *et al.*, 1989; Stoll *et al.*, 1999b). The substantial impact of gastrointestinal tissues on whole-body metabolism is a function of their relatively high rate of protein synthesis and oxygen consumption. Numerous studies with domestic animals have demonstrated that the fractional rate of protein turnover in the intestinal tissues exceeds that of peripheral tissues, such as muscle, by at least tenfold in growing animals and by as much as 30-fold in

adults (Lobley *et al.*, 1980, 1992; Attaix *et al.*, 1986; Burrin *et al.*, 1992). Given the high rates of protein and energy metabolism, it is logical that the nutrient requirements of the gut tissues are equally high, and hence consume a significant proportion of the whole-body nutrient needs. Yet there is limited quantitative information describing the specific nutrient needs of the gut tissues, particularly under normal feeding conditions. A complicating factor in the measurement of gut nutrient utilization is that the intestinal mucosa receives nutrients from two sources, the diet (brush-border membrane) and the systemic circulation (basolateral membrane). However, in recent years, development of the arterio-venous organ balance approach coupled with infusion of isotopic tracers has provided an *in vivo* model with which to

assess simultaneously the gastrointestinal utilization of nutrients derived from the diet and from systemic circulation.

Measuring Gut Nutrient Utilization

Net portal balance

To begin to understand nutrient utilization by the gut tissues, it is perhaps best first to examine the estimates that express the net nutrient balance as a proportion of dietary intake (Table 19.1). Net portal nutrient balance is determined from the rate of portal venous blood flow and the difference in substrate concentrations in the portal vein and artery. Comparisons among pigs at various ages indicate that these estimates are not uniform for all amino acids, but some general patterns are apparent.

The first is that less than 100% of the amino acids ingested appears in the portal venous output. This observation implies either that a substantial proportion of the dietary amino acid intake is utilized by the portal-drained visceral tissues, or that a substantial portion of the ingested proteins passes through the small intestine undigested. In regard to the latter possibility, although there is a wide range in the appar-

ent ileal digestibility among all protein sources, in many instances the true digestibility is 85–90%; this value is essentially 100% in the case of milk proteins (Rerat, 1993). Among the essential amino acids, the proportional net portal balance is relatively lower in young pigs (40–60% of intake) than in heavier, older pigs (65–90% of intake). This latter observation could be attributed to differences in methodology and experimental conditions; however, it is consistent with the fact that visceral tissue mass, and hence metabolism, represents a decreasing proportion of body weight with increasing age and body weight. A particularly critical observation among the essential amino acids is that the proportional net portal balance of threonine is especially low: approximately 40–50%, regardless of age. Another consistent pattern among these data is that the net portal balance of glutamate and aspartate is essentially zero, whereas that of glutamine is negative, indicating net utilization of arterial glutamine. Moreover, the net portal balance of some amino acids, namely alanine, tyrosine and arginine, is greater than 100%, suggesting net synthesis of these amino acids by the gut. Thus, the intestinal metabolism of amino acids, particularly some of the limiting essential amino acids, is nutritionally

Table 19.1. Proportional net portal balance (% of intake) of dietary nutrients in pigs.

Nutrient	Body weight (kg)			
	8 ^a	21 ^b	40 ^c	65 ^d
Lysine	53	61	68	69
Threonine	47	39	44	52
Methionine	54	61	98	95
Leucine	59	52	79	80
Phenylalanine	55	51	79	72
Arginine	118	58	100	92
Tyrosine	119	59	79	50
Alanine	259	161	205	215
Aspartate	4	ND	2	28
Glutamate	4	ND	ND	18
Glutamine ^e	–10	ND	–17	–8
Glucose	85	65	85	70

Recalculated from ^aReeds *et al.* (1996) and Stoll *et al.* (1998), ^bDeutz *et al.* (1998), ^cvan der Muelen *et al.* (1997), ^dRerat *et al.* (1992), using standard food analysis tables and the protein composition of the diet.

^eExpressed as a proportion of the arterial input. ND, not determined.

significant. A final observation is that the net portal balance of glucose is relatively high, ranging from 65% to 85%. Taken together, these data suggest that the gut consumes a much greater proportion of dietary amino acid intake than glucose.

Utilization of luminal versus arterial amino acids

Given the evidence that the gut is a net consumer of dietary amino acids, an important question is: what are the metabolic fates of these amino acids? In order to address this issue, it is important to realize that measurements of net portal nutrient balance represent not only the result of 'first-pass' metabolism of luminal amino acids by the mucosal enterocytes, but also the 'second-pass' metabolism of arterial amino acids by both the epithelial and non-epithelial tissue layers of the small intestine, as well as the stomach, pancreas, spleen and large intestine. In order to distinguish the relative metabolism of substrates derived from the luminal and arterial inputs into the PDV tissues, it is necessary to use a combined approach in which isotopically labelled substrates are infused enterally or intravenously, or preferably via both routes, and compare the measurements of portal tracer balance. This combined *in vivo* approach was pioneered over 20 years ago by Bergman and Heitman (1978) to study gastrointestinal substrate metabolism in sheep, and is now being used with increasing frequency to

investigate the nutritional significance of gut metabolism (Cappelli *et al.*, 1997; MacRae *et al.*, 1997a; Deutz *et al.*, 1998; Stoll *et al.*, 1998). These studies have shown that there is significant utilization of both luminal and arterial amino acids, and the relative rates of metabolism from these two inputs appear to be different in monogastric and ruminant animals. In our pigs fed a milk-based formula, when expressed relative to the intake, the utilization of some dietary essential amino acids via the luminal route (18–50%) is proportionally greater than from the arterial circulation (7–20%) (Table 19.2). Thus, from a relative perspective, this suggests that intestinal tissues preferentially utilize luminal versus arterial amino acids. Moreover, gut tissues preferentially use dietary amino acids rather than glucose.

We speculate that this phenomenon is a function of the inherent amino acid and glucose transport capacities of the apical (luminal) and basolateral (arterial) surfaces of intestinal enterocytes. However, in absolute molar terms, the uptake of some amino acids from the arterial and luminal routes is nearly equal, because the rate of arterial amino acid input is more than five times greater than that from the diet. Thus, the gut requirements for some amino acids are met equally from the diet and arterial supply. In stark contrast, the intestinal glucose needs are derived largely from the arterial circulation (85% total) versus the diet (15%). An additional distinction can be made between ruminants and monogastric animals regarding amino acids, in that

Table 19.2. Utilization of luminal and arterial amino acids by the PDV^a.

	LYS ^b	LEU ^c	PHE ^d	GLU ^e	GLN ^f	Glucose ^f
Dietary intake	299	486	135	585	219	3,400
Luminal uptake	75	81	68	562	147	217
% intake	25	18	50	96	67	6.4
Arterial input	1,239	1,423	320	731	934	18,874
Arterial uptake	99	170	21	80	191	1,227
% input	8	12	7	11	21	6.5

^aRates expressed as $\mu\text{mol kg}^{-1} \text{h}^{-1}$. ^bJ.B. van Goudoever, D.G. Burrin, B. Stoll, and P.J. Reeds, unpublished data.

Recalculated from ^cStoll *et al.* (1998); ^dStoll *et al.* (1999a), ^eReeds *et al.* (1997) and ^fStoll *et al.* (1999b).

studies in sheep suggest that the gastrointestinal tissues may derive a larger proportion of their amino acids from the arterial circulation than in pigs (MacRae *et al.*, 1997b). In the sheep, the net portal balance of dietary essential amino acids appearing in the mesenteric vein suggests 100% efficiency of absorption, whereas only approximately 60% of the amino acids appear in the portal venous outflow. This observation likely reflects the fact that, in ruminants, the stomach and large intestinal tissues represent a relatively greater proportion of gastrointestinal tract mass; and because these tissues presumably have limited exposure to luminal amino acids in a free form, they must rely on arterial amino acids.

Metabolic Fate of Nutrients Used by the Gut

The metabolic fate of the nutrients (either dietary or systemic) is a critical factor in interpreting the nutritional significance of their utilization by the gut. Once taken up by the intestinal tissues, amino acids can be utilized for three major metabolic purposes: (i) incorporation into protein; (ii) conversion via transamination into other amino acids, metabolic substrates and biosynthetic intermediates; and (iii) complete oxidation to CO_2 . In the first two pathways, amino acids can be deposited and recycled by the body for purposes of growth or other biological functions. In the case of some amino acids, namely threonine and cysteine, incorporation into endogenous secretions that are eventually fermented in the large intestine represents a net nutritional loss. Likewise, if the amino acids are completely oxidized to CO_2 by the mucosal cells, this is also a nutritional loss, especially in the case of essential amino acids. In the case of glucose, there are also numerous possibilities, but there are three major fates: (i) metabolism to lactate and alanine; (ii) complete oxidation to CO_2 ; and (iii) biosynthesis into amino-sugars and lipids.

Protein synthesis and amino acid metabolism

Given the high rate of turnover and secretion, one might predict that most of the dietary amino acids utilized by the gut are used for protein synthesis. Moreover, the pathways of amino acid transamination and catabolism, at least for the essential amino acids, have historically been considered to be largely confined to the liver and other peripheral tissues. On the contrary, our studies in piglets (Stoll *et al.*, 1998) and others' studies in dogs (Yu *et al.*, 1992) suggest that although the gut utilizes as much as 35–40% of the dietary essential amino acid intake, only about a third (i.e. 10%) of this is incorporated into mucosal protein. The proportionally low incorporation of dietary amino acids into mucosal protein may be a consequence of the preferential channelling of arterial amino acids into mucosal protein. In formula-fed pigs, we found that the incorporation of arterial phenylalanine into mucosal protein was 60% higher than dietary phenylalanine (Stoll *et al.*, 1999a). In the case of threonine, the gut utilizes nearly 60% of the dietary intake, and we (Stoll *et al.*, 1998) and others (Bertolo *et al.*, 1998) have speculated that much of the intestinal threonine uptake is used for the synthesis of secretory mucins, since these proteins have a relatively high threonine content. However, we have found that only about 10% of the dietary threonine is incorporated into mucosal protein, suggesting that if most of the threonine is incorporated into mucins, then it was secreted into the luminal contents during our 6 h infusions and not recovered in mucosal tissue. Alternatively, it is conceivable that there is substantial mucosal threonine metabolism, perhaps either to glycine or completely to CO_2 ; however, our preliminary results suggest that threonine oxidation by the gut is negligible (see Table 19.4). There is also evidence from our piglet studies suggesting intestinal hydroxylation of dietary phenylalanine into tyrosine, given that the net portal tyrosine balance is 167% of intake (Stoll *et al.*, 1998). Moreover, despite the substantial intestinal utilization rate

(65–95% of intake) of several dietary non-essential amino acids, particularly glutamate, aspartate, glutamine and glycine, a relatively small proportion (approximately 10% of intake) of these amino acids is incorporated into mucosal protein. Instead, these amino acids serve as precursors for the biosynthesis of other amino acids, such as arginine and proline (Murphy *et al.*, 1996; Wu *et al.*, 1998; Stoll *et al.*, 1999b), glutathione (Reeds *et al.*, 1997) and nucleic acids (Perez and Reeds, 1998). It is also important to note from these studies that some of these products are preferentially synthesized from luminal, as opposed to arterial, glutamate.

Non-essential amino acid and glucose oxidation

The extent to which dietary amino acids are completely catabolized to CO₂ and urea represents an obligatory loss, and is of nutritional importance to the animal. In this regard, it has been known for more than 25 years that there is substantial utilization of glutamine and glutamate by the intestine and that much of the glutamine is derived from the arterial circulation (Windmueller and Spaeth, 1975, 1980). More important is the fact that the *in situ* studies (Windmueller and Spaeth, 1980) showed that at least 50% of the dietary glutamate and glutamine taken up by the gut is completely oxidized to CO₂, which has been confirmed in our piglet studies *in vivo* (Stoll *et al.*, 1999b). Indeed, our recent data

(Stoll *et al.*, 1999b) (Table 19.3), based on *in vivo* infusions of [U-¹³C] glutamate, glutamine and glucose, together with direct measurements of ¹³CO₂ in the portal blood, led us to conclude that dietary glutamate and aspartate account for about 50% of the oxidative energy generated in the small intestinal mucosa.

These results are important for further reasons. Firstly, since the original report by Windmueller and Spaeth, it has been widely held that glutamine is the primary oxidative fuel for the gut. However, recent studies, as well as those of Windmueller and Spaeth (1975, 1980), actually demonstrate that glutamine, glutamate and aspartate ingested in the diet are perhaps equally important intestinal fuels. Secondly, if virtually all systemic aspartate, glutamate and glutamine derive from synthesis *de novo*, then the adenosine triphosphate used in this process represents an important energetic cost to the animal (Reeds *et al.*, 1998). Finally, it has been widely reported, largely from studies with short-term cultures of isolated enterocytes, that glucose is a major intestinal oxidative fuel. Although our results indicate that significant quantities of glucose are indeed oxidized by the visceral tissues, they demonstrate a preferential utilization of arterial rather than dietary glucose. It is important to note that, although glucose represents a major oxidative fuel (29%) in terms of total PDV CO₂ production, the proportion of glucose oxidized completely to CO₂ is substantially less than that of either glutamate or glutamine. This implies

Table 19.3. Oxidation of different substrates by the portal-drained viscera of 4-week-old piglets fed sow's milk replacer (Stoll *et al.*, 1999b).

Substrate	Portal utilization μmol kg ⁻¹ h ⁻¹	Percentage uptake oxidized ^a	Contribution to portal CO ₂ production (%)
Enteral glutamate	562	52 ± 3	36 ± 3
Arterial glutamine	191	70 ± 8	15 ± 2
Enteral glucose	217	2 ± 0.5	6 ± 2
Arterial glucose	1227	27 ± 9	29 ± 3

^aExpressed as a proportion of enteral delivery for enteral substrates and percentage of uptake from the mesenteric artery for arterial substrates.

that glutamine and glutamate are preferentially channelled towards mitochondrial oxidation, whereas most of the glucose is utilized for other metabolic or biosynthetic purposes. A small percentage of the portal glucose utilization is metabolized to alanine (7.5%) and lactate (5.9%), which leaves approximately 60% unaccounted for. We speculate that a significant proportion of the portal glucose uptake may be incorporated into amino sugars used for mucosal mucins synthesis.

Essential amino acid oxidation

The potential contribution of the catabolism of essential amino acids, despite its obvious nutritional implications, has received little recent attention, in part because early attempts to demonstrate the presence of the appropriate catabolic enzymes in the small intestinal mucosa were uniformly negative (Wu, 1998). However, recent studies suggest that dietary essential amino acids may indeed be oxidized by the intestinal mucosa. For example, in our studies (Stoll *et al.*, 1998), we have been unable to account for more than 25% of the first-pass utilization of enteral [U-¹³C] threonine, leucine, lysine and phenylalanine in mucosal protein incorporation. Furthermore, we found that the portal outflow of nitrogen in end products of amino acid metabolism (i.e. ammonia, alanine, arginine and citrulline) is greater than the calculated catabolism of dietary aspartate, glutamate, glutamine, glycine and serine (Stoll *et al.*, 1999b). This implies that other dietary amino acids, including essential amino acids, are being catabolized in the small intestine. Studies in sheep (Pell *et al.*, 1986; Cappelli *et al.*, 1997; Yu *et al.*, 2000) and dogs (Yu *et al.*, 1992) have found that approximately 5% of whole-body leucine flux is oxidized by the PDV. In pigs, lysine is considered to be the first limiting dietary amino acid and, as such, we have been investigating the extent of dietary lysine oxidation by the gut. Our preliminary evidence, obtained from duodenal infusions of [U-¹³C] lysine in piglets

(van Goudoever, unpublished data) (Table 19.4), suggests that about 5% of the dietary lysine is oxidized in first pass by the intestine. This seems to be a small proportion, but this intestinal oxidation of dietary lysine accounted for about 30% of whole-body lysine oxidation. Interestingly, although about 10% of the arterial flux of lysine was taken up by the PDV, none of this was oxidized, suggesting a preferential oxidation of dietary lysine. In the context of a single amino acid, oxidation of only 5% of the dietary intake may appear inconsequential; yet the collective oxidation of several essential amino acids may indeed represent a significant obligatory cost to the animal. Indeed, we have evidence in young pigs that the approximately 10–15% of the whole-body leucine and phenylalanine flux is oxidized by the PDV tissues. However, threonine seems to be unique among the essential amino acids, in that there is negligible ¹³C-threonine oxidation when given either enterally or systemically to young piglets. This is further evidence for metabolic channelling of amino acids within the gut and also indicates that there is heterogeneity in the metabolic fate among essential amino acids.

Impact of Gut Metabolism on Whole-body Nutrient Requirements

Effect of reduced gut mass

From the previous discussion, it would seem logical that those factors that are known to affect intestinal mass would also influence intestinal metabolism of dietary amino acids and hence their systemic availability. A number of factors have been shown to influence the relative mass of the intestine, including age, nutrient intake, feed additives and environmental factors. However, the most profound influence on intestinal mass is oral nutrient intake. Our studies in neonatal pigs (Burrin *et al.*, 1994) and those of others have shown that the provision of nutrients exclusively via the parenteral or intravenous route (i.e. total

Table 19.4. Contribution of portal-drained visceral tissues to whole-body tissues and threonine utilization in piglets.

	Lysine		Threonine	
	Rate ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	% Intake	Rate ($\mu\text{mol kg}^{-1} \text{h}^{-1}$)	% Intake
Dietary intake	518	–	934	
Whole-body oxidation	53 ± 15	10	75 ± 6	8
Total net portal utilization	241 ± 12	47	794 ± 49	85
Portal utilization – enteral AA	-15 ± 63	0	459 ± 53	49
Portal oxidation – enteral AA	24 ± 6	5	16 ± 13	0
Portal utilization – arterial AA	229 ± 71	44	497 ± 93	53
Portal oxidation – arterial AA	0	0	-21 ± 33	0

Preliminary unpublished results, J.B. van Goudoever, B. Stoll, D.G. Burrin and P.J. Reeds.

parenteral nutrition, TPN) maintains the whole-body growth rate, but markedly (approximately 50%) decreases the intestinal mass. We have preliminary evidence which suggests that the 52% reduction in intestinal mass observed after 7 days of TPN is associated with a 30% increase in the net portal absorption of enterally administered leucine (Burrin *et al.*, 1999) (Table 19.5). Moreover, the increased absorption was largely due to reduced portal utilization of arterial leucine, secondary to the reduced intestinal mass. This finding indicates that a 50% reduction in gut mass does not compromise the digestion and absorption of dietary nutrients, and actually increases their net systemic availability for growth. The results also provide a metabolic explanation for the phenomenon of compensatory growth, which occurs when

animals are fed *ad libitum* following periods of restricted food intake. Previous studies, largely in ruminants, have shown that the reduction in the mass and energy expenditure of the visceral organs during nutrient restriction results in increased dietary energy availability for compensatory growth (Ferrell, 1988). Similarly, it is plausible that the increased systemic amino acid availability resulting from a reduction in intestinal mass and amino acid metabolism also could lead to enhanced efficiency of dietary protein use and growth rate, typical of compensatory growth.

Effect of reduced dietary protein intake

The nutrient requirements of the gut can have a potentially critical impact on the

Table 19.5. Chronic TPN affects intestinal mass and portal leucine metabolism in neonatal pigs refed enterally after 7 days^a.

	Enteral	% Intake ^b	TPN	% Intake ^b
Small intestinal mass (g kg ⁻¹ BW)	47 ± 6	–	23 ± 2	–
Leucine intake	444 ± 20	–	459 ± 35	–
Total net portal absorption	237 ± 46	53	308 ± 68	67
Total net portal utilization	208 ± 25	47	151 ± 46	33
Portal utilization – enteral leucine	-3.9 ± 6.9	0	0.5 ± 4.0	0
Portal utilization – arterial leucine	220 ± 27	50	80 ± 10	17

^aPreliminary unpublished results (D.G. Burrin *et al.*, 1999) (leucine fluxes expressed as $\mu\text{mol kg}^{-1} \text{h}^{-1}$). Pigs were fed either sow's milk replacer enterally (Enteral) or an elemental nutrient solution intravenously (TPN) for 6 days. On day 7, both groups were fed enterally the same intake of sow's milk replacer for 6 h. ^bRates expressed as percentage of leucine intake on day 7.

systemic availability of nutrients, especially when the dietary intake is low or marginal. It is well known that protein malnutrition reduces whole-body growth rate. However, although our previous study conducted with neonatal pigs (Ebner *et al.*, 1994) demonstrated that protein malnutrition (15% dietary protein) significantly reduced whole-body growth, this was attributed mainly to reduced carcass growth, whereas gastrointestinal tissue growth was maintained. As a result, protein malnutrition actually increased the protein mass of the gut relative to the whole body, raising the possibility that gut nutrient needs may have limited growth. We tested this question in our most recent study by measuring the PDV metabolism of ¹³C-lysine and ¹³C-threonine infused both enterally and intravenously in young pigs fed either a high (25%) or low (10%) protein diet for 5 days. Low-protein feeding decreased the piglet growth rate by 50%, yet the relative mass of the gut was unchanged (Table 19.6). In pigs fed low protein, the net PDV lysine utilization increased to 85% of the dietary intake, compared with 47% in pigs fed high protein. Interestingly, on the high-protein diet, all of the PDV lysine utilization was derived from the arterial circulation, whereas on the low-protein diet the gut derived its lysine equally from the enteral and arterial inputs. These findings demonstrate that, under conditions of chronic low

protein intake, the lysine requirements of the gut remain relatively high and are preferentially met by first-pass metabolism from the diet, which limits the systemic availability of lysine for lean tissue growth.

Effect of enteric pathogens

It is known that infestation with pathogenic microbial and viral organisms adversely affects both intestinal structure and function, as well as the animal growth rate. The evidence from various domestic livestock species indicates that increased antigen exposure resulting from both pathogenic and non-pathogenic organisms imparts a protein metabolic cost to the animal associated with stimulation and maintenance of the pro-inflammatory, acute-phase response (MacRae, 1993; Johnson, 1997). There is considerable evidence that the pro-inflammatory response leads to increased catabolism and net loss of muscle protein. In contrast, studies show that pro-inflammatory insults, such as endotoxin and cytokine treatment, stimulate protein synthesis and endogenous protein secretion (MacRae, 1993; Higashiguchi *et al.*, 1994). A recent study by Yu *et al.* (2000) in sheep demonstrated that parasitic infection increased the rate of leucine utilization and oxidation by PDV tissues (Table 19.7). The increased PDV leucine utilization associated with infec-

Table 19.6. Chronic low-protein feeding affects PDV lysine metabolism in piglets^a (van Goudoever *et al.*, 2000).

	High protein	% Intake ^b	Low protein	% Intake ^b
Body growth rate (g kg ⁻¹ day ⁻¹)	45 ± 2		24 ± 2*	
Small intestinal mass (g kg ⁻¹ BW)	44 ± 2		42 ± 2	
Intake	518		207	
Whole-body oxidation	53 ± 15	10	18 ± 5*	9
Total net portal utilization	241 ± 12	47	174 ± 63*	85
Portal utilization – enteral lysine	-15 ± 63	0	91 ± 36*	44
Portal oxidation – enteral lysine	24 ± 6	5	9 ± 26	0
Portal utilization – arterial lysine	229 ± 71	44	111 ± 47*	54

^aPreliminary unpublished results, J.B. van Goudoever, B. Stoll, D.G. Burrin and P.J. Reeds (lysine fluxes expressed as μmol kg⁻¹ h⁻¹).

^bExpressed as percentage of lysine intake.

*P < 0.05 low versus high protein.

tion was likely used for endogenous protein secretion as well as oxidation, which together reduced by 20–30% the systemic availability of dietary amino acids.

The results of Yu *et al.* (2000) illustrated a fundamental mechanism of how reducing the gastrointestinal microflora and the incidence of pathogenic infection, using antimicrobial agents, can enhance the growth rate in domestic animals. Antimicrobial compounds have been widely used in animal feeds for several decades to improve animal growth. Since the advent of this practice, it has been shown that, by suppressing microbial activity, antimicrobials decrease the luminal concentration and associated toxic insult of ammonia and thereby reduce the thickness and mass of the intestinal mucosa and associated lymphoid tissue (Vissek, 1978). Additional evidence indicates that much of the luminal ammonia originates from bacterial hydrolysis of urea and deamination of dietary amino acids. Given that the gut may be an important site of dietary amino acid catabolism, it raises the question of whether this activity is associated with the luminal microbes or the cell populations of the mucosa. Although it is conceivable and likely that both possibilities exist, the end result is the catabolism and loss of dietary amino acids, especially those that are essential, that otherwise would be used for the synthesis and deposition of body protein. With the increased pressure to discontinue the use of feed-grade antibiotics, it will become increasingly important to develop alternative approaches, such as segregated early weaning of neonatal pigs and the use of

probiotics, to minimize the impact of pathogenic and non-pathogenic organisms on gut amino acid metabolism in order to enhance animal growth.

Implications for nutrient requirements

The substantial intestinal metabolism of dietary amino acids may also have nutritional significance with respect to the essentiality of some amino acids, namely arginine, threonine, methionine and cysteine. The extensive intestinal metabolism of dietary glutamate and glutamine observed in neonatal pigs (Stoll *et al.*, 1999b) has been linked to the synthesis of arginine and proline (Murphy *et al.*, 1996; Wu, 1998). In healthy suckling pigs, this intestinal synthesis of arginine provides only about half of the animal's needs for growth; thus, arginine is required in the diet of young pigs. Sow's milk is relatively low in arginine and the net intestinal synthesis of arginine declines substantially during the late suckling period (Wu, 1998). These observations raise the possibility that both the endogenous (via gut synthesis) and dietary arginine supply could become limiting for optimum growth in suckling piglets. Although proline is not considered an essential amino acid, maintaining the supply of proline is important for collagen synthesis under conditions of injury or stress. Moreover, the intestine is an important site of proline synthesis, which is derived preferentially from dietary precursors such as glutamate (Murphy *et al.*, 1996). Therefore, an important consideration may be whether the

Table 19.7. PDV leucine utilization in response to parasitic infection in sheep (Yu *et al.*, 2000).

	Control	Weeks infected		
		6	12	18
Portal leucine utilization				
Dietary	10	12	16	12
Arterial	59	74	72	61
Systemic leucine availability	76	54	51	61
% control	100	71	67	80

Rates of portal leucine metabolism expressed as mmol day⁻¹.

dietary requirements for arginine and proline for piglets are increased under conditions that compromise intestinal growth and function, such as weaning, and whether dietary supplementation is warranted. In the rat, an animal that normally does not require dietary arginine, small intestinal resection renders arginine a nutritionally essential amino acid (Wakabayashi *et al.*, 1995).

Dietary threonine and cysteine utilization by the intestinal mucosa for mucin and glutathione synthesis also may influence their systemic availability. From a nutritional perspective it is important to distinguish that, although threonine is considered essential, cysteine is not. However, cysteine can be synthesized from the essential amino acid, methionine; therefore, increased metabolism of methionine to meet cysteine needs may become limiting for growth and nutritionally significant. The secretory mucins play a key role in the innate immune defence of the mucosa, and the core protein of the major intestinal mucins contains a large amount of threonine and cysteine (van Klinken *et al.*, 1997). Likewise, cysteine is a component of the tripeptide antioxidant glutathione, which is critical for the maintenance of the structural integrity and barrier function of the intestinal mucosa (Martensson *et al.*, 1991). Given that the rates of mucosal synthesis and secretion of glutathione, and presumably mucins, are likely significant, it follows that their production by the gut could have a quantitatively significant impact on the animal's dietary requirement for threonine, cysteine and perhaps methionine. For threonine, but not cysteine, there is evidence to support this hypothesis. Firstly, based on net portal utilization, as much as 60–85% of dietary threonine is utilized by the gut (Stoll *et al.*, 1998; J.B. Van Goudoever, unpublished results) (Table 19.5). More conclusive was a study (Bertolo *et al.*, 1998) demonstrating that the threonine requirement of piglets maintained by parenteral nutrition (a treatment that certainly lowers gut mass and probably lowers mucin secretion) was nearly 60% lower than that of piglets receiving enteral feeding.

Moreover, preliminary studies (R.O. Ball *et al.*, unpublished results) indicated that feeding threonine-deficient diets to piglets significantly reduced intestinal mass and goblet cell numbers, and this suppression of intestinal growth could not be fully restored by providing threonine parenterally. Furthermore, studies with early-weaned piglets indicated that supplementing up to 0.75% threonine to a high-protein (26%) corn-soybean-meal diet increased growth rate (see Chapter 25). Another report, from the University of Alberta group (Shoveller *et al.*, 2000), showed that the methionine requirement of piglets maintained by parenteral nutrition was 35% lower than that of enterally fed piglets. This value is lower than the value of 46% based on our measurements of net portal utilization of dietary methionine. Nevertheless, both of these results suggest that the gut utilizes substantial amounts of dietary methionine and raises the question as to whether methionine is being metabolized to cysteine and used for intestinal mucin synthesis.

Regulation of Gut Growth

Given that the growth and metabolism of the gut significantly affects whole-body nutrient requirements, it is especially critical to understand those factors that regulate gut growth. It is well known that enteral nutrient intake is the primary stimulus for gut growth and that luminal nutrients are essential for mucosal epithelial cell proliferation and survival. A number of studies have quantified the relationship between various aspects of gut growth and both the amount and composition of nutrient intake (Spector *et al.*, 1977; Weser *et al.*, 1986; Shulman, 1988; Buts *et al.*, 1990; Zaloga *et al.*, 1992; Sax *et al.*, 1996; Adegoke *et al.*, 1999). Other studies suggest that glutamine, in particular, may play an especially important role in stimulating cell proliferation and inhibiting apoptosis (Papaconstantinou *et al.*, 1998; Rhoads, 1999). However, there is limited information regarding the optimum quantity and composition of enteral nutrients necessary

to maintain normal gut growth in the neonate. To address this question, we measured small intestinal growth in seven groups of neonatal pigs given equal total nutrient intakes for 7 days, such that different groups received 0, 10, 20, 40, 60, 80 or 100% of the total nutrient intake enterally, with the remainder being given parenterally (i.e. intravenously) (Burris *et al.*, 2000; Stoll *et al.*, 2000). We found that the minimal enteral nutrient intake necessary to increase mucosal mass was 40% of total, while 60% enteral nutrition was required to sustain normal mucosal proliferation and growth (Fig. 19.1). Moreover, we found that there is net intestinal protein loss at 0% enteral intake, protein balance at 20% enteral intake and maximal intestinal protein accretion at 60% enteral intake (Fig. 19.2). It is interesting to note that the amounts of enteral nutrient intake required to maintain intestinal growth (i.e. 40–60% of total) are generally similar to our previous estimates of gut nutrient utilization based on substrate metabolism.

In addition to their role as substrates for growth, enteral nutrients stimulate the local expression and release of numerous peptide hormones that could potentially act as gut trophic factors. In our study examining the relationship between the level of enteral nutrient intake and gut

growth, we measured the circulating concentrations of several putative gut growth factors, including insulin-like growth factor-I, insulin, gastrin, gastric-inhibitory peptide, peptide YY (PYY) and glucagon-like peptide-2 (GLP-2). We found that two of the gut-derived peptides, GLP-2 and PYY, were highly correlated with various endpoints of intestinal growth and cell proliferation (Fig. 19.3). Given that both GLP-2 and gut growth were correlated with the level of enteral nutrient intake, we postulated that GLP-2 may mediate the effects of enteral nutrient intake. In recent studies, we have demonstrated that infusion of GLP-2 to TPN-fed piglets significantly increased intestinal growth and largely reproduced the intestinal trophic effects observed in piglets fed sow's milk (see Chapter 10). Whether these gut peptides are trophic when given at physiological concentrations remains to be determined. However, these results strongly suggest that GLP-2 may indeed be an enterally derived signal that mediates the intestinal trophic effects of enteral nutrients. In future studies, it will be important to establish how other gut-derived peptides and trophic hormones affect not only the intestinal nutrient needs and metabolism, but also functional aspects of the gut under healthy and diseased conditions.

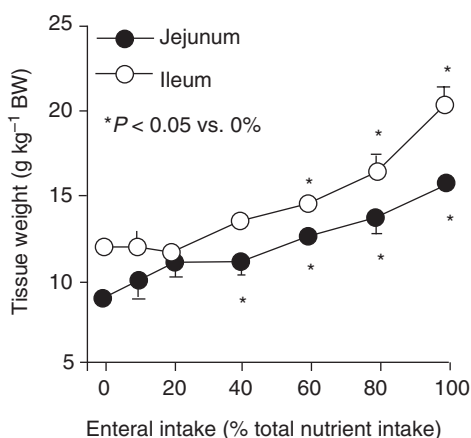


Fig. 19.1.

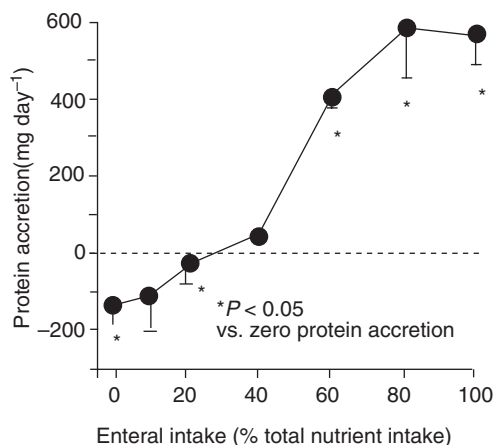


Fig. 19.2.

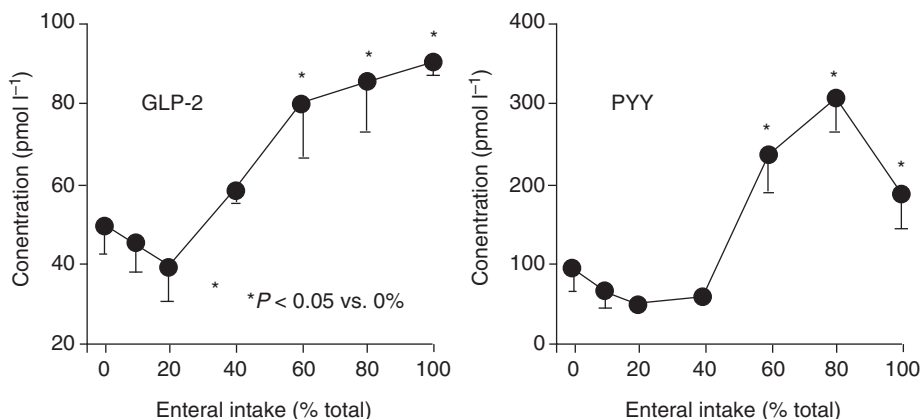


Fig. 19.3.

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References

- Adegoke, O.A., McBurney, M.I., Samuels, S.E. and Baracos, V.E. (1999) Luminal amino acids acutely decrease intestinal mucosal protein synthesis and protease mRNA in piglets. *Journal of Nutrition* 129, 1871-1878.
- Attaix, D., Manghebat, A., Grizard, J. and Arnal, M. (1986) Assessment of *in vivo* protein synthesis in lamb tissues with [³H] valine flooding doses. *Biochimica Biophysica Acta* 882, 389-397.
- Bergman, E.N. and Heitmann, R.N. (1978) Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. *Federation Proceedings* 37, 1228-1232.
- Bertolo, R.F., Chen, C.Z., Law, G., Pencharz, P.B. and Ball, R.O. (1998) Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. *Journal of Nutrition* 128, 1752-1759.
- Burrin, D.G., Ferrell, C.L., Eisemann, J.H., Britton, R.A. and Nienaber, J.A. (1989) Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. *British Journal of Nutrition* 62, 23-34.
- Burrin, D.G., Shulman, R.J., Reeds, P.J., Davis, T.A. and Gravitt, K.R. (1992) Porcine colostrum and milk stimulate visceral organ and skeletal muscle synthesis in neonatal piglets. *Journal of Nutrition* 122, 1205-1213.
- Burrin, D.G., Shulman, R.J., Langston, C. and Storm, M.C. (1994) Supplemental alanine/glutamine, organ growth and nitrogen metabolism in neonatal pigs fed by total parenteral nutrition. *Journal of Parenteral and Enteral Nutrition* 18, 313-319.
- Burrin, D.G., Stoll, B., Chang, X., Yu, H. and Reeds, P.J. (1999) Total parenteral nutrition does not compromise digestion or absorption of dietary protein in neonatal pigs. *FASEB Journal* 13, A1024.
- Burrin, D.G., Stoll, B., Jiang, R., Chang, X., Hartmann, B., Holst, J.J., Greeley, G.H. Jr and Reeds, P.J. (2000) Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough? *American Journal of Clinical Nutrition* 71, 1603-1610.

- Buts, J.P., Vijverman, V., Barudi, C., De Keyser, N., Maldague, P. and Dive, C. (1990) Refeeding after starvation in the rat: comparative effects of lipids, proteins and carbohydrates on jejunal and ileal mucosal adaptation. *European Journal of Clinical Investigation* 20, 441–452.
- Cappelli, F.P., Seal, C.J. and Parker, D.S. (1997) Glucose and [^{13}C] leucine metabolism by the portal-drained viscera of sheep fed on dried grass with acute intravenous and intraduodenal infusions of glucose. *British Journal of Nutrition* 78, 931–946.
- Deutz, N.E., Bruins, M.J. and Soeters, P.B. (1998) Infusion of soy and casein protein meals affects inter-organ amino acid metabolism and urea kinetics differently in pigs. *Journal of Nutrition* 128, 2435–2445.
- Ebner, S., Schoknecht, P., Reeds, P.J. and Burrin, D.G. (1994) Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. *American Journal of Physiology* 266, R1736–R1743.
- Ferrell, C.L. (1988) Contribution of visceral organs to animal energy expenditures. *Journal of Animal Science* 66, 23–34.
- Higashiguchi, T., Noguchi, Y., O'Brien, W., Wagner, K., Fischer, J.E. and Hasselgren, P.O. (1994) Effect of sepsis on mucosal protein synthesis in different parts of the gastrointestinal tract in rats. *Clinical Science* 87, 207–211.
- Johnson, R.W. (1997) Inhibition of growth by pro-inflammatory cytokines: an integrated view. *Journal of Animal Science* 75, 1244–1255.
- Lobley, G.E., Milne, V., Lovie, J.M., Reeds, P.J. and Pennie, K. (1980) Whole body and tissue protein synthesis in cattle. *British Journal of Nutrition* 43, 491–502.
- Lobley, G.E., Harris, P.M., Skene, P.A., Brown, D., Milne, E., Calder, A.G., Anderson, S.E., Garlick, P.J., Nevison, I. and Connell, A. (1992) Responses in tissue protein synthesis to sub- and supra-maintenance intake in young growing sheep: comparison of large-dose and continuous-infusion techniques. *British Journal of Nutrition* 68, 373–388.
- MacRae, J.C. (1993) Metabolic consequences of intestinal parasitism. *Proceedings of the Nutrition Society* 52, 121–130.
- MacRae, J.C., Bruce, L.A., Brown, D.S. and Calder, A.G. (1997a) Amino acid use by the gastrointestinal tract of sheep given lucerne forage. *American Journal of Physiology* 273, G1200–G1207.
- MacRae, J.C., Bruce, L.A., Brown, D.S., Farningham, D.A. and Franklin, M. (1997b) Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. *Journal of Animal Science* 75, 3307–3314.
- Martensson, J., Jain, A. and Meister, A. (1991) Glutathione is required for intestinal function. *Proceedings of the National Academy of Sciences USA* 87, 1715–1719.
- Murphy, J.M., Murch, S.J. and Ball, R.O. (1996) Proline is synthesized from glutamate during intra-gastric infusion but not during intravenous infusion in neonatal piglets. *Journal of Nutrition* 26, 878–886.
- Papaconstantinou, H.T., Hwang, K.O., Rajaraman, S., Hellmich, M.R., Townsend, C.M. Jr and Ko, T.C. (1998) Glutamine deprivation induces apoptosis in intestinal epithelial cells. *Surgery* 124, 152–159.
- Pell, J.M., Calderone, E.M. and Bergman, E.N. (1986) Leucine and ketoisocaproate metabolism and interconversion in fed and fasted sheep. *Metabolism* 35, 1005–1016.
- Perez, J.F. and Reeds, P.J. (1998) A novel stable isotopic approach enables the simultaneous determination of protein and RNA synthesis *in vivo*. *Journal of Nutrition* 128, 1562–1569.
- Reeds, P.J., Burrin, D.G., Jahoor, F., Wykes, L., Henry, J. and Frazer, E.M. (1996) Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. *American Journal of Physiology* 270, E413–E418.
- Reeds, P.J., Burrin, D.G., Stoll, B., Jahoor, F., Wykes, L.J., Henry, J. and Frazer, M.E. (1997) Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. *American Journal of Physiology* 273, E408–E415.
- Reeds, P.J., Burrin, D.G., Davis, T.A. and Stoll, B. (1998) Amino acid metabolism and the energetics of growth. *Archives of Tierernahrung* 51, 187–197.
- Rerat, A. (1993) Nutritional supply of proteins and absorption of their hydrolysis products: consequences on metabolism. *Proceedings of the Nutrition Society* 52, 335–344.
- Rerat, A., Simoes-Núñez, C., Mendy, F., Vaissade, P. and Vaugelade, P. (1992) Splanchnic fluxes of amino acids after duodenal infusion of carbohydrate solutions containing free amino acids or oligopeptides in the non-anaesthetized pig. *British Journal of Nutrition* 68, 111–138.

- Rhoads, M. (1999) Glutamine signaling in intestinal cells. *Journal of Parenteral and Enteral Nutrition* 23, S38–S40.
- Sax, H.C., Ilig, K.A., Ryan, C.K. and Hardy, D.J. (1996) Low-dose enteral feeding is beneficial during total parenteral nutrition. *American Journal of Surgery* 171, 587–590.
- Shoveller, A.K., Ball, R.O. and Pencharz, P.B. (2000) The methionine requirement is 35% lower during parenteral versus oral feeding in neonatal piglets. *FASEB Journal* 14, A558.
- Shulman, R.J. (1988) Effect of different total parenteral nutrition fuel mixes on small intestinal growth and differentiation in the infant miniature pig. *Gastroenterology* 95, 85–92.
- Spector, M.H., Levine, G.M. and Deren, J.J. (1977) Direct and indirect effects of dextrose and amino acids on gut mass. *Gastroenterology* 72, 706–710.
- Stoll, B., Burrin, D.G., Henry, J., Jahoor, F. and Reeds, P.J. (1997) Intestinal and hepatic phenylalanine utilization in fasted and fed piglets determined with intravenous and intragastric tracers. *American Journal of Physiology* 273, G1208–G1217.
- Stoll, B., Henry, J., Reeds, P.J., Yu, H., Jahoor, F. and Burrin, D.G. (1998) Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *Journal of Nutrition* 128, 606–614.
- Stoll, B., Burrin, D.G., Yu, H., Henry, J., Jahoor, F. and Reeds, P.J. (1999a) Utilization of enteral and arterial phenylalanine for mucosal and hepatic constitutive protein synthesis in pigs. *American Journal of Physiology* 276, G49–G57.
- Stoll, B., Burrin, D.G., Henry, J., Yu, H., Jahoor, F. and Reeds, P.J. (1999b) Substrate oxidation by the portal drained viscera of fed piglets. *American Journal of Physiology* 277, E168–E175.
- Stoll, B., Chang, X., Fan, M.Z., Reeds, P.J. and Burrin, D.G. (2000) Enteral nutrient intake determines the rate of intestinal protein synthesis and accretion in neonatal pigs. *American Journal of Physiology* 279, G288–G294.
- van Goudoever, J.B., Stoll, B., Henry, J.F., Burrin, D.G. and Reeds, P.J. (2000) Adaptive regulation of intestinal lysine metabolism. *Proceedings of the National Academy of Sciences USA* 97, 11620–11625.
- van der Meulen, J., Bakker, J.G., Smits, B. and de Visser, H. (1997) Effects of source of starch on net portal flux of glucose, lactate, volatile fatty acids and amino acids in the pig. *British Journal of Nutrition* 78, 533–544.
- van Klinken, B.J., Dekker, J., Buller, H.A. and Einerhand, A.W. (1995) Mucin gene structure and expression: protection vs. adhesion. *American Journal of Physiology* 269, G613–G627.
- Visek, W.J. (1978) The mode of growth promotion by antibiotics. *Journal of Animal Science* 46, 1447–1469.
- Wakabayashi, Y., Yamada, E., Yoshida, T. and Takahashi, N. (1995) Effect of intestinal resection and arginine-free diet on rat physiology. *American Journal of Physiology* 269, G313–G318.
- Weser, E., Babbitt, J., Hoban, M. and Vandeventer, A. (1986) Intestinal adaptation. Different growth responses to disaccharides compared with monosaccharides in rat small bowel. *Gastroenterology* 91, 1521–1527.
- Windmueller, H.G. and Spaeth, A.E. (1975) Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. *Archives of Biochemistry and Biophysics* 171, 662–672.
- Windmueller, H. and Spaeth, A.E. (1980) Respiratory fuels and nitrogen metabolism *in vivo* in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. *Journal of Biological Chemistry* 255, 107–112.
- Wu, G. (1998) Intestinal mucosal amino acid catabolism. *Journal of Nutrition* 128, 1249–1252.
- Yen, J.T., Nienaber, J.A., Hill, D.A. and Pond, W.G. (1989) Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. *Proceedings of the Society for Experimental Biology Medicine* 190, 393–398.
- Yu, F., Bruce, L.A., Calder, A.G., Milne, E., Coop, R.L., Jackson, F., Horgan, G.W. and MacRae, J.C. (2000) Subclinical infection with the nematode *Trichostrongylus colubriformis* increases gastrointestinal tract leucine metabolism and reduces availability of leucine for other tissues. *Journal of Animal Science* 78, 380–390.
- Yu, Y.-M., Burke, J.F., Vogt, J.A., Chambers, L. and Young, V.R. (1992) Splanchnic and whole body L-[1-¹³C, ¹⁵N] leucine kinetics in relation to enteral and parenteral amino acid supply. *American Journal of Physiology* 262, E687–E694.
- Zaloga, G.P., Ward Black, K. and Prielipp, R. (1992) Effect of rate of enteral nutrient supply on gut mass. *Journal of Parenteral and Enteral Nutrition* 16, 39–42.

20 Intestinal Metabolism of Methionine Significantly Affects Requirement and Proportion Spared by Cysteine

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Early-weaned piglets had a lower requirement for the sulphur amino acids when the diet was supplied intravenously (IV) and the gut was bypassed. The sparing capacity of cysteine (Cys) on the requirement for methionine (Met) was similar between IV diet infusion and intragastric (IG) diet infusion. These results indicate that Met is utilized by the gut during first-pass metabolism and that route of diet administration does not affect the sparing capacity of Cys.

Introduction

The weaning age for piglets is becoming younger in order to raise overall production. However, little research examining the amino acid (AA) requirements of piglets is available. Recent evidence has suggested that the gut utilizes a substantial proportion of dietary AAs (Bertolo *et al.*, 1998; Stoll *et al.*, 1998). The National Research Council (NRC, 1998) suggested that methionine (Met) could meet the total requirement for the sulphur AAs but recommended that they be supplied in a 50:50 ratio. Research in human nutrition has suggested that cysteine (Cys) may be conditionally essential in the premature infant (Zlotkin and Anderson, 1982). Because the term piglet is born with similar maturity as a premature human infant, there is a possibility that Cys may also be conditionally essential for the neonatal piglet. No evidence was found in the literature in which the Met requirement and Cys sparing capacity were determined directly in intra-

gastric (IG) and intravenously (IV) fed neonatal piglets. IV feeding allows for a requirement to be established when the gut is bypassed and thus relatively inactive. The objectives of this study were: (i) to determine the Met requirement when piglets were fed IG or IV; and (ii) to elucidate the capacity for Cys sparing in both routes of feeding.

Material and Methods

The indicator AA oxidation technique uses radiolabelled L-[1-¹⁴C] phenylalanine (Phe) as the indicator AA to measure the level of AA oxidation. During a constant infusion of the isotope, both breath and blood samples are taken and analysed for specific radioactivity. At a deficient concentration of the test AA, more of the indicator AA will be oxidized. As the test AA approaches requirement, the indicator AA oxidation will decline proportionally. The requirement for the test AA is defined as

the point where the oxidation becomes low and constant, indicating maximum rate of protein synthesis.

Fifty-six piglets were taken from the sow (1–2 days old; 1.4–1.7 kg) and implanted with infusion catheters and sampling catheters in the jugular and femoral veins, respectively. In the IG experiments an infusion catheter was inserted into the stomach. Piglets were fed identical complete diets continuously for 5 days and were randomized to two of seven test levels of Met, either with or without Cys (Table 20.1). After 24 h of test diet infusion, an indicator AA oxidation measurement was completed. Piglets were placed back on a complete diet for a washout period. Two days later, the second test diet was initiated for 24 h and a second indicator AA oxidation measurement was completed.

In study 1 ($n = 14$) and study 2 ($n = 14$), piglets received test diets, with no dietary Cys, and two of the seven diet levels of Met (Table 20.1) by IV or IG feeding, respectively. In study 3 ($n = 14$) and study 4 ($n = 14$), piglets received test diets, with excess Cys ($55 \text{ g kg}^{-1} \text{ day}^{-1}$), and two of seven diet levels of Met (Table 20.1), by IV or IG infusion, respectively. Dietary Cys intake was chosen to exceed the requirement estimated in study 1 by 30%.

Phe kinetics were determined over 4 h by measuring $^{14}\text{CO}_2$ expired and plasma specific radioactivity following a primed ($185:\text{Ci kg}^{-1}$), constant infusion ($129:\text{Ci kg}^{-1} \text{ h}^{-1}$) of L-[1- ^{14}C] Phe. Details of the infusion protocol, $^{14}\text{CO}_2$ and blood collection have been previously described (Bertolo *et al.*, 1998).

Plasma AAs and the specific radioactivity (SRA) of plasma Phe and tyrosine were mea-

sured by reverse-phase HPLC and fractions were collected to measure radioactivity as previously described (Bertolo *et al.*, 1998).

The percentage of dose oxidized was calculated by using the mean corrected VCO_2 at plateau. The data was partitioned between two regression lines based on the model that produced the highest regression coefficients for the dependent variable, dietary level of Met. In all four experiments, the Met requirement was determined by using a two-phase linear regression crossover model, as previously described (Bertolo *et al.*, 1998).

Results and Discussion

The piglets in each of the trials remained healthy. The Met requirement for all four studies appears in Table 20.2.

In study 1, when IV Met was fed without Cys, Phe oxidation decreased linearly ($P < 0.01$) between 0.05 and 0.29 g kg^{-1} Met intake per day and then remained low and constant. In study 2, when IG Met was fed without Cys, Phe oxidation decreased linearly ($P < 0.01$) between 0.05 and 0.42 g kg^{-1} Met intake per day and then remained low and constant. Using breakpoint analysis, the requirement of Met was determined to be 0.29 and 0.42 g kg^{-1} per day for studies 1 and 2, respectively. These data show that the IV requirement for Met, without Cys, is 69% of the IG requirement. These data closely agree with those of Stoll *et al.* (1998) who concluded, using mass balance techniques, that approximately 30% of dietary Met disappears in first-pass metabolism.

In study 3, when IV Met with 0.55 g kg^{-1} Cys per day was fed, Phe oxidation

Table 20.1. Dietary intakes of methionine.

Study	Diet ($\text{g kg}^{-1} \text{ day}$)	Method	Methionine intake ($\text{g kg}^{-1} \text{ day}^{-1}$)						
1	Met, no Cys	IV	0.05	0.1	0.2	0.3	0.4	0.6	1.0
2	Met, no Cys	IG	0.1	0.2	0.4	0.5	0.6	0.7	1.0
3	Met, 0.55 Cys	IV	0.025	0.05	0.1	0.15	0.2	0.3	0.6
4	Met, 0.55 Cys	IG	0.025	0.05	0.15	0.25	0.35	0.45	0.6

Solutions were made isonitrogenous by altering L-alanine concentrations.

Table 20.2. Methionine requirements (g kg⁻¹ day⁻¹).

Route of feeding	Met without Cys	Met with (0.55 g kg ⁻¹ day ⁻¹) Cys	Sparing capacity of Cys
Orally	0.42	0.25	40%
Intravenously	0.29	0.18	38%

decreased linearly ($P < 0.01$) between 0.025 and 0.18 g kg⁻¹ Met intake per day and then remained low and constant. In study 4, when IG Met was fed with excess Cys, Phe decreased linearly ($P < 0.01$) between 0.025 and 0.25 g kg⁻¹ Met intake per day and then remained low and constant. Using breakpoint analyses the requirement for Met, when excess Cys was supplied, was determined to be 0.18 and 0.25 g kg⁻¹ day⁻¹, for studies 3 and 4, respectively. These data show that the capacity of Cys to spare the requirement of Met is relatively similar between orally (40%) and intravenously (38%) fed piglets.

Implications

The difference in the dietary requirement for Met in IV vs. IG piglets must be due to

uptake of the sulphur AAs by the small intestine during first-pass metabolism. The disappearance of Met in first-pass metabolism may be accounted for by Met acting as a methyl donor, Met being converted to Cys and further metabolites, or by the incorporation of Met into intestinal proteins. In addition, the incorporation of Cys into intestinal proteins or the use of Cys for glutathione synthesis may account for the disappearance of sulphur AAs in first-pass metabolism.

In conclusion, by using the indicator AA oxidation technique, a mean Met intake for neonatal piglets receiving a diet IV or IG, with or without excess Cys, has been defined (Table 20.2). Our data support the concept that Met and Cys are highly utilized by the gut and that Cys is capable of sparing the requirement for Met both IV and IG.

References

- Bertolo, R.F.P., Chen, C.X.L., Law, G.K.L., Pencharz, P.B. and Ball, R.O. (1998) Threonine requirement of neonatal pigs receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. *Journal of Nutrition* 128, 1752–1759.
- National Research Council (1998) *Nutrient Requirements of Swine*, 10th revised edition. National Academy of Science, Washington, DC, 17 pp.
- Stoll, B., Henry, J., Reeds, P.J., Yu, H., Jahoor, F. and Burrin, D.G. (1998) Catabolism dominates the first pass intestinal metabolism of dietary essential amino acids in milk fed piglets. *Journal of Nutrition* 128, 606–614.
- Zlotkin, S.H. and Anderson, G.H. (1982) The development of cystathionase activity during the first year of life. *Pediatric Research* 16, 65–68.

21 Effect of Fermentable Components in the Diet on the Portal Flux of Glucose and Volatile Fatty Acids in Growing Pigs

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A study was performed to determine the portal flux of glucose and volatile fatty acids (VFA) (acetic acid, propionic acid and butyric acid) of two isocaloric diets differing in the content of fermentable carbohydrates (diet I based on maize and maize starch and diet II with 150 g wheat bran kg⁻¹ and 150 g sugarbeet pulp kg⁻¹).

The calculated portal flux for glucose was 2738 and 1783 mmol kg⁻¹ for diets I and II ($P = 0.06$), being 88 and 86% of the oral intake of glucose equivalents, respectively. For total VFA the portal flux was 762 and 1365 mmol kg⁻¹ for diets I and II, respectively ($P < 0.05$).

The contribution of VFA resulting from fermentation in the gastrointestinal tract to the energy supply in pigs is largely dependent on the diet composition and may be up to 30% of the energy requirement for maintenance of the growing pig.

Introduction

The measurement of the portal flux of nutrients in pigs provides a rather direct estimate of the amount of nutrients available for maintenance and production after absorption from the gastrointestinal tract. Moreover, it can provide information of the digestive and absorptive kinetics of nutrients, and allows estimation of the quantitative absorption of end products of fermentation in the gastrointestinal tract (e.g. volatile fatty acids).

A study was performed to determine the portal flux of glucose and volatile fatty acids (VFA) (acetic acid, propionic acid and butyric acid) from two isoenergetic diets differing in content of fermentable carbohydrates.

Materials and Methods

Diet I was based on maize (350 g kg⁻¹), maize starch (322 g kg⁻¹), maize gluten meal (70 g kg⁻¹), soybean meal (70 g kg⁻¹), sunflower meal (40 g kg⁻¹) and meat and bone meal (63 g kg⁻¹) and diet II on maize (350 g kg⁻¹), maize starch (80 g kg⁻¹), maize gluten meal (70 g kg⁻¹), soybean meal (70 g kg⁻¹), sunflower meal (40 g kg⁻¹), wheat bran (150 g kg⁻¹) and sugarbeet pulp (150 g kg⁻¹). Diets I and II contained 561 and 375 g glucose equivalents kg⁻¹, both 162 g crude protein kg⁻¹ and 9.9 MJ net energy kg⁻¹. The calculated content of fermentable carbohydrates was 42 and 145 g kg⁻¹ in diets I and II, respectively. Four pigs (castrates; 47 kg body weight at the start of the first pre-experimental period) were used, fitted with

a catheter in the carotid artery and in the portal vein (van Leeuwen *et al.*, 1995). The study was carried out in a changeover design with two experimental periods (P1 and P2), and pre-experimental periods of 8–9 days. Pigs were fed two times per day (0800 and 2000 h) at a level of 2.6 times the maintenance requirement for energy ($419 \text{ kJ ME kg}^{-0.75} \text{ day}^{-1}$) using automatic feeders. The mean feed intake in P1 and P2 was 1376 and 1568 g day^{-1} , respectively.

Arterial and portal blood samples were taken 0, 1, 2, 3, 4, 6, 8 and 10 h after the morning feeding on two consecutive days. Plasma samples were analysed for glucose content (dry slide technique, Ektachem 950 IRC system) and VFA (gas chromatography with a capillary column). Portal fluxes of glucose and VFA were calculated assuming a constant portal blood flow rate of $38 \text{ ml kg}^{-1} \text{ min}^{-1}$. Data per time point or overall mean values per treatment were analysed statistically using analysis of variance, with diet as experimental factor.

Results and Discussion

The mean portal (P) plasma glucose concentrations were 7.31 (I) and 6.15 (II), and they were 5.15 (I) and 4.76 (II) mmol l^{-1} for the arterial (A) concentrations (Table 21.1). The mean P-A values for glucose were 2.17 and 1.39 mmol l^{-1} for groups I and II, respectively. Assuming no difference in portal blood flow between the treatments,

the mean portal glucose flux was lower for diet II than for diet I (Table 21.2; $P = 0.06$). The difference can be fully attributed to the difference in intake of starch/glucose equivalents between both treatments. For diet I, portal glucose concentration peaked 1 h after feeding and gradually diminished over the remaining period between two meals. For diet II, the portal glucose flux was much more steady over the between-meal interval.

Relative to the dietary intake of glucose equivalents, 88 and 86% of the glucose was calculated to be recovered in the portal blood plasma for diets I and II, respectively. The results suggest that the portal appearance of glucose and its time course after feeding depends on the content, origin and form of the starch in the diet and on further diet composition, such as dietary fibre level.

The mean arterial and portal concentrations of VFA in blood plasma are given in Table 21.1. The mean P-A concentrations for acetic acid, propionic acid and butyric acid were 0.36, 0.16 and 0.04 mmol l^{-1} for group I and 0.61, 0.31 and 0.06 mmol l^{-1} for group II (Table 21.1). The arterial and portal plasma concentrations of VFA were rather steady between two meals. This indicates that VFA absorption mainly takes place in the hindgut, with a much more steady filling than the small intestine, when feeding twice per day. The data also indicate a much higher fermentation activity in the intestine of the pig when using

Table 21.1. Effect of diet composition on the mean portal (P) and arterial (A) concentration of volatile fatty acids (mmol l^{-1}).

	Diet I	Diet II	P^a	SED
Acetic acid				
P	0.931	1.259	0.03	0.112
A	0.574	0.646	0.29	0.057
Propionic acid				
P	0.202	0.360	0.01	0.045
A	0.040	0.048	0.66	0.017
Butyric acid				
P	0.046	0.071	0.20	0.017
A	0.005	0.007	0.38	0.002

^aEffect of diet (I vs. II); SED = standard error of the difference.

Table 21.2. Effect of diet composition on the calculated portal flux of glucose and volatile fatty acids (mmol kg⁻¹ diet).

	Diet I		Diet II		<i>P</i>	SED
	mmol kg ⁻¹	(%)	mmol kg ⁻¹	(%)		
Glucose	2738		1783		0.06	419
Acetic acid	480	(63)	846	(62)	0.03	129
Propionic acid	224	(29)	429	(31)	0.01	56
Butyric acid	57	(8)	90	(7)	0.21	23
Total VFA	761	(100)	1365	(100)	0.02	191

(%), molar proportion of total VFA.

diet II, with wheat bran and sugarbeet pulp, compared with diet I. The estimated portal flux of VFA was about 80% higher for diet II (1365 mmol kg⁻¹ diet) compared with diet I (762 mmol kg⁻¹ diet) (Table 21.2) (*P* < 0.05).

For both diets the portal flux was highest for acetic acid, followed by propionic acid and butyric acid. The relative proportion of the flux of individual VFA did not differ largely between the two diets (Table 21.2). VFA absorbed from the gastrointestinal tract into the portal blood, based

on data of the present study, could cover up to 30% of the calculated energy requirements for maintenance of growing pigs.

It can be concluded from the present study that the portal flux of glucose and volatile fatty acids is largely affected by diet composition. Presence of fermentable carbohydrates in the diet largely increases fermentation in the digestive tract, resulting in an increase in the portal flux of VFAs. These can be used as energetic substrates for the pig.

Reference

van Leeuwen, P., Leuvenink, H.G.D., Haasbroek, W.M., Priem, G., Bosch, M. and van Kleef, D.J. (1995) A portal vein catheterization technique in pigs and sheep, and postprandial changes of pO₂, pCO₂, pH, urea, ammonia and creatinine and proteins in portal and arterial blood measured in pigs. *Journal of Animal Physiology and Animal Nutrition* 73, 38–46.

22 Arginine and Proline Synthesis Occurs Primarily on First-pass Metabolism by the Small Intestine in Piglets

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We hypothesized that the interconversion of proline and arginine occurs in the small intestine. Ten piglets received either intraportal (IP) or intragastric (IG) infusions of labelled arginine, ornithine and proline; this infusion protocol isolated small intestinal first-pass metabolism. Fluxes of arginine, proline and ornithine were lower during IP infusion, indicating substantial synthesis by the gut. During IP infusion, percentage conversions from proline to ornithine, ornithine to arginine and ornithine to proline were much lower than during IG infusion. Thus, in situations where gut metabolism is bypassed or compromised, arginine and proline are individually essential because their biosyntheses are negligible.

Introduction

In a previous experiment, we demonstrated in piglets that arginine-induced hyperammonaemia was ameliorated when proline was included in the diet (Brunton *et al.*, 1999). We concluded that: (i) the piglet cannot synthesize sufficient arginine to dispose of ammonia via urea; (ii) the piglet cannot synthesize sufficient proline to maintain plasma concentrations; and (iii) arginine synthesis from proline is dependent on gut metabolism. The present multi-isotope experiment was designed to determine whether proline and arginine interconversion is dependent on small intestinal first-pass metabolism.

Materials and Methods

Animals

Ten piglets (1–3 days old; 1.3–1.8 kg) were grouped between two treatments: intrapor-

tally (IP) or intragastrically (IG) infused piglets. All piglets were fitted with stomach catheters for feeding and femoral vein catheters for blood sampling; for tracer infusion, umbilical vein catheters were also implanted in IP pigs. An elemental and complete diet was gastrically infused for 6 days supplying 15 g amino acids kg⁻¹ day⁻¹ and 1.1 MJ ME kg⁻¹ day⁻¹.

Radiotracers and calculations

On day 5, piglets received a primed (111 kBq kg⁻¹), constant (185 kBq kg⁻¹ h⁻¹ × 6 h) infusion of L-[guanido-¹⁴C]arginine; the urea pool was primed with [¹⁴C]urea intravenously (463 kBq kg⁻¹). On day 6, piglets received primed (481 kBq kg⁻¹, 370 kBq kg⁻¹ h⁻¹, respectively), constant (370 kBq kg⁻¹ h⁻¹ × 6.5 h) infusions of L-[¹⁴C(U)]ornithine and L-[2,3-³H]proline. Blood was sampled every 30 min.

Plasma amino acid concentrations and specific radioactivities (SRA) were measured

by HPLC with fraction collection. Flux (arginine, ornithine, proline) = constant infusion rate ÷ SRA at steady state. The small intestinal first-pass contribution (%) = $(\text{Flux}_{\text{IG}} - \text{Flux}_{\text{IP}}) \div \text{Flux}_{\text{IG}} \times 100$. To address the role of small intestinal first-pass metabolism on amino acid conversions, we used SRA ratios (%) at steady state (i.e. relative conversion) = $\text{SRA}_{\text{product}} \div \text{SRA}_{\text{infused AA}} \times 100$. Data were analysed by a paired sample *t*-test and were different if *P* < 0.05.

Results

Fluxes (μmol kg⁻¹ h⁻¹) during IP infusion were lower for ornithine (IG, 280 ± 46; IP, 137 ± 12), proline (IG, 493 ± 37; IP, 426 ± 29) and arginine (IG, 457 ± 77; IP, 292 ± 30), resulting in small intestinal percentage contributions of 49 ± 13 (ornithine), 13 ± 4 (proline) and 40 ± 1 (arginine).

Discussion

In this study, we infused amino acid tracers into the portal vein or stomach, thus separating the respective absence or presence of intestinal first-pass metabolism. This study has clearly demonstrated that small intestinal metabolism is necessary for the conversion of proline to arginine; almost no conversion was evident during IP infusion, compared with 16% during IG infusion (i.e. 42.2% × 38.9%). Furthermore, the conversion of proline to arginine appears to occur almost entirely on first-pass metabolism, because our models do not preclude the arterial extraction of pre-

cursors (i.e. proline) for the synthesis of arginine. These data confirm our earlier observations that parenteral proline does not alleviate arginine deficiency-induced hyperammonaemia (Brunton *et al.*, 1999). Total arginine synthesis was significantly lower during IP infusion, as indicated by arginine flux measurements. Because protein breakdown was similar and dietary arginine intake was identical between groups, the difference in arginine flux (amino acid synthesis + protein breakdown + dietary intake) between IP and IG (165 μmol kg⁻¹ h⁻¹) reflects *de novo* arginine synthesis by the small intestine.

Proline synthesis from arginine was also dependent on small intestinal metabolism. Proline flux differences indicate that the small intestinal contribution to proline synthesis (13%) was less than that for arginine synthesis (40%). The relative conversion of arginine to ornithine was not different between IP and IG; however, the conversion of ornithine to proline appeared to be localized to the gut, because a 75% lower relative conversion was observed during IP infusion. The relative conversion for the reverse pathway (proline to ornithine) was > 98% lower when small intestinal metabolism was bypassed. These data support the conclusion that the synthesis of arginine from proline is more dependent on first-pass metabolism than the synthesis of proline from arginine. Because ornithine was not present in the diet and is not a component of protein, ornithine synthesis is equal to ornithine flux. Consequently, small intestinal first-pass metabolism is responsible for half of the ornithine synthesized in the body.

Table 22.1. Relative conversions between P5C amino acids.

	IG	IP		IG	IP
Orn —Arg	38.9 ± 8.1	10.9 ± 6.2*	Pro —Orn	42.2 ± 12.0	1.0 ± 1.0*
—Pro	18.3 ± 5.6	4.5 ± 0.5*	—OHPro	14.1 ± 7.8	14.6 ± 6.7
—Glu	6.7 ± 1.9	4.2 ± 0.7*	—Glu	17.7 ± 5.1	22.2 ± 7.2
—Urea	4.7 ± 2.4	2.8 ± 1.4			
—OHPro	24.2 ± 10.9	10.1 ± 4.5*	Arg —Urea	53.3 ± 20.5	44.5 ± 10.8
—Gln	8.0 ± 2.0	5.1 ± 0.7			

*, *P* < 0.05.

One of the goals of our multiple tracer infusion protocol was to identify the limiting step in the pathway that results in arginine synthesis being dependent on gut metabolism. As shown in Table 22.1, the gut-dependent pathway for the syntheses of proline and arginine was the interconversion between ornithine and proline. Therefore, the gut-localized enzymes are either ornithinamin-transferase (OAT) or P5C reductase and proline oxidase (Fig. 22.1; underlined values are different). However, proline oxidase activity was not dependent on the gut, since IP and IG infusions gave similar relative conversions to glutamate (22 vs. 18%, respectively, $P = 0.20$). OAT is also involved in the conversion from ornithine to glutamate and this conversion was lower during IP infusion (4.2 vs. 6.7%, $P = 0.04$). Thus, we conclude that OAT activity must be predominantly localized to the small intestine; this explains the negligible interconversion between arginine and proline when gut metabolism is bypassed.

By infusing amino acid radioisotopes via the gastric or portal venous route in orally fed piglets, our results directly demonstrate that small intestinal first-pass metabolism is necessary for the intercon-

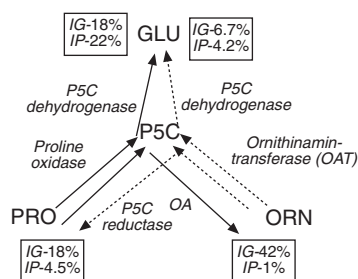


Fig. 22.1. P5C amino acid conversions and enzymes.

version between arginine and proline. In situations where first-pass gut metabolism is bypassed or compromised (i.e. total parenteral nutrition, gut resection, intestinal disease or weaning-induced gut stress), arginine and proline are individually essential because their biosyntheses are negligible.

Acknowledgements

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Reference

- Brunton, J.A., Bertolo, R.F.P., Pencharz, P.B. and Ball, R.O. (1999) Proline ameliorates arginine deficiency during enteral but not during parenteral feeding in neonatal piglets. *American Journal of Physiology* 277 (Endocrinology and Metabolism 40), E223–E231.

23 Effect of Diet Composition on Organ Size and Energy Expenditure in Growing Pigs

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Catheters were placed in the portal vein, the ileal vein and the carotid artery of eight pigs trained to receive their feed mixed with water (1 ml g⁻¹ of feed) twice daily (0800 and 1600 h). Daily feed allowance was 2.6 × maintenance digestible energy (DE) requirement (110 kcal DE kg⁻¹ BW^{0.75}). After pigs had consumed a barley–canola meal (BCM) diet (*n* = 4, BW = 39 kg) or a casein–cornstarch (CC) diet (*n* = 4, BW = 40 kg) for 14 days, measurements of O₂ consumption by the portal-drained viscera (PDV) and the whole animal were conducted following the 16 h fast (just before the 0800 h feeding) and during the 6 h postprandial period. Compared with CC pigs, BCM pigs had higher fasting O₂ consumption by the PDV and by the whole animal. There were no differences between BCM and CC pigs in the mean 6 h postprandial O₂ consumption by the PDV and by the whole animal. Expressed as a percentage of BW, weights of the PDV and the liver but not the heart and kidneys were higher for BCM than for CC pigs.

Introduction

Endogenous gut nitrogen losses (ENL) in growing pigs are affected by diet composition. A decrease in ENL will lower metabolic cost for synthesizing endogenous gut protein and associated ENL, and thus improve the efficiency of utilizing dietary amino acids and energy for lean growth in pigs. Measurements on the energy cost associated with ENL in pigs, however, have not been performed. Nyachoti *et al.* (1997) reported that pigs fed a barley–canola meal (BCM) diet had high ENL whereas those fed a casein–cornstarch (CC) diet had very low ENL. Pigs fed BCM diet were further found to have greater weights of the colon and the liver than those fed CC diet (Nyachoti *et al.*,

2000). Porcine portal-drained viscera (PDV), including the gastrointestinal tract, spleen and pancreas, are metabolically highly active and account for 20–25% of the whole-animal O₂ consumption, although they represent only 5% of the total animal weight (Yen *et al.*, 1989; Yen, 1997).

The objective of the present study was to quantify responses in organ size and the energy expenditure of the PDV in growing pigs fed a BCM or CC diet that produces high or low ENL, respectively.

Materials and Methods

The high and low ENL diets for the present study were, respectively, the BCM

and CC diets used in our previous studies (Nyachoti *et al.*, 1997). A total of eight Yorkshire $\times$ Landrace crossbred barrows (23.2 kg initial body weight (BW)) were trained to receive their feed mixed with water (1 ml g⁻¹ of feed) twice daily (0800 and 1600 h). The daily feed allowance of the pig was $2.6 \times$ maintenance digestible energy (DE) requirement (110 kcal DE kg⁻¹ of BW^{0.75}). A feed containing 50% BCM diet and 50% CC diet was given to pigs from 7 days prior to surgery until 10–14 days after surgery. At surgery, catheters were placed in the portal vein, the ileal vein and the carotid artery of pigs as described previously (Yen and Killefer, 1987).

On days 10–14 post-surgery, pigs (31.4 kg) were placed in open-circuit calorimeters for pre-assignment measurements. Following the measurements, pigs were assigned to receive 100% BCM diet ($n = 4$) or 100% CC diet ($n = 4$) for 14 days. They were then placed into calorimeters again for post-assignment measurements. Both the pre- and post-assignment measurements were conducted following the 16 h fast (just before the 0800 feeding) and dur-

ing the 6 h postprandial period. The measurements included O₂ consumption by the whole animal and that by the PDV, as described previously (Yen *et al.*, 1989). At the time of post-assignment measurements, the BW of BCM and CC pigs were 39.0 and 40.0 kg, respectively.

Upon completion of post-assignment measurements of O₂ consumption, pigs were killed for measurements of their visceral organ size.

Results and Discussion

Table 23.1 shows that the fasting O₂ consumption by the PDV of BCM pigs was greater ($P = 0.08$) than that of CC pigs. The fasting O₂ consumption by the whole animal tended ($P = 0.16$) to be greater for BCM pigs than for CC pigs. The mean 6 h postprandial O₂ consumption by the PDV did not differ ($P = 0.74$) between BCM and CC pigs. There were also no differences ($P = 0.48$) between BCM and CC pigs in the postprandial whole-animal O₂ consumption.

When the organ weight was expressed as a percentage of BW, the weight of the

Table 23.1. Fasting and 6 h postprandial oxygen consumption by the portal-drained viscera (PDV) and by the whole animal and visceral organ weights in four growing pigs fed barley–canola meal (BCM) or casein–cornstarch (CC) diet.

Item	Diet		P value
	BCM	CC	
Average body weight (BW) (kg)	39.00	40.00	0.60
O ₂ consumption (ml min ⁻¹ kg ⁻¹ BW)			
Fasting PDV	1.40	0.90	0.08
Fasting whole-animal	7.97	5.91	0.16
Postprandial PDV	1.87	1.80	0.74
Postprandial whole-animal	8.51	9.02	0.48
Organ weight (% of BW)			
PDV	5.73	4.58	< 0.01
Stomach	0.78	0.63	< 0.05
Small intestine	2.31	2.06	0.28
Caecum	0.20	0.17	0.16
Colon + rectum	1.70	1.16	< 0.01
Pancreas	0.17	0.17	0.91
Spleen	0.56	0.39	0.30
Liver	2.90	2.35	< 0.01
Heart	0.42	0.44	0.67
Kidneys	0.52	0.51	0.86

PDV was greater ($P < 0.01$) for BCM pigs than for CC pigs. The BCM and CC pigs differed ($P < 0.05$) in weights of the stomach, the colon plus rectum and the liver but not ($P \geq 0.28$) the small intestine, the pancreas, the spleen, the heart and kidneys.

The greater weights of the colon plus rectum and the liver in BCM pigs than in CC pigs observed in the present study substantiate our previous findings (Nyachoti *et al.*, 2000). The present study also shows that the BCM pigs had increased stomach weight and a greater weight in the PDV compared with the CC pigs. As a reflection of an increased PDV weight and associated energy expenditure, the fasting O_2 con-

sumption by the PDV in BCM pigs was greater than that in CC pigs. Porcine liver is metabolically as active as the PDV, and the hepatic O_2 consumption may be as high as that by the PDV (Yen, 1997). An increased O_2 consumption by the PDV and a greater weight of the liver in BCM pigs of the present study resulted in a tendency ($P = 0.16$) for a greater fasting O_2 consumption by the whole animal compared with CC pigs.

It is concluded that feeding a diet producing high ENL to growing pigs increases their weights of the PDV and the liver as well as maintenance energy requirement. Further studies are under way to evaluate effects of feeding a variety of diets on fasting heat production in growing pigs.

References

- Nyachoti, C.M., de Lange, C.F.M. and Schulze, H. (1997) Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. *Journal of Animal Science* 75, 3206–3213.
- Nyachoti, C.M., de Lange, C.F.M., McBride, B.W., Leeson, S. and Schulze, H. (2000) Dietary influence on organ size and *in vitro* oxygen consumption by visceral organs of growing pigs. *Livestock Production Science* (in press).
- Yen, J.T. (1997) Oxygen consumption and energy flux of porcine splanchnic tissues. In: Laplace, J.P., Fevrier, C. and Barbeau, A. (eds) *Digestive Physiology in Pigs. Proceedings of the 7th International Symposium on Digestive Physiology in Pigs*. EAAP Publication No. 88, pp. 260–269.
- Yen, J.T. and Killefer, J.A. (1987) A method for chronically quantifying net absorption of nutrients and gut metabolites into hepatic portal vein in conscious swine. *Journal of Animal Science* 64, 923–934.
- Yen, J.T., Nienaber, J.A., Hill, D.A. and Pond, W.G. (1989) Oxygen consumption by portal vein-drained organs and by whole animal in conscious growing swine. *Proceedings of the Society of Experimental Biology and Medicine* 190, 393–398.

24 α -Ketoglutaric Acid Reduces Nitrogen Losses in Rats Fed Nitrogen-free Diet

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The purpose of this study was to investigate the nitrogen-sparing effect of α -ketoglutaric acid (AKG) in rats fed a nitrogen-free diet with AKG added (0, 3, 6 g kg⁻¹ of feed) for 14 days. All three diets resulted in a loss of body weight. AKG reduced N urinary excretion by 18% regardless of the dose ($P < 0.05$), whereas only 6 g AKG kg⁻¹ feed was effective in reducing blood concentration of essential amino acids by -22.2% ($P < 0.05$). Enterocyte length increased by 25 and 49% at 3 and 6 g of AKG kg⁻¹ diet, respectively ($P < 0.01$). These data further substantiate the role of AGK in modulating intestinal metabolism and nitrogen turnover.

Introduction

Glutamine, glutamine analogues, α -ketoglutaric acid (AKG) are anabolic molecules protecting muscle protein from catabolism in surgical, burned, septic and polytrauma patients, thus stabilizing the amino acids blood profile and diminishing blood urea nitrogen. The physiological role of AKG has attracted growing interest in the area of human clinical nutrition. The potential benefit of using it as feed additive is to be investigated.

Materials and Methods

Animals and diet

The research was carried out using 30 Sprague Dawley male rats weighing 138 g (± 7.26) liveweight, reared in single metabolic cages. Animals were allotted in three homogeneous groups and fed for 14 days on a nitrogen-free diet (Table 24.1) with added AKG at 0, 3 or 6 g kg⁻¹ of feed.

Animals were weighed at 0, 8 and 14 days from the beginning of the trial. The nitrogen ingesta-excreta balance was measured daily throughout 12 days of the experimental period, following 2 days of adaptation. Prior to this study, animals were fed a diet providing (g kg⁻¹) 890 of dry matter, 230 of crude protein, 41 of ether extract, 73 of crude fibre, 112 of ash, and 546 of N-free extractives. At the end of the trial, ileum mucosa was sampled from six rats per group and subjected to scanning electron microscopy (SEM) measurements. Blood amino acid (AA) concentration was determined on six rats per group. The present study was conducted in compliance with animal welfare and protection (Directive No. 86/609/EEC and Italian law Act Decreto Legislativo No. 116, issued on 27 January 1992). The research farm CERZOO, where the study was carried out, is authorized to conduct animal studies, according to Section 12 of the above Act No. 116, by the Italian Ministry of Health (Decreto Ministeriale No. 253/95-A, issued on 18 August 1995).

Table 24.1. Compositions and proximate analysis of nitrogen-free diet.

Ingredients	g kg ⁻¹	Chemical composition	g kg ⁻¹
Starch	420	Dry matter	918.2
Sucrose	420	Crude protein	0.5
Hydrogenated oil	100	Ether extract	100.0
Cod-liver oil	20	Crude fibre	20.3
Mineral premix ^a	30	Ash	26.7
Vitamin premix ^b	10	N-free extractives	770.7

^aMineral premix supplying (kg⁻¹ diet): calcium phosphate, 15 mg; sodium chloride, 2.22 mg; potassium citrate monohydrate, 6.66 mg; potassium sulphate, 1.56 mg; magnesium oxide, 0.72 mg; manganese carbonate, 0.1 mg; ferric citrate, 0.18 mg; zincum carbonate, 0.05 mg; copper carbonate, 0.01 mg; potassium iodate, 0.3 µg; sodium selenite, 0.3 µg; chromium potassium sulphate, 0.02 mg.

^bVitamin premix supplying (kg⁻¹ diet): vitamin A, 4000 IU; vitamin D₃, 1000 IU; vitamin E, 50 IU; vitamin K, 0.05 mg; thiamine, 6 mg; riboflavin, 6 mg; pyridoxine, 7 mg; cyanocobalamin, 0.01 mg; niacin, 30 mg; biotin, 0.2 mg; calcium pantothenate, 16 mg; folic acid, 2 mg.

Ingesta–excreta balance

Animals were kept in individual metabolic cages (3700MO, Tecniplast, Varese, Italy) at 22°C, 55% relative humidity, 12 h light, with free access to water and feed. The observation period was split into two phases of 6 days each. Urine was collected in a sulphuric acid solution (30 g l⁻¹) and kept at 0°C prior to analysis. The N content of faeces and urine was determined according to Kjeldahl (AOAC, 1990).

Chemical analysis of feed and blood

Feed analyses were performed according to AOAC (1990). After 12 h of fasting, rats were anaesthetized with intraperitoneal injection of 0.1 mg g⁻¹ body weight of ketamine HCl (Ketavet 50, Gellini, Latina, Italy) and heart blood was sampled with 3 ml single-use syringes (Becton Dickinson Acute Care, USA) containing lithium heparin. Samples were analysed for free AA according to Stein and Moore (1954).

Statistical analysis

Weight parameters, blood AA concentrations and intestinal morphology data were analysed by analysis of variance (ANOVA),

using the General Linear Model procedure according to SAS 6.12 (1996) methods; the differences among means of groups were performed using Student's *t*-test based on the variance derived from ANOVA. Differences were considered statistically significant at *P* < 0.05.

Results and Discussion

All three nitrogen-free diets resulted in a loss of body weight that averaged 3.33 g per head day⁻¹ and 0.93 g per head day⁻¹ during the first 8 days and last 6 days of the trial, respectively. AKG-added diets tended to result in a lower body weight loss by approximately 6–7% per day. AKG did not affect N faecal excretion, but it reduced N urinary excretion by 18.2% and 20% when included in the diet at 3 and 6 g kg⁻¹, respectively (Table 24.2), with no difference between the two doses. Conversely, AKG at 6 g kg⁻¹ diet was the only dose that significantly reduced circulating total AA (–12%; *P* < 0.05) and essential AA both in terms of blood concentration (–22%; *P* < 0.05) and in relation to total AA (–12%; *P* < 0.05; data not shown). SEM observation of the ileum showed no difference among treatments relative to mucosa thickness (0.464 ± 0.156 mm), villi height (350.413 ± 84.589

μm) and microvilli height ($0.949 \pm 0.095 \mu\text{m}$). Nevertheless, AKG linearly increased enterocyte length (EL) ($y = 1.48x + 18.23$; $r^2 = 0.9998$; where y is the EL, and x is the AKG dose), resulting in longer enterocytes by 25 and 49% at 3 and 6 g AKG kg^{-1} diet, respectively ($P < 0.01$; Fig. 24.1). These data further substantiate the role of AKG in modulating intestinal metabolism and nitrogen turnover.

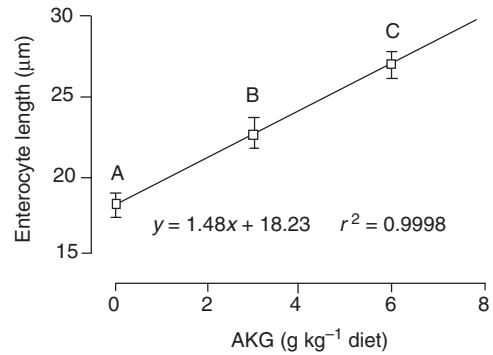


Fig. 24.1. AKG dose-dependent increase of enterocyte length (A, B, C: $P < 0.01$).

Table 24.2. Faecal and urinary endogenous nitrogen.

		Days	Control	AKG 3 g kg^{-1}	AKG 6 g kg^{-1}
Faecal N	(mg per rat)	0–6	51.40 $\pm$ 19.12	42.70 $\pm$ 21.00	36.78 $\pm$ 10.59
		7–12	46.49 $\pm$ 10.78	46.13 $\pm$ 16.68	53.94 $\pm$ 19.42
		0–12	97.89 $\pm$ 25.44	88.83 $\pm$ 26.50	90.72 $\pm$ 26.09
Urinary N	(mg per rat)	0–6	175.00 $\pm$ 32.98 ^b	141.82 $\pm$ 31.68 ^a	148.96 $\pm$ 37.74 ^{ab}
		7–12	60.35 $\pm$ 39.65 ^b	50.74 $\pm$ 39.52 ^a	49.79 $\pm$ 29.56 ^a
		0–12	235.35 $\pm$ 65.01	192.56 $\pm$ 64.99	188.79 $\pm$ 54.16
Total N	(mg per rat)	0–12	333.25 $\pm$ 77.42	281.39 $\pm$ 81.46	279.50 $\pm$ 62.98
	(mg per rat per day)	0–12	27.77 $\pm$ 6.45	23.45 $\pm$ 6.78	23.29 $\pm$ 5.25

^{ab} $P < 0.05$.

References

- AOAC (1990) *Official Methods of Analysis*, 15th edn (ed. Helrich K.). Association of Official Analytical Chemists, Arlington, Virginia.
- Stein, W.H. and Moore, S. (1954) Procedure for chromatographic determination of amino acids on four per cent cross-linked sulphonated polystyrene resins. *Journal of Biological Chemistry* 211, 893–906.

25 Dietary Supplementation of Synthetic L-Threonine Differentially Affects the Gastrointestinal Tract and Whole-body Growth in Early-weaned Piglets Fed Maize and Soybean Meal-based Diets

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Thirty-six piglets were weaned from sows at the age of 10 days, randomly allocated to six experimental diets and individually housed in floor pens for 12 days. The six diets were maize and soybean meal-based (26% crude protein from 15.6% soybean meal and 56% maize) with six levels of threonine (Thr) supplementation (0, 0.25, 0.50, 0.75, 1.00 and 1.25%). Adding (Thr) at the level of 0.25–0.75% appeared to increase weight gain and feed conversion efficiency and slightly enhanced feed intake. However, when expressed as per unit of body weight, the piglets with Thr supplementation between 0.25 and 0.75% seemed to have a relatively smaller gastrointestinal tract (GIT) to support whole-body growth more efficiently compared with the control and the diets with much higher Thr supplementation (1.00–1.25%). Plasma urea nitrogen contents paralleled the relative weights of GIT between treatment groups. In conclusion, dietary supplementation of synthetic Thr in maize and soybean meal-based diets is effective in improving growth performance and in enhancing the GIT functions in early-weaned piglets.

Introduction

The weaning of piglets is characterized by slower growth, which manifests itself symptomatically by depressed growth with frequent diarrhoea and physiologically by intestinal mucosal atrophy. Weaning removes piglets from sow's milk that is very rich in free-form trophic nutrients such as amino acids, nucleosides and polyamines. Thus, improving early-weaned piglet nutrition and decreasing

feeding cost have been a major challenge to researchers and the pig industry. Although maize and soybean meal-based starter diets are relatively economical, piglets weaned on these diets usually experience slow growth. It has been shown that about 60% of enteral threonine (Thr) is first-pass utilized by portal-drained visceral organs (Stoll *et al.*, 1997; Bertolo *et al.*, 1998). Enteral rather than systematic Thr is essential for the synthesis of protective mucin proteins for the gut mucosa

(Ball *et al.*, 1999). Enteral Thr is also essential for protein synthesis in differentiating epithelia of the small intestine, thus essential for mucosal growth. In this study, we hypothesize that piglets weaned on maize and soybean meal-based diets, formulated according to National Research Council guidelines (NRC, 1998), are deficient in available Thr supply; thus dietary supplementation of synthetic Thr may improve gut functions and improve whole-body growth in early-weaned piglets.

Materials and Methods

Thirty-six Yorkshire piglets (18 male and 18 female), weaned from sows at the age of 10 days, were randomly assigned to one of the six experimental diets and fed for an additional 12 days. The piglets were individually housed in floor pens and had free access to water and diets. The six diets were maize and soybean meal-based (26% crude protein from 15.6% soybean meal plus 56% maize) with six levels of Thr supplementation (0, 0.25, 0.50, 0.75, 1.00

and 1.25%). The surrounding temperature was adjusted to about 26°C with heating lamps. Initial and final body weights as well as feed intake were measured. At the end of feeding trials, the piglets were sacrificed to collect blood plasma samples and measure the fresh weights of other visceral organs.

Results and Discussion

Data reported in Figs 25.1 and 25.2 were mean $\pm$ SE ($n = 6$). No differences in plasma Thr content (μM) were detected ($P > 0.05$) between the level of 0.25% supplementation and the control group (Thr1 diet), suggesting that the amount of supplied Thr has been primarily used in the first pass by the visceral organs (Fig. 25.1). Dietary supplementation of Thr improved whole-body growth, feed conversion efficiency (g gain g^{-1} feed), and slightly increased feed intake (Fig. 25.1). When expressed in per unit of body weight, the gastrointestinal tract (GIT) weight was relatively lower in the diets with Thr

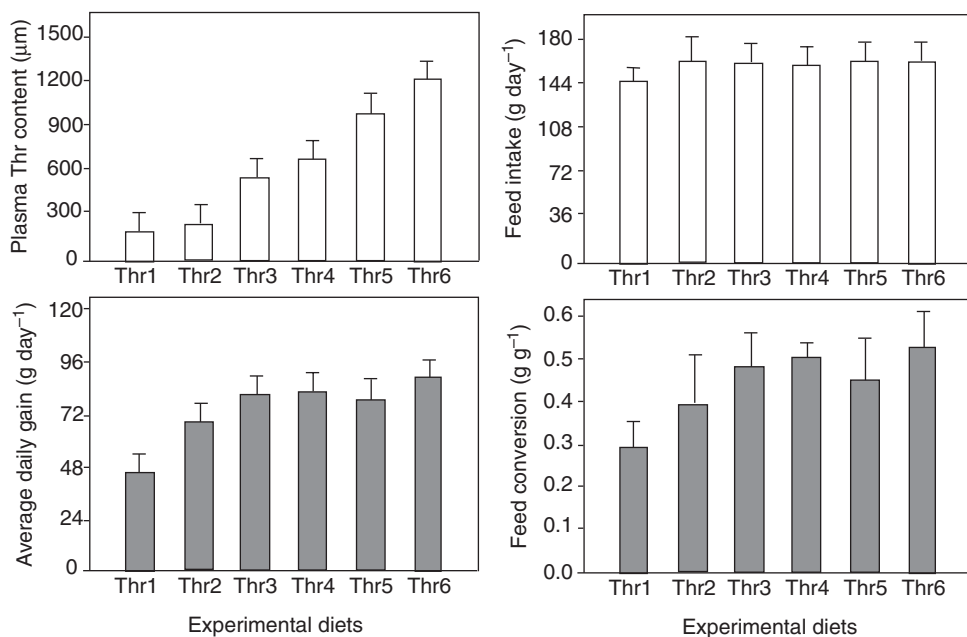


Fig. 25.1. L-Threonine improves feed intake, average daily gain and feed efficiency.

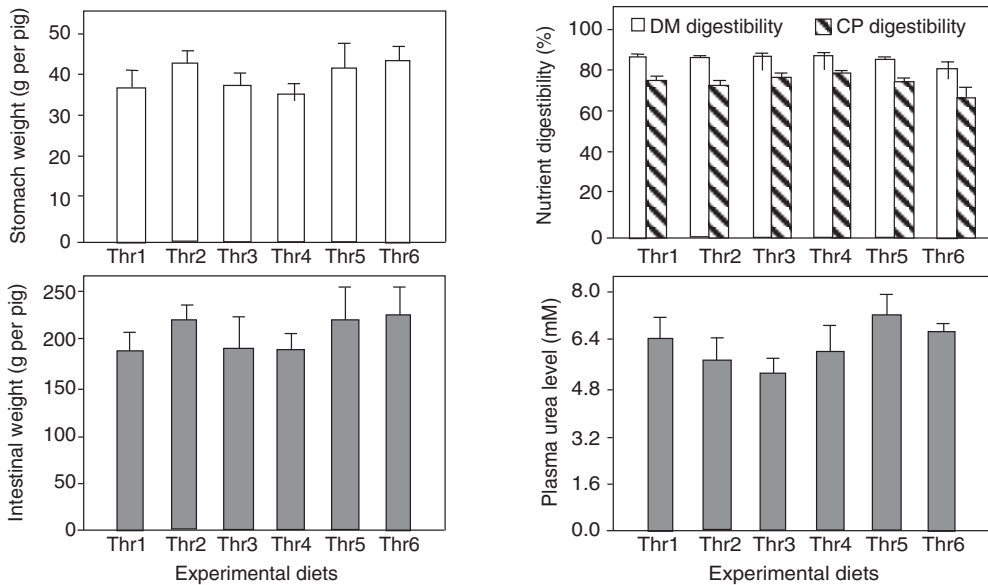


Fig. 25.2. L-Threonine affects GIT growth, nutrient digestion and protein metabolism.

contents between 0.25 and 0.75% in comparison with the control (Thr1 diet) and the higher Thr groups (1.00–1.25%) (Fig. 25.2). Plasma urea nitrogen concentrations followed a similar trend (Fig. 25.2). These results suggest that dietary supplementation of Thr at the levels of 0.25–0.75% is beneficial to improve growth performance and likely the GIT functions. In summary, weaning of piglets between 10 and 22 days on maize and soybean meal-based diets

likely triggered enhanced demand for Thr (1.25–1.80%), which is much higher than the NRC (1998) recommended level (0.98% total Thr). Dietary supplementation of synthetic Thr (0.25–0.75%) to maize and soybean meal-based diets likely improves growth, feed conversion efficiency and the gastrointestinal functions. We are still repeating the current study to increase the number of observations in order to draw firm conclusions.

References

- Ball, R.O., Law, G., Bertolo, R.F.P. and Pencharz, P.B. (1999) Adequate oral threonine is critical for mucin production and mucosal growth by the neonatal piglet gut. In: Lobley, G.E., White, A. and MacRae, J.C. (eds) *Protein Metabolism and Nutrition – Book of Abstracts of the VIIIth International Symposium on Protein Metabolism and Nutrition*. Aberdeen, UK, p. 31.
- Bertolo, R.F.P., Chen, C.Z.L., Law, G., Pencharz, P.B. and Ball, R.O. (1998) Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. *Journal of Nutrition* 128, 1752–1759.
- NRC (1998) *Nutrient Requirements for Swine*, 10th edn. National Academy of Sciences, Washington, DC.
- Stoll, B., Henry, J., Reeds, P.J., Yu, H., Jahoor, F. and Burrin, D.G. (1998) Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *Journal of Nutrition* 128, 606–614.

Part IV

Digestive Processes

26 Intestinal Degradation of Dietary Carbohydrates – from Birth to Maturity

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Dietary carbohydrates constitute a major fraction of the diet for pigs and consist of mono-, di- and oligosaccharides and two broad classes of polysaccharides – starch and non-starch polysaccharides (NSP). The activity of the various carbohydrate-degrading enzymes adapts to the age of the animal and to changes in dietary composition. This ensures in most cases an efficient pre-caecal digestion of lactose in the suckling period and of sucrose and starch after weaning. The diverse population of anaerobic bacteria in the large intestine ferments carbohydrates (mostly NSP) that are not degraded in the small intestine. Factors that influence the breakdown of NSP in the large intestine include the source of NSP, solubility, degree of lignification, level of inclusion in the diet, transit time, age and weight of the animal and microbial composition. The large intestine grows progressively larger than the small intestine in older animals, which enables sows to have a higher capacity than growing pigs to degrade NSP components.

Introduction

Dietary carbohydrates provided as lactose in sow's milk and mono-, di-, oligo- and polysaccharides in solid feeds constitute a major fraction of diets for pigs. It is now clear that dietary carbohydrates are a diverse group of substances with varied fates in the intestinal tract and varied physiological properties. Starch and disaccharides are mainly broken down by a combination of salivary, pancreatic and mucosal enzymes in the small intestine, with the end products (glucose, galactose and fructose) absorbed to the portal vein. In contrast, no enzymes are present in the small intestine of pigs to cleave the bondings in some oligosaccharides and non-starch polysaccharides (NSP). These carbohydrates, along with some starch

(resistant starch, RS), however, can be broken down by microbial fermentation primarily in the large intestine (caecum and colon). The principal end products of microbial fermentation of carbohydrates are short-chain fatty acids (SCFA) and lactic acids (LA), which are also absorbed to the host.

The composition of carbohydrates ingested by pigs from birth to maturity varies widely: the carbohydrates in sow's milk are mainly lactose, while diets provided for growing pigs and adult sows have a far more complex composition in terms of chemical structure and organization. Consequently, the pig's intestinal tract has to adapt its morphological structure, digestive enzyme activity and microbial hydrolytic activity in response to these dietary changes. The main purpose of this

paper is to give an overview of the dietary carbohydrates present in the pig diet and of the intestinal degradation of carbohydrate components from birth to maturity.

Classification of Feed Carbohydrates

In the classical feedstuff analysis according to Weende, the carbohydrate fraction is characterized by the content of crude fibre and nitrogen-free extracts (NFE), the latter being calculated as the difference between 100 and the percentage of ash, protein ($N \times 6.25$), fat and crude fibre. With modern analytical techniques, the carbohydrates can be classified more specifically based on their chemical constituents (Theander *et al.*, 1989; Bach Knudsen, 1997). In the present paper a chemical classification is used according to degree of polymerization (DP), identity of constituent sugars, and type(s) of glycosidic linkages. The sugar fraction (DP 1–2) constitutes mono- and disaccharides. Lactose is the major carbohydrate component of sow's milk and in dairy by-products (whey), while sucrose is the most abundant sugar in plant products. The content of monosaccharides is usually low (Bach Knudsen, 1997). Oligosaccharides (DP 3–10) are composed of three to ten monomers linked together and are present in seeds and by-products of many legumes, mallow, composite and mustard species (Bach Knudsen, 1997). Polysaccharide (DP > 10) starch is the most abundant carbohydrate in most plant materials, particularly cereals (Åman and Hesselman, 1984; Bach Knudsen, 1997). Starch is a mixture of the linear $\alpha(1-4)$ -linked amylose and the branched $\alpha(1-4), (1-6)$ -linked amylopectin (Gallant *et al.*, 1992). The two α -glucans are present in various proportions within plant cells as discrete granules and with a size and form characteristic for the individual plant species. The starch can further be divided into types A, B or C starch, according to its X-ray diffraction pattern. Starch of type A is present in cereals and has in general an open structure, while type B starch is more compact and found in tubers, e.g. potato (Biliaderis, 1991). Starch

of type C is a combination of A and B starch and is present in peas and beans. The plant cell walls consist of a series of NSP often associated and/or substituted with proteins and phenolic compounds, in some cells together with the phenolic polymer lignin (McDougall *et al.*, 1996). The main polysaccharides of the plant cell walls are cellulose, arabinoxylans, mixed linked β -glucan, xyloglucans, rhamnogalacturonans and arabinogalactans, to mention the major ones (Selvendran, 1984; Theander *et al.*, 1989). Some plant materials may also contain intracellular NSP, but their occurrence is low, with the exception of fructans in Jerusalem artichoke and mannans in palm and coconut cake.

Lignin is not a carbohydrate but is tightly associated to cell wall polysaccharides. Lignin can be described as a very branched network built up by phenylpropane units and is probably partly linked to cell wall hemicellulose polysaccharides (Liyama *et al.*, 1994). Lignification serves two main functions. It cements and anchors the cellulose microfibrils and other matrix polysaccharides and in this way stiffens the walls. The term dietary fibre (DF) is used in most recent animal literature for cell wall and storage NSP and lignin and can be measured by either enzymatic-gravimetric or enzymatic-chemical methods (Southgate, 1995; Bach Knudsen *et al.*, 1997). In older literature it is also common to use crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest, 1985). Although these older gravimetric methods underestimate the fibre content to various extents, they have proved valuable as an index for the digestibility of energy (Just *et al.*, 1984).

Development of the Gastrointestinal Tract in Response to Age and Feeding

The dimension of the gastrointestinal tract, like other organs, changes with development (age). This is seen in a study by McCance (1974), who measured the effect of age on the weight and length of pigs intes-

tine from 0 to 1200 days of life. In the early weeks of life there was a much more rapid growth in the length of the small intestine; as the animals get older, the large intestine grows progressively more than the small intestine. These differences may be explained on a functional basis: the diet of the piglets during suckling is very nutritious and provides no residue; as the animals get older, an increased amount of fibre provides the stimulus for the large intestine.

The weaning process is known to be associated with gross changes in intestinal structure and function. It is generally found that the villus length decreases and crypt depth increases in weaned as compared with suckling pigs, and the feeding of different levels of creep feed does not appear to influence intestinal morphology (Hampson and Kidder, 1986; Miller *et al.*, 1986; Kelly, 1990). Kelly (1990) suggested that the morphological and biochemical changes in the small intestine in pigs at weaning are directly attributable to precocious maturation of the gut as a result of withdrawal of intrinsic factors in sow's milk. Other factors that have been suggested as causing morphological and biochemical changes in the period around weaning are poor feed consumption, inflammatory reactions in response to bacterial metabolites, influence of rotavirus and hypersensitivity to antigenic components of the diet (Kenworthy, 1976; Hampson and Kidder, 1986).

Distinct growth-stage and region-related differences in the epithelial structure and proliferation of caecum and colonic tissues (Brunsgaard, 1997) have also been observed. Around the time of weaning, the large intestine undergoes morphological changes manifested in an increase in crypt size and a decrease in proliferative activity (Brunsgaard, 1997), presumably reflecting a decrease in stimuli of luminal origin, i.e. SCFA. In the period up to 4 months after birth, an increase in crypt height and proliferative activity has been observed (Brunsgaard, 1997).

The carbohydrate composition has a pronounced impact on the intestinal tract. The physicochemical properties of the fibre,

such as water-binding capacity and viscosity, exert diverse physiological actions along the gastrointestinal tract. Physicochemically induced changes in the luminal environment are presumably responsible for altered intestinal cell turnover, and morphology in the small intestine of growing pigs fed high-fibre diets (Jin *et al.*, 1994). Increased intestinal cell turnover, longer length and higher weight have also been observed in the large intestine of growing pigs fed on high-fibre diets (Jin *et al.*, 1994; Jørgensen *et al.*, 1996). It was further found that the feed particle size affects the mucosal architecture, epithelial cell proliferation and the production and composition of the mucins in the large intestine of pigs (Brunsgaard, 1998).

Development of Carbohydrase Activity

The transport processes in the intestinal enterocyte cannot accommodate anything larger than monosaccharides; thus dietary carbohydrates, which are mainly present as polymers, have to be degraded to low molecular weight compounds. For disaccharides and starch this is done in a process that involves the α -amylase secreted in salivary and pancreatic juice, and various oligosaccharidases, sucrase and lactase present on the intestinal brush border (Kidder and Manners, 1980; Gray, 1992). The group of oligosaccharidases includes: glucoamylase, which is capable of removing single glucose residues from the non-reducing end of the α -(1–4) chain; sucrase, which shortens α -(1–4)-linked oligosaccharides, particularly maltotriose and maltose; and α -dextrinase, which has the capacity to cleave the non-reducing terminal α -(1–6)-link from amylopectin once it becomes uncovered (Gray, 1992). The activity of α -amylase is low at birth but increases rapidly, particularly after about 4 weeks of age (Corring *et al.*, 1978). Hartman *et al.* (1961) reported that pancreatic α -amylase increased similarly with age in both unweaned and weaned piglets and piglets weaned at 1 week of age. Pigs weaned at 35 days of age showed a sharp increase in α -amylase activity when the diet was changed from milk to a high starch diet.

In contrast, α -amylase activity in saliva has been found to be low in both newborn and older pigs. Moreover, salivary α -amylase is acid labile and is rapidly degraded in the stomach; it therefore contributes insignificantly to the degradation of starch. Maltase and glucoamylase activities are low at birth (Dahlquist, 1961; Hartman *et al.*, 1961) but in addition to the age-related increase in activity there also appears to be a substrate-induced increase in activity that is superimposed on the age-related increase in activity (McCracken, 1984; McCracken and Kelly, 1984; Kelly *et al.*, 1991). Kidder and Manners (1980) reported an increase in glucoamylase activity up to 200 days of age and then a levelling off.

In most mammals, including the pig, lactase activity in the small intestine is high at birth and begins to decrease from about 1 week of age (Manners and Stevens, 1972; Kidder and Manners, 1980). However, as the animals get older, the variability in lactase activity is much higher than that of other carbohydrases (Kidder and Manners, 1980). Several studies with piglets have shown that the activity of sucrase increases with the age of the animals (Walker, 1959; Dahlquist, 1961; Manners and Stevens, 1972). Walker (1959) and Manners and Stevens (1972) found an almost linear increase in sucrase activity from birth to 3 weeks of age. Similarly, the relative digestive capacity for sucrose was significantly linear correlated ($y = 2.11 + 6.09x$, $r^2 0.983$) with the age of the animals in the period 2–15 days of age (Bird and Hartmann, 1996). Moreover, Kidder and Manners (1980) reported a close relationship between sucrase activity and the log age of pigs covering the period from 19 days to 7.5 years of age.

Digestion of Carbohydrates

Sugars

Lactose is the principal carbohydrate constituent of the sow's milk. Although the intestinal lactase activity falls rapidly during the first 2 weeks and then more gradu-

ally to reach adult level by 8 weeks of age, there is no indication of lactose malabsorption in piglets up to 18 days of age (Bird *et al.*, 1995). This is in contrast to older pigs, which have a limited capacity to digest lactose, as revealed by studies of ileo-cannulated pigs (Oksbjerg *et al.*, 1988) and catheterized pigs (Rérat *et al.*, 1984). Hydrolysed lactose, however, was absorbed better but there was, at the same time, an increased excretion of galactose in urine (Oksbjerg *et al.*, 1988). Accordingly, the limiting factor probably involves the hydrolysis of lactose in the small intestine. Fructose, which is absorbed from the small intestine by passive diffusion, is also to a limited extent absorbed from the small intestine (Ly, 1992). The capacity to digest sucrose and absorb glucose is high (Ly, 1992); these two sugars are digested and absorbed more rapidly than starch (Rérat *et al.*, 1984).

Oligosaccharides

The intestinal mucosa of pigs lacks the enzymes capable of cleaving a number of oligosaccharides that are naturally present in plant materials (i.e. raffinose oligosaccharides, fructo-oligosaccharides) or used as feed additives (i.e. neosugar, transgalacto-oligosaccharides). Oligosaccharides were earlier considered as an antinutritional factor, which potentially could accumulate in the small intestine, cause osmotic diarrhoea and, because of their rapid fermentation and high gas production (Krause *et al.*, 1994), cause discomfort for the animals. However, studies with raffinose oligosaccharides (from soybean, lupin and peas), fructo-oligosaccharides and transgalacto-oligosaccharides all show a high pre-caecal digestibility (Gdala *et al.*, 1994; Canibe and Bach Knudsen, 1997b; Gdala *et al.*, 1997; Houdijk *et al.*, 1999), with only trace levels being detected beyond the caecum (Gdala *et al.*, 1997). There has been a growing interest in oligosaccharides because of their possibly prebiotic properties, i.e. stimulation of the growth and/or activity of one or a limited

number of bacteria in the colon and, thus, improved host health (Houdijk, 1998; Nemcová *et al.*, 1999). Information on the beneficial effects of oligosaccharides in pigs is limited.

Starch

Salivary and pancreatic amylase activity is almost negligible at birth but increases rapidly during the first 3–5 weeks. These conditions limit the digestion of starch in young animals and raw starch was found to be less well digested and more slowly absorbed than glucose, maltose and a soluble starch preparation (Cunnigham, 1959). However, the total tract digestibility of starch was not influenced by the type of starch ingested (Cunnigham, 1959) and various thermal processes (irradiation or extrusion) have shown inconsistent results in terms of improved growth rates and total tract digestibility in piglets weaned at 10–21 days of age (Van der Poel *et al.*, 1989; Hongtrakul *et al.*, 1998).

The site of starch degradation has been investigated using both slaughter technique and pigs fitted with cannulas at various points of the small intestine. In a study involving pigs with re-entrant cannulas placed in the duodenum, jejunum or ileum, it was found that up to 50% of the overall absorption of carbohydrates and more than half the overall absorption of total reducing substances and glucose occurred in the small intestine, anterior to the mid-jejunum (Sambrook, 1979). In studies with double re-entrant cannulated pigs, Johansen and Bach Knudsen (1994b) found that 17–25% of starch in oat flour and oat bran was absorbed 2.5 m distal to the pylorus and 24–37% was absorbed 4.6 m distal to the pylorus (Johansen and Bach Knudsen, 1994a). Noah *et al.* (1999) studied the digestion of native and pregelatinized maize starch in the proximal intestine and found that for both starch preparations approximately 25% of the ingested starch was assimilated above the duodenal cannula (positioned 75 cm beyond the pylorus). Similarly, Bach

Knudsen *et al.* (1993a), using slaughter technique, found that 64% of an oat flour-based diet and 49% of an oat bran-based diet disappeared in the proximal third of the small intestine.

The open structure of cereal starch enables easy access to hydrolysis by α -amylases and most studies with ileo-cannulated pigs show that most of the starch has been absorbed by the time the digesta reaches the end of the small intestine. On average, the digestibility of starch from 38 trials (Just *et al.*, 1985; Graham *et al.*, 1986a,b, 1989; Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.*, 1993a,b; Jørgensen *et al.*, 1996, 1997; Gdala *et al.*, 1997; Pettersson and Lindberg, 1997), in which the pigs were fed raw cereals, was 96.2% (range 83.7–100%). Heat treatment, baking or extrusion cooking will alter the native structure of starch and make the starch from cereal even more digestible. Thus extrusion cooking increased the digestibility of starch in barley from 83.7% to 96.9% (Fadel *et al.*, 1988), baking increased the digestibility of starch from 94.8% to 96.9–98.6% (Fadel *et al.*, 1989) and breads made from wheat, rye and oats have all demonstrated starch digestibility above 98% (Glitsø *et al.*, 1998; Bach Knudsen and Canibe, 2000). A factor that may reduce the digestibility of starch in the small intestine is the particle size of the feed, as coarse particles encapsulate intracellular nutrients and thereby withhold the content from digestion by endogenous enzymes. This was demonstrated by Livesey *et al.* (1995), who recovered 16.5% from flaked barley and 0.9% from milled barley in the ileal effluent of ileostomy subjects. Studies performed at our institute confirm the results from humans that coarse particles reduce the digestibility of starch in the small intestine of pigs.

Types B and C starch are usually less digestible than cereal starch. For instance, substitution of cereal by pea starch generally reduces the digestibility of starch by 0.8–3.3% (abs.) (Graham and Åman, 1987; Gdala *et al.*, 1997). In studies with pea starch, the digestibility of dried pea starch was found to be 88.9–92.9% and of toasted

starch 85.7–94.2% (Canibe and Bach Knudsen, 1997b). The reasons for the relatively low digestibility of pea starch are: the entrapment of starch in fibrous thick-walled cells (Würsch *et al.*, 1986); the crystal structure of the C pattern pea starch granules (Gallant *et al.*, 1992); and the relatively high amylose:amylopectin ratio (Ring *et al.*, 1988). Even lower digestibilities of starch are found for raw type B tuber starch (e.g. potato). Just *et al.* (1985) found that the ileal apparent digestibility of starch and sugars (soluble carbohydrates) was much lower after substitution of cereal starch by raw potato starch. Raw potato starch, however, is only occasionally used in practical pig production.

Non-starch polysaccharides (fibre)

Although there are no endogenous enzymes secreted to the stomach and small intestine or present at the intestinal brush border that are able to hydrolyse the glycosidic linkages in NSP, some disappearance occurs in the upper intestinal tract. This degradation is caused by the microflora colonizing these sites of the gastrointestinal tract. Jensen and Jørgensen (1994) reported a gradual increase in total anaerobic bacteria from 10^7 to 10^9 viable counts in stomach to 10^9 viable counts in distal small intestine. Significant levels of LA and SCFA have also been reported in digesta collected from the stomach and various sites of the small intestine (Argenzio and Southworth, 1974; Bach Knudsen *et al.*, 1991). The organic acids present at these sites of the gastrointestinal tract derive not only from fermentation of NSP but also from degradation of other carbohydrates – sugars, oligosaccharides and starch.

Based on results reported from 51 digestibility trials using ileo-cannulated pigs, the average digestibility of NSP up to the end of the small intestine was found to be 24%, with large variations between experiments (range –10 to +62%) (Graham *et al.*, 1986a,b, 1989; Graham and Åman, 1987; Fadel *et al.*, 1988, 1989; Bach

Knudsen and Hansen, 1991; Abrahamsson *et al.*, 1993; Bach Knudsen *et al.*, 1993a,b; Jørgensen *et al.*, 1996; Canibe and Bach Knudsen, 1997b; Gdala *et al.*, 1997; Glitsø *et al.*, 1998; Bach Knudsen and Canibe, 2000). The large variation in digestibility of NSP at this site of the gastrointestinal tract is certainly not only influenced by the type of NSP; technical factors (feeding level, sampling technique, marker type, etc.) are undoubtedly also partly responsible for the big variation.

The results obtained with cereal diets consistently show a higher digestibility of the linear and relatively soluble β -glucan with values in the range 17–73% in oats (Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.*, 1993a,b) and 70–97% in barley (Graham *et al.*, 1986b, 1989; Fadel *et al.*, 1988, 1989). In contrast, branched-chain arabinoxylans from wheat, rye and oats are more or less quantitatively recovered in the ileal effluents with digestibility values ranging from –10 to 12% in wheat products (Bach Knudsen and Hansen, 1991), –8 to 11% in oat products (Bach Knudsen and Hansen, 1991; Bach Knudsen *et al.*, 1993a,b; Bach Knudsen and Canibe, 2000) and –10 to 19% in rye products (Glitsø *et al.*, 1998). In barley-based diets, the digestibility of arabinoxylans varied from –7 to 51% and cellulose from 21 to 50% (Graham *et al.*, 1986b, 1989; Fadel *et al.*, 1988, 1989). The digestibility of pectic polysaccharides may also be expected to be high, as judged from the high loss anterior to the ileum of uronic acids and galactose in swede (Millard and Chesson, 1984) and uronic acids and arabinose in peas (Canibe and Bach Knudsen, 1997b). However, other studies have found a low digestibility of pectic polysaccharides (Jørgensen *et al.*, 1996).

The main site for NSP degradation is the large intestine. At this site of the gastrointestinal tract, digesta is retained for prolonged periods of time (generally 20–40 h), which allows prolific bacterial growth: 10^{10} – 10^{11} viable counts g^{-1} fresh materials. The most common isolates in an extensive study of pig faecal bacteria by Moore *et al.* (1987) were in the *Streptococcus* genus,

representing 27.5% of all isolates. *Lactobacillus*, *Fusobacterium*, *Eubacterium*, *Bacteroides* and *Peptostreptococcus* were the next most common bacteria. The outcome of fermentation of NSP and other carbohydrates in the large intestine is production of SCFA, mainly acetate, propionate and butyrate, and the gases H_2 , CO_2 and CH_4 . When the dietary level of NSP is raised, more substrates enter the large intestine, giving rise to a higher activity of the microflora (Bach Knudsen *et al.*, 1991; Jensen and Jørgensen, 1994), increased production of SCFA (Giusi-Perier *et al.*, 1989) and increased production of gases (Jensen and Jørgensen, 1994). The SCFA are rapidly absorbed from the large intestine and may provide up to 24% of the maintenance energy requirement for growing pigs (Yen *et al.*, 1991) and potentially even more for adults.

The most important factors influencing total tract digestibility of NSP include the source of NSP, solubility, degree of lignification, the level of inclusion in the diet, transit time, the age and weight of the animal and the microbial composition. The importance of the source of NSP as a factor determining its total tract digestibility is documented in several studies with cereals, legumes and forage. In these studies the total tract digestibility of cellulose has been found to vary 23–65% in whole grain barley (Graham *et al.*, 1986b, 1989; Fadel *et al.*, 1989), 24–60% in whole-grain wheat and wheat fractions (Bach Knudsen and Hansen, 1991; Goodlad and Mathers, 1991; Bach Knudsen and Canibe, 2000), 10–84% in whole-grain rye and rye fractions (Glitsø *et al.*, 1998), 78–83% in rolled oats and oat bran (Bach Knudsen *et al.*, 1991), 13–42% in bran and hulls of wheat, maize and oats (Stanogias and Pearce, 1985), 2–84% in hulls of soybean, lupins and peas (Stanogias and Pearce, 1985) and 21–47% in forage (Keys and DeBarthe, 1974). Solubility is another factor that clearly influences the degradability of NSP. Swelling and high water-binding capacity of soluble NSP result in an increased surface area, which encourages easy colonization of the residue. These conditions are

responsible for the ready degradation of soluble NSP. Thus, the bulk of β -glucan in oats (Bach Knudsen *et al.*, 1993a), pectic polysaccharides in peas and sugarbeet pulp (Canibe and Bach Knudsen, 1997a; Petkevicius *et al.*, 1997) and soluble arabinoxylans from rye (Glitsø *et al.*, 1999) are broken down already in the caecum and proximal colon. Other cell wall polysaccharides, although not measured analytically as soluble, may also be extensively degraded during passage through the large intestine. For instance, a linear xylan present in the aleurone cells of rye was found to be slowly but completely fermented (Glitsø *et al.*, 1999). Moreover, it is a common feature that the hemicellulose and non-cellulosic polysaccharides are, with few exceptions, better digested than cellulose.

The role of lignin as a factor limiting the degradation of NSP is documented in several studies. Thus, the digestibility of cellulose and arabinoxylans is much higher for cell wall materials from non-lignified materials (wheat flour, rolled oats, rye flour, oat bran, sugarbeet pulp) than from lignified materials (pericarp/testa from rye or wheat, wheat bran or oat hulls) (Bach Knudsen and Hansen, 1991; Petkevicius *et al.*, 1997; Glitsø *et al.*, 1998). The digestibility of cellulose and hemicellulose is also clearly lower in lignified hulls and bran of wheat, oats and maize than non-lignified hulls of lupin and soybean (Stanogias and Pearce, 1985). Moreover, because of the close association of polysaccharides and lignin the whole polysaccharide–lignin complex becomes very insoluble and the main cell wall polysaccharides are virtually degraded to the same degree, in contrast to non-lignified materials, where cellulose is less well digested compared with hemicellulose polysaccharides (Bach Knudsen and Hansen, 1991; Glitsø *et al.*, 1998).

The impacts of the inclusion of cell walls on the digestibility of fibre components have given conflicting results. The level of fibre inclusion was found to depress the digestibility of cellulose (Farrell and Johnson, 1970) and NSP (Longland *et al.*, 1993), while Stanogias and Pearce

(1985) failed to demonstrate any consistent relationship between the fibre level and the digestibility of its own components.

The length of time digesta remains in the digestive tract of the animal and exposed to digestive enzymes and microbial degradation largely influences the extent to which the feeds are digested. In 46 species of herbivorous mammals fed grass-type fibre, Van Soest (1985) reported a linear relationship between mean retention time and the digestibility of cellulose. However, although Stanogias and Pearce (1985) showed reduced transit time in response to increased fibre inclusion, it was concluded that there was no strong association between transit time and the total tract digestibility of NDF.

Several factors favour a more extensive degradation of fibrous components in sows or adult animals compared with piglets and growing pigs. Adult animals usually have a lower feed intake per unit of body weight, a slower digesta transit, a greater intestinal volume and a higher cellulolytic activity. Varel (1987) reported that adult animals had 6.7 times more cellulolytic bacteria in the colon than growing pigs and trials have shown a generally higher digestibility of crude fibre and higher content of metabolizable energy in sows than in growing pigs (Fernández *et al.*, 1986; Shi and Noblet, 1993). The most significant differences between the two groups of animals were seen for some cereal by-products and most roughage (Fernández *et al.*, 1986). In a study by Jørgensen *et al.* (1978) using three diets with increasing levels of insoluble fibre fed to pigs weighing 20, 90 and 225 kg, respectively, an increased total tract digestibility of energy in response to age was seen for the medium and high-fibre diets but not for the diet with a normal level of fibre. Noblet and Bach Knudsen (1997) also found that the digestibility of cellulose and non-cellulosic polysaccha-

rides was consistently higher in sows than in growing pigs. A recent study indicated an improved utilization of the fibrous diets in pregnant sows in the range of 25% (Olesen, 1999).

A long-term effect of feeding high-fibre diets may also be seen. Longland *et al.* (1993) concluded that pigs fed high-fibre diets may adapt to the diets in terms of N and energy balance after 1 week, but 3–5 weeks may be necessary before adapting to the digestibility of resistant NSP residues. This is supported by data on the number of cellulolytic bacteria in the colon of growing pigs and adult animals. In a study where four diets were fed – a control diet and diets with increasing levels of fibre deriving from either 20% maize cobs, 40% lucerne or 96% lucerne, the number of cellulolytic bacteria increased by 200% relative to the control diet when diets containing 40 and 96% lucerne were fed (Varel and Pond, 1985). In contrast, there was no effect on the level of cellulolytic bacteria when a 20% maize-cob diet was fed. Overall, the level of cellulolytic bacteria may represent 10% of the culturable flora when high-fibre diets are fed.

Implication

The intestinal tract of pigs has a high capacity to adapt its morphological structure and enzymatic and microbial activity to variation in dietary carbohydrate composition from birth to maturity. This ensures in most cases an efficient digestion in the small intestine of lactose in the suckling period and of sucrose and starch after weaning. As the animal gets older, the large intestine grows progressively more than the small intestine, enabling sows to have a higher capacity than growing pigs to degrade fibre components.

References

- Abrahamsson, M., Graham, H., Dandanell Daveby, Y. and Åman, P. (1993) Ileal and faecal digestibility of light- or darkcoloured peas (*Pisum sativum*) in growing pigs. *Animal Feed Science and Technology* 42, 15–24.

- Åman, P. and Hesselman, K. (1984) Analysis of starch and other main constituents of cereal grains. *Swedish Journal of Agricultural Research* 14, 135–139.
- Argenzio, R.A. and Southworth, M. (1974) Sites of organic acid production and absorption in gastrointestinal tract of the pig. *American Journal of Physiology* 228, 454–460.
- Bach Knudsen, K.E. (1997) Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology* 67, 319–338.
- Bach Knudsen, K.E. and Canibe, N. (2000) Breakdown of plant carbohydrates in the digestive tract of pigs fed on wheat or oat based rolls. *Journal of the Science of Food and Agriculture* 80, 1253–1261.
- Bach Knudsen, K.E. and Hansen, I. (1991) Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. *British Journal of Nutrition* 65, 217–232.
- Bach Knudsen, K.E., Jensen, B.B., Andersen, J.O. and Hansen, I. (1991) Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *British Journal of Nutrition* 65, 233–248.
- Bach Knudsen, K.E., Jensen, B.B. and Hansen, I. (1993a) Digestion of polysaccharides and other major components in the small and large intestine of pigs fed diets consisting of oat fractions rich in β -D-glucan. *British Journal of Nutrition* 70, 537–556.
- Bach Knudsen, K.E., Jensen, B.B. and Hansen, I. (1993b) Oat bran but not a β -glucan-enriched oat fraction enhances butyrate production in the large intestine of pigs. *Journal of Nutrition* 123, 1235–1247.
- Bach Knudsen, K.E., Johansen, H.N. and Glitsø, V. (1997) Methods for analysis of dietary fibre – advantage and limitations. *Journal of Animal and Feed Sciences* 6, 185–206.
- Biliaderis, C.G. (1991) The structure and interactions of starch with food constituents. *Canadian Journal Physiology* 69, 60–78.
- Bird, P.H. and Hartmann, P.E. (1996) Changes in the concentration of fructose in the blood of piglets of different ages after doses of fructose, fructose plus glucose and sucrose. *British Journal of Nutrition* 76, 399–407.
- Bird, P.H., Atwood, C.S. and Hartmann, P.E. (1995) The responses of blood galactose to oral doses of lactose, galactose plus glucose and milk to piglets. *British Journal of Nutrition* 73, 753–761.
- Brunsgaard, G. (1997) Morphological characteristics, epithelial cell proliferation, and crypt fission in caecum and colon of growing pigs. *Digestive Diseases and Science* 42, 2384–2393.
- Brunsgaard, G. (1998) Effects of cereal type and feed particle size on morphological characteristics, epithelial cell proliferation, and lectin binding patterns in the large intestine of pigs. *Journal of Animal Science* 76, 2787–2798.
- Canibe, N. and Bach Knudsen, K.E. (1997a) Apparent digestibility of non-starch polysaccharides and short-chain fatty acids production in the large intestine of pigs fed dried or toasted peas. *Acta Agriculturae Scandinavica* 47, 106–116.
- Canibe, N. and Bach Knudsen, K.E. (1997b) Digestibility of dried and toasted peas in pigs. 1. Ileal and faecal digestibility of carbohydrates. *Animal Feed Science and Technology* 64, 293–310.
- Corring, T., Aumaitre, A. and Durand, G. (1978) Development of digestive enzymes in the piglet from birth to 8 weeks. I. Pancreas and pancreatic enzymes. *Nutrition and Metabolism* 22, 231–243.
- Cunnigham, H.M. (1959) Digestion of starch and some of its degradation products by newborn pigs. *Journal of Animal Science* 18, 964–974.
- Dahlquist, A. (1961) Intestinal carbohydrases of new-born pig. *Nature* 190, 31–32.
- Fadel, J.G., Newman, C.W., Newman, R.K. and Graham, H. (1988) Effects of extrusion cooking of barley on ileal and faecal digestibilities of dietary components in pigs. *Canadian Journal of Animal Science* 68, 891–897.
- Fadel, J.G., Newman, R.K., Newman, C.W. and Graham, H. (1989) Effects of baking hullless barley on the digestibility of dietary components as measured at the ileum and in feces in pigs. *Journal of Nutrition* 119, 722–726.
- Farrell, D.J. and Johnson, K.A. (1970) Utilization of cellulose by pigs and its effects on caecal function. *Animal Production* 14, 209–217.
- Fernández, J.A., Jørgensen, H. and Just, A. (1986) Comparative digestibility experiments with growing pigs and adult sows. *Animal Production* 43, 127–132.
- Gallant, D.J., Bouchet, B., Buléon, A. and Pérez, S. (1992) Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition* 46 (Suppl. 2), S3–S16.

- Gdala, J., Buraczewska, L., Jansman, A.M.J., Wasilewko, J. and Leeuwen, P. (1994) Ileal digestibility of amino acids and carbohydrates in lupins for young pigs. In: Souffrant, W.-B. and Hagemesiters, H. (eds) *VIIth International Symposium on Digestive Physiology in Pigs*. EAAP Publication No. 89, Bad Doberan, pp. 93–96.
- Gdala, J., Johansen, H.N., Bach Knudsen, K.E., Knap, I., Wagner, P. and Jørgensen, O.B. (1997) The digestibility of carbohydrates, protein and fat in the small and large intestine of piglets fed non-supplemented and enzyme supplemented diets. *Animal Feed Science and Technology* 65, 15–33.
- Giusi-Perier, A., Fiszlelewicz, M. and Rérat, A. (1989) Influence of diet composition on intestinal volatile fatty acids and nutrient absorption in unanesthetized pigs. *Journal of Animal Science* 67, 386–402.
- Glitsø, L.V., Brunsgaard, G., Højsgaard, S., Sandström, B. and Bach Knudsen, K.E. (1998) Intestinal degradation in pigs of rye dietary fibre with different structural characteristics. *British Journal of Nutrition* 80, 457–468.
- Glitsø, L.V., Gruppen, H., Schols, H.A., Højsgaard, S., Sandström, B. and Bach Knudsen, K.E. (1999) Degradation of rye arabinoxylans in the large intestine of pigs. *Journal of the Science of Food and Agriculture* 79, 961–969.
- Goodlad, J.S. and Mathers, J.C. (1991) Digestion by pigs of non-starch polysaccharides in wheat and raw peas (*Pisum sativum*) fed in mixed diets. *British Journal of Nutrition* 65, 270–295.
- Graham, H. and Åman, P. (1987) Whole-crop peas. II. Digestion of early- and late-harvested crops in the gastrointestinal tract of pigs. *Animal Feed Science and Technology* 17, 33–43.
- Graham, H., Hesselman, K. and Åman, P. (1986a) The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. *Journal of Nutrition* 116, 242–251.
- Graham, H., Hesselman, K., Jonsson, E. and Åman, P. (1986b) Influence of β -glucanase supplementation on digestion of a barley-based diet in the pig gastrointestinal tract. *Nutritional Reports International* 34, 1089–1096.
- Graham, H., Fadel, J.G., Newman, C.W. and Newman, R.K. (1989) Effect of pelleting and β -glucanase supplementation on the ileal and faecal digestibility of a barley-based diet in the pig. *Journal of Animal Science* 67, 1293–1298.
- Gray, G.M. (1992) Starch digestion and absorption in nonruminants. *Journal of Nutrition* 122, 172–177.
- Hampson, J. and Kidder, D.E. (1986) Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. *Research in Veterinary Science* 40, 24–31.
- Hartman, P.A., Hays, V.W., Baker, R.O., Neagle, L.H. and Catron, D.V. (1961) Digestive enzyme development in the young pig. *Journal of Animal Science* 20, 114–123.
- Hongtrakul, K., Goodbrand, R.D., Behnke, K.C., Nelssen, J.L., Tokach, M.D. and Bergström, J.R. (1998) The effects of extrusion processing of carbohydrate sources on weanling pig performance. *Journal of Animal Science* 76, 3034–3042.
- Houdijk, J. (1998) Effects of non-digestible oligosaccharides in young pig diets. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands, 184 pp.
- Houdijk, J.G.M., Bosch, M.W., Tamminga, S., Verstegen, M.W.A., Berenpas, E.B. and Knoop, H. (1999) Apparent ileal and total-tract nutrient digestion by pigs as affected by dietary nondigestible oligosaccharides. *Journal of Animal Science* 77, 148–158.
- Jensen, B.B. and Jørgensen, H. (1994) Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. *Applied and Environmental Microbiology* 60, 1897–1904.
- Jin, L., Reynolds, L.P., Redmer, D.A., Caton, J.S. and Crenshaw, J.D. (1994) Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. *Journal of Animal Science* 72, 2270–2278.
- Johansen, H.N. and Bach Knudsen, K.E. (1994a) Effects of reducing the starch content in oat-based diets with cellulose on jejunal flow and absorption of glucose over an isolated loop of jejunum in pigs. *British Journal of Nutrition* 72, 717–729.
- Johansen, H.N. and Bach Knudsen, K.E. (1994b) Effects of wheat-flour and oat mill fractions on jejunal flow, starch degradation and absorption of glucose over an isolated loop of jejunum in pigs. *British Journal of Nutrition* 72, 299–313.
- Jørgensen, H., Just, A. and Fekadu, M. (1978) Fodermidlers værdi til svin. 9. Formalingsgradens og træstofkoncentrationens indflydelse på foderets fordøjelighed hos svin af forskellig alder (vægt). *Meddelelse nr. 230 fra Statens Husdyrbrugsforsøg*, København, 4 pp.

- Jørgensen, H., Zhao, X.-Q. and Eggum, B.O. (1996) The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *British Journal of Nutrition* 75, 365–378.
- Jørgensen, H., Gabert, V.M. and Eggum, B.O. (1997) The nutritional value of high-lysine barley determined in rats, young pigs and growing pigs. *Journal of Science Food and Agriculture* 73, 287–295.
- Just, A., Jørgensen, H. and Fernández, J.A. (1984) Prediction of metabolizable energy for pigs on the basis of crude nutrients in the feeds. *Livestock Production Science* 11, 105–128.
- Just, A., Jørgensen, H. and Fernández, J.A. (1985) Correlations of protein deposition in growing female pigs to ileal and faecal digestible crude protein and amino acids. *Livestock Production Science* 12, 145–159.
- Kelly, D. (1990) Effect of creep feeding on structural and functional changes of the gut of early weaned pigs. *Research in Veterinary Science* 48, 350–356.
- Kelly, D., Smyth, J.A. and McCracken, K.J. (1991) Digestive development of the early-weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. *British Journal of Nutrition* 65, 169–180.
- Kenworthy, R. (1976) Observations on the effects of weaning in the young pig. Clinical and histopathological studies of intestinal function and morphology. *Research in Veterinary Science* 21, 69–75.
- Keys, J.E. and DeBarthe, J.V. (1974) Cellulose and hemicellulose digestibility in the stomach, small intestine and large intestine of swine. *Journal of Animal Science* 39, 53–56.
- Kidder, D.E. and Manners, M.J. (1980) The level and distribution of carbohydrases in the small intestine mucosa of pigs from three weeks of age to maturity. *British Journal of Nutrition* 43, 141–153.
- Krause, D.O., Easter, R.A. and Mackie, R.I. (1994) Fermentation of stachyose and raffinose by hind-gut bacteria of the weanling pig. *Letters in Applied Microbiology* 18, 349–352.
- Livesey, G., Wilkinson, J.A., Roe, M., Faulks, R., Clark, S., Brown, J.C., Kennedy, H. and Elia, M. (1995) Influence of the physical form of barley grain on the digestion of its starch in the human small intestine and implications for health. *American Journal of Clinical Nutrition* 61, 75–81.
- Liyama, K., Lam, T.B.-T. and Stone, B.A. (1994) Covalent cross-links in the cell wall. *Plant Physiology* 104, 315–320.
- Longland, A.C., Low, A.G., Quelch, D.B. and Bray, S.P. (1993) Adaptation to the digestion of non-starch polysaccharide in growing pigs fed on cereal or semi-purified basal diets. *British Journal of Nutrition* 70, 557–566.
- Ly, J. (1992) Studies of the digestibility of pigs fed dietary sucrose, fructose or glucose. *Archives of Animal Nutrition* 42, 1–9.
- Manners, M.J. and Stevens, J.A. (1972) Changes from birth to maturity in the pattern of distribution of lactase and sucrase activity in the mucosa of the small intestine of pigs. *British Journal of Nutrition* 28, 113–127.
- McCance, R.A. (1974) The effect of age on the weights and lengths of pigs' intestines. *Journal of Anatomy* 117, 475–479.
- McCracken, K.J. (1984) Effect of diet composition on digestive development of early-weaned pigs. *Proceedings of the Nutrition Society* 43, 109A.
- McCracken, K.J. and Kelly, D. (1984) Effect of diet and post-weaning food intake on digestive development of early-weaned pigs. *Proceedings of the Nutrition Society* 43, 110A.
- McDougall, G.J., Morrison, I.M., Stewart, D. and Hillman, J.R. (1996) Plant cell walls as dietary fibre: range, structure, processing and function. *Journal of the Science of Food and Agriculture* 70, 133–150.
- Millard, P. and Chesson, A. (1984) Modification to swede (*Brassica napus* L.) anterior to the terminal ileum of pigs: some implications for the analysis of dietary fibre. *British Journal of Nutrition* 52, 583–594.
- Miller, B.G., James, P.S., Smith, M.W. and Bourne, F.J. (1986) Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *Journal Agricultural Science Cambridge* 107, 579–589.
- Moore, W.E.C., Moore, L.V.H., Cato, E.P., Wilkins, T.D. and Kornegay, E.T. (1987) Effect of high-fiber and high-oil diets on the fecal flora of swine. *Applied and Environmental Microbiology* 53, 1638–1644.

- Nemcová, R., Bomba, A., Gancarciková, S., Herich, R. and Guba, P. (1999) Study of the effect of *Lactobacillus paracasei* and fructooligosaccharides on the faecal microflora in weaning piglets. *Berliner und Münchener Tierärztliche Wochenschrift* 112, 225–228.
- Noah, L., Lecannu, G., David, A., Kozłowski, F. and Champ, M. (1999) Digestion of starch and glycaemic response to mixed meals in pigs. *Reproduction Nutrition Development* 39, 245–254.
- Noblet, J. and Bach Knudsen, K.E. (1997) Comparative digestibility of wheat, maize and sugar beet pulp non-starch polysaccharides in adult sows and growing pigs. In: Laplace, J.P., Fevrier, C. and Barbeau, A. (eds) *Digestive Physiology in Pigs*. INRA, Saint Malo, France, pp. 571–574.
- Oksbjerg, N., Jørgensen, H., Mortensen, H.P., Fernández, J.A. and Madsen, A. (1988) The feeding value of hydrolysed permeate lactose in growing and finishing pigs. *Acta Agriculturae Scandinavica* 38, 253–260.
- Olesen, C.S. (1999) Energy metabolism and utilization of high fibre diets in pigs – fibre sources: wheat bran and sugar beet pulp. MSc thesis, The Royal Veterinary and Agricultural University, Copenhagen and Danish Institute of Agricultural Sciences, Foulum.
- Petkevicius, S., Bach Knudsen, K.E., Nansen, P., Roepstorff, A., Skjøth, F. and Jensen, K. (1997) The impact of diets varying in carbohydrates resistant to endogenous enzymes and lignin on population of *Ascaris suum* and *Oesophagostomum dentatum* in pigs. *Parasitology* 114, 555–568.
- Pettersson, Å. and Lindberg, J.E. (1997) Ileal and total tract digestibility in pigs of naked and hulled barley with different starch composition. *Animal Feed Science and Technology* 66, 97–109.
- Rérat, A.A., Vaissade, P. and Vaugelade, P. (1984) Absorption kinetics of some carbohydrates in conscious pigs. 2. Quantitative aspects. *British Journal of Nutrition* 51, 517–529.
- Ring, S.G., Gee, J.M., Whittam, M., Orford, P. and Johnson, I.T. (1988) Resistant starch: its chemical form in foodstuffs and effect on digestibility *in vitro*. *Food Chemistry* 28, 97–109.
- Sambrook, I.E. (1979) Studies on digestion and absorption in the intestines of growing pigs. 7. Measurements of the flow of total carbohydrate, total reducing substances and glucose. *British Journal of Nutrition* 42, 267–277.
- Selvendran, R.R. (1984) The plant cell wall as a source of dietary fibre: chemistry and structure. *American Journal of Clinical Nutrition* 39, 320–337.
- Shi, X.S. and Noblet, J. (1993) Digestible and metabolizable energy values of ten feed ingredients in growing pigs fed *ad libitum* and sows fed at maintenance level; comparative contribution of the hindgut. *Animal Feed Science and Technology* 42, 223–236.
- Southgate, D.A.T. (1995) *Dietary Fibre Analysis*. The Royal Society of Chemistry, Cambridge, 174 pp.
- Stanogias, G. and Pearce, G.R. (1985) The digestion of fibre by pigs. 1. The effects of amount and type of fibre on apparent digestibility, nitrogen balance and rate of passage. *British Journal of Nutrition* 53, 513–530.
- Theander, O., Westerlund, E., Åman, P. and Graham, H. (1989) Plant cell walls and monogastric diets. *Animal Feed Science and Technology* 23, 205–225.
- Van der Poel, A.F.B., den Hertog, L.A., van den Abeele, T., Boer, H. and van Zuilichem, D.J. (1989) Effect of infrared irradiation or extrusion processing of maize on its digestibility in piglets. *Animal Feed Science and Technology* 26, 29–43.
- Van Soest, P.J. (1985) Definition of fibre in animal feeds. In: Cole, D.J.A. and Haresign, W. (eds) *Recent Advances in Animal Nutrition – 1985*. Butterworths, London, pp. 55–70.
- Varel, V.H. (1987) Activity of fiber-degrading microorganisms in the pig large intestine. *Journal of Animal Science* 65, 488–496.
- Varel, V.H. and Pond, W.G. (1985) Enumeration and activity of cellulolytic bacteria from gestating swine fed various levels of dietary fibre. *Applied Environmental Microbiology* 49, 858–862.
- Walker, D.M. (1959) The development of the digestive system of the young animal. II. Carbohydrate enzyme development in the young pig. *Journal of Agricultural Science* 52, 357–363.
- Wüsch, P., Del Vedovo, S. and Koellreutter, B. (1986) Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *American Journal of Clinical Nutrition* 43, 23–29.
- Yen, J.T., Nienaber, J.A., Hill, D.A. and Pond, W.G. (1991) Potential contribution of absorbed volatile fatty acids to whole-animal energy requirement in conscious swine. *Journal of Animal Science* 69, 2001–2012.

27 Dietary Oligosaccharide Supplements: Effects on Digestion in Pigs

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Ileo-cannulated pigs weighing 21 kg were fed a semi-synthetic control diet or this diet supplemented with either 2% fructo-oligosaccharides (FOS) or 2% α -galacto-oligosaccharides (GOS). Feeding FOS and GOS did not affect apparent total-tract digestibility of dry matter; it tended to lower apparent ileal protein digestibility, but did not influence nitrogen retention. Fe and Zn, but not Ca, Mg, P and Cu, showed increased apparent ileal and total-tract digestibility. In the small intestine, oligosaccharide degradation ranged between 40 and 50%. Oligosaccharides contributed to fermentation in the distal gut and were not detected in the faeces.

Introduction

Fructo-oligosaccharides (FOS) and α -galacto-oligosaccharides (GOS) belong to the carbohydrates that are not enzymatically digested in the gut, but are fermented by the microflora. GOS have been considered as antinutritional factors since they may decrease metabolizable energy (Leske *et al.*, 1993) and cause diarrhoea. FOS have been claimed to behave as prebiotics, as they would increase acidity in the gut and positively change microbial ecology.

In the present study, GOS and FOS were tested as feed additives in young ileo-cannulated pigs with regard to the effects on protein and mineral utilization as well as intestinal fermentation.

Materials and Methods

GOS were obtained by extraction of soybeans. The dried extract consisted of 44% sucrose, 9.8% raffinose and 38.1% stachyose. FOS were obtained by hydrolysis of chicory inulin. The mixture showed

an average polymerization degree of 10. The control diet contained 16% casein, 57% maize starch, 15.2% dextrose, 2.5% maize oil, 5% cellulose, 0.3% Cr_2O_3 , 2% celite and 2% amino acid–vitamin–mineral mixture. The GOS source was included in the semi-synthetic diet at the expense of mainly celite in order to yield 0.4% raffinose and 1.6% stachyose. The FOS mixture was supplemented at a 2% level in exchange of celite. Cr_2O_3 served as a marker to measure faeces production and the amount of chyme leaving the small intestine.

The experiment was carried out with 12 castrated male ileo-cannulated pigs which were individually housed in metabolism cages. Each diet was fed *ad libitum* to four animals from surgery onward. The digestibility experiment was started 12 days later. At that time mean body weight was 21 kg. For 5 consecutive days, feed intake was measured and faeces and urine were collected. Chyme leaving the ileum was continuously collected for 3 h each day, using plastic bags connected to the cannula. Bags were replaced every hour to

minimize fermentation and were frozen immediately. At the end of the digestibility experiment the collections from each pig were pooled and a sample was freeze-dried. The animals were killed in the non-fasted state and the digesta in the proximal and distal part of the small intestine as well as in the first 10 cm of the proximal colon were sampled. The pH, NH_3 and short-chain fatty acids in fresh digesta were determined as reported earlier (De Schrijver *et al.*, 1999). Oligosaccharides in diets, freeze-dried chyme and faeces samples were analysed using high-performance anion-exchange chromatography with pulsed amperometric detection. Ca, Mg, Fe, Zn and Cu were quantified by atom-absorption spectrophotometry. Phosphorus was measured colorimetrically.

Results and Discussion

Apparent faecal digestibility of dry matter was not significantly influenced by dietary GOS and FOS supplementation. Apparent faecal protein digestibility tended ($P < 0.1$) to decrease in the GOS and FOS groups. Supplemented raffinose, stachyose and inulin were not detected in faeces samples, indicating total fermentative degradation of these carbohydrates in the gut. Total-tract digestibility of Ca, Mg, P and Cu was not significantly affected by the dietary treatments. When compared with the control group, the GOS group showed higher total-tract digestibility of Fe ($P < 0.05$) and Zn ($P < 0.1$); in the FOS group only Fe digestibility tended to increase ($P < 0.1$).

Apparent ileal digestibility of dry matter, protein, Ca, Mg, P and Cu was not significantly changed by GOS and FOS intake. Similar to total-tract digestibility, ileal digestibility of Zn and Fe was significantly ($P < 0.05$) improved or tended ($P < 0.1$) to improve when oligosaccharides were provided. A substantial degradation of the supplemented oligosaccharides in the small intestine was found, ranging between 30 and 50%.

N-retention in the control group and the GOS and FOS groups averaged 75.2%, 74.7% and 77.1% of N-intake, respectively. These results were not significantly different. Thus the slightly elevated faecal N-output in the animals fed the GOS and FOS diets was compensated by less urinary N-loss, confirming our previous results in experiments with rats and pigs fed diets containing 6% inulin (Van Hoof and De Schrijver, 1996). The increased faecal N-excretion might be caused by stimulated microbial activity when oligosaccharides are fed, resulting in more conversion of NH_3 into microbial protein. This was confirmed by the significantly lower colonic NH_3 concentrations when GOS and FOS were fed (Table 27.1).

In the proximal small intestine, chyme characteristics such as pH, NH_3 concentration and viscosity were not influenced by oligosaccharide intake (Table 27.1). In the distal part of the small intestine, lower NH_3 concentrations were found in the GOS and FOS groups ($P < 0.1$). Moreover, mainly the GOS group showed decreased intestinal pH values. Although these effects were not always statistically significant at the $P < 0.05$ level, they point to elevated fermentation in the distal small intestine caused by the oligosaccharides. This was confirmed by the measured oligosaccharide degradation in the small intestine. Concomitantly, the production of short-chain fatty acids tended to increase as shown by the elevated contents in the ileal digesta. Similar effects from consumption of GOS and FOS on short-chain fatty acid contents were observed in the colonic digesta. Moreover, lactic acid concentration was increased, especially in the GOS group. As a consequence, the degree of acidity was increased in the colonic digesta.

In conclusion, the digestibility experiments showed that 2% dietary GOS or FOS supplementation did not substantially affect protein and mineral utilization, but resulted in a significant change in intestinal fermentation.

Table 27.1. Characteristics of intestinal digesta.

	Control group	GOS group	FOS group
Proximal small intestine			
pH	6.1	6.3	6.1
NH ₃ (μmol g ⁻¹)	4.9	6.5	6.8
Viscosity (cP)	1.25	1.20	1.30
Distal small intestine			
pH	6.8	6.3*	6.5
NH ₃ (μmol g ⁻¹)	9.6	7.4*	6.7*
Viscosity, cP	1.29	1.38	1.34
Ileal digesta			
Acetate (μmol g ⁻¹)	7.8	9.1*	10.7*
Propionate (μmol g ⁻¹)	2.2	5.9*	5.5*
Butyrate (μmol g ⁻¹)	0.5	0.7	1.0
Total (μmol g ⁻¹)	10.5	15.7*	17.2*
Colonic digesta			
Acetate (μmol g ⁻¹)	88	118*	115
Propionate (μmol g ⁻¹)	30	40*	42*
Butyrate (μmol g ⁻¹)	6	19*	16*
Total (μmol g ⁻¹)	124	177*	173*
Lactic acid (μmol g ⁻¹)	0.05	0.12*	0.09
pH	5.6 ^a	5.1 ^b	5.2 ^b
NH ₃ (μmol g ⁻¹)	35.2 ^a	25.8 ^b	23.7 ^b

^{a,b}Means in the same row with a different letter are significantly different ($P < 0.05$). *Significantly different from control group at $P < 0.1$.

References

- De Schrijver, R., Vanhoof, K. and Vande Ginste, J. (1999) Effect of enzyme resistant starch on large bowel fermentation in rats and pigs. *Nutrition Research* 19, 927–936.
- Leske, K.L., Jevne, C.J. and Coon, C.N. (1993) Effect of oligosaccharide additions on nitrogen-corrected true metabolizable energy of soy protein concentrate. *Poultry Science* 72, 664–668.
- Vanhoof, K. and De Schrijver, R. (1996) Nitrogen metabolism in rats and pigs fed inulin. *Nutrition Research* 16, 1035–1039.

28 Endogenous N Contribution to Digesta of the Small Intestine of Growing Pigs Fed Semi-synthetic and Cereal-based Diets Causing Various Digesta Viscosities

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The effect of digesta viscosity on endogenous N flow in the small intestine was studied in 16 male cannulated and ¹⁵N-labelled pigs of about 24 kg body weight. Two purified diets without or with carboxymethylcellulose and two rye–wheat-based diets without or with addition of xylanase were used. Carboxymethylcellulose increased the flow of endogenous N up to 45% at the duodenum. Addition of xylanase to rye–wheat diet decreased the endogenous N flow to a small extent.

Introduction

Increased contents of soluble non-starch polysaccharides impair the apparent ileal digestibility of protein and amino acids in pigs (Mosenthin *et al.*, 1994; Van Barneveld, 1999). No reports have been published yet on the effect of digesta viscosity on endogenous N in digesta of the small intestine of pigs fed protein-containing diets. The goal of the experiment was to quantify the endogenous N in the digesta of duodenum and ileum in ¹⁵N-labelled piglets receiving diets which caused various digesta viscosity.

Material and Methods

Animals, diets, feeding

Sixteen male pigs, on average 24.4 kg body weight (BW), fitted with both a duodenal

re-entrant cannula and a post-valvular T-shape caecum (PVTC) cannula were used. Four dietary treatments were applied. Of two purified diets, one contained 5% crystalline cellulose (C) and the other 2% carboxymethylcellulose of high viscosity (CMC). Two other diets were based on rye and wheat, one without (RW) and one with addition of enzyme (RWE). The enzyme (ZY 68), containing 1192 IU of xylanase activity per gram enzyme preparation, was supplied at a level of 0.4 g kg⁻¹. All diets provided equal amounts of apparent ileal digestible crude protein (100 g kg⁻¹) and contained soybean protein concentrate and Lys, Met, Thr and Try to cover at least 75% of the requirements of apparent digestible amino acids. The diets were fed in equal portions at 0800 and 2000 h (2.7 times the requirement of energy for maintenance, assumed to be 459 kJ ME kg⁻¹ BW^{0.75} day⁻¹). Water was restricted to 3 times feed intake.

Experimental procedure

The pigs were housed individually in metabolism cages. After cannulation they received gradually increased amounts of the experimental diets and, after 1 week, 2.3 g ^{15}N -labelled yeast (95% enrichment) daily per animal over 10 days. On days 11 to 15, spot samples of ileal digesta were taken (9 h after the morning meal). Ileal and duodenal digesta were collected on days 16 and 17, respectively, for 1 h daily. Collection of urine and faeces was carried out during the whole experiment. The ^{15}N -abundance of urinary N was used as an indicator for isotope enrichment of endogenous N-secretion.

Results and Discussion

After the labelling period, the decrease in atom % ^{15}N excess of urinary N was paralleled by that in ileal digesta (Fig. 28.1).

This shows that the intake of unlabelled dietary N caused a similar ^{15}N -dilution in both N pools. On day 18 (slaughter of animals), the ^{15}N -enrichment in urine corresponded well with those in TCA-soluble N of blood plasma, bile and tissues of the small intestine.

The inclusion of carboxymethylcellulose instead of crystalline cellulose increased significantly the viscosity of duodenal and ileal digesta (pooled over 12 h) from 0.90 to 2.32 mPa s and from 1.82 to 649.5 mPa s, respectively. In cereal-based diets, viscosity of duodenal digesta did not differ between RW and RWE diets (1.52 and 1.42 mPa s, respectively), while viscosity of ileal digesta was reduced significantly over the whole collection period by the xylanase supplementation (Table 28.1).

Diet C resulted in the lowest N flow of endogenous origin (Table 28.2). In contrast to the ileum, the endogenous N flow at the duodenum was up to 45%

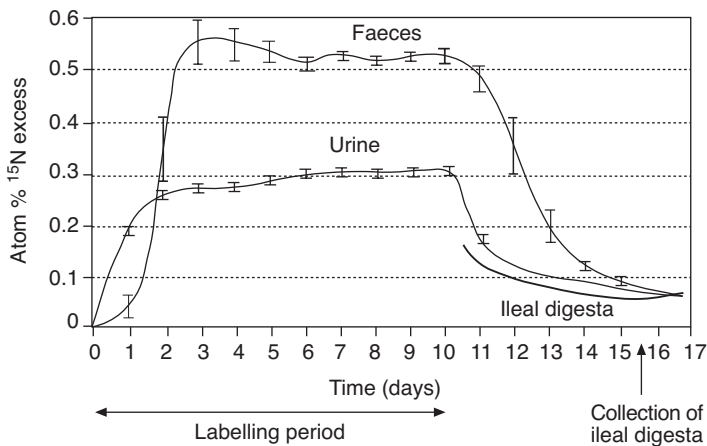


Fig. 28.1. Time course of the average atom % ^{15}N excess in N of faeces, urine and ileal digesta in pigs fed on diets C and CMC (mean $\pm$ SE, $n = 8$).

Table 28.1. Viscosity (mPa s) in ileal digesta of cereal-based diets (mean $\pm$ SE, $n = 4$).

Time after feed (h)	Diet RW	Diet RWE
3	4.95 $\pm$ 0.98 ^a	2.06 $\pm$ 0.36 ^b
6	11.79 $\pm$ 2.67 ^a	3.18 $\pm$ 0.44 ^b
9	13.38 $\pm$ 2.66 ^a	4.96 $\pm$ 1.33 ^b
12	12.91 $\pm$ 7.34	2.82 $\pm$ 0.56

^{ab}Means between diets with different superscript differ ($P < 0.05$).

Table 28.2. Total and endogenous N flow (mg kg⁻¹ BW^{0.75} per 12 h) at the duodenum and distal ileum (mean ± SE; n =4).

	Diet			
	DM intake (g kg ⁻¹ BW ^{0.75} 12 h ⁻¹) /N intake (mg kg ⁻¹ BW ^{0.75} 12 h ⁻¹)			
	C	CMC	RW	RWE
	42.2/863	42.1/872	42.2/994	42.2/992
Duodenum				
Total N	816.9 ± 18.3 ^a	920.4 ± 31.4 ^a	1131.4 ± 38.5 ^b	1159.2 ± 16.9 ^b
Endogenous N	159.2 ± 6.9 ^a	231.6 ± 17.1 ^{ab}	307.6 ± 24.9 ^b	275.6 ± 25.1 ^b
g kg ⁻¹ DM intake	3.8 ± 0.2 ^a	5.5 ± 0.4 ^{ab}	7.3 ± 0.6 ^b	6.5 ± 0.6 ^b
% of total N	19.5 ± 1.1 ^a	25.1 ± 1.3 ^{ab}	27.2 ± 1.8 ^b	23.8 ± 2.3 ^{ab}
Ileum				
Total N	128.1 ± 10.9 ^a	129.2 ± 8.9 ^a	232.8 ± 8.5 ^b	225.5 ± 26.1 ^b
Endogenous N	95.4 ± 8.5 ^a	108.2 ± 9.4 ^a	159.8 ± 10.3 ^b	152.2 ± 28.1 ^{ab}
g kg ⁻¹ DM intake	2.3 ± 0.2 ^a	2.6 ± 0.2 ^a	3.8 ± 0.2 ^b	3.6 ± 0.6 ^{ab}
% of total N	74.5 ± 1.2 ^{ab}	83.4 ± 2.0 ^b	68.5 ± 2.4 ^a	66.1 ± 4.0 ^a

^{abc}Means or medians between diets with different superscript differ (*P* < 0.05).

higher for diet CMC as compared with diet C.

In spite of similar intake of dry matter and apparent digestible N, the rye–wheat-based diets resulted in significantly higher amounts of endogenous N as compared with purified diets. Addition of xylanase reduced to some extent these amounts at the duodenum but not at the ileum. This result is in agreement with findings of Dänicke *et al.* (2000), who observed a non-significant decrease of endogenous N flow at the ileum of pigs fed a rye–wheat-based diet supplemented with xylanase, compared with the unsupplemented diet.

Conclusions

It seems that the gel-forming carboxymethylcellulose influenced the secretion and/or absorption of endogenous N to a higher extent in the stomach–duodenum than in the jejunum–ileum section. Xylanase did not effectively decrease endogenous N losses at the distal ileum in pigs receiving a rye–wheat-based diet.

Acknowledgements

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References

Dänicke, S., Kluge, H., Dusel, G. and Jeroch, H. (2000) Effect of NSP-hydrolyzing enzymes on endogenous N-losses in pigs. *Proceedings of the Society of Nutrition Physiology* 9, 62. (In German.)
Mosenthin, R., Sauer, W.C. and Ahrens, F. (1994) Dietary pectin's effect on ileal and faecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. *Journal of Nutrition* 124, 1222–1229.
Van Barneveld, R.J. (1999) Chemical and physiological characteristics of grain related to variability in energy and amino acid availability in pigs: a review. *Australian Journal of Agriculture Research* 50, 667–668.

29 Isolated Pectins Vary in their Functional Properties in the Gut of Piglets

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Seven different pectins at equal concentration in the diet were fed to weaning piglets and compared regarding their influence on luminal viscosity and effect on ileal digestibility of organic matter. Pure solutions of the pectins displayed markedly different viscosity. The viscosity in the small intestine was also very different, but the order of magnitude was not the same as for the pure solutions. The differences in intestinal viscosity were not related to the differences in ileal digestibility of organic matter.

Introduction

Soluble dietary fibres affect the gastrointestinal environment with possible positive and negative effects on growth performance and health. On one site, the soluble fibre can stimulate the growth of a healthy gut flora, leading to increased production of short-chain fatty acids and a lower pH in the large intestine (Bach Knudsen *et al.*, 1991), and may protect against colonization by pathogenic bacteria in the small intestine. On the other site, soluble fibres increase luminal viscosity, leading to slower absorption with a risk of reduced ileal digestibility (Johansen *et al.*, 1997). The influence on the gastrointestinal environment is dependent on dose and structure of the polysaccharides. Isolated pectins vary in their structure and molecular weight. External factors such as pH, ion strength, concentration of divalent ions, soluble solids, etc., also play an important role for the viscosity elevating properties of structurally different pectins. This is expected to lead to differences in *in vivo*

responses when fed to, for example, piglets or chickens (Langhout and Schutte, 1996). An ongoing project aim was to provide basic information about the interaction between type and level of pectin, and the functional properties and ability to stabilize the gastrointestinal environment in piglets.

Materials and Methods

Four citrus pectins with different degrees of esterification (DE) were used: DM80 was highly methylated pectin with a DE of approximately 80%; DM60, DM46 and DM5 had DEs of approximately 60, 46 and 5%, respectively. Finally, two citrus pectins classified as calcium-sensitive (CAS) and calcium-insensitive (CAN) and pectin from sugarbeet (SB) were tested. CAS was highly methylated but with blocks of unmethylated galacturonic acids. The pectins were compared at a level of 32 g kg⁻¹ diet.

The pigs ($n = 8$ per group, $n = 32$ for

control animals) were weaned at 4 weeks of age. The experimental diets were fed *ad libitum* for 3 weeks and 2 g chromic oxide kg^{-1} diet was used as digestibility marker. The piglets were killed at 7 weeks of age, the gastrointestinal tracts were taken out and divided into the stomach, three equal parts of the small intestine, the caecum and three equal parts of the large intestine. The gastrointestinal contents were weighed and analysed. Unfractionated digesta were analysed for the content of dry matter, ash and chromic oxide. The digesta from the stomach and small intestine was separated in a liquid phase and sediment by centrifugation at 12,000 g. The viscosity was measured in the liquid phase with a Brookfield Digital LVTDV-IICP viscometer and presented at shear rate 45 s^{-1} (Johansen *et al.*, 1997). The concentration of uronic acids (Bach Knudsen, 1997) was measured in both fractions. For comparison of types of pectin, only values of viscosity for the distal small intestine are given.

Results and Discussion

Viscosity of isolated pectins in water and in gut contents

When dissolved in water at similar concentration (0.96 g l^{-1}), CAS gave the highest

viscosity, followed by DM80 and CAN. DM60, DM46 and SB led to similar viscosity in water, and DM5 elicited the lowest viscosity of all the tested pectins (Fig. 29.1). Differences in molecular weight and chemical structure explain the differences between the pectins.

In the upper gastrointestinal tract, the order of viscosity level between pectin types was different. The highest values were obtained for DM80 and DM60. Intermediate viscosities were obtained with CAS, DM46, CAN and SB, while DM5 did not create viscosity significantly different from the control group (2.3 mPa s). The dissimilarity in order of viscosity-elevating properties seen in water and in the gut contents seems to be due to their structure and different sensitivity and reaction with components in the gastrointestinal environment.

The proportion of uronic acids that was present in the liquid phase decreased with decreasing DE, with DM80 $61 \pm 6\%$, DM60 $45 \pm 6\%$, DM46 $26 \pm 5\%$, DM5 $5 \pm 5\%$, although the total concentration of uronic acids in the distal small intestinal was not significantly different between the four dietary treatments (approximately 10 g kg^{-1} digesta). Hence, although the viscosity of pure solutions of DM60, DM46 and DM5 at the same concentration (9.6 g l^{-1}) was almost identical, decreasing viscosities in

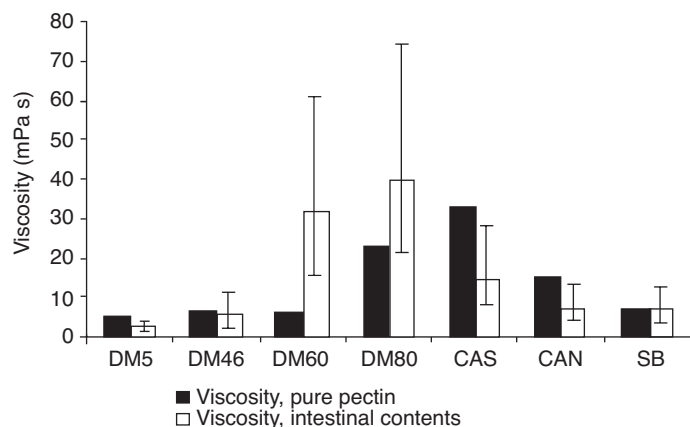


Fig. 29.1. The viscosity of different pectins in water at a concentration of 9.6 g l^{-1} (black bars) and in the distal third of the small intestine of 7-week-old piglets (white bars, mean values with their 95 % confidence interval, $n = 8$).

gut contents were seen with lower DE. This phenomenon is presumably due to increased gel formation with calcium and other divalent ions in the gastrointestinal environment with lower DE of the pectin. The gels would not stay in solution after centrifugation and therefore would not contribute to the measured viscosity. Laboratory tests have confirmed that especially DM46, DM5 and CAS form strong gels with Ca^{2+} .

Nutritional relevance and effect on animal health

The ileal digestibility of organic matter (%) was significantly reduced only with inclusion of DM80 (60 ± 2.4 , $n = 8$) and DM46 (63 ± 2.4 , $n = 8$) compared to the control diet without pectin (70 ± 1.2 , $n = 32$). The organic matter digestibility with inclusion of DM5, DM60, CAS, CAN and SB was

66–68 (± 2.4), and was not significantly different from the control group.

We were not able to detect any positive effect of the pectins on the prevalence of diarrhoea, because the health status of the piglets in general was very good.

Conclusion

It is concluded that the functional properties of pectins in the gastrointestinal tract deviate from the rheological behaviour in water. The digestion process is a dynamic process with changing environmental conditions of pH, ionic strength, microbial enzymatic degradation, etc. Therefore, the rheological behaviour of pectins in the gut and their potential influence on physiological and nutritional parameters cannot be predicted for direct extrapolation from their rheological behaviour in a simple solution.

References

- Bach Knudsen, K.E. (1997) Carbohydrate and lignin contents of plant material used in animal feeding. *Animal Feed Science and Technology* 67, 319–338.
- Bach Knudsen, K.E., Jensen, B.B., Andersen, J.O. and Hansen, I. (1991) Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *British Journal of Nutrition* 65, 233–248.
- Johansen, H.N., Bach Knudsen, K.E., Wood, P.J. and Fulcher, R.G. (1997) Physico-chemical properties and the digestibility of oat bran polysaccharides in the gut of pigs. *Journal of the Science of Food and Agriculture* 73, 81–92.
- Langhout, D.J. and Schutte, J.B. (1996) Nutritional implications of pectins in chicks in relation to esterification and origin of pectins. *Poultry Science* 75, 1236–1242.

30 Factors of Buffering of Large Intestine Digesta in the Pig

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Stimulation of ileocaecal digesta flow by dietary fibre is related to a reduction of the dry matter concentration of the digesta. Levels of sodium bicarbonate, Na and K passing to the large intestine rise. With limited dietary Na, an equimolar intraluminal exchange of Na and K and high levels of aldosterone in the serum were found. Exogenous intravenous aldosterone moderately affected levels of Na and K in the caecum. The effect of angiotensine was limited.

Introduction

The pH-homeostasis and iso-osmolality in the colon of monogastric animals depend on bicarbonate secreted to the lumen, short-chain fatty acids (SCFA), ammonia, soluble compounds of the feed, endogenous protein and electrolytes secreted. The regulation of pH and iso-osmolality, prerequisites for eubiosis, is complex. This forms the basis of the physiological gut functions: storage of digesta, separation of gas, transport by peristalsis and excretion, fermentation to transfer undigestible matter to absorbable SCFA and ammonia, reincorporation of SCFA and N to bacterial matter, and absorption of water, Na, SCFA and ammonia.

These functions depend on an adapted microflora, optimal pH, osmolality, dry matter and availability of nutrients. Bicarbonate and osmolality depend on Na and K levels in the intestinal fluid. It can be assumed that aldosterone is the main hormone regulating electrolyte equilibrium in the colon. At the beginning of the experiments, the question arose how dietary fibre, low levels of water and Na would

affect buffering components in the caecal digesta and aldosterone levels in the blood. Additionally it was of interest how intravenously applied aldosterone and angiotensine would affect K and Na concentrations in the caecal chyme.

Material and Methods

Eight minipigs were kept in metabolism cages, fitted with a catheter in the vena jugularis and a caecal cannula, and fed twice daily a mineralized control diet (maize/soybean meal with 20% crude protein, 0.39% Na and 2.7% crude fibre), or a fibre-rich diet (Fibre +) was fed with 18.5% crude fibre resulting from a change of maize to lucerne meal. Dest. water was offered *ad libitum* and an intake of 2.2 to 4 l day⁻¹ resulted. A variant received not more than 600 ml day⁻¹ (Water -). A third variant received low Na supply (Na -) with a diet with less than 0.18 g Na kg⁻¹. Caecal chymus was collected at feeding and 2, 4 and 6 h postprandial, blood was taken 4 h postprandially. Aldosterone levels were examined in the low sodium variant (Na -).

Results and Discussion

The water content of caecal digesta rises postprandially (Table 30.1). This most likely is due to high digesta flow 2–4 h after feed intake. Dietary crude fibre significantly stimulates postprandial waterflow. Its maximum is found 4 h after feed intake. With low water supply, effects for ileocaecal water flow were small as recycling to the small intestine is important. With limited Na supply a nearly normal ileocaecal water and Na flow was observed.

The sum of Cl and bicarbonate (Table 30.2) showed a postprandial rhythm with a maximum 2 h postprandial. High fibre stimulates concentration of Cl and bicarbonate; low water supply has limited effects but restriction of Na reduces the concentration significantly (especially of bicarbonate). Fibre causes an augmentation of the total anion flow, linked with the dry matter flow and a stimulation of the ileocaecal buffer

flow. The limited Na causes a decline of buffers in the caecum. This might explain aspects of fermentative disorders after low Na supply in monogastric animals.

The counterparts of the anions are the cations Na and K, which play an important role for the resulting osmolality. Additionally, these cations are partners in the bicarbonate buffer system. The sum of the molar concentrations of K and Na in caecal chymus is quite constant, varying between 150 and 160 mmol l⁻¹ (Table 30.3). The proportion of K is 12–15%. Postprandially only a tendency concerning some higher concentrations 2–4 h after feed intake was present. The mean pH in the control was 6.3 and postprandial variations were small.

Dietary fibre depresses cation concentration (especially K) and stimulates the pH. This might be due to retarded fermentation of caecal fibre-rich digesta. The ileocaecal flow and bicarbonate increase markedly; this is

Table 30.1. Water content (mean $\pm$ SD) in caecal digesta of minipigs ($n = 18$).

Diet	Sampling time (h postprandial)			
	0	2	4	6
Control	85.2 $\pm$ 1.2	86.2 $\pm$ 1.4	86.2 $\pm$ 1.4	86.0 $\pm$ 1.2
Fibre +	86.0 $\pm$ 1.3	88.4* $\pm$ 1.4	87.0* $\pm$ 1.8	86.7* $\pm$ 1.4
Water –	84.5* $\pm$ 1.0*	85.1* $\pm$ 1.6	85.2* $\pm$ 1.7	84.8* $\pm$ 1.5
Na –	86.3 $\pm$ 0.8	86.8 $\pm$ 1.0	87.0 $\pm$ 0.6	86.6 $\pm$ 0.8

*Significant difference (95%) between variant and control.

Table 30.2. Concentration of bicarbonate and chlorine in caecal chymus: sum of both anions (nmol l⁻¹, $\pm$ SD), and bicarbonate as % of the sum.

Diet	Sampling time (h postprandial)			
	0	2	4	6
Control	118 $\pm$ 18 30%	133 $\pm$ 19 34%	114 $\pm$ 16 32%	108 $\pm$ 16 31%
Fibre +	150 $\pm$ 30* 15%	210 $\pm$ 35* 29%	155 $\pm$ 26* 21%	162 $\pm$ 27* 21%
Water –	120 $\pm$ 20 28%	115 $\pm$ 28 31%	122 $\pm$ 12 33%	107 $\pm$ 18 27%
Na – (after 16 days of deprivation)	80 $\pm$ 11* 14%	87 $\pm$ 12* 20%	88 $\pm$ 14* 28%	71 $\pm$ 12* 20%

*Significant difference (95% level) between variant and control.

Table 30.3. Sum of sodium and potassium (mmol l⁻¹, ± SD) in the fluid of caecal digesta.

Diet	pH	Sampling time (h postprandial)			
		0	2	4	6
Control	6.33 ± 0.2	150 ± 11	152 ± 10	159 ± 14	150 ± 13
K (%)		15	14	12	13
Fibre +	6.49 ± 0.2*	141 ± 7	143 ± 9	146 ± 19	149 ± 11
K (%)		10	8	9	9
Water –	6.51 ± 0.25*	184 ± 22*	184 ± 20*	186 ± 17*	179 ± 21*
K (%)		12	11	10	11
Na –	5.85 ± 0.3*	133 ± 14*	145 ± 17*	143 ± 17*	140 ± 20*
K (%)		28	27	24	28

*Significant difference (95% level) between variant and control.

responsible for high buffering capacity. Low water supply, however, stimulates the concentration of cations, especially of Na, significantly. The pH rises significantly in parallel with the remarkable concentration of bicarbonate. Low dietary Na induced a depression of the pH and an exchange between Na and K but the sum of both was maintained at a nearly 'normal' level. The depression of the pH exceeded optimal buffering of the digesta.

Caecally fistulated animals were fitted with catheters in the jugular vein and used in a switchover experiment (Table 30.4).

An aldosterone-dependent reduction of Na in caecal chyme results, with rising concentrations. Burdiek (1992) infused aldosterone into animals receiving low- and high-fibre diets, demonstrating that in the latter this exchange was far more pronounced than in low-fibre diets.

Table 30.4. Aldosterone infusion and Na/K relations in caecal chymus (mmol kg⁻¹, ± SD) (n=3).

	Hours postprandial					
	Control			+ Aldosterone		
	0	4	8	0	4	8
Sodium	118 ± 2	124 ± 4	115 ± 5	110 ± 2	112 ± 2	94 ± 5*
Potassium	11.5 ± 2	9.2 ± 1	8.5 ± 3	13 ± 1	11 ± 1	14.5 ± 1.5
Sum	129.5	133.2	123.5	123	123	108.5
% potassium	8.9	6.9	6.9	10.6	8.9	13.4

Initial dose 33.4 µg kg⁻¹ BW; remainder of dose 66.6 µg kg⁻¹ during 5 h infusion period. Total dose 100 µg aldosterone per kg⁻¹ BW.

*Significant difference (95% level) between variant and control.

Reference

Burdiek, H. (1992) Wirkungen faserreicher Fütterung- in Wechselwirkung zu intra-venösen Aldosterongaben- auf ausgewählte Parameter von Caecumchymus und Blut beim Göttinger Miniaturschwein. Diss.med.vet. Hannover.

31 Consequences of Ileal Endogenous Losses on Body Protein Retention and Feedstuff Evaluation

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An experiment was carried out to measure nitrogen, lysine and threonine real digestibility and ileal endogenous losses using the ^{15}N -amino acid isotope dilution method. Three pea cultivars (Solara, Eiffel and Madria) and the effect of micro-grinding (Solara) were tested using ileorectal anastomosed pigs. Solara and Eiffel had the same standardized digestibility. Solara induced lower ileal endogenous losses and had a lower real digestibility than Eiffel. Moreover, our data showed a negative correlation between ileal endogenous losses and body protein retention. We propose to derive from digestibility data an availability coefficient taking into account the total cost of endogenous losses, including metabolic losses.

Introduction

The standardized digestibility (SD) of amino acid (AA) involving the correction of the ileal flow by basal endogenous losses is currently used to formulate pig diets. However, additional ileal endogenous losses can be induced specifically by raw materials. Therefore, for a better estimation of the nutritive value of feedstuffs, assessment of total endogenous losses leading to determination of real digestibility is important. This is currently made with the isotope dilution technique, either with labelled animals or with labelled diet. Although the latter technique underestimates both values due to recycling of the isotope (Hess *et al.*, 2000) it gives information on individual AA. It was combined in the present experiment with nitrogen balance in order to assess the impact of endogenous losses of the limiting AA on their availability.

Materials and Methods

Three different pea cultivars were labelled (Solara, Eiffel and Madria) using ^{15}N -labelled ammonium nitrate. The three crops were ground through a 2.5 mm screen. One batch of the Solara cultivar was micro-ground to a particle size of 25–50 μm diameter. For each batch, two pea/maize-starch-based diets were formulated, one with the unlabelled pea and one double labelled with ^{15}N and 0.3% chromic oxide. These diets (Table 31.1) and a protein-free diet (PF, 80% maize starch and 5% wood cellulose) were fed to ileorectal anastomosed pigs (35.4 kg body weight) according to a Latin square design. For dietary and endogenous flow determinations, the experimental and analytical procedures were as previously described (Hess *et al.*, 2000). The real digestibilities of N or AA (RDN(AA)) were calculated as the ^{15}N or ^{15}N -AA digestibilities on the

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basis of the analytical data (chromic oxide and N and AA concentrations, ¹⁵N enrichments) from both the labelled diet and a sample of digesta collected for 9 h after a labelled test meal. Total ileal endogenous losses of N and AA [N(AA)endo] were determined as the apparently indigestible quantity (measured during the previous 2-day collection period, when pigs were offered the unlabelled diet) minus the real indigestible quantity. The specific endogenous losses of N or AA [N(AA)endospe] from each batch of pea were calculated as the total minus the basal endogenous losses measured with the PF diet, both expressed per kg dry matter intake (DMI). Body N retention (Nr) was calculated as N intake minus N in digesta and in urine during the 2-day collection period. The following model described the partition between the total cost of the endogenous losses and the total cost of body Nr, under the hypothesis that dietary N was limiting N accretion:

$$\text{RDN} = \text{Nr}/\text{Kb} + \text{Nendo}/\text{Ke}$$

The coefficients Ke and Kb were determined using a non-linear statistical procedure. The true availability coefficients of N and AA [ACN(AA)] were calculated as:

$$\text{ACN(AA)} = (\text{RDN(AA)}/100) - [\text{N(AA)endospe}/\text{Ke}]/\text{N(AA)},$$

where N(AA) was the dietary N or AA kg⁻¹ DMI and assuming that Ke determined with N data also applied to the limiting AA.

Results and Discussion

The standardized and real digestibilities were lower for Madria than for the two other cultivars (Table 31.2). Micro-grinding improved significantly the standardized and real digestibilities of the Solara cultivar but did not influence the ileal endogenous losses. With Solara, the ileal endogenous losses were less important than with the two other cultivars, which induced highly significant specific ileal endogenous losses in excess of the basal losses. However, we measured the same standardized digestibility for Solara and Eiffel as a result of a greater stimulation of the ileal endogenous losses combined with a higher real digestibility with Eiffel.

The equation describing the relationship between retained N and the endogenous N losses, both expressed per g RDN, was $y = 0.82 - 1.90x$ ($r^2 = 0.81$) (Fig. 31.1). The efficiencies of RDN for body protein retention and the ileal endogenous protein flow were $\text{Kb} = 0.844 \pm 0.122$ and $\text{Ke} = 0.415 \pm 0.122$, respectively.

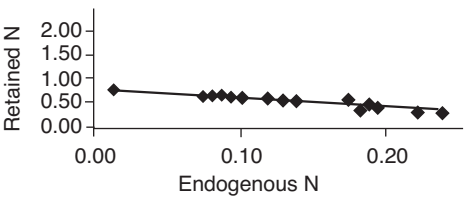


Fig. 31.1. N retention and ileal endogenous losses.

Table 31.1. Chemical composition of the experimental diets (per 100 g, as fed).

	Protein-free	Solara	Micro	Madria	Eiffel
Dry matter (g)	89.3	89.2	89.0	88.1	88.0
Crude protein (g)	0.44	15.3	14.4	14.8	14.8
Digestible energy (Mcal)	3.11	3.21	3.22	3.21	3.22
Neutral detergent fibre (g)	4.1	7.9	–	11.5	8.8
Acid detergent fibre (g)	2.8	4.7	–	5.2	4.0
Crude fibre (g)	–	4.2	–	4.4	3.7
10 ³ TIU	–	309	–	319	265
Lys (g)	–	1.16	1.10	1.16	1.11
Thr (g)	–	0.63	0.59	0.60	0.59

Table 31.2. Standardized (SD) and real (RD) digestibilities, total ileal endogenous losses (Losses) and availability coefficient (AC) for total N, lysine and threonine.

		Protein-free	Solara	Micro	Madria	Eiffel	SEM
SD	N	–	76.1 ^x	90.2 ^z	66.5 ^y	75.1 ^x	1.4
	Lys	–	82.0 ^x	92.6 ^z	76.6 ^y	81.5 ^x	0.8
	Thr	–	74.7 ^x	87.3 ^z	66.7 ^y	73.1 ^x	1.2
Losses	N	1.87 ^w	2.84 ^{wx}	1.78 ^w	4.60 ^y	3.73 ^{xy}	0.50
	Lys	0.35 ^w	0.42 ^{wxy}	0.57 ^{wx}	0.82 ^{xy}	1.07 ^y	0.17
	Thr	0.58 ^w	0.89 ^{xy}	0.81 ^{wx}	1.36 ^z	1.30 ^{yz}	0.11
RD	N	–	81.3 ^{xy}	90.1 ^z	76.5 ^x	83.2 ^y	2.2
	Lys	–	82.6 ^{xy}	94.5 ^z	80.1 ^x	81.7 ^y	1.8
	Thr	–	79.8 ^{xy}	90.3 ^z	77.7 ^x	83.6 ^y	1.3
AC	N	–	70.0 ^y	91.7 ^x	57.0 ^z	66.0 ^y	2.8
	Lys	–	82.1 ^y	89.1 ^x	71.6 ^z	73.5 ^z	2.1
	Thr	–	67.6 ^y	83.0 ^x	51.1 ^z	58.1 ^z	2.7

^{wxyz}Means in the same row followed by a different letter differ at $P < 0.05$.

The fact that Ke was less than 1 clearly indicated that a metabolic cost was associated with the ileal endogenous losses. Basal endogenous losses are part of the pig requirement that needs to be met with available amino acids. Therefore, in order to evaluate the truly available N or AA for protein deposition, only the specific

endogenous losses and their metabolic cost must be subtracted from the real digestible fraction. It is noteworthy that, despite similar SD and RD, Eiffel AA were significantly less available than those of Solara. Because it was clearly the limiting AA, this conclusion probably applied to threonine rather than to lysine.

Reference

- Hess, V., Ganier, P., Thibault, J.N. and Sève, B. (2000) Comparison of the isotope dilution method for determination of the ileal endogenous amino acid losses with labelled diet and labelled pigs. *British Journal of Nutrition* 83, 123–130.

32 Ileal Flow of Endogenous Amino Acids in Relation to Energy Source: Consequences on the Evaluation of Ileal Digestibility of Protein in Growing Pigs

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Six growing pigs, fitted with an ileorectal anastomosis, were fed three protein-free diets, in which energy was mainly supplied either by maize starch, starch and sucrose (1:1), or starch with soy oil to increase the lipid content from 4.5 to 7.5% and, alternatively, three equivalent diets with soybean meal. The ileal endogenous flow of amino acids was generally higher with oil, lower with sucrose and intermediate with starch. Standardized true digestibility of amino acids, calculated with the endogenous values of the corresponding protein-free diet, was unchanged between starch and sucrose but higher with oil for cysteine, isoleucine, tyrosine and valine.

Introduction

The assessment of the true (standardized) digestibility of amino acids (ITD) in a feed-stuff requires knowledge of the endogenous amino acid flow at the ileum. The latter can be obtained in routine experiments using protein-free diets. The optimal duration of the period of protein deprivation, amount and composition of fibre, and mineral and vitamin composition have been extensively studied (Mariscal-Landin *et al.*, 1995; Mroz *et al.*, 1996). Nevertheless, the composition of the protein-free diet is not standardized and varies between laboratories and sometimes between experiments within laboratories. The current experiment was designed to assess the effects of non-fibre energy source on the ileal endogenous flow of amino acids and consequences on the estimation of ITD.

Material and Methods

Six castrated pigs, with an initial body weight of 55 kg and surgically prepared with an ileorectal anastomosis, were fed six different diets during six consecutive weeks. Three protein-free diets were based either on starch (PFMS), or on equal parts of starch and sucrose (PFSC), or as PFMS but with added soy oil (PFOL). Three other diets (SBMS, SBSC and SBOL) were based on the same energy sources, respectively, but included soybean meal to reach a protein content of 16%. All diets contained 5% of wood cellulose, 4.5% of minerals and vitamins and a minimum 5% of sucrose. Soy oil was used to reach a lipid content of 7.5% in PFOL and SBOL and 4.5% in other diets. Diets were given in order to supply 2.5 times the energy requirement for maintenance. Normal and

protein-free diets were given alternatively, according to a Latin square design. Other procedures were carried out as in Mariscal-Landin *et al.* (1995). One pig on SBMS had to be removed due to erratic values. Chemical analyses were carried out according to AFNOR procedures and amino acids were determined by HPLC (Waters) after hot hydrolysis (6 N HCl) in sealed flasks. Statistical analysis was performed according to SAS procedures.

Results and Discussion

Ileal endogenous flow was, for most of the amino acids, lowest for SC, highest for OL and intermediate for MS. This reflected the residual nitrogen content in the diets, but not individually for each amino acid as presented in Table 32.1. The largest difference was obtained for tyrosine, namely 0.234, 0.167 and 0.148 g kg⁻¹ of dry matter intake (DMI), with OL, MS and SC, respectively. In contrast, lysine was less affected with 0.245, 0.217 and 0.192 g kg⁻¹ DMI, in the same order.

For most of the amino acids, the apparent digestibility and ITD (the latter calculated with the corresponding protein-free diets) were not affected by the energy source (Table 32.2). Only the IDT of nitrogen and four amino acids (cysteine, isoleucine, tyrosine and valine) were significantly higher with SBOL than with SBMS and SBSC, but did not differ between the latter two, except for tyrosine (std = 0.51%). The digestibility of cysteine (std = 1.97%) was 5.3% higher with OL than with MS or SC. Lysine (std = 0.52%), methionine (std = 0.99%) and threonine (std = 1.02%) were not significantly affected.

Conclusion

With the exception of four amino acids, with high-fat diets the non-fibre energy source in protein-free diets has a small effect on the subsequent evaluation of the true standardized ileal digestibility of amino acids, despite differences in the endogenous flow.

Table 32.1. Endogenous flow of amino acids according to the energy source.

	PFMS	PFSC	PFOL	<i>P</i> > <i>F</i>
N intake (g day ⁻¹)	1.593	0.972	1.266	
Excretion (g kg ⁻¹ DM)				
Total nitrogen	1.048	0.994	1.154	0.1322
Cysteic acid	0.111 ^b	0.105 ^c	0.120 ^a	0.0001
Alanine	0.227 ^{ab}	0.209 ^b	0.259 ^a	0.0782
Arginine	0.218 ^{ab}	0.191 ^b	0.237 ^a	0.0896
Aspartic acid	0.388 ^a	0.341 ^b	0.408 ^a	0.0063
Glutamic acid	0.450 ^{ab}	0.408 ^b	0.501 ^a	0.0840
Glycine	0.305	0.285	0.399	0.1365
Histidine	0.097 ^{ab}	0.087 ^b	0.115 ^a	0.0488
Isoleucine	0.189 ^a	0.165 ^b	0.206 ^a	0.0132
Leucine	0.287 ^b	0.255 ^c	0.316 ^a	0.0073
Lysine	0.217	0.192	0.245	0.1208
Methionine	0.062 ^{ab}	0.055 ^b	0.067 ^a	0.0717
Phenylalanine	0.175 ^b	0.153 ^c	0.193 ^a	0.0031
Proline	0.305	0.313	0.314	0.9710
Serine	0.255 ^b	0.230 ^c	0.270 ^a	0.0001
Threonine	0.282 ^b	0.256 ^c	0.304 ^a	0.0021
Tyrosine	0.167 ^{ab}	0.148 ^b	0.234 ^a	0.0283
Valine	0.241 ^a	0.212 ^b	0.263 ^a	0.0086
Total amino acids	3.975 ^{ab}	3.584 ^b	4.450 ^a	0.0505

^{ab}Values with the same letter differ at the level of probability *P* > *F*.

Table 32.2. ITD coefficients (%) from soybean meal according to energy source.

	Apparent digestibility				True standardized digestibility			
	SBMS	SBSC	SBOL	$P > F$	SBMS	SBSC	SBOL	$P > F$
N	81.2 ^b	80.6 ^b	82.5 ^a	0.025	84.6 ^b	83.8 ^b	86.2 ^a	0.008
Cys	76.0 ^b	74.1 ^b	79.3 ^a	0.016	80.3 ^b	78.1 ^b	83.4 ^a	0.015
Ala	84.4	85.3	84.4	0.421	87.0	87.5	87.6	0.704
Arg	90.9	91.2	91.0	0.314	92.4	92.4	92.6	0.418
Asp	83.9	84.2	84.2	0.546	85.7	85.6	86.0	0.480
Glu	88.5	88.1	88.7	0.280	89.8	89.2	90.1	0.086
Gly	78.2	78.6	78.6	0.644	81.7	81.7	83.2	0.089
His	86.4	86.8	86.2	0.184	88.4	88.4	88.6	0.473
Ile	87.2 ^b	87.8 ^a	87.6 ^{ab}	0.019	89.1 ^b	89.4 ^{ab}	89.7 ^a	0.024
Leu	87.6	87.9	87.5	0.111	89.5	89.5	89.7	0.244
Lys	87.0	87.2	87.5	0.269	88.5	88.4	89.2	0.084
Met	88.3	89.2	89.3	0.387	90.9	91.2	91.8	0.389
Phe	88.0	88.4	88.1	0.114	89.7	89.8	90.0	0.125
Pro	87.9	87.5	87.5	0.992	90.8	90.4	90.6	0.976
Ser	87.4 ^a	87.3 ^a	85.7 ^b	0.061	90.1	89.6	88.8	0.221
Thr	82.5	82.3	81.0	0.135	86.3	85.6	85.3	0.556
Tyr	87.2 ^b	88.8 ^a	88.2 ^a	0.005	89.5 ^c	90.4 ^b	91.2 ^a	0.004
Val	85.0 ^b	85.9 ^a	86.3 ^a	0.016	87.4 ^b	87.8 ^b	88.8 ^a	0.013
Total AA	85.7	85.9	86.0	0.506	88.1	87.9	88.6	0.159

^{ab}Values with the same letter differ at the level of probability $P > F$.

However, the protein digestibility of high-fat feedstuffs could be underestimated if the protein-free diet used for the calculation of ITD is not devised on the same basis of lipid content. Considering these exceptions, it is suggested that, when ITD is determined, the ileal endogenous flow should be assessed simultane-

ously, in order to take into account not only the variability between pigs but also the effect of the composition of the non-protein fraction of the feedstuff. From a physiological point of view, it would be of interest to know if the endogenous flow is affected by microbial activity or by enzymatic secretions.

References

- Mariscal-Landin, G., Sève, B., Colléaux, Y. and Lebretton, Y. (1995) Endogenous amino nitrogen collected from pigs with end-to-end ileorectal anastomosis is affected by the method of estimation and altered by dietary fiber. *Journal of Nutrition* 125, 136–146.
- Mroz, Z., Bakker, G.C., Jongbloed, A.W., Dekker, R.A., Jongbloed, R. and van Beers, A. (1996) Apparent digestibility of nutrients in diets with different energy density, as estimated by direct and marker methods for pigs with or without ileo-cecal cannulas. *Journal of Animal Science* 74, 403–412.

33 Dietary Fibre: Effect on Gastric Emptying in Pregnant Sows

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The effects of feeding two very differently composed fibre-rich diets and a low dietary fibre (DF) diet on the rate of gastric emptying were examined in six gastric-cannulated pregnant sows. The stomach contents were evacuated through the gastric cannula once daily. Increasing the content of DF in the diet led to higher recoveries of liquid digesta but did not have any effect on the gastric emptying of dry matter (DM) and dietary components. The type of DF in the diet did not have a major influence on the results of the study.

Introduction

Most of the present knowledge on the effect of dietary fibre (DF) on gastric emptying has been achieved from studies with DF isolates and not under normal meal conditions, where the effects are often confounded by changes in other dietary components. The objective of the present study was to examine, under normal meal conditions, the effects of varying the quantity and type of DF on the rate of gastric emptying in pregnant sows. Additionally, it was examined whether any effect could be related to the viscosity or to the water-binding capacity (WBC) of digesta.

Materials and Methods

Three diets were formulated to provide the same levels of fat, protein, minerals and vitamins but different levels and types of DF. The concentrated low DF (150 g kg⁻¹ DM) control diet (diet C) was based on soybean meal, barley and wheat. The two fibre-rich diets (approximately 245 g kg⁻¹ DM) were formulated as a combination of

the control diet and a fibre source. The fibre-rich diets contained added soluble DF from sugarbeet pulp (SBP) and insoluble DF from wheat bran (WB), respectively.

Six pregnant sows were fitted with a gastric cannula in the fundic region according to the procedure described by Low *et al.* (1985). The experiment was carried out in two blocks, both designed as 3 × 3 Latin squares, with three sows fed one of the three diets for 1 week during three consecutive weeks per block. Water intake was restricted to 4 l at each feeding time.

The gastric digesta of each pig was evacuated through the gastric cannula once daily in a randomized order just before and 0.5, 1, 2, 3 and 5 h after feeding the morning meal. The digesta that flowed freely out of the stomach after opening the cannula was collected, weighed and sampled. In order to remove any residual digesta remaining in the stomach, the stomach was rinsed with a 38°C physiological solution of saline until the outflow appeared free of particles. When all the digesta had been removed, the undiluted digesta was returned to the stomach through the gastric cannula.

Results and Discussion

Gastric emptying of digesta was very similar for diets SBP and WB (Fig. 33.1) and was significantly slower than for diet C ($P < 0.01$ and $P < 0.05$, respectively). Thus, higher amounts of digesta were recovered from the stomach when the level of DF in the diet increased. Despite apparent differences in the velocity of emptying, there was no statistically significant difference in the recovery of gastric DM among the studied diets (Fig. 33.1). The digesta evacuated from the stomach comprises the remaining portion of the meal, saliva and gastric secretion. The fact that the DM content of digesta was not significantly different among the three diets suggests that the effect of a higher level of DF in the diet was either to increase the volume of gastric contents by additional secretion or to delay the emptying of the liquid phase (but not the solid phase) of digesta, or a

combination of both. The viscosity elevating properties of soluble DF are generally accepted as being responsible for delayed gastric emptying. Results from previous studies in which soluble DF isolates have been shown to increase *in vitro* viscosity suggested that SBP diet would raise viscosity of digesta considerably. In the present study, however, viscosity of digesta was not statistically significantly different among diets (Fig. 33.1). Plant materials need to solubilize in order to increase viscosity. In this experiment the concentration of non-starch polysaccharides (NSP) was very low in the liquid phase of digesta, meaning that only a small fraction of NSP was solubilized in the stomach (Table 33.1). The large fraction of intact and only partly dispersed cell walls does not contribute to the viscosity of the liquid phase of digesta but might influence the bulkiness of digesta by the increased ability to hold water (Johansen *et al.*, 1996).

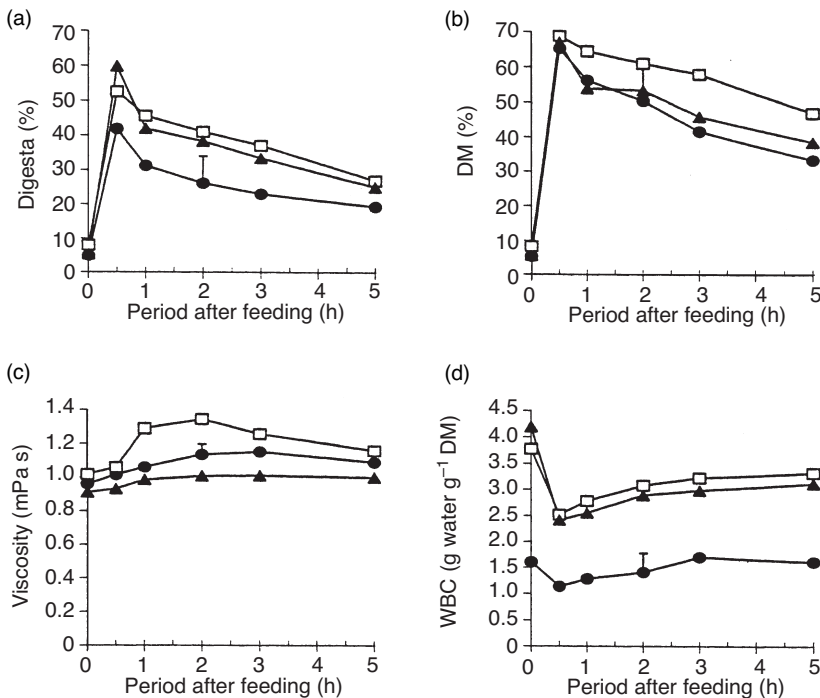


Fig. 33.1. Recovery of (a) digesta and (b) dry matter. (c) Viscosity (mPa s) and (d) water-binding capacity of evacuated gastric digesta before and 0.5, 1, 2, 3 and 5 h after feeding. Control (●), wheat bran (▲) and sugarbeet pulp (□) diets.

WBC of the two fibre-rich diets studied was significantly higher than the WBC of the C diet (Fig. 33.1), indicating that diets SBP and WB retained more water per gram DM than diet C. It seems likely that the higher volume of water bounded to NSP might partly explain that more liquid was retained in the stomach for the diets containing higher amounts of NSP, i.e. the SBP and WB diets.

Conclusions

The present study showed that feeding diets with a high content of DF, rich in

either soluble DF from SBP or insoluble DF from WB, reduced the rate of gastric emptying of the liquid phase of digesta but had no effect on the gastric emptying of the DM and dietary components. The slowed gastric emptying of the liquid digesta could not be related to the viscosity of the liquid phase but could be partly related to the increased ability of the fibres to retain water, i.e. to their water-binding capacity. No effect could be attributed to differences in the type of fibre added, indicating that other factors, such as solubility of the cell wall polysaccharides and interaction between dietary components, may have an effect in complex meals.

Table 33.1. Concentration (g 100 g⁻¹ DM) of insoluble NSP and soluble NSP in the digesta recovered from the stomach (values are least square means for six sows).

Diet	Time after feeding (h)											
	Insoluble NSP						Soluble NSP					
	0.5	1	2	3	5	15.5	0.5	1	2	3	5	15.5
C	12.3 ^{aA}	13.6 ^{aAB}	14.9 ^{aBC}	15.4 ^{aBC}	15.2 ^{aBC}	16.2 ^{aC}	0.15 ^{aA}	0.16 ^{aA}	0.18 ^{aA}	0.18 ^{abA}	0.16 ^{abA}	0.08 ^{aB}
WB	21.4 ^{bA}	21.0 ^{bA}	24.0 ^{bB}	24.7 ^{bB}	24.7 ^{bB}	26.6 ^{bB}	0.14 ^{aA}	0.15 ^{aA}	0.16 ^{aA}	0.16 ^{aA}	0.13 ^{aA}	0.09 ^{abA}
SBP	22.0 ^{bA}	21.7 ^{bA}	24.0 ^{bA}	23.8 ^{bA}	23.5 ^{bA}	26.9 ^{bB}	0.17 ^{aA}	0.21 ^{bBC}	0.24 ^{bC}	0.22 ^{bC}	0.18 ^{bAB}	0.13 ^{bD}
Pooled (SEM)			2.18						0.03			

^{abc}Values with different superscript letters within a column were significantly different ($P < 0.05$).

^{ABCD}Values with different superscript within a row were significantly different ($P < 0.05$).

References

- Low, A.G., Pittman, R.J. and Elliot, R.J. (1985) Gastric emptying of barley-soyabean diets in the pig: effects of feeding level, supplementary maize oil, sucrose or cellulose, and water intake. *British Journal of Nutrition* 54, 437–447.
- Johansen, H.N., Bach Knudsen, K.E., Sandström, B. and Skjøth, F. (1996) Effects of varying contents of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs. *British Journal of Nutrition* 75, 339–351.

34 Effect of Converting Lysine in Barley and Canola Meal into Homoarginine on Nutrient Composition and Ileal Amino Acid Digestibilities in Growing Pigs

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Effects of guanidinating lysine in barley (B) and canola meal (CM) on nutrient composition and ileal amino acid (AA) digestibility were investigated. Guanidination did not change nutrient composition of B ($P > 0.10$) but it increased levels of crude protein (CP) (38.4 vs. 49.0%), crude fibre (10.2 vs. 16.0%), acid detergent fibre (30.0 vs. 43.4%) and neutral detergent fibre (29.8 vs. 49.4%) in CM ($P < 0.05$). Guanidination did not affect ($P > 0.05$) apparent ileal digestibility of dry matter (DM), CP and AA in four 33.6 kg barrows fitted with a simple T-cannula at the terminal ileum and fed a barley–canola meal diet. True ileal lysine, threonine and isoleucine digestibilities were 88.1%, 97.1% and 89.0%, respectively.

Introduction

The conversion of dietary lysine into homoarginine (HA) in a guanidination reaction with methylisourea (MIU) is used as a method for measuring endogenous amino acid (AA) losses in monogastric animals fed protein-containing diets (Schmitz *et al.*, 1991; Nyachoti *et al.*, 1997; Moughan *et al.*, 1998). Although most of the assumptions for using this technique have been validated using purified dietary protein sources, it is not known whether these assumptions apply to practical feedstuffs. Furthermore, the effect of the guanidination process on nutrient content and digestibility in the resulting material has not been fully investigated.

The objective of the current study was

to determine the effect of guanidination on the chemical composition of barley (B) and canola meal (CM) and on apparent ileal digestibility of dry matter (DM), crude protein (CP) and AA in a 16% CP barley–canola meal (BCM)-based diet fed to growing pigs. In addition, true ileal digestibility of CP and AA were estimated.

Materials and Methods

Batches of B and CM containing 200 g of CP were soaked in 1 l of distilled water, mixed with 1 l of 0.5 MIU solution and incubated at 4°C for 6 days, at a pH of 10.5. Material was then centrifuged at 4000 × g and 4°C to recover the guanidinated protein. The material was then washed and

centrifuged three times with water whose pH was adjusted to the isoelectric point of each protein source, to remove excess MIU, and freeze-dried. Four 33.6 kg Yorkshire barrows were fitted with a simple T-cannula at the terminal ileum and fed a 16% CP non-guanidinated BCM-based diet for four consecutive 14-day periods. On day 12, ileal digesta was collected for a 24 h period, to determine apparent digestibilities. On day 14, a second 24 h ileal digesta collection was conducted, after feeding a meal of a diet in which 50% of the protein sources in the diet were replaced with guanidinated material. Chromic oxide (0.5%) was included in both diets as an indigestible marker for determining apparent digestibility, while titanium oxide (0.5%) was added to the guanidinated diet for determining true digestibility (Nyachoti *et al.*, 1997). Digesta samples were pooled by pig and by 24 h period to give 16 observations per diet.

Results

Dry matter recovery following guanidination was 77.2% ($n = 8$, $SD = 2.7$) and 74.2% ($n = 7$, $SD = 4.2$) for B and CM, respectively. Lysine to HA conversions were 82% and 76% for B and CM, respectively. Guanidination did not change nutrient

composition of B ($P > 0.10$), but it increased CP (38.4 vs. 49.0%), crude fibre (10.2 vs. 16.0%), acid detergent fibre (ADF) (30.0 vs. 43.4%) and neutral detergent fibre (NDF) (29.8 vs. 49.4%) levels in CM ($P < 0.05$). Guanidination did not affect ($P > 0.05$) apparent ileal digestibility values for DM, CP and AA in a BCM diet fed to growing pigs, despite the composition changes observed in CM (Table 34.1). True ileal lysine, threonine and isoleucine digestibilities were 88.1%, 97.1% and 89.0%, respectively.

Discussion

The material lost during guanidination likely consists of representative material lost during homogenization of the material between washings and material solubilized in the washing solution. Observed DM losses and lysine conversion rates cannot fully explain the composition changes in CM. Based on guanidination of lysine, increases in CP content of 0.3 and 1.5 percentage units were expected for B and CM, respectively. Calculations based on the sum of AA nitrogen of all the AA in the non-guanidinated and guanidinated B and CM gave increases of 1.9% and 15.3% in the CP of B and CM, respectively. The additional CP in CM could be attributed to

Table 34.1. Apparent ileal dry matter, crude protein and amino acid digestibilities (%) in pigs fed a barley–canola meal-based diet with or without guanidinated proteins.

Item	Diet		SEM
	Non-guanidinated	Guanidinated	
Dry matter	57.7	56.4	1.6
Crude protein	68.8	68.0	1.1
Amino acids			
Arginine	82.8	82.8	0.8
Histidine	73.3	73.4	2.6
Isoleucine	71.6	73.9	1.4
Leucine	73.8	76.2	1.3
Lysine	73.9	74.5	1.4
Phenylalanine	77.2	78.2	1.3
Threonine	68.8	65.7	2.0
Valine	73.0	73.7	1.5

SEM, pooled standard error of the mean.

a selective removal of non-protein material during the guanidination process or an incomplete removal of excess MIU during washing.

Contents of crude fibre, ADF and NDF were increased by 5.2, 12.1 and 17.5 percentage units in CM, but no such changes were observed in B. Based on a DM loss of approximately 26% following guanidination, the maximum increase in content of any fibre fraction was 35%, which was lower than the observed increase in crude fibre and NDF contents. Apparently, guanidination interferes with analyses of fibre fractions.

The apparent ileal DM, CP and AA digestibility for the non-guanidinated and guanidinated diets were within the range of reported values for a BCM-based diet (Nyachoti *et al.*, 1997; NRC, 1998). The observed apparent ileal digestibility of lysine (approximately 74%) was similar to

that reported previously (e.g. Nyachoti *et al.*, 1997). These results indicate that the process of guanidination *per se* does not alter the apparent digestibility of dietary protein and AA. These observations corroborate results of others using purified proteins (Schmitz *et al.*, 1991). Results of true ileal AA digestibilities were similar to those of Nyachoti *et al.* (1997) but slightly higher for most AA than those reported by NRC (1998). This is to be expected, because NRC (1998) values were determined by feeding a protein-free diet to estimate endogenous AA, a technique known to underestimate endogenous AA flow when feeding practical diets (Moughan *et al.*, 1998).

It is concluded that guanidination does not interfere with digestion and therefore the HA method is a useful technique for routine determination of true ileal AA digestibilities in pigs fed practical diets.

References

- Moughan, P.J., Souffrant, W.B. and Hodgkinson, S.M. (1998) Physiological approaches to determining endogenous amino acid flows in the mammal. *Archives Animal Nutrition* 51, 237–252.
- NRC (1998) *Nutrient Requirements of Swine*, 10th edn. National Academy Press, Washington, DC.
- Nyachoti, C.M., de Lange, C.F.M. and Schulze, H. (1997) Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. *Journal of Animal Science* 75, 3206–3213.
- Schmitz, M., Hagemeister, H. and Erbersdobler, H.F. (1991) Homoarginine labelling is suitable for determination of protein absorption in miniature pigs. *Journal of Nutrition* 121, 1575–1580.

35 Effect of Enzyme Supplementation of Different Quality Hullless Barley on Apparent (Ileal and Overall) Digestibility of Nutrients in Young Pigs

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The effect of an enzyme cocktail supplementation of five varieties of hullless barley (HB) on apparent ileal (AID) and overall digestibility (AOD) of nutrients was investigated in 20 Cotswold pigs with an initial mean body weight of 15 kg. Positive ($P < 0.05$) effects of enzyme addition were observed for AID of non-starch polysaccharide, dry matter, gross energy, crude protein and most of the essential amino acids, with the magnitude greater for diets based on Condor and Buck. Due to the improved AID of nutrients, there was a reduction in hindgut fermentation.

Introduction

Hullless barley (HB) differs from hulled barley in that the hull is less firmly attached to the kernel and consequently detached after threshing, leading to higher level of valuable nutrients and increased volume density. However, the crop contains a highly viscous carbohydrate fraction known as β -glucan at levels ranging between 40 g and 70 g kg⁻¹, consistently higher than in hulled barley (30–45 g kg⁻¹) and other non-starch polysaccharide (NSP) sugars, e.g. xylose (Baidoo and Liu, 1998). The objectives of this study were to investigate the effects of adding an enzyme mixture on apparent ileal digestibility (AID) and apparent overall digestibility (AOD) of nutrients of the complete feeds, each containing one of the five HB varieties plus canola meal as protein supplement.

Materials and Methods

HB (774 g kg⁻¹) varieties, Phoenix (P), Falcon (F), Silky (S), Condor (C) and Buck (B) were separately formulated with 200 g canola meal kg⁻¹ without or with the addition of 2 g enzyme cocktail kg⁻¹ (β -glucanase, activity 600 units; xylanase, activity 745 units; and protease, activity 500 units kg⁻¹ diet). In each diet, chromic oxide was included at 2 g kg⁻¹ as indigestible marker. Twenty Cotswold castrate pigs of 15 kg body weight, surgically fitted with post-valvular T caecum cannulas were used. The cannulated pigs were randomly allocated to individual pens according to a randomization design of the HB diet treatments. The experiment was based on a crossover design, with each pig undergoing three balance periods, and pigs were fed twice a day at 2.6 times maintenance

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energy requirement at each balance period. After a 5-day adaptation period, 5-day faeces collection was followed by 2-day collection of ileal digesta according to Yin *et al.* (2000). Amino acids were analysed using an LKB 4151 Alpha AA analyser (LKB Biochrom Ltd, Cambridge). Chromic oxide analysis was carried out according to Saha and Gilbreath (1991). NSP analysis was carried out according to Slominski and Campbell (1990). Analyses of β -glucanase and viscosity *in vitro* were carried out according to Inborr *et al.* (1993). Data were

statistically analysed using GLM procedures of SAS (1991).

Results

The contents of dietary fibre and the viscosity were highest in B and C and lowest in P. For example, the β -glucan content was 61.5, 58.8, 48.3, 46.1 and 40.2 g kg⁻¹, respectively, for diets B, C, S, F and P. The AID of DM, GE and CP were significantly ($P < 0.05$) higher for diet P followed by F, S

Table 35.1. Effect of enzyme addition on apparent ileal (I), hindgut (H) and overall (O) digestibility (%) of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and total non-starch polysaccharides (NSP) of pigs fed diets based on Phoenix (P), Falcon (F), Silky (S), Condor (C) and Buck (B).

	Diet						Enzyme		
	P	F	S	C	B	SEM	–	+	SEM
IDM	63.4 ^a	61.4 ^{ab}	60.0 ^{bc}	60.6 ^{bc}	58.3 ^c	1.56	58.4 ^b	63.1 ^a	1.89
IGE	64.3 ^a	62.3 ^{ab}	60.6 ^{bc}	61.9 ^{bc}	59.0 ^c	1.45	59.0 ^b	64.4 ^a	1.57
ICP	70.6 ^a	67.4 ^{ab}	66.1 ^b	57.1 ^c	57.3 ^c	1.99	62.1 ^b	66.4 ^a	1.49
INDF	17.2	17.5	17.5	18.8	15.9	3.98	13.0 ^b	20.0 ^a	4.89
INSP	29.3	30.1	31.6	27.8	31.7	4.89	25.5 ^b	33.6 ^a	5.32
HDM	17.2	17.4	16.6	16.2	16.8	1.86	18.7 ^a	15.3 ^b	1.09
HGE	15.6	15.0	14.8	15.3	15.7	1.90	16.9 ^a	14.0 ^b	1.11
HCP	4.1 ^b	4.4 ^b	5.1 ^b	8.4 ^a	9.8 ^a	1.87	9.0 ^a	6.8 ^b	1.69
HNDF	34.4	33.0	31.2	31.6	36.3	2.00	34.8 ^a	23.8 ^b	3.45
ODM	80.6 ^a	77.2 ^{ab}	76.2 ^b	78.6 ^{ab}	75.7 ^b	1.34	76.6 ^b	79.0 ^a	1.12
OGE	79.9 ^a	76.0 ^b	75.4 ^b	76.6 ^b	76.5 ^b	1.50	75.3 ^b	78.9 ^a	1.06
OCP	74.7 ^a	71.8 ^b	71.2 ^b	72.4 ^b	72.3 ^b	1.39	71.4	73.4	1.45
ONDF	51.6	52.0	56.4	54.8	52.2	4.20	50.4 ^b	56.5 ^a	3.24

^{abc}Values in the same row with different superscript letters differ ($P < 0.05$).

Table 35.2. Effect of enzyme addition on apparent ileal digestibility (%) of amino acids in pigs fed diets based on Phoenix (P), Falcon (F), Silky (S), Condor (C) and Buck (B).

	Diet						Enzyme		
	P	F	S	C	B	SEM	–	+	SEM
Arg	75.7 ^a	75.6 ^a	74.3 ^{ab}	73.1 ^{ab}	73.0 ^b	1.06	73.4	76.3	1.98
Iso	73.1 ^a	72.9 ^a	72.5 ^a	67.1 ^b	67.8 ^b	1.0	66.9 ^b	72.7 ^a	1.56
Leu	74.6 ^a	73.5 ^a	71.7 ^a	71.7 ^a	66.4 ^b	1.83	68.9 ^b	73.4 ^a	1.89
Lys	72.0 ^a	70.7 ^a	69.8 ^a	64.7 ^b	64.0 ^b	1.25	64.8 ^b	70.8 ^a	1.23
Met	77.6 ^a	75.8 ^a	73.7 ^{ab}	71.3 ^b	71.4 ^b	2.66	71.9	75.9	2.80
Phe	74.2 ^a	73.6 ^{ab}	73.0 ^{ab}	71.8 ^{ab}	70.6 ^b	1.18	69.9 ^b	75.4 ^a	1.25
Thr	65.1 ^a	64.8 ^a	63.6 ^a	59.5 ^b	58.2 ^b	1.79	59.1 ^b	64.3 ^a	1.11
Val	72.7 ^a	72.0 ^a	70.8 ^a	62.6 ^b	60.4 ^b	1.22	66.4	68.7	1.99
Ala	67.1 ^a	66.2 ^a	65.6 ^a	58.7 ^b	56.0 ^b	1.79	60.4 ^b	65.0 ^a	1.89
Tyr	63.6 ^a	66.5 ^a	62.7 ^{bc}	66.2 ^a	59.4 ^c	1.0	61.6 ^b	65.8 ^a	1.11

^{abc}Values in the same row with different superscript letters differ ($P < 0.05$).

and C, and lowest for diet B. Similarly to the AID, the AOD of DM, GE and CP were highest for diet P. Enzyme addition significantly ($P < 0.05$) increased both AID and AOD of nutrients but reduced the hindgut digestibility of DM, GE, CP and NDF (Table 35.1). The AID of AA was highest in diet P followed by F, S and C and lowest in B. Enzyme addition significantly ($P < 0.05$) increased the AID for most AA (Table 35.2).

Discussion

The reason for lower digestibility of nutrients in HB may be due to the higher level

of β -glucans. The measured mean β -glucans (51 g kg^{-1}) are 51, 82 and 70% higher, respectively, than that in hulled barley reported by Baidoo and Liu (1998) and Yin *et al.* (2000). The reasons for the differences in digestibility between the HB may be due to the different β -glucan contents. β -glucan is the main antinutritional factor in HB, as discussed previously; therefore, the enzyme treatment has relatively more impact in HB genotypes containing high levels of β -glucan. It is concluded that enzyme addition may improve digestibility of nutrients for young pigs fed HB-based diets. The magnitude of responses, however, appeared to depend on HB variety.

References

- Baidoo, S.K. and Liu, Y.G. (1998) Hulless barley for swine: ileal and fecal digestibility of proximate components, amino acids and non-starch polysaccharides. *Journal of Science of Food and Agriculture* 76, 397–403.
- Baidoo, S.K., Liu, Y.G. and Yungblut, D. (1998) Effect of microbial enzyme supplementation on energy, amino acid digestibility and performance of pig fed hulless barley for swine. *Canadian Journal of Animal Science* 78, 202–210.
- Saha, D.C. and Gilbreath, R.L. (1991) Analytical recovery of chromium from diet and faeces determined by colorimetry and atomic absorption spectrophotometry. *Journal of Science of Food and Agriculture* 55, 433–446.
- SAS (1991) *SAS User's Guide*, Version 6.03. SAS Institute, Cary, North Carolina.
- Slominski, B.A. and Campbell, L.D. (1990) Non-starch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. *Journal of Science of Food and Agriculture* 53, 175–184.
- Yin, Y.L., McEvoy, J.D.G., Schulze, H. and McCracken, K.J. (2000) Studies on cannulation and alternative indigestible markers and the effect of food enzyme supplementation in barley-based diets on ileal and overall digestibility in growing pigs. *Animal Science* 70, 63–72.

36 Relationships Between Nutrient Digestibility, β -Glucan Content and Ileal Digesta Viscosity in Pigs Fed Different Australian Barley Cultivars

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Experiments were conducted to define the relationship between barley β -glucan content, digestible energy (DE) content and true ileal digestible lysine (TIDL) content with ileal digesta viscosity. A significant correlation was found between DE content ($P < 0.05$) and TIDL content ($P < 0.05$) to ileal digesta viscosity, but there was no relationship ($P > 0.05$) between these parameters and β -glucan content of the barley cultivars examined. The results suggest that non-starch polysaccharide (NSP) structure rather than the content of specific components has more influence on the digestibility of key dietary nutrients.

Introduction

The major non-starch polysaccharides (NSP) found in the plant cell walls of barley are mixed-link β -(1–3),(1–4) glucans, arabinoxylans and cellulose; however, the β -glucan fraction may possess antinutritive properties. Reports pertaining to the antinutritive properties of barley β -glucans in growing pigs and interrelationships with viscosity are scarce and, where reported, the data are equivocal. This may be due to: (i) the twofold variation in total β -glucan content that exists and/or (ii) the greater digestibility of β -glucan in the gastrointestinal tract (Pluske *et al.*, 1999). The soluble β -glucan fraction is degraded considerably at the terminal ileum (67%) compared with the insoluble β -glucan fraction (21%). At the faecal level there was nearly complete disappearance of soluble β -glucan but considerable recovery of insoluble β -glucan (Bach Knudsen and Canibe, 1997). A greater ileal digestibility

of soluble β -glucan may produce less-viscous chyme that, in turn, may result in higher nutrient digestibility. The aim of this experiment was to assess the digestible energy (DE) content and true ileal amino acid digestibility (TIAAD) of some Australian barleys, and to determine whether the β -glucan content and subsequent viscosity of the ileal digesta influences these values.

Materials and Methods

Six barley varieties grown in Australia in 1996/97 were used in a preliminary study to assess the relationship between DE content, true ileal digestible lysine (TIDL), β -glucan contents and ileal digesta viscosity. Diets used were cold press pelleted and contained (kg^{-1}) 945.05 g test barley, 30 g dicalcium phosphate, 2.75 g salt, 1.2 g minerals and vitamins, 1 g choline chloride and 20 g Celite® (acid-insoluble ash indigestible marker).

The DE content of the six barley varieties was determined by total faecal collection using 24 Large White $\times$ Landrace male pigs (35–40 kg). Faeces were frozen immediately at -20°C and, after thawing, DE content of each barley variety was determined with bomb calorimetry and acid-insoluble ash measurements (van Barneveld *et al.*, 1994). The TIDL content was determined in Large White $\times$ Landrace male pigs (35–40 kg) that were surgically fitted with simple T-piece ileal cannulas (van Barneveld *et al.*, 1994). Diets were allocated based on a 5×5 Latin square design in two experiments, utilizing a common control in each experiment and three test barleys per experiment. An additional diet of (kg^{-1}) 125 g enzymatically hydrolysed casein (EHC), 300 g raw sugar, 520.05 g starch, 30 g dicalcium phosphate, 2.75 g salt, 1.2 g minerals and vitamins, 1 g choline chloride and 20 g Celite[®] was also included so that endogenous amino acid losses could be determined for true ileal digestibility calculations.

Test diets were introduced gradually over 3 days following a surgery recovery period of 7 days. Each feeding period consisted of 7 days, with pigs fed at three times their estimated maintenance requirement for energy (i.e. $0.5 \times \text{BW}^{0.75}$). On days 6 and 7 of each feeding period, ileal digesta was collected on to ice from each pig over two consecutive days at 2 h intervals (0800, 1000, 1200, 1400 and 1600 h). Ileal digesta were frozen immediately at -20°C after collection. Amino acids were analysed following hydrolysis for 24 h

with 6N HCl using ion-exchange chromatography and post-column derivatization with ninhydrin. The enzyme hydrolysed protein/ultrafiltration method of Butts *et al.* (1991) was used to determine endogenous flows of amino acids. Viscosity was determined using digesta supernatant prepared after centrifugation at 12,000 g for 12 min at 4°C . Supernatant viscosity was measured using a Brookfield DV-III viscometer. Barley β -glucan content was determined using AOAC method 995.16.

Results and Discussion

Ileal viscosity values were low and ranged from 1.61 to 2.05 cP. Total β -glucan content ranged from 3.29 to 4.80%. Barley DE content ranged from 11.38 to 12.99 MJ kg^{-1} (air-dry) while TIDL content ranged from 2.13 to 3.27 g kg^{-1} (air-dry basis; Table 36.1).

The DE content was negatively and significantly correlated with ileal digesta viscosity ($y = -2.637x + 16.426$; $r^2 = 0.547$; $P < 0.05$). The TIDL content was also negatively and significantly correlated with ileal viscosity ($y = -4.622x + 9.584$; $r^2 = 0.736$; $P < 0.05$). These data suggest that, despite the narrow range of viscosities recorded (0.44 cP), the increases in viscosity were of sufficient magnitude to depress energy digestibility and TIDL.

There was no correlation between DE content and β -glucan content, or ileal digesta viscosity and barley β -glucan content, respectively (DE vs. β -glucan: $y =$

Table 36.1. Digestible energy, TIDL, β -glucan and ileal digesta viscosity contents of six Australian barleys fed to pigs.

Barley cultivar	Digestible energy (MJ kg^{-1})	True ileal digestible lysine (g kg^{-1})	β -Glucan (%)	Ileal viscosity (cP)
Tantangara	12.64	2.70	3.68	1.61
Galleon	12.64	2.13	3.96	1.36
Schooner (Region 1)	12.90	3.07	3.52	1.34
Skiff	12.99	3.27	3.74	1.38
Schooner (Region 2)	11.38	2.97	3.29	1.65
Grimmet	12.09	2.98	4.80	1.73

$0.0267x + 12.338$; $r^2 = 0.0005$; $P > 0.05$; ileal digesta vs. β -glucan: $y = 0.1222x + 1.0433$; $r^2 = 0.142$; $P > 0.05$). We have shown previously that there is no relationship between DE content and the TIDL content of the barley cultivars (van Barneveld *et al.*, 1999).

In cases where there are only subtle differences in the content of dietary NSP, the digestibility of energy and amino acids in growing pigs appears to be influenced more by the structure of the NSP (which in turn

influences digesta viscosity) rather than by the content of specific NSP, such as β -glucan. While the suppressive influence of dietary fibre has been demonstrated on many occasions, preliminary results from the current experiment suggest that an improved understanding of the specific influences of NSP structure and content on the nutritional quality of feed ingredients is required if nutritionists are to account accurately for variation in nutritional value between ingredients.

References

- Bach Knudsen, K.E. and Canibe, N. (1997) Digestion of carbohydrates in the small and large intestine of pigs fed on wheat or oat based rolls. In: Laplace, J.-P., F  vrier, C. and Barbeau, A. (eds) *Proceedings of the VIIth International Symposium on Digestive Physiology in Pigs*. EAAP Publication No. 88, St Malo, France, pp. 562–566.
- Butts, C.A., Moughan, P.J. and Smith, W.C. (1991) Endogenous amino acid flow at the terminal ileum of the rat determined under peptide alimentation. *Journal of the Science of Food and Agriculture* 5, 175–187.
- Pluske, J.R., Pethick, D.W., Durmic, Z., Hampson, D.J. and Mullan, B.P. (1999) Non-starch polysaccharides in pig diets and their influence on intestinal microflora, digestive physiology and enteric disease. In: Garnsworthy, P.C. and Wiseman, J. (eds) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, pp. 189–226.
- van Barneveld, R.J., Batterham, E.S. and Norton, B.W. (1994) The effect of heat on amino acids for growing pigs. 1. A comparison of ileal and faecal digestibilities of amino acids in raw and heat-treated field peas (*Pisum sativum* cv. Dundale). *British Journal of Nutrition* 72, 221–241.
- van Barneveld, R.J., Ru, Y.R., Szarvas, S.R., Wyatt, G.F., Dunshea, F.R. and Pluske, J.R. (1999) Range in digestible energy and true ileal digestible lysine content of Australian barley samples. In: Cranwell, P.D. (ed.) *Manipulating Pig Production VII*. Australasian Pig Science Association, Victoria, Australia, p. 267.

37 *In Vitro* Release of Medium-chain Fatty Acids (MCFA) from Selected Fat Sources with Selected Exogenous Lipolytic Enzymes in Pig Gastric Simulated Conditions

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Seeking a valuable alternative to nutritional antibiotics used in piglet nutrition, the present study investigated whether appropriate amounts of medium-chain fatty acids (MCFA) could be liberated in simulated gastric conditions (pH 3–6; 3 h; 37°C; synthetic diet; shaking water bath) from four selected MCFA-containing fats (coconut oil, MCTAG1, MCTAG2, butter oil) and six lipases. Depending on the conditions applied, a degree of lipolysis up to 20% was obtained in the medium. The *in vitro* protocol used offers an excellent tool for screening the numerous combinations of MCFA-containing fat sources and lipolytic enzymes for their usefulness as pig feed supplements with potential stabilizing or suppressive effects on the gut flora.

Introduction

Although only 15% of the antibacterials used in Europe are applied as feed additives, concerns about the possible risks (e.g. selection of antibiotic-resistant microorganisms and cross-resistance) for humans have led to a search for alternatives. Following the recent EU ban on the majority of these in-feed antibiotics, alternative measures (management, feeding, hygiene) and/or products have been (or will be) worked out. Examples are probiotics, prebiotics, enzymes, herbs and plant extracts, prefermented feeds and organic acids (Thomke and Elwinger, 1998). However, for unknown reasons, the zootechnical improvements with classical organic acids are not always consistent. The antimicrobial properties of various free fatty acids, especially medium-chain fatty acids (MCFA) (C4–C12), and their fatty

acid monoglycerol esters have been known for many years (for a review, see Kabara, 1978). Moreover, medium-chain triacylglycerols (MCTAG) elicit nutritional and physiological responses in mammals that differ from those of long-chain triacylglycerols (LCTAG) (Odle, 1999). However, an excess of MCFA can have important unwanted side-effects: they can be narcotic when applied as a force-fed lipid emulsion bolus to piglets shortly after birth. Besides, MCFA are a strong stimulus of cholecystokinin and perhaps other intestinal hormones with a pronounced satiating effect that could interfere with gastric emptying and feed intake. A lower feed intake could also be the result of the strong goat-like odour and aversive taste of MCFAs. To avoid those side-effects, a concept was set up for the combined in-feed use of selected MCTAGs and selected lipases in order to liberate MCFAs gradually in the upper

intestinal parts, which we believe to be the most likely site for control of the microbial load.

Materials and Methods

The selected lipolytic enzymes, provided by Kemin Europa (B), were coded L1, L2, L3, L5 (microbial origin), L4 (porcine pancreas) and L6 (calf pancreas). The fat sources used were coconut oil, MCTAG1, MCTAG2 and butter oil and they contained 65.2%, 97.2%, 93.6% and 12.6% MCFA, respectively.

For the incubation system used in series I, the medium was made up of the following ingredients: 0.25 g of the selected fat source (molten, if necessary); 2.25 g of a synthetic diet; 10 ml buffer solution of pH 3, pH 4, pH 5 or pH 6; 0.5 ml pepsine solution (50 mg 100 ml⁻¹ distilled water); and 0.5 ml of a lipolytic enzyme suspension to provide 10,000 ppm in fat. Incubation was in duplicate in tightly closed vessels for 180 min at 37°C in a shaking water bath, simulating the mean retention time of feed particles and pH in the stomach of the pig (Cranwell *et al.*, 1976; Furuya *et al.*, 1978). To stop the lipolysis, 1 ml 3M HCl was added immediately to reach pH 1. In series II, the same incubation conditions as in series I were used, except that the fat source was dispersed, before introduction into the vessels, as follows: 10 g of molten fat, 10 ml aq. d. and 20 ml of a dispersion solution (glycerol/gum arabic) (Ultra Turrax, 20,500 rpm, 1 min).

Fatty acids (individual, total and free) were analysed as isopropyl esters by capillary gas-liquid chromatography. All analyses were carried out in duplicate on each incubation sample.

Results and Discussion

The degree of fat hydrolysis was assessed by analysing the free fatty acid (FFA) content after the incubation experiment. No other fat digesta products – diacylglycerols (DAG) or monoacylglycerols (MAG)

– were analysed because, in general, lipases from microbial origin show a very broad or a *sn*-1,3 specificity with no pronounced fatty acid specificity. Thin-layer chromatography analysis of lipid classes did indeed reveal that the primary products of microbial lipolysis were FFA, as also reported by Linfield *et al.* (1984). The sequence of fat digestion products with pancreatic lipases (L4, L6) is TAG > 1,2 (2,3) DAG > 2-MAG and FFA, with relatively little hydrolysis of the fatty acid at the 2 position. Without using any dispersion in the medium, for all fat sources the best results were obtained with L2 and L5, while hydrolytic activity was highest between pH 3 and 4 with each enzyme, fitting well with the pH normally occurring *in vivo* in the stomach of young pigs (Cranwell *et al.*, 1976). With these enzymes, total lipolysis (FFA as % of total fatty acids present) reached at pH 3 a maximum of 9%, 18%, 15% and 5% for coconut oil, MCTAG1, MCTAG2 and butter oil, respectively. L3 also had a high activity on MCTAG2 and butter oil. The amount of released FFA seems to depend on the amount present in the fat source, so the enzymes used showed low substrate specificity. In incubation series I, the amount of released MCFA (as % of total fatty acids present) was 3.5% for coconut oil, 10–15% for MCTAG1 and MCTAG2 and 0.5% for butter oil with L2 and L5.

In order to increase the screening capacity of the incubation system for the different lipolytic enzymes, a new experiment was set up in series II where fats were dispersed in a glycerol/gum arabic medium. It is known that during digestion in the stomach of non-ruminants, a coarse dispersion of fat (droplets of 200–50,000 Å) is already formed, due to the churning action of the stomach (Glickman, 1983), upon which preduodenal lipases may act. In series II again the highest rates of lipolysis were noted with L2 and L5. With these lipases, the amounts of total fatty acids released were raised to about 16–18% for coconut oil, 14–18% for MCTAG1 and MCTAG2 and 8% for butter oil, corresponding with a release of 9%

total free MCFA for coconut oil, 16–18% for MCTAG1, 16% for MCTAG2 and 0.85% for butter oil.

It can be concluded that, depending on the conditions applied, a degree of lipolysis up to 20% can be obtained in the medium. The *in vitro* protocol used offers an excellent tool for the screening of the numerous combinations of MCFA-containing fat sources and lipolytic enzymes for their usefulness as feed supplements with a potential stabilizing or suppressive effect on the gut flora. Results of further research carried out *in vitro* and *in vivo* with gastro-cannulated piglets, and of zootechnical experiments to validate the concept of the combined use of triacylglycerols containing

MCFA and exogenous lipolytic enzymes for controlled release in the foregut of MCFA, as an alternative for nutritional antibiotics in piglet nutrition, will be published in the near future. A patent has been applied for by the authors and the financiers for the protection of the concept.

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References

- Cranwell, P., Noakes, D. and Hill, K. (1976) Gastric secretion and fermentation in the suckling pig. *British Journal of Nutrition* 36, 71–86.
- Furuya, S., Sakamoto, K., Asano, T., Takahashi, S. and Kameoka, K. (1978) Effects of added dietary sodium acrylate on passage rate of markers and apparent digestibility by growing pigs. *Journal of Animal Science* 47, 159–165.
- Glickman, R. (1983) Fat absorption and malabsorption. *Clinical Gastroenterology* 12, 323–334.
- Kabara, J. (1978) Fatty acids and derivatives as antimicrobial agents – a review. In: Kabara, J. (ed.) *The Pharmaceutical Effects of Lipids*. AOCS, Champaign, Illinois, pp. 1–14.
- Linfield, W., Barauskas, R., Sivieri, L., Serota, S. and Stevenson, R. (1984) Enzymatic fat hydrolysis and synthesis. *Journal of the American Oil Chemists' Society* 61, 191–195.
- Odle, J. (1999) Medium-chain triglycerides: a unique energy source for neonatal pigs. *Pig News and Information* 20, 25N–32N.
- Thomke, S. and Elwinger, K. (1998) Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. *Annales de Zootechnie* 47, 245–271.

38 Ileal Digestibility Determination of Different Diets by Two Fistulation Techniques (T-cannula vs. Steered Ileocaecal Valve Cannula) and Two Markers (Chromic Oxide vs. Titanium Oxide)

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T-cannulation was compared with SICV cannulation in ileal digestibility experiments with five diets. Chromic oxide and titanium oxide were used as markers. Digestibility determinations were not influenced by cannulation technique. However, standard deviations in SICV cannulation were smaller than those in T-cannulation. Repeatability of the determination of apparent digestibility of dry matter, crude protein and lysine as a weighted deviation of the mean of diets (residual mean square error) was 2.5–3.3 for SICV cannulation and 5.4–6.2 for the T-cannulation.

Introduction

Several reviews (Fuller, 1991; Danfær and Fernández, 1999) have examined the merits of the different methodologies employed in the last 30 years to measure ileal digestibilities. None of them found enough evidence to indicate the overall superiority of any one procedure. T-cannulation allows only partial sampling of digesta. Apart from being dependent on the use of markers, the technique also depends on the assumption that the partial sample obtained is perfectly representative of the total flow. Mroz *et al.* (1994) developed a technique for the collection of digesta through a single large T-cannula placed in the caecum opposite the ileocaecal valve (SICV). This last approach is promising in the sense that the surgery required is less

traumatic than most other methods and preserves the integrity of the small intestine. However, the technique is still dependent on markers. The T-cannulation technique has been used at the Danish Institute of Agricultural Sciences for many years but results have often been vitiated by unreasonably large deviations. Therefore, a comparison with the SICV technique was performed in order to test its adequacy for a selected number of feed-stuffs with varying chemical composition and using two markers.

Materials and Methods

One pig (35 kg) from each of five litters was surgically fitted with either a T-cannula (16 mm diameter) or an SICV-cannula. After a

recovery period of 14 days, the pigs were fed one of five diets according to a double 5×5 Latin square arrangement. The experimental diets consisted of an N-free mixture or soybean meal, sunflower meal, peas or rapeseed cake diluted to about 16% protein with the N-free mixture. Two markers, chromic oxide (0.5 g kg^{-1} diet) and titanium oxide (1.5 g kg^{-1} diet), were added. The pigs were fed three times daily at intervals of exactly 8 h. Each experimental period lasted 14 days in total. For the first 4 days the pigs were fed a Foulum Standard grower diet, followed by 10 days on the experimental diet. Two 8 h quantitative collections of ileal digesta from the pigs were conducted at intervals of 1 day to avoid dehydration and/or electrolyte depletion. Water was provided with the feed ($2.5 \text{ l water kg}^{-1}$ feed). Sampling of ileal digesta was performed in the pens by means of plastic bags attached to the cannula. Differences between determinations for each cannulation technique were tested by analysis of variance, with technique, diet, technique $\times$ diet, experimental period and animal within technique as independent variables. The reliability of the determinations by each technique, expressed as the 'repeatability' (RMSE, residual mean square error), was estimated by variance analysis within technique or marker, after the model $X_{ij} = \mu_i + \epsilon_{ij}$, where X_{ij} = the j^{th} replicate determination of the i^{th} sample, μ_i = mean of the i^{th} sample and ϵ_{ij} = deviation from the mean.

Results and Discussion

The apparent ileal digestibility of four diets (results of the N-free diet excluded) with the two cannulation techniques is shown in Table 38.1. Digestibility determinations were not significantly influenced by cannulation technique. In general, there was good agreement between values obtained with the two techniques except for the case of soybean meal, where values obtained with the SICV-cannula were about 5–6 percentage units higher than those obtained with the T-cannula.

The repeatability of the determination of the apparent digestibility of dry matter, protein and lysine – by each cannulation technique – is shown in Table 38.2. Generally, determinations obtained with SICV cannulation showed substantially less variation than those obtained with the T-cannula, which indicates that although the techniques yield similar digestibility estimates, it can be expected that repeated measurements with SICV cannulation will be more consistent than those obtained with T-cannulation. This is most probably because digesta samples obtained by SICV cannulation are more homogeneous than those obtained by T-cannulation.

The digestibility of four diets as determined with chromic oxide (Cr) or with titanium oxide (Ti) is presented in Table 38.3. Chromic oxide determinations were in all cases a little higher than those obtained with titanium oxide. The differences –

Table 38.1. The apparent ileal digestibility of diets (% of intake) determined by SICV or T-cannulation and chromic oxide.

	Dry matter		Crude protein		Lysine	
	SICV	T	SICV	T	SICV	T
Diet						
Soybean meal	80	75	79	75	88	83
Sunflower meal	67	68	68	70	68	70
Peas	74	76	79	84	91	92
Rapeseed cake	68	67	66	72	77	78
P-value						
Cannula	0.776		0.162		0.970	
Diet	0.001		0.001		0.001	
Cannula $\times$ diet	0.317		0.153		0.337	

Table 38.2. The repeatability of apparent ileal digestibility determinations with SICV- or T-cannulation and chromic oxide ($n = 5$).

	Dry matter		Crude protein		Lysine	
	SICV	T	SICV	T	SICV	T
RMSE	3.12	5.39	2.46	6.24	3.30	5.70

Table 38.3. The apparent ileal digestibility of diets (% intake) determined with chromic oxide (Cr) or titanium oxide (Ti).

	Dry matter		Crude protein		Lysine	
	Cr	Ti	Cr	Ti	Cr	Ti
Diet						
Soybean meal	77.4	76.7	77.0	76.3	85.8	85.3
Sunflower meal	67.4	66.6	69.1	68.4	68.7	68.0
Peas	74.8	73.6	81.4	80.5	91.2	90.8
Rapeseed cake	67.4	66.8	69.0	68.4	77.4	77.0
Mean (Cr – Ti)	0.8		0.7		0.5	
Marker <i>t</i> -test	0.001		0.001		0.002	
<i>P</i> -value						
Diet	0.772		0.967		0.840	
Cannula	0.043		0.061		0.068	
Diet × cannula	0.519		0.399		0.478	

although significant – were not of practical relevance. In contrast, Yin *et al.* (2000) found that determinations with Cr were substantially lower than those with Ti.

In summary, comparison of SICV and T-cannulation and two markers with diets of different chemical composition did not

reveal any marked difference between the cannulae or between markers. Because of the simplicity in surgery and subsequent cannula handling, T-cannulation should be preferred. However, in cases where the precision of each determination is crucial, SICV cannulation should be the option.

References

Danfær, A. and Fernández, J.A. (1999) Developments in the prediction of nutrient availability in pigs: a review. *Acta Agriculturae Scandinavica, Section A, Animal Science* 49, 73–82.

Fuller, M.F. (1991) Methodologies for the measurement of digestion. In: Verstegen, M.W.A., Huisman, J. and den Hartog, L.A. (eds) *Vth International Symposium on Digestive Physiology in Pigs*. EAAP Publication no. 54, 273–288.

Mroz, Z., Bakker, G.C.M., Dekker, R.A., Jongbloed, R. and Jongbloed, A.W. (1994) Application of the steered ileo-caecal valve cannulation (SICV) technique to measure digesta kinetics and ileal digestibility in pigs fed high fibrous diets (3). In: Souffrant, W.-B. and Hagemeister, H. (eds) *Vth International Symposium on Digestive Physiology in Pigs*. EAAP Publication no. 80, 57–59.

Yin, Y.-L., McEvoy, J.D.G., Schulze, H. and McCracken, K.J. (2000) Studies on cannulation method and alternative indigestible markers and the effects of food enzyme supplementation in barley-based diets on ileal and overall apparent digestibility in growing pigs. *Animal Science* 70, 63–72.

39 Soluble Saccharides, Volatile Fatty Acids and Lactic Acid in Stomach and Ileum of Pigs Fed Wheat Bran-based Diets with and without Enzyme Treatment

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Pigs were fed diets containing 40% wheat bran incubated (control C) with a cellulase (Cel-*i*) or xylanase (Xyl-*i*) preparation, or with addition of the cellulase (Cel-*a*) or xylanase (Xyl-*a*) preparation immediately before feeding. Incubation of wheat bran reduced neutral detergent fibre and increased soluble starch, β -glucans and saccharides, especially for the cellulase preparation. Gastric and ileal arabinose and xylose concentrations were higher for most enzyme-treated diets but there were no significant differences in volatile fatty acid and lactic acid concentrations. It can be concluded that the amount of soluble saccharides in stomach and small intestine may be increased by cell wall-degrading enzyme preparations.

Introduction

The main non-starch polysaccharides (NSP) of wheat are arabinoxylans. When crude cellulase or xylanase preparations are used in pig diets with 40% wheat bran, the β -glucanase and cellulase activities in stomach and ileum increase (Inbarr *et al.*, 1999). In this study the effect of treating wheat bran with those fibre-degrading enzymes on the degradation of NSP and on the concentrations of volatile fatty acids (VFA) and lactic acid in the upper part of the gastrointestinal tract is reported. Two methods of enzyme application were employed: either pretreatment of wheat bran with the enzyme preparations or adding the enzyme preparations just before feeding.

Material and Methods

Five crossbred barrows of approximately 31 kg live weight were fitted with stomach and ileal cannulae and fed five diets for five 2-week periods in a 5×5 Latin square design. The diets contained wheat bran (40%) as the only source of NSP. The wheat bran was incubated with a water/acetic acid mixture at 39°C and pH 5.0 for 3.5 h (control diet, C). For treatments Cel-*i* and Xyl-*i* the wheat bran was incubated in the same conditions with either a crude cellulase or a xylanase preparation, respectively. For treatments Cel-*a* and Xyl-*a*, either the cellulase or the xylanase preparation, respectively, was added to wheat bran (treated in the same way as in the control diet) immediately

before feeding. The pigs were fed twice daily. Samples from the stomach (collected 0, 2, 4, 6 and 8 h after feeding) and from the ileum (collected in six 2 h intervals after feeding) were analysed for soluble saccharides, VFA and lactic acid. Saccharides were also determined in the urine.

Results

Incubation of wheat bran without enzymes resulted in a small reduction of NDF and an increase in the amount of soluble starch, β -glucans and saccharides. Higher amounts of soluble saccharides were measured for incubations with the enzyme preparations, especially the cellulase preparation. After all incubations, 62% of the soluble saccharides were monosaccharides.

Two hours after feeding diets Cel-*i*, Cel-*a* and Xyl-*i*, concentrations of arabinose and xylose in the stomach were higher compared with diet C, with 69% monosaccharides for diet C and 51% for diets Cel-*i*, Cel-*a* and Xyl-*i*. Six hours after feeding, the concentration of saccharides in the stomach was about seven times lower than 2 h after feeding and the portion of monosaccharides averaged 32%.

Glucose, xylose, arabinose, fructose and traces of galactose were present in the ileum 2–4 h after feeding. Xylose and arabinose concentrations were higher after feeding the enzyme-treated diets, but this was only significant for diet Cel-*i*. The portion of monosaccharides averaged 50%. In the period 6–8 h after feeding, the saccharide concentration was on average 0.7 times the concentration 2–4 h after feeding. The xylose and arabinose concentrations were higher for the enzyme-treated diets, and the portion of monosaccharides averaged 30%.

After feeding diet C, small traces of glucose, xylose, galactose and fructose and slightly more arabinose were found in the urine. The excretion of monosaccharides increased after feeding the enzyme-treated diets, with the exception of diet Xyl-*a*. The higher urinary excretion of monosaccha-

rides for the cellulase-treated diets and for diet Xyl-*i* was mainly caused by a higher excretion of xylose and arabinose.

In the stomach, highest VFA concentration was measured shortly after feeding and thereafter VFA concentration declined with time. The relative contributions of acetic and propionic acids were inversely related. Shortly after feeding, acetic acid accounted for 0.88 and this proportion declined to 0.76 at 8 h after feeding, while propionic acid accounted for 0.06 shortly after feeding and this proportion increased to 0.14 at 8 h after feeding. Shortly after feeding, lactic acid concentration in the stomach averaged 7.6 mmol l⁻¹ and increased up to 22.7 mmol l⁻¹ 4 h after feeding. Thereafter lactic acid concentration decreased. There were no significant differences in VFA and lactic acid concentration in the stomach between the diets.

The VFA concentration in the ileum remained reasonably constant with time after feeding (acetic acid 0.73, propionic acid 0.20, butyric acid 0.05). From 4 h after feeding, the VFA concentration tended to be higher for diets Cel-*i* and Xyl-*i*. In the first 2 h after feeding, ileal lactic acid concentration averaged 2.4 mmol l⁻¹. Highest concentrations were measured 4–6 h after feeding, after which the concentration declined. There were no differences in lactic acid concentration between the diets.

Discussion

The cellulase preparation was active not only at pH 5 (during the incubations) but at wider ranges of pH as found in the stomach (Inborr *et al.*, 1999). This resulted in an increase in the concentration of soluble saccharides in the stomach, both for diets Cel-*i* and Cel-*a*. However, reductions of released oligosaccharides in the stomach seemed to be less than during incubation, since in the stomach only about half of all soluble saccharides were monosaccharides. The amount of soluble saccharides released by the xylanase preparation was less compared with the cellulase preparation. This

may be expected, since not only the β -glucanase activity but also the xylanase activity of cellulase-treated wheat bran was higher than that of xylanase-treated wheat bran, notwithstanding the aim to supply the same amounts of xylanase activity (Inborr *et al.*, 1999).

The xylose:arabinose ratio in the ileum was lower than in the stomach. This may be caused by a difference in absorption. Xylose disappears almost completely from the small intestine (Schutte *et al.*, 1991), while 30% of ingested arabinose enters the large intestine (Schutte *et al.*, 1992). Moreover, a proportion of all saccharides entered the large intestine, not only xylose and arabinose but also glucose. The slightly higher arabinose concentration found in the urine after feeding diet C may reflect the effect of incubation of the wheat bran. The higher urinary excretion of monosaccharides for the cellulase-treated diets and for diet Xyl-*i* reflects the effect of these cell wall-degrading enzymes, since in pigs a dose-dependent urinary excretion exists for both xylose and arabinose (Schutte *et al.*, 1991, 1992).

In the stomach there was no effect of the higher soluble saccharide concentration upon fermentation and there was no more than a tendency to increased VFA concentrations for the diets incubated with the cellulase or xylanase preparation. The observations that *in vitro* mono- and oligosaccharides stimulate lactic acid fermentation (Dierick and Decuypere, 1995) and that dietary L-arabinose and D-xylose result in higher ileal VFA concentrations (Schutte *et al.*, 1991, 1992) may be related to the much higher dosages used. The relatively constant VFA concentration in the ileum measured with time after feeding is in agreement with earlier observations of Argenzio and Southworth (1975).

It can be concluded that using cell wall-degrading enzyme preparations may increase the amount of soluble saccharides in stomach, small intestine and urine. This effect depends upon the nature of the enzyme preparation used and the method of enzyme application. However, using cell wall-degrading enzyme preparations only slightly affects fermentation in the small intestine.

References

- Argenzio, R.A. and Southworth, M. (1975) Sites of organic acid production and absorption in gastrointestinal tract of the pig. *American Journal of Physiology* 228, 454–460.
- Dierick, N.A. and Decuypere, J.A. (1995) Advances in the use of enzymes in pig nutrition. In: van Hartingsveldt, W., Hessing, M., van der Lugt, J.P. and Somers, W.A.C. (eds) *Second European Symposium of Feed Enzymes*. TNO Nutrition and Food Research Institute, Zeist, pp. 23–29.
- Inborr, J., Puhakka, J., Bakker, J.G.M. and van der Meulen, J. (1999) β -Glucanase and xylanase activities in stomach and ileum of growing pigs fed wheat bran based diets with and without enzyme treatment. *Archives of Animal Nutrition* 52, 263–274.
- Schutte, J.B., de Jong, J., Polziehn, R. and Verstegen, M.W.A. (1991) Nutritional implications of D-xylose in pigs. *British Journal of Nutrition* 66, 83–93.
- Schutte, J.B., de Jong, J., van Weerden, E.J. and Tamminga, S. (1992) Nutritional implications of L-arabinose in pigs. *British Journal of Nutrition* 68, 195–207.

40 Is the Rat a Reliable Model for the Growing Pig for Estimating Standardized Digestibility of Protein and Amino Acids?

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The potential for using the rat as a suitable and relatively inexpensive *in vivo* model for the growing pig is investigated. The standardized digestibility of protein and amino acids was determined in barley, wheat, soybean meal and rapeseed meal. Two *in vivo* methods with pigs and rats, respectively, and one *in vitro* method were compared. The *in vivo* determination with pigs was performed with cannulated growing pigs. The determination with rats was performed by a slaughter method. The *in vitro* digestibility was determined by a two-step pepsin/pancreatin method. Generally, a good agreement between all three methods was found, indicating that the rat may serve as a reliable model for the growing pig in confirming results from *in vitro* measurements of standardized digestibility of protein and amino acids in feedstuffs without *in vivo* data.

Introduction

In modern feed evaluation it is essential to use actual and reliable figures on protein and amino acid digestibility. To ensure that the values are unaffected by experimental conditions, standardized digestibility should be used. Furthermore, it is valuable to take into account the variation of each feed batch. As *in vivo* methods are time consuming and expensive, it is necessary to use *in vitro* methods for the determination of standardized digestibility. A two-step pepsin/pancreatin method corrected for the feed-specific endogenous loss would be suitable (Boisen, 1998). For validation of the *in vitro* method it is relevant to investigate whether a relatively cheap *in vivo* model with rats can be used. This will in particular be useful for *in vitro* results obtained with more unusual feedstuffs that have not been investigated directly for pigs.

Material and Methods

In vivo method – rats

A total of 98 male Wistar rats, with an average body weight of 200 g, were housed individually under standardized conditions. The maximum particle size of raw materials was 1 mm. Experimental diets based on barley or wheat were supplied with vitamins and minerals and fed without further preparation, while diets based on rapeseed meal or soybean meal were diluted with an N-free mixture before the addition of vitamins and minerals. For determination of the basal endogenous loss of protein and amino acids, a group of rats was fed an N-free diet. The number of rats allocated to different treatments was as follows: barley, 20; wheat, 20; rapeseed meal, 16; soybean meal, 24; and N-free diet, 18. The rats were adapted to the test diets for 3 days and slaughtered on day 4, approximately 4 h

after they were fed the test diets. Sampling was done by flushing the content of the last quarter of the small intestine with isotonic water. The standardized digestibility of protein and amino acids in barley and wheat was determined by the N-free method; for rapeseed meal and soybean meal a regression method was used.

In vivo method – pigs

Twenty-four female ileo-cannulated growing pigs were housed individually. Sampling was performed when body weights of the pigs corresponded to approximately 45, 60 and 75 kg. Four treatments and three replicates were used. Each feedstuff was fed in six dilutions. Dilutions were prepared using an N-free mixture. All diets were supplemented with vitamins and minerals. Standardized digestibility of barley, wheat, rapeseed meal and soybean meal was determined by regression.

In vitro method

Real digestibility of crude protein and amino acids was determined by a two-step pepsin/pancreatin method, basically according to the experimental procedure described by Boisen and Fernández (1995). Four samples of each raw material of about 0.5 g were used for crude protein determi-

nation. For amino acids the sample size was modified to 2 g. Four samples were used for each raw material. Two samples were used for determination of nitrogen, and a pooled sample of the other two samples was used for amino acid analyses. Standardized digestible crude protein and amino acids were calculated according to Boisen (1998). Because of the modification of sample size, the content of amino acids was corrected for sample size before calculating standardized digestibility.

All diets and samples used in the trials were made from the same batch of each raw material. In both *in vivo* experiments, chromium oxide was used as a marker.

Results and Discussion

Data for *in vitro* and *in vivo* standardized ileal digestibility of crude protein and amino acids in different raw materials are shown in Table 40.1.

With the actual set-up of the trial it was not possible to perform a statistical evaluation when comparing figures on digestibility obtained with different methods. Thus, the current results can only be indicative. Data obtained for pigs and rats show differences. Some of the differences are great, but they are not consistent. It is expected that these differences may be reduced after improvements in the rat assay. Critical points are the sampling procedure and the

Table 40.1. Standardized ileal crude protein (CP) and amino acid digestibility (%) determined *in vitro* and *in vivo* on rat and pig.

	Barley			Wheat			Rapeseed meal (high fat)			Soybean meal		
	<i>In vitro</i>	Rat	Pig	<i>In vitro</i>	Rat	Pig	<i>In vitro</i>	Rat	Pig	<i>In vitro</i>	Rat	Pig
CP	77.8	74.8	79.0	79.8	86.5	85.8	80.1	69.6	78.7	89.1	87.8	86.9
Lys	74.1	75.4	78.1	81.6	83.6	84.9	80.8	78.8	83.6	92.0	89.3	90.6
Met	79.3	82.0	80.8	84.7	89.7	88.7	86.0	89.0	88.1	92.6	91.5	91.0
Cys	78.0	82.2	79.3	85.5	91.8	86.2	80.5	76.6	78.2	84.2	86.6	81.8
Thr	68.8	72.3	72.6	77.6	83.4	79.7	74.5	73.2	74.7	89.4	84.6	84.4
Iso	78.1	79.4	79.9	85.4	87.1	86.9	76.5	75.6	81.2	88.6	85.8	88.5
Leu	80.0	83.7	81.4	87.2	90.7	88.5	79.6	83.7	83.6	87.4	87.4	88.0
His	76.7	75.1	79.8	85.2	84.2	88.0	83.4	78.7	85.4	91.3	84.8	89.2
Phe	83.1	84.8	82.9	88.0	90.4	83.0	79.9	83.5	83.0	87.7	88.7	88.4
Val	77.1	78.9	76.8	83.6	86.2	83.9	77.7	76.5	78.8	89.1	86.5	86.6

use of chromium as a non-digestible marker.

The *in vitro* data illustrate that the standardized amino acid digestibility is different from that of crude protein. This was also the case when real digestibility was calculated. In a recent proposal for a practical application, it was assumed that real *in vitro* digestibility of amino acid was equal to real digestibility of crude protein (Boisen, 1998). Thus, the difference between standardized digestibility of crude protein and of the amino acids relies on only an estimated figure on the feed-specific endogenous loss. Because of actual analyses on amino acids, the results in Table 40.1 consider the feed-specific endogenous loss and actual differences in real digestibility as well. For routine methods it is unrealistic to determine digestibility of amino acids. Therefore, specific

correction factors should be estimated and validated by *in vivo* methods, eventually a rat assay. It is important to note that the factors used for correction for the feed-specific endogenous loss were originally based on the composition of the non-specific endogenous loss. For future calculations the acceptability of this procedure should be evaluated.

When evaluating the actual *in vitro* figures, the low number of samples has to be considered. In any case, the values obtained by the *in vitro* method correspond with the *in vivo* results. Therefore, it seems reasonable to continue development of the *in vitro* method and to consider the rat method for validation. Taking into account the possibility of improvement of the rat method, the results indicate that the rat may serve as a reliable model for the growing pig.

References

- Boisen, S. (1998) A new protein evaluation system for pig feeds and its practical application. *Acta Agriculturae Scandinavica Section A, Animal Science* 48, 1–11.
- Boisen, S. and Fernández, J.A. (1995) Prediction of the apparant ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. *Animal Feed Science and Technology* 51, 29–43.

41 Faecal and Ileal Digestibility of Casein- and Soybean-based Diets in Growing Pigs

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A study was conducted to determine the effects of ileorectal anastomosis (IR) and protein source on apparent digestibility in growing pigs. Intact animals fed casein diet had significantly higher crude protein (CP) and neutral detergent fibre (NDF) digestibilities than IR pigs. Intact animals fed soybean diet presented significantly higher dry matter (DM), organic matter (OM), CP, NDF, acid detergent fibre (ADF) and gross energy (GE) digestibilities than IR pigs. Intact pigs fed casein diet presented significantly higher DM, OM, CP, NDF and GE, and lower total lipid (TL) digestibilities than those fed soybean diet. IR pigs fed casein diet had higher DM, OM, CP, NDF, ADF and GE digestibility values than the ones fed soybean diet.

Introduction

This study was included in a project aiming to investigate lipid metabolism and the effects of protein source and ileorectal anastomosis on apparent digestibility of nutrients in growing pigs.

Material and Methods

Two groups of 12 growing crossbred Duroc × (Large White × Landrace) male pigs ($\pm$ 30 kg live weight) penned in individual metabolism cages were used. All animal and experimental procedures followed the regulations and ethical guidelines of the Portuguese Animal Welfare Commission.

In the first 2 weeks (adaptation period), all pigs were fed with a semi-purified casein-based diet (CD; casein, 18%; sucrose, 10%; starch, 46.4%; straw, 10%; soybean oil, 10%; methionine, 0.1%; salt and minerals, 5.2%; premix, 0.3%). After this period, six animals from each group

were submitted to ileorectal anastomosis (IR). Pigs from group 1 continued to be fed with CD and pigs from group 2 began to be fed with a diet (SD) where 60% of the protein supplied by casein was replaced by protein from extruded soybean (casein, 7.2%; extruded soybean, 24.67%; sucrose, 10%; starch, 36.57%; straw, 10%; soybean oil, 7%; methionine, 0.1%; salt and minerals, 4.16%; premix, 0.3%). Both diets had similar amounts of gross energy (GE) (4.05 vs. 4.10 kcal g⁻¹ DM), crude protein (15.99 vs. 15.35% DM) and lysine, methionine and cystine. Diets were supplied at a rate of 50 g kg⁻¹ live weight (LW) divided into two meals. Animals had free access to water. IR animals were supplemented daily with 20 g NaCl and 20 g sodium bicarbonate. Diet refusals and spillage were controlled and measured twice daily.

After a 15-day period for surgery recovery and diet adaptation (group 2, SD), both groups were submitted to a 5-day collection period. Faeces were collected twice daily and ileal digesta eight times a day,

with 3 h intervals. Faecal and ileal digesta were immediately stored at -20°C until freeze-dried, and were then ground and chemically analysed.

Data were analysed according to a multivariate analysis of variance, following the IR + protein source (PS) + (IR $\times$ PS) model.

Results and Discussion

There were no differences ($P > 0.05$) in average intake of CD (99.37 and 86.69 $\text{g kg}^{-1} \text{LW}^{0.75} \text{day}^{-1}$ for intact and IR pigs, respectively) and SD (100.82 and 104.83 $\text{g kg}^{-1} \text{LW}^{0.75} \text{day}^{-1}$) and of intact animals (99.37 and 100.82 $\text{g kg}^{-1} \text{LW}^{0.75} \text{day}^{-1}$ for CD and SD pigs, respectively). IR pigs consumed significantly less ($P \leq 0.05$) CD than SD (86.69 and 104.83 $\text{g kg}^{-1} \text{LW}^{0.75} \text{day}^{-1}$, respectively). Effects of IR on apparent digestibility of nutrients are presented on Figs 41.1 and 41.2.

Intact CD pigs presented significantly higher ($P \leq 0.05$) digestibilities than IR CD pigs only in crude protein (CP) (93.39 vs.

89.41%) and neutral detergent fibre (NDF) (45.04 vs. 39.88%). These results could be justified by the CD's semi-purified form, which led to an easier enzymatic access to the diet nutrients. Intact SD pigs presented higher dry matter (DM) (82.08 vs. 76.42%), organic matter (OM) (85.11 vs. 81.34%), CP (84.88 vs. 78.39%) ($P \leq 0.001$), NDF (39.84 vs. 35.82%) ($P \leq 0.01$), acid detergent fibre (ADF) (21.11 vs. 18.20%) ($P \leq 0.05$) and GE (85.98 vs. 82.51%) ($P = 0.000$) digestibilities than IR SD pigs, probably reflecting the presence of the caecum-colon microbial digestion. The caecum-colon bypass led to an important decrease of digestibility values in IR animals, especially the ones consuming SD. The decrease in NDF (-11.43% in CD and -10.11% in SD) and ADF (-4.27% in CD and -13.78% in SD) digestibilities show that significant quantities of plant cell walls can be degraded in the pig's large intestine (Varel *et al.*, 1988). PS effects on nutrient digestibilities in intact and IR pigs are presented in Figs 41.3 and 41.4.

Significant differences were detected

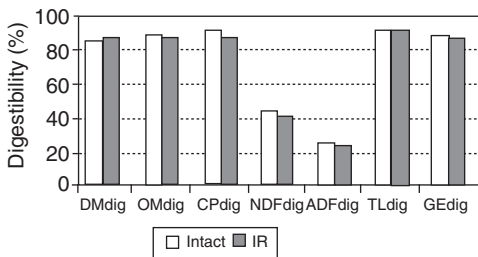


Fig. 41.1. IR Effect on CD nutrient digestibilities.

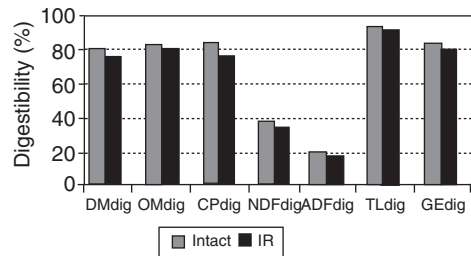


Fig. 41.2. IR Effect on SD nutrient digestibilities.

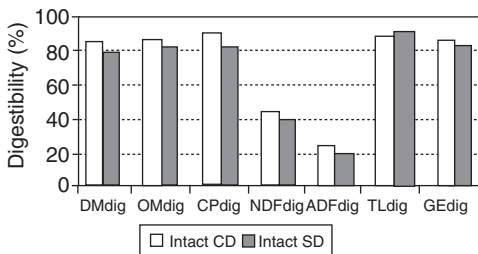


Fig. 41.3. PS effect on digestibilities in intact animals.

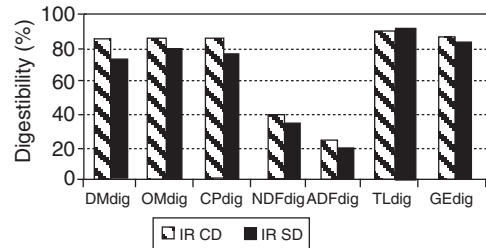


Fig. 41.4. PS effect on digestibilities in IR animals.

between DM (88.06 vs. 82.08%), OM (90.47 vs. 85.11%), CP (93.39 vs. 84.88%) ($P = 0.000$), NDF (45.04 vs. 39.84%), total lipids (TL) (92.82 vs. 95.09%) ($P < 0.01$) and GE (90.47 vs. 85.98%) ($P = 0.000$) digestibilities for CD and SD in intact animals. IR pigs consuming CD presented higher digestibility values for DM (86.56 vs. 76.42%), OM (89.25 vs. 81.34%), CP (89.41 vs. 78.39%) ($P = 0.000$), NDF (39.88 vs. 35.82%) ($P \leq 0.05$), ADF (24.68 vs. 18.20%) ($P \leq 0.01$) and GE (89.16 vs. 82.51%) ($P = 0.000$) than the ones consuming SD. These results seem to be in accordance with the hypothesis presented for the differences detected between nutrient digestibilities in intact and IR animals, i.e. the fact that the CD's semi-purified form would permit easier or more efficient digestion of their nutrient compounds, when compared with SD. On the other hand, the soybean protein structure and the presence of antinutritive factors (anti-trypsin activity = 13,717.5 TIA g^{-1} fresh weight) in extruded soybean and

the higher NDF content of SD (8.20 vs. 11.41% DM) could have affected CP (Grala *et al.*, 1999) and GE (Cauwenberghe *et al.*, 1997) digestibilities.

The higher SD fibre content seems to have negatively influenced the digestibility values of non-fibrous dietary components, particularly in IR pigs. Similar observations were made by Stanogias and Pearce (1985). This could be the result of a faster rate of passage of food through the alimentary tract (Gargallo and Zimmerman, 1981) and an increased excretion of dietary components bound or physically entrapped in the bulk of the bolus of the fibrous digesta (Eastwood and Kay, 1979).

In conclusion, this study is in agreement with several others by pointing to a lower digestibility of SD when compared with a semi-purified CD. Furthermore, the presence of a caecum-colon microbial digestion showed a marked effect on the digestibility of fibrous and non-fibrous dietary components.

References

- Cauwenberghe, S. van, Jondreville, C., Beaux, M.F., Williatte, I. and Gatel, F. (1997) Estimation de la valeur énergétique des aliments et des matières premières chez le porcelet en post-sevrage et chez le porc charcutier. *Journées de la Recherche Porcine en France* 29, 205–212.
- Eastwood, M.A. and Kay, R.M. (1979) An hypothesis for the action of dietary fibre along the gastrointestinal tract. *American Journal of Clinical Nutrition* 32, 364–367.
- Gargallo, J. and Zimmerman, D.R. (1981) Effect of dietary cellulose level on intact and cecectomized pigs. *Journal of Animal Science* 53, 395–402.
- Grala, W., Verstegen, M.W.A., Jansman, A.J.M., Huisman, J. and Leeuwen, P. van (1999) Apparent protein digestibility and recovery of endogenous nitrogen at the terminal ileum of pigs fed diets containing various soybean products, peas or rapeseed hulls. *Animal Feed Science and Technology* 80, 231–245.
- Stanogias, G. and Pearce, G.R. (1985) The digestion of fiber by pigs. 1. The effects of amount and type of fiber on apparent digestibility, nitrogen balance and rate of passage. *British Journal of Nutrition* 53, 513–530.
- Varel, V.H., Jung, H.G. and Pond, W.G. (1988) Effects of dietary fiber of young adult genetically lean, obese and contemporary pigs: rate of passage, digestibility and microbiological data. *Journal of Animal Science* 66, 707–712.

42 The Effect of Different Levels of Fat Inclusion and Cereal Type on Digestibility Parameters for Growing Pigs

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Fourteen pigs, with post-valve T-caecum cannulae, were randomized to seven diets (five by-product with increasing fat level and two cereal diets) using a three-period crossover design. Seven-day faecal collections were followed by two 12 h ileal collections using TiO_2 as a marker. Significant increases in ileal digestibility (ID) of dry matter (DM) (4.7%), oil (18.9%) and energy (5.9%) and increases (NS) of ID for crude protein (CP) (3.2%) and neutral detergent fibre (5.6%) occurred with 10% fat compared with the basal diet. The barley- and maize-based diets resulted in higher overall digestibility of DM (9.3%), CP (6.4%) and energy (9.1%) and 46% less faeces (DM basis) than by-product diets.

Introduction

Diets based on high-fibre ingredients are less well digested than those based on cereals, and the higher proportion of hindgut fermentation also tends to reduce the efficiency of utilization of absorbed energy. On the other hand, fats are normally highly digestible and converted to body tissue with higher efficiency. Therefore, it could be expected that the use of high-fibre, low-energy ingredients in combination with added fat would provide a diet as equally suited to the needs of pigs as a cereal-based diet. However, this remains an area of debate. The aim of this study was to examine the effect of enhancing digestible energy (DE) levels with fat, as opposed to reducing fibre by use of cereal sources, on digestibility values and sites of digestion in growing pigs.

Materials and Methods

Fourteen male crossbred (Large White $\times$ Landrace) pigs, weighing approximately 20 kg each, were fitted with post-valve T-caecum cannulae (van Leeuwen *et al.*, 1991) and randomized to seven commercially steam-pelleted diets (80°C for 3 min), using a three-period partially unbalanced crossover design. Each period comprised a 5-day pre-feed, 7-day faecal collection and 2-day (2 $\times$ 12 h) ileal collections. Five diets were formulated by diluting a low-energy, high-by-product basal diet (A) with fat (vegetable oil) added at 19, 38, 57 and 76 g kg^{-1} (diets B, C, D and E, nominally described as containing 5%, 6.5%, 8% and 10% dietary fat, respectively). Two cereal diets were formulated using barley, wheat and maize (designated barley and maize) to supply levels of digestible energy equiva-

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lent to those in by-product diets C and E, respectively.

Samples of faeces and ileal digesta were freeze-dried and pooled within period and pig for each dietary treatment. The proximate analysis was carried out according to AOAC (1984). Gross energy was determined in an oxygen bomb calorimeter (Parr, Model 1271). TiO_2 was measured according to Leone (1973). Overall apparent digestibility (OD) was determined as the difference between total feed intake and faecal output, with ileal apparent digestibility (ID) measured using the TiO_2 marker. The results were subjected to ANOVA according to the crossover design using the GENSTAT program (1993).

Results and Discussion

Analyses of diet compositions are shown in Table 42.1. These are in good agreement with values calculated from the diet formulation, except for diet B, which contained more oil ($70.9 \text{ g kg}^{-1} \text{ DM}$) than calculated ($47 \text{ g kg}^{-1} \text{ fresh weight}$). This showed that an error had occurred in diet B preparation, and thus it has been excluded from further discussion.

One of the most striking differences between diets A to E and the cereal diets was the faecal output, which was much higher (47%) when pigs were fed the by-product diets. This would be of major importance in production systems that need to reduce the level of waste products.

Highly significant effects ($P < 0.001$) of diet treatment on ID and OD and hindgut fermentation (expressed as a proportion of OD) occurred (Table 42.2). ID of DM and energy were significantly higher (4.7 and 5.9%) and ID of protein numerically higher (3.2%) with the high-fat diet (E) compared with the basal diet (A). However, the proportion of hindgut fermentation was reduced with increasing fat content, with the result that OD of DM, CP and energy were similar across all levels of fat inclusion. It would be expected that the efficiency of utilization of energy for growth would tend to increase from diet A to diet E as a consequence of the reduced hindgut fermentation and the increased energy contribution from fat.

There was a marked difference between the two cereal diets, with higher ID of DM and energy (8%) for the maize diet but no significant difference in OD due to the higher proportion of hindgut fermentation occurring with the barley diet. However, assuming typical values for the utilization of absorbed glucose (ID) and volatile fatty acids (Van Es, 1987), the calculated difference in efficiency of energy utilization would be less than 2%.

Comparing the cereal diets with diets A to E, a higher proportion of digesta DM entering the hindgut was fermented with the cereal diets (0.49 vs. 0.38, $P < 0.001$, SEM 0.023) although the proportion contributed to OD was not different. Thus, the overall efficiency of utilization of energy with the cereal and by-product diets would seem to be similar.

Table 42.1. Dry matter (DM), crude protein (CP), oil, neutral detergent fibre (NDF) and gross energy (GE) analysis of the diets ($\text{g kg}^{-1} \text{ DM}$).

	A	B	C	D	E	Barley	Maize
DM	885.8	890.8	884.5	879.0	886.1	883.3	891.6
CP	223.4	217.5	212.7	212.2	206.3	218.0	214.9
Oil	38.4	70.9	78.3	93.5	110.6	26.1	25.8
NDF	240.3	228.9	232.8	222.0	227.1	179.7	177.2
GE (MJ)	18.43	19.36	19.33	19.75	20.18	18.06	18.28

Table 42.2. Effect of diet on OD, ID and hindgut fermentation of dry matter (DM), crude protein (CP), oil, neutral detergent fibre (NDF) and gross energy (GE).

	A	B	C	D	E	Barley	Maize	P	SEM
Total faeces (g day ⁻¹)									
Wet	1094 ^c	–	987 ^{bc}	1002 ^{bc}	1019 ^{bc}	666 ^a	682 ^a	< 0.001	39.2
Dry	273.4 ^b	–	266.8 ^b	271.9 ^b	272.9 ^b	182.5 ^a	183.2 ^a	< 0.001	7.57
Overall digestibility									
DM	0.799 ^{ab}	–	0.785 ^{bc}	0.765 ^a	0.778 ^{ab}	0.848 ^d	0.858 ^d	< 0.001	0.0062
CP	0.794 ^{ab}	–	0.796 ^{ab}	0.793 ^{ab}	0.784 ^a	0.850 ^c	0.841 ^c	< 0.001	0.0060
Oil	0.714 ^b	–	0.793 ^c	0.768 ^c	0.759 ^c	0.648 ^a	0.666 ^a	< 0.001	0.0129
NDF	0.514 ^{ab}	–	0.515 ^{ab}	0.454 ^a	0.508 ^{ab}	0.590 ^{cd}	0.615 ^d	< 0.001	0.0216
GE	0.779 ^{ab}	–	0.785 ^{ab}	0.767 ^a	0.775 ^{ab}	0.847 ^c	0.854 ^c	< 0.001	0.0093
Ileal digestibility									
DM	0.660 ^a	–	0.668 ^a	0.649 ^a	0.691 ^b	0.697 ^b	0.753 ^c	< 0.001	0.0071
CP	0.739 ^{ab}	–	0.749 ^{ab}	0.741 ^{ab}	0.763 ^{bc}	0.744 ^{ab}	0.777 ^c	NS	0.0097
Oil	0.713 ^{bc}	–	0.788 ^{cd}	0.849 ^d	0.848 ^d	0.579 ^a	0.694 ^b	< 0.001	0.0318
NDF	0.427 ^{ab}	–	0.406 ^{ab}	0.384 ^a	0.451 ^b	0.466 ^b	0.534 ^c	< 0.001	0.0319
GE	0.674 ^a	–	0.686 ^{ab}	0.673 ^a	0.714 ^c	0.704 ^{bc}	0.757 ^d	< 0.001	0.0073
Hindgut fermentation*									
DM	0.166 ^{bc}	–	0.166 ^{bc}	0.155 ^{ab}	0.122 ^a	0.187 ^c	0.132 ^a	< 0.001	0.0098
NDF	0.178	–	0.211	0.126	0.095	0.220	0.112	NS	0.0594
GE	0.150 ^{bcd}	–	0.140 ^{bc}	0.124 ^b	0.087 ^a	0.177 ^d	0.122 ^b	< 0.001	0.0103

^{a,b,c}Values within a row without a common superscript are significantly different.

*Fermentation as a proportion of OD, i.e. (OD – ID)/OD.

References

- AOAC (1984) *Official Methods of Analysis*, 14th edn. Association of Official Analytical Chemists, Washington, DC.
- Genstat 5 Release 3 Manual (1993) Genstat 5 Committee, Clarendon Press, Oxford, UK.
- Leone, J.L. (1973) Collaborative study of the quantitative determinations of titanium dioxide in cheese. *Journal Association of Analytical Chemists* 56, 535–537.
- Van Es, A.J.H. (1987) Energy utilization of low digestibility carbohydrates. In: Leegwater, D.C., Feron, V.J. and Hermus, R.J.J. (eds) *Low Digestibility Carbohydrates*. Pudoc, Wageningen, The Netherlands.
- van Leeuwen, P., van Kleef, D.J., van Kempen, G.J.M., Huisman, J. and Verstegen, M.W.A. (1991) The post-valve T-caecum cannulation technique in pigs applied to determine the digestibility of amino acids in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* 65, 183–193.

43 Effects of Dietary Xylanase Inclusion and Level of Steam Conditioning on Ileal and Overall Digestibility Parameters in the Growing Pig

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Twelve pigs, with post-valve T-caecum cannulae, were randomized to eight diets in a 4 × 2 factorial design, the factors being heat (cold-pelleted; steam-treated at 70°C; 80°C; 90°C) and xylanase (absent; present), using a four-period crossover design. Seven-day faecal and two 12 h ileal collections were made using TiO₂ as a marker. Xylanase addition significantly increased ileal digestibility (ID) of neutral detergent fibre (NDF) (17%) and overall digestibility (OD) of protein (1%). Dietary heat treatment reduced OD of dry matter, crude protein and energy (1.2 to 2%) and increased OD and ID of NDF (5.3 and 17.6%), although the effects on diet nutritive value were small.

Introduction

Steam conditioning is widely used in commercial pig feed production. Whilst severe diet heat treatment may adversely affect nutrient utilization, even modest heat (80°C for 120 s) may result in reduced ileal apparent digestibility of wheat-based diets fed to growing pigs (Lewis *et al.*, 1999). In addition, a recent study by BOCM-Pauls (cited by McCracken, 2000) saw higher gain:feed of broilers fed diets heated at 85°C for 20 s, rather than for 2–3 min (0.66 vs. 0.62, respectively, $P < 0.001$). The effects of degree of steam conditioning on digestibility parameters in growing pigs are therefore also of importance. Benefits of addition of dietary xylanase to wheat-based

diets for growing pigs are inconsistent, with improvements in ileal digestibility of nutrients reported in some, but not all, trials (Bedford *et al.*, 1992; Thacker *et al.*, 1992). Thus, this study examined the effect of the degree of steam conditioning on digestibility of wheat-based diets for growing pigs, with and without the addition of exogenous xylanase.

Materials and Methods

Twelve male crossbred pigs (approximately 20 kg) were fitted with post-valve T-caecum cannulae (van Leeuwen *et al.*, 1991) and randomized to eight diets in a 4 × 2 factorial design, the factors being heat

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(cold-pelleted; steam-treated at 70°C; 80°C; 90°C) and enzyme (xylanase, *T. longibrachiatum*, Finnfeeds Ltd: absent; present). Heat conditioning was effected by low-pressure direct steam injection for 90, 120 or 150 s, respectively; enzyme was added where appropriate (1 g kg⁻¹ feed), prior to mixing. Diets contained (g kg⁻¹): wheat 550, barley 100, soybean meal 258, tallow 50, minerals, etc. 42, and were pelleted (3 mm die) and fed using a four-period partially balanced crossover design. Each period comprised a 5-day pre-feed, 7-day faecal collection and 2-day (2 × 12 h) ileal collections. Samples of faeces and ileal digesta were freeze-dried and pooled within period and pig for each diet treatment. Proximate analysis was done according to AOAC (1984), gross energy was determined in a bomb calorimeter (Parr, Model 1271) and TiO₂ measured according to Leone (1973). Overall apparent digestibility (OD) was determined as the difference between total feed intake and faecal output, with ileal apparent digestibility (ID) measured using the marker (TiO₂). The results were subjected to ANOVA according to the crossover design using the GENSTAT program (1993).

Results and Discussion

The mean composition of the diets was (kg⁻¹ DM): crude protein 223.9 g, oil 67.1 g

and NDF 147.7 g and gross energy 19.27 MJ. The OD of dry matter (DM), protein and energy decreased ($P < 0.05$) with increased temperature (Table 43.1), although effects were small (< 2%). The ID of DM, protein and energy were unaffected by heat treatment or enzyme addition (Table 43.2), but ID of NDF increased with heat treatment ($P < 0.01$) and with enzyme addition ($P < 0.001$).

In line with a previous study (Lewis *et al.*, 1999), xylanase addition increased OD of protein ($P < 0.05$) but effects for DM and energy were not significant. Improvements in ID of NDF were of a similar size by enzyme inclusion (0.576 vs. 0.492) or by steam conditioning at 90°C (0.574 vs. 0.488), with no further increases seen when both treatments were applied to diets, suggesting that these processes acted on the same component of the diet. The treatments may have disrupted the endosperm cell walls (Saunders *et al.*, 1969) and allowed greater exposure to microbial degradation. No effect on ID of protein occurred in the present trial, indicating that there were no negative effects on protein quality of these treatments.

The results confirm previous results in our laboratory of small effects of xylanase inclusion with wheat-based diets, and suggest that under the conditions of heat treatment applied here, there was little effect on the nutritive value of the diets. However, in view of the trend towards higher tempera-

Table 43.1. Effect of heat treatment on overall and ileal digestibility of dry matter (DM), crude protein (CP), energy (GE) and neutral detergent fibre (NDF).

	Heat treatment				<i>P</i>	SEM
	Cold	70°C	80°C	90°C		
Overall						
DM	0.878 ^{bc}	0.882 ^c	0.873 ^{ab}	0.866 ^a	< 0.001	0.0029
CP	0.889 ^{bc}	0.885 ^{bc}	0.878 ^{ab}	0.871 ^a	0.004	0.0038
GE	0.882 ^b	0.883 ^b	0.875 ^{ab}	0.871 ^a	0.012	0.0029
NDF	0.679 ^a	0.715 ^b	0.694 ^{ab}	0.706 ^b	0.009	0.0081
Ileal						
DM	0.750	0.749	0.746	0.742	NS	0.0083
CP	0.796	0.792	0.794	0.790	NS	0.0086
GE	0.768	0.764	0.765	0.762	NS	0.0081
NDF	0.488 ^a	0.519 ^{ab}	0.554 ^{bc}	0.574 ^c	0.003	0.0175

^{abc}Least square means in same row with different superscripts, significant ($P < 0.05$).

Table 43.2. Effect of enzyme supplementation on overall and ileal digestibility of dry matter (DM), crude protein (CP), energy (GE) and neutral detergent fibre (NDF).

	Non-enzyme	Plus enzyme	P	SEM
Overall				
DM	0.872	0.877	NS	0.0021
CP	0.877	0.885	0.043	0.0027
GE	0.875	0.880	NS	0.0021
NDF	0.669	0.728	< 0.001	0.0057
Ileal				
DM	0.741	0.752	NS	0.0059
CP	0.790	0.796	NS	0.0061
GE	0.759	0.770	NS	0.0057
NDF	0.492	0.576	< 0.001	0.0124

tures in commercial practice, McCracken (2000) advised a need for more research, using defined experimental conditions, to be conducted.

References

- AOAC (1984) *Official Methods of Analysis*, 14th edn. Association of Official Analytical Chemists, Washington, DC.
- Bedford, M.R., Patience, J.F. and Classen, H.L. (1992) The effect of dietary enzyme supplementation of rye and barley-based diets on digestion and subsequent performance in weanling pigs. *Canadian Journal of Animal Science* 72, 97–105.
- Genstat 5 Release 3 Manual (1993) Genstat 5 Committee, Clarendon Press, Oxford, UK.
- Leone, J.L. (1973) Collaborative study of the quantitative determinations of titanium dioxide in cheese. *Journal Association of Analytical Chemists* 56, 535–537.
- Lewis, F.J., McCann, M.E.E., Schulze, H., McEvoy, J. and McCracken, K.J. (1999) The effects of wheat variety, dietary heat treatment and enzyme inclusion on digestibility parameters for growing pigs. *Proceedings of the British Society of Animal Science*, p. 165.
- McCracken, K.J. (2000) Effects of physical processing on the nutritive value of poultry diets. *Proceedings 26th Poultry Science Symposium*, June 1999.
- Saunders, R.M., Walker, H.G. Jr and Kohler, G.O. (1969) Aleurone cells and the digestibility of wheat milled feed. *Poultry Science* 48, 1497–1503.
- Thacker, P.A., Campbell, G.L. and Groot-Wassink, J.W.D. (1992) Effect of salinomycin and enzyme supplementation on nutrient digestibility and the performance of pigs fed barley or rye based diets. *Canadian Journal of Animal Science* 72, 117–125.
- van Leeuwen, P., van Kleef, D.J., van Kempen, G.J.M., Huisman, J. and Verstegen, M.W.A. (1991) The post-valve T-caecum cannulation technique in pigs applied to determine the digestibility of amino acids in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* 65, 183–193.

44 The Effect of Variety and Location of Production on the Nutritive Value of Barley Fed to Growing Pigs

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Two barley varieties (Dandy, Riviera) from Northern Ireland (NI) and two (Lamba, Crusader) from the Republic of Ireland (ROI) were obtained from each of six locations in each country. The samples were formulated into 24 diets containing 650 g barley kg⁻¹ and fed to two sets of 12 pigs, in a partially balanced four-period crossover design. Titanium dioxide was used as an indigestible marker. Overall apparent digestibility (OD) and ileal apparent digestibility (ID) were calculated. Specific weight had a poor relationship to OD and ID ($r^2 < 0.20$). Starch content of barley was positively correlated with OD of dry matter and energy ($r^2 = 0.40$ and 0.36 , respectively). Variety and location of production significantly affected digestibility.

Introduction

Chemical composition and nutritive value of cereal grains are affected by variety and location of production (Valaja *et al.*, 1997). Spring barley is the most widely produced cereal in Northern Ireland (NI) and in the Republic of Ireland (ROI) and is a major ingredient in pig diets. The main objective of this study was to examine the effect of variety and location of production on the nutritive value of selected varieties of locally produced spring barley for growing pigs. A further aim was to examine the relationship between specific weight (SW) and chemical composition and nutritive value of barley. Specific weight is traditionally used to predict nutritive value of grain but a number of reports (e.g. Stewart *et al.*, 1997) have found a poor relationship

between SW and either chemical composition or nutritive value.

Materials and Methods

Two barley varieties (Riviera, Dandy) were obtained from six locations in NI and two (Crusader, Lamba) were obtained from six locations in ROI. Twenty-four pelleted diets, containing (g kg⁻¹): barley 650, soy-bean meal 283, tallow 30, limestone 12, dicalcium phosphate 9, salt 3.3, titanium dioxide 1.5, minerals/vitamins 11, were produced and 12 (three of each variety) were used in each of two trials. Post-valve T-caecum (PVTc) cannulation (van Leeuwen *et al.*, 1991) was carried out on two sets of 12 male pigs at 8 weeks old. Each set of pigs was randomized to the 12

diets using a partially balanced four-period crossover design. In each period a 7-day faecal collection and a 2-day (2×12 h) ileal collection followed a 5-day pre-feed. The results were analysed by analysis of variance using GENSTAT 5. Simple and multiple regressions were calculated between digestibility and chemical and physical parameters.

Results

Overall digestibility (OD) ranged from 0.81 to 0.87 for dry matter (DM); 0.82–0.88 for crude protein (CP); 0.64–0.84 for oil; 0.49–0.71 for neutral detergent fibre (NDF); and 0.82–0.88 for energy. Ileal digestibility (ID) ranged from 0.63 to 0.69 for DM; 0.67–0.76 for CP; 0.24–0.52 for NDF; and 0.64–0.71 for energy. OD of DM and energy were lower ($P < 0.05$) for Dandy. OD of oil was also lower ($P < 0.05$) for Dandy compared with Crusader and Lamba (Table 44.1). There were no significant effects of variety on ID (Table 44.2). Digestible energy (DE) ranged between 14.4 and 16.2 MJ kg⁻¹ DM, with the DE content of Dandy being lower ($P < 0.05$) than that of Riviera and Crusader (Table 44.1).

Riviera (site 1) gave higher ($P < 0.05$) OD of DM (0.870), energy (0.875), CP (0.877), oil (0.836) and NDF (0.645) than Riviera (site 4) (0.821, 0.822, 0.823, 0.644 and 0.538, respectively). OD of DM (0.825), energy (0.825) and CP (0.816) for Crusader (site 4) were lower than the values at the other sites. Lamba (site 6) gave lower OD of DM (0.814), energy (0.819), CP (0.824) and NDF (0.488) than Lamba at all other sites. ID of DM (0.628) for Dandy (site 4) was lower ($P < 0.05$) than for Dandy (site 1) (0.679). OD of DM and energy were positively correlated with starch and negatively correlated with CP and NDF content ($r^2 = 0.40, 0.50$ and 0.48 , respectively, for DM and $0.36, 0.45$ and 0.41 for energy). There were no strong relationships between ID and chemical composition ($r^2 < 0.1$ for all parameters). SW was not strongly correlated with OD or ID ($r^2 < 0.2$). DE was positively correlated with starch ($r^2 = 0.31$) and negatively correlated with CP, NDF and ash ($r^2 = 0.32, 0.33, 0.35$). A combination of GE and SW (easily measured parameters) gave a poor relationship (Table 44.3) and even multiple regressions based on chemical composition were little better than a number of the simple regressions.

Table 44.1. The effect of variety on OD and DE content (MJ kg⁻¹ DM) (d.f. = 23).

Variety	DM	CP	B-Oil	NDF	Ash	Energy	DE
Riviera	0.845 ^b	0.856	0.753 ^{ab}	0.616	0.557 ^a	0.847 ^b	15.5 ^b
Dandy	0.833 ^a	0.850	0.734 ^a	0.608	0.556 ^a	0.834 ^a	15.0 ^a
Crusader	0.845 ^b	0.853	0.790 ^c	0.604	0.583 ^b	0.847 ^b	15.5 ^b
Lamba	0.842 ^b	0.853	0.767 ^{bc}	0.596	0.572 ^{ab}	0.844 ^b	15.4 ^{ab}
SED	0.004	0.005	0.016	0.013	0.012	0.004	0.130

^{abc}Means without a common superscript are significantly different ($P < 0.05$).

Table 44.2. The effect of variety on ID (d.f. = 23).

Variety	DM	CP	NDF	Ash	Energy
Riviera	0.653	0.719	0.387	0.228	0.671
Dandy	0.651	0.729	0.407	0.230	0.671
Crusader	0.664	0.728	0.404	0.253	0.679
Lamba	0.654	0.726	0.383	0.251	0.673
SED	0.010	0.012	0.020	0.020	0.011

Table 44.3. Multiple regression relationships between DE and chemical composition.

	Starch	CP	NDF	Ash	GE	SW	r^2
1					*	*	0.17
2	*	*	*		*		0.38
3	*	*	*	*	*		0.40

*Parameter included in multiple regression.

Discussion

The low OD and DE content of Dandy (Table 44.1) can be attributed, at least partly, to the lower level of starch and higher level of NDF (Taverner *et al.*, 1981). However, the correlations determined between DE and chemical composition (Table 44.3) indicate that none of the parameters taken individually or collectively

gives strong enough relationships to enable their use as predictors of DE. This concurs with the report of Batterham *et al.* (1980), but the correlation between GE and SW and DE content ($r^2 = 0.17$) determined in this study is much poorer than that of Batterham *et al.* (1980) ($r^2 = 0.71$). The poor relationship between SW and digestibility contrasts with Brand and Swart (1999) but supports the results of Stewart *et al.* (1997).

References

- Batterham, E.S., Lewis, C.E., Lowe, R.F. and McMillan, C.J. (1980) Digestible energy content of cereals and wheat by-products for growing pigs. *Animal Production* 31, 259–271.
- Brand, T.S. and Swart, C.J. (1999) Relationship between various physical and chemical measurements on barley grain and its nutritive value measured by *in sacco* and *in vitro* methods. *Journal of Applied Animal Research* 15, 153–163.
- Stewart, A.H., Acamovic, T., Taylor, A.G. and Fraser, H. (1997) An evaluation of wheat specific weight as a determinant of nutritive value for pigs and poultry. *Proceedings of the British Society of Animal Science*, p. 66.
- Taverner, M.R., Hume, I.D. and Farrell, D.J. (1981) Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. *British Journal of Nutrition* 46, 149–152.
- Valaja, J., Suomi, K., Alaviuhkola, T. and Mela, T. (1997) Effects of variety, soil type and nitrogen fertilizer supply on the nutritive value of barley for growing pigs. *Agricultural and Food Science in Finland* 6, 295–303.
- van Leeuwen, P., van Kleef, D.J., van Kempen, G.J.M., Huisman, J. and Verstegen, M.W.A. (1991) The post-valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* 65, 183–193.

45 Dietary Fat Supplementation Affects Apparent Ileal Digestibility of Amino Acids and Digesta Passage Rate of Rapeseed Meal-based Diet

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The effect of dietary fat supplementation on the apparent ileal digestibility of amino acids and the passage rate of digesta was evaluated in six ileo-cannulated castrated male pigs (40.8–83.3 kg). Increasing levels of rapeseed oil or raffinated tallow (7% or 14% of dry matter) were added to the rapeseed meal diet. Tallow improved the apparent ileal digestibility of most amino acids, whereas rapeseed oil had no effect on amino acid digestibilities. Both fat sources increased digesta mean retention time (MRT) measured with ytterbium by 45 min ($P < 0.05$), but had no effect on digesta MRT estimated with cobalt.

Introduction

Dietary fat is a valuable energy source in pig diets. When low-energy raw materials are used, supplementary fat is added to increase dietary energy content. Both animal and vegetable fats are highly digestible in pigs. Dietary fat may also affect digestibility of other nutrients. Li and Sauer (1994) observed that rapeseed oil linearly increased apparent ileal amino acid digestibility of soybean meal in piglets. In some studies, however, such effects have been relatively minor (Imbeah and Sauer, 1991). The present study was conducted to study the effects of rapeseed oil and tallow on apparent ileal digestibility of amino acids and the mean retention time of digesta in pigs fed rapeseed meal diets.

Material and Methods

Six ileo-cannulated castrated male pigs (Yorkshire × Landrace) between 40.8 kg (SE 0.65) and 83.3 kg (SE 1.71) live weight were used. A steered ileocaecal valve (SICV) cannula was fitted into the caecum of the pigs at an average live weight of 33 kg. Animals were assigned to five experimental diets according to a 6 × 4 cyclic crossover design. The semi-purified control diet contained low-glucosinolate rapeseed meal as the sole protein source (41.5% of diet). Increasing levels of rapeseed oil or raffinated tallow (7% or 14% of diet) were added to the rapeseed meal diet at the expense of wheat starch. Animals were fed twice daily (at 0600 and 1800 h) according to live weight (W) (88 g feed kg⁻¹ W^{0.75}

day⁻¹). Chromium-mordanted straw was used as a marker (1.7 g kg⁻¹ feed). Experimental periods lasted 14 days, which included a 6-day adaptation period, 4 days' total faeces collection and a total of 24 h ileal digesta collection from 0600 to 1800 h on days 11 and 14. The ileal digesta passage rate was measured during the second day of ileal digesta collection. At morning feeding (0600 h), pigs were given a single dose of ytterbium-mordanted rapeseed meal (10 g kg⁻¹) and liquid cobalt-EDTA (20 ml kg⁻¹ feed). Sixteen digesta samples obtained during a 24 h period were collected hourly from 0600 to 1600 h, once every 2 h from 1600 to 2400 h and once every 3 h from 2400 to 0600 h. Mean retention time (MRT) of digesta was calculated, based on both markers, according to Thielemans *et al.* (1978). Data were subjected to analysis of variance using a model that accounted for the effects of animal, period and diet.

Results and Discussion

Raffinated tallow improved the apparent ileal digestibility of crude protein and most amino acids in the rapeseed meal-based diet, whereas rapeseed oil had no effect on amino acid digestibilities (Table 45.1). The apparent ileal digestibility of methionine ($P < 0.05$), cystine ($P < 0.05$), arginine ($P < 0.05$) and threonine ($P < 0.10$) was increased by 2–3.5 percentage units with a 14% dietary inclusion of tallow. Li and Sauer (1994) observed a similar increase in apparent ileal amino acid digestibility in piglets when 12.2% of rapeseed oil was added to soybean meal diet. Fat supplementations had no effect on the apparent ileal digestibility of lysine and histidine, but increased digesta MRT estimated with ytterbium small digesta particle marker by 45 min ($P < 0.05$). Dietary fat inclusion had no effect on digesta MRT measured with cobalt, considered as a digesta liquid phase

Table 45.1. Effect of rapeseed oil (RO) and raffinated tallow (TA) on the apparent ileal digestibility of amino acids and mean ileal digesta retention time in pigs fed a low-glucosinolate rapeseed meal-based diet (least square means).

	Diet					SEM ^a	Contrasts ^b			
	1	2	3	4	5		C1	C2	C3	C4
Fat source	Cont	RO	TA	RO	TA					
Fat level %	0	7	7	14	14					
N	5	5	5	5	4					
Crude protein	64	62	66	64	66	1.1	NS	*	NS	NS
Amino acids										
Lysine	74	74	75	74	75	1.1	NS	NS	NS	NS
Threonine	62	62	64	62	65	1.3	NS	t	NS	NS
Methionine	76	76	78	77	79	0.8	NS	*	NS	NS
Cystine	70	70	71	69	73	1.1	NS	*	NS	NS
Arginine	82	81	83	82	84	0.6	NS	*	NS	NS
Histidine	78	77	80	78	80	1.2	NS	NS	NS	NS
Isoleucine	66	66	68	66	69	1.2	NS	t	NS	NS
Leucine	70	69	72	70	73	1.0	NS	*	NS	NS
Phenylalanine	71	70	72	71	74	1.2	NS	t	NS	NS
Valine	63	62	64	62	65	1.2	NS	t	NS	NS
MRT ^{Co} (h)	6.8	7.1	7.4	7.5	7.2	0.32	NS	NS	NS	NS
MRT ^{Yb} (h)	10.6	9.8	10.1	10.8	10.6	0.30	NS	NS	*	NS

MRT^{Co}, mean retention time of cobalt; MRT^{Yb}, mean retention time of ytterbium.

^aSEM of diet 5 is table value multiplied by 1.128.

^bC1, control vs. fat supplementations (1 vs. 2, 3, 4 and 5); C2, rapeseed oil vs. raffinated tallow (2 and 4 vs. 3 and 5); C3, fat level 7% vs. 14% (1 and 3 vs. 4 and 5); C4, interaction C2 × C3 (2 and 5 vs. 3 and 4).

NS, not significant; t, $P < 0.10$; * $P < 0.05$.

marker. In contrast to our results, Imbeah and Sauer (1991) found no differences in the ileal passage rate of digesta with 10% inclusion of rapeseed oil. On the other hand, Low *et al.* (1985) found that gastric emptying was delayed when maize oil was fed to growing pigs.

In conclusion, dietary tallow improved the apparent ileal digestibility of protein and amino acids in rapeseed meal, in part due to the longer retention time of digesta

in the stomach and small intestine. Rapeseed oil had a similar effect on the ileal retention time of digesta but, for an unknown reason, had no effect on ileal amino acid digestibilities.

Acknowledgement

Appreciation is expressed to Rehuraisio Ltd, Finland, for financial support.

References

- Imbeah, M. and Sauer, W.C. (1991) The effect of dietary level of fat on amino acid digestibilities in soybean meal and canola meal and on rate of passage in growing pigs. *Livestock Production Science* 29, 227–239.
- Li, S. and Sauer, W.C. (1994) The effect of dietary fat content on amino acid digestibility in young pigs. *Journal of Animal Science* 72, 1737–1743.
- Low, A.G., Pittman, R.J. and Elliot, R.J. (1985) Gastric emptying of barley–soya-bean diets in the pig: effects of feeding level, supplementary maize oil, sucrose or cellulose, and water intake. *British Journal of Nutrition* 54, 437–447.
- Thielemans, M.-F., Francois, E., Bodardt, C. and Thewis, A. (1978) Mesure du transit gastrointestinal chez le porc à l'aide des radioanthanides. Comparaison avec le mouton. *Annales de Biologie Animale Biochimie Biophysique* 18, 237–247.

46 The Role of the Exocrine Pancreas in Pig Performance and Amino Acid Absorption

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In order to elucidate the effect of exocrine pancreatic secretion on performance and on the blood plasma amino acid concentrations in pigs, the following pilot experiment was performed. Exocrine pancreatic insufficiency was induced in three pigs (16–21 kg) by ligation of the main pancreatic duct. Adding pancreatic enzymes to the feed resulted in an increase in daily feed intake (1014 vs. 721 g) and daily weight gain (540 vs. 263 g). The concentrations of free amino acids in the blood plasma decreased when enzymes were added to the feed.

Introduction

It is generally accepted that the exocrine pancreatic secretion is not a limiting factor for pig performance and that the quantity of enzymes secreted is theoretically sufficient to hydrolyse 10–100 times the amount of feed usually ingested (Zebrowska *et al.*, 1983; Corring *et al.*, 1989). However, we detected a positive correlation between exocrine pancreatic secretion and daily weight gain (DWG) (Botermans and Pierzynowski, 1999). In order to elucidate the effect of exocrine pancreatic secretion on performance and free amino acid (FAA) concentrations in the blood plasma, the following pilot experiment was performed.

Materials and Methods

Animals and management

Three crossbred barrows, (Swedish Landrace × Yorkshire) × Hampshire, were used in this study. The pigs weighed 16–21 kg at the start of the experiment. Exocrine pancreatic insufficiency was induced by ligation of the main pancreatic duct. The pigs were supplied with catheters in the jugular vein, allowing blood sampling. Then the pigs were housed individually in 1.0 × 2.0 m pens in a climate-controlled unit.

The pigs were fed a commercial cereal-based pelleted diet for growing/finishing pigs (Slaktsvinsfoder, Singel pelletskross,

lantmännen, Sweden; content kg⁻¹: metabolizable energy, 12.6 MJ; crude protein, 140 g; crude fat, 35 g; crude fibre, 49 g; lysine, 8.5 g; methionine and cystine, 5.4 g; treonine 5.5 g). The pigs had *ad libitum* access to feed between 0900 and 1000 h and between 1600 and 1700 h, and the daily feed intake was recorded.

Experimental plan

For the first period after surgery (10 days) the pigs were fed the commercial feed. For the second period (14 days), the pigs were given the same commercial feed and a supplement of 12 g pancreatic enzymes per feeding per pig (pancreatin, Creon, Solvay GmbH, Germany). Daily weight gain was recorded.

Blood sampling and analyses

At the end of each testing period (at day 10 and day 24), blood samples were taken from the jugular vein at 1200 h. The blood plasma samples were deproteinized and then analysed for FAA (Reverter *et al.*, 1997).

Results

The daily feed intake (1014 vs. 721 g) and DWG (540 vs. 263 g) were higher when pancreatic enzymes were added to the feed. The concentrations of FAA in the blood plasma are presented in Table 46.1.

Discussion

As expected, supplying pancreatic enzymes to the feed resulted in an increase in the DWG of the pigs. Even the daily feed intake increased when pancreatic enzymes were added to the feed. The lower feed intake of the pigs when no enzymes were added to the feed might have been due to an imbalance in essential amino acids (EAA) in the blood as reported by Henry (1985).

The lower concentrations of FAA in the jugular vein when enzymes were added to the feed can be explained in different ways. A normal balance between the different FAA in the blood might have facilitated an increase in DWG, which then resulted in lower concentrations of FAA in the jugular vein. Another possibility is that the presence of pancreatic enzymes resulted in a shift from FAA to peptide absorption in the small intestine. It is known that different ratios between types of enzymes (e.g. cholesterol ester lipase and lipase ratios) result in different metabolic products (Hernell and Bläckberg, 1994), indicating that the exocrine pancreas might have a regulatory function, affecting the metabolites that are available for absorption in the jejunum. It has been shown that protein can be absorbed in FAA form and as short peptides (Daniel *et al.*, 1994). The mechanisms involved in the absorption of peptides to the enterocytes and partial transference to the blood have not yet been elucidated, but the exocrine pancreas might play a crucial role. Peptides are directly utilized by the productive (periph-

Table 46.1. Concentrations ($\mu\text{mol l}^{-1}$) of essential (EAA) and non-essential (NEAA) free amino acids (FAA) in the blood plasma of pancreatic duct ligated pigs fed a commercial diet with or without pancreatic enzymes.

	With pancreatic enzymes	Without pancreatic enzymes
EAA	750	1695
NEAA	1355	1856
Total FAA	2105	3551

eral) tissues (Pierzynowski *et al.*, 1997). Therefore a more profound exploration of the described phenomenon is planned in the near future.

Acknowledgements

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References

- Botermans, J.A.M. and Pierzynowski, S.G. (1999) Relations between body weight, feed intake, daily weight gain, and exocrine pancreatic secretion in chronically catheterized growing pigs. *Journal of Animal Science* 77, 450–456.
- Corring, T., Juste, C. and Lhoste, E.F. (1989) Nutritional regulation of pancreatic and biliary secretions. *Nutrition Research Reviews* 2, 161–180.
- Daniel, H., Boll, M. and Wenzel, U. (1994) Physiological importance and characteristics of peptide transport in intestine epithelial cells. *VIIth International Symposium on Digestive Physiology in Pigs*. EAAP Publication No. 80, pp. 1–7.
- Henry, Y. (1985) Dietary factors involved in feed intake regulation in growing pigs: a review. *Livestock Production Science* 12, 339–354.
- Hernell, O. and Bläckberg, L. (1994) Human milk bile salt-stimulated lipase: functional and molecular aspects. *Journal of Pediatrics* 125, 56–61.
- Pierzynowski, S.G., Puchala, R. and Sahlu, T. (1997) Effects of dipeptides administered to a perfused area of the skin in angora goats. *Journal of Animal Science* 75, 3052–3056.
- Reverter, M., Lundh, T. and Lindberg, J.E. (1997) Determination of free amino acids in pig plasma by precolumn derivatization with 6-*N*-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate and high-performance liquid chromatography. *Journal of Chromatography B*, 696, 1–8.
- Zebrowska, T., Low, A.G. and Zebrowska, H. (1983) Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pig. *British Journal of Nutrition* 49, 401–410.

47 Ileal and Faecal Digestibility of Nutrients in Piglets Fed Non-supplemented and Enzyme-supplemented Barley Diets

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A study was carried out to determine the effect of enzyme supplementation of a barley-based diet on the ileal and faecal digestibilities of dry matter, crude protein, gross energy, crude fibre, acid detergent fibre, neutral detergent fibre and amino acids. Six piglets, weaned at 4 weeks of age, were fitted with a simple T-cannula at the terminal ileum and housed individually in metabolism cages. After recovery from surgery, the pigs were fed the barley diet alone or supplemented with Rovabio™ Excel (xylanase + β -glucanase), according to a two-period crossover design. Chromium oxide was used as indigestible marker. During each period, faeces and ileal digesta were obtained from all the pigs. Enzyme supplementation slightly improved the ileal and faecal digestibilities of most nutrients and amino acids, but this improvement was not statistically significant ($P > 0.05$).

Introduction

Pigs are not able to secrete the enzymes necessary to hydrolyse the non-starch polysaccharides (NSP) found in the cell wall of some plant materials used in animal feeding. This can result in an inferior utilization of some nutrients, especially in NSP-rich diets. The NSP fraction of the cell walls in the endosperm of barley is particularly rich in β -glucans, which may restrict access to the intracellular nutrients, starch and protein (Chesson, 1993). Furthermore, in broiler chickens, soluble β -glucans have been shown to increment the viscosity of the luminal content, reducing the exposure of substrates to the endogenous enzymes. Therefore, the supplementation of barley diets with enzyme preparations containing β -glucanase activity should improve the utilization of its nutrients. Several studies have been developed to evaluate the effect

of enzyme supplementation on nutrient digestibility in pigs. However, animal response to enzyme utilization reported in the literature tends to vary.

The aim of the present investigation was to study the effect of enzyme supplementation on pig performance and the ileal and faecal digestibilities of nutrients in weaned piglets fed diets based on barley.

Materials and Methods

Animals and housing

Six female piglets (Landrace $\times$ Large White) were weaned at 4 weeks of age, with an average weaning weight of 8.17 ± 0.96 kg. The pigs were surgically modified (at weaning) with a T-cannula at the terminal ileum, as described by Walker *et al.* (1986). The pigs were housed individually in 1 m^2

metabolism cages and temperature was maintained constant at 25°C.

Experimental diets

The main components of the experimental diet were barley (50%), full-fat soybean (15%), sweet whey (9%), soybean 48 (8.6%), fishmeal (5%), skimmed milk (4%) and lard (4%). Chromium oxide was included in the diets as a marker (0.3%). The diet was fed either alone (control diet T-1) or supplemented with Rovabio™ Excel (Aventis Animal Nutrition) (0.1 g kg⁻¹; diet T-2). The enzyme is a mixture of xylanase and β-glucanase. The β-glucanase activity, measured by a colorimetric method (Cosson *et al.*, 1999), was 44,566 U g⁻¹. The diet was fed *ad libitum* in mash form, and water was continuously available.

Experimental procedure

After a 1-week recovery period, the six piglets were offered the two diets (control and with enzyme) in two different experimental periods, according to a crossover design. In each experimental period the piglets were adapted to the diets and then ileal digesta was collected for 3 days. The adaptation phase of the first period lasted 7 days and that of the second period lasted 4 days. Faeces were collected for 24 h on the last day of adaptation and ileal digesta was continuously collected into bags during 3 days from 8 to 18 h. Piglets were weighed

at the beginning of each period and before the start of the ileal collection. Feed intake was also controlled.

Results and Discussion

As shown in Table 47.1, pigs fed the enzyme-supplemented diet gained weight at a slightly faster rate than those on the control diet. In addition, there was a small improvement in average daily intake and feed/gain ratio. However, none of these parameters reached statistical significance ($P > 0.05$).

The effect of enzyme supplementation on the ileal and faecal digestibilities of nutrients are presented in Table 47.2. Enzyme addition seems to have a positive effect by slightly improving the digestibility of most nutrients, but this did not turn out to be statistically significant ($P > 0.05$). This is probably due to the reduced number of pigs used. Nevertheless, the agreement between the performance and the digestibility results points towards a positive effect of the enzyme supplementation of barley diets for weaning pigs.

Acknowledgements

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Table 47.1. Effect of Rovabio™ Excel supplementation on pig performance.

Parameter	T-1 control	T-2 enzyme	STD
Average daily gain (g)	401	414	84.1
Average daily intake (g)	509	539	60.9
Feed/gain ratio	1.31	1.29	0.279

Means are not statistically different ($P > 0.05$).

Table 47.2. Effect of Rovabio™ Excel on ileal and faecal apparent digestibilities (%).

Nutrient	Ileal digestibility			Faecal digestibility		
	T-1 Control	T-2 Enzyme	STD	T-1 Control	T-2 Enzyme	STD
Dry matter	66	68	1.6	80	81	0.8
Crude protein	72	73	2.5	81	83	1.3
Crude energy	68	69	2.1	80	80	1.5
Crude fibre	4	5	7.8	25	31	8.2
Neutral detergent fibre	26	28	4.5	50	53	4.8
Acid detergent fibre	10	9	5.8	36	40	7.0
Aspartic acid	71	74	2.3	85	86	2.0
Threonine	73	75	2.4	86	87	1.3
Serine	73	76	3.0	87	88	1.4
Glutamic acid	82	83	1.6	91	92	0.8
Proline	78	79	1.3	90	91	0.6
Glycine	66	68	2.3	82	83	1.0
Alanine	70	72	3.4	82	83	1.2
Cystine	60	63	5.2	83	82	1.6
Valine	72	72	2.7	83	84	0.8
Methionine	82	83	3.7	86	86	0.8
Isoleucine	77	78	2.2	85	86	1.1
Leucine	77	78	2.2	86	87	1.2
Phenylalanine	78	78	2.4	84	85	1.5
Tyrosine	77	78	1.7	86	86	1.1
Histidine	77	79	1.0	89	90	1.2
Lysine	79	80	2.3	88	89	1.1
Arginine	83	83	1.2	89	90	1.1

Means are not statistically different ($P > 0.05$).

References

- Chesson, A. (1993) Feed enzymes. *Animal Feed Science and Technology* 45, 65–79.
- Cosson, T., Pérez Vendrell, A.M., González Teresa, B., Reñé, D., Taillade, P. and Brufau, J. (1999) Enzymatic assays for xylanase and β -glucanase feed enzymes. *Animal Feed Science and Technology* 77, 345–353.
- Walker, W.R., Morgan, G.L. and Maxwell, C.V. (1986) Ileal cannulation in baby pigs with a simple T-cannula. *Journal of Animal Science* 62, 407–411.

48 Apparent Ileal Digestibility of Protein and Amino Acids in Wheat Supplemented with Enzymes for Growing Pigs

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A trial was conducted to evaluate the effect of two different enzymes on the ileal digestibility of dry matter, protein and amino acids of wheat for growing pigs. The supplementation of wheat with enzymes improved on average about 4–6 percentage units the apparent ileal digestibility of dry matter, crude protein and amino acids. These improvements were statistically significant ($P < 0.05$) for most amino acids. No differences were observed between the two enzyme preparations. Additionally the freeze-dried ileal contents were examined microscopically to quantify the different cell types containing undigested starch or protein. It was observed that enzymes reduced the number of cells containing undigested protein or starch.

Introduction

The supplementation of pig diets with enzymes tries to overcome some enzymatic limitations of the pig's own digestive secretions. It is well known that wheat cell walls are particularly rich in arabinoxylans and that these non-starch polysaccharides cannot be hydrolysed by the digestive enzymes of the pig. The present study was conducted to evaluate the effect of two different sources of xylanase on the apparent ileal digestibility of wheat protein and amino acids for growing pigs.

metabolism cages in order to collect ileal juices quantitatively.

Diets and feeding

The experimental diets were prepared using 94.5% wheat (ground at 3 mm) and 5.5% of a vitamin/mineral premix. The diets were presented in mash form and were offered to the pigs twice a day mixed with water. Drinking water was continuously available.

Materials and Methods

Animals and housing

Six 30 kg male pigs (Landrace × Large White) were used. They were surgically modified with an end-to-end ileorectal anastomosis and were kept in adjustable

Experimental treatments

There were three experimental treatments: T-1 was the control diet, without enzymes; T-2 consisted of the same diet with the addition of 500 ppm of Enzyme 1 (premix of 50% strength, GrindazymTM PF; Finnfeeds); and T-3 consisted of the same diet with the addition of 1000 ppm of

Enzyme 2 (Grindazymy™ ProCerea 2000; Finnfeeds). Grindazym PF had an activity of 6000 FXU g⁻¹ xylanase and 2500 BGU g⁻¹ β-glucanase; ProCerea 2000 had an activity of 10,000 TLXU g⁻¹ xylanase.

Experimental design and procedures

The six pigs were offered all dietary treatments in three experimental periods, following a crossover design. Each experimental period consisted of 5 days of adaptation followed by 2 days of quantitative collection. Ileal digesta was collected twice a day and kept refrigerated. At the end of the collection period the digesta of each pig was homogenized, sampled, freeze-dried and analysed for protein and amino acids. The freeze-dried samples of ileal digesta were also examined microscopically to quantify the number of different cell types that contained undigested protein or starch. The values obtained for each treatment were compared statistically, con-

sidering the effects of the pig and of the experimental period.

Results and Discussion

Both enzymes improved the apparent ileal digestibility of dry matter, crude protein and all the amino acids (Table 48.1). This improvement was statistically significant ($P < 0.05$) for most essential amino acids, and ranged between 2 and 6% digestibility units. Notably, improvements in digestibility of around 6% were observed for lysine and the sulphur amino acids. No differences were observed between the two xylanase sources.

The number of wheat cells in ileal digesta containing undigested protein was significantly reduced to 40–60% by the addition of enzymes (Fig. 48.1). This reduction was statistically significant ($P < 0.05$) for embryo leaf and aleurone cells. Additionally, endosperm cells containing undigested starch were quantified (not

Table 48.1. Ileal apparent digestibility (%) of wheat protein and amino acids.

Nutrient	T-1 Control	T-2 Enzyme 1	T-3 Enzyme 2	Standard error	<i>P</i> value T1 vs. T2 and T3
Dry matter	75 ^a	80 ^b	79 ^b	1.0	0.0063
Crude protein	79 ^a	83 ^b	82 ^b	1.0	0.0169
Aspartic acid	76	80	81	1.4	0.0424
Threonine	73	78	78	1.6	0.0503
Serine	84 ^a	87 ^b	87 ^{ab}	0.8	0.0272
Glutamic acid	93 ^a	94 ^b	94 ^b	0.3	0.0034
Proline	91 ^a	93 ^b	91 ^{ab}	0.7	0.1328
Glycine	77 ^a	82 ^{ab}	82 ^b	1.5	0.0324
Alanine	69 ^a	74 ^b	74 ^b	1.6	0.0253
Cystine	80 ^a	86 ^b	86 ^b	1.2	0.0028
Valine	77 ^a	82 ^b	80 ^{ab}	1.0	0.0208
Methionine	78	80	81	1.4	0.1447
Tryptophan	74	81	77	2.1	0.1156
Isoleucine	82 ^a	85 ^b	85 ^b	0.7	0.0058
Leucine	83 ^a	86 ^b	86 ^b	0.6	0.0044
Phenylalanine	80 ^a	83 ^b	82 ^{ab}	0.9	0.0392
Tyrosine	87 ^a	89 ^b	88 ^b	0.5	0.0157
Histidine	86 ^a	89 ^b	88 ^b	0.4	0.0010
Lysine	72 ^a	78 ^b	76 ^{ab}	1.5	0.0159
Met + Cys	79 ^a	85 ^b	85 ^b	0.9	0.0009
Arginine	84 ^a	87 ^b	87 ^b	0.5	0.0014

^{ab}Values in the same row with different letters are significantly different ($P < 0.05$).

shown in the figure). There was a significant ($P < 0.05$) reduction from 3.5 to 1.1 and 1.6 cells per photoframe for Enzymes 1 and 2, respectively.

It can be concluded that on average the

use of xylanase improved the amino acid apparent ileal digestibility of wheat by around 4% digestibility units. Its action appears to be equally effective in all the different cell types studied.

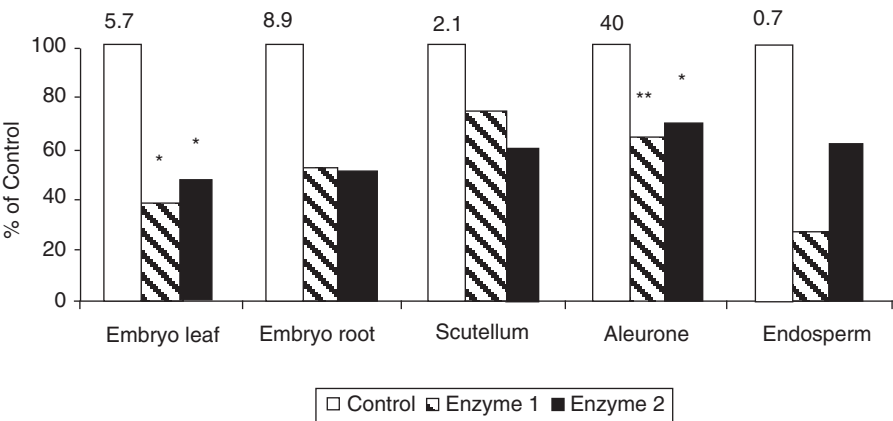


Fig. 48.1. Effect of enzyme supplementation of wheat for pigs on the number of cells containing undigested protein at ileal level (value on top of control bar indicates the number of cells per photoframe).

49 Apparent Ileal Digestibility of Protein and Amino Acids and Digestible Energy in Barley Supplemented with Enzymes for Growing Pigs

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In a first trial the effect of enzymes on the ileal digestibility of protein and amino acids of barley for growing pigs was evaluated. The enzyme improved the apparent ileal digestibility of all the amino acids by between 1 and 6 percentage units and this was statistically significant ($P < 0.05$) for most essential amino acids. The freeze-dried ileal digesta were examined microscopically and it was observed that the enzyme reduced the number of endosperm cells containing either undigested protein or undigested starch. In a second trial, conducted with intact pigs, it was observed that the enzyme improved the digestible energy content of barley from 3112 to 3205 kcal kg⁻¹.

Introduction

Barley contains certain non-starch polysaccharides (NSP) (e.g. β -glucans) which cannot be hydrolysed by the pig's own digestive enzymes. The presence of these NSP on the cell walls may prevent the access of the digestive enzymes into the cells and thus the digestion of the nutrients. We conducted two studies to assess to what degree the supplementation of barley with NSP-degrading enzymes improves the digestion of nutrients and to quantify the improvement in the nutritive value.

Materials and Methods

Animals and housing

Eight and six 30 kg male pigs (Landrace $\times$ Large White) were used in Trial 1 and Trial 2, respectively. For Trial 1 they were surgically modified with an end-to-end ileo-

rectal anastomosis and for Trial 2 they were intact. Pigs were kept in adjustable metabolism cages in order to collect ileal juices or faeces.

Diets and feeding

The experimental diets were prepared using only barley (ground at 3 mm) and the corresponding vitamin/mineral premix. Due to differences in the vitamin/mineral premixes the inclusion of barley was 94.5% and 97.3% for Trials 1 and 2, respectively. The diets were presented in mash form and were offered to the pigs twice a day mixed with water. Drinking water was continuously available.

Experimental treatments

There were two experimental treatments: T-1 was the control diet, without enzymes, and

T-2 consisted of the same diet with the addition of an enzyme product (Grindazym™ GV FEED; Finnfeeds). The enzyme product was included at a dose of 200 ppm and 300 ppm for Trials 1 and 2, respectively. The enzyme product incorporates two cellulases, which are β -glucanase ($10,000 \text{ BGU g}^{-1}$) and xylanase (4000 FXU g^{-1}).

Experimental design and procedures

In Trial 1 the eight pigs were offered both dietary treatments in two experimental periods following a crossover design. Each experimental period consisted of 5 days of adaptation followed by 2 days of quantitative collection. Ileal digesta was collected twice a day and kept refrigerated. At the end of the collection period the digesta of each pig was homogenized, sampled and freeze-dried. The protein and amino acid contents were analysed. The freeze-dried samples were examined microscopically and the number of endosperm cells that contained undigested protein or starch was quantified. In Trial 2 a 7-day pre-experimental period was conducted, after which the six pigs were offered both dietary treatments in a two-period crossover design. Each experimental period consisted of 3 days of adaptation followed by 4 days of collection. Faeces were collected twice a day and kept frozen. Samples were freeze-dried and the energy content was analysed.

Results and Discussion

The enzyme improved the apparent ileal digestibility of dry matter, crude protein and all the amino acids (Table 49.1). This improvement was statistically significant ($P < 0.05$ or greater) for most essential amino acids, and was between 1 and 6% digestibility units.

The number of endosperm cells containing undigested protein or starch also was significantly reduced ($P < 0.05$) by the addition of enzyme (Fig. 49.1). In the presence of enzyme the number of endosperm cells containing undigested protein or starch was 39% or 45%, respectively, of those in the control group.

The enzyme also improved the apparent faecal digestibility of dry matter and energy (Table 49.1). This represented a 3% improvement in the digestive energy value of barley.

From the results presented it can be concluded that, on average, the use of enzymes improved the apparent ileal digestibility of the essential amino acids of barley by between 4 and 5% (with respect to the control). Similarly, the energy value of barley was improved by 3% with the use of enzymes. The microscopy observations suggest that these improvements are due to an improvement of digestion of the nutrients inside the endosperm cells, which could be explained by an improvement of the permeability of the cell walls to the digestive enzymes.

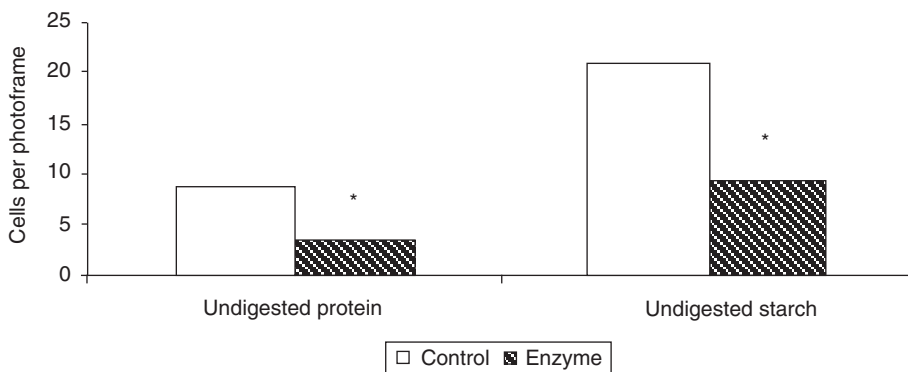


Fig. 49.1. Effect of enzyme supplementation of barley for pigs on the number of endosperm cells containing undigested protein or starch at ileal level.

Table 49.1. Ileal and faecal apparent digestibility (%) of barley nutrients.

Nutrient	T-1 Control	T-2 Enzyme	Standard error	P value T-1 vs. T-2
Ileal apparent digestibility				
Dry matter	64	68	1.3	0.1101
Crude protein	94	95	0.1	0.0626
Aspartic acid	73	75	1.1	0.1832
Threonine	69	73	1.0	0.0392
Serine	76	79	0.6	0.0272
Glutamic acid	85	87	0.5	0.0231
Proline	74	78	1.5	0.1412
Glycine	62	67	0.9	0.0182
Alanine	65	69	1.2	0.1058
Cystine	61	67	1.4	0.0258
Valine	71	75	0.9	0.0558
Methionine	72	76	0.7	0.0126
Tryptophan	71	75	1.0	0.0787
Isoleucine	75	78	0.6	0.0276
Leucine	77	80	0.6	0.0269
Phenylalanine	73	76	0.7	0.0359
Tyrosine	79	82	0.5	0.0188
Histidine	79	82	0.4	0.0064
Lysine	76	77	0.9	0.4954
Arginine	79	81	0.4	0.0202
Faecal apparent digestibility				
Dry matter	80	82	0.4	0.0524
Gross energy	79	81	0.5	0.0462
DE of barley (kcal kg ⁻¹)	3112	3205	18.2	0.0231

50 Effects of Heat Treatment or Pelleting on the Nutritional Value of a Cereal-based Diet for Piglets

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A same cereal-based diet, presented either in a mash form, or pelleted, or after mild or heavy heat treatment with a steam heat conditioner, was fed to piglets weighing between 11 and 18 kg. Heat treatment reduced ileal digestibility of most nutrients and increased the percentage of the digestibility that occurred in the hindgut. Pelleting increased ileal digestibility of most nutrients and led to the best total-tract digestible energy and overall nutritional values.

Introduction

Heat treatment has been developed in France in order to improve the flowing properties of mash and to kill salmonella in the diets of poultry and, by extension, in those of pigs. Many field observations have shown that steaming a meal diet, fed as mash, can reduce piglet asymptomatic diarrhoea and therefore this could be an alternative to the systematic use of antibiotics in animal feeding (Taffin, 1999). Although a reduction of digestibility values at the ileal level and a slight improvement at the faecal level has been demonstrated (Lewis *et al.*, 1999), the effect of heat treatment on the digestibility parameters of piglets remains unclear.

Material and Methods

In order to compare the effect of steaming versus pelleting on digestibility, 40-day-old piglets ($n = 24$) weighing 12 kg were fitted with a post valve T-caecum cannula (van Leeuwen *et al.*, 1991). After a 7-day recovery period, piglets were randomly assigned to cereal-based diets (wheat 27%, barley 20%, triticale 10%, soybean meal 18% and peas 12%) (Table 50.1). This diet was either: kept in a mash form (MF), steam pelleted (PEL: 60°C), mild heat treated (MHT: 30–90°C, 120 s) or heavy heat treated (HHT: 95°C, 185 s) in a CTID 520 × 15 Stolz heat conditioner (Stolz Sequipag, Pontivy, France). Chromium oxide was

introduced before treatments as an indigestible marker. The experimental period consisted of an ileal period (7 days) followed by a faecal period (12 days). The ileal period included 4 days of adaptation followed by 3 days of juice collection (3×12 h). The faecal period comprised a 9-day adaptation period followed by a 3-day faeces collection period.

Results and Discussion

PEL significantly improved the ileal digestibility of dry matter (DM), organic matter (OM), energy (E), starch (St), ether extract (EE) and the ileal digestible energy (IDE), compared with MF (Table 50.2). On the other hand, it lowered the crude fibre (CF) and phosphorus (P) ileal digestibility of the diet. At the faecal level, only OM digestibility was increased by PEL.

HHT significantly decreased E, EE, sugars (Su) and P ileal digestibility as well as IDE content of the diet compared with MF but no differences were found at the faecal level. As opposed to MF, MHT only decreased CF and P ileal digestibility.

Compared with PEL, HHT and MHT diets reduced ileal digestibility of DM, OM, E, St, EE and the IDE content of the diet. But whereas HHT decreased crude protein (CP), Su and P and increased CF ileal digestibility, MHT increased P and had no effect on CP, Su and CF digestibility. At the faecal level, only the EE digestibility was significantly reduced with HHT.

Table 50.1. Proximate analysis of experimental diets (g kg⁻¹ DM).

Diet	Mash form	Steam-pelleted	Mild heat-treated	Heavy heat-treated
Crude protein	225	225	233	231
Crude fibre	41	37	37	40
Enzymatic starch	446	434	461	436
Ether extract	53	52	49	42
Organic matter	936	935	939	938
Gross energy (MJ kg ⁻¹ DM)	18.6	18.6	18.5	18.4
Sugars	49	49	49	39
Phosphorus	7	7	7	7
Phytic phosphorus	2	2	2	2
Phytasic activity (IU kg ⁻¹)	405	431	206	21

Table 50.2. Ileal and faecal apparent digestibility (%); digestible energy (MJ kg⁻¹ DM).

Diets	Mash form	Steam-pelleted	Mild heat-treated	Heavy heat-treated	P	RSD
Ileal						
Juices pH	6.4 ^{ab}	5.7 ^b	6.9 ^a	6.1 ^b	**	0.5
Dry matter	72 ^b	74 ^a	71 ^b	71 ^b	***	1
Crude protein	82 ^{ab}	83 ^a	81 ^{ab}	81 ^b	*	2
Organic matter	74 ^b	77 ^a	73 ^b	73 ^b	***	1
Energy	74 ^b	76 ^a	72 ^{bc}	72 ^c	***	1
Enzymatic starch	95 ^b	99 ^a	95 ^b	94 ^b	***	1
Ether extract	79 ^b	84 ^a	77 ^b	73 ^c	***	2
Sugars	74 ^a	71 ^a	76 ^a	57 ^b	**	8
Crude fibre	15 ^a	4 ^b	8 ^b	14 ^a	**	5
Phosphorus	70 ^a	64 ^c	67 ^b	59 ^d	***	2
Digestible energy	13.7 ^b	14.1 ^a	13.4 ^{bc}	13.2 ^c	***	0.2
Faecal						
Dry matter	89	91	90	91	NS	1
Crude protein	90	92	91	91	NS	1
Organic matter	91 ^b	92 ^a	92 ^{ab}	92 ^{ab}	*	1
Energy	89	91	89	90	NS	1
Enzymatic starch	100	100	100	100	NS	0.1
Ether extract	75 ^{ab}	81 ^a	76 ^{ab}	72 ^b	*	4
Sugars	100	100	100	100	NS	0.2
Crude fibre	62	65	61	65	NS	5
Phosphorus	72	69	72	68	NS	5
Digestible energy	16.5	16.8	16.6	16.6	NS	0.2

P-values: NS, $P > 0.10$; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

RSD, residual standard deviation.

^{abcd}Values within the same row without a common superscript are significantly different ($P < 0.05$).

Conclusion

Our results demonstrated that heat treatment (steaming) reduced ileal digestibility of most nutrients and increased the percentage of the digestibility that occurred in the hindgut, as almost no difference was found at the end of the digestive tract. Total tract DE was then barely or not reduced, compared with the untreated con-

trol diet. However, as metabolic efficiency of nutrients was expected to be lower when digested in the hindgut (fermented) rather than when digested in the small intestine (hydrolysed), nutritional value of heat-treated diets should be reduced in terms of net energy. Pelleting increased ileal digestibility of most nutrients and led to the best total-tract digestible energy and overall nutritional values.

References

- Lewis, F.J., McCann, M.E.E., Schulze, H., McEvoy, J. and McCracken, K.J. (1999) The effects of wheat variety, dietary heat treatment and enzyme inclusion on digestibility parameters for growing pigs. In: *Proceedings of the British Society of Animal Science*. British Society of Animal Science, Penicuik, Midlothian, UK, p. 165.
- Taffin, J.P. (1999) Conséquences sur le terrain des traitements thermiques en production porcine. In: *Proceedings of the Colloque Annuel*. CRITT Valicentre, INRA, Nouzilly, France, pp. 41–45.
- van Leeuwen, P., van Kleef, D.J., van Kempen, G.J.M., Huisman, J. and Verstegen, M.W.A. (1991) The Post valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* 65, 183–193.

51 Effects of Adding Potassium Diformate and Phytase Excess for Weaned Piglets

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Adding potassium diformate (KDF) significantly lowered the pH, coliform and streptococci counts in the stomach at 0.9% ($P < 0.05$) but coliform only in the colon at 1.8% level ($P < 0.02$). Ammonia was decreased ($P < 0.01$) in the stomach, as the residual KDF increased significantly. In the absence of phytase, KDF tended to improve the digestibility of dry matter and nitrogen (1.7 and 2.1%, NS). However, simultaneous and excessive addition of phytase (1450 UI kg⁻¹) reversed the tendency, as well as that for energy (interactions, $P = 0.12$ and 0.08, respectively). Phosphorus digestibility was always improved by the addition of phytase ($P < 0.03$).

Introduction

The newly weaned piglet is faced with numerous nutritional and microbiological challenges occurring after withdrawal of milk and its replacement by more complex and less digestible feedstuffs. The ban of antibiotics from starter diets has led to the search for alternative solutions to control digestive disturbance and to allow the highest potential of nutrient digestibility at weaning. Organic acids and their salts, such as potassium diformate (KDF) (Formi®; Norsk Hydro ASA), have been recently proposed as efficient candidates (Roth *et al.*, 1998). At the same time, the reduction of pollutant phosphorus will lead to a larger use of phytase, and even to overdosage. The following experiment considers this eventuality.

Material and Methods

Forty-two piglets weaned at 27 ± 1 days and 8 ± 1 kg of live weight, individually caged, were randomly allotted to six treatments. The starter diet (buffering capacity

540 mEq HCl kg⁻¹ at pH 3) was supplied with KDF (0, 0.9 and 1.8%) in the absence or the presence of microbial phytase : 1450 FTU kg⁻¹, level which is three times the reasonable one. Diet was low in Ca (0.92%) and in P (0.52%) (71% and 65%, respectively, of the French recommendations). After 5 days of adaptation, a 7-day digestibility trial was carried out, after which fresh samples of contents from stomach, duodenum, jejunum, jejuno-ileum, caecum and proximal and distal colon were collected for immediate pH measurements and microbiological counts. Dry matter, residual KDF, ammonia and lactic acid levels were determined on fresh or deep-frozen samples and other components on lyophilized samples. All analyses were performed according to AOAC or AFNOR recommendations.

Results and Discussion

The addition of KDF tended to lower the pH of the stomach (Table 51.1). In the stomach and the proximal colon, coliform

counts decreased ($P < 0.05$) when KDF level increased, as demonstrated by Kirchgessner *et al.* (1992) and Gabert *et al.* (1995) with formic acid. In the stomach, streptococci counts were significantly lowered while the dominant flora of lactobacilli was not affected by the treatment. *Clostridium perfringens* counts were low in the stomach, but increased in the jejunum and in the colon, independently of the KDF level, in agreement with the data of Risley *et al.* (1992) in the presence of organic acids. Sulphur-reducing bacteria tended to decrease in piglets supplied KDF, mainly in the ileum and in the proximal colon.

In the stomach, residual KDF increased following the KDF supply, as well as in the first third of the small intestine, then the product was completely absorbed (Table 51.2). KDF decreased the level of ammonia in the stomach ($P < 0.01$) but not in the ileal digesta, in agreement with Gabert *et al.* (1995). The level of ammonia was generally

lower in the chyme of the small intestine (11 mg g^{-1} dry matter) than in the stomach (16 mg g^{-1}), but tripled in the hindgut, particularly in the caecum (47 mg g^{-1}).

In the absence of phytase, KDF tended to improve the apparent digestibility of all nutrients and N retention in piglets in agreement with Partanen and Mroz (1999) and Roth *et al.* (1998). Also KDF was readily absorbed, as confirmed by the absence of residual values in the chyme at the end of the small intestine. The digestibility of phosphorus ($P < 0.001$) and that of calcium ($P < 0.04$) were significantly improved by the addition of phytase for diets supplying a reduced level of these minerals. However, KDF at the highest level impaired the effect of the phytase, and this later tended to impair the improvement of digestibility with KDF alone, for dry matter (DM) ($P < 0.07$), minerals ($P < 0.07$) and energy ($P < 0.08$), phosphorus and calcium ($P < 0.13$) and to a lesser extent for nitrogen (Table 51.3).

Table 51.1. Effect of Formi® on pH and selected bacterial counts and segments.

Segment	KDF %	pH	Coliforms	Faecal strepto.	<i>Clostridium perfringens</i>	Lactobacilli
Stomach	0.0	5.27	2.2×10^6	5.5×10^5	1.8×10^2	4.5×10^6
	0.9	4.92	1.1×10^6	1.2×10^5	6.9×10^1	1.2×10^8
	1.8	5.12	2.3×10^5	1.7×10^5	3.5×10^2	1.9×10^6
$P > F$		0.08	0.05	0.04	0.58	0.40
Colon	0.0	6.54	1.3×10^8	8.0×10^4	2.7×10^5	8.2×10^8
	0.9	6.62	5.1×10^7	2.3×10^5	8.2×10^2	7.7×10^8
	1.8	6.65	7.4×10^6	1.3×10^5	2.7×10^3	9.7×10^8
$P > F$		0.53	0.02	0.52	0.16	0.15

$P > F$, probability of equivalence between treatments.

Table 51.2. Formate and ammonia contents (mg kg^{-1} dry matter) in selected segments.

	KDF (%) $\pm$ phytase			Significance		
	0	0.9	1.8	KDF	Phytase	Interaction
Formate						
Stomach	1.61	12.73	27.87	< 0.001	0.378	0.886
Duodenum	0.08	0.53	0.70	0.034	0.416	0.550
Ammonia						
Stomach	20.1	16.0	15.52	0.008	0.336	0.281
Caecum	41.8	56.0	41.9	0.442	0.355	0.661

Table 51.3. Digestibility coefficients of main nutrients (%).

	KDF (%) (without phytase)			KDF (%) (with phytase)			Significance ($P > F$)		
	0.0	0.9	1.8	0.0	0.9	1.8	KDF	Phytase	Interaction
DM	86.4 ^b	88.1 ^a	87.0 ^{ab}	88.4 ^a	88.1 ^a	87.1 ^{ab}	0.168	0.090	0.066
N	80.5 ^b	83.5 ^{ab}	83.6 ^{ab}	85.1 ^a	85.1 ^a	83.3 ^{ab}	0.417	0.041	0.122
Energy	85.1 ^{ab}	86.9 ^{ab}	85.9 ^{ab}	87.2 ^a	86.7 ^{ab}	85.5 ^{ab}	0.219	0.279	0.081
Ca	64.9 ^b	68.3 ^{ab}	66.1 ^{ab}	72.0 ^a	69.6 ^{ab}	66.8 ^{ab}	0.301	0.035	0.130
P	55.0 ^c	59.2 ^{bc}	57.0 ^c	70.9 ^a	67.7 ^a	64.6 ^{ab}	0.448	0.001	0.130
N eff	68.9	71.1	73.9	73.46	70.8	70.9	0.646	0.745	0.081

N eff, N retained/N absorbed %.

^{abc}Values with different superscripts are significantly different, $P < 0.05$.

Conclusion

As a general conclusion, KDF supply was beneficial to the digestive process of the weaned piglets. This conclusion is based on measurements of microbial counts all along the digestive tract, ammonia level in the intestinal chyme and digestibility values. This occurred despite a high variability in the values observed soon after weaning. The interaction of KDF with a

high supply of microbial phytase is more confusing and should be further investigated in the near future.

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References

Gabert, V.M., Sauer, W.C., Schmitz, M., Ahrens, F. and Mosenthin, R. (1995) The effect of formic acid and buffering capacity on the ileal digestibilities of amino acids and bacterial populations and metabolites in the small intestine of weanling pigs fed semipurified fish meal diets. *Canadian Journal of Animal Science* 75, 615–623.

Kirchgesner, M., Gedek, B., Wiehler, S., Bott, A., Eidelsburger, U. and Roth, F.X. (1992) Influence of formic acid, calcium formate and sodiumhydrogen-carbonate on the microflora in different segments of the gastrointestinal tract. *Journal of Animal Physiology and Animal Nutrition* 68, 73–81.

Partanen, K. and Mroz, Z. (1999) Organic acids for performance enhancement in pig diets. *Nutrition Research Review* 12, 117–145.

Risley, C.R., Kornegay, E.T., Lindemann, M.D., Wood, C.M. and Eigel, W.N. (1992) Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. *Journal of Animal Science* 70, 196–206.

Roth, F.X., Windisch, W. and Kirchgesner, M. (1998) Effect of potassium diformate (FormiTM LHS) on nitrogen metabolism and nutrient digestibility in piglets at graded dietary lysine supply. *Agribiological Research* 51, 167–175.

52 Measuring Ileal Basal Endogenous Losses and Digestive Utilization of Amino Acids Through Ileorectal Anastomosis in Pigs: Ring Test Between Three Laboratories

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The ileal digestibility of amino acids from three feedstuffs (barley, pea and soybean meal) were measured in pigs fitted with ileorectal anastomosis in three different sites. Since the basal endogenous losses obtained with a protein-free diet differed significantly between sites, site-specific values were used for calculation of true standardized digestibilities. Significant differences were found between feedstuffs, but also between laboratories and sites. Inter-laboratory crossed analyses revealed site and site $\times$ feedstuff effects independent of laboratory. We conclude that, in practice, the use of mean values from several sites may be recommended.

Introduction

Three organizations, located in France, measured the ileal digestibility of amino acids (AA) using the same surgical technique, i.e. ileorectal end-to-end pre-valvular anastomosis (IRA) (Laplace *et al.*, 1994). The data generated in these three sites were reinterpreted within a vast programme aimed at publishing unified standardized ileal digestibility values of AA in feedstuffs for pigs. A ring test was organized to dissociate inter-laboratory associated with laboratory procedures from inter-site variations associated with animal rearing and environmental parameters.

Material and Methods

Four diets – three made of tested feedstuffs (barley, pea, soybean meal) combined with starch, sugar and maize oil, and a protein-free (PF) diet made of the three latter ingredients in similar proportions – were successively offered in different order to each of four pigs for 1-week periods. The adaptation:collection combinations of duration (in days) were 4:3 in site A and 5:2 in sites B and C. During measurements, the average weight (BW) of pigs (about 45 kg) and the level of feeding (about 80 g dry matter kg⁻¹ BW) were very similar in the three sites. Other parameters

(genotype of the pigs and sampling procedures) were as usual and differed between sites. The laboratory associated with each site made analyses of the diets and of all local digesta samples according to its routine procedures. In addition, for each diet,

Table 52.1. Composition of tested feedstuffs and diets, (100 g⁻¹ dry matter).

	Feedstuff		
	Barley	Pea	Soybean meal
Dry matter (% as fed)	87.6	88.5	87.9
Crude protein	9.8	26.0	50.5
Lysine	0.40	1.78	3.11
Threonine	0.35	0.92	1.96
Methionine	0.17	0.23	0.68
SAA	0.39	0.54	1.45
Tryptophan	0.14	0.24	0.73
Isoleucine	0.36	1.08	2.42
Valine	0.54	1.20	2.50
Crude fibre	5.0	5.8	6.9
Neutral detergent fibre	18.6	13.4	12.5
Acid detergent fibre	5.8	6.7	7.5
TIU (g ⁻¹)		3400	

Table 52.2. Basal endogenous losses (g kg⁻¹ dry matter intake) (means ± SD).

	Site		
	A	B	C
Crude protein	8.7 ± 1.1 ^b	7.2 ± 1.2 ^c	9.7 ± 0.3 ^a
Lysine	0.29 ± 0.04 ^b	0.24 ± 0.02 ^b	0.41 ± 0.05 ^a
Threonine	0.33 ± 0.03 ^b	0.27 ± 0.04 ^c	0.39 ± 0.02 ^a
Methionine	0.08 ± 0.01 ^b	0.05 ± 0.01 ^c	0.13 ± 0.02 ^a
SAA	0.26 ± 0.02 ^a	0.16 ± 0.01 ^b	0.30 ± 0.03 ^a
Tryptophan	0.11 ± 0.01 ^b	0.09 ± 0.01 ^b	0.17 ± 0.01 ^a
Isoleucine	0.26 ± 0.03 ^b	0.18 ± 0.02 ^c	0.33 ± 0.03 ^a
Valine	0.34 ± 0.03 ^b	0.25 ± 0.04 ^c	0.48 ± 0.04 ^a

^{abc}Values followed by different letters differed at $P < 0.05$.

Table 52.3. Amino acid apparent digestibilities for diets, and standardized digestibilities for feedstuffs, obtained with local analyses (sites A, B and C) (means ± SD).

		Apparent digestibility (%)			True digestibility (%)		
		A	B	C	A	B	C
Crude protein	Barley	65 ± 0.3	66 ± 1.6	68 ± 1.6	70 ± 2.3	75 ± 3.8	75 ± 2.3
	Pea	77 ± 2.9	84 ± 1.6	77 ± 2.0	82 ± 2.3	88 ± 2.6	83 ± 2.2
	Soybean	84 ± 0.9	87 ± 2.5	84 ± 2.5	89 ± 0.9	91 ± 0.7	89 ± 2.4
Lysine	Barley	63 ± 3.7	63 ± 5.4	60 ± 5.0	70 ± 2.9	69 ± 5.2	71 ± 4.8
	Pea	84 ± 2.4	88 ± 1.3	83 ± 1.7	87 ± 2.1	90 ± 1.4	87 ± 2.0
Threonine	Soybean	89 ± 0.9	90 ± 1.0	87 ± 1.7	91 ± 0.7	92 ± 1.0	91 ± 1.3
	Barley	55 ± 1.0	58 ± 4.0	56 ± 2.3	66 ± 0.8	67 ± 3.1	68 ± 2.7
	Pea	75 ± 2.3	80 ± 1.5	74 ± 2.2	80 ± 1.9	85 ± 1.6	81 ± 2.5
	Soybean	83 ± 0.6	83 ± 2.5	83 ± 2.3	88 ± 0.2	87 ± 2.3	89 ± 2.1

Table 52.4. Crude protein and amino acid standardized digestibilities of feedstuffs as influenced by site (SA, SB, SC) or laboratory (LA, LB, LC) (means of sites).

	Feed (F)	Site (S)			<i>P</i> (S × F)	Laboratory (L)			<i>P</i> (L × F)
		SA	SB	SC		LA	LB	LC	
Crude protein	Barley	70	72	74		72	71	72	
	Pea	80	88	82	0.001	84	82	84	NS
	Soybean	89	91	88	0.015	90	89	89	NS
Lysine	Barley	67	72	70		71	66	72	
	Pea	86	91	86	0.001	88	87	87	0.010
	Soybean	91	92	90	0.080	92	91	91	0.028
Threonine	Barley	61	67	69		82	81	80	
	Pea	74	85	80	0.001	69	63	65	0.010
	Soybean	86	89	88	0.050	89	87	87	NS

pooled samples of digesta from the four pigs were prepared for inter-laboratory crossed AA analyses in order to dissociate the respective contributions of laboratory and site characteristics in the observed differences in digestibility for each tested feedstuff.

Results and Discussion

With local analyses, basal endogenous losses measured with the PF diet differed significantly; they were lower in site B, intermediate in site A and higher in site C (Table 52.2). Therefore the apparent digestibility was standardized into true digestibility using site-specific basal endogenous losses (Table 52.3). Few diet × site interactions were significant on the

apparent or true digestibilities. The expected differences between feedstuffs were obtained in the three sites. The values were significantly higher in site B than in sites A and C. Inter-laboratory crossed analyses revealed differences between laboratories for the true digestibility of most AA (Table 52.4). The laboratory × site interactions were generally not significant (results not shown). However, the effects of the laboratory × feed interaction were much less significant than those of the site × feed interaction. Genotype or environmental differences might explain these results. Although we did not observe any outbreak of digestive disease, differential health status might have occurred between sites. It is interesting that site differences were higher for pea, intermediate for barley and lowest for soybean meal.

Reference

- Laplace, J.P., Souffrant, W.B., Hennig, U., Chabeauti, E. and Février, C. (1994) Measurement of pre-caecal dietary protein and plant cell wall digestion in pigs; comparison of surgical procedures for ileorectal anastomosis. *Livestock Production Science* 40, 313–328.

53 The Ileal Digestion of Semipurified Diets Containing Increasing Levels of Casein and the Use of the Regression Method to Estimate the Endogenous Flow of Amino Acids at the Distal Ileum of Growing Pigs

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An experiment was carried out to investigate the effect of increasing the level of casein in a semipurified diet on the ileal digestibilities of crude protein (CP) and amino acids (AA) and on AA flow at the terminal ileum in growing pigs. The confounding effect of body weight (BW) and dry matter intake (DMI) was avoided by using pigs with equal initial BW. The level of CP inclusion ranged from 156 to 252 g kg⁻¹ DM and the observed apparent ileal digestibilities were not greatly affected by the level of dietary protein. Apparent ileal digestibilities increased slightly and this facilitated the use of the regression method to calculate the flows of endogenous AA.

Introduction

Quantification of endogenous amino acids (AA) is necessary in order to obtain true ileal AA digestibilities. Endogenous AA are both animal and bacterial in nature and originate from pancreatic enzymes, mucin, bacterial protein and AA from sloughed epithelial cells. Free AA, peptides or proteins that originate from the intestine or from bacteria and are not reabsorbed before reaching the distal ileum are referred to as endogenous AA. In the present study the crude protein (CP) range was chosen to cover and maintain normal physiological conditions with optimal protein retention.

Material and Methods

Sixteen crossbred barrows, from four litters (four pigs from each), with an initial body weight (BW) of approximately 35 kg, were surgically fitted with a simple T-cannula at the distal ileum. Four protein levels were used and pigs from a different litter were used in each period. The average initial BW of the pigs at the start of each of the four experimental periods were 37.8, 37.2, 38.3 and 35.6 kg for periods 1, 2, 3 and 4, respectively. The same amount of feed (dry matter intake (DMI)) was fed during each experimental period to avoid any confounding effects of DMI on endogenous AA

flow. The pigs were fed 1.35 kg day⁻¹ in three meals of equal amount at 0700 h, 1500 h and 2300 h, respectively. Four diets were formulated to contain 13, 16, 19 or 22% CP on an as-fed basis; spray-dried casein was used as the sole source of dietary protein. Other ingredients in the diets included soybean oil (6%), cellulose (4%), sucrose (10%) and a vitamin and mineral premix; the remainder of the diet was adjusted to 100% with wheat starch. Chromic oxide (0.2%) was used as an indigestible marker. Each experimental period comprised 12 days: 5 days adaptation to the experimental diets and 5 days quantitative collection of faeces and urine, followed by 2 days of ileal collection. Digesta were continuously collected on day 11 and day 12, from 0700 to 1500 h on each day. These samples were freeze-dried and pooled prior to analyses.

Results and Discussion

The range of selected inclusion levels of casein was assumed to be just below and mainly at the plateau level of apparent ileal digestibility. From Table 53.1 it can be seen that this was also obtained. The apparent ileal CP digestibilities for the 13, 16, 19 and 22% CP diets were 90.6, 93.3, 93.7 and 93.1%, respectively, and likewise apparent ileal lysine digestibilities were 96.6, 97.4, 97.3 and 97.7%.

The daily CP intake ranged from 193 to 314 g day⁻¹ (Table 53.2); however, the relative output in faeces remained low and constant independent of dietary intake. The output in urine was not significantly different but was lowest at the lowest CP level, indicating optimal CP utilization.

The ileal digestibilities of CP or AA can be estimated by linear regression (Table

Table 53.1. Apparent ileal digestibilities of protein and essential amino acids.

	Diet (% protein)				RMSE	P-value
	13	16	19	22		
Protein (g kg ⁻¹ DM)	156	186	224	252		
Lysine (g kg ⁻¹ DM)	12.5	14.8	17.4	20.4		
Protein (N × 6.25)	91 ^b	93 ^a	94 ^a	93 ^a	1.0	0.003
Lysine	97 ^b	97 ^a	97 ^a	98 ^a	0.4	0.022
Methionine	96 ^b	97 ^a	98 ^a	98 ^a	0.5	0.011
Cystine	71 ^b	80 ^a	78 ^a	79 ^a	1.9	0.001
Threonine	86 ^b	90 ^a	90 ^a	89 ^a	1.3	0.001
Isoleucine	91 ^b	94 ^a	94 ^a	93 ^{ab}	1.7	0.073
Leucine	95 ^b	97 ^a	96 ^a	97 ^a	0.4	0.002
Histidine	95 ^b	97 ^a	97 ^a	97 ^a	0.4	0.003
Valine	92 ^b	95 ^a	95 ^a	94 ^a	1.1	0.044

RMSE, residual mean square error.

Values not sharing a superscript were significantly different ($P < 0.05$).

Table 53.2. Intake of protein (g day⁻¹) and output in faeces and urine (% of intake).

	Diet (% protein)				RMSE	P-value
	13	16	19	22		
Intake protein (g day ⁻¹)	193	231	278	314		
Output faeces (%)	5.6	4.1	3.4	3.3	1.0	0.182
Output urine (%)	23.7	24.5	33.3	37.8	12.6	0.433

Table 53.3. Endogenous ileal protein and amino acids in growing pigs estimated as the intercept from the equation^a $y = I + bx$.

	Intercept g kg ⁻¹ DM	Digestibility (b)	Standard error (intercept)	P > T (intercept)	Standard error (b)	P > T (b)
Protein (N × 6.25)	-8.63	0.97	3.03	0.013	0.015	0.001
Lysine	-0.27	0.99	0.10	0.016	0.006	0.001
Methionine	-0.11	0.99	0.04	0.014	0.007	0.001
Cystine	-0.13	0.90	0.04	0.005	0.036	0.001
Threonine	-0.44	0.94	0.20	0.044	0.023	0.001
Isoleucine	-0.23	0.95	0.30	0.354	0.027	0.001
Leucine	-0.40	0.98	0.18	0.030	0.009	0.001
Histidine	-0.16	0.99	0.04	0.007	0.007	0.001
Valine	-0.32	0.96	0.25	0.155	0.018	0.001

^a y = digested ileal protein/amino acids; I = Intercept (endogenous); b = digestibility; x = protein/amino acids intake (all units g kg⁻¹ DMI).

53.3). The digestibilities thus obtained can be termed true (Furuya and Kaji, 1989) because they are not influenced by endogenous CP or AA. The intercept of the linear regression provides the estimated levels of CP or AA of endogenous origin (Fan *et al.*, 1995). The endogenous flows of CP and AA were as follows: CP, 8.63 g kg⁻¹ DMI; lysine, 0.27 g kg⁻¹ DMI; methionine, 0.11 g kg⁻¹ DMI; cystine, 0.13 g kg⁻¹ DMI; and threonine, 0.44 g kg⁻¹ DMI. Both the ileal digestibility of CP and individual AA and the estimated endogenous amount were comparable to values obtained by Furuya and Kaji (1989) in a similar study. However, graded levels of casein, from 0 to 22%, were used by Furuya and Kaji (1989). In the present study the inclusion levels of casein used ranged from 15 to 26%. From a statistical perspective, the closer the graded levels of CP or AA are to the origin of the coordinate, the more reliable (lower

standard error) is the estimation of the endogenous output. Differences in abundance among the endogenous AA in ileal digesta result mainly from differences in the concentrations in various endogenous secretions into the digestive tract. Studies by Lien *et al.* (1997) showed that mucin output represented 30% of the endogenous threonine and Gabert *et al.* (1996) found relative high abundance of leucine in porcine pancreatic juice. These were also the two endogenous AA at highest concentrations in this study. It can be concluded from the current study that increasing the level of CP as casein within normal physiological conditions in a semipurified diet does not greatly affect apparent ileal AA digestibilities. Therefore, endogenous AA flows may not be greatly affected by protein (AA) intake. In addition, the regression method can be readily used to estimate the ileal flow of endogenous CP and AA.

References

- Fan, M.Z., Sauer, W.C. and McBurney, M.I. (1995) Estimation by regression analysis of endogenous amino acid levels in digesta collected from the distal ileum of pigs. *Journal of Animal Science* 73, 2319–2328.
- Furuya, S. and Kaji, Y. (1989) Estimation of the true ileal digestibility of amino acids and nitrogen from their apparent values for growing pigs. *Animal Feed Science and Technology* 26, 271–285.
- Gabert, V.M., Sauer, W.C., Li, S. and Fan, M.Z. (1996) Exocrine pancreatic secretions in young pigs fed diets containing faba beans (*Vicia faba*) and peas (*Pisum sativum*): concentrations and flows

of total, protein-bound and free amino acids. *Journal of the Science of Food and Agriculture* 70, 256–262.

Lien, K.A., Sauer, W.C. and Fenton, M. (1997) Mucin output in ileal digesta of pigs fed a protein-free diet. *Zeitschrift für Ernährungswissenschaft* 36, 182–190.

54 The Ileal and Faecal Digestion of Monosaccharides in 12 Different Pea Samples Determined in Growing Pigs

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Two experiments were carried out to investigate the digestion of carbohydrates in 12 different samples of peas. In each experiment, six growing pigs were fitted with a simple T-cannula at the distal ileum, and ileal and faecal digestibilities of monosaccharides were measured.

Introduction

Dry peas are used in swine diets in North America and Europe as a source of protein and digestible energy (Savage and Deo, 1989). Peas contain a relatively high content of non-starch polysaccharides (Graham and Åman, 1987) and identifying varieties that contain relatively digestible (fermentable by bacteria) non-starch polysaccharides would result in a reduction in solid waste production as well as improved energy utilization. In addition, there may be enhanced digestion and absorption of other nutrients. The objective of this study was to measure ileal and faecal digestibilities of monosaccharides in 12 different samples of peas and the disappearance of monosaccharides in the caecum and large intestine.

Material and Methods

Twelve samples of dry peas were obtained from 12 spring varieties that are commonly grown in Alberta, Canada. Two experiments were conducted and six varieties were used in each. In each experiment, six crossbred barrows, with an initial body weight of approximately 35 kg, were surgically fitted with a simple T-cannula at the distal ileum. Each experiment was conducted according to a 6 × 6 Latin square design. Ileal digesta were collected in the first experiment. In the second experiment, ileal digesta and faeces were collected. There were six experimental periods and the pigs were fed 1.6 kg day⁻¹ in two meals of equal amount at 0800 and 2000 h, respectively. In each experiment, six experimental diets were formulated to contain

16% CP on an as-fed basis; peas were used as the sole source of dietary protein. Other ingredients in the diets included 10% dextrose, 3% canola oil and a vitamin and mineral premix. The remainder of the diet was adjusted to 100% with maize starch. Chromic oxide (0.3%) was used as an indigestible marker.

In the first experiment, there were 5 days of adaptation to the experimental diets and 2 days of digesta collection. Digesta were continuously collected on day 8 and day 9, for a total of 24 h, at 2 h intervals. In the second experiment, each experimental period comprised 7 days: 5 days of adaptation to the experimental diets and 2 days of faecal collection. Digesta and faeces were frozen immediately after collection and were freeze-dried prior to chemical analyses. The ingredients, diets, digesta and faeces were analysed for monosaccharides according to Blakeney *et al.* (1983) and Kraus *et al.* (1990), following a two-step hydrolysis procedure with sulphuric acid. The alditol acetate derivatives of the monosaccharides were quantified by gas chromatography.

Results and Discussion

There were many differences ($P < 0.05$) in the ileal digestibilities between the pea varieties. Negative ileal digestibilities of

arabinose and xylose in pigs fed diets containing peas were also observed by Graham and Aman (1987). The high digestibilities observed for the Express pea diet are difficult to explain and need to be interpreted with caution.

Express peas contained more ribose and contained lower levels of rhamnose, fucose, arabinose, xylose and mannose than the other varieties. Ileal digesta from pigs fed the Express pea diet contained proportionally less arabinose and xylose. Based on the relatively high ileal digestibilities of galactose (Tables 54.1 and 54.2), a large amount of the oligosaccharides (raffinose, stachyose and verbascose; Savage and Deo, 1989) in peas was fermented by bacteria in the small intestine. Negative digestibilities could have been due to limited monosaccharide degradation in the small intestine and the presence of endogenous carbohydrates. Several of the monosaccharides occur at relatively low levels in peas, therefore small changes in monosaccharide levels could have large effects on digestibility. Faecal digestibilities (Table 54.3) were substantially higher than ileal digestibilities and there was a large disappearance of monosaccharides in the caecum and large intestine, as indicated by the large differences between ileal and faecal digestibilities. Similar results were observed by Graham and Aman (1987). In conclusion, there were

Table 54.1. Ileal digestibilities (%) of dry matter and monosaccharides in different varieties of peas fed to growing pigs in Experiment 1.

Item	Progreta Pea 1	Radley	Century	Tara	Express	Trapper	SEM ($n = 6$)
Dry matter	69.5	69.5	65.0	66.1	68.6	64.8	1.20
Ribose	7.0 ^b	12.0 ^b	-4.5 ^b	5.8 ^b	85.8 ^a	-1.0 ^b	10.47
Rhamnose	49.2 ^b	49.7 ^b	26.5 ^c	43.1 ^b	94.1 ^a	43.6 ^b	4.52
Fucose	-76.6 ^b	-75.7 ^b	-135.5 ^c	-79.4 ^b	64.7 ^a	-72.7 ^b	14.82
Arabinose	15.4 ^b	16.2 ^b	-9.7 ^c	11.0 ^b	86.9 ^a	20.4 ^b	5.74
Xylose	-3.3 ^b	-9.8 ^b	-24.8 ^b	-8.9 ^b	85.7 ^a	4.3 ^b	7.37
Mannose and fructose	54.8 ^b	36.7 ^{bc}	18.5 ^c	16.3 ^c	84.3 ^a	48.1 ^{bc}	8.13
Glucose	71.0 ^b	74.7 ^b	61.4 ^c	69.6 ^b	85.4 ^a	72.4 ^b	1.92
Galactose	66.4 ^b	76.2 ^b	68.5 ^b	69.9 ^b	91.7 ^a	70.0 ^b	3.30

^{abc}Means within a row with different superscript letters differ ($P < 0.05$).

Table 54.2. Ileal digestibilities (%) of dry matter and monosaccharides in different varieties of peas fed to growing pigs in Experiment 2.

Item	Titan Pea 1	Stegholt	Tipu	Miranda	Princess	Victoria	SEM (n = 6)
Dry matter	66.0 ^a	56.3 ^b	56.3 ^b	63.6 ^a	63.2 ^a	63.5 ^a	1.53
Ribose	-30.7 ^b	-11.2 ^{ab}	-36.5 ^b	7.3 ^{ab}	30.1 ^a	-17.6 ^{ab}	12.85
Rhamnose	37.2 ^a	29.2 ^{ab}	48.0 ^a	29.0 ^{ab}	7.1 ^b	38.1 ^a	6.22
Fucose	-117.4 ^c	-112.1 ^c	-93.0 ^{bc}	-60.6 ^{ab}	-101.4 ^c	-51.0 ^a	11.15
Arabinose	-5.8 ^b	-12.5 ^b	-4.1 ^{ab}	19.9 ^a	-0.8 ^{ab}	9.3 ^{ab}	5.98
Xylose	-24.3 ^{abc}	-40.6 ^c	-5.1 ^{ab}	-0.01 ^a	-27.7 ^{bc}	-0.9 ^a	6.49
Mannose and fructose	56.0 ^a	65.4 ^a	63.7 ^a	65.7 ^a	5.4 ^b	48.9 ^a	6.83
Glucose	61.4 ^b	64.8 ^b	64.7 ^b	76.8 ^a	67.9 ^b	67.1 ^b	2.54
Galactose	70.1 ^a	66.9 ^a	58.9 ^b	71.0 ^a	72.9 ^a	73.7 ^a	2.00

^{abc}Means within a row with different superscript letters differ ($P < 0.05$).

Table 54.3. Faecal digestibilities (%) of dry matter and monosaccharides in different varieties of peas fed to growing pigs in Experiment 2.

Item	Titan Pea 1	Stegholt	Tipu	Miranda	Princess	Victoria	SEM (n = 6)
Dry matter	93.6 ^a	91.4 ^b	91.9 ^b	92.5 ^b	92.3 ^b	92.4 ^b	0.28
Ribose	29.7 ^b	47.0 ^b	33.8 ^b	65.2 ^a	69.3 ^a	34.1 ^b	4.87
Rhamnose	69.0	56.3	72.6	73.7	66.9	50.0	7.98
Fucose	44.2 ^b	47.8 ^b	49.7 ^b	61.7 ^a	53.2 ^{ab}	55.3 ^{ab}	2.87
Arabinose	99.2	99.1	99.5	99.5	99.0	99.1	0.13
Xylose	97.7	93.5	96.0	95.3	95.9	89.6	3.13
Mannose and fructose	94.2 ^{ab}	95.6 ^a	94.9 ^a	95.4 ^a	92.3 ^b	93.5 ^{ab}	0.58
Glucose	99.4	98.7	98.9	99.3	98.8	98.5	0.34
Galactose	96.9 ^b	96.4 ^c	96.8 ^b	97.7 ^a	97.3 ^b	97.1 ^b	0.14

^{abc}Means within a row with different superscript letters differ ($P < 0.05$).

large differences in the ileal digestibilities of monosaccharides between different samples of peas and these differences were lessened at the faecal level due to the equalizing effects of the bacteria in the caecum and colon.

References

- Blakeney, A.B., Harris, P.J., Henry, R.J. and Stone, B.A. (1983) A simple and rapid preparation of alditol acetates for monosaccharide analyses. *Carbohydrate Research* 113, 291–299.
- Graham, H. and Åman, P. (1987) Whole-crop peas. II. Digestion of early- and late-harvested crops in the gastrointestinal tract of pigs. *Animal Feed Science and Technology* 17, 33–43.
- Kraus, R.J., Shinnick, F.L. and Marlett, J.A. (1990) Simultaneous determination of neutral and amino sugars in biological materials. *Journal of Chromatography* 513, 71–81.
- Savage, G.P. and Deo, S. (1989) The nutritional value of peas (*Pisum sativum*). A literature review. *Nutrition Abstract Reviews Series A* 59, 65–87.

55 Additivity of Ileal Endogenous Losses and Real Digestibilities of Amino Acids Determined by Means of the ^{15}N -labelled Diets Technique in Growing Pigs Fed Various Feedstuffs

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The ileal endogenous losses and real digestibilities of protein and amino acids from wheat, barley, pea, faba bean and rapeseed meal were determined in growing pigs by means of the ^{15}N -labelled diet technique. The ileal real digestibilities were additive when the feedstuffs were mixed together, in contrast to the ileal endogenous losses which, apart from endogenous lysine excretion, did not show any additivity.

Introduction

In pigs, the total ileal endogenous losses (EL) of amino acids (AA) are composed of a constant basal fraction (bEL) at a fixed body weight (BW) and a variable, feed-specific fraction (fsEL). The assessment of the AA supply to pigs is more accurate when apparent ileal digestibility (AID) data are corrected for bEL, giving rise to true ileal digestibility (TID) values, which are additive (Furuya and Kaji, 1991). A further refinement of that accuracy may theoretically be expected with the use of ileal real digestibility (IRD) values, i.e. AID data corrected for the total EL (bEL + fsEL). As far as diet formulation is concerned, the additivity of IRD and fsEL (i.e. the additivity of EL, since bEL is constant) should concomitantly be verified. But such an improvement requires distinction between the endogenous nitrogenous compounds and the dietary ones in ileal digesta. That distinction is currently possible by means of

the ^{15}N -dilution technique (labelled diets or animals). However, it has so far been obtained at the nitrogen (N) level only. The aim of the present work was to realize such a distinction at the AA level and verify the additivity of the EL and IRD data for various feedstuffs currently used in pig feeding.

Material and Methods

A total of 42 ileorectal anastomized (IRA) and 30 ileo-cannulated (PVTC) male pigs (22–26 kg body weight) were used. They were fed 90 g dry matter (DM) $\text{kg}^{-1} \text{W}^{0.75-1}$ day⁻¹, in two meals, with diets based on either wheat (W), barley (B), pea (P), faba beans (FB) or rapeseed meal (RM) as the sole protein source, or a combination of those ingredients: W + FB + RM (diet 1) and B + P + RM (diet 2) (Table 55.1). The ileal daily flows and the AID of the nitrogenous compounds were determined on IRA

pigs (five to eight pigs per diet) whereas the EL of N and AA were recorded by means of the ^{15}N -labelled diet technique from PVTC pigs (four to five pigs per diet). Diets and ileal digesta were analysed for DM, total N (Kjeldahl method) and AA (HPLC – fluorimetry) as well as for the ^{15}N -enrichment of total N (EA-IRMS) and AA (GC-C-IRMS). Additivity was examined by comparing data recorded with mixed diets to values expected from single ingredient characteristics.

Results and Discussion

Although W, B, P and RM induced lower EL of N than data reported by Souffrant (1991), the EL and IRD of N, Lys and Thr values from the five protein sources (Table 55.2) were overall of the same magnitude as those reported by Leterme *et al.* (1996) and Hess *et al.* (2000). However, B led to higher N EL and lower N IRD than recorded by Leterme *et al.* (2000), and P gave rise to N and Lys EL which were

Table 55.1. Ingredients (%) of the diets fed to growing pigs.

Ingredient	W	B	P	FB	RM	Diet 1	Diet 2
Wheat (W)	90	—	—	—	—	58	—
Barley (B)	—	84	—	—	—	—	44
Pea (P)	—	—	65	—	—	—	30
Faba bean (FB)	—	—	—	54	—	27	—
Rapeseed meal (RM)	—	—	—	—	42	9	15
Maize starch	—	—	24	35	44	—	—
Sucrose	4	4	5	5	5	—	—
Vegetal oil	—	7	—	—	4	—	5
Mineral/vitamin mixture	6	5	6	6	5	6	6

Table 55.2. Total ileal endogenous losses (g kg^{-1} DM intake) and ileal real digestibility (%) of nitrogen and essential amino acids in growing pigs.

	DM of the protein source					DM of the mixed protein sources			
	W	B	P	FB	RM	Diet 1	O/E	Diet 2	O/E
Endogenous losses									
Nitrogen	1.98	3.01	2.22	2.77	2.40	3.96	176	3.91	148
Isoleucine	0.41	0.56	0.34	0.40	0.24	0.80	206	0.79	184
Leucine	0.80	1.00	0.68	0.91	0.88	1.40	167	1.26	145
Lysine	0.94	1.40	0.51	0.77	1.06	0.85	94	0.96	92
Phenylalanine	0.50	0.60	0.32	0.64	0.55	0.83	153	0.76	152
Threonine	0.92	0.96	1.19	1.44	1.32	1.35	122	1.50	136
Tryptophan	0.44	0.65	0.42	0.41	1.16				
Valine	0.62	0.78	0.56	0.67	0.82	0.97	148	0.96	135
Ileal real digestibility									
Nitrogen	86	87	89	89	86	83	96	82	93
Isoleucine	89	92	88	89	87	87	98	86	95
Leucine	92	89	89	90	90	88	96	86	94
Lysine	85	91	91	91	87	89	102	88	98
Phenylalanine	94	92	89	90	92	86	94	86	95
Threonine	89	89	89	90	85	83	94	83	94
Tryptophan	94	94	82	82	90				
Valine	90	91	87	88	87	85	96	83	94

W, wheat; B, barley; P, pea; FB, faba bean; RM, rapeseed meal. O/E = [(Observed data) / (Expected values)] $\times$ 100.

lower than those observed by Leterme *et al.* (1996), but similar to those recorded with the Solara cultivar by Hess *et al.* (2000). Contrary to the IRD, the EL of N and AA varied greatly from one protein source to another (Table 55.2). It might therefore be suggested that the EL may greatly explain the variations in AID observed among different batches of the same feedstuff (Hess *et al.*, 2000). It also means that an identical ranking of dietary ingredients cannot be attained when moving from one AA to another. But cereals systematically led to high EL of Lys, Thr, Leu and Val; P and FB led to high EL of Thr, Leu, Val and Lys; and RM led to high EL of Thr, Trp, Lys and Leu (Table 55.2). Moreover, whatever the mixed diet, EL (i.e. also fsEL) of N and AA were clearly non-additive, with the exception of the endogenous lysine excretion (diets 1 and 2) (Table 55.2). By contrast, no major

dietary interactions on IRD could be observed.

Conclusion

The present results confirm the good additivity of IRD in diet formulation for pigs. On the contrary, the EL are not additive and this questions the possibility of integrating EL data in the formulation of pig diets.

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References

- Furuya, S. and Kaji, Y. (1991) Additivity of the apparent and true ileal digestible amino acid supply in barley, maize, wheat or soya-bean meal based diets for growing pigs. *Animal Feed Science and Technology* 32, 321–331.
- Hess, V., Ganier, P., Thibault, J.N. and Sève, B. (2000) Comparison of the isotope dilution method for determination of the ileal endogenous losses with labelled diet and labelled pigs. *British Journal of Nutrition* 83, 123–130.
- Leterme, P., Théwis, A., François, E., van Leeuwen, P., Wathélet, B. and Huisman, J. (1996) The use of ¹⁵N-labeled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. *Journal of Nutrition* 126, 2188–2198.
- Leterme, P., Souffrant, W.B. and Théwis, A. (2000) Effect of barley fibres and barley intake on the ileal endogenous nitrogen losses in piglets. *Journal of Cereal Science* (in press).
- Souffrant, W.B. (1991) Endogenous nitrogen losses during digestion in pigs. *EAAP Publication* 54, 147–166.

56 The Effect of Encapsulated Acidifiers on Antroduodenal Myoelectrical Activity in Pigs

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The aim of the study was to investigate the effect of an encapsulated acidifier (Aciprol®) on gastroduodenal electromyography (EMG) using a newly developed method of telemetry measurement in freely moving pigs. Three bipolar silver electrodes were sutured on the stomach antrum, on the duodenum just after the pylorus, and after the pancreatic duct opening. After recording several complete duodenal migrating myoelectrical complexes (MMC), Aciprol® was given with a portion of morning food, and recording continued for 24 h. Aciprol® increased the number of MMC cycles per day and shortened the postprandial pattern.

Introduction

Supplementation of pig diets with organic acids is believed to improve digestive processes in the stomach by increasing pepsin activity in a lower pH. This is the reasoning behind including organic acids in the diet, especially for weanling pigs. The favourable influence of acidifiers on growth of beneficial bacteria in the gastrointestinal (GI) tract is not questioned; however, their role in reducing the undesirable bacteria count is less convincing (Risley *et al.*, 1992; Gabert and Sauer, 1994). Organic acids do not influence the gastric emptying rate (Partanen and Mroz, 1999), but using inorganic acids alone may result in depression of food intake and GI tract motility (Roth and Kirchgessner, 1998). Aciprol® (Soda, Monaco) is a feed additive consisting of encapsulated organic and inorganic acids, which are slowly released in the GI tract and are believed not to influence endogenous enzyme activities.

The present report shows the preliminary results on the effect of Aciprol® on antroduodenal electromyography (EMG) in weaned pigs using telemetry (Gacsalyi *et al.*, 2000).

Material and Methods

Three castrated male Landrace × Duroc crossbred male pigs of average 18 kg body weight (BW) were used in the experiment, performed in compliance with European Community regulations on animal experimentation. The animals were allowed to adapt for about 7 days before surgery. The pigs were fed commercial diets twice a day (2% of BW per meal). After intramuscular premedication with azaperone (Stresnil, Janssen & Cilag Pharma, Vienna, Austria) at 5 mg kg⁻¹ BW, the animals were anaesthetized intravenously with pentobarbital at 10–20 mg kg⁻¹ BW and ketamine at 10 mg kg⁻¹ BW. A right flank laparotomy was

performed and three bipolar silver electrodes were sutured to the serosal layer on the stomach antrum, on the proximal duodenum just after the pylorus, and after the pancreatic duct opening. A three-channel telemetry transmitter implant (D70EEE, DSI, Oregon, Minnesota, USA) was fixed extraperitoneally between the layers of abdominal muscles. During the recovery period of 5–7 days, amoxycillin (ClamoxylTM L.A., Pfizer, England) was administered intramuscularly every second day at a dose of 15 mg kg⁻¹. The signals were received by a receiver placed over the cage coupled to an analogue output (Fig. 56.1). Signals were filtered and amplified by an analogue/digital converter, and analysed by a computer. After recording several complete duodenal migrating myoelectrical complexes (MMC), at the end of phase 1 and beginning of phase 2 of MMC, Aciprol[®] (2% feed) was given with a portion of morning food, and recording continued for 24 h.

Results and Discussion

The EMG signal in control recordings showed typical characteristics in the

antrum and duodenum in terms of duration, amplitude and frequency of the spikes and MMC phases (Ruckebusch and Bueno, 1976; Lesniewska *et al.*, 1998). There was a tendency towards an increase in the total number of MMC cycles (23 vs. 21 cycles) following administration of Aciprol[®] as compared with the control. This was caused by a shortening of postprandial patterns in the morning (78 vs. 158 min) and in the evening (75 vs. 130 min). Phase I of the MMC was shortened in the evening and night after administration of Aciprol[®]. Phase II was prolonged just after Aciprol[®] treatment, and markedly shortened in the evening. The migration speed of spikes was 5.7 cm s⁻¹ in the control and 5.1 cm s⁻¹ after Aciprol[®] was given. In conclusion, bolus administration of encapsulated acidifiers had a modest but long-lasting (24 h) effect on antroduodenal myoelectrical activity in pigs.

Acknowledgements

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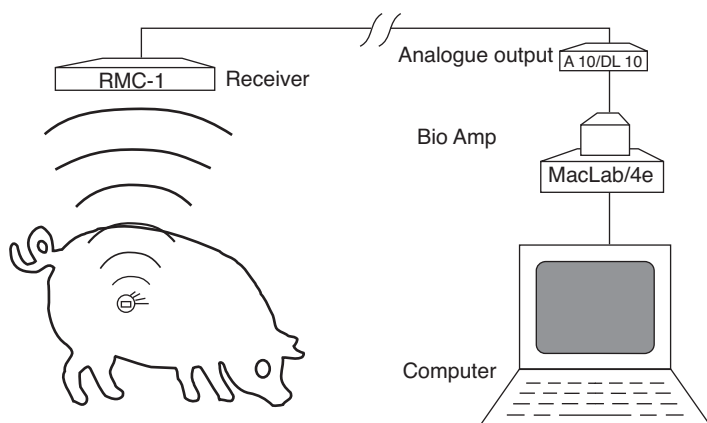


Fig. 56.1. Schematic presentation of the recording system used for EMG telemetry.

References

- Gabert, V.M. and Sauer, W.C. (1994) The effects of supplementing diets for weanling pigs with organic acids. A review. *Journal of Animal and Feed Sciences* 3, 73–87.
- Gacsalyi, U., Zabielski, R. and Pierzynowski, S.G. (2000) Telemetry facilitates long-term recording of gastrointestinal myoelectrical activity in pigs. *Experimental Physiology* 85, 239–241.
- Lesniewska, V., Pierzynowski, S.G., Nygaard Johansen, H., Skou Jensen, M. and Jensen, B.B. (1998) Weaning in pigs: duodenal myoelectrical activity during the change from sow's milk to solid feed. *Journal of Animal and Feed Sciences* 7 (Supplement), 267–270.
- Partanen, K.H. and Mroz, Z. (1999) Organic acids for performance enhancement in pigs diet. *Nutritional Research Review* 12, 117–145.
- Risley, C.R., Kornegay, E.T., Lindemann, M.D., Wood, C.M. and Eigel, W.N. (1992) Effect of feeding organic acids on selected intestinal content measurements at varying times postweaning in pigs. *Journal of Animal Science* 70, 196–206.
- Roth, F.X. and Kirchgeßner, M. (1998) Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. *Journal of Animal and Feed Sciences* 7 (Supplement), 25–33.
- Ruckebusch, Y. and Bueno, L. (1976) The effect of feeding on the motility of the stomach and the small intestine in the pig. *British Journal of Nutrition* 35, 397–405.

57 The Influence of Intraduodenally Infused Fats on Exocrine Pancreatic Secretions and on Plasma Levels of Peptide YY and Cholecystokinin in Growing Pigs

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Six growing pigs were fitted with permanent pancreatic duct cannulas and jugular vein catheters. A medium-chain triglyceride (C 8:0), a long-chain triglyceride (16:0) or saline was infused intraduodenally over a period of 1 h, beginning 1 h postprandially. The infusions of different fats into the duodenum evoked different responses. It can be assumed that the chain length of the fat infused will have an influence on the release of cholecystokinin and therefore on exocrine pancreatic secretions. There is no clear evidence that peptide YY is mediating the regulation of exocrine pancreatic secretions with respect to digestion of fats differing in chain length.

Introduction

The gastrointestinal hormones peptide YY (PYY) and cholecystikinin (CCK) are considered to be major regulative hormones of the exocrine pancreas. Several authors have shown that dietary fat stimulates the release of PYY and of CCK as well, but the effect of dietary fat on plasma CCK levels in pigs remains controversial. The objective of the present study was to examine the effect of purified fat sources differing in chain length (glyceroltricaprylate, C 8:0, vs. glyceroltristearate, C 18:0) on the spontaneous secretion of the exocrine pancreas and on plasma levels of CCK and PYY in pigs.

Materials and Methods

Six growing pigs (13.6 kg) were surgically prepared with silicon pancreatic duct catheters, duodenal re-entrant T-cannulas and a catheter in the external jugular vein. The animals were fed twice daily at a rate of 2% of body weight (BW). After a 7-day post-surgical recuperation period, beginning with the morning feeding, a medium-chain triglyceride (MCT) (glyceroltricaprylate, C 8:0), a long-chain triglyceride (LCT) (glyceroltristearate, C 16:0) or saline as a control was infused intraduodenally according to a 3 × 2 Latin square design at a total rate of 0.1% of BW over a period of 1 h. Pancreatic juice was

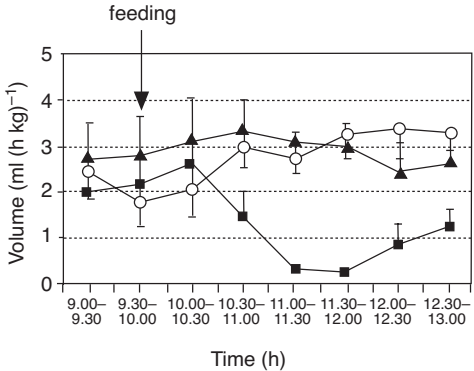


Fig. 57.1. The diurnal pattern of volume of secretion of pancreatic juice after intraduodenal infusion of saline (○), MCT (■) and LCT (▲); mean + SEM.

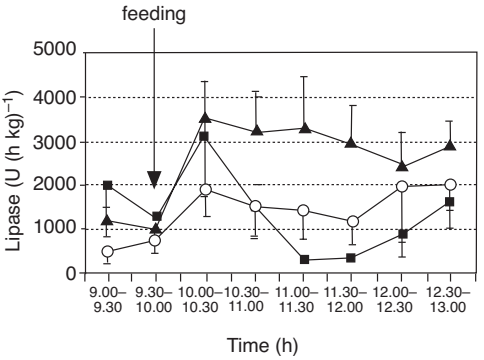


Fig. 57.2. The diurnal pattern of lipase output in pancreatic juice after intraduodenal infusion of saline (○), MCT (■) and LCT (▲); mean + SEM.

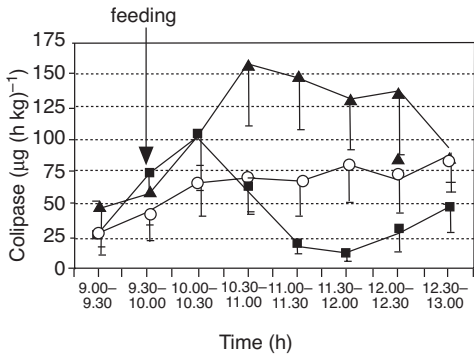


Fig. 57.3. The diurnal pattern of colipase output in pancreatic juice after intraduodenal infusion of saline (○), MCT (■) and LCT (▲); mean + SEM.

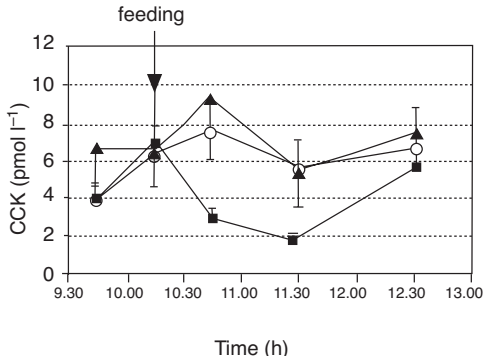


Fig. 57.4. The diurnal pattern of CCK release in plasma after intraduodenal infusion of saline (○), MCT (■) and LCT (▲); mean + SEM.

collected every 30 min over a period of 4 h, beginning 1 h preprandially (0900 h) until 3 h postprandially (1300 h); blood samples were obtained 15 min preprandially and 15, 45, 90 and 150 min postprandially. Data were analysed using repeated-measures ANOVA. The level of significance was set at $P < 0.05$.

Results and Discussion

The infusion of LCT evoked a change in the trend of the curve for the volume of secretion. Moreover, both LCT and MCT infusions induced a change ($P < 0.05$) in the trends of the curves for protein and trypsin output. Content and output of lipase and colipase were influenced by MCT infusions ($P < 0.05$). There were no changes ($P > 0.05$) in the trends of the curves for CCK and PYY levels. A difference ($P < 0.05$) between the trends of the curves for the saline and MCT treatment was observed for the volume of secretion (Fig. 57.1), protein output, lipase content and output (Fig. 57.2), trypsin and colipase output (Fig. 57.3). Moreover, a difference ($P < 0.05$) in the trends of the curves between MCT and LCT was obtained for the outputs of protein and colipase (Fig. 57.3) as well as for lipase outputs (Fig. 57.2). Plasma CCK levels were decreased ($P < 0.05$) in the MCT treatment as compared with the saline and LCT treatment (Fig. 57.4). For plasma PYY levels, no differences ($P > 0.6$) were observed between the diurnal patterns of all three treatments.

All variables determined, except for colipase contents, showed an immediate response of the exocrine pancreas to feed intake and infusions of fat. This prandial response to feed intake is reflected by an immediate increase in the volume of secretion (Fig. 57.1), in enzyme activities such as lipase (Fig. 57.2) and in plasma CCK levels (Fig. 57.4). Protein and trypsin outputs showed very similar diurnal patterns, which confirms that trypsin is a major component of the protein fraction in pan-

creatic juice of pigs (Harada *et al.*, 1982). Comparison of treatments based on lipase contents in pancreatic juice did not reveal a significant ($P > 0.05$) effect of fats differing in chain length on pancreatic lipase secretion. Based on total lipase outputs, however, three different trends of the curves for the three different infusion treatments were obtained.

The different infusion treatments had only minor effects on plasma PYY levels. It cannot be excluded that in the present study the absolute amounts of triglycerides infused intraduodenally were not sufficient to stimulate the PYY release at the ileal level where the receptors are located. The plasma CCK levels for the saline and LCT treatments did not differ from each other, which confirms earlier observations in pigs (Corring and Chayvialle, 1987). However, in the present study, the plasma CCK concentrations decreased after the start of the MCT infusions. This decrease followed the same diurnal pattern as was obtained for the outputs of pancreatic enzymes.

Since Furuse *et al.* (1992) demonstrated that MCT are absorbed via both the blood and the lymphatic system, whereas LCT are absorbed exclusively via the lymphatic system, these differences in absorption pathways could mediate different hormonal feedback mechanisms which, in turn, may affect the rate of secretion of pancreatic juice and enzymes. Furthermore, MCT might have been absorbed at a higher rate than LCT, thus resulting in lower quantities reaching the receptor area of the ileum.

In conclusion, the infusions of different fats into the duodenum under prandial conditions evoked different responses. It can be assumed that the chain length of the fat infused will have an influence on the release of CCK and therefore on exocrine pancreatic secretions. There is no clear evidence that PYY is mediating the regulation of exocrine pancreatic secretions with respect to digestion of fat differing in chain length.

References

- Corring, T. and Chayvialle, J.A. (1987) Diet composition and the plasma levels of some peptides regulating pancreatic secretion in pigs. *Reproduction, Nutrition, Development* 27, 967–977.
- Furuse, M., Choi, Y.H., Mabayo, R.T. and Okumura, J. (1992) Feeding behaviour in rats fed diets containing medium chain triglyceride. *Physiology and Behavior* 52, 815–817.
- Harada, E., Nakagawa, K. and Kato, S. (1982) Characteristic secretory response of the exocrine pancreas in various mammalian and avian species. *Comparative Biochemistry and Physiology /A* 73, 447–453.
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58 Secretion of Nitrogen Compounds in the Small Intestine of Pigs Fed Diets with Different Protein Levels

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Five pigs with temporarily isolated loops (1.3–1.7 m) of the upper part of the small intestine were fed diets containing 6, 12 or 18% protein. The loops were perfused with 0.9% NaCl at the rate of 600 ml h⁻¹ and the flushed-out intestinal juice was analysed for N and amino acid contents. Neither the total N secretion per metre of the intestine (775–860 mg N daily), nor the amino acid composition, expressed as percentage of the sum of 17 amino acids, was affected by dietary protein level. Among the amino acids, high proportions of threonine (5.91–5.96%) and lysine (7.06–7.33%) were found.

Introduction

Determination of the 'true' ileal digestibility of proteins fed to pigs demands information on the net flow of endogenous protein (N) through the small intestine and especially the outflow from the ileum. Protein secretion into the small intestinal lumen changes with the nature and quantity of the diet, which was proved by a large number of non-isotopic methods and by use of isotopic labels. A useful approach to determining minimum values for secretory outflow is the use of a protein-free diet

(Corring *et al.*, 1984; de Lange *et al.*, 1989); however, there is every reason to suppose that the presence of dietary protein will alter the rate and recycling of the secretions (de Lange *et al.*, 1990). When isolated loops were used for estimating N-compounds secreted in intestinal juice, it was found that a protein-free diet reduced secretion to about 50% of that found after feeding pigs with a diet containing 17% crude protein (Buraczewska, 1979).

The aim of the present work was to investigate whether different protein levels

(6, 12 and 18%) in diets fed to pigs would alter intestinal secretion of nitrogen compounds, measured in temporarily isolated loops.

Materials and Methods

Animals

The studies were carried out on five pigs of 30–45 kg body weight (BW) with temporarily isolated loops (1.3–1.7 m long) of the upper part of the small intestine, about 50 cm from the pylorus. The techniques of cannulation and isolation of the loops and the procedure of their perfusion with 0.9% NaCl (600 ml h⁻¹) were as described earlier (Buraczewska, 1979). The aim of the perfu-

sion was to flush out the intestinal juice to minimize absorption of its compounds.

Diets

The pigs were fed three diets containing 6, 12 or 18% protein of the same amino acid composition, using the changeover design. The high-protein (HP) diet was composed of 35% wheat, 33% maize, 19% soybean meal, 10% rapeseed meal and 3% of mineral/vitamin mixture. The diets containing 12 and 6% protein were prepared by dilution of the HP diet with a protein-free diet, based on maize starch.

Metabolizable energy (ME) and lysine concentrations in the HP diet were 12.6 MJ and 8.96 g kg⁻¹, respectively, and propor-

Table 58.1. Amounts of protein (N) secreted in the upper part of the small intestine of pigs fed diets containing 6, 12 or 18% protein (mg m⁻¹ day⁻¹).

	Protein in diets (%)		
	6	12	18
Total N (mg)	775 ± 97	860 ± 154	853 ± 144
Protein N (% of total N)	67.9 ± 3.9	59.7 ± 7.4	56.7 ± 6.9

Table 58.2. Amino acid composition of intestinal juice in pigs fed diets containing 6, 12 or 18% protein. Values are expressed in per cent of the sum of 17 amino acids (*n* = 4).

Amino acid	Protein in diets (%)			SD	<i>P</i> <
	6	12	18		
Asp	9.72	9.92	9.58	0.26	0.153
Thr	5.96	5.91	5.96	0.24	0.943
Ser	6.03	6.19	6.12	0.22	0.624
Glu	13.57	13.96	13.17	0.78	0.399
Pro	5.45	5.55	5.50	0.18	0.751
Gly	4.87	4.85	4.91	0.10	0.699
Ala	5.26	5.15	5.21	0.90	0.313
Val	6.39	6.41	6.51	0.20	0.682
Ile	4.12	4.18	4.14	0.13	0.810
Leu	9.28	9.69	9.28	0.46	0.394
Tyr	4.80	4.70	5.00	0.45	0.671
Phe	5.00	4.93	5.01	0.10	0.589
His	2.53	2.38	2.40	0.15	0.316
Lys	7.27	7.06	7.33	0.38	0.603
Arg	6.47	6.20	6.24	0.31	0.463
Cys	2.09	1.99	2.18	0.19	0.425
Met	1.29	1.25	1.25	0.09	0.811

tions of threonine, methionine and tryptophan to lysine were 0.62, 0.30 and 0.18, respectively. The daily amount of feed given to each pig was 2.8 times its assumed maintenance requirement for metabolizable energy ($1.4 \text{ MJ ME kg}^{-1} \text{ BW}^{0.75}$). The pigs were fed, twice daily at 0800 and 2000 h, on diets mixed with water in the ratio 1:1. Drinking water was freely available. Each diet was given to each pig for 7 days. On days 6 and 7 the loops were isolated and perfused during 12 h day^{-1} , after the morning meal.

Analytical procedures

The effluent was analysed for total N, protein N and amino acids. Protein fraction was separated as precipitable in 3% sulphosalicylic acid. Nitrogen was determined by the Kjeldahl method. Amino acid analyses were performed with a Beckman 6300 amino acid analyser.

Results and Discussion

The results given in Table 58.1 show that different levels of dietary protein did not

affect intestinal N secretion. The proportion of protein N in total N ranged on average from 57 to 68%, and was not influenced by the diets. This may result from the high rate of protein synthesis of mucosal protein with the contribution of not only dietary (luminal) but also arterial amino acids (Reeds *et al.*, 1999).

Amino acid composition of the juice, expressed per 16 g N, varied widely between daily samples of the effluent, irrespective of the protein level of the diet. It seems that different proportions of non-amino acid N in total N were responsible for the variability (Buraczewska, 1979). However, at all dietary protein levels, the amino acid composition of the juice was similar when expressed as a percentage of the sum of 17 determined amino acids (Table 58.2).

Characteristic for the composition is the large proportion of threonine and lysine. Among secretory components, intestinal mucins are most quantitatively significant, as they play a key role in the defence of the mucosa. The core proteins of the major intestinal secretory mucins contain high quantities of threonine and also cysteine, proline and serine (van Klinken *et al.*, 1998).

References

- Buraczewska, L. (1979) Secretion of nitrogenous compounds in the small intestine of pigs. *Acta Physiologica Polonica* 30, 319–326.
- Corring, T., Calmes, R., Rérat, A. and Geugneau, A.M. (1984) Effects of short-term feeding of a protein-free diet on endogenous secretion: exocrine pancreas secretion in the pig. *Reproduction, Nutrition, Développement* 24, 495–506.
- de Lange, C.F.M., Sauer, W.C. and Souffrant, W.B. (1989) The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. *Journal of Animal Science* 67, 755–762.
- de Lange, C.F.M., Souffrant, W.B. and Sauer, W.C. (1990) Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N -isotope dilution technique. *Journal of Animal Science* 68, 409–418.
- Reeds, P.J., Burrin, D.G., Stoll, B. and van Goudoever, J.B. (1999) Consequences and regulation of gut metabolism. In: Lobley, G.E., White, A. and MacRae, J.C. (eds) *Protein Metabolism and Nutrition*. Wageningen Pers, Wageningen, pp. 127–153.
- van Klinken, B.J., Einerhand, A.W., Buller, H.A. and Dekker, J. (1998) Strategic biochemical analysis of mucins. *Analytical Biochemistry* 265, 103–116.

59 Effect of Intraduodenal Infusion of Lactic and Formic Acid on Pancreatic Juice Secretion and Antroduodenal Myoelectrical Activity in Piglets After Weaning

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In weaned piglets fed standard weaning diet supplemented with lactic or formic acid, we compared the effect of infusion of equimolar lactic or formic acid solutions on exocrine pancreatic secretion and gastrointestinal myoelectrical activity.

Supplementation of the diet with lactic or formic acid had no effect on basal pancreatic secretion. Infusion of acid solutions increased outflow of pancreatic juice and bicarbonate output. This stimulation was greater after infusion of lactic than formic acid. Infusion of acid solutions inhibited antral myoelectrical activity. The secretory and motility response to acid infusions was not affected by the diet.

Introduction

Feed acidifiers appear to be potential alternatives to prophylactics such as feed antibiotics in piglets after weaning. However, the performance response of weaned piglets to dietary acids is often variable, which can be related to the different type and doses of acids used and therefore the response of the gastrointestinal tract.

Exocrine pancreatic secretion and gastrointestinal motility are two physiological factors that are directly responsible for digestion. In pigs, as in other animals and in humans, pancreatic secretion is periodic. It is manifested by cyclically recurring fluctuations in the secretion of pancreatic juice concomitant with the gastroduodenal motility pattern. In piglets at weaning, the pattern of gastrointestinal motility undergoes abrupt changes

(Lesniewska *et al.*, 1998) and the concentration of enzymes in the pancreatic tissue decreases (Jensen *et al.*, 1997). So far, little is known about the influence of dietary acidification on gastrointestinal motility and exocrine pancreatic secretion. Thaela *et al.* (1999) observed that supplementation of the feed of weaned pigs with lactic acids increased the exocrine pancreas secretion in volume, total protein and trypsin activity when compared with a diet without supplementation. Whether other dietary acidifiers have a similar effect is not known. However, it has been shown that intestinal acidification regulates gastroduodenal motility and elevates exocrine pancreatic secretion. Harada *et al.* (1986) showed that the intestinal infusion of equimolar solutions of different monocarboxylic acids induced different (qualitatively and quantitatively) responses from exocrine pancreas in anaesthetized pigs.

Therefore the aim of our study was to compare the effect of lactic and formic acid on exocrine pancreatic secretion and gastroduodenal motility in weaned piglets.

The collection of pancreatic juice and recording of gastrointestinal myoelectrical activity were conducted based on periodic pancreatic secretion (PPS) and migrating myoelectric complex (MMC).

Materials and Methods

Fifteen 5-week-old pigs were surgically prepared with a pancreatic duct catheter, a duodenal cannula and three bipolar electrodes (implanted on distal stomach and proximal and distal duodenum). The pigs were weaned 1 week before surgery and divided into three experimental groups depending on the diet: group C (standard weaning diet); group L (standard weaning diet + 3.6% of lactic acid); and group F (standard weaning diet + 1.8% of formic acid). The acids were mixed with water, which was then added to the feed just before feeding. The feed:water ratio was 1:2.5. The pH of diets C, L and F was 6, 3.7 and 3.7, respectively. Experimental recordings of myoelectrical activity and collections of exocrine pancreatic secretion started on days 5 and 6 post-surgery. To register gastrointestinal myoelectrical activity, a MacLab digital-analogue system was used. Pancreatic juice was sampled at 5 min intervals. The volume of each sample was measured and 100 µl was taken for the analysis of total protein and bicarbonate. Three interdigestive control MMC/PPS cycles were recorded, starting after 12 h of

fasting. At the beginning of the following phase II of MMC, 2 h of intraduodenal (ID) infusion of lactic or formic acid solution was performed. The solutions of acids were equal in molar concentration (200 mM), pH 2.21 and flow rate (10 ml kg⁻¹ body weight (BW) h⁻¹). The acid infusions were performed on two separate experimental days with a minimum interval of 2 days between experiments. We compared the volume of pancreatic juice (ml kg⁻¹ BW), output of total protein (mg kg⁻¹ BW) and bicarbonate (mmol kg⁻¹ BW) as well as frequency of electrical response activity (ERA) from 30 min after the beginning of each phase II of MMC before infusion (basal secretion) and 30 min from the beginning of the infusion period. The effect of treatments was calculated per animal as a percentage of the basal response. Statistical evaluations were accomplished using two-factor ANOVA without interaction, which were not significant (*P* < 0.05).

Results

Pancreatic juice secretion

Addition of lactic or formic acid to the diet had no effect (*P* > 0.05) on basal secretion of pancreatic juice in volume, total protein and bicarbonate outputs.

The ID infusion of acid solutions increased outflow of pancreatic juice and bicarbonate output but had no effect on total protein output. The outflow of pancreatic juice volume and output of bicarbonate were significantly higher after infusion of

Table 59.1. The effect of intraduodenal (ID) infusion of lactic and formic acid on exocrine pancreatic secretion in weaned piglets. Data presented as percentage of basal pancreatic secretion.

	ID Lactic acid (mean + SE)	ID Formic acid (mean + SE)	<i>P</i> =
Volume	166 + 12	109 + 12	0.003
Protein	113 + 11	87 + 11	0.1
Bicarbonate	179 + 20	118 + 20	0.04

lactic when compared with the formic acid solution (Table 59.1). The response to acids infusions was not affected by the diet.

Gastrointestinal myoelectrical activity

The frequency of ERA in gastric antrum decreased during the infusion of acid solutions. Infusion of lactic acid solution decreased antral ERA by 75% (SE 2) which tended ($P = 0.05$) to be greater than inhibition evoked by formic acid (48%, SE 8). The frequency of ERA in the duodenum was unaffected by the acid infusions.

Conclusions

The basal pancreatic secretion and the secretory response to duodenal acidification were not significantly affected by acidification of the diet. Therefore we suggest that addition of the lactic or formic acid to the diet has no effect on the secretory capacity of exocrine pancreas in pigs after weaning. On the other hand, when comparing the effect of ID infusion of lactic or formic acid, our results clearly indicate that lactic acid is a more effective stimulant of pancreatic secretion and gastrointestinal motility.

References

- Harada, E., Nijjama, M. and Syuto, B. (1986) Comparison of pancreatic secretion via endogenous secretin by intestinal infusion of hydrochloric and monocarboxylic acid in anesthetized piglets. *Japanese Journal of Physiology* 36, 843–856.
- Jensen, M.S., Jensen, S.K. and Jacobsen, K. (1997) Development of digestive enzymes in pigs with emphasis on lipolytic activity in the stomach and pancreas. *Journal of Animal Science* 75, 437–445.
- Lesniewska, V., Pierzynowski, S.G., Johansen, H.N., Jensen, M.S. and Jensen, B.B. (1998) Weaning of pigs: duodenal myoelectrical activity during change from sow's milk to solid feed. *Journal of Animal and Feed Sciences* 7, 267–270.
- Thaela, M.-J., Swiech, E., Hedemann, M.S., Jacob, S., Pierzynowski, S.G. and Jensen, B.B. (1999) Effect of dietary lactic acid on the pancreatic secretion of weaned piglets. In: Krogdahl, A., Mathiesen, S.D. and Pryme, I.F. (eds) *Proceedings of the Seventh Scientific Workshop: Effects of Antinutrients on the Nutritional Value of Legume Diets*. Office of Official Publications of the European Communities, Luxembourg, 8, 19–22.

60 Influence of Luminal Pancreatic Juice and Bile on the Secretion of Pancreatic Juice and Bile in Weaned Pigs

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A chronic model was developed to collect pure pancreatic juice and bile in weaned pigs. Effects of pancreatic juice and bile diversion and reintroduction into the duodenum were studied under preprandial and prandial conditions. Diversion of pancreatic juice increased pancreatic secretion in both of these periods. Diversion of bile increased pancreatic secretion but did not affect bile flow. Diversion of both reduced bile secretion. In conclusion, there is preprandial and prandial feedback control of pancreatic juice secretion and no bile feedback control in weaned pigs. Bile flow is influenced by pancreatic juice diversion.

Introduction

Regulation of pancreatic secretion by the amount of pancreatic juice (PJ) in the lumen of the duodenum (feedback control) has been intensively studied, in particular in rats (Fushiki *et al.*, 1999). In pigs, pancreatic feedback control has been observed in the interdigestive and prandial period (Houe *et al.*, 1997) or only in the prandial period (Rådberg *et al.*, 1997). According to Houe *et al.* (1997) this mechanism does not depend on the quantity of pancreatic enzymes introduced into the duodenum or on the concentration of cholecystokinin (CCK) in the peripheral blood. Bile feedback control and the influence of luminal bile on pancreatic secretion remain obscure

(Vantini *et al.*, 1982; Koop, 1990). The aim of the present study was to develop a pig model for simultaneous collection of PJ and bile, and to clarify the involvement of the two body fluids on the regulation of preprandial and prandial PJ and bile secretion in conscious pigs.

Materials and Methods

Treatments and experiments were conducted according to European Community regulations concerning the protection of experimental animals. Nine weaned pigs (15 ± 5 kg body weight, 8 weeks old) were prepared with pancreatic duct catheters and duodenal cannulae (first surgery). In

four animals the bile duct was catheterized (second surgery) approximately 4 weeks later. After the operations and between collections, the bile and pancreatic catheters and the corresponding duodenal cannulae were externally interconnected to allow bile and pancreatic juice flow into the duodenum. The pigs received a standard pig diet (2% of body weight per meal), between 1000 and 1100 h, and between 1530 and 1630 h; water was allowed *ad libitum*. The pigs recovered well from the surgery; after the first operation the body weight (BW) gain was 300–450 g day⁻¹; after the second it was 150–300 g day⁻¹. The secretion studies started after a 1 week postsurgical recovery period. Morning preprandial and prandial collections of pancreatic juice with and without reintroduction of PJ and/or bile were performed on freely moving pigs. PJ samples were kept frozen (–20°C) until analysis. The pancreatic juice was analysed for volume, total protein content and trypsin activity; bile was analysed for volume.

Results and Discussion

After the first operation, diversion of PJ in the preprandial period led to a significant rise in the PJ protein output, whereas in the prandial period it significantly increased both the PJ protein and trypsin outputs (Table 60.1). This is in agreement with the study by Houe *et al.* (1997) but not with our previous study (Rådberg *et al.*, 1997). We think the difference was

generated by lower feed intake (only 2% of BW per meal) in the present study. The other experimental procedures (e.g. surgery, animal maintenance) were identical to those reported earlier. This means that stimulation by the remnants that still occupy the upper gut before morning feeding may mask the preprandial feedback control.

The preprandial PJ secretion after the second operation was twofold higher compared with that before bile cannulation. Feeding stimulated the PJ protein and trypsin outputs after the first and second surgeries; the PJ response to food tended to be higher in pigs with pancreatic and bile duct cannulae as compared with pigs with only pancreatic duct cannulae (not significant). This is the first study to report such a phenomenon, since catheterization in earlier studies was performed in one step and we cannot give a definite explanation at present. Presumably, a reduction of the circulating bile acid pool due to loss of some bile during the recovery period after the second operation could have contributed. This, in turn, could activate a secretin-dependent mechanism controlling duodenal acidity (Houe *et al.*, 1997). Further studies are necessary.

Bile diversion (with PJ reintroduction) did not affect the bile flow in the preprandial period, indicating the absence of bile feedback control in this period (Table 60.2). In the prandial period, a tendency towards reduction of bile flow after bile diversion was observed (not significant). Thus, feedback control of bile flow in the

Table 60.1. Pancreatic juice secretion in pigs with and without return of PJ into the duodenum (mean $\pm$ SD).

Flow/output (h ⁻¹ kg ⁻¹ BW)	PJ return	PJ diversion	P ^a
Preprandial			
Juice flow (ml)	2.4 $\pm$ 1.6	2.9 $\pm$ 2.0	0.11
Protein output (mg)	5.4 $\pm$ 4.9	7.2 $\pm$ 5.6	0.04
Trypsin output (U)	4.0 $\pm$ 4.0	4.9 $\pm$ 5.4	0.28
Prandial			
Juice flow (ml)	4.0 $\pm$ 1.4	4.6 $\pm$ 2.6	0.55
Protein output (mg)	17.9 $\pm$ 6.8	29.6 $\pm$ 4.1	0.02
Trypsin output (U)	10.3 $\pm$ 4.2	21.2 $\pm$ 12.2	0.05

^aUnpaired Student's *t*-test.

prandial period was not confirmed statistically. Interestingly, the diversion of both bile and PJ led to a significant reduction of bile flow before a meal and a tendency to decrease it after eating (Table 60.2), and agrees with earlier studies on fistulated dogs (Davies *et al.*, 1985). In conclusion,

present results suggest that there is a preprandial and prandial feedback mechanism controlling the secretion of pancreatic juice but not the secretion of bile. Bile flow is influenced by pancreatic juice diversion, but the mechanism behind it needs further investigation.

Table 60.2. Preprandial and prandial bile flow (ml h⁻¹ kg⁻¹ BW) with and without PJ and bile diversion in pigs; (+) reintroduction, (-) diversion (mean ± SD).

	+ Bile, +PJ	- Bile, +PJ	- Bile, - PJ
Preprandial	4.1 ± 1.8 ^a	4.2 ± 1.9 ^a	2.7 ± 0.9 ^b
Prandial	3.4 ± 1.5	2.3 ± 2.0	1.7 ± 1.2

^{ab}Letters indicate significant differences in row (*P* < 0.05).

References

Davies, H.A., Wheeler, M.H., Psaila, J., Rhodes, J., Newcombe, R.G., Jones, J.M., Procter, D., Adrian, T.E. and Bloom, S.R. (1985) Bile exclusion from the duodenum. Its effect on gastric and pancreatic function in the dog. *Digestive Diseases Science* 30, 954.

Houe, T., Saetre, S., Svendsen, S., Olsen, O., Rehfeld, J.F. and Schaffalitzky de Muckadell, O.B. (1997) Feedback regulation of pancreatic exocrine secretion in minipigs. *Scandinavian Journal of Gastroenterology* 32, 324–379.

Fushiki, T., Tsuzuki, S. and Pierzynowski, S.G. (1999) Feedback regulation of pancreatic secretion. In: Pierzynowski, S.G. and Zabielski, R. (eds) *Biology of the Pancreas in Growing Animals*. Elsevier, Amsterdam, pp. 249–260.

Koop, I. (1990) Role of bile acids in the control of pancreatic secretion and CCK release. *European Journal of Clinical Investigation* 20, Supplement 1, S51–S57.

Rådberg, K.M., Lundin, P.D.P., Pierzynowski, S.G. and Weström, B.R. (1997) Effects of diversion of pancreatic juice on exocrine pancreatic secretion and intestinal molecular absorption in pigs. In: Laplace, J.P., Fevier, C. and Barbeau, A. (eds) *Proceedings of the VIIth International Symposium on Digestive Physiology in Pigs*. St Malo, France, pp. 544–548.

Vantini, I., Pederzoli, P., Maffezzoli, G.F., Brocco, G., Cavallini, G., Pilon, T., Ederle, A., Piubello, W., Benini, L., Ferrini, S., Marchiori, N. and Scuro, L.A. (1982) Presence of duodenopancreatic feedback in minipigs and possible interference from bile. *American Journal of Gastroenterology* 77, 154–157.

61 Effects of Stress on Exocrine Pancreatic Secretion

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The effects of competition, frustration, 'anti-stress' and stress on the exocrine pancreatic secretion were studied in six pigs with chronic catheters in the pancreatic duct, duodenum and jugular vein. No effects of competition were seen. During frustration the volume of pancreatic juice increased to postprandial levels while the protein output did not rise until the pigs were actually fed. After an injection of amperozide the secretion practically ceased. Three and 4 days after an injection of ACTH, when the serum concentration of cortisol was back down to normal, an increased output of protein and trypsin was seen.

Introduction

The effects on the exocrine pancreas of mixing pigs with and without the neuroleptic amperozide, frustration and simulation of chronic stress, have been studied by Botermans *et al.* (1999). In that study, aggression and stress lowered preprandial amylase activity and tended to reduce preprandial secretion volume but no other effects could be detected. An injection of amperozide decreased food intake as well as preprandial secretion and tended to decrease the postprandial secretion. Frustration led to decreased output of protein and trypsin. Injection of ACTH (simulation of chronic stress) resulted in both an increased secretion and an increased feed consumption.

The aim of the present study was to investigate further the effects of stress on the exocrine pancreatic secretion by using partly different stressors, to check the effect of the 'anti-stress' drug amperozide and to eliminate the effect of the amount of feed consumed in the ACTH experiment.

Thus, the effects of competition, frustration, 'anti-stress' and stress on exocrine pancreatic secretion were examined.

Material and Methods

Six pigs were surgically fitted with chronic catheters in the pancreatic duct, duodenum and jugular vein (Pierzynowski *et al.*, 1988). Two pigs were excluded from the experiment, one due to loss of a pancreatic duct catheter and one due to signs of pancreatitis. The pigs were able to move freely at all times, including during the collection of pancreatic juice. Juice was collected between 0800 and 1200 h and the volume was measured every 30 min. A small sample (approximately 2 ml) was removed and frozen; the rest of the pancreatic juice was infused into the duodenum in order not to interfere with feedback regulation mechanism or digestion. The pancreatic juice samples were analysed for protein content and trypsin activity. Blood was collected twice daily, 1 h before feeding and 1 h after

feeding. The blood samples were analysed for the level of cortisol. The pigs were housed in individual pens and during the competition and frustration treatments there was an opening, large enough to allow some degree of fighting, in the partition where the feed bowl was located. For the competition and frustration treatments, one pig was fed while the neighbouring pig had to wait 1 h for feed. The following day the treatments were reversed. The 'anti-stress' treatment consisted of an intramuscular injection of amperozide (1 mg kg⁻¹, Hogpax® vet, Pharmacia & Upjohn, Sweden) and the 'stress' treatment of a single intramuscular injection of ACTH (15 µg kg⁻¹, Synacthen® Depot, Ciba-Geigy, Switzerland) at 0600 h. The control values were recorded on control days before and after each experiment. As the injection of amperozide resulted in reduced feed intake, the feed given on the control day was limited to the amount eaten by each pig on the experimental day.

but the protein content and trypsin activity did not increase until the pig was actually fed, 1 h later (Fig. 61.1).

The injection of amperozide caused a very marked reduction of the volume of postprandial pancreatic secretion (9.50 vs. 76.1 ml h⁻¹, amperozide vs. control day). The preprandial volume was also lower on the day of the amperozide injection (31.0 vs. 83.5 ml h⁻¹). The output of protein and trypsin was reduced by amperozide. No changes in pancreatic secretion were apparent during the day of ACTH injection, despite the massive increase in plasma cortisol (246 vs. 112 nmol l⁻¹ ACTH vs. control). However, 3 and 4 days after the ACTH injection, when the serum cortisol levels were back down to normal, an increase in output of postprandial protein (1.24 vs. 0.857 g h⁻¹) and trypsin (11.1 × 10⁵ vs. 7.59 × 10⁵ U h⁻¹) was seen in all pigs.

Discussion

Results

No effects of competition on the exocrine pancreatic secretion could be detected. During the frustration treatment, when the neighbouring pig was fed, the volume of the secretion rose to postprandial levels

The rise in volume of pancreatic output that was observed during the frustration was not observed by Botermans *et al.* (1999). The fact that the protein output of the juice only slightly increased during frustration and did not reach its maximum until the pigs were actually fed suggests

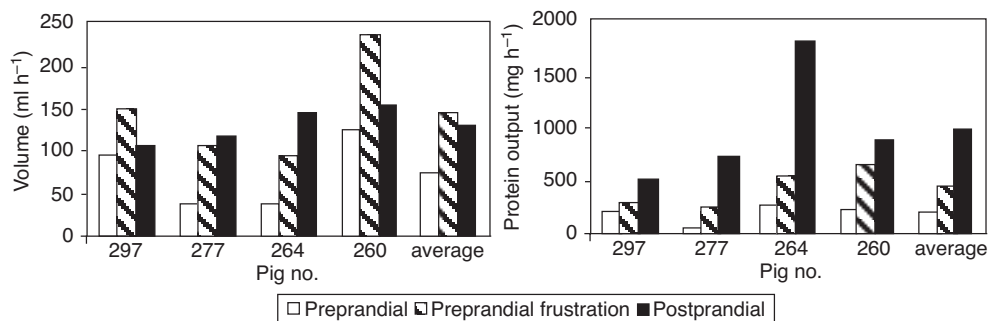


Fig. 61.1. The volume and protein concentration of exocrine pancreatic secretion during the frustration study. The frustration sample was taken at normal feeding time, when the neighbouring pig was fed. The postprandial sample was taken 1 h later, when the experimental pig was fed.

that there is a difference in regulation mechanisms between the different components of the pancreatic juice.

The effect of amperozide was already seen during the preprandial period even if it was more pronounced during the postprandial period. This effect is probably due not to a low level of stress but more likely to a direct effect of the drug on the nervous system that regulates pancreatic exocrine secretion (Rådberg *et al.*, 1999).

The fact is that 3 and 4 days after a single injection of ACTH, and a corresponding peak in plasma cortisol, the increased output of protein and trypsin in the pancreatic juice was similar to that reported by Botermans *et al.* (1999). In the latter study the pigs were injected four times during the 48 h preceding the experiment and on the experimental day an increased preprandial secretion of protein and volume of pancreatic output was seen. Whether the observed simultaneous increase in feed

intake was the cause for the increased secretion was discussed. In the present experiment the pigs were only allowed the same amount of feed as consumed during the control days before the ACTH experiment. Thus, the observed increase in pancreatic secretion of proteins did not appear to be dependent on an increased feed intake. In summary, cortisol or ACTH having an effect on the synthesis of pancreatic proteins rather than the secretion could cause the late response observed in both studies.

Acknowledgements

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References

- Botermans, J.A.M., Svendsen, J., Weström, B. and Pierzynowski, S.G. (1999) The effect of stress conditions on exocrine pancreatic secretions in growing pigs. *Journal of Animal Physiology and Animal Nutrition* 82, 150–162.
- Pierzynowski, S.G., Weström, B.R., Karlsson, B.W., Svendsen, J. and Nilsson, B. (1988) Pancreatic cannulation of young pigs for long-term study of exocrine pancreatic function. *Canadian Journal of Animal Science* 68, 953–959.
- Rådberg, K., Botermans, J., Weström, B.R. and Pierzynowski, S.G. (1999) Depressive effects of anesthesia or sedation on exocrine pancreatic function in pigs. *Laboratory Animal Science* 49, 662–664.

62 An Increased Hindgut Fermentation Promoted Major Changes on the VFA Profile but not on the Total VFA Concentration or the Digesta Contents

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We studied the volatile fatty acids (VFA) contents in caecum, colon and rectum of finishing Landrace and Iberian pigs, fed on a maize- or sorghum-acorn-based diet. As referred to in Chapter 63, Iberian pigs promoted an increased amount of organic matter (OM) available for hindgut fermentation, but no differences were observed in the VFA digesta content. Significant changes were observed on the profiles of main VFA (acetate, propionate, butyrate and branched-chain VFA). Differences in the pattern of fermentation appear to be related to breed and diet characteristics.

Introduction

Volatile fatty acids (VFA), principally acetate, propionate and butyrate, are produced in the caecum-colon of pigs as end products of the microbial fermentation.

Concentration of VFA at different gastrointestinal sites is a direct function of the microbial growth; thus, VFA concentrations and profiles are considered, respectively, as an index of the amount of organic matter (OM) fermented and of the relative different substrate fermentation. In this sense, the present abstract explores the changes observed for the caecum-colon parameters (digesta content, VFA concentrations and profile) of Landrace and Iberian finishing pigs, fed on different sources of carbohydrates (maize- vs. sorghum-acorn-based diets), for which we have reported changes in the amount of OM fermenting in the hindgut compartment (see Chapter 63).

Material and Methods

Forty-eight castrated male pigs (24 Landrace, 24 Iberian; body weight 88.1 ± 6.4 kg) were used in a finishing and slaughter experiment, as described elsewhere. Briefly, animals were allocated in 16 pens (three animals each) and assigned to two dietary treatments: diet C (maize-based) and diet S (sorghum-acorn-based). At an average final weight of 107–108 kg, six animals from each breed and diet, without previous fasting, were slaughtered, and whole-gastrointestinal excised; samples were taken of digesta from the caecum, the proximal, medial and distal colon and the rectum and were acidified with H_3PO_4 (approximately 4 g fresh weight digesta ml^{-1} of 5% H_3PO_4 , 50 mM 4-methyl valerate as internal standard) and stored at -20°C until the analysis of centrifuged samples for VFA concentration by gas-liquid chromatography, following the method proposed by Jouany (1982).

Results and Discussion

Experimental treatments promoted significant ($P < 0.01$) changes in the amount of OM flowing to the hindgut compartment (1.30 and 1.10 kg for Iberian, and 0.62 and 0.87 kg for Landrace pigs, fed on diets C and S, respectively) (Table 62.1). An increase in the OM flowing into the large intestine in Iberian pigs was not associated with significant increases in the caecum–colon digesta content; and Iberian pigs showed significantly lower digesta contents than Landrace, either in caecum (222 vs. 335 g; $P = 0.06$) and colon (1347 vs. 2132 g; $P < 0.01$).

Figure 62.1 shows VFA concentrations in different large intestine compartments. Changes in OM fermentation were not associated with significant changes in the VFA concentration; average concentrations ($\mu\text{mol g}^{-1}$) were: caecum, 167; proximal colon, 183; medial colon, 176; distal colon, 144; and rectum, 112.

Figure 62.2 shows the evolution of the acetate, propionate, butyrate and branched-chain VFA (BCVFA) profiles in the caecum, the proximal, medium and distal colon and the rectum. In the proximal colon, Iberian pigs showed, vs. Landrace, higher propionic percentages (31 vs. 24.4%), and concomitant decreases in the acetate (56.1 vs. 60.1%; $P < 0.05$) and butyrate (10.8 vs. 12.7%, $P = 0.10$) percentages. No significant differences were observed between diets. Differences between breeds likely reflect an increase in the starch reaching the hindgut compartment of Iberian pigs, favouring the development of propionate-

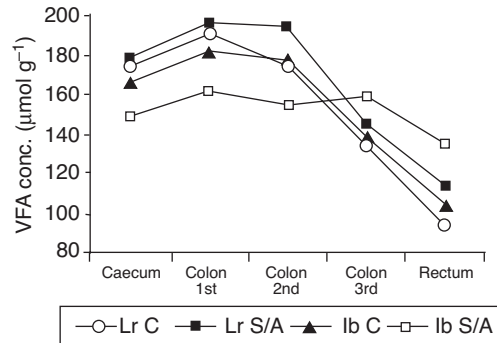


Fig. 62.1. VFA concentrations in different digestive compartments. LR, Landrace; IB, Iberian.

producing bacteria at the expense of acetate. Propionate differences were progressively reduced from proximal to medial and distal colon, reflecting a progressive run-out of rapidly fermentable substrates. However, the butyrate percentage in the medial colon was affected by breed (14.9% in Landrace and 12.1% in Iberian; $P < 0.01$) and diet (12.2% in diet C and 14.7% in diet S; $P < 0.01$). Under certain conditions, the development of a large protozoal population accompanies an increase in butyrate. Branched-chain VFA percentages increased from the lowest values in caecum (0.87%) and the proximal colon (1.01%) to 2.24, 3.70 and 4.6% in the medial and distal colon and rectum, respectively. Among experimental treatments, and irrespective of hindgut compartment, Iberian pigs showed lower BCVFA than Landrace ($P < 0.05$) and diet S than diet C ($P < 0.05$).

Differences were observed between Landrace and Iberian pigs on the pattern of

Table 62.1. Average values of daily OM intake and daily OM flowing at the ileum, and full weight of the caecum–colon compartment.

	Landrace		Iberian		SE	Probability		
	Diet C	Diet S	Diet C	Diet S		Breed	Diet	Int.
OM intake (kg)	2.82	2.97	3.71	3.79	0.22	**	NS	NS
Ileum OM flows (kg)	0.63	0.87	1.30	1.10	0.17	**	NS	NS
Full caecum (g)	426	579	369	393	59.1	*	NS	NS
Full colon (g)	3399	3968	2932	2792	274	**	NS	NS

Probability: Int = interaction; NS, not significant; * $P < 0.05$; ** $P < 0.01$.

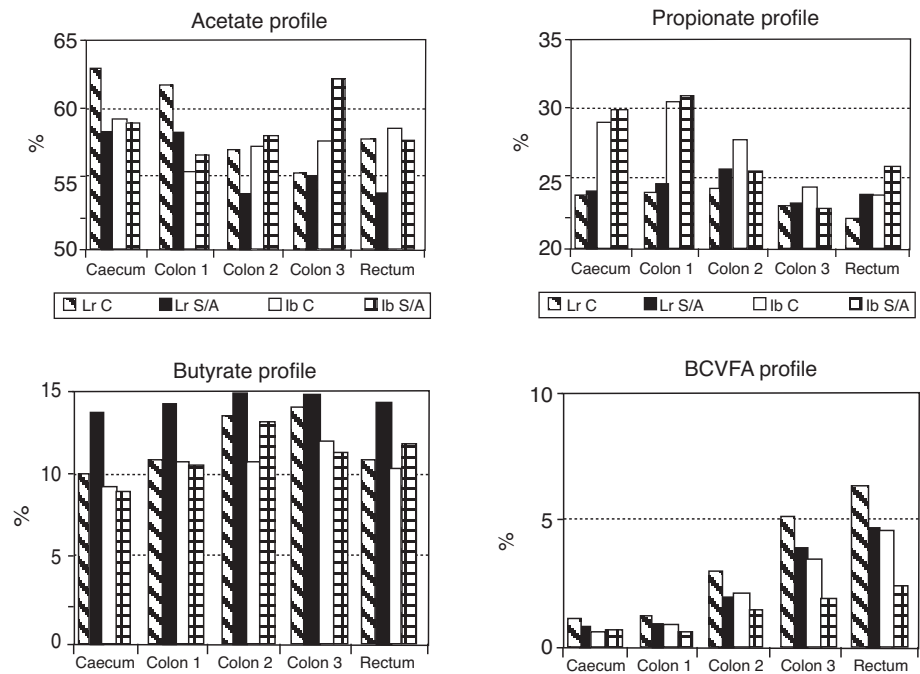


Fig. 62.2. Evolution of acetate, propionate, butyrate and BCFVA profiles (%). Lr, Landrace; Ib, Iberian.

fermentation and VFA profiles, associated with maize- or sorghum–acorn-based diets, which could suggest specific microbial

adaptions. An inverse correlation between BCFVA percentages and the amount of OM fermented in the hindgut is suggested.

Reference

Jouany, J.P. (1982) Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Science des Aliments* 2, 131–144.

63 Comparative Digestibility and Productive Performances Between Landrace and Iberian Pigs Fed on a Maize- or a Sorghum–Acorn-based Diet

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An experiment was carried out to study differences between finishing Landrace and Iberian pigs in their mechanisms for digesting ingredients such as maize or sorghum–acorn. Forty-eight neutered males (24 Landrace and 24 Iberian) were fed on two experimental diets, with a similar nutrient composition, based on maize or sorghum–acorn. Results of this trial showed that digestibility was affected by breed characteristics and dietary ingredients. Landrace pigs showed a lower dry matter intake and higher ileum digestibility. Hindgut organic matter fermentation contributed extensively to whole-tract digestibility, partially compensating ileum digestibility differences between Landrace and Iberian pigs.

Introduction

Digestible energy (DE) values are available for most of the commonly used feeds, these being mainly predicted by their chemical composition. Among the compositional parameters used, DE is calculated from crude protein (CP), ether extract (EE) contents and an approximate index of carbohydrate quality, and either crude fibre (CF) or neutral detergent fibre (NDF) (Noblet and Perez, 1993).

The present paper explores other likely effects affecting the digestibility of energy, such as animal characteristics (lean vs. fat breed-line) and dietary ingredients used

(different sources of carbohydrates, maize- vs. sorghum–acorn-based diets).

Material and Methods

Forty-eight castrated male pigs (24 Landrace, a lean line; 24 Iberian, a breed not selected against fatness; body weight 88.1 ± 6.4 kg) were used in a finishing and slaughter experiment. Animals were allocated to 16 pens (three animals in each) and assigned to two dietary treatments: diet C (maize, 75.0%; soybean meal, 19.7%; olive oil, 1.2%; acorn shell, 1.5%) and diet S (maize, 37.2%; sorghum, 27.5%; acorn,

Table 63.1. Chemical composition of the experimental diets (% dry matter).

	Diet C	Diet S
Crude protein	16.19	17.19
NDF	14.20	16.80
Crude fat	5.24	5.33
Gross energy (kcal kg ⁻¹)	3973	3938

12.5%; soybean meal, 19.5%; soybean oil, 0.7%), administered *ad libitum*. Each diet included 1.5 g kg⁻¹ Cr₂O₃ as a digestibility marker. Table 63.1 presents the chemical composition of the experimental diets.

Following a 10-day period of adaptation, productive performance and whole-tract digestibility parameters were evaluated during 14-day periods until animals reach an average final weight of 107–108 kg. At this weight, six animals from each breed and diet were slaughtered, without previous fasting, and ileum digesta samples were obtained for digestibility measurements. Chromium III oxide concentration was determined following the method of Williams *et al.* (1962).

Results and Discussion

Table 63.2 presents the production parameters (voluntary feed intake, daily BW

gains and feed:gain ratios) determined for the whole period. Voluntary intake was significantly higher in Iberian (3.93 kg) than in Landrace pigs (3.03 kg). However, no significant differences were obtained for the average daily gain (0.770 and 0.763 g day⁻¹, respectively) and feed:gain ratios were higher for Iberian than for Landrace pigs (5.31 vs. 4.03, *P* < 0.05). No significant differences were observed between diets.

Table 63.3 presents the digestible energy content and the apparent digestibility coefficients for energy, organic matter (OM), CP and NDF determinations, in the small intestine and the whole digestive tract.

Digestible energy predicted by the method of Noblet and Perez (1993) was 3307 kcal kg⁻¹ for diet C and 3167 kcal kg⁻¹ for diet S. The calculated values were significantly lower for both diets: 3140 and 3157 kcal kg⁻¹ for diet C and 3109 and 2902 kcal

Table 63.2. Effect of maize- and sorghum–acorn-based diets on voluntary intake (kg day⁻¹), weight gain (kg day⁻¹) and feed:gain ratios for Landrace and Iberian pigs.

	Landrace		Iberian		SE	Probability		
	Diet C	Diet S	Diet C	Diet S		<i>P</i> Breed	<i>P</i> Diet	<i>P</i> Int.
Initial body weight	88.7	91.0	86.1	87.7	1.84	NS	NS	NS
Feed intake	2.95	3.12	3.89	3.97	0.22	**	NS	NS
Daily gain	0.76	0.77	0.81	0.74	0.04	NS	NS	NS
Feed:gain	4.02	4.05	4.85	5.76	0.55	*	NS	NS

Probability: *P* Int. = interaction; NS, not significant; **P* < 0.05; ***P* < 0.01.

Table 63.3. Effect of maize- and sorghum–acorn-based diets on the ileum and faecal digestibility coefficients determined for OM, CP, NDF and DE in Landrace and Iberian finishing pigs.

	Landrace		Iberian		SE	Probability		
	Diet C	Diet S	Diet C	Diet S		<i>P</i> Breed	<i>P</i> Diet	<i>P</i> Int.
Faecal digestibility								
OM	0.862	0.850	0.850	0.815	0.009	**	*	NS
CP	0.787	0.744	0.775	0.673	0.021	*	***	NS
NDF	0.518	0.682	0.547	0.589	0.035	NS	**	*
DE								
(DE/GE)	0.790	0.790	0.795	0.737	0.015	NS	*	0.066
(kcal kg ⁻¹)	3140	3109	3157	2902	60.40	NS	*	0.067
Ileum digestibility								
OM	0.726	0.684	0.670	0.581	0.034	*	*	NS

Probability: *P* Int. = interaction; NS, not significant; **P* < 0.05; ***P* < 0.01; *P* < 0.001.

kg⁻¹ for diet S, estimated for Landrace and Iberian pigs, respectively; differences appear to be dependent on the breed.

Whole-tract digestibility of OM showed differences associated with the experimental treatments (breed and diet), being significantly higher ($P < 0.01$) for Landrace (0.856) than Iberian (0.832) and for diet C (0.856) than S (0.832). Differences in OM digestibility were associated with differences in the whole-tract digestibilities of CP and NDF. A higher OM intake by Iberian pigs appeared to promote decreases (vs. Landrace) on the digestibility parameters, these decreases being higher with diet S than diet C.

Differences between diets and breeds could be associated with differences in the foregut and hindgut digestibilities. Significant differences were observed between breeds and diets for ileum digestibility of OM, being higher ($P < 0.05$) for Landrace than Iberian, and for diet C

than diet S. Hindgut digestibility, calculated from whole-tract and ileum digestibility parameters, contributed extensively to whole-tract digestibility (from 15.8% in Landrace fed on diet C to 31.7% in Iberian fed on diet C); this compensated, except in Iberian pigs fed on diet S, for most differences observed in ileum digestibility. In particular, lack of compensation in Iberian pigs fed on diet S was mainly associated with significant decreases in NDF digestibility (0.589 vs. 0.682 in Iberian and Landrace pigs, respectively; $P < 0.05$).

The present results suggest a breed effect on the digestibility parameters, mainly in the diet with the lowest small intestine digestibility coefficients (sorghum–acorn). Although no significant differences were observed (interaction between breed and diet; $P > 0.05$), present results reflect main differences between Iberian and Landrace pigs on the methods used to digest maize- and sorghum–acorn-based diets.

References

- Noblet, J. and Pérez, J.M. (1993) Prediction of digestibility of nutrient and energy values of pig diets from chemical analysis. *Journal of Animal Science* 71, 3389–3398.
- Williams, C.H., David, D.J. and Lismaa, O. (1962) The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *Journal of Agricultural Science* 59, 381–385.

64 The Influence of Dietary Protein Level on the Amino Acid Composition of Ileal Endogenous Protein Losses in Pigs

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The amino acid composition of ileal endogenous protein loss was investigated in growing pigs fed seven different experimental diets, all fed at six different protein levels. For diets with low protein or imbalanced protein, the contribution of proline was generally high. The occurrence of high proline was closely counteracted by a decrease in glutamic acid and appears to be mainly from secreted free proline.

Introduction

Digesta collected at the terminal ileum contain large quantities of endogenous protein, which may influence measurements of protein and amino acid digestibility considerably. Therefore, the composition of the endogenous protein loss needs to be known for calculating values of standardized digestibility. However, according to the literature, the amount of proline and glutamic acid appear to be highly variable (Boisen and Moughan, 1996).

Material and Methods

The ileal amino acid flow was determined in T-cannulated growing pigs by using chromic oxide as a marker. Seven experimental diets based on four individual common protein sources (barley, wheat, soybean meal and rapeseed meal) and three complete diets with varying composition of the same four protein sources were investigated. Each diet was fed at six different protein levels, obtained after dilution with a 1:1 mixture of sucrose and dextrose

together with adequate vitamins and minerals. The diets were fed for 14 days and ileum digesta were collected during the last 3 days according to a standard collection scheme.

Results

The contribution of amino acid N (amide-N not included) in the total N flow at ileal level was found to be below 50% of total N. However, after correction for undigested dietary N and amino acids as well, based on results from *in vitro* digestibility analysis according to Boisen and Moughan (1996), a relative increase in the contribution was found for almost all amino acids (Table 64.1). Exceptions were threonine and tryptophan, which were constant, while glycine and proline were reduced at high and low dietary protein level, respectively. The contribution of proline in particular was variable and increased on average from 6% to 13% when the dietary protein level was decreased from 17% to 6% across the different experimental diets. Further extrapolation to protein level at

Table 64.1. Influence of dietary protein level on the amino acid composition (g per 160 g N) of ileal flow of total crude protein and endogenous protein, respectively.

Crude protein in diet (%) ^b	Endogenous protein flow ^a		Total protein flow	
	0	17	0	17
Essential amino acids				
Lysine	18	10	23	32
Methionine	4	2	8	9
Cysteine	9	14	13	16
Threonine	40	42	38	40
Tryptophan	10	10	10	11
Isoleucine	16	20	21	32
Leucine	26	32	37	53
Histidine	9	8	13	17
Phenylalanine	18	21	25	35
Tyrosine	12	8	18	22
Valine	30	38	36	45
Non-essential amino acids				
Arginine	18	−2	25	31
Alanine	31	38	34	40
Aspartic acid	45	60	50	75
Glutamic acid	12	12	66	110
Glycine	58	65	55	54
Proline	210	52	168	56
Serine	33	40	36	45
AA-N, per cent of total N	51	38	57	60
Endogenous protein loss	12.9	19.4	14.3	39.4

^aCorrected for undigested dietary protein.^bExtrapolation to 0% and 17% dietary protein, respectively.

zero led to a proline contribution of 17%. This value is, like the general amino acid composition, in good agreement with data from literature on the composition of endogenous protein loss after feeding N-free diets. The relative decrease of proline was closely counteracted by the increase in glutamic acid, strongly indicating a close inverse relationship between the concentration of these two amino acids.

Furthermore, a close relationship was

found between high proline loss and the amount of surplus amino acids, relative to the ideal amino acid pattern, in the seven experimental diets (Table 64.2). The relationship between proline and glutamic acid is further presented schematically in Fig. 64.1.

In a supplementary study, selected ileal digesta samples identified for high proline contents were further analysed. In one sample high in proline, despite the pig

Table 64.2. Endogenous loss of proline and glutamic acid (g per 160g N) after feeding seven different diets.

	Proline	Glutamic acid	Pro + Glu	Pro:Glu ratio
Wheat	35 ± 22	19 ± 3	54 ± 23	1.88
Barley	58 ± 31	39 ± 8	97 ± 33	1.48
Rapeseed meal	38 ± 19	26 ± 7	64 ± 20	1.46
Soybean meal	26 ± 25	23 ± 10	49 ± 25	1.13
Mixture 1	28 ± 10	32 ± 10	60 ± 12	0.86
Mixture 2	28 ± 13	29 ± 7	57 ± 12	0.95
Mixture 3	30 ± 17	33 ± 8	63 ± 22	0.90

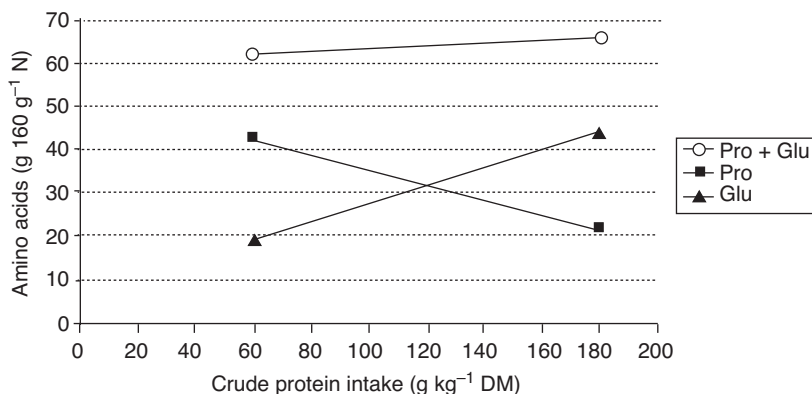


Fig. 64.1. Schematic presentation of the influence of crude protein intake on the endogenous losses of proline, glutamic acid, and proline + glutamic acid, respectively (average for seven different diets – see text).

being fed a diet with 18% protein from soybean meal, the contribution of free proline was found to be 30% of total proline. In other ileal digesta samples, collected after feeding N-free diets, a considerable portion of proline (up to 64%) was found to be free, indicating that the extra proline content in ileal digesta is mainly from secreted free proline. These results suggest that proline excretion in ileal digesta is an alternative excretion route in the elimination of surplus N from the gut protein metabolism, in particular during inadequate dietary amino acid supply.

Conclusion

High ileal endogenous proline loss, generally observed after feeding protein-free or protein-low diets, can also occur after feeding imbalanced protein. The amino acid composition of ileal endogenous protein loss is influenced by the varying losses of proline but is relatively constant when correcting for these losses. The occurrence of high proline was closely counteracted by a decrease in glutamic acid and appeared to be mainly from secreted free proline.

Reference

- Boisen, S. and Moughan, P.J. (1996) Dietary influences on endogenous ileal and amino acid loss in the pig – a review. *Acta Agriculturae Scandinavica, Section A, Animal Science* 46, 154–164.

65 Influence of Dietary *Lupinus luteus*, *Vicia sativa* and *Lathyrus cicera* on Immune Response and Pancreatic Digestive Enzymes of Early-weaned Pigs

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This study was designed to measure the digestive and immune responses of piglets, weaned at 28 days, fed one of four diets based on either control dry skim milk, or leguminous seeds of *Lupinus luteus* (LL), *Vicia sativa* (VS) or *Lathyrus cicera* (LC). Immunoblotting analysis detected the presence of antibodies against β -conglutinin of *Lupinus luteus*, vicilin of *Vicia sativa* and vicilin of *Lathyrus cicera* in the serum of piglets fed on the LL, VS or LC diets, respectively. However, no storage protein was found in the serum of any piglet fed either on LL, VS or LC diets. No differences were observed in amylase, lipase, trypsin or chymotrypsin total activity expressed as IU g⁻¹ of pancreatic tissue. Similarly, there were no significant differences in the weight of the liver, the pancreas and the spleen.

Introduction

The presence of antinutritional factors (ANF) in legume seeds can form complexes with the digestive enzymes, reducing their activity in the intestine and interfering with their biosynthesis. The presence of tannins in *Vicia faba* decreases the specific activity of trypsin but stimulates the synthesis of chymotrypsin, apparently through positive feedback mechanisms (Jansman *et al.*, 1994). The inactivation of the anti-trypsinic factors (ATF) of peas by heat treatment increases the activity of chymotrypsin and trypsin in the pancreatic tissue (Bengala Freire *et al.*, 1991). Furthermore, the presence of allergenic storage proteins (e.g. glycinin and conglycinin from soybean; vicilin and legumin from peas) can cause hypersensitivity reactions, damaging the small intestine of piglets and resulting in inadequate levels

of digestive enzymes and reduced absorptive capacity (Stokes *et al.*, 1987).

Materials and Methods

Twenty-four crossbred (Duroc $\times$ Landrace) male piglets, weaned at 4 weeks of age, were allotted randomly to four groups. For 4 weeks, each group received either a 20% crude protein control diet based on dry skim milk or a diet prepared by replacing 30% of the protein content with the protein of *Lupinus luteus*, *Vicia sativa* and *Lathyrus cicera* seeds, respectively. Piglets were slaughtered at an average live weight of 10 kg and the digestive organs and blood samples were collected. Measurement of pancreatic amylase, lipase, trypsin and chymotrypsin total activity was performed according to Bengala Freire *et al.* (1991). The globulin fraction of *L. luteus*, *V. sativa*

and *L. cicera* seeds was isolated by the method described by Franco *et al.* (1997). Total globulin samples were fractionated using FPLC anion exchange chromatography. Immunoblotting analysis was performed on the piglets' plasma for the detection of the isolated proteins and their specific antibodies. Data on the weight of digestive organs and the activity of pancreatic digestive enzymes were compared by analysis of variance.

Results and Discussion

Results showed that the replacement of 30% of the protein content of the control diet by the protein of *L. luteus*, *V. sativa* and *L. cicera* had no significant effect either on the weight of the digestive organs or on the total activity of the pancreatic digestive enzymes (Table 65.1). It is well known that an adaptation of the pancreatic digestive enzymes to dietary levels of starch, protein and lipids occurs in piglets (Peiniau *et al.*, 1996). In the present study, once the experimental diets were closely balanced in starch, protein and lipids, a change in pancreatic digestive enzymes due to the dietary level of those constituents could not be expressed. Furthermore, the level of ATFs and tannins in *V. sativa* and *L. cicera* was not high enough to change the synthesis of trypsin and chymotrypsin.

Concerning the detection of the storage

proteins' antibodies (Fig. 65.1), several bands of β -conglutin of *L. luteus*, vicilin and legumin of *V. sativa* and 2s-type and vicilin of *L. cicera* were recognized. However, the presence of bands of legumin of *V. sativa* and of 2s-type protein of *L. cicera* in control immunoblots, probed with plasma from piglets fed the control diet, indicated that for these proteins the observed reaction was due to non-specific interactions. Thus it was considered that only the results concerning the presence of antibodies against β -conglutin of *L. luteus*, vicilin of *V. sativa* and vicilin of *L. cicera* were truly positive. On the contrary, no storage protein was found in the serum of the piglets. Stokes *et al.* (1987) observed that the amount of antigenic protein from soybean present in the serum of piglets that were fed this legume seed declined to non-detection levels over the 20 days after weaning. Once the piglets' serum was collected at 28 days after weaning, there could have been a decline to non-detection levels of the antigenic proteins. This was possibly due to the development of oral tolerance or mechanisms of immune exclusion, as suggested by Stokes *et al.* (1987). In conclusion, the detection of IgG anti-storage proteins indicated that, although no storage proteins were detected in the piglets' serum at 56 days of age, at least β -conglutin from *L. luteus* and vicilin from *V. sativa* and *L. cicera* were absorbed in an antigenically intact form, through the small intestine.

Table 65.1. Effect of different diets on the relative weight (g kg^{-1} live weight) of the digestive organs and on the total pancreatic digestive enzymes activity (IU g^{-1} of tissue).

	Control	<i>Lupinus luteus</i>	<i>Vicia sativa</i>	<i>Lathyrus cicera</i>	Analysis of variance	Residual SD
Liver	26.26	25.88	26.30	26.47	NS	1.84
Pancreas	2.65	2.57	2.63	2.60	NS	0.33
Spleen	2.36	2.28	2.52	2.52	NS	0.69
Gall bladder	1.68 ^a	0.99 ^b	1.11 ^{ab}	1.3 ^{ab}	*	0.33
Amylase (10^3)	180.4	140.6	131.3	136.3	NS	47.6
Lipase	7083	7355	5484	7658	NS	2187
Trypsin	4878	3738	4430	3496	NS	1603
Chymotrypsin	1630	1690	1682	1860	NS	300

^{ab} Means with the same superscript are not significantly different ($P > 0.05$). * $P < 0.05$; NS, not significant.

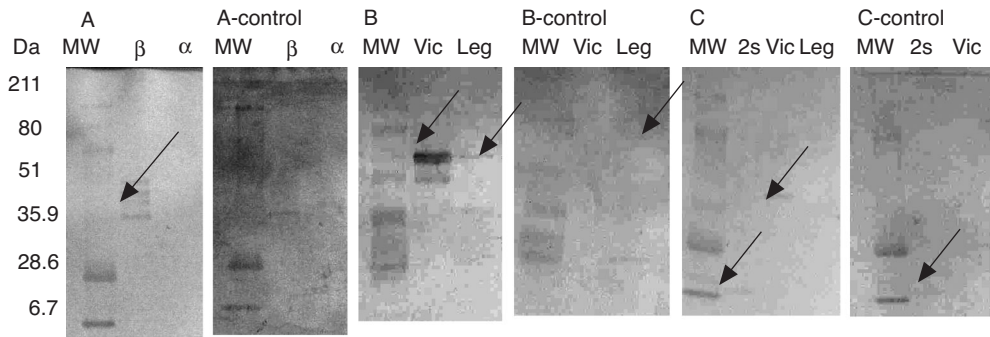


Fig. 65.1. Most representative membranes of immunoblotting analysis of the main storage proteins of *Lupinus luteus*, *Vicia sativa* and *Lathyrus cicera* in the serum of the piglets. (A) *Lupinus luteus*. (B) *Vicia sativa*. (C) *Lathyrus cicera*. β, β-conglutin; α, α-conglutin; Vic, vicilin; Leg, legumin; 2s, 2s type protein; MW, molecular weight. Arrows indicate positive reactions.

References

- Bengala Freire, J.P., Aumaitre, A. and Peiniau, J. (1991) Effects of feeding raw and extruded peas on ileal digestibility pancreatic enzymes and plasma glucose and insuline in early weaned pigs. *Journal of Animal Physiology and Animal Nutrition* 65, 154–164.
- Franco, E., Ferreira, R.B. and Teixeira, A.R. (1997) Utilization of an improved methodology to isolate *Lupinus albus* conglutins in the study of their sedimentation coefficients. *Journal of Agriculture Food Chemistry* 45, 3908–3913.
- Jansman, A.J.M., Enting, H., Verstegen, M.W.A. and Huisman, J. (1994) Effect of condensed tannins in hulls of faba beans (*Vicia faba* L.) on the activities of trypsin (EC 2.4.21.4.) and chymotrypsin (EC 2.4.21.1.) in digesta collected from the small intestine of pigs. *British Journal of Nutrition* 71, 627–641.
- Peiniau, J., Aumaitre, A. and Lebreton, Y. (1996) Effects of dietary protein sources differing in solubility on total tract and ileal apparent digestibility of nitrogen and pancreatic enzymes activity in early weaned pigs. *Livestock Production Science* 45, 197–208.
- Stokes, C.R., Miller, B.G., Bailey, M., Wilson, A.D. and Bourne, F.J. (1987) The immune response to dietary antigens and its influence on disease susceptibility in farm animals. *Veterinary Immunology and Immunopathology* 17, 413–423.

66 Influence of NSP-degrading Enzymes on Plasma Urea Nitrogen Level, Gastrointestinal Tract pH, Xylanase Activity, Viscosity and Nutrient Digestibility in Weaned Pigs

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The effects of β -glucanase and xylanase supplementation of diets based on two hull-less barley varieties on plasma urea nitrogen (PUN), gastrointestinal tract (GIT) pH, xylanase activity, viscosity and non-starch polysaccharide (NSP) and nutrient digestibility in the third section of small intestine (TSSI) were studied in 30 weaned pigs. Six pigs were euthanased on day 0 and 24 on day 10, and the GIT was rapidly removed and divided by ligature into stomach, three sections of the small intestine and caecum. The digesta from the above parts were collected for chemical analysis. Enzyme addition significantly ($P < 0.05$) reduced the PUN level and digesta viscosity and increased the digestibility of NSP, crude protein (CP) and energy in the TSSI.

Introduction

The influence of non-starch polysaccharide (NSP) on digestion in the gastrointestinal tract (GIT) includes viscosity in the small intestine, the absorption of nutrients, and modification of transit time and microbial fermentation. NSP-degrading enzymes are used in poultry and pig production to improve feed conversion. Many studies have been performed to measure the influence of NSP-hydrolysing enzymes on performance of non-ruminants. However, the mechanism by which the enzyme works is not clear for pigs (Baidoo *et al.*, 1998; Yin *et al.*, 2000). The objective of this study was to determine the effects of carbohydrase supplementation of hull-less barley-based diets on blood PUN level and GIT physiological status in weaned pigs.

Materials and Methods

Thirty pigs (Cotswold) with an initial body weight of 5 kg were fed a normal maize-soybean meal diet and were divided into five groups of six. Six pigs were euthanased on day 0 and the remaining 24 were randomly allocated to four diets. Diets were based on hull-less barley varieties Buck (B) or Falcon (F) (700 g B or F, 170 g soybean meal, 60 g fish meal, 40 g tallow, 28 g premix, 2 g Cr_2O_3 kg^{-1}) without or with addition of 1 g kg^{-1} of β -glucanase and xylanase. After 10 days, pigs were euthanased and the GIT was rapidly removed and divided into stomach, three sections of the small intestine and caecum. The digesta from the above parts were collected for chemical analysis. Blood sample was taken from the jugular vein on days 0

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and 10 for plasma urea nitrogen (PUN) analysis. The results were subjected to ANOVA according to the 2×2 factorial design, using the procedures of SAS (1991).

Results

Buck-based diets have higher β -glucans (59 g kg^{-1}) and total NSP (103 g kg^{-1}) values than Falcon-based diets, in which β -glucans were 45 g kg^{-1} and total NSP was 97 g kg^{-1} . The pH and viscosity were highest in the third section of small intestine (TSSI) and lowest in the stomach. Neither diet nor enzyme treatment significantly affected the pH. Enzyme addition significantly reduced the viscosity in the TSSI (Table 66.1). The viscosity in all parts of the GIT measured at day 0 was significantly ($P < 0.05$) lower than those measured at day 10. Assessment of GIT β -glucanase activity was not completed, due to difficulties in analysis. Xylanase activity in the TSSI was significantly ($P < 0.01$) higher for the enzyme-treated diets than for that of the unsupplemented diets (Table 66.2). Falcon-based diet had a significantly higher crude protein (CP) and energy digestibility in the TSSI than those of Buck-based diet.

Enzyme addition significantly increased the apparent digestibility of CP, energy, arabinose, xylose, glucose and total NSP in the TSSI (Table 66.2). Enzyme addition significantly ($P < 0.05$) decreased PUN level, but there was no difference ($P > 0.05$) between the two barley-based diets (Table 66.2). PUN level at day 10 was significantly ($P < 0.05$) higher than that at day 0.

Discussion

Feed enzymes work within a particular pH range and are themselves potentially open to digestion by the endogenous proteins. This is a problem in the pig stomach. A pH value of 3 observed in this study in the stomach could have a detrimental effect on exogenous enzyme activity. It is essential, therefore, that enzymes are protected against proteolysis but available at the jejunum and ileum. The measured high level of xylanase activity confirms that the exogenous enzymes still survived in the small intestine.

The results of this study demonstrated that the weaned pigs immediately changed their intestinal physiological status when a normal weaned diet was changed to a hull-less barley-based diet. The viscosity in the

Table 66.1. GIT pH and viscosity (cP) for pigs fed hull-less barley diets with (+) and without (–) addition of feed enzymes.

	Buck		Falcon		SEM		<i>P</i>	
	–	+	–	+	–	+	Barley	Enzyme
pH								
Stomach	3.01	3.02 ^c	3.03 ^c	3.08 ^c	0.598	0.664	NS	NS
Small intestine								
First section	5.70	5.72 ^b	5.69 ^b	5.73 ^b	0.689	0.700	NS	NS
Second section	6.10	6.20 ^{ab}	6.23 ^{ab}	6.20 ^{ab}	0.890	0.725	NS	NS
Third section	6.81	6.78 ^a	6.77 ^a	6.81 ^a	0.689	0.698	NS	NS
Caecum	5.90	5.89 ^b	5.80 ^b	5.79 ^b	0.678	0.782	NS	NS
SEM	0.835	0.783	0.772	0.825				
Viscosity								
Stomach	1.57	1.54 ^b	1.50 ^c	1.48 ^c	0.569	0.498	NS	NS
Small intestine								
First section	2.32	1.59 ^b	2.08 ^b	1.87 ^c	0.668	0.672	NS	NS
Second section	2.67	1.95 ^b	2.40 ^b	2.06 ^b	0.789	0.720	NS	NS
Third section	3.81	2.52 ^a	3.13 ^a	2.42 ^{ab}	0.567	0.553	NS	< 0.05
Caecum	2.65	2.03 ^b	2.53 ^b	2.00 ^b	0.590	0.623	NS	NS
SEM	0.569	0.521	0.499	0.623				

^{abc}Values in the same column with different superscript letters differ ($P < 0.05$).

Table 66.2. Xylanase activity (units kg⁻¹ dry matter) in third section of small intestine and the effect of barley varieties with (+) and without (–) enzyme addition on NSP, crude protein and energy digestibility (%) in the third section of small intestine and blood PUN level (mg dl⁻¹).

	Buck		Falcon		SEM		P	
	–	+	–	+	–	+	Barley	Enzyme
Xylanase activity	< 100	1200	< 100	1290	105.5	200.0	NS	< 0.01
Arabinose	16.3	23.9	18.1	23.3	6.12	4.81	NS	< 0.05
Xylose	14.6	20.8	13.6	18.2	3.26	1.19	NS	< 0.05
Mannose	19.7	20.0	25.5	30.2	4.61	4.32	< 0.05	NS
Galactose	40.1	39.0	33.6	34.7	5.60	5.20	NS	NS
Glucose	20.2	28.1	22.3	24.3	4.39	4.89	NS	< 0.05
Total	20.1	26.2	21.3	25.9	2.39	3.82	NS	< 0.05
Crude protein	62.0	66.3	65.9	68.9	1.09	1.00	< 0.05	< 0.05
Energy	65.9	72.5	69.2	73.1	1.15	1.00	< 0.05	< 0.05
PUN	18.00	14.21	17.59	15.55	3.874	3.007	NS	< 0.05

TSSI was only 2.61 cP for the pigs at day 0 but it increased to 5.30 cP at day 10. A significantly increased PUN (from 9.13 to 18.00 mg dl⁻¹) when feeding the hull-less barley-based diets indicated that amino acids from the digested hull-less barley was poorly utilized without exogenous enzymes. The decrease in ileal digesta viscosity and the increase in ileal digestibility of NSP may explain the increase in ileal

digestibility of nutrients observed in this study and others (Baidoo *et al.*, 1998; Yin *et al.*, 2000).

It is concluded that the high levels of β -glucans and xylose in hull-less barley do affect ileal digesta viscosity, digestion and protein utilization in weaned pigs. Carbohydrase addition may improve the GIT physiological status and increase nutrient digestion and absorption.

References

- Baidoo, S.K., Liu, Y.G. and Yungblut, D. (1998) Effect of microbial enzyme supplementation on energy, amino acid digestibility and performance of pig fed hullless barley for swine. *Canadian Journal of Animal Science* 78, 202–210.
- SAS (1991) *SAS User's Guide, Version 6.03*. SAS Institute, Cary, North Carolina.
- Yin, Y.L., McEvoy, J.D.G., Schulze, H. and McCracken, K.J. (2000) Studies on cannulation and alternative indigestible markers and the effect of food enzyme supplementation in barley-based diets on ileal and overall digestibility in growing pigs. *Animal Science* 70, 63–72.

67 Ileal Fatty Acid Digestibility of Different Oilseeds*

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In this study, the fatty acid ileal and faecal digestibility of different oilseeds was investigated. Four diets were tested, each based on barley as the main ingredient. Barley was mixed with starch, soybean, linseed or rapeseed. Feed, chymus and faecal samples were analysed for crude fat and fatty acids. Ileal and faecal crude fat digestibilities were similar, but ileal were inferior to faecal polyunsaturated fatty acid digestibilities. Stearic acid had a negative faecal digestibility, indicating its formation in the hindgut. In conclusion, fatty acids appeared to be less digestible than generally assumed on the basis of crude fat digestibility.

Introduction

Vegetal instead of animal fat sources in pig feed are becoming increasingly popular. Many vegetal oils are characterized by an unsaturated fatty acid profile. Eeckhout and De Paepe (1987) showed that the faecal crude fat digestibility of vegetal oil was higher than 90%, when added as such to the feed. When measuring faecal digestibility, however, the possible microbial transformation of fatty acids in the hindgut is not taken into account. The aim of this study was to investigate and compare the ileal and faecal fatty acid digestibility of rapeseed, soybean and linseed.

Materials and Methods

The diets (Table 67.1) were formulated to have a similar crude fat content (8%). Celite 545 was added to the diets as a marker. Linseed and rapeseed were milled

together with barley through a 2 mm sieve. A control feed, to estimate the fatty acids of barley origin and possibly of endogenous origin, was included as well. The crude fat and fatty acid digestibility of the oilseeds could thus be calculated.

Four ileo-cannulated pigs (Decuyper *et al.*, 1977) of about 50 kg body weight received one of the feeds, following a Latin square design. After an adaptation period of 5 days, chyme and faecal samples were collected on two consecutive days. The feedstuffs and diets were analysed for crude fat (with acid hydrolysis), crude protein, crude fibre (EC methods), ash and fatty acids (no oven drying prior to fatty acid analysis). Chyme and faecal samples were freeze-dried and then finely ground (2 mm sieve). Subsequently, 4 mol l⁻¹ HCl insoluble ash, crude fat (EC method) and fatty acid pattern (Sukhija and Palmquist, 1988), using nonadecanoic acid as an internal standard, were determined on the chyme and faecal samples.

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Table 67.1. Ingredient composition (%) of the experimental diets.

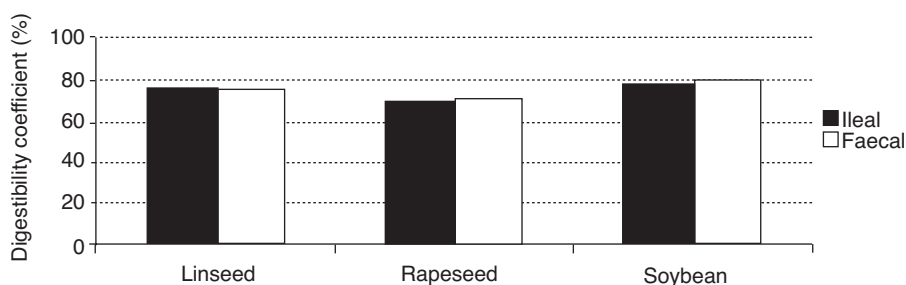
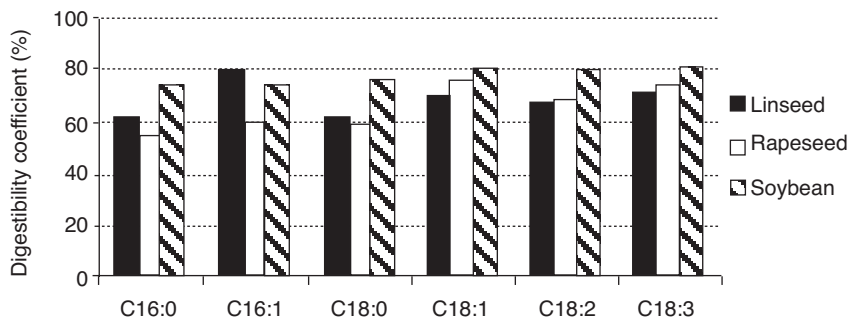
Ingredients	Control diet	Linseed diet	Rapeseed diet	Soybean diet
Barley	57.0	57.0	57.0	57.0
Maize starch	40.0	23.0	18.1	–
Linseed	–	17.0	–	–
Rapeseed	–	–	21.9	–
Soybean	–	–	–	40.0
Mineral/vitamin mix	3.0	3.0	3.0	3.0

Results and Discussion

The ileal and faecal crude fat digestibility were very similar for each of the oilseeds (Fig. 67.1), presumably very little absorption of fatty acids occurs in the hindgut. However, the ileal and faecal digestibilities of the individual fatty acids were quite different (Figs 67.2 and 67.3). Overall, the ileal fatty acid digestibilities amounted maximally to 80%, even for the (poly)unsaturated fatty acids, which are supposed to be highly digestible. In contrast, the faecal polyunsaturated fatty acid

digestibility was more than 80%. For stearic acid (C18:0), the faecal fatty acid digestibility was negative, which means that C18:0 was formed in the hindgut. Presumably, a shift from the polyunsaturated fatty acids mainly to stearic acid took place in the hindgut. Apparently hydrogenation predominantly yielded C18:0, as the faecal and ileal palmitic acid (C16:0) digestibility coefficients were very similar.

In conclusion, ileal polyunsaturated fatty acid digestibilities appear to be much lower than what might be expected on the basis of their faecal digestibilities.

**Fig. 67.1.** Ileal and faecal digestibility of different oilseeds.**Fig. 67.2.** Ileal fatty acid digestibility of different oilseeds.

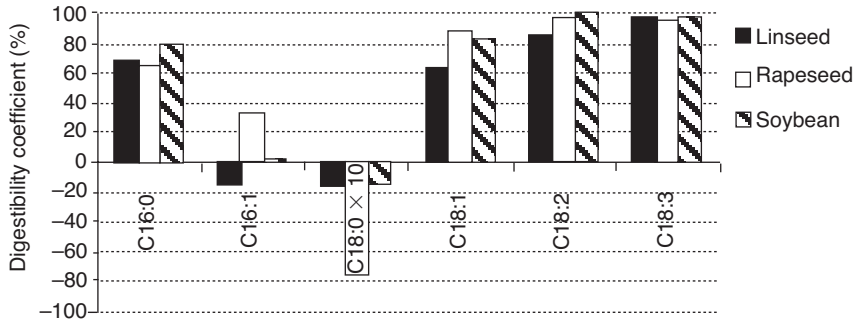


Fig. 67.3. Faecal fatty acid digestibility of different oilseeds.

References

- Decuyper, J.A., Vervaeke, I.J., Henderickx, H.K. and Dierick, N.A. (1977) Gastro-intestinal cannulation in pigs: a simple technique allowing multiple replacements. *Journal of Animal Science* 46, 463–468.
- Eeckhout, W. and De Paepe, M. (1987) Aspecten van de verteerbaarheid van sojaolie, rundvet en hun mengsels bij mestvarkens. *Communication no. 690 of the Department of Animal Nutrition and Husbandry*, Centre Agricultural Research-Ghent, 110 pp.
- Sukhija, P.S. and Palmquist, D.L. (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and faeces. *Journal of Agricultural and Food Chemistry* 36, 1202–1206.

68 The Availability of the B-vitamins Thiamin and Vitamin B₆ from Different Feed Sources for Pigs

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The aim of this investigation was to determine the availability of native thiamin and vitamin B₆ from different feeds. As a valuable measure for this, the determination of pre-caecal digestibility was used. The tested pig feeds were barley, wheat, rye, maize, wheat bran, boiled potatoes, soybean meal, milk powder and brewer's yeast.

Precaecal digestibility for thiamin from all tested feedstuffs was within a range of 81–94%. The feeds of animal origin and the plant products showed on average a nearly equal digestibility for thiamin (90% vs. 87%). Precaecal digestibility of vitamin B₆ from all tested food sources ranged from 51 to 87%. The digestibility of vitamin B₆ from plant products was on average 14% lower when compared with animal products.

Introduction

The availability (especially bioavailability) of nutrients, as for example of the B-vitamins, is very important in the nutrition of animals in regard to health and growth development. The precaecal digestibility of native B-vitamins has been well established as a measure for the availability of these nutrients in pigs (Roth-Maier *et al.*, 1998). Therefore, in the present study a technique of ileorectal anastomosis (IRA) in an end-to-end version was used to determine the precaecal digestibility of thiamin and vitamin B₆ from different pig feeds of plant and animal origin. The most important advantage of IRA for measuring B-vitamin digestibility is that vitamin synthesis by the intestinal flora is circumvented because this surgical technique bypasses the colon, causing digesta to move straight from the ileum to the rectum (Roth-Maier *et al.*, 1998). The tested feeds were barley, wheat, rye, maize, wheat bran, boiled pota-

atoes, soybean meal, milk powder and brewer's yeast.

Materials and Methods

For the purpose of the present investigation, growing pigs with an initial body weight of 33–40 kg were used in four experiments of Latin square design so that each animal was fed each diet in the experiment, respectively. The animals were provided surgically with an IRA in an end-to-end technique with preserved ileocaeco-colic valve. After surgery, the animals were allowed a 3-week recuperation period before starting the metabolic trials. Then the pigs were fed diets that provided them with a daily dry matter (DM) intake of 1350 to 1400 g, an energy amount of 21.75 MJ ME and 225 g crude protein per animal per day, and optimal mineralization and supplementation of fat-soluble vitamins. They were fed the individual experi-

mental diets for 12 days. The diets consisted of the tested feedstuff in the highest possible amount, which was the only source of the B-vitamins, and a thiamin- and vitamin B₆-free supplementary diet. The digesta were collected twice a day quantitatively during the final 5 days of the experimental period. Thiamin was determined in feeds and chyme by a fluorometric assay and vitamin B₆ was measured by HPLC. For more details see Roth-Maier *et al.* (1998, 1999, 2000).

Results and Discussion

The vitamin concentrations of the feedstuffs are represented in Table 68.1. The daily thiamin intake with these feeds was in the range of 1.4–5.4 mg and thiamin concentrations in chyme were 0.7–3.6 µg g⁻¹ DM. Therefore 170–780 µg thiamin were excreted daily. The vitamin B₆ intake was in the range of 1.8–5.6 mg, whereas

vitamin B₆ concentrations in chyme were 2.2–5.6 µg g⁻¹ DM. This resulted in daily vitamin B₆ excretions of 315–1480 µg.

The results of the precaecal digestibility of thiamin and vitamin B₆ are shown in Table 68.2. Precaecal digestibility for thiamin from all tested feedstuffs was within a range of 81%–94%, with the highest value from barley and the lowest value from maize. In comparison with the feeds of animal origin, the plant products showed on average a nearly equal precaecal digestibility for thiamin (87% vs. 90%). Moreover, all tested feedstuffs exhibited a rather good availability of thiamin. Regarding the availability of vitamin B₆ from all tested feeds, precaecal digestibilities were found between 51% and 87% in the following order: boiled potatoes > milk powder > brewer's yeast > soybean meal > wheat > maize > barley > wheat bran > rye. Comparing the plant products to the products of animal origin, they showed on average a 14% lower precaecal digestibility for

Table 68.1. Thiamin and vitamin B₆ concentrations of the feedstuffs (µg g⁻¹ DM).

Feedstuff	Thiamin	Vitamin B ₆	Feedstuff	Thiamin	Vitamin B ₆
Barley	6.50	3.60	Potatoes, boiled	4.39	8.54
Wheat	5.87	3.61	Soybean meal	6.48	5.31
Rye	4.94	1.96	Milk powder	2.93	2.93
Maize	5.49	4.06	Brewer's yeast, dried	53.05	30.76
Wheat bran	8.83	8.29			

Table 68.2. Precaecal digestibility of native thiamin and vitamin B₆ from different feeds (%).

Feedstuff	Number of observations	Precaecal digestibility	
		Thiamin	Vitamin B ₆
Barley	6	94 ± 2	63 ± 6
Wheat	5	87 ± 4	69 ± 4
Rye	3	84 ± 1	51 ± 11
Maize	2	81 ± 0	67 ± 1
Wheat bran	6	92 ± 2	56 ± 5
Potatoes, boiled	5	84 ± 4	87 ± 3
Soybean meal	3	89 ± 4	75 ± 4
Milk powder	6	88 ± 2	84 ± 2
Brewer's yeast, dried	3	91 ± 2	78 ± 6

vitamin B₆ (67% versus 81%) although boiled potatoes had the highest precaecal digestibility for vitamin B₆ determined in this study.

The reason for this is that many foods of plant origin contain a significant proportion of their vitamin B₆ in glycosylated form, predominantly as pyridoxine-5'- β -D-glucoside, which was shown to have an incomplete bioavailability (Gregory, 1993). In conclusion, thiamin seems to have a higher avail-

ability than vitamin B₆ in the tested feeds; therefore, special care should be taken over the supply of vitamin B₆ in pig nutrition, because in some fields of pig production, the requirement is not always met optimally.

Acknowledgement

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References

- Gregory, J.F. (1993) Nutritional properties of pyridoxine- β -glucosides. In: Eson, A. (ed.) *The Biochemistry and Molecular Biology of β -Glucosidases*. American Chemical Society, Washington, DC, pp. 113–131.
- Roth-Maier, D.A., Kirchgessner, M., Erhardt, W., Henke, J. and Hennig, U. (1998) Comparative studies for the determination of precaecal digestibility as a measure for the availability of B-vitamins. *Journal of Animal Physiology and Animal Nutrition* 79, 198–209.
- Roth-Maier, D.A., Wild, S.I., Erhardt, W., Henke, J. and Kirchgessner, M. (1999) Investigations on the intestinal availability of native thiamin in selected foods and feedstuffs. *European Journal of Nutrition* 38, 241–246.
- Roth-Maier, D.A., Kettler, S.I. and Kirchgessner, M. (2000) Availability of vitamin B₆ from different food sources. *International Journal of Food Sciences and Nutrition* (in press).

Part V

Econutrition and Health Maintenance

69 Dietary Manipulation of Enteric Disease

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This chapter gives examples of how experimental manipulation of ingredients and physical forms of pig diets has been used to alter susceptibility to selected enteric bacterial pathogens. Specifically, experiments conducted in our laboratory investigating interactions between diet and bacteria in the stomach, the small intestine and the large intestine are reviewed. The long-term aim of this work has been to develop practical cost-effective diets or dietary treatments which create environmental conditions in targeted parts of the gastrointestinal tract that in turn inhibit the local growth or deleterious activity of the common enteric bacterial pathogens of pigs.

Introduction

Enteric bacterial infections cause extensive morbidity and loss of production in the pig industry. A number of distinct bacterial species are involved, and each of these pathogens tends to colonize and cause disease in different regions of the gastrointestinal tract. Furthermore, each of the associated diseases occurs predominantly in pigs of certain ages or classes (e.g. *Escherichia coli* diarrhoea in sucklers and/or weaners; swine dysentery in growers and finishers, etc.). Antimicrobial agents are the main tool used for control of these infections and are given to pigs to treat overt disease, to provide prophylaxis in situations where disease is liable to occur, and to improve growth rates in the absence of disease. Unfortunately, problems are arising over the use of antimicrobials in the pig industry. Their long-term use eventually selects for the survival of resistant bacterial species or strains, and genes encoding this resistance can also be transferred to other formerly susceptible bacteria. Currently a variety of bacterial pathogens of pigs is showing resistance to a range of antimicrobial drugs. Not only is

this reducing the number of antimicrobials available to control bacterial diseases in pigs, but this resistance also poses risks to human health. Risks include the transfer of multidrug-resistant zoonotic pathogens such as *Salmonella* spp. and *Campylobacter* spp. from pigs to humans, the direct or indirect transfer of resistance genes from the porcine intestinal microflora to human bacterial strains, and the presence of antimicrobial drug residues in pig meat. Public concern about these issues is leading to reduced availability of antimicrobial agents for use in pig production. Consequently it is important to develop means of both controlling bacterial infections and promoting growth in pigs without recourse to the use of antimicrobials.

Alternative methods of control of bacterial pathogens that do not require antimicrobials include modifying management practices to limit exposure to pathogens and to minimize stress, using and improving available vaccines, undertaking selective breeding of animals for resistance to infectious diseases, and improving the pig's immune responses through the use of cytokines and other immunomodulatory agents. Strategies for selective destruction

of bacterial pathogens in the gastrointestinal tract include such things as the oral application of organic acids, or inorganic chemicals such as zinc oxide, and the use of specific bacteriophages or bacteriocins.

Another approach focuses on excluding the growth of pathogens in the gut by encouraging the growth of other bacteria that are considered to take up microniches in the intestine and compete with the pathogens at those sites. This approach has been termed competitive exclusion and has long been used in the poultry industry, where components of the normal intestinal microflora are given to young chicks to inhibit colonization by *Salmonella* serovars (Nurmi and Rantala, 1973). The principle has recently been extended to control of *Yersinia* spp. infections in pigs (Asplund *et al.*, 1996). One form of this approach is to feed pigs specialized strains of certain probiotic bacteria, especially *Lactobacillus* spp. and *Bifidobacterium* spp., that are selected because they are considered to promote gut health and exclude pathogens. Such probiotics probably have most promise for use in controlling infections in young pigs, for example in the period immediately after weaning, when the resident intestinal microflora is not yet stable.

A variation on the probiotic approach has been to feed specific dietary components that act as substrate for natural populations of protective bacteria, such that these proliferate and more effectively exclude pathogens. For example, in the case of *Clostridium difficile* infection, different dietary fibre sources have been investigated to optimize inhibition by the resident microbial flora through its production of specific short-chain fatty acids (May *et al.*, 1994). Similarly, so-called prebiotic dietary supplements such as fructose-containing oligosaccharides have been used to selectively increase numbers of *Bifidobacterium* spp. in the large intestine, the presence of which in turn is thought to result in an inhibition of colonization by certain pathogens (Gibson *et al.*, 1995). Recently, specific metabolites from plants have been identified which when fed may interact with short-

chain fatty acids to create inhibitory conditions for pathogens such as *E. coli* strain O157 (Duncan *et al.*, 1998).

Rational attempts to optimize these approaches have been impeded by the extreme diversity and complexity of the intestinal microflora in the pig, which varies quantitatively and qualitatively at different intestinal sites and at different stages in the life of the pig. There have been relatively few detailed studies on the intestinal microflora of the large intestine of pigs (e.g. Robinson *et al.*, 1981, 1984), mainly because of a lack of appropriate culture techniques suitable for many of the fastidious anaerobic microorganisms that inhabit this part of the tract. Fortunately, in the near future, results of studies using newer molecular techniques will provide much more information about the composition of this microflora.

The main source of growth substrate for the gastrointestinal microflora comes from the diet, although endogenous secretions can also be utilized by different classes of bacteria. Simple sugars tend to act as the main growth substrate in the upper part of the gastrointestinal tract, whilst in the large intestine, where the main bacterial biomass is located, dietary fibre serves as the major bacterial substrate. Dietary fibre is defined as plant materials that are not digested in the small intestine, and can be broadly divided into material that is fermented rapidly and material that is fermented more slowly. It is known that different forms of fibre in the diet can broadly influence the composition and metabolic activity of the large intestinal microflora in pigs (Varel *et al.*, 1982; Varel and Pond, 1985; Bach Knudsen *et al.*, 1991; Jensen and Jorgensen, 1994; Reid and Hillman, 1999). Currently, even where addition of appropriate substrate is known to stimulate proliferation of specific groups of resident bacteria, little is known about the way in which these bacteria interact with pathogenic species of bacteria. This lack of information makes it difficult to predict how a given dietary component could be used to influence indirectly a given enteric pathogen.

Besides influencing the normal gastrointestinal microflora, diet could also influence colonization by pathogens through other routes. For example, it could act by modulating the amount of specific substrate available for the pathogen at a given site, by influencing viscosity of the intestinal contents and hence altering accessibility of receptor sites and/or affecting intestinal motility, and by direct or indirect effects on the intestinal mucosa. As an example of the latter effect, different cereal types and particle size have been shown to alter epithelial cell proliferation and lectin binding patterns of the epithelium in the large intestine of pigs (Brunsgaard, 1998). Similar changes may occur in specific colonization sites or bacterial receptors on the enterocytes. The diet also might influence intestinal function; for example, components in boiled rice inhibit secretion in the small intestine, and hence reduce the magnitude of secretory diarrhoea due to pathogens such as enterotoxigenic *E. coli* (Mathews *et al.*, 1999).

Despite a lack of detailed knowledge, the general contention that dietary components can in some way influence colonization or disease expression by pathogens is consistent with reports from the field, where it is often observed that changes to the diet result in either increased or decreased enteric disease. For example, it has been reported that units adopting liquid feeding of by-products or using fermented wet feed have a lower incidence of salmonellosis than herds using dry feed (Stege *et al.*, 1997; van der Wolf *et al.*, 1999). Nevertheless, because other issues apart from specific dietary components may be contributing to the effects, information from such field studies needs to be confirmed by careful experiments conducted under controlled conditions. In the case of liquid feeding and salmonellosis, the relative hygiene of the various diets may be influencing the infectious dose presented to the pigs, rather than the diet itself having protective effects in the gastrointestinal tract.

In view of the effects that diet can have on the intestinal environment, including

its resident microflora, and because of the need to develop new means for control of specific enteric bacterial pathogens, attempts have been made to select or manipulate pig diets under experimental conditions to determine whether this helps to control some of these important infections. For these experiments the diet itself should have the correct optimal balance of macro- and micro-nutrients to support normal pig growth, and should not contain any toxic components (e.g. mycotoxins) that may increase susceptibility to pathogenic bacteria.

The hypothesis that we have been examining is that diet can be manipulated to influence the intestinal environment such that interactions between pathogens and the host are modified, and specific diseases prevented. Aspects of these studies have previously been reviewed (Hampson *et al.*, 1999).

Stomach

Ulceration of the pars oesophagea

The pars oesophagea of the stomach of pigs is commonly found to contain erosions and ulcers at slaughter, and these lesions are thought to be responsible for reduced growth rates (Ayles *et al.*, 1996). Ulcers may also lead to perforation of the stomach wall and to peritonitis, as well as to haemorrhage into the stomach lumen. Such complications are a common cause of sudden death in grower pigs and sows (Friendship, 1999). The condition is known to be responsive to diet, with many factors such as the fineness of dietary grind, pelleting, maize starch content, gelatinization, and unknown factors in wheat all having been implicated in its aetiology (Friendship, 1999). Workers in Brazil demonstrated a link between the presence of the spiral bacterium *Helicobacter heilmannii* in the stomach and the occurrence of ulcers (Barbosa *et al.*, 1995; Queiroz *et al.*, 1996) – a situation that parallels the confirmed involvement of *Helicobacter pylori* in the aetiology of human stomach ulceration.

Studies in our laboratory have looked for a possible link between diet and bacterial activity in the aetiology of ulceration in the pars oesophagea. A weaner model of stomach ulceration was developed in which weaners fed finely ground wheat developed quite severe ulceration after 2–3 weeks, whilst pigs fed the same wheat that had been subjected to high pressure and temperature extrusion did not develop lesions (Accioly *et al.*, 1998). In initial experiments, urease activity was detected in the stomachs of some of the pigs with ulcers, and this was thought to indicate the involvement of a urease-producing bacterium (likely to be *H. heilmannii*). In this model, by some as yet unknown mechanism, extrusion of the wheat resulted in the apparent absence of the organism and of ulcers. When this experiment was repeated, ulcers again occurred predominantly in pigs fed the finely ground wheat diet (Table 69.1). When PCR tests were applied to stomach samples from the pigs, *Helicobacter* spp. were found in only a few pigs on both diets, whilst *Campylobacter* spp. were significantly more common in the pigs fed extruded wheat and lacking ulcers (Phillips, 1999). This suggests a link between diet, *Campylobacter* spp. and pro-

tection from ulcers. A similar but even more highly significant association between the presence of *Campylobacter* spp. and lack of ulcers was seen in a recent Western Australian abattoir survey of pigs from nine farms, though unfortunately details of the diets fed on these farms are not yet available (Phillips, 1999). These results suggest either that *Campylobacter* spp. may in some way protect from ulcers, or that these bacteria are part of the normal microflora of the stomach that is displaced in the presence of ulcers, or by the same dietary factors that predispose to ulcers.

At this stage the role of *H. heilmannii* in the aetiology of ulcers is uncertain. For example, in the above survey, a positive association between ulcers and *Helicobacter* spp. was found only on one of the nine farms. Similarly, a recent study in gnotobiotic swine failed to produce ulceration of the pars oesophagea when the animals were inoculated with *H. heilmannii* and fed a carbohydrate-enriched liquid diet (Krakowa *et al.*, 1998). In contrast, pigs that were fed this diet and inoculated with *Lactobacillus* and *Bacillus* spp. did develop ulcers. In this case it was suggested that fermentation by these latter bacterial species was encouraged by the

Table 69.1. Occurrence of ulcers and colonization by *Helicobacter* spp. and *Campylobacter* spp. in weaner pigs fed diets containing finely ground wheat (W) or the same wheat after extrusion (EW) (after Phillips, 1999).

Diet	Pig	Ulcers	<i>Helicobacter</i> spp.	<i>Campylobacter</i> spp.
W	1	–	+	+
W	2	+	+	+
W	3	+	–	+
W	4	+	–	+
W	5	+	–	–
W	6	+	–	–
W	7	+	–	–
W	8	+	–	–
EW	9	+	–	+
EW	10	–	+	+
EW	11	–	+	+
EW	12	–	–	+
EW	13	–	–	+
EW	14	–	–	+
EW	15	–	–	+
EW	16	–	–	+

presence of readily available dietary substrate, and that the acidic short-chain fatty acids produced as end products of the fermentation were damaging to the epithelium. Regardless of whether or not *H. heilmannii* is a primary pathogen in the stomach, or whether other bacteria may contribute to damage to the epithelium of the stomach, both possibilities provide links between diet, enteric bacteria and disease. Similarly, the links between *Campylobacter* spp., diet and an absence of ulceration hint at possible protective interactions. Knowledge about such links will provide new opportunities for the control of ulceration of the pars oesophagea in pigs.

Small intestine

Postweaning colibacillosis

The growth checks and diarrhoea that regularly occur in many piggeries in the first 5–10 days after weaning are a serious industry problem. Colonization of the small intestine by enterotoxigenic strains of *E. coli* in this period results in a severe secretory diarrhoea: postweaning colibacillosis (PWC). Besides mortalities and the requirement for antimicrobial medication, the associated growth checks result in overall increases in the time pigs take to reach market weight (Hampson, 1994).

PWC is a multifactorial condition and there are various dietary influences on the disease (Hampson, 1987). For example,

diets containing high concentrations of protein (21%) have been shown to predispose to the condition (Prohaszka and Baron, 1980). Some highly digestible and milk-based weaner diets have been associated with reduced postweaning diarrhoea (English, 1981), whilst conversely it has been suggested that the inclusion of fibre sources to weaner diets will reduce the incidence and severity of PWC (Bertschinger *et al.*, 1978; Bolduan *et al.*, 1988). The mechanisms of protection are uncertain but may relate to reduced availability of substrate for the *E. coli* within the lumen of the small intestine.

To investigate these observations further, pigs were fed a highly digestible cooked rice/animal protein diet, or the same diet supplemented with guar gum as a source of additional soluble non-starch polysaccharide (sNSP), and their growth rates and faecal excretion of haemolytic *E. coli* were recorded (McDonald *et al.*, 1997, 1999). Pigs fed the basic rice/animal protein diet for 2 weeks after weaning were heavier, had lighter large intestines, and had less fermentation at this site than the pigs fed the same diet containing guar gum. When pigs on the two diets were challenged with enterotoxigenic *E. coli*, significantly more of these organisms were recovered from the small intestine of the pigs on the rice diet supplemented with sNSP than on the other diet (Table 69.2). Pigs fed a commercial wheat–lupin-based diet had significantly more of the pathogens isolated than did the pigs on the rice/animal protein diet (data not shown).

Table 69.2. Recovery of haemolytic *Escherichia coli* from the intestinal tract of infected weaner pigs fed either cooked white rice or the same diet supplemented with guar gum (after McDonald *et al.*, 1999).

Item	Diet type		Statistics
	RA	RGG	
Mean CFU g ⁻¹ in small intestine	1.3 × 10 ⁴	8.0 × 10 ⁹	<i>P</i> < 0.05
No. positive sites in small intestine	0.53	1.63	<i>P</i> < 0.01
CFU g ⁻¹ in colon	1.4 × 10 ⁷	3.7 × 10 ¹⁰	NS
No. positive sites in colon	1.71	1.69	NS

Diet RA: 74% cooked white rice + 20% animal protein + 4% soybean meal.

Diet RGG: 64% cooked white rice + 10% guar gum + 20% animal protein + 4% soybean meal.

Maximum number of possible positive sampling sites in small intestine was three.

This experiment suggests that the presence of sNSP in weaner diets is detrimental in terms of both piglet growth and proliferation of enterotoxigenic *E. coli* in the small intestine. It also indicates that there are benefits in feeding a highly digestible rice-based diet to weaners. The mechanism(s) involved in the protection from PWC is/are not certain, but may be related to the reduced availability of substrate for the bacteria in the small intestine of pigs fed the rice-based diet compared with the rice/guar gum diet and wheat-based diet. Addition of guar gum increases the viscosity of the digesta in the small intestine, and may lead to stasis in the unstirred layer immediately above the epithelium, allowing the bacteria trapped here to proliferate and attach to the epithelium. If dietary sNSP are confirmed as a predisposing factor in PWC, then careful selection of ingredients to minimize these components and/or treatment with exogenous enzymes may be helpful in controlling the condition.

Large Intestine

Swine dysentery

Swine dysentery (SD) causes substantial economic loss to the pig industry in many parts of the world. The disease results from infection of the caecum and colon with an anaerobic intestinal spirochaete, which was recently renamed *Brachyspira* (*Serpulina*) *hyodysenteriae* (Ochiai *et al.*, 1997). The spirochaete colonizes the mucus layer and crypts of the large intestine and induces a severe mucohaemorrhagic colitis and dysentery. The disease is most usually seen in grower and finisher pigs, and can result in severe growth depression and variable mortality. Experimental infection studies in gnotobiotic pigs have shown that both colonization by the spirochaete and lesion formation are enhanced by the presence of other species of anaerobic bacteria, particularly certain *Bacteroides* spp. and

Fusobacterium spp. (Meyer *et al.*, 1975; Whipp *et al.*, 1979). Vaccines developed to prevent the infection have been relatively ineffective, and control of SD on infected piggeries is mainly achieved through the prophylactic and/or therapeutic use of antimicrobials, or by eradication of the spirochaete through depopulation and/or medication (Hampson *et al.*, 1997).

There have been a number of anecdotal accounts of dietary influences on SD. According to Harris and Lysons (1992), in the era before antimicrobials were developed pigs with SD were treated by feeding them oats soaked in salt water or sodium hydroxide solution, on the assumption that the high fibre content of the diet was beneficial. Prohaszka and Lukacs (1984) reported a field study where the diet of pigs with SD was changed from one based on maize to one based on maize silage. This change lowered the pH values of the digesta in the large intestine, and was associated with an inhibition of growth of *B. hyodysenteriae* and protection against disease. These observations led Siba *et al.* (1996) to test the hypothesis that diets rich in highly fermentable fibre would generate an acidic environment in the large intestine which would protect from colonization by *B. hyodysenteriae*. Groups of weaner pigs were fed either a typical Australian wheat/lupin diet that contained rapidly fermentable fibre sources, or an experimental diet composed of cooked white rice and animal protein that was designed to contain low levels of dietary fibre. On the basis of lower volatile fatty acid concentrations and elevated pH values of the digesta in the large intestine, the cooked rice diet apparently did reduce the extent and rate of bacterial fermentation in the large intestine compared with the wheat diet. Contrary to the original hypothesis, however, most of the pigs fed wheat/lupin developed SD after experimental challenge, whilst none of the pigs fed the rice diet developed SD, although a few showed evidence of transient colonization by the spirochaete. When either the cooked rice or the animal protein was

mixed with either lupin or wheat, disease occurred after challenge (Table 69.3). This indicated that there were no specific inhibitory substances in either the rice or the animal protein. The protective effects of the rice diet were considered most likely to be due to reduced fermentation in the large intestine, and associated suppression of members of the resident microflora that normally facilitate colonization by the spirochaete. The possibility that the protection was associated with other physical changes in the large intestine associated with reduced fermentation (e.g. drier digesta) was not excluded. In a subsequent study of pigs fed the protective rice-based diet, an analysis of the bacterial flora in the large intestines showed some evidence for there being reductions in populations of bacteria that have been reported to act synergistically with *B. hyodysenteriae*, notably *Fusobacterium necrophorum* and *F. nucleatum* (Durmic *et al.*, 1998b).

In subsequent work, parboiled rice was found to be unsuitable as a substitute for cooked rice and it was shown that it was important for the rice to be fully cooked with a ratio of two volumes of water per volume of rice in order for fermentation in the large intestine to be minimized (Siba, 1997). Workers in Canada (R.N. Kirkwood, 1998, personal communication) and Denmark (R.H. Lindecrona, 1999, personal communication) have reported difficulty in

reproducing the protective effect of the rice diet. This may have been associated with the way the rice was processed, or it may reflect differences in the composition of the intestinal microflora of pigs at the different study sites. If the latter is the case, it adds further complexity to the objective of developing means to control SD through the diet.

Generally it is neither practical nor economical to feed pigs cooked rice to control SD, except perhaps for a short period as part of the lead-up to an eradication programme on a piggery. An experiment where the rice-based diet was fed to pigs that already had clinical SD did not diminish the duration of excretion or disease in these animals, perhaps because it takes some time before the effects of a change in diet are reflected in a change of environment in the large intestine (Durmic, 2000).

A study was undertaken to examine a variety of different cereal grains, either heat-processed or not, in order to identify practical alternatives to feeding rice and to help to identify components of the diet that might predispose to colonization by the spirochaete (Pluske *et al.*, 1996). Feeding either steam-flaked maize or sorghum was found to reduce the incidence of disease. An analysis of all the diets tested identified sNSP and resistant starch (RS) as being important dietary components that promoted fermentation in the large intestine

Table 69.3. Pooled results showing faecal shedding of spirochaetes and the incidence of swine dysentery in pigs fed different diets following oral challenge with *B. hyodysenteriae* (after Siba *et al.*, 1996).

	Diet type			
	RA	RL	WA	WL
No. of pigs challenged	16	6	6	16
No. of pigs shedding <i>B. hyodysenteriae</i> in faeces	3	6	5	13
Mean duration (days) of shedding in faeces	4.6	5.4	5.6	8.5
No. of pigs developing SD	0	5	3	10
Incidence of disease (%)	0	83.3	60	62.5

Diet RA: 77% cooked white rice + 18% animal protein.

Diet RL: 64% cooked white rice + 13% animal protein + 15% dehulled Australian sweet lupins.

Diet WA: 75% wheat + 17% animal protein.

Diet WL: 62% wheat + 11.5% animal protein + 15% dehulled Australian sweet lupins.

and which were associated with a high incidence of SD. To investigate this further, sNSP and RS were added in pure form, either by themselves or together, to the original protective cooked rice diet (Pluske *et al.*, 1998). Retrograde maize starch was used as the source of RS and guar gum was used as the source of sNSP. Pigs fed these supplemented diets became colonized and developed dysentery, whilst those on the base cooked rice diet did not. Consistent with the hypothesis that rapid fermentation associated with sNSP and/or RS is required to facilitate colonization and disease, when oat chaff was added to the protective rice diet as a source of predominantly insoluble NSP, this diet remained protective. Based on measured fermentation parameters (pH of digesta, gut weight and volatile fatty acid (VFA) and ATP content), the chaff did not stimulate fermentation to the same extent as seen with sNSP or RS. In turn, the correct predisposing conditions for colonization by *B. hyodysenteriae* were not generated.

Wheat is an important cereal grain for feeding to pigs in Australia, but does contain significant (and variable) levels of fermentable substrate. To try to develop wheat-based diets that are protective against SD, attempts were made to increase the digestibility of wheat in the small intestine by the addition of exogenous enzymes and/or by cereal grain extrusion (Durmick *et al.*, 1997, 1998a). Dietary enzymes used with the wheat included xylanase, to reduce the viscous effects of sNSP, and various mixtures of amylase and/or protease to help to increase digestion of starch in the small intestine. Extrusion was used to achieve starch gelatinization and consequently to improve its digestibility in the small intestine. The raw wheat diets were hammer milled (8 mm screen) and animal protein was used as the protein source in all diets. Unfortunately neither extrusion nor addition of enzymes influenced the microflora or fermentation parameters in the large intestine sufficiently to prevent colonization by *B. hyodysenteriae* (Durmick *et al.*, 2000). Addition of enzyme actually

resulted in an increased rate of fermentation in the caecum, with reduced fermentation being recorded only in the distal part of the colon. The increased caecal fermentation may have been a result of liberation of smaller highly fermentable oligosaccharides from the NSP in the wheat, or may have been due to more rapid transit of the digesta through the small intestine as a consequence of its reduced viscosity following degradation of the sNSP. Extrusion of the wheat led to some reduced indices of fermentation in the large intestine, but also failed to significantly reduce the expression of SD, even when combined with enzyme treatment. A possible explanation could be that the relatively high temperatures associated with extrusion strengthened bonds within the starch granules, making them less susceptible to enzyme action. Alternatively, heating may have increased the ratio of sNSP to insoluble NSP in the wheat grain (Pluske *et al.*, 1996).

Since sorghum is inherently low in sNSP and is available as a stock food in parts of Australia, attempts were then made to develop this cereal grain as the basis of a protective diet. Sorghum grain has a relatively high starch content, so treatments were mainly aimed at increasing starch digestion in the small intestine. Overall, inclusion of dietary enzymes based on xylanase, amylase and protease and/or extrusion of the sorghum in a diet based on sorghum and animal protein had relatively few influences on the microflora of the large intestine (Durmick *et al.*, 2000). Furthermore, there were few obvious effects of the treatments on fermentation parameters in the large intestine, nor on the expression of SD in experimentally infected pigs (Durmick, 2000). Extrusion of the sorghum actually significantly ($P < 0.05$) increased the expression of SD compared with animals fed raw sorghum, but this was complicated by the fact that only one of six of the latter pigs developed SD. The relatively low expression of disease in the pigs fed raw sorghum may have been related to the fact that the sorghum grain

used in these experiments was finely milled through a 1.2 mm screen using an air-assisted hammer mill. Fine grinding results in improved digestibility in the small intestine (Wandra *et al.*, 1995; Mavromichalis and Hancock, 1999), and this should result in the reduced availability of substrate for fermentation in the large intestine. In these experiments, various fermentation indices (weight of the large intestine, pH and ATP, VFA and starch content of the digesta) suggested that finely ground sorghum resulted in rates of fermentation as low as those found with extruded sorghum. Further analysis of grind size comparing results for 54 infected pigs in different experiments identified a significant response to fine grinding ($P < 0.05$), with $63 \pm 8\%$ of pigs fed coarsely ground wheat or sorghum developing SD compared with $35 \pm 8\%$ of pigs fed finely ground wheat or sorghum.

To obtain an overview of results from the various experiments, data from 204 pigs were examined. These comprised data obtained from 102 uninfected pigs on various experimental diets, with the outcome in relation to occurrence of SD based on what happened to a matching 102 pigs on the same diets that were experimentally infected with *B. hyodysenteriae*. Initially, simple linear regression analyses were conducted between dietary characteristics and indices of fermentation recorded in the large intestine of uninfected pigs against the percentage of infected pigs showing

clinical expression of the disease (Table 69.4). The percentage of variation in SD in the infected pigs that was explained by individual indices of fermentation in the uninfected pigs was generally low (0.3 to 18%) (Table 69.4). When a multiple regression model incorporating these individual indices of fermentation was used, however, this explained 51% ($P < 0.001$) of the total variation in the percentage of experimentally challenged pigs that developed SD, as follows: percentage of experimentally challenged pigs that developed SD = $151.4 + 7.1$ (sNSP in diet, %) + 39.3 (weight of full caecum, % body weight) – 21.7 (pH caecum) + 3.8 (ATP in proximal colon, nmol g⁻¹ digesta) – 0.14 (total VFA in large intestine, mmol); $r^2 = 0.51$.

The resultant model accounted for a high proportion of the variability in disease encountered (51%), and was heavily driven by the sNSP level of the diet (Table 69.4). Given that dietary interventions designed to counter some of the effects of sNSP were not effective in protecting against SD, the model clearly points to the need to select grains (diets) inherently low in sNSP to achieve better protection against the disease. The other components of the model mainly relate to fermentation indices in the caecum and proximal colon, which in turn are related to the fermentability of the diet (Table 69.4). This overall analysis supports the contention that controlling or reducing fermentation in the first part of the large intestine is the

Table 69.4. Correlations between diet characteristics and fermentative indices in the large intestine, the multiple regression model, and the percentage of experimentally challenged pigs that developed SD.

Model component	Individual correlation with % disease expression		Multilinear regression	
	Correlation (r^2)	P value	Contribution to final r^2 (%)	P value for model
Dietary sNSP	0.17	0.001	42	< 0.001
Full caecum	0.18	< 0.001	17	< 0.001
pH caecum	0.16	< 0.001	11	0.001
ATP proximal colon	0.04	0.04	5	0.029
Total [VFA] in large intestine	0.003	NS	25	< 0.001

major factor in reducing the expression of SD. Reducing fermentation in this part of the large intestine is difficult, however, since even small amounts of undigested material passing from the terminal ileum will be available for fermentation.

Although a cheap and easy means of treating all common cereal grains to make them protective against SD has not been identified, some progress has been made, and sorghum and maize have been identified as being appropriate grains on which to concentrate investigation. Further work is needed to verify the effects of grind size on large intestinal fermentation and disease with these and wheat-based diets. The cooked rice diet is exceptional in its ability to protect infected pigs from expressing SD, and it clearly results in the lowest rates of fermentation in the large intestine when compared with all other diets examined to date. Although expensive, it could be used prophylactically in outbreaks of SD, or to reduce shedding of *B. hyodysenteriae* prior to medication and/or destocking, as part of an SD eradication programme.

Porcine intestinal spirochaetosis

Porcine intestinal spirochaetosis (PIS) is a chronic diarrhoeal disease of weaner and grower/finisher pigs, resulting from colonization by the anaerobic intestinal spirochaete *Brachyspira* (*Serpulina*) *pilosicoli* (Trott *et al.*, 1996; Ochiai *et al.*, 1997). As with the closely related *B. hyodysenteriae*, *B. pilosicoli* colonizes the caecum and colon but, unlike *B. hyodysenteriae*, which is chemotactic to mucus and moves deep into the crypts, *B. pilosicoli* remains largely in the lumen of the intestine, or may attach by one cell end to the epithelium adjacent to the intestinal lumen (Hampson and Trott, 1995).

Before the description of PIS and its association with *B. pilosicoli*, certain cases of what was almost certainly PIS have been described as grower scour/non-specific colitis (Smith and Nelson, 1987). This con-

dition was reported to be influenced by diet, with pelleting of the diet being said to predispose to the condition (Connor, 1992). Interestingly, finely ground pelleted food is also believed to predispose to salmonellosis (Schwartz, 1999), and to ulceration of the pars oesophagea (Friendship, 1999).

In view of the physical and genetic similarity of *B. pilosicoli* and *B. hyodysenteriae*, their very similar habitats in the large intestine, and reports from the field of dietary influences on PIS, an investigation was undertaken to determine whether the cooked rice diet that protects from SD might also protect from PIS (Hampson *et al.*, 2000). In this study two groups of weaner pigs were fed either a standard commercial wheat/lupin weaner diet ($n = 8$), or the rice-based diet described above ($n = 6$) for 3 weeks after weaning. All pigs were then challenged orally over 3 days with 10^{10} active mid-log phase cells of a Western Australian field strain of *B. pilosicoli* (strain 95/1000). The pigs were killed 3–4 weeks post inoculation. All animals became colonized with *B. pilosicoli* strain 95/1000, but this occurred significantly later (mean of 10 days post inoculation compared with 3 days) and lasted for significantly less time (mean of 5 days compared with 16 days) in the pigs fed rice compared with those fed wheat. One pig fed the wheat diet developed an acute and severe erosive colitis with severe watery diarrhoea within 3 days post inoculation and was euthanased. All the other pigs on both diets developed a mild transient diarrhoea, lasting only 2–3 days. Small areas of mild patchy colitis were observed grossly at post-mortem, but no spirochaete attachment to the epithelium was detected.

This study demonstrated that, as with *B. hyodysenteriae*, colonization by *B. pilosicoli* can be influenced by diet. In this case the rice-based diet did not prevent colonization, but only retarded its occurrence. This result suggests that the two spirochaete species have different environmental requirements and constraints on their ability to colonize the large intestine

of pigs. The basis of these differences is unknown, and may or may not be associated with the different micro-niches that they occupy in the large intestine, with differences in their metabolism and physiological requirements, or with other factors. The generally mild nature of PIS, together with the lack of complete protection using the rice-based diet, means that the prospects for an economically viable means of controlling PIS in piggeries by using the rice-based diet alone are poor. Given the differences between the response of the two spirochaete species, however, it would be instructive to investigate whether other diets may have a relatively greater influence on the proliferation of *B. pilosicoli*, perhaps acting via different mechanisms than those postulated for *B. hyodysenteriae*.

Conclusions

The studies described provide examples of connections between the pig's diet and the presence and/or extent of proliferation of certain pathogenic enteric bacteria. These pathogens inhabit different parts of the gastrointestinal tract (stomach, small intestine, large intestine) and are themselves quite distinct organisms. Nevertheless, in each case, reducing sNSP and/or resistant starch content of the diet appears to reduce proliferation of pathogens. Such dietary effects on infection may not be restricted to bacterial enteric pathogens; for example, there is evidence that carriage of the parasitic nematode *Oesophagostomum dentatum* in pigs is enhanced by diets rich in insoluble fibre (Petkevicius *et al.*, 1997).

The basis of the protective effects observed is incompletely understood, nor is it clear whether different mechanisms with the same outcome are involved in the different infections in different parts of the gastrointestinal tract. The protection may be related to reduced availability of substrate for the pathogen, alterations to the normal microflora, changes in the epithe-

lium, or physicochemical changes in the intestinal environment, such as reduced viscosity or hydration of the digesta. Under these changed conditions the exogenous pathogenic bacteria may face increased constraints in colonizing and proliferating. Even though no clear explanation has been found for the protection achieved, this work suggests the possibility of new approaches to the control of enteric pathogens. The principles involved may also apply to other bacterial populations in the gut; for example, by reducing sNSP levels in the finisher diet it may be possible to minimize the subclinical intestinal carriage of pathogenic bacteria such as *Salmonella* spp. and *Campylobacter* spp. which can contaminate meat, and which present a threat to human health. The work also may be relevant to intestinal infections in other monogastric species, particularly poultry and human beings.

A better understanding of the underlying mechanisms of dietary protection should help in the rational development of other control measures for these infections. For example, pre-fermentation of pig diets is being investigated as a means to limit colonization of the intestinal tract by pathogenic bacteria. The postulated mode of action has concentrated on antibacterial effects of the resultant acidity of the diet. However, pre-fermentation would also be predicted to lower the sNSP content of the diets and this may contribute to any protective effects achieved. Knowledge of this alternative mechanism should allow the pre-fermentation to be designed with the object of minimizing the sNSP content of the diet, rather than just generating an acidic environment.

In summary, the diet consumed influences a number of parameters in the gut, including proliferation of certain pathogenic bacterial species. Making modifications to the diet may help to control disease and hence reduce the need to use antimicrobials. A major challenge is to develop protective diets that are cost effective. Whilst it may be justifiable to feed expensive special-

ized diets to pigs for a short period after weaning, or during a disease clean-up operation, generally it is not cost effective to feed these sorts of diets for long periods. Furthermore, it is unlikely that one type of protective diet would be suitable for all enteric infections, as dietary effects are likely to be very different in different parts of the gastrointestinal tract, and the interactions with different pathogenic bacteria may differ. For example, fine grinding of selected cereal grains to increase starch digestibility may reduce the susceptibility of pigs to SD but may predispose them to other conditions, particularly ulceration of the pars oesophagea. Therefore, a dietary approach

to control needs to be conducted on a disease-by-disease basis but also needs to take into account other potential interactions.

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References

- Accioly, J.M., Durmic, Z., McDonald, D.E., Oxberry, S.L., Pethick, D.W., Mullan, B.P. and Hampson, D.J. (1998) Dietary effects on the presence of ulcers and urease-producing organisms in the stomach of weaner pigs. *Proceedings of the 15th International Pig Veterinary Society Congress*, Birmingham, England, 3, 242.
- Asplund, K., Hakkinen, M., Bjorkroth, J., Nuotio, L. and Nurmi, E. (1996) Inhibition of the growth of *Yersinia enterocolitica* O:3 by the microflora of porcine caecum and ileum in an *in vitro* model. *Journal of Applied Bacteriology* 81, 217–222.
- Ayles, H.L., Friendship, R.M. and Ball, R.O. (1996) Effects of dietary particle size on gastric ulcers, assessed by endoscopic examination, and relationship between ulcer severity and growth performance of individually fed pigs. *Swine Health and Production* 4, 211–216.
- Bach Knudsen, K.E., Jensen, B.B., Andersen, J.O. and Hansen, I. (1991) Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *British Journal of Nutrition* 65, 233–248.
- Barbosa, A.J.A., Silva, J.C.P., Nogueira, A.M.M.F., Paulino, E. Jr and Miranda, C.R. (1995) Higher incidence of *Gastrosprillum* sp. in swine with gastric ulcers of the *pars oesophagea*. *Veterinary Pathology* 32, 134–139.
- Bertschinger, H.U., Eggenberger, E., Jucker, H. and Pfirter, H.P. (1978) Evaluation of low nutrient, high fibre diets for the prevention of porcine *Escherichia coli* enterotoxaemia. *Veterinary Microbiology* 3, 281–290.
- Bolduan, G., Jung, H., Schnabel, E. and Schneider, R. (1988) Recent advances in the nutrition of weaner piglets. *Pig News and Information* 9, 381–385.
- Brunsgaard, G. (1998) Effects of cereal type and feed particle size on morphological characteristics, epithelial cell proliferation, and lectin binding patterns in the large intestine of pigs. *Journal of Animal Science* 76, 2787–2798.
- Connor, J.F. (1992) Nonspecific colitis. *Australian Association of Pig Veterinarians Proceedings*, Adelaide, Australia, pp. 79–80.
- Duncan, S.H., Flint, H.J. and Stewart, C.S. (1998) Inhibitory activity of gut bacteria against *Escherichia coli* O157 mediated by dietary plant metabolites. *FEMS Microbiology Letters* 164, 283–288.
- Durmic, Z. (2000) Evaluation of dietary fibre as a contributory factor in the development of swine dysentery. PhD thesis, Murdoch University, Murdoch, Western Australia.
- Durmic, Z., Pethick, D.W., Mullan, B.P., Schulze, H. and Hampson, D.J. (1997) The effects of extrusion and enzyme addition in wheat-based diets on fermentation in the large intestine and expression of swine dysentery. In: Cranwell, P.D. (ed.) *Manipulating Pig Production VI*. Australasian Pig Science Association, Werribee, Victoria, Australia, p. 180.

- Durmic, Z., Pethick, D.W., Mullan, B.P., Schulze, H., Accioly, J.M. and Hampson, D.J. (1998a) Evaluation of some dietary treatments designed to reduce the incidence of swine dysentery. *Proceedings of the 15th International Pig Veterinary Society Congress*, Birmingham, UK, 3, 134.
- Durmic, Z., Pethick, D.W., Pluske, J.R. and Hampson, D.J. (1998b) Changes in bacterial populations in the colon of pigs fed different sources of dietary fibre, and the development of swine dysentery after experimental infection. *Journal of Applied Microbiology* 85, 574–582.
- Durmic, Z., Pethick, D.W., Mullan, B.P., Schulze, H., Accioly, J. and Hampson, D.J. (2000) Extrusion of wheat or sorghum and/or addition of exogenous enzymes to pig diets influences the large intestinal microbiota but does not prevent swine dysentery following experimental infection. *Journal of Applied Microbiology* 89, 678–686.
- English, P.R. (1981) Establishing the early weaned pig. *Proceedings of the Pig Veterinary Society* 7, 29–37.
- Friendship, R.M. (1999) Gastric ulcers. In: Straw, B.E., Mengeling, W.L., D'Allaire, S. and Taylor, D.J. (eds) *Diseases of Swine*, 8th edn. Blackwell Scientific Publications, Oxford, UK, pp. 685–694.
- Gibson, G.R., Beatty, E.R., Wang, X. and Cummins, J.H. (1995) Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108, 975–982.
- Hampson, D.J. (1987) Dietary influences on porcine postweaning diarrhoea. In: Barnett, J.L., Batterham, E.S., Cronin, G.M., Hansen, C., Hemsworth, P.H., Hennessy, D.P., Hughes, P.E., Johnston N.E. and King R.H. (eds) *Manipulating Pig Production*. Australasian Pig Science Association, Werribee, Australia, pp. 202–214.
- Hampson, D.J. (1994) Postweaning *Escherichia coli* diarrhoea in pigs. In: Gyles, C.J. (ed.) *Escherichia coli in Domestic Animals and Humans*. CAB International, Wallingford, UK, pp. 171–191.
- Hampson, D.J. and Trott, D.J. (1995) Intestinal spirochaete infections of pigs: an overview with an Australian perspective. In: Hennessy, D.P. and Cranwell, P.D. (eds) *Manipulating Pig Production V*. Australasian Pig Science Association, Werribee, Australia, pp. 139–169.
- Hampson, D.J., Atyeo, R.F. and Combs, B.G. (1997) Swine dysentery. In: Hampson, D.J. and Stanton, T.B. (eds) *Intestinal Spirochaetes in Domestic Animals and Humans*. CAB International, Wallingford, UK, pp. 175–209.
- Hampson, D.J., Pluske, J.R. and Pethick, D.W. (1999) Can diet be used as an alternative to antibiotics to help control enteric bacterial infections of pigs? In: Cranwell, P.D. (ed.) *Manipulating Pig Production VII*. Australasian Pig Science Association, Werribee, Australia, pp. 210–219.
- Hampson, D.J., Robertson, I.D., La, T., Oxberry, S.L. and Pethick, D.W. (2000) Influences of diet and vaccination on colonisation of pigs with the intestinal spirochaete *Brachyspira (Serpulina) pilosicoli*. *Veterinary Microbiology* 73, 75–84.
- Harris, D.L. and Lysons, R.J. (1992) Swine dysentery. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S. and Taylor, D.J. (eds) *Diseases of Swine*. Iowa State University Press, Ames, Iowa, pp. 599–616.
- Jensen, B.B. and Jorgensen, H. (1994) Effect of dietary fibre on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. *Applied and Environmental Microbiology* 60, 1897–1904.
- Krakowa, S., Eaton, K.A., Rings, D.M. and Argenzio, R.A. (1998) Production of gastroesophageal erosions and ulcers (GEU) in gnotobiotic swine monoinfected with fermentative commensal bacteria and fed high-carbohydrate diet. *Veterinary Pathology* 35, 274–282.
- Mathews, C.J., MacLeod, R.J., Zheng, S.X., Hanrahan, J.W., Bennett, H.P. and Hamilton, J.R. (1999) Characterization of the inhibitory effect of boiled rice on intestinal chloride secretion in guinea pig crypt cells. *Gastroenterology* 116, 1342–1347.
- Mavromichalis, I. and Hancock, J.D. (1999) Wheat grain, co-products in swine feeds examined. *Feedstuffs* 71, 13–17.
- May, T., Mackie, R.I., Fahey, G.C., Cremin, J.C. and Garleb, K.A. (1994) Effect of fiber source on short-chain fatty acid production and on the growth and toxin production by *Clostridium difficile*. *Scandinavian Journal of Gastroenterology* 19, 916–922.
- McDonald, D.E., Pluske, J.R., Pethick, D.W. and Hampson, D.J. (1997) Interactions of dietary nonstarch polysaccharides with weaner pig growth and postweaning colibacillosis. In: Cranwell, P.D. (ed.) *Manipulating Pig Production VI*. Australasian Pig Science Association, Werribee, Australia, p. 179.
- McDonald, D.E., Pethick, D.W., Pluske, J.R. and Hampson, D.J. (1999) Adverse effects of soluble non-starch polysaccharide (guar gum) on piglet growth and colibacillosis immediately after weaning. *Research in Veterinary Science* 67, 245–250.

- Meyer, R.C., Simon, J. and Byerly, C.S. (1975) The etiology of swine dysentery. III. The role of selected gram negative obligate anaerobes. *Veterinary Pathology* 12, 46–54.
- Nurmi, E. and Rantala, M. (1973) New aspects of *Salmonella* infection in broiler production. *Nature* 241, 210–211.
- Ochiai, S., Mori, K. and Adachi, Y. (1997) Unification of the genera *Serpulina* and *Brachyspira*, and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. *Microbiology and Immunology* 41, 445–452.
- Petkevicius, S., Bach Knudsen, K.E., Nanse, P., Roepstorff, A., Skjoth, F. and Jensen, K. (1997) The impact of diets varying in carbohydrates resistant to endogenous enzymes and lignin on populations of *Ascaris suum* and *Oesophagostomum dentatum* in pigs. *Journal of Parasitology* 114, 555–568.
- Phillips, N.D. (1999) Molecular detection and identification of gastric bacteria in pigs. Honours thesis, Murdoch University, Western Australia.
- Pluske, J.R., Siba, P.M., Pethick, D.W., Durmic, Z., Mullan, B.P. and Hampson, D.J. (1996) The incidence of swine dysentery in pigs can be reduced by feeding diets that limit fermentation in the large intestine. *Journal of Nutrition* 126, 2920–2933.
- Pluske, J.R., Durmic, Z., Pethick, D.W., Mullan, B.P. and Hampson, D.J. (1998) Confirmation of the role of non-starch polysaccharides and resistant starch in the expression of swine dysentery in pigs following experimental infection with *Serpulina hyodysenteriae*. *Journal of Nutrition* 128, 1737–1744.
- Prohaszka, L. and Baron, F. (1980) The predisposing role of high protein supplies in enteropathogenic *Escherichia coli* infections of weaned pigs. *Zentralblatt für Veterinärmedizin B* 27, 222–232.
- Prohaszka, L. and Lukacs, K. (1984) Influence of the diet on the antibacterial effect of volatile fatty acids and on the development of swine dysentery. *Zentralblatt für Veterinärmedizin B* 31, 779–785.
- Queiroz, D.M.M., Rocha, G.A., Mendes, E.N., Moura, S.B., Oliveira, A.M.R. and Miranda, D. (1996) Association between *Helicobacter* and gastric ulcer disease on the *pars oesophagea* in swine. *Gastroenterology* 111, 19–27.
- Reid, C.-A. and Hillman, K. (1999) The effects of retrogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. *Animal Science* 68, 503–510.
- Robinson, I.M., Allison, M.J. and Bucklin, J.A. (1981) Characterization of the cecal bacteria of normal pigs. *Applied and Environmental Microbiology* 41, 950–955.
- Robinson, I.M., Whipp, S.C., Bucklin, J.A. and Allison, M.J. (1984) Characterization of predominant bacteria from the colons of normal and dysenteric pigs. *Applied and Environmental Microbiology* 48, 964–969.
- Schwartz, K.J. (1999) Salmonellosis in swine is a challenging opponent. *Worldwide Pig Progress* June, pp. 20–22.
- Siba, P.M. (1997) Dietary control of swine dysentery. PhD thesis, Murdoch University, Murdoch, Western Australia.
- Siba, P.M., Pethick, D.W. and Hampson, D.J. (1996) Pigs experimentally infected with *Serpulina hyodysenteriae* can be protected from developing swine dysentery by feeding them a highly digestible diet. *Epidemiology and Infection* 116, 207–216.
- Smith, W.J. and Nelson, E.P. (1987) Grower scour/non-specific colitis. *Veterinary Record* 121, 334.
- Stege, H., Dahl, J., Christensen, J., Baggesen, D.L., Nielsen, J.P. and Willeberg, P. (1997) Subclinical *Salmonella* infections in Danish finishing pig herds: risk factors. *Proceedings of the 2nd International Symposium on the Epidemiology and Control of Salmonella in Pork*, Copenhagen, Denmark, pp. 148–152.
- Trott, D.J., Stanton, T.B., Jensen, N.S., Duhamel, G.E., Johnson, J.L. and Hampson, D.J. (1996) *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. *International Journal of Systematic Bacteriology* 46, 206–215.
- van der Wolf, P.J., Bongers, J.H., Elbers, A.R.W., Franssen, F.M.M.C., Hunneman, W.A., van Exsel, A.C.A. and Tielen, M.J.M. (1999) *Salmonella* infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. *Veterinary Microbiology* 67, 263–275.
- Varel, V.H. and Pond, W.G. (1985) Enumeration and activity of cellulytic bacteria from gestating swine fed various levels of dietary fibre. *Applied and Environmental Microbiology* 49, 858–862.

- Varel, V.H., Pond, W.G., Pekas, J.C. and Yen, J.T. (1982) Influence of high-fibre on bacterial populations in gastrointestinal tracts of obese and lean genotype pigs. *Applied and Environmental Microbiology* 44, 107–112.
- Wandra, K.J., Hancock, J.D., Behnke, K.C., Hines, R.H. and Stark, C.R. (1995) Effects of particle size and pelleting on growth performance, nutrient digestibility and stomach morphology in finishing pigs. *Journal of Animal Science* 73, 757–763.
- Whipp, S.C., Robinson, I.M., Harris, D.L., Glock, R.D., Mathews, P.J. and Alexander, T.J.L. (1979) Pathogenic synergism between *Treponema hyodysenteriae* and other selected anaerobes in gnotobiotic pigs. *Infection and Immunity* 26, 1042–1047.

70 Protective Effect of Processed Soybean During Perfusion of ETEC-infected Small Intestinal Segments of Early-weaned Piglets

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After weaning, piglets frequently have diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) and rotavirus. In the small intestine segment perfusion (SISP) model, we studied the effect of processed and mould-fermented soybean products on net absorption of fluid. All processed soybean products appeared to protect against the fluid loss upon ETEC infection. Net fluid absorption was highest for cooked soybean followed by autoclaved soybean and soybean tempe. ETEC infection hardly affected net sodium and chloride absorption in segments with soybean tempe. Processed soybean products are beneficial in maintaining fluid balance during ETEC infection, and in case of tempe also sodium and chloride losses are reduced. Therefore mould-fermented soybean in particular may be beneficial in case of postweaning diarrhoea.

Introduction

After weaning, piglets frequently have diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) and rotavirus (Nabuurs, 1998). Tempe, a mould-fermented soybean product, has been shown to inhibit *E. coli* infection in rabbits (Karmini *et al.*, 1997) and has been reported to contain an

antibacterial substance (Wang *et al.*, 1972). In the small intestine segment perfusion (SISP) model (Nabuurs *et al.*, 1993) it is possible to perfuse small intestinal segments within one piglet simultaneously in the absence and in the presence of ETEC. We have used the SISP model to study the effect of processed and mould-fermented soybean products on net absorption of fluid.

Material and Methods

Segments (length $\pm$ 20 cm) were fitted in the middle of the small intestine in 6-week-old anaesthetized piglets, weaned at 3 weeks of age. Odd-numbered segments were injected with 5 ml ETEC (5×10^9 colony-forming units O149:K91:K88^{ac}, producing heat-labile and heat-stable toxin) and even-numbered segments with 5 ml PBS, whereupon the segments were perfused over 8 h.

Saline, predigested raw soybean, predigested autoclaved soybean, predigested cooked soybean, and predigested mould-fermented soybean (= soybean tempe) were tested in 12 piglets, each with five pairs of segments (an uninfected and an adjacent ETEC-infected). Net fluid absorption was calculated from the difference between the volumes of inflow and outflow divided by the surface area (length $\times$ circumference) of the segment.

Results

During perfusion with saline, net fluid absorption decreased by more than 50%

upon ETEC infection. All processed soybean products appeared to protect against fluid loss upon ETEC infection (Fig. 70.1), although ETEC was still present in large quantities in the segments after perfusion (data not shown).

In ETEC-infected as well as uninfected segments, net fluid absorption was highest for cooked soybean followed by autoclaved soybean and soybean tempe (Fig. 70.1). ETEC infection only slightly affected net sodium and chloride absorption in segments perfused with soybean tempe (Table 70.1).

Discussion

The sequence in net fluid absorption for the three processed soybean products may be related to the difference in osmolality of the products, being highest for soybean tempe (data not shown). Since large quantities of ETEC were found after perfusion of processed soybean products, there is no evidence for any antibacterial activity. Although ETEC was applied to the segments before perfusion, it is possible that certain soybean compounds may interfere

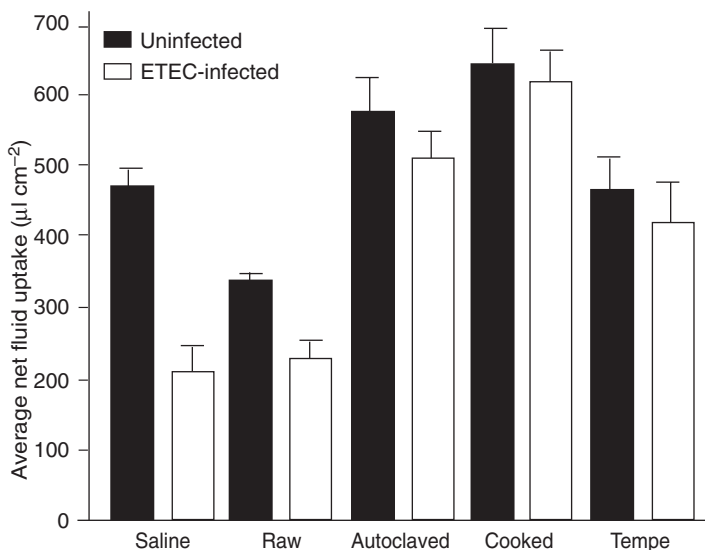


Fig. 70.1. Average net fluid uptake during perfusion with saline, raw soybean, autoclaved soybean, cooked soybean and soybean tempe. Average $\pm$ standard error of the mean is shown.

Table 70.1. Average net uptake of dry matter, Na and Cl. Average $\pm$ standard error of mean is shown both for uninfected and ETEC-infected segments.

Product	Dry matter (mg cm ⁻²)		Na (μ mol cm ⁻²)		Cl (μ mol cm ⁻²)	
	Uninfected	+ETEC	Uninfected	+ETEC	Uninfected	+ETEC
Saline	1.5 $\pm$ 0.8	-4.1 $\pm$ 1.0	74 $\pm$ 5	41 $\pm$ 6	66 $\pm$ 5	47 $\pm$ 6
Raw	7.6 $\pm$ 0.8	1.2 $\pm$ 1.4	-5 $\pm$ 2	-27 $\pm$ 2	7 $\pm$ 1	0 $\pm$ 1
Autoclaved	13.2 $\pm$ 1.3	12.9 $\pm$ 1.8	21 $\pm$ 3	1 $\pm$ 3	33 $\pm$ 4	23 $\pm$ 3
Cooked	13.3 $\pm$ 1.5	11.5 $\pm$ 1.5	21 $\pm$ 2	12 $\pm$ 3	22 $\pm$ 3	17 $\pm$ 2
Tempe	16.1 $\pm$ 1.7	15.0 $\pm$ 2.0	24 $\pm$ 3	18 $\pm$ 4	29 $\pm$ 4	24 $\pm$ 4

with the attachment of ETEC to the epithelial cells. The reduced fluid losses by processed soybean products may on the other hand be explained by scavenge of toxins by soybean compounds. It can be concluded that processed soybean prod-

ucts are beneficial in maintaining fluid balance during ETEC infection, and, in the case of tempe, sodium and chloride losses are also reduced. Therefore mould-fermented soybean in particular may be beneficial in case of postweaning diarrhoea.

References

- Karmini, M., Affendi, E., Hermana, Karyadi, D. and Winarno, F.G. (1997) The inhibitory effect of tempe on *Escherichia coli* infection. In: Sudarmadji, S., Suparmo and Raharjo, S. (eds) *Proceedings International Tempe Symposium*. Indonesian Tempe Foundation, Bali, Indonesia, pp. 157–162.
- Nabuurs, M.J. (1998) Weaning piglets as a model for studying pathophysiology of diarrhea. *Veterinary Quarterly* 20, S42–45.
- Nabuurs, M.J., Hoogendoorn, A., van Zijderveld, F.G. and van der Klis, J.D. (1993) A long-term perfusion test to measure net absorption in the small intestine of weaned pigs. *Research in Veterinary Science* 55, 108–114.
- Wang, H.L., Ellis, J.J. and Hesseltine, C.W. (1972) Antibacterial activity produced by moulds commonly used in oriental food fermentations. *Mycologia* 64, 218–221.

71 The Dose–Response Effect of Fermented Liquid Wheat on Growth Performance and Gastrointestinal Health of Weaned Piglets

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The addition of 0%, 15%, 30% and 45% fermented liquid wheat to liquid diets for weaned piglets favoured daily gain (+ 6.1%; $P = 0.14$) and feed conversion ratio (4.6 to 6.7%; $P < 0.001$) as compared with a diet without fermented wheat. Between the diets with 15%, 30% and 45% fermented liquid wheat, no differences in daily gain and feed conversion ratio were observed ($P > 0.10$). The diet with fermented wheat seemed to be favourable for villus height and ratio of villus height:crypt depth in the first part of the small intestine, compared with the diet without fermented wheat.

Introduction

Fermented liquid feeds (FLF) seem to improve growth performance and health of pigs compared with non-fermented liquid feeds (Scholten *et al.*, 1999). The favourable results with FLF may be caused by a lower pH, increased levels of organic acids and a different microbiological status of the gastrointestinal tract. Moreover, villus architecture may be affected positively by feeding FLF (Scholten *et al.*, 1999). The effects of different levels of fermented liquid wheat on the growth performance and gastrointestinal parameters of weaned piglets were studied. Some preliminary results are published in this chapter.

Material and Methods

A total of 248 piglets were weaned at 28 days of age, weighing about 8 kg. After weaning the piglets received one of four liquid diets during a period of 35 days. All four diets had the same composition, except for the proportion of the wheat (45% of the total diet) that was fermented. Diet 1 consisted of 0% fermented wheat, diet 2 of 15%, diet 3 of 30% and diet 4 of 45%. Non-fermented wheat is exchanged for fermented wheat. Fermented wheat was prepared by adding water (30°C; ratio 2.2:1) to hammer-milled wheat 24 h prior to feeding. One hour before feeding, the fermented wheat was added to the supple-

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mentary compound feed, to which warm water (ratio 2.2:1) was also added. The diets contained no added organic acids and no antibiotic growth promoters and were low in copper and zinc content. The pigs were housed in groups of four, and fed restricted, twice a day (0800 h and 1600 h) by trough. Feeding level was calculated based on average metabolic weight per pen. Piglets were weighed at D0 (day of weaning) and D4, D8, D13, D20, D27 and D34 after weaning. In total 40 piglets were dissected: eight pigs directly at weaning (D0), 16 at D4 and 16 at D8. The piglets dissected at D4 and D8 were fed diet 1 or diet 4. The gastrointestinal tract was divided into seven segments: stomach, small intestine (three segments), colon, caecum and rectum. The chymus was analysed for pH, level of volatile fatty acids, lactic acid and microbiology (counts of yeasts, lactic acid bacteria, total coliforms, *E. coli*). From the three segments of the small intestine, biopsies were taken and the villus height, crypt depth, mitoses and number and type

of goblet cells were analysed. In this chapter only the results of growth performance and villus height/crypt depth of the small intestine are presented.

Results

The pH of the fermented liquid diets was 5.8, 5.3, 4.9 and 4.5 for diets 1, 2, 3 and 4, respectively, at the moment of feeding. Daily gain of the control group was lower than daily gain of the three groups fed fermented wheat (Table 71.1; $P = 0.14$). The feed conversion ratio of the control group was lower compared with the three diets with fermented wheat (Table 71.1; $P < 0.01$).

Some characteristics of villus architecture of the dissected piglets (0% and 45% fermented wheat) are presented in Table 71.2. The piglets fed fermented wheat seemed to have higher villus height and a higher ratio of villus height: crypt depth, both at D4 and D8, compared with the piglets fed diet 1 (Table 71.2).

Table 71.1. Growth performance of piglets fed diets with 0, 15, 30 or 45% fermented wheat during 5-week period after weaning.

	0%	15%	30%	45%	SEM
Number of piglets	52	52	52	52	
Number of pens	13	13	13	13	
Initial weight (kg)	8.1	8.1	8.1	8.2	
Daily gain (g d ⁻¹)	280	297	297	297	6.1
Feed intake (g d ⁻¹)	540	535	548	539	8.0
Feed conversion ratio	1.94 ^a	1.81 ^b	1.85 ^b	1.83 ^b	0.02

^{ab} Data within row with different superscripts differ, $P < 0.01$.

Table 71.2. Daily gain, villus height and crypt depth in the first part of the small intestine of piglets ($n = 8$) directly after weaning (D0), day 4 (D4) or day 8 (D8) after weaning, fed diet 1 or diet 4.

Day	Diet (% fermented wheat)	Daily gain (g day ⁻¹)	Villus height (µm)	Crypt depth (µm)	Villus: crypt ratio
D0	—	—	447	193	2.32
D4	0%	-12	278	236	1.18
D4	45%	+27	321	223	1.44
D8	0%	+32	296	275	1.08
D8	45%	+66	398	260	1.53

Daily gain is based on the first 4 (D4) or 8 days (D8) after the start of the trial.

Conclusions

- The addition of fermented liquid wheat to liquid diets favoured feed conversion ratio (4.6 to 6.7%) compared with a liquid diet without fermented wheat fed to pigs during 5 weeks after weaning.
- Between the diets with 15%, 30% and 45% fermented liquid wheat, no significant difference in feed conversion ratio was observed.
- The diet with fermented wheat seemed to be favourable for villus height and the ratio of villus height: crypt depth in the first part of the small intestine, compared with the diet without fermented wheat.

Reference

Scholten, R.H.J., van der Peet-Schwering, C.M.C., Verstegen, M.W.A., den Hartog, L.A., Schrama, J.W. and Vesseur, P.C. (1999) Fermented co-products and fermented compound diets for pigs: a review. *Animal Feed Science and Technology* 82, 1–19.

72 Influence of Liquid Feed, Fermented Liquid Feed, Dry Feed and Sow's Milk Fed *ad libitum*, on the 'Ecophysiology' of the Terminal Ileum, Caecum and Colon of the Postweaned Piglet

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This study examined the potential of fermented liquid feed (FLF) to suppress coliform numbers in the terminal ileum, caecum and colon of the pig. FLF changed the ratio of *Lactobacilli* to coliforms in the gastrointestinal (GI) tract. Coliform populations ($\log_{10}$ CFU ml^{-1}) were reduced to below detectable limits (< 3.0) at the terminal ileum when piglets were fed the FLF, and were highest in pigs given dry feed (8.5). Overall there was a significant reduction in coliform population in the GI tract when piglets were fed FLF compared with dry feed.

Introduction

Until recently, the industry managed digestive problems in the young pig by the use of prophylactic antibiotics. Some of these antimicrobials are now prohibited for pro-

phylactic use in the EU and others may be banned in the near future. Therefore, alternatives are being sought. Numerous studies have shown that the performance of weaner pigs is improved when fed on a liquid diet (reviewed by Jensen and

Mikkelsen, 1998). When liquid diets are fed *ad libitum*, the maintenance of food hygiene and palatability is a major problem. Enteropathogens and other spoilage bacteria rapidly colonize the liquid diet. This problem can be overcome by fermenting the diet with lactic acid bacteria (LAB). Improvements have been reported in the dry matter feed intake and growth rate of weaner piglets offered fermented liquid feed (FLF) (Brooks *et al.*, 1996). Piglets fed FLF appeared to be capable of maintaining their dry matter feed intake even when fed diets of very low dry matter concentration (Geary *et al.*, 1996). In the aforementioned studies, the fermentation of the diet with LAB proved to be an effective means of preventing the colonization of the feed with spoilage microflora, limiting the growth of *Escherichia coli* and maintaining the palatability of the diets. The objective of the study reported here was to examine the effect of feed form on the ecophysiology of the young pig's gut.

Materials and Methods

Thirty-two Duroc (12.5%) × Landrace (50%) × Large White (37.5%) piglets, initial weight 6.2 ± 1.25 kg, were obtained from a minimal-disease herd. Twenty-four piglets, weaned at 23 ± 2 days of age, were individually housed in flat deck pens. The piglets were randomly allocated (eight per treatment) to one of three dietary treatments: non-fermented liquid feed (NFLF), fermented liquid feed (FLF) or dry feed (DF). A further eight piglets were suckled by their dam (S). The experimental diet was based on milk and cereals (175 mg kg^{-1}). NFLF was prepared daily by mixing feed in the ratio of 1 kg dry meal to 5 l of water. FLF was prepared by inoculating the liquid diet with *Lactobacillus plantarum* (Alltech Inc., Kentucky, USA) and steeping for 5 days at 25°C before feeding. A continuous fermentation was maintained by retaining 50% of FLF each day to inoculate new feed entering the system. All feeds were fed *ad libitum* for 14 days.

Gastrointestinal (GI) tracts were

removed under terminal anaesthesia. Samples of small intestine, caecum and colon were removed for analysis of short-chain fatty acid concentration, pH and enumeration of lactobacilli and coliform populations. Lactobacilli and coliform populations were determined by plate count techniques. Short-chain fatty acid separations were performed with HPLC using an Aminex HPX-87H (30 cm × 6.8 mm) cation exchange column (Bio-Rad, Herts, UK). Microbial counts were $\log_{10}$ transformed prior to analysis. Data were analysed by a GLM-ANOVA and comparison between means was performed using Tukey's post-hoc test. The statistical analyses were undertaken using Minitab v.10.2 (Minitab Inc., Pennsylvania, USA, 1994).

Results

No coliform bacteria ($< 3.0 \log_{10} \text{ CFU ml}^{-1}$) were detected in the terminal ileum of pigs fed FLF, compared with 8.5, 8.1 and 6.0 $\log_{10} \text{ CFU ml}^{-1}$ digesta in DF, NFLF and S pigs, respectively (Table 72.1). Significant ($P < 0.01$) differences in the concentrations of acetic, formic and propionic acids were found in the digesta in the terminal ileum of the pigs fed FLF and NFLF, compared with DF and S pigs (Table 72.1).

There was a significant ($P < 0.001$) reduction in the number of coliforms in the caecum and colon of pigs fed FLF compared with DF (Table 72.2). The lactobacilli population was significantly greater ($P < 0.01$) in the terminal ileum, caecum and colon of the pigs fed FLF than DF. The pH of the digesta in the colon of S piglets was higher ($P < 0.01$) than in the other treatments.

Discussion

Fermenting liquid feed produced a 'biosafe' feed, which resulted in a reduction in coliform populations throughout the lower GI tract. FLF provided a feed with a low pH and high numbers of lactic acid bacteria numbers. FLF influenced

Table 72.1. Microbial counts ($\log_{10}$ CFU ml^{-1}), pH and short-chain fatty acid profile (mM g^{-1} wet weight digesta) at the terminal ileum of piglets fed different diets.

Diet	pH	Lactobacilli	Coliforms	Acetate	Formate	Propionate
FLF	6.1 ^a	8.8 ^a	< 3.0	179.6 ^a	195.2 ^a	23.6 ^a
LF	6.4 ^a	7.0 ^a	8.1 ^{ab}	153.2 ^a	135.9 ^a	27.0 ^a
DF	6.3 ^a	< 3.0	8.5 ^a	54.7 ^b	64.9 ^b	6.3 ^a
S	5.9 ^a	7.3 ^a	6.0 ^b	ND	6.4 ^c	0.7 ^b

^{abc} Within columns, means with the same superscript are not statistically different ($P > 0.05$).
ND, none detected.

Table 72.2. Microbial counts ($\log_{10}$ CFU ml^{-1}) and pH of the digesta in the caecum and colon of piglets fed different diets.

	FLF	LF	DF	S
Caecum				
pH	6.0 ^a	6.0 ^a	5.8 ^a	6.1 ^a
Lactobacilli	8.5 ^a	8.1 ^a	5.5 ^b	7.3 ^a
Coliforms	5.5 ^a	7.4 ^{ab}	8.4 ^b	7.5 ^{ab}
Colon				
pH	6.2 ^a	6.0 ^a	5.9 ^a	6.6 ^b
Lactobacilli	8.6 ^a	7.9 ^a	5.0 ^b	8.0 ^a
Coliforms	5.6 ^a	8.1 ^a	8.6 ^b	7.3 ^a

^{ab} Within rows, means with the same superscript are not statistically different ($P > 0.05$).

short-chain fatty acid concentrations in the terminal ileum and resulted in a significant reduction in coliform populations. This was reflected in a more favourable ratio of lactobacilli to coliforms in the GI tract.

This study supports work previously published by Jensen and Mikkelsen (1998). These results have implications in terms of piglet health and dietary prevention of enteric diseases.

References

Brooks, P.H., Geary, T.M., Morgan, D.T. and Campbell, A. (1996) New developments in liquid feeding. *Pig Journal* 36, 43–63.

Geary, T.M., Brooks, P.H., Morgan, D.T., Campbell, A. and Russell, P.J. (1996) Performance of weaner pigs fed *ad libitum* with liquid feed at different dry matter concentrations. *Journal of Food and Agriculture* 72, 17–24.

Jensen, B.B. and Mikkelsen, L.L. (1998) Feeding liquid diets to pigs. In: Garnsworthy, P.C. and Wiseman, J. (eds) *Recent Advances in Animal Nutrition 1998*. Nottingham University Press, Nottingham, UK, pp. 107–126.

73 Effects of Oligosaccharides in Weanling Pig Diets on Performance, Microflora and Intestinal Health

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Specific oligosaccharides in weanling pig diets may help to temper the transition from milk diets to dry feed, though performance may not reach the levels found when antibiotics are included in the diet. Within the normal stresses of a production type environment, use of fructo-oligosaccharide (FOS) and sugarbeet pulp (BP), either separate or combined, helped in maintaining a healthy gastrointestinal tract (GIT) environment through greater colonization of bifidobacteria or reduction of *Escherichia coli* in the intestinal system. Further research is needed to determine whether changing levels of various oligosaccharides can also provide consistent efficient pig gains and feed conversion.

Materials and Methods

The objective of this study was to investigate the effects of adding fructo-oligosaccharide (FOS) and sugarbeet pulp (BP) to weanling pig diets on performance, intestinal volatile fatty acid (VFA) patterns, intestinal microbial counts and general health status of the pig. A group feeding study was conducted consisting of two trials with 175 pigs each, in two different nurseries. One of the nurseries was cleaned and disinfected before the experimental group was brought in; the other one was not. Twenty-five pens were used within each nursery and five dietary treatments were assigned at random to five replicate pens blocked within the room. Each treatment and pen was balanced for sex of pigs at similar initial weights. The pigs were weaned at 26–28 days of age. Five diets were formulated: (i) negative control diet (NEG) without any of the experimental

ingredients; (ii) antibiotic diet (AB) containing Virginiamycin (Stafac) at 0.05% of the diet; (iii) fructo-oligosaccharide diet (FOS) containing FOS at 5% of the diet; (iv) beet pulp diet (BP), containing dried sugarbeet pulp at 10% of the diet; and (v) combination diet (COM), containing a mixture of FOS at 2.5% and sugarbeet pulp at 5% of the diet.

The animals were fed their respective diets for a period of 4 weeks. The weight of individual pigs and pen feed intake data were recorded weekly. Incidence of diarrhoea was monitored. At the end of the trials, one animal from each pen was selected and sacrificed. Lungs, liver, spleen, ileocaecal lymph nodes and the gastrointestinal tract (GIT) were evaluated for gross pathology. GIT samples were taken from the terminal ileum, proximal colon and distal colon. These sample sites were selected because they present an image of bacterial and fermentation characteristics

throughout the GIT. The samples were analysed for *Escherichia coli*, bifidobacteria and total anaerobic bacterial counts, as well as VFA composition. Statistically, each trial was analysed separately.

Results

Average daily gains (ADG) and serum IGF-1 levels were not significantly changed by dietary treatment in either the clean or dirty nursery trials (Table 73.1). A comparison of the COM and BP fed pigs showed a trend towards an increased ADG of pigs fed the COM diet in week 4 of the clean nursery trial. Overall the AB diet, FOS diet and COM diet increased gains by 16%, 9% and 6%, respectively, compared with the NEG control, in the clean nursery trial. In the dirty nursery trial, feed efficiency was improved 14% with the FOS diet compared with the NEG diet. However, the FOS diet led to reduced feed intakes (24%) compared with the NEG diet in the dirty nursery trial.

There were few differences in diarrhoea incidence or the apparent health status of the organs in the pigs, based upon gross necropsy at the end of each trial. Although not significant, there was a trend towards lower *E. coli* concentrations in terminal ileal contents for pigs fed the FOS and BP diets in the clean nursery trial. FOS was associated with increased bifidobacteria in proximal colon samples of pigs fed FOS in

both the clean and dirty nurseries (Table 73.1). *E. coli* was reduced in the proximal colon of pigs fed FOS and BP diets in the clean nursery and tended to be reduced in the dirty nursery. The greatest fermentation activity in the proximal colon was in pigs fed the BP and COM diets, with acetic acid levels higher than other dietary treatments, especially in pigs fed the COM diet.

FOS tended to reduce the *E. coli* concentrations and increased bifidobacterial concentrations in the distal colon of the pigs, especially in the clean nursery trial (Table 73.1). BP fed pigs also had lower *E. coli* levels in the distal colon. Total anaerobic bacteria concentrations were also elevated in the distal colon with the FOS diet. There were few differences in VFA composition in the distal colon, though there was a numerical trend towards higher total fermentation acid concentrations with pigs fed the BP and COM diets.

Acknowledgements

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Further Reading

- Houdijk, J. (1998) Effects of non-digestible oligosaccharides in young pig diets. PhD thesis, Wageningen Agricultural University, The Netherlands.
- Koopman, J.P., Mroz, Williams, B.A. and others (1999) Voeding en gezondheid van het maag-darmkanaal, Onderzoeksreeks 4, Productschap diervoeder.

Table 73.1. Performance (average daily gain, average daily food intake, food conversion ratio), serum IGF-1, volatile fatty acids (VFA) and microbial results ($\log^{10}$) of the proximal and distal colon of pigs fed experimental diets in a clean nursery room and a dirty nursery room.

Trait	Clean nursery					Dirty nursery				
	NEG	AB	FOS	BP	COM	NEG	AB	FOS	BP	COM
ADG (kg day^{-1})	0.24	0.28	0.26	0.24	0.25	0.26	0.23	0.23	0.27	0.27
ADFI (kg day^{-1})	0.45	0.48	0.46	0.45	0.48	0.38 ^{ab}	0.34 ^{ab}	0.29 ^b	0.40 ^a	0.39 ^a
FCR	1.89	1.72	1.77	1.90	1.89	1.43 ^{ab}	1.44 ^{ab}	1.23 ^b	1.48 ^a	1.46 ^a
Serum IGF-1 (ng ml^{-1})	98.1	107.8	101.8	70.9	103.3	99.1	86.4	99.1	91.3	112.9
Proximal colon										
<i>E. coli</i>	6.30 ^{ab}	6.71 ^a	5.05 ^b	5.0 ^b	5.94 ^{ab}	5.99	6.74	5.72	5.37	6.26
Bifidobacteria	8.10	8.27	8.64	8.09	8.27	8.70 ^{ab}	8.81 ^{ab}	9.28 ^a	8.44 ^b	8.52 ^{ab}
Total anaerobes	9.72	9.79	10.12	9.57	9.99	10.30 ^{ab}	10.08 ^{ab}	10.51 ^a	10.00 ^b	10.05 ^b
VFA (mmol l^{-1})										
Acetic acid	89.6 ^{ab}	84.9 ^b	90.6 ^{ab}	97.3 ^{ab}	116.5 ^a	84.6	83.7	77.7	100.2	99.8
Propionic acid	33.6	42.2	39.0	35.0	44.5	32.5	30.6	40.9	37.1	42.1
Butyric acid	12.8	18.9	20.2	12.4	16.2	15.7	13.9	13.0	15.8	21.4
Isobutyric acid	1.4	1.9	1.3	0.8	0.9	2.2 ^a	2.1 ^a	1.0 ^b	1.3 ^{ab}	1.4 ^{ab}
Isovaleric acid	0	0.6	1.6	0	0	1.3	1.1	0	0.4	0.9
Valeric acid	3.2	2.5	4.9	2.3	2.3	4.0	3.8	3.0	3.5	3.3
Total VFA	140.6	150.9	157.5	147.9	180.4	140.3	135.3	135.6	158.2	168.9
Distal colon										
<i>E. coli</i>	6.09 ^a	6.73 ^a	4.78 ^{ab}	3.95 ^b	5.65 ^{ab}	5.55	6.61	5.31	4.96	6.34
Bifidobacteria	8.01 ^b	8.26 ^{ab}	9.25 ^a	8.43 ^{ab}	8.27 ^{ab}	8.45	8.42	9.07	8.42	8.63
Total anaerobes	9.71 ^b	9.73 ^b	10.26 ^a	9.71 ^b	9.64 ^b	10.08	9.90	10.20	9.98	9.95
VFA (mmol l^{-1})										
Acetic acid	47.5	46.7	50.0	69.6	71.0	69.9	55.3	55.3	76.4	80.0
Propionic acid	16.1	25.0	16.7	21.4	22.1	26.0	17.2	20.9	25.5	27.0
Butyric acid	4.9	4.8	5.5	12.8	11.5	10.8	7.3	4.7	9.8	12.8
Isobutyric acid	1.1	6.1	2.1	1.9	1.9	2.2	2.2	1.4	1.6	2.3
Isovaleric acid	0	0	0	0.8	0	0.7	1.1	0.8	0.9	0.9
Valeric acid	1.3	1.0	0	2.6	2.1	2.8	2.0	1.9	2.8	2.9
Total VFA	70.9	83.6	74.2	109.1	108.6	112.5	84.9	85.0	117.0	125.9

^{ab}Means in a row with different superscripts differ by $P < 0.05$

74 A Porcine Intestinal Organ Culture Model to Study the Adhesion of *Salmonella* and *E. coli* *in vitro*

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The methodology used to develop a porcine intestinal organ culture model is described. Pathogenic bacteria have highly developed specific adhesion mechanisms. Feed components are likely to affect intestinal receptor expression and they may also have a direct effect on the pathogen and on the competitive microflora in the lumen. In order to study these interactions it is essential to have an appropriate model. The study of reduced pathogen adherence is an appropriate endpoint for the study of the effectiveness of these treatments. In this model more non-pathogenic *Escherichia coli* were associated with the jejunum than *Salmonella* and more *Salmonella* were associated with the ileum than *E. coli*.

Introduction

Current understanding of bacterial adherence is derived mainly from studies using cultured epithelial cells and *in vivo* models. Although the use of cultured cells is convenient, the relevance of results obtained must be confirmed *in vivo*. However, studies *in vivo* are demanding in terms of both time and expenditure. The porcine intestinal organ culture (PIOC) model can allow testing of the efficacy of feed components before undergoing an infection study *in vivo*. This model is particularly relevant in determining feed structure effects and morphological changes to the gut epithelium that may have taken place *in vivo* in animals fed such diets. Intestinal tissue can be taken from the experimental animals fed particular feed components and then challenged with different bacterial inocula.

Materials and Methods

Animals

Danish Landrace × Yorkshire crossbred 9–10-week-old pigs (18–20 kg) were chosen at random from the herd at the Danish Institute of Agricultural Science, Foulum, Denmark. Pigs were fasted for 12 h prior to the experiment. The pigs were killed with a lethal injection of pentobarbital sodium (200 g l⁻¹). Animal experimentation and care of experimental animals complied with the regulations of the Danish Ministry of Justice, Law no. 726 (December 1993).

Bacterial strain and preparation of inoculum

Salmonella typhimurium S986 has been well characterized in previous studies (Naughton *et al.*, 1996, 2000). In order to

distinguish the *Escherichia coli* inoculum from indigenous strains, a naladixic acid marker was used. *Salmonella* and *E. coli* were retrieved as required from cultures stored at -80°C , plated on MacConkey agar plates, and incubated overnight. Bacteria were transferred from MacConkey agar by a sweep (three colonies) to 40 ml of Luria broth (trypticase, Merck, 10 g l^{-1} ; yeast extract, Merck, 5 g l^{-1} ; NaCl 5 g l^{-1} ; pH 7.5) incubated statically for 16 h at 37°C . The culture was centrifuged (1500 g , 15 min at 4°C) and the pellet resuspended in DMEM (Gibco).

Experimental procedure

The method was adapted from Bolton *et al.* (1999). Immediately after slaughter, the abdominal wall was opened by a midline incision and the gastrointestinal tract was removed. Six lengths of tissue were taken with 5 cm spaces between segments. Three lengths (11 cm each) were taken aseptically from the ileum (30 cm from the ileocaecal valve) and a further three lengths (11 cm each) were taken from the mid-jejunum and immersed in DMEM (Gibco). Mesenteric membrane was carefully removed from the segments. Two pieces of polyethylene tubing (Siltube, Eurpharm; diameter 6 mm) were inserted into either end of the tissue segment and a suture was applied (USP 3; Kruuse) to keep the tubing in place. The tissue was washed through with 100 ml of phosphate-buffered saline (PBS; pH 7.2) using a FillMaster pump (Type 311, Delta Scientific Medical, DK) (flow rate 7.7 cm s^{-1}) to remove any intestinal contents. A 21 gauge needle was then connected to the open end of the tubing and 10 ml DMEM alone (control) or DMEM containing either non-pathogenic *E. coli* or *Salmonella typhimurium* S986 was inoculated and the segment sealed using teflon plugs (diameter 5 cm). The organ culture was immersed in DMEM in a 300 ml infusion bottle in a shaking water bath (150 rpm) at 37°C in a 10% CO_2 atmosphere.

After 60 min the tissue was removed and washed with 100 ml of PBS to remove non-adherent bacteria.

The tissues were weighed and homogenized with a Janke-Kunkel Ultra-Turrax T25 homogenizer (The Netherlands) at 20,000 rpm on ice for 20 s in PBS plus Triton X (1%). A tenfold dilution series was prepared in triplicate from the homogenates to a final dilution of 1×10^{-6} . Samples were plated out according to the spread-plate method (Collins and Lyne, 1989). Lactose and non-lactose fermentors were enumerated on MacConkey agar (Merck; 1.05410), *Salmonella* were isolated on BPLS agar (Merck; 1.10747) and *E. coli* were isolated on Luria agar (Tryptone, Merck, 10 g l^{-1} ; yeast extract, Merck, 5 g l^{-1} ; NaCl 5 g l^{-1} ; agar, Merck, 15 g l^{-1} ; pH 7.5) with naladixic acid at $25\text{ }\mu\text{g ml}^{-1}$. Plates were incubated for 16 h at 37°C . Specific antisera (Behring, Marburg, Germany) and biochemical tests confirmed the presence of *Salmonella*.

Data were analysed by unpaired *t*-test. Results were expressed as the mean $\pm$ SD.

Results

In terms of percentage recovery from the tissue, significantly ($P < 0.05$) higher numbers of *Salmonella* (22.8 ± 5.6) were recovered from the ileum than *E. coli* (7.9 ± 2.3), and significantly ($P < 0.05$) higher numbers of *E. coli* were recovered from the jejunum (16.4 ± 4.2) in comparison with the ileum (7.9 ± 2.3). The results suggest that *E. coli* associates more avidly in the jejunum and that *Salmonella* associate more avidly in the ileum. This model system should be appropriate for the study of the effects of feed components on host resistance to opportunist pathogens such as *S. typhimurium* and pathogenic *E. coli*.

Acknowledgements

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References

- Bolton, A.J., Osborne, M.P., Wallis, T.S. and Stephen, J. (1999) Interaction of *Salmonella choleraesuis*, *Salmonella dublin* and *Salmonella typhimurium* with the porcine and bovine terminal ileum *in vivo*. *Microbiology* 145, 2431–2441.
- Collins, C.H. and Lyne, P.M. (1989) Cultural methods. In: Collins, C.H., Lyne, P.M. and Grange, J.M. (eds) *Microbiological Methods*. Butterworths, London, p. 84.
- Naughton, P.J., Grant, G., Spencer, R.J., Bardocz, S. and Pusztai, A. (1996) A rat model of infection by *Salmonella typhimurium* or *Salmonella enteritidis*. *Journal of Applied Bacteriology* 81, 651–656.
- Naughton, P.J., Grant, G., Bardocz, S. and Pusztai, A. (2000) Modulation of *Salmonella* infection by the lectins of *Canavalia ensiformis* (Con A) and *Galanthus nivalis* (GNA) in the rat model *in vivo*. *Journal of Applied Microbiology* 88, 720–727.
- Sojka, M.G., Dibb-Fuller, M. and Thorns, C.J. (1996) Characterisation of monoclonal antibodies specific to SEF 21 fimbriae of *Salmonella enteritidis* and their reactivity with other *Salmonellae* and *Enterobacteria*. *Veterinary Microbiology* 48, 207–221.

75 Effects of Virginiamycin on Histology of the Small Intestinal Mucosa in Piglets

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The objective of this study was to determine the histological effects of Virginiamycin (VM), given as a feed additive, on the small intestinal mucosa in piglets. The study consisted of two trials both with a control (C) group fed a diet without antibiotics and an experimental group fed a diet with 40 mg kg⁻¹ VM. A moderate oral challenge with *Escherichia coli* K88 was given to the two groups of five piglets in Trial 1. The groups in Trial 2 consisted of 16 piglets each. Dimensions of villi and crypts, the number of crypt goblet cells and mucin types were determined.

In Trial 1, 6 days after the challenge, crypt depths were significantly ($P = 0.05$) decreased and the number ($n \mu\text{m}^{-1}$) of crypt goblet cells was significantly increased ($P = 0.01$) in the VM group compared with those of the C group. In Trial 2, the villus lengths of the VM group were increased ($P = 0.06$) while crypt depths were unchanged. Further litter effects regarding crypt depths and mucin type were seen.

Introduction

Mechanisms by which antibiotics enhance animal growth and feed efficiency are

poorly understood (Commission on Antimicrobial Feed Additives, 1997; Anderson *et al.*, 1999). Two of the suggested mechanisms are: (i) inhibition of

subclinical infections and less translocation of pathogens; and (ii) changing the small intestinal mucosa villi to a more slender villi structure which may enhance the uptake of nutrients from the digesta. The first mechanism is related to a disturbed microbial equilibrium in the intestines of the animals, while the second mechanism is related to a normal balanced microflora.

The objective of the present study was to determine the effects of Virginiamycin (VM) in piglets after a moderate subclinical challenge of *Escherichia coli* K88 (Trial 1), and in piglets kept according to practical standards with a normal balanced microflora (Trial 2).

Materials and Methods

The two trials comprised a control group (C) fed a diet without antibiotics, and a group fed the same diet with 40 mg kg⁻¹ Virginiamycin (VM). The experiments were conducted with conventional crossbred piglets weaned at 21 days of age and equal numbers of littermates were allocated to each group.

In Trial 1, the piglets were derived from sows vaccinated against *E. coli* K88 and housed in metabolism cages. The feed intake of the piglets was restricted to 2.9 times the requirement of metabolizable energy for maintenance and water was freely available. Seven days after weaning, five piglets were allocated to each of the two experimental groups. At day 31 post weaning, a moderate challenge with *E. coli*

K88 (O149K91) (1.5 ml × 10⁹ CFU) was given orally (Meijer *et al.*, 1997). The experiment was finished at day 38 post weaning.

In Trial 2, the piglets were derived from sows who were not vaccinated against *E. coli*. The piglets were pen-housed and feed and water were freely available. The trial started at day 14 post weaning with the allocation of 16 piglets each to the C and VM groups and ended at day 28 post weaning.

Piglets of both trials were dissected under general anaesthesia. Laparotomy was performed and samples 1.5 cm wide were taken from the mid-jejunum, 5.5 m distal from the ligament of Treitz, for histological determinations as described by van Leeuwen *et al.* (1995). Sections (5 µm) were cut and stained with the periodic acid Schiff method (PAS). Those of Trial 1 were also stained with a combination of high iron diamide and alcian blue (HID-AB) for staining the sulphated mucins (sulphated mucins are brown = HID⁺) (Bancroft *et al.*, 1990).

Each parameter was statistically analysed using analysis of variance with diet type and litter as factors.

Results and Discussion

In Trial 1, villus heights of both groups of piglets were similar while crypt depths were significantly ($P = 0.05$) smaller, and the number of goblet cells was significantly ($P = 0.01$) higher in the VM group compared with the C group (Table 75.1). A tendency

Table 75.1. Histological characteristics of the small intestinal mucosa of piglets at day 38 post weaning, fed a diet without (C) or with 40 mg Virginiamycin (VM) kg⁻¹ in Trial 1.

	C (n = 5)	VM (n = 5)	SEM	P value	
				Groups	Litters
Villus height (µm)	490	473	43	NS	NS
Crypt depth (µm)	250	218	12	0.05	0.1
Villus/crypt ratio	2.0	2.2	0.3	NS	NS
Crypt goblet cells (n 100 µm ⁻¹)	6.4	8.7	0.5	0.01	0.1
HID ⁺ goblet cells (%)	60	52	7.7	NS	0.1

was observed that the villus/crypt (V/C) ratio was increased in the VM group compared with the ratio in the C group. Van Beers (1996) found clear increased crypt depths and villus shortening in the small intestinal mucosa of piglets due to weaning stress. However, 7 days later, the differences in villus height were already minimal whereas the crypt depths did not increase until 14 days post weaning. The significant smaller crypt depths of the VM group indicate a smaller histological effect of the *E. coli* challenge on the piglets in the VM group.

In Trial 2, the villus heights of the VM group were significantly increased ($P =$

0.06) compared with the C group and crypt depths of the VM group were slightly smaller (Table 75.2). This result agrees with the reviewed findings (Commission on Antimicrobial Feed Additives, 1997; Anderson *et al.*, 1999). They proposed a more slender villus structure after addition of antibiotics, which may enhance the uptake of nutrients from the digesta.

Litter effects ($P < 0.1$) were observed regarding crypt depths, numbers of goblet cells and mucin type in the crypt goblet cells. This may imply that variation in the small intestinal architecture is at least partly related to the genetic background of the piglets.

Table 75.2. Histological characteristics of the small intestinal mucosa of piglets at day 28 post weaning, fed a diet without (C) or with 40 mg Virginiamycin (VM) kg⁻¹ in Trial 2.

	C (n = 16)	VM (n = 16)	SEM	P value	
				Groups	Litters
Villus height (µm)	395	436	30	0.06	Ns
Crypt depth (µm)	329	315	18	NS	0.1
Villus/crypt ratio	1.3	1.4	0.1	NS	NS
Crypt goblet cells (n 100 µm ⁻¹)	6.9	7.0	0.4	NS	NS

References

Anderson, D.B., McCracken, V.J., Aminov, R.I., Simpson, J.M., Mackie, R.I., Verstegen, M.W.A. and Gaskins, H.R. (1999) Gut microbiology and the mechanisms of action of growth-promoting antibiotics in swine. *Pig News and Information* 20(4), 115N–122N.

Bancroft, J.D., Stevens, A. and Turner, D.R. (1990) *Theory and Practice of Histological Techniques*, 3rd edn. Churchill Livingstone, Edinburgh.

Commission on Antimicrobial Feed Additives (1997) *Anti Microbial Feed Additives*. Government Official reports, SOU 1997: 132, Ministry of Agriculture, Stockholm, Sweden.

Meijer, J.C., van Gils, L., Blokland, B., de Geus, B., Harmsen, M., Nabuurs, M., and van Zijderveld, F. (1997) Weaned piglets orally challenged with *E. coli* following stress as a model of post weaning diarrhoea. In: *Proceedings of the Symposium on Gastrointestinal Disorders*, 15 September 1997, ID- DLO, Lelystad, The Netherlands.

Van Beers, H.M.G. (1996) The changes in the function of the large intestine of weaned pigs. PhD thesis, University of Utrecht, The Netherlands.

Van Leeuwen, P., Jansman, A.J.M., Wiebinga, J., Koninkx, J.F.J.G. and Mouwen, J.M.V.M. (1995) Dietary effects of (*Vicia faba* L.) tannins on the morphology and function of the small-intestinal mucosa of weaned pigs. *British Journal of Nutrition* 73, 31–39.

76 Net Absorption of Fluid in Uninfected and ETEC-infected Piglet Small Intestine: Effect of Osmolality

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After (early) weaning, piglets frequently have diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) and rotavirus. Using the small intestine segment perfusion model with uninfected and ETEC-infected segments, we have investigated the effect of osmolality on net fluid absorption in piglets. Increased osmolality resulted in a decreased net fluid absorption. Regression analysis showed a linear relationship between osmolality and net fluid absorption both for uninfected and ETEC-infected segments. ETEC infection resulted in a decrease in net fluid absorption independent of the osmolality. Although hypotonic solutions do not eliminate the decreased net fluid absorption observed in ETEC infection, it is shown that low osmolality may promote net fluid absorption in piglets with diarrhoea.

Introduction

Clinical studies show that reducing the osmolality of oral rehydration solutions reveals beneficial effects on the clinical course of acute diarrhoea in children (Thillainayagam *et al.*, 1998). After (early) weaning, piglets frequently have diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) and rotavirus (Nabuurs, 1998). Using the small intestine segment perfusion model (Nabuurs *et al.*, 1993) with uninfected and ETEC-infected segments, we have investigated the effect of osmolality on net fluid absorption in piglets.

Material and Methods

Segments (length $\pm$ 20 cm) were fitted in the middle of the small intestine in

6-week-old anaesthetized piglets, weaned at 3 weeks of age. The odd-numbered segments were injected with 5 ml ETEC (5×10^9 colony-forming units O149:K91:K88^{ac}, producing heat-labile and heat-stable toxin) and the even-numbered segments with 5 ml phosphate-buffered saline (PBS), whereupon the segments were perfused over 8 h with 64 ml.

In the first experiment, four different sodium chloride based solutions containing amino acids (1 g l^{-1}) and glucose (1 g l^{-1}) in the range of 150–375 mOsmol kg^{-1} were tested in eight piglets, each with four pairs of segments (an uninfected and an adjacent ETEC-infected). For the second experiment the range in osmolality was enlarged and five different solutions in the range of 0–600 mOsmol kg^{-1} were tested in five piglets, each with five pairs of segments.

Net fluid absorption was calculated from the difference between the volumes of inflow and outflow divided by the surface area (length $\times$ circumference) of the segment.

Results

Increased osmolality resulted in a decreased net fluid absorption (Fig. 76.1). Regression analysis showed a linear relationship between osmolality and net fluid absorption both for uninfected and ETEC-infected segments in the first experiment.

The slopes of the regression lines for uninfected and ETEC-infected segments were not different, but the elevations were significantly different.

In the second experiment an osmolality of 600 mOsmol l⁻¹ resulted in a net fluid secretion even in the uninfected segments, and an osmolality beyond 150 mOsmol l⁻¹ did not result in a further increase of the net fluid absorption (Fig. 76.2).

In both experiments ETEC infection resulted in a decrease in net fluid absorption of about 400 μ l cm⁻² independent of the osmolality of the perfusion fluid used.

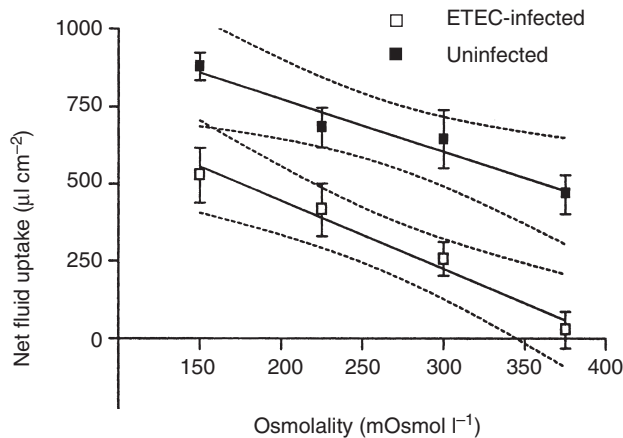


Fig. 76.1. Average net fluid absorption ($n = 8$, $\pm$ SEM) in uninfected and ETEC-infected segments perfused with solutions with different osmolality (150–375 mOsmol l⁻¹). Linear regression lines with 95% confidence intervals are shown.

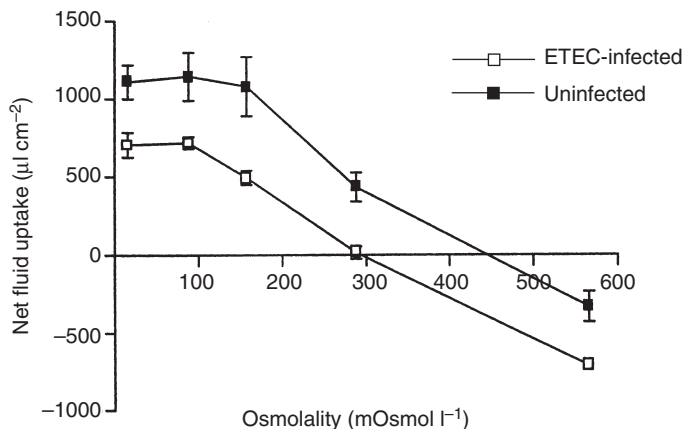


Fig. 76.2. Average net fluid absorption ($n = 5$, $\pm$ SEM) in uninfected and ETEC-infected segments perfused with solutions with different osmolality (0–600 mOsmol l⁻¹).

Discussion

The linear decrease in net fluid absorption when perfusion fluids of increased osmolality were used corresponds well with earlier results in rats (Thillainayagam *et al.*, 1993). A similar relationship was shown for ETEC-infected segments as has been observed before for cholera toxin-induced

secreting rat intestine (Pillai *et al.*, 1994). Below 150 mOsmol l⁻¹ there was no longer any positive effect of osmolality on net fluid absorption. Although hypotonic solutions do not eliminate the decreased net fluid absorption observed in ETEC infection, it is clear that low osmolality may promote net fluid absorption in piglets with diarrhoea.

References

- Nabuurs, M.J. (1998) Weaning piglets as a model for studying pathophysiology of diarrhea. *Veterinary Quarterly* 20, S42–45.
- Nabuurs, M.J., Hoogendoorn, A., van Zijderveld, F.G. and van der Klis, J.D. (1993) A long-term perfusion test to measure net absorption in the small intestine of weaned pigs. *Research in Veterinary Science* 55, 108–114.
- Pillai, G.V., Brueton, M.J., Burston, D. and Sandhu, B.K. (1994) Evaluation of the effects of varying solute content on the efficacy of oral rehydration solutions in a rat model of secretory diarrhoea. *Journal of Pediatric Gastroenterology and Nutrition* 18, 457–460.
- Thillainayagam, A.V., Carnaby, S., Dias, J.A., Clark, M.L. and Farthing, M.J. (1993) Evidence of a dominant role for low osmolality in the efficacy of cereal based oral rehydration solutions: studies in a model of secretory diarrhoea. *Gut* 34, 920–925.
- Thillainayagam, A.V., Hunt, J.B. and Farthing, M.J. (1998) Enhancing clinical efficacy of oral rehydration therapy: is low osmolality the key? *Gastroenterology* 114, 197–210.

77 Soluble Non-starch Polysaccharides from Pearl Barley Exacerbate Experimental Postweaning Colibacillosis

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The performance and health of weaner pigs ($n = 40$) was compared between pigs fed either a rice-based weaner pig diet or the same diet with pearl barley included at 50% weight of the air-dry diet. Within each dietary treatment, two-thirds of the pigs were infected with enterotoxigenic *Escherichia coli* O8:K88:K87 at 48, 72 and 96 h post-weaning; the remaining third were healthy controls. Compared with those fed the barley diet, pigs fed the rice-only diet grew faster, had less intestinal proliferation of *E. coli*, and exhibited less severe diarrhoea. It appears that the soluble non-starch polysaccharides (sNSP) in barley are detrimental to the performance of both healthy weaner pigs and those with postweaning colibacillosis (PWC).

Introduction

Increasing restrictions on the use of antimicrobials in pig feeds has accentuated the need for alternative strategies when managing outbreaks of postweaning colibacillosis (PWC). Associated with a proliferation of enterotoxigenic *Escherichia coli* in the small intestine, PWC occurs in piglets during the first 1–2 weeks after weaning, causing weight loss, diarrhoea and death. Environmental, managemental and genetic factors all influence the establishment and progression of this disease, particularly the composition and intake of the weaning diet (Bertschinger, 1999; McDonald *et al.*, 1999). The aim of this study was to investigate the effects of feeding a diet high in soluble non-starch polysaccharide (sNSP) content, in the form of pearl barley, on whole-body and intestinal growth and development, and intestinal proliferation

of enterotoxigenic *E. coli* in both healthy newly weaned piglets and those experimentally infected with an enterotoxigenic strain of *E. coli*.

Materials and Methods

Forty Large White $\times$ Landrace pigs were weaned at 21 days of age and allocated, according to live weight (LW), to one of two dietary treatments. Diet R consisted of cooked white rice, balanced for weaner nutritional requirements with an animal protein supplement (bloodmeal, meat and bonemeal, fishmeal). The second diet, B50, comprised cooked white rice plus an animal protein supplement, with the addition of hammer-milled dehulled (pearl) barley comprising 50% of the total weight of the air-dry diet. The amount of rice in the B50 diet was reduced and minor adjustments

were made in the amounts of ingredients in the animal protein supplement to compensate for the addition of barley. There were minimal amounts of sNSP in the rice diet (0.4%) whereas the barley diet contained around 2.5% sNSP.

Six pigs from each dietary group were kept as healthy uninfected controls whilst the rest were orally inoculated with 10 ml of a broth culture of 10^{10} ml⁻¹ *E. coli* O8:K87:K88:StaP:Stb:LT at 48, 72 and 96 h post weaning. The pigs in this trial constituted part of a larger study, where healthy pigs were housed individually and infected pigs were housed in groups to facilitate transmission of *E. coli*. Four of the infected pigs were excluded, as they became ill and were euthanased early in the trial. All pigs were fed *ad libitum* for 7–9 days after weaning and then euthanased to record gut measurements. The stomach, small intestine, caecum and colon were weighed empty and full, and digesta samples were taken for determination of pH values, volatile fatty acid (VFA) concentration, intestinal viscosity and intestinal proliferation of *E. coli* O8:K87:K88. Liveweights and voluntary food intake were measured daily during the trial.

Results and Discussion

The LW gain of pigs fed the rice-only diet (R) was greater than that of pigs fed the barley–rice diet (B50) (Table 77.1), but this increase was not statistically significant. When the weight of the full gut was removed it was apparent that pigs fed the rice-only diet had greater carcass gain, indicating that the energy was being focused into muscle growth rather than intestinal growth. This was further supported by finding that the full large intestine comprised a larger proportion of the body weight in those pigs fed the barley diet.

Greater microbial fermentation in the colon of pigs fed the barley–rice diet was indicated by higher VFA concentrations and more acidic pH values, especially in the distal half of the colon. These results demonstrate the adaptation of the piglet to the fermentation and utilization of dietary sNSP.

In the ileum of the healthy pigs, VFA concentrations were higher in pigs fed diet R whereas in the infected pigs the VFA concentrations were greater in those fed diet B50, resulting in a significant effect of

Table 77.1. The effect of diet on mean intestinal weights, fermentation, viscosity and intestinal proliferation of haemolytic *E. coli* in pigs 1 week after weaning.

	Healthy pigs		Infected pigs		SEM	P-value	
	R (n = 6)	B50 (n = 6)	R (n = 11)	B50 (n = 13)		Diet	Disease
Live weight gain (g day ⁻¹)	143	94	11	−8	38	NS	***
Carcass gain (g day ⁻¹)	74	26	−28	−56	36	*	***
Weight of full large intestine (% LW)	2.7	3.8	2.6	3.2	0.6	**	NS
pH distal colon	6.8	6.1	6.8	6.5	0.3	**	NS
Distal colon VFA (mmol l ⁻¹)	84	114	60	78	20	**	**
Ileal VFA (mmol l ⁻¹)	18	9	12	14	5	*	
Ileal viscosity (cP)	2.1	2.8	1.6	2.3	1.13	*	*
<i>E. coli</i> in jejunum (log ₁₀ CFU g ⁻¹)	0	0	0.9	4.2	2.4	*	
<i>E. coli</i> in colon (log ₁₀ CFU g ⁻¹)	0	0	3.2	6.2	1.8	**	

Diet R: rice + animal protein.

Diet B50: rice + animal protein + barley.

Carcass = live weight minus full weight of gut.

P* < 0.05; *P* < 0.01; *** *P* < 0.001; NS, not significant.

interaction of diet and disease. This suggests that the site of fermentation moves distally when barley is added to a rice diet in healthy weaner pigs, and that there is greater ileal microbial fermentation in infected pigs when barley is added to the diet. The presence of barley in the ileum also significantly increased the viscosity of the digesta in healthy and infected pigs.

All the infected pigs lost weight after inoculation. Pigs fed the rice-only diet appeared to recover quite rapidly, gaining weight (relative to their weaning weight) by the end of the trial. Pigs offered the barley-rice diet continued to lose weight, which greatly affected their carcass gain. There was less proliferation of intestinal *E. coli* O8:K87:K88 in pigs fed the rice-only diet and this was evident clinically, as these pigs continued to eat well and had less severe diarrhoea than those fed the barley diet.

Conclusions

The addition of barley to the diet altered the physicochemical conditions in the intestines, increasing the ileal viscosity and moving microbial fermentation more distally in the tract in healthy pigs. The energy expended in adapting the intestinal tract for digestion of sNSP resulted in a depression in carcass growth, which was exacerbated by the presence of postweaning colibacillosis. The association of sNSP with increased viscosity and intestinal bacterial proliferation has been shown in poultry (Choct *et al.*, 1996) but is less clear-cut in pigs. Interactions between dietary sNSP and intestinal pathophysiology are complex and require further elucidation. However, it appears that a diet low in sNSP is beneficial for the performance of both healthy weaner pigs and pigs with PWC.

References

- Bertschinger, H.U. (1999) Postweaning *Escherichia coli* diarrhoea and oedema disease. In: Straw, B.E., D'Allaire, S., Mengeling, W.L. and Taylor, D.J. (eds) *Diseases of Swine*, 8th edn. Iowa State University Press, Iowa, pp. 441–454.
- Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G. (1996) Increased small intestinal fermentation is partly responsible for the antinutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* 37, 609–621.
- McDonald, D.E., Pethick, D.W., Pluske, J.R. and Hampson, D.J. (1999) Adverse effects of soluble non-starch polysaccharide (guar gum) on piglet growth and experimental colibacillosis immediately after weaning. *Research in Veterinary Science* 67, 245–250.

78 The Correlation between Coliform Populations Collected from Different Sites of the Intestinal Tract of Pigs

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Fingerprinting of rectal flora was concluded to be a relevant method to mirror enteric microflora since microbial diversity was similar in all segments investigated. However, the composition of coliform flora somewhat differed between different segments of the intestine.

Introduction

To understand the functional status of intestinal flora, assessing the microbial diversity has been assumed to constitute an adequate approach (Baquero *et al.*, 1988). In practice, this has been from rectal swabs. However, due to the divergence of the ingesta and density of bacteria in different parts of the intestine, these rectal samples may not necessarily reflect the flora of other parts of the intestine. The aim of the present study was to compare the coliform flora of different sites in the gut of pigs.

Material and Methods

Six conventional pigs (Hampshire × Yorkshire–Landrace–Duroc) that had been weaned at age 35 days, and thereafter offered naked barley, were included in the study. On day 63 the principals were sacrificed and samples were collected from six different sites of the gut; the proximal jejunum (2 m distal from the pylorus); the distal jejunum (2 m proximal from the

ileum); the ileum; the caecum; the colon (at the turn of the helix); and the rectum.

The number of colony-forming units of coliforms g⁻¹ ingesta was made by cultivation on violet red bile (VRB) agar. A metabolic fingerprint of coliform flora was made from another fraction of the samples. The PhenePlate (PhP) system evaluates the kinetics of bacterial metabolism of highly discriminating liquid substrates and is executed in microtitre plates (Möllby *et al.*, 1993). Twenty-four colonies from each sample were randomly picked and inoculated on PhenePlates comprising 11 dehydrated reagents, specifically chosen to differentiate between coliforms (PhP-RS plates; Biosys, Stockholm, Sweden) (Kühn and Möllby, 1993). The diversity (Di) of coliforms within each sample were calculated as diversity indexes (Hunter and Gaston, 1988). The homogeneity (H) between different floras from the same sampling site was calculated and presented as a mean correlation coefficient. The population similarity coefficient (Sp) between bacterial floras collected at different locations of the intestinal tract was calculated

for all piglets and clustered according to the single linkage clustering method (Sneath and Sokal, 1973).

Results

The number of coliforms g^{-1} ingesta increased through the digestive channel (Fig. 78.1). The mean D_i of these floras was high (0.95) and only minor differences between the different locations within each pig were recorded. The mean D_i of the six defined locations of the intestinal tract that were sampled ranged from 0.91 (colon) to 0.95 (ileum). The D_i in the rectum was 0.92.

The homogeneity between coliform populations of different pigs was low ($r_{\text{mean}} = 0.55$) at the first sampling sites (i.e. jejunum). However, the homogeneity between coliform populations of different pigs increased through the passage of the intestine (Fig. 78.1).

The S_p values obtained indicated different coliform floras in the different sites of the intestine (Fig. 78.2).

Discussion and Conclusion

Despite the coliform floras being somewhat differed between the different segments of

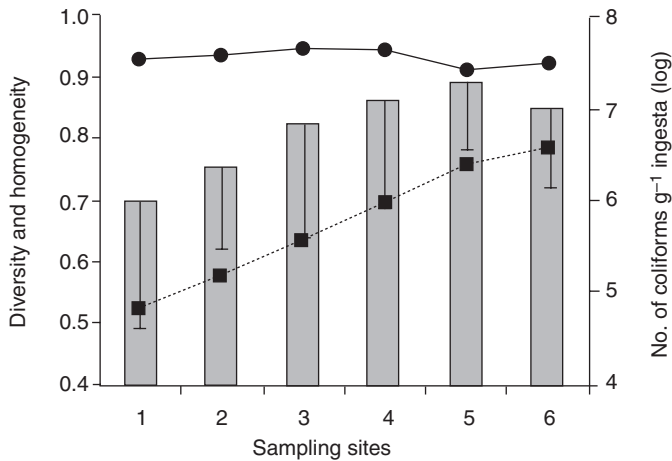


Fig. 78.1. The number of coliforms g^{-1} ingesta (bars) in different sites of the intestine of pigs. These sites comprised: (1) proximal jejunum; (2) distal jejunum; (3) ileum; (4) caecum; (5) colon, (6) rectum. The figure also show the diversity of these flora (circles) and the homogeneity between the flora of the pigs at each site (squares).

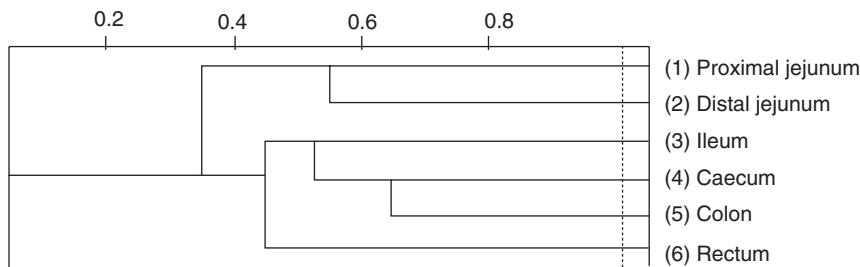


Fig. 78.2. The S_p between coliform floras of different parts of the intestine.

the intestine, fingerprinting of the rectal flora was concluded to be a relevant method to mirror the enteric microflora, since the microbial diversity was similar in all segments investigated. The density of

microbes was highest in the rectal samples, presumably indicating a more stable flora. Therefore results obtained when fingerprinting the rectal flora possibly should be counted as the most secure.

References

- Baquero, F., Fernandez-Jorge, M., Vicente, M.F., Alos, J.I. and Reig, M. (1988) Diversity analysis of the human intestinal flora: a simple method based on bacterial morphotypes. *Microbial Ecology in Health and Disease* 1, 101–108.
- Hunter, P.R. and Gaston, M.A. (1988) Numeric index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology* 26, 2465–2466.
- Kühn, I. and Möllby, R. (1993) The PhP RS system – a simple microplate method for studying coliform bacterial populations. *Journal of Microbiological Methods* 17, 101–107.
- Möllby, R., Kühn, I. and Katouli, M. (1993) Computerised biochemical fingerprinting – a new tool for typing of bacteria. *Reviews in Medical Microbiology* 4, 231–241.
- Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy*. W.H. Freeman, New York.

79 Bifidobacteria in Piglets

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The population of *Bifidobacterium* spp. in the gastrointestinal tract of suckling piglets was investigated and three selective agars (Beerens, Raffinose-Bifidobacterium and Modified Wilkins-Chalgren agar) were evaluated with regards to enumeration of bifidobacteria from piglet samples. Isolated bifidobacteria from the piglets were assigned to *B. boum*, *B. suis* and an unidentified species. The population varied between piglets and constituted in all piglets less than 1% of the total bacterial population. The results demonstrated the inadequacy of the selective agars for enumeration of bifidobacteria in piglet faecal and intestinal samples.

Introduction

Composition of the microflora colonizing the gastrointestinal tract of pigs plays an essential role for animal health, animal

nutrition and performance. Feed additives such as non-digestible oligosaccharides (NDOs) are believed to influence intestinal microbial fermentation and contribute to a beneficial intestinal microflora. NDOs have

been shown to stimulate the growth of bifidobacteria in the gastrointestinal tract of humans and these bacteria are generally accepted as being beneficial and health promoting. The aim of the present study was to investigate the population of *Bifidobacterium* spp. in piglets and to evaluate three selective agars with regard to enumeration of this group of bacteria. This was performed in order to investigate the effects of NDOs on the intestinal microflora of weanling piglets in later animal trials.

Materials and Methods

Faecal and intestinal samples obtained from suckling piglets (age 2–4 weeks) were diluted and used to inoculate the three selective agars: Beerens (Beerens, 1990), Raffinose-Bifidobacterium (RB) (Hartemink *et al.*, 1996) and Modified Wilkins-Chalgren (MW) (Rada *et al.*, 1997). In addition, the total number of anaerobic bacteria was enumerated using Wilkins-Chalgren agar (Oxoid, CM619). For each sample, 30 colonies from each of the three selective agars were picked at random. Isolates belonging to the genus *Bifidobacterium* were identified by demonstration of F6PPK activity and PCR using genus-specific primers, and were then differentiated by

amplified ribosomal DNA restriction analysis (ARDRA). Non-bifidobacterial isolates were divided into groups based on their SCFA profiles from glucose fermentation. Representatives of each group were identified by comparative 16S rRNA gene sequence analysis using the BLAST database search programs (www.ncbi.nlm.nih.gov/BLAST/).

Results

On average, bacterial numbers of 8.38, 8.60 and 7.18 log CFU g⁻¹ were shown from the piglet samples using Beerens, RB and MW agars, respectively (data not shown). In only two samples out of ten (eight pigs), 7% and 3% of the isolates from RB and 7% and 50% of the isolates from Beerens were identified as bifidobacteria (Fig. 79.1). Improved selectivity was seen for MW agar with 17%, 97%, 33% and 3% of the isolates from four samples, respectively, belonging to the genus *Bifidobacterium* (Fig. 79.1). It should be mentioned that in samples F2, S4, SI4 and C4 no colonies at all appeared on MW agar (data not shown).

Thus, the average bifidobacterial population level detected from MW in the four samples was 7.21 log CFU g⁻¹ (6.48–7.77

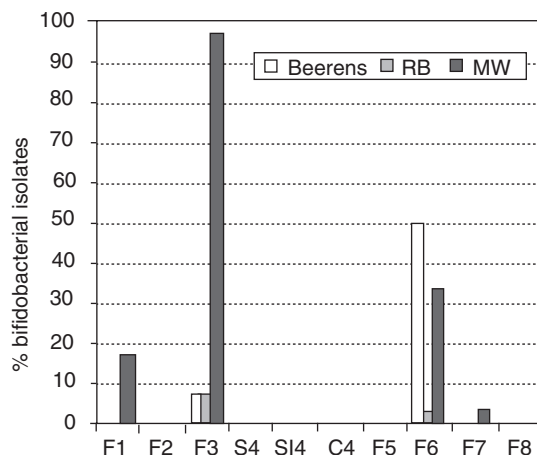


Fig. 79.1. Percentage of bifidobacteria among isolates from each sample using three selective agars. Samples: F, faecal; S, stomach; SI, small intestine; C, colon.

log CFU g⁻¹) (Fig. 79.2) and this level constituted less than 1% of the total anaerobic bacteria population (9.62 log CFU g⁻¹) in these samples (data not shown). In the remaining six samples (four pigs), the bifidobacterial population level was below the detection limit (5.27–6.66 log CFU g⁻¹) (Fig. 79.2).

ARDRA profiles divided the bifidobacteria into three groups (data not shown) and sequencing of the 16S rDNA identified isolates of these groups as *B. boum* (19 isolates), *B. suis* (25 isolates) and a possible new species (17 isolates) (Table 79.1). Only MW agar enabled isolation of the new *Bifidobacterium* sp. (Table 79.1). Division of non-bifidobacterial isolates by their SCFA profiles and partial 16S rDNA sequences demonstrated that members of the genera *Lactobacillus*, *Streptococcus*,

Enterococcus and *E. coli* appeared primarily on Beerens and RB agars, while colonies isolated from MW agar mainly belonged to *Clostridium*, *Bacteroides* and *Actinomyces* (data not shown).

Conclusion

In conclusion, the present study demonstrated that the population of bifidobacteria is variable in piglets and constitutes less than 1% of the total bacterial population. Beside *B. boum* and *B. suis*, a possible new bifidobacterial species was isolated. It was also demonstrated that current selective agars for enumeration of bifidobacteria in piglet faecal and intestinal samples are inadequate. The identification of non-bifidobacterial isolates, however, may con-

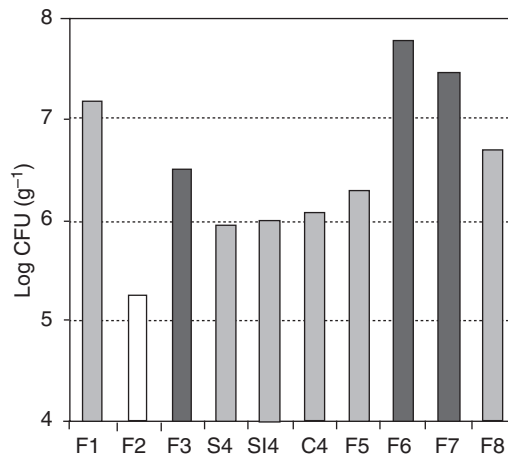


Fig. 79.2. Number of bifidobacteria in each sample calculated from counts on MW agar and the percentages of bifidobacterial isolates. Black column: estimated bifidobacterial number; grey column: detection limit for bifidobacteria (no bifidobacteria were isolated from these samples); samples: F, faecal; S, stomach; SI, small intestine; C, colon.

Table 79.1. Bifidobacteria species isolated from the three selective agars.

Bifidobacterial isolates	Selective agars		
	Beerens	RB	MW
<i>B. boum</i>	2	2	15
<i>B. suis</i>	15	1	9
<i>B. sp.</i>	0	0	17

tribute to the formulation of an improved selective media for the enumeration of bifidobacteria. Alternatively, oligonucleotide

probes could be designed from 16S rDNA sequences and used in conjunction with colony hybridization to increase reliability.

References

- Beerens, H. (1990) An elective and selective isolation medium for *Bifidobacterium* spp. *Letters in Applied Microbiology* 11, 155–157.
- Hartemink, R., Kok, B.J., Weenk, G.H. and Rombouts, F.M. (1996) Raffinose-Bifidobacterium (RB) agar, a new selective medium for bifidobacteria. *Journal of Microbiological Methods* 27, 33–43.
- Rada, V., Sirotek, K. and Petr, J. (1999) Evaluation of selective media for bifidobacteria in poultry and rabbit caecal samples. *Journal of Veterinary Medicine* 56, 369–373.

80 Effect of FormiTM-LHS on Digesta and Faecal Microbiota, and on Stomach Alterations of Piglets

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Addition of 1.8% FormiTM-LHS (potassium diformate) to a starter piglet diet decreased the counts of total anaerobic bacteria, lactic acid bacteria, yeasts and, to a lesser extent, coliform bacteria in faeces and digesta samples from the stomach, distal small intestine, caecum and middle segment of the colon of piglets. The pH along the gastrointestinal tract of the piglets was not significantly affected by Formi-LHS. No effect of Formi-LHS on the epithelium of the stomach was observed.

Introduction

Addition of organic acids to piglet diets has been reported to increase performance of the animals and decrease the concentration of coliform bacteria, known to be involved in digestive disorders, and other microorganisms in the gastrointestinal tract

(Roth and Kirchgessner, 1998). These results have made organic acids possible candidates as substitutes for antibiotics in starter diets for piglets. Also the use of organic acid salts has received attention during recent years.

The aim of this investigation was to study the effect of addition of FormiTM LHS

to a starter piglet diet on the microbiota in faeces and in the gastrointestinal tract of piglets, and on alterations of the stomach epithelium.

Materials and Methods

Two diets were formulated: a standard starter piglet diet containing 160 units of copper; and the same diet supplemented with 1.8% Formi™-LHS. Thirty-six piglets (male and female) weaned at 4 weeks of age were used. Two littermates were housed in a pen and received the control diet; their corresponding littermates were housed together and received the Formi-LHS diet. Fresh faecal samples were collected from the rectum on days 0, 1, 3, 5, 7, 14, 21 and 28 post weaning. On days 7 and 29, one animal per pen was sacrificed and the gastrointestinal tract was divided into eight segments: stomach; three equal segments of the small intestine; caecum; and three equal segments of the colon, including the rectum. The stomach epithe-

lium of the pars proventricularis was visually examined for alterations.

Calculations were performed with the GLM procedure, using the SAS statistical program. Results are expressed as least square means and standard error. Comparison of the stomach epithelium between diets was carried out using the probit procedure.

Results

Total anaerobic bacteria concentration in faeces was significantly reduced on day 21 in the pigs receiving Formi-LHS (Fig. 80.1a), and the same tendency was observed on days 3 and 28 ($P = 0.09$). The concentration of lactic acid bacteria was significantly ($P < 0.05$) lower on days 5, 7 and 28 in the faecal samples of pigs receiving Formi-LHS in the diet (Fig. 80.1b). The same tendency was measured on days 3 ($P = 0.05$) and 21 ($P = 0.06$). On days 7, 14, 21 and 28, numerically lower number of coliforms were measured in the faeces of pigs

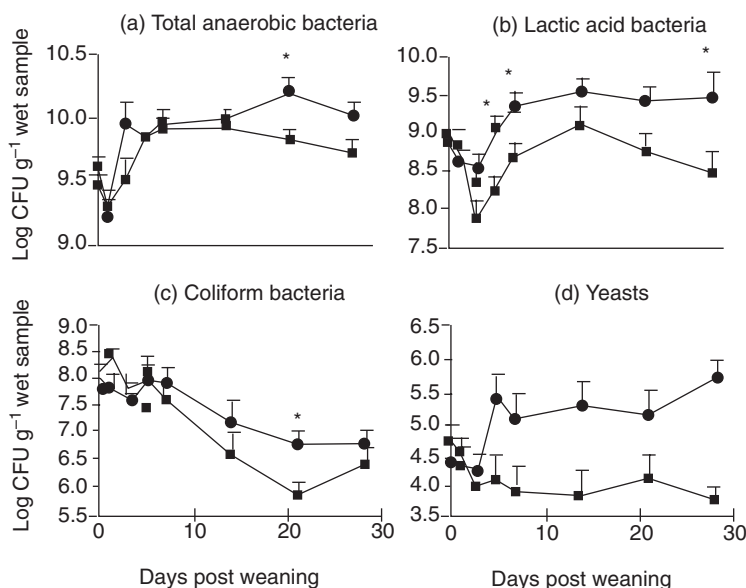


Fig. 80.1. Microbiology (log CFU g⁻¹ wet sample) in faeces post weaning: ● control diet, ■ Formi-LHS diet. Values are means and standard errors. * $P < 0.05$.

fed the Formi-LHS diet, but significance was only reached on day 21 (Fig. 80.1c). The concentration of yeasts was lower from day 3 post weaning when feeding Formi-LHS (Fig. 80.1d). However, some values were below the detection level, which makes it difficult to draw categorical conclusions.

On day 7, the numbers of total anaerobic bacteria, lactic acid bacteria and coliform bacteria were lower in the stomach, the last segment of the small intestine, the caecum and the middle segment of the colon of pigs fed Formi-LHS compared with the control pigs. However, none of the differences was significant ($P > 0.05$). Due to values below detectable levels in both groups, no evidence of significant differences in coliform counts does not necessarily mean that differences do not exist. Yeast counts were also lower in all segments after feeding Formi-LHS, but statistics could not be assessed. However, there were more samples with counts below detectable levels when Formi-LHS was fed, compared with feeding the control diet.

On day 29, addition of Formi-LHS significantly decreased the concentrations of total anaerobic bacteria in the proximal segments of the gastrointestinal tract, and of lactic acid bacteria in the stomach ($P = 0.05$), small intestine and caecum ($P < 0.05$) and colon ($P = 0.14$). Coliforms were present at lower levels in all segments of the gastrointestinal tract measured after feeding Formi-LHS. Significance was only reached in the colon but, as previously described, it should be pointed out that the real values from the Formi-LHS group are lower than those presented. Yeasts were present at significantly lower levels in all segments of the gastrointestinal tract of the pigs receiving Formi-LHS in the diet.

The concentration of formic acid was higher in pigs fed the Formi-LHS diet. Although the pH value in the stomach was slightly lower in pigs fed Formi-LHS both on day 7 and on day 29, no significant differences ($P > 0.05$) were detected in this or other segments of the gastrointestinal tract between the two groups of pigs.

Addition of Formi-LHS did not affect the stomach epithelium of pigs either on day 7 or on day 29 after weaning.

Discussion

The positive effects of organic acid supplementation to weanling pig diets have been attributed to, among other mechanisms, a lowering of pH, mainly in the stomach, which controls the growth of pathogenic bacteria (Partanen and Mroz, 1999). However, studies with formic acid have shown an antimicrobial effect of this acid with no pronounced effect, or no effect at all, on the stomach or intestinal pH. It has been suggested that the benefits of including organic acids should rather be attributed to the antimicrobial properties of the protons and anions into which formic acid is divided after passing the bacterial cell wall and which have a disruptive effect on bacterial protein synthesis (Partanen and Mroz, 1999). Addition of Formi-LHS did not significantly affect pH along the gastrointestinal tract in the present study. However, the levels of various bacteria decreased when Formi-LHS was added to the diet. This antimicrobial effect may be attributed to dissociation of formic acid inside the cells with the corresponding inhibition of bacterial enzymes, which places bacterial cells under considerable stress and makes them unable to replicate.

References

- Partanen, K.H. and Mroz, Z. (1999) Organic acids for performance enhancement in pig diets. *Nutrition Research Reviews* 12, 1–30.
- Roth, F.X. and Kirchgessner, M. (1998) Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. *Journal of Animal and Feed Sciences* 7, 25–33.

81 The Effect of Fermentation and/or Sanitization of Liquid Diets on the Feeding Preferences of Newly Weaned Pigs

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The objective of this study was to examine the potential of chlorine dioxide to suppress bacterial contamination and hence produce a 'biosafe' feed for the young piglet. Chlorine dioxide at a concentration of 300 ppm proved to be effective in eliminating coliforms from the feed and did not affect the palatability of the diet or feed intake of piglets. The study improves our understanding of the effects of controlling the preparation of 'biosafe' feed, maintaining feed intake of the piglet and hence reducing postweaning growth check and improving the welfare and health of the pig.

Introduction

It has been demonstrated that liquid feeding can improve the feed intake, growth, feed conversion and health of weaner piglets (Brooks *et al.*, 1996). However, in some instances feeding fermented feed has resulted in reduced feed intake due to poor palatability. Steeping of feed in water enables the proliferation of epiphytic coliforms. The objective of this study was to examine the potential of chlorine dioxide (ClO_2) to suppress bacterial contamination in liquid feed and hence produce a 'biosafe' feed for the young piglet. Chlorine dioxide is a strong oxidizing and sanitizing agent with a broad antimicrobial spectrum. It has recently received attention due to its potential advantages over other chlorine-based sanitizers (Berg *et al.*, 1986). It has potential applications in the food industry (Foschino *et al.*, 1968; Beauchat *et al.*, 1998) and is also one of the most effective disinfectants for use in water, as it is active against bacteria and viruses (Junli *et al.*, 1997). Chlorine dioxide is not considered

to represent a health and welfare risk to the young pigs.

Materials and Methods

An experiment was conducted using a two-factor factorial design. Factor 1 was the concentration of ClO_2 (Sanitech; Alltech, Inc., Kentucky, USA) added at time 0 (0, 100, 200, 300, 400, 500 ppm). Factor 2 was the time at which coliforms were enumerated (0, 3, 6, and 24 h). Feed was prepared using a commercial weaner pig diet (2.5 feed:1 water) and was steeped at 30°C for 24 h. Coliforms were enumerated on violet red bile agar (Oxoid, England) using a serial dilution and pour plate technique. Data were log transformed and analysed by GLM-ANOVA and comparison of means was performed by Tukey's post-hoc test. The pigs experiment was according to a randomized incomplete block design with six paired dietary treatments and four replicates. Each pig was offered, *ad libitum*, a choice of two liquid feeds from

four different feed treatments. The four dietary treatments were: freshly prepared liquid feed; freshly prepared liquid feed sanitized with 300 ppm ClO₂; fermented liquid feed; and fermented liquid feed sanitized with 300 ppm ClO₂. Feed intake was measured daily for 14 days. The results were analysed in two ways: (i) feed intake was quantified and analysed by a one-way ANOVA and means were compared by Tukey's post-hoc test; (ii) the data were normalized by calculating percentage preferences and analysed by performing a two-tailed paired-sample *t*-test on each feed choice combination. Statistical analyses were performed using Minitab v.12 (Minitab Inc., USA).

Results

The effect of ClO₂ on coliform populations is shown in Table 81.1. At ClO₂ concentrations of 200 ppm or less, coliform counts (log₁₀ CFU ml⁻¹) increased over a period of 24 h. At 300 ppm ClO₂ and above, no coliforms were detected (< 2.0 log₁₀ CFU ml⁻¹) at any sampling time. The interaction between ClO₂ concentration and length of steeping time was highly significant (*P* < 0.001). Pigs had a significant preference (*P* < 0.01) for non-fermented liquid feed over fermented liquid feed. However, sanitization treatment did not affect the preference or actual feed intake (Fig. 81.1). There was

Table 81.1. Effects of ClO₂ and time on coliform populations (log₁₀ CFU ml⁻¹) in liquid feed.

Time	Chlorine dioxide concentration (PPM)						Time of main effect	SED
	0	100	200	300	400	500		
0	3.2 ^a	3.2 ^{a1}	2.7 ^{a1}	< 2.0	< 2.0	< 2.0	2.8 ¹	0.12
3	4.3	3.3 ¹	2.5 ¹	< 2.0	< 2.0	< 2.0	3.0 ^{1,2}	
6	4.9	3.7 ¹	2.4 ¹	< 2.0	< 2.0	< 2.0	3.3 ²	
24	8.2	6.3	4.0	< 2.0	< 2.0	< 2.0	5.1	
Main effect								
ClO ₂	5.1	4.1	2.9	<2.0	<2.0	<2.0		
SED	0.10			SED of the interaction = 0.11				

^{abc}Within rows, means with the same superscript are not significantly different (*P* > 0.05).
^{1,2,3}Within columns, means with the same superscript are not significantly different (*P* > 0.05).

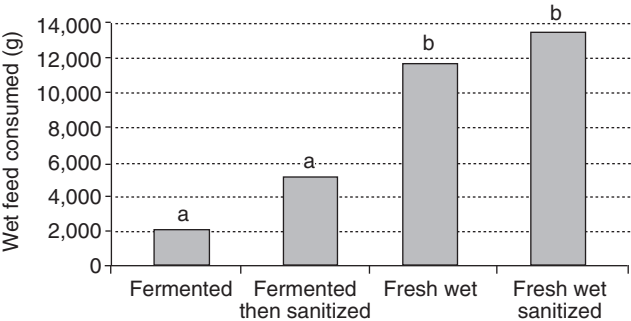


Fig. 81.1. Average feed intake per pig as defined by dietary preparation.
^{a,b} Means with the same superscript are not significantly different (*P* > 0.05) (Tukey's post-hoc test).

no significant difference in total consumption of fermented and non-fermented feed when no choice was given.

Discussion

Animal feed is a recognized source of pathogenic microorganisms for farm livestock. Infectious diarrhoea is one of the most common and economically devastating conditions encountered in the animal agriculture industry. Among the bacterial causes of diarrhoea in animals, *Escherichia coli* and *Salmonella* spp. are the most common and economically important (Holland, 1990). The demonstration that chlorine dioxide is able to inactivate coliform populations in liquid feed creates the possibility of producing 'biosafe' liquid feed for young piglets. A mechanisms by which chlorine

dioxide kills *E. coli* cells was reported by Berg *et al.* (1986), who found that loss of permeability control of the outer bacterial membrane was the primary lethal event at the physiological level. In our experiments, the inclusion of 300 ppm ClO_2 in liquid feed immediately reduced the coliforms to below detectable levels and produced a feed that remained free of enteropathogens for at least 24 h. Importantly, it achieved this without adversely affecting the palatability of the diet or the intake of feed by the piglets. Therefore, from these results, 300 ppm ClO_2 is the recommended level for inclusion in liquid feed preparations.

This study demonstrates that it is possible to produce a 'biosafe' liquid feed that is well accepted by piglets. Such a diet may help to reduce the postweaning growth check and in so doing improve the welfare and health of the newly weaned pig.

References

- Berg, J.D., Roberts, P.V. and Matin, A. (1986) Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. *Journal of Applied Bacteriology* 60, 213–220.
- Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S. (1998) Efficacy of spray application of chlorinated water in killing pathogenic bacteria on row apples, tomatoes, and lettuce. *Journal of Food Protection* 61, 1305–1311.
- Brooks, P.H., Geary, T.M., Morgan, D.T. and Campbell, A. (1966) New development in liquid feeding. *Pig Veterinary Journal* 36, 43–64.
- Foschino, R., Nerveña, I., Motta, A. and Galli, A. (1998) Bactericidal activity of chlorine dioxide against *Escherichia coli* in water and on hard surfaces. *Journal of Food Protection* 61, 668–672.
- Holland, R.E. (1990) Some infectious causes of diarrhoea in young farm animals. *Clinical Microbiology Review* 3, 345–375.
- Junli, H., Li, W., Nenqi, R., L.X., Fun, S.R. and Guanle, Y. (1997) Disinfection effect of chlorine dioxide on viruses, algae and animal planktons in water. *Water Research* 31, 455–460.

82 Influence of Zinc Oxide on Faecal Coliforms of Piglets at Weaning

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The effects of postweaning supplementation with 2500 ppm zinc oxide (ZnO) on the performance and enteric microflora of 19 healthy conventional piglets were studied. No difference between the groups in the number of coliforms, enterococci or *Clostridium perfringens* excreted g⁻¹ faeces was recorded. In contrast, the decrease in diversity of the faecal coliform flora postweaning was less pronounced among the ZnO-treated pigs. Further, in the second week postweaning, ZnO-treated pigs gained 1.6 times more weight than the controls ($P < 0.001$).

Introduction

Postweaning diarrhoea (PWD) is a major cause of losses in pig production and is associated with a spectrum of concurrent environmental, infectious and physiological factors (Spencer *et al.*, 1989). Feed supplementation with 2500 ppm ZnO has been proved to reduce PWD, but the mechanism of action is unclear (Holm, 1998). The aim of this study was to investigate the influence of such a feed on weaned piglets as monitored by clinical observations including daily weight gain (DWG), by quantification of target bacteria, and by determination of diversity of the rectal coliform flora.

Material and Methods

Nineteen conventional piglets from two litters were examined from partus until the age of 63 days. The litters were mixed at weaning (day 35). The controls ($n = 9$) were given a conventional dry feed containing 155 ppm zinc; the experimental animals (n

= 10) were given a dry feed of the same brand supplemented with 2500 ppm ZnO. The quantification of target bacteria was performed after consecutive tenfold steps of dilution followed by incubation on selective media (Melin *et al.*, 1997). Determination of diversity according to Hunter and Gaston (1988) was made using an automated system for biochemical fingerprinting (PhenePlate®, Biosys, Stockholm, Sweden) (Möllby *et al.*, 1993).

Results

All piglets remained healthy and neither rotavirus nor *Brachyspira* spp. were found. Further, there were no differences between the groups in the number of coliforms, enterococci or *Clostridium perfringens* excreted g⁻¹ faeces. The content of *E. coli* g⁻¹ faeces declined from a level of 10⁹ at 1 week post weaning to 10⁶ at weaning and 10⁵ on day 63. No difference between the groups was observed (Fig. 82.1).

In contrast, the decrease in diversity of the faecal flora postweaning was more pro-

nounced and remained longer among the control animals compared with the ZnO-treated pigs (Fig. 82.2). In the second week postweaning, the ZnO-treated pigs gained 1.6 times more weight than the controls ($P < 0.001$). No difference in daily weight gain was recorded at any other time.

Discussion and Conclusion

The gut is a dynamic ecosystem of major complexity, and gastrointestinal health can be defined as the ability to maintain a balance within this system. Further, there is believed to be a positive correlation between environmental stability, high diversity and high community stability. Therefore, a flora with a high diversity is considered to reflect a stable microbial community with a higher colonization resistance. Weaning introduces a number of factors that could be looked upon as environmental instability, resulting in a decreased diversity of the enteric microflora. The reduced decrease in the faecal coliform diversity among ZnO-treated piglets postweaning indicates a positive effect on the stability of the

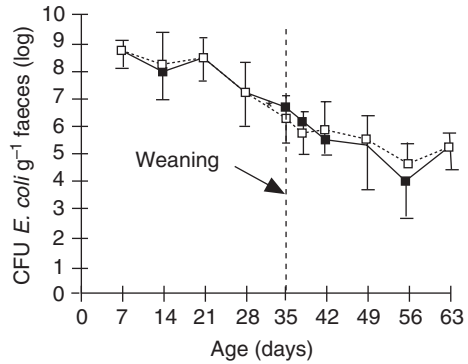


Fig. 82.1. Number of *E. coli* g⁻¹ faeces in ten piglets treated with ZnO (filled symbols) and nine control animals (open symbols).

enteric flora by supplementation with 2500 ppm ZnO from weaning. However, this effect was limited to the first 2 weeks postweaning. Further, signs of intoxication have been seen after oral exposure to ZnO in high concentrations (Jensen-Waern *et al.*, 1998). Moreover, environmental aspects of spreading faeces with a high content of ZnO must be considered (Witter, 1992). Therefore, feed supplementation with ZnO at weaning should not exceed 2 weeks.

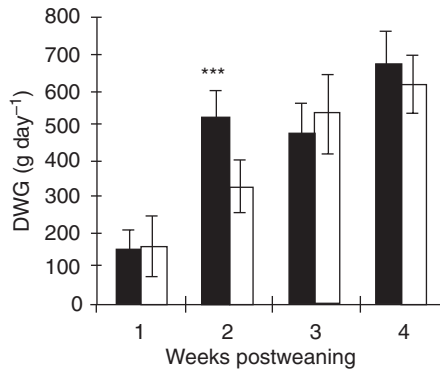
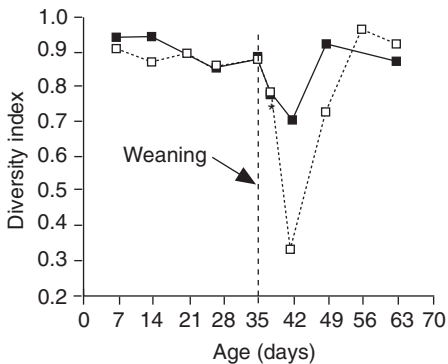


Fig. 82.2. Comparison of diversity and daily weight gain (DWG) between ten piglets treated with ZnO (filled symbols) and nine control animals (open symbols).

References

- Holm, A. (1998) *Escherichia coli*-associated post-weaning diarrhoea in piglets. Zinc oxide added to feed as an antibacterial agent? *Dansk Veterinaertidsskrift* 71, 1118–1126.

- Hunter, P.R. and Gaston, M.A. (1988) Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology* 26, 2465–2466.
- Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L. and Wallgren, P. (1998) Dietary zinc oxide in weaned pigs – effects on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. *Research in Veterinary Science* 64, 225–231.
- Melin, L., Jensen-Waern, M., Johannisson, A., Ederoth, M., Katouli, M. and Wallgren, P. (1997) Development of selected faecal microfloras and of phagocytic and killing capacity of neutrophils in young pigs. *Veterinary Microbiology* 54, 287–300.
- Möllerby, R., Kühn, I. and Katouli, M. (1993) Computerised biochemical fingerprinting – a new tool for typing of bacteria. *Reviews in Medical Microbiology* 4, 231–241.
- Spencer, B.T., Howell, P.G., Hillman, K., Murdoch, T.A., Spencer, R.J. and Stewart, C.S. (1989) Some husbandry factors influencing weaning stresses in piglets. *Journal of South African Veterinary Association* 60, 62–64.
- Witter, E. (1992) Heavy metal concentrations in agricultural soils critical to microorganisms. *Swedish Environmental Protection Agency*, Report 4079.

83 Comparison between the Ileocaecal and Rectal Microflora in Pigs

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When pigs were offered a standard feed, the diversity of the coliform flora at the ileocaecal ostium was similar to that of the rectum. Surgical implantation of a T-cannula in the caecum followed by intramuscular treatment with tetracycline did not alter the diversity of the coliform flora at any of these sites. However, changes in the diet influenced the diversity of the coliform flora. Close to a switch in diet, differences in diversity were observed between the two sampling sites.

Introduction

In many situations the rectum would be the preferred site for sample collection in order to assess the effect of nutrition and environment on gut microflora. This would reduce the need to sacrifice and to modify pigs surgically in order to collect the samples wanted. Further, it would allow the

use of larger groups of pigs, and to study pigs kept under farm-like conditions.

It has been shown that marked changes occur in the gut environment (i.e. pH, organic acids) and microbial activity along the gastrointestinal (GI) tract of pigs (Bach Knudsen *et al.*, 1991, 1993). Differences in diet composition can impose further changes, and may thus have implications

for the diversity of the gut microflora at different sites along the GI tract.

The aim with the present study was to compare the site of sampling of enteric coliform flora in pigs after treatment with antibiotics and when fed different diets.

Material and Methods

Animals, housing and feeding

A total of seven Swedish Yorkshire barrows were surgically fitted with a T-cannula (PVTC) in the caecum at 30 kg live weight (LW). After surgery the pigs received one intramuscular injection with a long acting oxytetracycline (20 mg kg⁻¹ LW; Tetramycin vet. Prolongatum, Pfizer, New York, USA). They were given a convalescence period of 3 weeks (21 ± 1 days) before the feeding trial was initiated.

The pigs were fed twice daily at 4% of the mean LW in the group until they reached 70 kg LW. Thereafter they were offered 2.8 kg feed per day. The pigs were kept in separate pens in rearing facilities with 12 h light per day. From the beginning of the feeding trial all straw was removed.

Experimental design and diets

The effect of oxytetracycline treatment on enteric coliform flora was followed continuously, from surgery until the start of the feeding trial, by sampling the group of seven pigs subjected to PVTC cannulation. The pigs were fed a standard commercial pig grower diet during this period.

The effect of diet composition on gut environment and enteric coliform flora was studied in a 5 × 5 unbalanced Latin square design, using five of the PVTC-cannulated pigs. Each experimental period lasted for 17 days. The cereal-based diets were: Standard (SF; 150 g non-starch polysaccharides (NSP) kg⁻¹ dry matter (DM)); NSP low (NSP_L; 100 g NSP kg⁻¹ DM, 13% soluble NSP); NSP low with high proportion soluble (+S) NSP (NSP_{L+S}; 27% soluble NSP); NSP high (NSP_H; 200 g NSP kg⁻¹

DM, 13% soluble NSP); and NSP high +S (NSP_{H+S}; 27% soluble NSP). The diets were iso-nitrogenous (130 g crude protein kg⁻¹ DM) and contained 14.3 MJ ME (diets SF, NSP_H and NSP_{H+S}) and 15.3 MJ ME (NSP_L and NSP_{L+S}).

Sample collection and culturing

Ileocaecal and rectal samples for culturing were collected at surgery and during the convalescence period before the feeding trial (i.e. at 3, 7, 14 and 20 days post surgery). During the feeding trial, ileocaecal and rectal samples were collected on days 9 and 17 in each period. Ileocaecal samples were also collected on days 15 and 17 to study the gut environment.

Microbiological samples were cultured on blood agar plates. After culturing, the samples were frozen until analysed. Analyses were performed using the metabolic fingerprinting technique, the Phene Plate (PhP) system (Ph Plate, Stockholm AB, Sweden).

Results

Oxytetracycline treatment

There was no alteration in the diversity of the enteric coliform flora post surgery, in ileocaecal or faecal samples (Fig. 83.1a). Also, the diversity was similar at both sites of sampling.

Diet composition

The ileocaecal coliform diversity was decreased 9 days after introducing feed with high amounts of NSP, but was restored at day 17 (Fig. 83.1b). For diets with low NSP, the ileocaecal coliform diversity gradually decreased from day 0 to day 17. The faecal coliform diversity was less clear and could not be related to the dietary content of NSP. The diversity was different at the two sites of sampling. The ileocaecal pH was lower ($P < 0.05$) in the

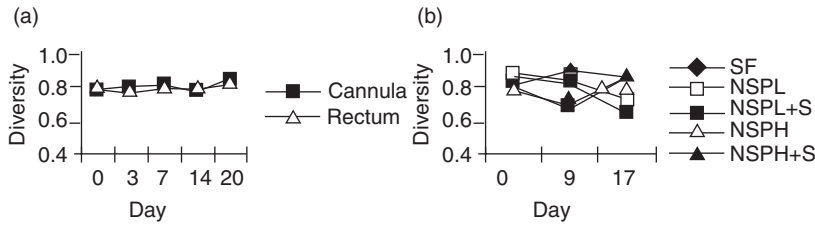


Fig. 83.1. Median values of the microbial diversity (a) in ileocaecal and rectal samples collected post surgery, and (b) in ileocaecal samples from diets with differing NSP content.

diets with high NSP than in the other diets (Table 83.1). Diet NSP_H showed the lowest pH and the highest values ($P < 0.05$) for acetic, propionic and total organic acids. Lactic acid varied between diets and could not be related to the dietary NSP content.

Discussion

The diversity of enteric coliform flora in pigs offered a standard diet was not changed after gut surgery and treatment with oxytetracycline. Further, the diversity of coliform flora in the rectum corresponded well with that at the ileocaecal ostium, indicating that rectal flora mirrors the flora of the intestine well. In contrast, when the dietary content of NSP altered, the diversity of enteric coliform flora in the rectum did not correspond to the flora at the ileocaecal site of sampling. This suggests limitations in the general use of rectal

flora to describe the flora in the intestine close to modifications in dietary composition.

A high microbial diversity implies a high number of different strains, which could be beneficial for the animal when challenged with various pathogens. The present data indicates that diets with a medium to high content of NSP may result in a higher microbial diversity in the small intestine. Consequently, feed composition appears to have a potential as an important tool in the prevention of enteric disorders. As shown by Bach Knudsen *et al.* (1991), the supply of fermentable carbohydrates with the diet will stimulate gut microbial activity and will result in the production of organic acids. This is supported by the present study, but the profile of individual acids in the ileocaecal samples indicates that the dominating strains may differ due to diet carbohydrate composition.

Table 83.1. Ileocaecal values on pH, individual (HLA, lactic; HPr, propionic) and total (OA_{total}) organic acids (mmol l⁻¹).

	Diet					SEM	Significance
	SF	NSP _L	NSP _{L+S}	NSP _H	NSP _{H+S}		
pH	7.47 ^a	7.58 ^a	7.50 ^a	7.05 ^c	7.26 ^b	0.07	***
HLA	36.8 ^a	12.6 ^c	20.7 ^b	38.1 ^a	26.8 ^{ab}	4.4	**
HPr	4.0 ^a	3.7 ^a	4.4 ^a	11.1 ^c	7.1 ^b	0.5	***
OA _{total}	52.0 ^b	32.9 ^a	42.6 ^{ab}	76.7 ^c	53.0 ^b	4.5	***

*** Significant at $P < 0.001$; ** significant at $P < 0.01$.

References

- Bach Knudsen, K.E., Jensen, B.B., Andersen, J.O. and Hansen, I. (1991) Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *British Journal of Nutrition* 65, 233–248.
- Bach Knudsen, K.E., Jensen, B.B. and Hansen, I. (1993) Oat bran but not a β -glucan-enriched oat fraction enhances butyrate production in the large intestine of pigs. *Journal of Nutrition* 123, 1235–1247.
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84 Inulin Incorporation in the Weaned Pig Diet: Intestinal Coliform Interaction and Effect on Specific Systemic Immunity

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In the wider framework of the search for alternatives for the preventive use of antibiotics, the present experiments have shown that inulin could interfere *in vitro* with the adhesion of a pathogenic coliform to the intestinal porcine mucosa. Moreover, a first immunization experiment pointed towards a systemic specific immunomodulation (increase in IgA and IgM titres) by inulin after a boost with intramuscularly injected bovine thyroglobulin (BT).

Introduction

Weaning is a very delicate period in pig production. It often leads to digestive disorders that the preventive use of antibiotics in the diet is able to control. However, increasing concerns about the emergence of antibiotic-resistant strains of bacteria, the transfer of drug resistance between bacterial strains and the presence of possible

drug residues in meat could soon lead to total prohibition at European level. Consequently, alternative approaches for the control of enteric diseases are desirable.

The general aim of this study is to assess the possible use of inulin as an effective, profitable and lasting alternative for preventive use of some antibiotics in pig diets. From studies on humans and rats, inulin, a

natural polymer of fructose extracted from chicory, can be considered as a prebiotic (Gibson and Roberfroid, 1995; Roberfroid *et al.*, 1998).

Our previous experiments have established that inulin digestion in weaned piglets would mainly be of microbial origin and thus located in the hindgut, where it modifies the profile of fermentation metabolites towards an increase in intestinal *n*-valerate and propionate pools at the expense of acetate pools and also towards a decrease in intestinal ammonia pools (Rossi *et al.*, 1997).

The objectives of the experiments described in this chapter were twofold: (i) to assess the effect in the small intestine of inulin itself on an enteropathogenic coliform; and (ii) to study the presumed interaction between inulin and the immune response. In the latter respect, the effect of inulin ingestion on the systemic humoral specific response against intramuscularly injected bovine thyroglobulin (BT) was measured.

Material and Methods

Adhesion assay

An intestinal segment 15 cm long was excised from the mid-jejunum of piglets at the moment of slaughter. The segment was opened and washed in Krebs–Henseleit buffer (120 mM NaCl, 14 mM KCl, 25 mM NaHCO₃, 1 mM KH₂PO₄; pH 7.4) containing 1% (vol vol⁻¹) formaldehyde at 4°C; the villi were gently scraped from the mucosa with a glass slide and suspended in the same buffer. The villi were washed four times in Krebs–Henseleit without formaldehyde and suspended in phosphate buffered saline (PBS) supplemented with 1% (wt vol⁻¹) (D) mannose to prevent adhesion of *Escherichia coli* type-1 pili (F1). Subsequently 4×10^8 F4ac⁺ *E. coli* were added to an average of 50 villi in a volume of 1 ml and incubated for 1 h while being gently shaken. The adhesion of bacteria along a 50 µm villus brush border was examined at 20 different sites by phase-

contrast microscopy (magnification $\times 600$), after which the mean bacterial adhesion per 250 µm villus brush-border length was calculated.

To test the potential of inulin to inhibit adhesion, this sugar was added to the incubation solution at a final concentration of 5%, 1% or 0.1% and results were compared with those of the above-mentioned control. A further assay was performed by preincubating inulin with the coliforms or the villi and gently shaking the suspension at room temperature for 60 min. Subsequently, the preincubated coliforms or villi were washed in PBS to eliminate the non-adhering inulin; then native villi or coliforms, respectively, were added, incubated and counted as above.

Study of specific systemic immunomodulation

Two groups of ten BT-seronegative piglets (seven vaccinated and three placebos) were allocated on the basis of the sex, weight and littermate: one group received a diet containing 5% inulin (Fibruline®, Warcoing Industrie, Belgium) and the other group was used as control. The two diets containing soybean meal, maize, synthetic amino acids, soybean oil, minerals and vitamins were well balanced and isonutrient. Immediately after weaning (28 days of age), pigs were allowed to adapt to these diets for 3 weeks. All pigs, except the placebos, were then immunized by intramuscular injection of BT (1 mg in 1 ml PBS/incomplete Freund's adjuvant (50/50)) and a booster was given 100 days later. Serum was sampled regularly from the jugular vein and the antibody response was followed by measuring the BT-specific IgA and IgM via indirect ELISA.

Results and Discussion

Adhesion assay

Results of the *in vitro* adhesion assay with inulin directly added suggested that

inulin could be effective in partially inhibiting adhesion of the F4ac⁺ coliform to villus brush border ($52 \pm 17\%$ inhibition by 5% inulin). Moreover the preincubation assay showed that this interference would be due rather to the inulin interaction with the brush border of intestinal villi than with the F4⁺ *E. coli* (at 5% inulin: inhibition of 66 ± 16 and $25 \pm 20\%$, respectively). Because of a huge intra- and interanimal variability, those results have to be extended to more pigs and the suspected interaction between inulin and the brush border has to be further analysed.

Effect on specific systemic immune response

No differences appeared for the primary response but there was an increase (although non-significant) in the mean log₂ titre of the specific IgA and IgM antibodies at day 21 after a boost in the inulin group (30.3% and 15.0%, respectively, from the

value at day 0) as compared with the control group (Fig. 84.1).

Conclusion

Results suggest that inulin can block adhesion of F4⁺ *E. coli* to the small intestinal villi. The effect on a humoral immune response following parenteral immunization is less clear. However, the results need to be confirmed and deepened. In this respect, a challenge study using F4⁺ *E. coli* has to be performed in pigs and will have to combine several integrated aspects – health, digestive physiology and immunity – to understand the effect of inulin.

Acknowledgement

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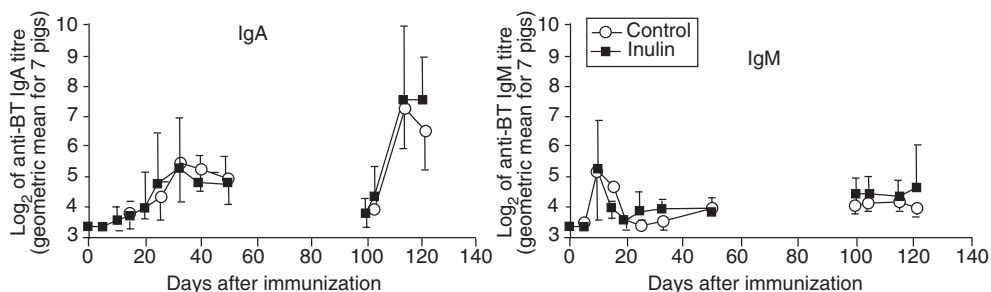


Fig. 84.1. Anti-BT IgA and IgM titres expressed as the geometric mean log₂ titre for seven pigs per alimentary treatment ($\pm$ SD).

References

- Gibson, G.R. and Roberfroid, M. (1995) Dietary modulation of the human colonic microbiota – introducing the concept of prebiotics. *Journal of Nutrition* 125, 1401–1412.
- Roberfroid, M., Van Loo, J. and Gibson, G.R. (1998) The bifidogenic nature of chicory inulin and its hydrolysis products. *Journal of Nutrition* 128(1), 11–19.
- Rossi, F., Ewodo, C., Fockedey, R., Thielemans, M.F. and Théwis, A. (1997) Digestibility and fermentation of inulin in the weaned piglet digestive tract. In: Hartemink, R. (compiler) *Proceedings of the International Symposium Non-digestible Oligosaccharides: Healthy Food for the Colon?* Wageningen, The Netherlands (4–5 December 1997), p. 143.

85 *In vitro* Methodology to Evaluate the Effect of Various Organic Acids on the Microflora in the Gastrointestinal Tract of Pigs

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An *in vitro* system was developed to study the effect of various feed additives on the microbiota in the gastrointestinal tract of pigs. The system was adjustable with regard to pH and temperature and an anoxic atmosphere was created. Bacteriological measurements, including bacterial colony count and quantification of fermentation products, were conducted in response to pH and various types and doses of organic acids. A pH-dependent microbial growth and fermentation pattern was observed. In addition, both type and dose level of the organic acids employed affected the magnitude of response.

Introduction

It is well established that the activity and metabolic end products of the gastrointestinal microbiota have a great influence on animal health and growth performance. Diet supplementation with organic acids or their salts has been shown to reduce the frequency of postweaning diarrhoea and to improve growth performance in piglets (Sutton *et al.*, 1991). However, little is known about their mode of action. In order to study the effects of various organic acids on the microbiota in the gastrointestinal tract of pigs, an *in vitro* system was established and standardized with respect to culture medium, pH level and relevant indicators of the microbial ecology in the gastrointestinal tract of pigs. Subsequently, the applicability of the *in vitro* system was tested, in terms of typical screenings such as dose-response and comparative experiments.

Materials and Methods

A batch culture system was used and involved several fermentors, each with a working volume of 800 ml. The culture medium in use was pooled digesta obtained from either the stomach or the proximal part of the small intestine of slaughter pigs. The digesta was diluted (1:1) in a sterile anaerobic salt medium and inoculated with a suspension of fresh faeces (1% final concentration) collected from piglets. The slurry was stirred magnetically and kept under anoxic conditions by sparging with high-purity nitrogen gas. The culture pH was automatically maintained at the desired value, using a pH controller, regulated by 5 M NaOH and 5 M HCl. The incubation temperature was maintained at 38°C by a circulating water bath. During the incubation period, samples were aseptically removed from the fermentors and taken for bacterial enumeration and analy-

sis of short-chain fatty acids (SCFA) and lactic acid.

The effect of potassium diformate (KDF) on the microbiota in the stomach and proximal part of the small intestine (SI) was investigated at pH 3, 4 and 5 and pH 5, 6 and 7, respectively. Moreover, a dose-response experiment was performed to study the microbial effects of various doses of KDF in stomach content (pH 4.5) and in SI content (pH 5.5). Finally, the microbial effects of six types of organic acids were compared, on equimolar basis, in stomach (pH 4.5) and SI (pH 5.5) content.

Results and Discussion

The density of coliforms and lactic acid bacteria in stomach content was significantly reduced ($P < 0.001$) in response to a decreased pH and addition of KDF (Fig. 85.1).

Growth of lactic acid bacteria was observed during the incubation period at pH 4 and 5. Coliforms were able to grow at pH 5, at least in the beginning of the incubation period, whereas they were killed at pH 3 and 4. Regardless of pH, KDF reduced the bacterial density at all sampling times; in particular, the count of coliforms was reduced. The highest concentrations and largest alterations in the fermentation pattern of SCFA and lactic acid occurred at pH 4 and 5 (data not shown). These results suggested that different bacterial populations existed and that bacteria able to grow at pH 5 further metabolized lactic acid to SCFA. Due to the very low bacterial presence, only low concentrations of fermentation products were found at pH 3. In accordance with the experiments involving stomach content, KDF exerted a growth-depressing effect ($P < 0.001$) on coliforms and lactic acid bacteria in SI content (pH 5, 6, 7). Moreover, the coliforms in SI content were significantly affected by pH ($P < 0.001$). In contrast, no effect of pH was demonstrated on the growth of lactic acid bacteria. Likewise, the bacterial metabolites were unaffected by pH level (data not shown).

The time required for the bacterial count to double (generation time) or halve (bacterial half-life) in response to various doses of KDF added to stomach content is given in Table 85.1.

It appears that the bacterial half-life of coliforms was reduced in a dose-dependent manner. The generation time of lactic acid bacteria was directly proportional to dose-level. Similarly, KDF exerted a dose-related anti-microbial action on coliforms in SI content at pH 5.5, whereas no apparent effect of dose was detected on the density of lactic acid bacteria (data not shown).

The efficiency of various acids in killing coliforms in stomach content was according to a diminishing scale: benzoic > fumaric > lactic > butyric > formic > propionic acid (data not shown). Benzoic and fumaric acid were the only acids influenc-

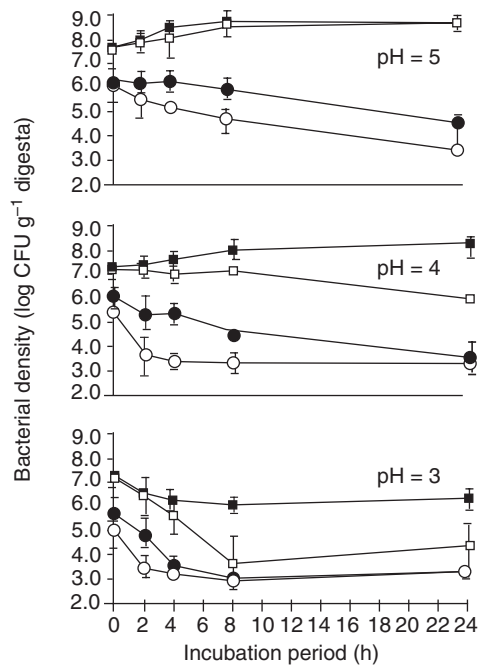


Fig. 85.1. Effect of KDF on the growth of coliforms (●) and lactic acid bacteria (■) at pH 3, 4 and 5. KDF was present in a concentration of 0.6%. Each symbol plotted with the standard deviation represents the mean value of three replicates (solid symbols: control; open symbols: KDF).

Table 85.1. Death and growth kinetics of coliform and lactic acid bacteria at various doses of KDF in stomach content (pH 4.5).

	Dose of KDF (% wt/vol.)					
	0	0.1	0.2	0.4	0.6	1.0
Bacterial half-life, min (coliforms)	150	120	90	66	60	54
Generation time, min (lactic acid bacteria)	102	138	144	162	210	930

ing the lactic acid bacteria. Accordingly, benzoic acid proved to be the most potential acid in reducing bacterial counts in SI content. Formic acid was the only acid killing coliforms without affecting lactic acid bacteria at pH 5.5. The remaining acids tested in SI content provoked no evident response in the bacterial count.

Conclusion

In conclusion, the *in vitro* system offers a reliable method to describe alterations in the microbiota in response to different environmental conditions and it provides a valid basis for the selection of product to be tested *in vivo*.

Reference

Sutton, A.L., Mathew, A.G., Scheidt, A.B., Patterson, J.A. and Kelly, D.T. (1991) Effects of carbohydrate sources and organic acids on intestinal microflora and performance of the weanling pig. In: Versteegen, M.W.A., Huisman, J. and den Hartog, L.A. (eds) *Proceedings of the 5th International Symposium on Digestive Physiology in Pigs*. Wageningen, The Netherlands, pp. 442–427.

86 Postprandial Flow Rates of Formic Acid and Potassium in Duodenal Digesta of Weaned Piglets Fed Graded Doses of Potassium Diformate

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Six duodenally cannulated gilts of 8.3 kg initial body weight were used to study kinetics and acidifying effects of supplemental potassium diformate (KDF) in graded doses (0.0, 0.9 and 1.8%) at 5, 35, 65, 95, 125, 185 and 245 min postprandially. This salt clearly acidified duodenal digesta until 65 min (by 0.3 to 0.5 pH units; $P < 0.01$ or 0.05), but afterwards the acidity was similar among the treatments. Addition of KDF had a positive linear effect ($P < 0.001$) on the concentration of formic acid (FA) in the duodenal digesta. The quantity of FA flowing into the duodenum was from 79 to 93% of formate dosed orally. Also, there was a clear positive interrelationship between the dose of KDF and the intraduodenal flow rate of potassium.

Introduction

For consumers' safety, numerous countries ban antibiotic growth promoters in diets for pigs. Among potential alternatives, potassium diformate (KDF) as a specifically produced salt of formic acid ($\text{HCOOH} \cdot \text{HCOOK}$) seems to be of particular interest. In contrast to formic acid, KDF is non-odorous and non-corrosive, which is of great importance for feed manufacturers and farmers. The growth-promoting effects of this salt have been widely documented (Paulicks *et al.*, 1996; Kirchgessner *et al.*, 1997; Øverland *et al.*, 2000). However, less is known about physiological processes and mechanisms modulating growth of pigs. Successful application of KDF in pig diets requires a better understanding of its *in vivo* mode of action. Therefore, the purpose of this experiment was to evaluate the

effect of supplemental KDF in graded doses (0.0, 0.9 and 1.8%) on postprandial changes in intraduodenal acidity and in flow rates of its composite elements, i.e. formic acid and potassium.

Materials and Methods

An experiment was carried out using six Yorkshire $\times$ (Yorkshire $\times$ Danish Landrace) crossbred sib gilts according to a double 3×3 Latin square design. They were weaned at 21 days of age and housed individually in metabolic pens (1.15×1.35 m). Two weeks later, a simple T-duodenal cannula was fitted 30 cm posterior to the pylorus of each piglet. After a 10 day recovery, the piglets were randomly grouped into three pairs and each pair was allotted to three treatments according to a

3 × 3 Latin square design, as follows: basal diet (BD); BD + 0.9% KDF; or BD + 1.8% KDF. BD was formulated with barley (49%), wheat (30%), extracted soybean meal (12%) and meat meal (5%) as major ingredients. No in-feed antibiotics were implemented. The contents of supplementary Zn and Cu were 50 and 30 ppm, respectively. These treatments were isoen-
ergetic (13.1 MJ ME or 9.3 MJ NE_f kg⁻¹) and isoproteinous (17.5% crude protein kg⁻¹ diet). Co-EDTA (2 g kg⁻¹ feed) was used as a liquid phase marker. The feeding level was adjusted weekly to 2.5 × maintenance requirement for ME (= 1 MJ ME kg⁻¹ BW^{0.75}). Daily rations were given twice (0700 and 1600 h) in a liquid form (3:1 water:feed). Each experimental period lasted 7 days: after 5-day adaptation to the respective diets, days 6 and 7 were assigned for the collection of duodenal digesta in seven postprandial intervals: 0–15, 30–45, 60–75, 90–105, 120–135, 180–195 and 240–255 min. In addition to acidity, concentrations of formic acid (FA), potassium (K) and cobalt (Co) both in diets and digesta were determined in order to calculate the postprandial presence and flow of FA and K in the duodenum. The orthogonal data on the postprandial changes of nutrient concentration in the duodenal digesta at any given time of sampling were subjected to ANOVA for Latin square designs, and the significance of means was tested using Student's *t*-test.

Results and Discussion

In general, no health problems or feed refusals were noted among the piglets throughout this experiment. Their growth rate (284 g day⁻¹) at the restricted feeding level for ME can be regarded as satisfactory. Analysed contents of (FA) in experimental treatments 1, 2 and 3 were 0.13, 5.86 and 11.61 g kg⁻¹, respectively. These respective treatments contained 7.1, 9.48 and 12.01 g K kg⁻¹. Postprandial changes of (FA) and pH in fresh (wet) duodenal digesta are presented in Table 86.1.

Postprandial changes in (FA) showed that the piglets receiving 0.9% KDF had in the duodenal digesta 79–88% of the quantity of consumed FA. By feeding 1.8% of this salt, the quantity of FA entering the duodenal digesta ranged from 81 to 93% of its dietary intake. This finding implies that, during the gastric transit of KDF, the rate of its dissociation is relatively low. It is well established that undissociated forms of organic acids are predominantly effective in inhibiting growth of intraluminal pathogens (Kirchgessner *et al.*, 1997). Until 65 min postprandially, duodenal digesta of piglets fed KDF was more acidic (by 0.3–0.5 pH units; *P* < 0.01 or 0.05), but afterwards the acidity was similar among the treatments. Irrespective of the treatment, the contents of K in the duodenal digesta have gradually increased up to threefold of its intake. This implies that

Table 86.1. Effect of adding potassium diformate (KDF) in graded doses (0.0, 0.9 and 1.8%) to a basal diet on the postprandial concentration of formic acid [FA] and acidity (pH) in fresh duodenal digesta.

Time (min)	[FA] (mg kg ⁻¹ fresh digesta)					pH of duodenal digesta				
	KDF (%)			SED	<i>P</i> -value	KDF (%)			SED	<i>P</i> -value
	0.0	0.9	1.8			0.0	0.9	1.8		
5	12	727	1410	69.9	< 0.001	5.49	5.01	4.93	0.199	0.058
35	16	783	1628	82.4	< 0.001	4.90	4.66	4.61	0.070	0.013
65	9	453	920	32.9	< 0.001	4.30	3.98	3.90	0.119	0.032
95	10	292	616	30.6	< 0.001	3.56	3.52	3.48	0.160	0.862
125	9	232	518	27.9	< 0.001	3.58	3.40	3.34	0.159	0.371
185	10	189	328	29.0	< 0.001	3.60	3.26	3.63	0.209	0.229
245	13	129	261	24.6	< 0.001	3.08	3.18	3.53	0.225	0.186

there was a meaningful contribution of endogenous K from pancreatic juice. Dietary intake in the morning meal and postprandial changes in the duodenal flow of FA and K as influenced by KDF doses in the basal diet are presented in Table 86.2.

The flow of both composite elements of KDF was usually proportional to its quantity in the diet. Based on the data illustrating the postprandial flow of Co, we found no effect of KDF on the patterns of gastric emptying.

Conclusions

Piglets had no health problems related to the doses of KDF (0.9 or 1.8%) in a cereal–soybean meal-based diet. With the graded doses of KDF, the postprandial concentrations or flows of FA and K in the duodenal digesta were linearly increasing ($P < 0.001$). The quantity of FA found beyond the stomach ranged from 81 to 93% of dietary intake. KDF lowered the pH of digesta by 0.3–0.5 units up to 65 min after feeding.

Table 86.2. Effect of intake of formic acid (FA) and potassium (K) from potassium diformate (KDF) in the morning meal on the postprandial flow of FA and K in the duodenum of piglets.

	Time (min)	FA (mg)					K(g)				
		KDF (%)				P-value	KDF (%)				P-value
		0.0	0.9	1.8	SED		0.0	0.9	1.8	SED	
Intake	0	33	1467	3001	88.9	< 0.001	1.79	2.37	3.10	0.040	< 0.001
Postprandial flow	5	4	284	530	31.3	< 0.001	0.22	0.40	0.51	0.035	< 0.001
	35	4	223	511	51.7	< 0.001	0.34	0.44	0.53	0.081	0.130
	65	2	113	256	14.3	< 0.001	0.26	0.32	0.41	0.023	0.002
	95	3	73	157	13.4	< 0.001	0.22	0.25	0.30	0.014	0.003
	125	2	57	136	8.7	< 0.001	0.20	0.21	0.28	0.031	0.075
	185	2	44	81	9.3	< 0.001	0.14	0.17	0.19	0.022	0.158
	245	3	31	60	6.9	< 0.001	0.13	0.15	0.16	0.023	0.547

References

- Kirchgessner, M., Paulicks, B.R. and Roth, F.X. (1997) Effects of supplementations of diformate complexes (FormiTM LHS) on growth and carcass performance of piglets and fattening pigs in response to application time. *Agribiological Research* 50, 1–10.
- Øverland, M., Granli, T., Kjos, N.P., Fjetland, O., Steien, S.H. and Stokstad, M. (2000) Effect of dietary formates on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing–finishing pigs. *Journal of Animal Science* 78, 1875–1884.
- Paulicks, B.R., Roth, F.X. and Kirchgessner, M. (1996) Dose effects of potassium diformate (FormiTM LHS) on the performance of growing piglets. *Agribiological Research* 49, 318–326.

87 Effect of Sodium Chlorate on *Salmonella* sv. Typhimurium Concentrations in the Pig Gut

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Salmonella species are of importance to the swine industry, potentially causing economic losses due to disease and compromised food safety. In this chapter, we report that oral administration of sodium chlorate to weaned pigs resulted in decreased caecal concentrations of *Salmonella* serovar Typhimurium but not potentially beneficial anaerobes. Thus, chlorate treatment may be a potential preharvest strategy to reduce gut concentrations of *Salmonella*.

Introduction

Salmonellae remain an important cause of human and animal disease in many regions of the world (Lax *et al.*, 1995). *Salmonella* infections of swine are common, resulting in clinical salmonellosis or asymptomatic infections (Lax *et al.*, 1995). The absence of clinical symptoms by apparently healthy carrier pigs and their propensity to shed *Salmonella* intermittently creates an inconspicuous source for future infections and for possible contamination of pork products during slaughter. Berends *et al.* (1996) reported that up to 30% of finished pigs may shed *Salmonella* and that higher incidences may occur during transport and lairage. Because the gut and affiliated lymph tissue are the primary reservoirs for *Salmonella* in market-ready pigs (Baird-Parker, 1990; Berends *et al.*, 1996), strategies are sought to reduce on-farm *Salmonella* colonization of pigs.

Salmonella, like most members of the family *Enterobacteriaceae*, possess respiratory nitrate reductase activity which also catalyses the intracellular reduction of chlorate, an analogue of nitrate, to cyto-

toxic chlorite (Stewart, 1988). Since most gastrointestinal anaerobes lack respiratory nitrate reductase, we conducted a study to determine whether chlorate may selectively kill *Salmonella* but not potentially beneficial anaerobes within the pig gut.

Materials and Methods

Weaned pigs 26 to 29 days old were orally infected with 8×10^7 colony-forming units (CFU) of a novobiocin- and naladixic acid-resistant strain of *Salmonella* sv. Typhimurium. The pigs were randomly placed into three treatment groups (15 pigs each) and each group was reared in a separate concrete floored pen. Chlorate treatments were administered at 8 h and again at 16 h post *Salmonella* challenge and were administered via oral gavage (10 ml) of 0, 100 or 200 mM sodium chlorate solutions, corresponding to 0 $\times$, 1 $\times$ or 2 $\times$ treatment, respectively. All treatments also contained 2.5 mM sodium nitrate as an inducer of nitrate reductase activity and 20 mM sodium lactate as reductant for chlorate reduction. Pigs were euthanased at 8 h

intervals (five per group) following the last treatment. Ileocolic lymph nodes and caecal contents (1–2 g) collected by necropsy were cultured for *Salmonella* as described earlier (Nisbet *et al.*, 1999). Concentrations of total culturable anaerobes were estimated via a three-tube most probable number method (AOAC, 1980). Data were analysed to test for differences in proportions of *Salmonella*-positive specimens using a chi-square test. Caecal concentrations of *Salmonella* and most probable numbers of total culturable anaerobes were analysed for treatment or time after treatment differences, using an analysis of variance.

Results and Discussion

Chlorate treatment reduced ($P < 0.05$) caecal concentrations of *Salmonella* sv. Typhimurium (Table 87.1) but not most probable numbers of total culturable anaerobes ($P > 0.05$), which ranged from 10.6 to 11.5 $\log_{10}$ cells g^{-1} of contents. These data suggest that chlorate treatment may be a practical preharvest intervention consistent with the proposal of Berends *et al.* (1996) that on-farm *Salmonella* control strategies take advantage of the natural colonization

resistance conferred to the host by their native gut flora. Davies *et al.* (1999) suggested that preharvest *Salmonella* control strategies may have greater impact if applied during the finishing period and it is reasonable to expect chlorate treatment to be effective in reducing concentrations of *Salmonella* in mature as well as in weaned animals. In such a setting, chlorate could easily be administered with the last meal prior to transport to the abattoir or in drinking water during lairage. An added benefit of such a terminal treatment is that cross-contamination often associated with transport and lairage (Baird-Parker, 1990) may be mitigated.

The use of chlorate in a preharvest *Salmonella* control programme will clearly depend on the development of administration protocols that are efficacious yet do not compromise pork quality or safety. At low concentrations, chlorate salts are used in veterinary and human medicine and their use in toothpastes at concentrations of up to 5% has been approved by the European Union (Cosmetic Ingredient Review Panel, 1995). At higher concentrations, however, chlorate is toxic to humans and animals, with LD_{50} values generally exceeding 1 g kg^{-1} body weight (Cosmetic Ingredient Review Panel, 1995). McCauley

Table 87.1. Effect of oral chlorate administration on recovery of *Salmonella* serovar Typhimurium from weaned pigs.

	Hours after last chlorate treatment								
	Qualitative recovery (% culture positive)						Quantitative recovery (mean log ₁₀ CFU g ⁻¹) ^a		
	Ileocolic lymph tissue			Caecal contents					
	8	16	24	8	16	24	8	16	24
ClO ₃ group									
0×	20	80	20	100	100	80	3.5	2.8	1.5
1×	0	20	20	80	60	60	2.4	0.6	1.6
2×	20	20	40	60	60	60	1.3	0.9	1.6
ClO ₃ effect	(P > 0.05)			(P > 0.05)			(P < 0.05)		
Time effect	(P > 0.05)			(P > 0.05)			(P > 0.05)		
Interaction	(P > 0.05)			(P > 0.05)			(P > 0.05)		

^aSEM = 0.66.

et al. (1995) reported moderate toxicity in Sprague-Dawley rats following 90 consecutive days of feeding 48 mM sodium chlorate and no observable adverse effects when feeding 3 mM sodium chlorate similarly. In the present study, each pig received a single dose of approximately 4 or 8 mM chlorate ion, based on 500 ml water consumption, following the collective administration of the 1× or 2× treatments, respectively, and symptoms of chlorate toxicity were not observed.

Although not significant, our data suggest an effect of time after treatment on caecal *Salmonella* concentrations and a possible interaction between chlorate and time after treatment (Table 87.1). Chlorate treatment did not significantly reduce the proportions of pigs yielding *Salmonella*-positive ileocolic lymph or caecal contents (Table 87.1). Clearly, further studies are needed to elucidate optimal concentrations, timing, and duration of treatments as well as other pharmacokinetic properties of chlorate.

References

- AOAC (1980) *Official Methods of Analysis*, 13th edn. Association of Official Analytical Chemists, Arlington, Virginia, 826 pp.
- Baird-Parker, A.C. (1990) Foodborne illness. *The Lancet* 336, 1231–1235.
- Berends, B.R., Urlings, H.A.P., Snijders, J.M.A. and Van Knapen, F. (1996) Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. *International Journal of Food Microbiology* 30, 37–53.
- Cosmetic Ingredient Review Panel (1995) Final report on the safety assessment of potassium chlorate. *Journal of the American College of Toxicology* 14, 221–230.
- Davies, P., Funk, J. and Morrow, W.E.M. (1999) Fecal shedding of *Salmonella* by a cohort of finishing pigs in North Carolina. *Swine Health and Production* 7, 231–234.
- Lax, A.J., Barrow, P.A., Jones, P.W. and Wallis, T.S. (1995) Current perspectives in salmonellosis. *British Veterinary Journal* 151, 351–377.
- McCauley, P.T., Robinson, M., Daniel, F.B. and Olson, G.R. (1995) The effects of subchronic chlorate exposure in Sprague-Dawley rats. *Drug and Chemical Toxicology* 18, 185–199.
- Nisbet, D.J., Anderson, R.C., Harvey, R.B., Genovese, K.J., DeLoach, J.R. and Stanker, L.H. (1999) Competitive exclusion of *Salmonella* serovar Typhimurium from the gut of early weaned pigs. In: Bahnson, P. (ed.) *Proceedings of the 3rd International Symposium on the Epidemiology and Control of Salmonella in Pork*. Biomedical Communications Center, University of Illinois, Urbana-Champaign, Illinois, pp. 80–82.
- Stewart, V. (1988) Nitrate respiration in relation to facultative metabolism in enterobacteria. *Microbiological Reviews* 52, 190–232.

88 Reduced *Campylobacter* Prevalence in Piglets Reared in Specialized Nurseries

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The purpose of the present study was to compare the effects of off-sow rearing to on-sow rearing on the prevalence of *Campylobacter* in neonatal pigs. In two replications, we removed piglets from sows within 24 h of birth, reared them in specialized nurseries for 21 days, cultured daily rectal swabs for *Campylobacter*, and compared *Campylobacter* status of these piglets with that of littermates reared on sows. Off-sow *Campylobacter* prevalence was 0% to 19%, whereas on-sow was 89% to 100%. Based on our results, we conclude that *Campylobacter* prevalence may be diminished in neonates that are reared off-sow in specialized nurseries.

Introduction

In recent years, *Campylobacter* has emerged as one of the most common causes of human enteric disease in developed countries. *Campylobacter* has been isolated from raw beef, pork, lamb, chicken, cooked meats and seafood (Stern *et al.*, 1985; Fricker and Park, 1989; Zanetti *et al.*, 1996). It is generally recognized that the predominant species that colonizes swine is *C. coli*, whereas the primary isolate from poultry is *C. jejuni* (Stern *et al.*, 1985; Weijtens *et al.*, 1993, 1997; Young *et al.*, 2000); however, individual swine farms can have a relatively high prevalence of *C. jejuni* (Harvey *et al.*, 1999). The mode of transmission in pigs is probably from dam to offspring, with colonization occurring at an early age (Weijtens *et al.*, 1993, 1997; Young *et al.*, 2000). The purpose of this study was to compare the effects of off-sow rearing with on-sow rearing on the prevalence of *Campylobacter* in neonatal pigs.

Materials and Methods

In two replications, we removed piglets from sows within 24 h of birth, reared them in specialized nurseries for 21 days, cultured daily rectal swabs for *Campylobacter*, and compared *Campylobacter* status of these piglets with that of littermates reared on sows. Sows were cultured for *Campylobacter* at the beginning and end of the 21 day period. There were 23 piglets (14 off-sow and 9 on-sow) in the first replicate and 44 piglets (29 off-sow and 15 on-sow) in the second. The nurseries consisted of wire-floored farrowing crates that were equipped with heat lamps, heating pads and self-feeders. A commercial milk replacer was fed three times daily. A maximum of five piglets were housed per pen. Rectal swabs were enriched in 10 ml Bolton's broth and incubated for 4 h at 37°C and 20 h at 42°C; 10 µl of broth were streaked on Campy-Cephex agar plates and incubated at 42°C

under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂) for 48 h. Colonies demonstrating typical *Campylobacter* morphology were then verified by use of a commercial latex agglutination test kit (INDX-Campy, Integrated Diagnostics, Baltimore, Maryland, USA). Data analyses were performed utilizing chi-square and Fisher's exact test ($P = 0.05$) (Cody and Smith, 1997).

Results and Discussion

In Replicate I, the *Campylobacter* prevalence of nursery-reared piglets was 13 of 14 (93%) on day 2 and 0 of 14 (0%) on day 20 (Table 88.1). *Campylobacter* prevalence of the sow-reared piglets was 8 of 9 (89%) from days 2 to 20. In Replicate II, 12 of 29 (41%) on day 2, and 5 of 26 (19%) on day 20, of the nursery-reared piglets were culture-positive for *Campylobacter*. Of the sow-reared piglets, *Campylobacter* status was 7 of 15 (47%) on day 1 and 15 of 15 (100%) on day 20. In both replications, nursery pens would often contain both *Campylobacter*-positive and *Campylobacter*-negative piglets, yet negative piglets did not convert to a positive status. We noted that positive piglets that converted had reduced colony-forming units g⁻¹ of faeces for 24–48 h prior to becoming negative. Sows were culture-positive for *Campylobacter* at the beginning and remained so throughout the study.

Concerns are increasing that *Campylobacter* spp. in poultry and other meats may be of importance to public

health (Stern *et al.*, 1985). In a recent study, we found that market-age pigs at slaughter had a *Campylobacter* prevalence of 92% (Harvey *et al.*, 1999). We conducted the present study to determine whether we could reduce the prevalence of *Campylobacter* in piglets. It is apparent from this study that piglets have *Campylobacter* present in their gastrointestinal tract as early as 24 h of age, and if left with the sow they will continue to be positive for the organism. It is felt that *C. coli* and *C. jejuni* are commensal organisms in pigs and the route of transmission is from dam to offspring (Weijtens *et al.*, 1997; Young *et al.*, 2000). In the present study, cohabitation of piglets with sows increased the prevalence of *Campylobacter* when compared with that of piglets reared off-sow. The prevalence rates from on-sow rearing in our study are similar to those observed by Young *et al.* (2000), in which piglets were reared off-sow but on the floor. Although not conclusive, the results of our study and those of others (Weijtens *et al.*, 1993, 1997; Young *et al.*, 2000) would suggest that successful permanent colonization of the gastrointestinal tract may be dependent upon continuous and/or repeated exposure to the faeces of *Campylobacter*-positive pigs.

With the increased number of cases of campylobacteriosis reported in the United States each year, public health agencies are concerned about food safety issues attributable to the prevalence of *Campylobacter* spp. in food-producing animals. Eventually, *Campylobacter* may come under regulatory control and methods to decrease

Table 88.1. Prevalence of *Campylobacter* in piglets from 1 to 20 days of age.

	1 day	2 days	20 days
Replicate I			
Nursery-reared	7/14 ^x (50%)	13/14 ^{bx} (93%)	0/14 ^{cx} (0%)
Sow-reared	6/9 ^x (67%)	8/9 ^{bx} (89%)	8/9 ^{by} (89%)
Replicate II			
Nursery-reared	8/29 ^x (28%)	12/29 ^b (41%)	5/26 ^{bx} (19%)
Sow-reared	7/15 ^{bx} (47%)	Not sampled	15/15 ^{cy} (100%)

^{bc}Values within the same row with different superscripts are significantly different.

^{xy}Values within the same column of the same replicate with different superscripts are significantly different.

the prevalence will be sought. We believe that our finding may have application for the production of pigs that have a reduced prevalence of *Campylobacter*, thus enhancing the safety of the pork food chain.

References

- Cody, R.P. and Smith, J.K. (1997) *Applied Statistics and the SAS Programming Language*, 4th edn. Prentice Hall, Upper Saddle River, New Jersey, 445 pp.
- Fricker, C.R. and Park, R.W.A. (1989) A two-year study of the distribution of 'thermophilic' campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *Journal of Applied Bacteriology* 66, 477–490.
- Harvey, R.B., Young, C.R., Ziprin, R.L., Hume, M.E., Genovese, K.J., Anderson, R.C., Droleskey, R.E., Stanker, L.H. and Nisbet, D.J. (1999) Prevalence of *Campylobacter* spp. isolated from the intestinal tracts of pigs raised in an integrated swine production system. *Journal of the American Veterinary Medical Association* 215, 1601–1604.
- Stern, N.J., Hernandez, M.P., Blankenship, L., Diebel, K.E., Doores, S., Doyle, M.P., Ng, H., Pierson, M.D., Sofos, N.J., Sveum, W.H. and Westhoff, D.C. (1985) Prevalence and distribution of *Campylobacter jejuni* and *Campylobacter coli* in retail meats. *Journal of Food Protection* 48, 595–599.
- Weijtens, M.J.B.M., Bijker, P.G.H., van der Plas, J., Urlings, H.A.P. and Biesheuvel, M.H. (1993) Prevalence of *Campylobacter* in pigs during fattening; an epidemiological study. *Veterinary Quarterly* 15, 138–143.
- Weijtens, M.J.B.M., van der Plas, J., Bijker, P.G.H., Urlings, H.A.P., Koster, D., van Logtestijn, J.G. and Huls, J.H.J. In't Veld (1997) The transmission of campylobacter in piggeries; an epidemiological study. *Journal of Applied Microbiology* 83, 693–698.
- Young, C.R., Harvey, R.B., Anderson, R.C., Nisbet, D.J. and Stanker, L.H. (2000) Enteric colonization following exposure to *Campylobacter* in pigs. *Research in Veterinary Science* 65, 75–78.
- Zanetti, F., Varoli, O., Stampi, S. and DeLuca, G. (1996) Prevalence of thermophilic *Campylobacter* and *Arcobacter butzleri* in food of animal origin. *International Journal of Food Microbiology* 33, 315–321.

Part VI

Free Communications

89 Digestive Physiology in the Pig: 40 Years of Research in France

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The contribution of the research group formed by A. Rérat in the early 1960s in Jouy-en-Josas, and then expanded in Rennes, is summarized. As the digestive processes are a major key to nutritional status in monogastric animals, original methodologies based on advanced experimental surgery were developed in the pig to quantify the yield of digestion and absorption, to learn their factors of variation and to understand their physiological and nutritional consequences. The group also extended its expertise to the control of food intake, to the role of nervous system and regulatory peptides, and to several gut-related biomedical topics.

Introduction

In order to base the improvement of overall growth on a better knowledge of nutritional requirements in growing pigs, A. Rérat headed from the 1960s the progressive enlargement of a specialized research team. As efficiency of the digestive processes is a major key to the nutritional status of monogastric animals, the group explored several digestive physiological functions. The initial studies were performed by Rérat, who obtained a first description of food passage in the digestive tract (Rérat and Lounnon, 1963). He initiated the study of pancreatic secretion (Rérat, 1962, cited by Aumaître *et al.*, 1964) and founded the bases for a direct quantitative measurement of nutrient absorption with the development of portal vein catheterization (Arsac and Rérat, 1962) and portal blood flow measurement (Rérat, 1971). These studies were then extended by the whole group through the development of original techniques based on advanced experimental surgery allowing physiological studies in conscious animals, such as gastrointestinal motility and

food passage, digestive enzyme secretions, luminal digestion and intestinal absorption, intestinal and hepatic handling of nutrients, neurohumoral control of food intake and digestive processes. Further developments concerned human biomedical research.

From a Myogenic Organization to a Pattern of Gastrointestinal Motility

Having been developed in sheep (Ruckebusch and Laplace 1967), electromyography of visceral smooth muscle was then used in conscious pigs by Laplace (1972a). Over the period 1970–1985 this technique allowed us to study the respective properties of the muscle cells or layers of the stomach and small intestine, i.e. the cellular and tissue electrical characteristics, and their myogenic organization to produce various motor events (Laplace, 1980a). On the gastric body, a periodic oscillation of the membrane potential (3.8 min^{-1}) occurs from pacemaker cells near the cardia and propagates at an increasing speed ($0.5\text{--}2 \text{ cm s}^{-1}$)

towards the pylorus. If there is a mechanical contraction, another characteristic electrical event occurs, but only in close temporal association with the oscillation. A permanent pacesetter electrical rhythm (slow waves) is also recorded from the intestinal muscle but along the small intestine there is a proximodistal decreasing gradient of the slow wave rhythm, from 17–18 min⁻¹ on the proximal duodenum to 11 min⁻¹ on the terminal ileum. Parallel to this, the slow waves propagate in the proximodistal direction at a decreasing rate (about 80–20 mm s⁻¹) and may elicit the discharge of spike potentials from the longitudinal and circular layers. Thus they act as a pacesetter for these bursts of spikes.

Due to this organization, the maximum frequency of gastric contractions at any point of the stomach, as well as their aboral propagation, is determined by the basic rhythm (i.e. 3.8 min⁻¹) and its aboral propagation. Therefore contractions, when they occur, may be either local or propagated on the gastric body and/or antrum at an increasing speed, accounting for the regular movements of mixing of gastric contents, for the propulsion of contents towards the distal antrum, and for the sudden closure of the pylorus when reached by the contraction. In the same way, the maximum frequency of the contractions of the small intestine at a given point is fixed by the local rhythm of the slow waves, and their apparent propagation occurs at a velocity determined by the rate of the slow-wave propagation (i.e. a few centimetres s⁻¹). These basic mechanisms can be organized in different ways to produce either isolated local contractions, or contractions propagated over a variable length (peristaltic waves) occurring within an irregular spiking activity (ISA), or multiple local rhythmic contractions forming a regular spiking activity (RSA) over a whole segment of intestine at a given time (rhythmic segmentation). These organized forms of motility occur within an overall pattern of sequences known as the myoelectric or motor migrating complex (MMC).

MMC Pattern, Digesta Flow and Coordination of Motility, pH and Secretions

We investigated the role of this MMC pattern of organization of motility along the time and over the whole tract in relation to feeding, and its relationship with the flow of digesta in normal or pathological conditions, to provide preliminary explanations for the general assumption that food passage results from gastrointestinal motility (see reviews by Laplace, 1980a, 1984). The MMC recurs regularly from the proximal duodenum and slowly migrates distally along the small intestine (from 300 mm min⁻¹ in proximal area to 50 mm min⁻¹ in distal area). An MMC includes a quiescent phase, an ISA phase, and a brief (3–4 min) RSA phase. Along the pig small intestine, two to three complexes are permanently present. Their average number per 24 h is around 20 on the duodenum, 16 in the jejunum and 11 in the ileum, as 40% of them do not migrate along the whole intestine. The average recurrence interval ranges around 70 min in the duodenum and 110–120 min in the ileum. During the overnight fast, the relative duration of quiescence increases at the expense of the ISA, while after a large meal a continuous ISA phase occurs on the proximal intestine for 2–3 h. Moreover, the basic pattern may be modified depending on the diet, suggesting a relationship between MMC pattern and food passage. We established that digesta flux occurs as batches of digesta are propelled within the length of the segment concerned by an ISA phase, while the forthcoming RSA acts as a barrier avoiding backflow. Therefore the proximodistal passage of digesta along the small intestine occurs grossly within 120 min, i.e. at an average rate determined by the migration rate of MMC, which acts as a cruise control system for digesta flow.

The digestive secretions contribute in part to the pH of digestive contents, which might in turn trigger various neurohumoral events. *In situ* studies of intragastric pH showed that the characteristics of the diet are of minor importance in comparison

with those of gastric acid secretion. Though overflowed during the postprandial phase in fed pigs, bile and pancreatic juice contribute highly to the neutralization of gastric acid effluent in fasted pigs, thus supporting the hypothesis of a close coordination between motility and secretions in the gastroduodenal area. Bile and pancreatic secretions, always minimal during duodenal quiescence, increase after the start of an ISA phase to reach a maximum at the onset of an RSA phase. Further experiments confirmed that a true biliary and pancreatic secretory component of the MMC exists in the pig. Nevertheless, gastric acid outflow was shown to be the determinant of the cyclical variation of intraduodenal pH, which takes place within a much more acid range during biliary and pancreatic diversion (Abello *et al.*, 1988). Looking for a possible mediation of some regulatory peptides, we found evidence in conscious fasting pigs of a temporal relationship between the successive phases of MMC and the cyclic variation of plasma motilin, pancreatic polypeptide and gastrin, but not somatostatin (Cuber *et al.*, 1988). The variations of plasma motilin only are of sufficient magnitude to result possibly in significant effects on duodenal motility in the pig.

Bile and Pancreatic Secretions: Development, Adaptations and Significance

The age at which sufficient amounts of the various digestive enzymes are produced is of importance in determining an optimal time for weaning. The progressive development of the enzymatic equipment in the piglet (Aumaître, 1971) occurs as an early appearance of pancreatic chymotrypsin (1 week) and lipase (2 weeks), followed later by amylase (1 month). However, considering their prominent role in the enzymatic digestion of food in growing pigs, pancreatic and biliary secretions received sustained attention. Most of the studies on these two secretions were mainly based on permanent fistulation

techniques, allowing continuous collection, sampling and restitution of pancreatic secretion (Corring *et al.*, 1972) or bile secretion (Juste *et al.*, 1979). The main results were summarized in review papers (Corring, 1977, 1980) and received confirmations under various feeding conditions (Aumaître *et al.*, 1995).

The adaptation of pancreatic enzymes to their substrates was first observed by Aumaître and Rérat (1966, cited by Aumaître, 1971), as the quantitative production of amylase increased in the presence of increased starch intake, the *in vitro* lipase activity varied according to the nature of lipid substrates, and the chymotrypsin production varied with protein quality and level in the diet. As a qualitative long-term regulation, this nutritional adaptation of exocrine pancreatic secretion establishes within 2–3 days and stabilizes within 5–7 days. Its occurrence was shown to depend on the relative amounts of hydrolysis products present in the small intestine, and to be probably mediated by a substance produced by the duodenal mucosa. The exocrine pancreatic secretion is also subjected to a quantitative and short-term regulation by negative feedback which may explain the kinetics of pancreatic secretion during the digestion of a meal. The exclusion of pancreatic enzymes from intestinal lumen stimulates total pancreatic secretion, while their restitution leads back to a normal secretion level. The binding of enzymes to dietary substrates, as well as antitryptic factors which significantly decrease the activity of proteases in the lumen, results in an increased biosynthesis of chymotrypsin (Corring, 1977, 1980).

The extracorporeal derivation of bile for quantitative and qualitative measurements, and its duodenal reinfusion to preserve the normal enterohepatic cycle, showed that the excretion of bile salts strongly increased within 2 h after intake of the morning and afternoon meals (300% higher than before the morning meal). This was the result of the excretion of a highly concentrated bile, while a marked increase in volume only occurred 2–3 h after the meal

intake. An efficient enterohepatic circulation of bile acids is ensured in the pig from the small intestine. The quantitative absorption of total bile acids and their 79% hepatic uptake were assessed by Legrand-Defretin *et al.* (1986). While bile salts were formerly considered to be reabsorbed from the distal intestine, it was possible to show that, in the pig, the proximal intestine plays a very important role in the recycling of bile salts (Juste *et al.*, 1988). In addition, a model of cholesterolic lithiasis was developed in pigs fed a diet enriched with cholesterol and cyclodextrine, while the beneficial effect of soy proteins was shown to result from a change in the bile acid pool leading to a reduced hydrophobicity.

The overall role of bile and pancreatic secretions in the digestion were evidenced. Deprivation of pancreatic juice for a long time leads to a strong decrease in nutrient digestibility, mainly for proteins (–35%), and depression of carbohydrate and amino acid absorption. Deprivation of bile led to a 23% decrease in apparent energy digestibility and a 32% decrease in total lipid apparent digestibility. This role was further supported in studying the responses of bile flow, biliary lipids and bile acid pool in the pig to quantitative variations in dietary fat (Juste *et al.*, 1983).

Kinetics of Digestion: Quantitative and Qualitative Approach of Luminal Digesta

To assess nutrition in pigs, we developed several methods suitable for conscious animals, to investigate the chronology as well as the qualitative and quantitative characteristics of gastric or intestinal contents. After Auffray *et al.* (1967), we tried to improve the overall knowledge of the mechanisms involved in the control of gastric outflow, using both an original duodenal trans-thoracic re-entrant fistulation technique and the modelling of the emptying curves (Laplace and Tomassone, 1970). The differential emptying of the main dietary constituents (starch, proteins) was studied, using either this duodenal fistulation or a gastric fistulation technique

(Cuber *et al.*, 1980). From studies with purified diet or with cereal diets, we confirmed that gastric emptying was highly responsible for the rate of absorption of carbohydrate and amino acids from the small intestine, but it was not possible to determine with certainty whether or not the rate of gastric emptying limited intestinal carbohydrate absorption.

In pigs, most nutrients are absorbed from the small intestine. It was thus of importance to quantify the losses of various nutrients escaping absorption by crossing the ileocaecal junction. For that purpose we developed a specific ileocolic postvalve fistulation technique, avoiding any mixing of the collected intestinal digesta with the colonic contents (Darcy and Laplace, 1980). This technique allowed for the first time the preservation of the functional role of the ileocolic valve and description in detail of the true kinetics of passage of the bulk of digesta from the small to the large intestine. The quantities of nitrogen and carbohydrates leaving the small intestine were measured according to the effects and interactions of dietary starch and protein sources (Darcy *et al.*, 1981). It was then possible to calculate a precaecal digestibility which, when applied to amino acids, was shown to be a better indicator of amino acid availability than the faecal one.

The ileocolic postvalve fistulation technique was used to determine the amino acid composition of digesta escaping the small intestine under different dietary protein sources, and to calculate the apparent digestibility of amino acids before any modification by colonic flora (Darcy-Vrillon and Laplace, 1984). A fairly repeatable precaecal amino acid hierarchy was found with very different diets, while large distortions do exist at the faecal level depending on the activity of the caecocolic flora. Therefore, a new technique to give an easier current estimation of amino acid availability was developed for feed formulation: the so-called ileorectal anastomosis (IRA) technique (Laplace *et al.*, 1985). The IRA-based method, carefully evaluated (Fuller *et al.*, 1994), became widely used in

the pig, for example to assess associative effects between different fibre sources on the digestibilities of amino acids but also energy and cell wall components (Laplace *et al.*, 1989). This improved our understanding of the physicochemical processes involved in digestion of dietary proteins. Indeed, the concept of ileal or precaecal digestible amino acids gained major importance in the explanation of protein efficiency and deposition in the pig.

Intestinal Absorption and Recycling, and Hepatic Handling of Nutrients

The apparent digestibility (disappearance from the lumen) does not indicate an effective absorption or in which form it is liable to appear in the organism. The nutrient considered may be used or modified by the microflora; it may be metabolized and/or retained by the cells of the gut wall during its absorption. Other nutrients may appear such as amino acids, derived from secretions or synthesized by microorganisms. Thus, a methodology was developed for a qualitative approach based on measurement of blood or lymph concentrations of nutrients, and a quantitative approach of the kinetics of their absorption, to appreciate the real nutritive value of feeds (see reviews by R  rat *et al.*, 1980).

The initial qualitative data concerned the changes in lipid composition of intestinal lymph (Raulin *et al.*, 1966), or in the porto-arterial differences of nutrient concentrations (relative enrichment of the efferent portal blood): minerals (Gueguen and R  rat, 1967), amino acids (Pion and R  rat, 1967) or sugars (Auma  tre *et al.*, 1969). Then a quantitative description of the absorption was obtained by measuring the blood flow rate in the portal vein (R  rat, 1971), while blood samples were obtained by permanent catheterizations of the portal vein (Arsac and R  rat, 1962) and the carotid artery. Though this does not account for metabolization processes in the gut wall, it was possible to calculate an apparent absorption according to the formula $Q = (C_p - C_a) \cdot D \cdot dt$, where (C_p -

C_a) represents the porto-arterial differences in the nutrient concentrations, D is the blood flow rate in the portal vein, and Q is the amount absorbed within the time interval dt .

Numerous important results have been obtained by this methodology (see review by R  rat, 1988). The absorption kinetics of total amino acids from some cereals (e.g. barley, maize) is slower than what happens with other feeds (e.g. fishmeal, wheat). A variable absorption rate of individual amino acids was observed according to the feedstuff, as well as a differential absorption rate of hexoses and amino acids. The absorption rate of total and individual amino acids issued from purified proteins (rapeseed, casein) was faster than when issued from untreated feeds (Galibois *et al.*, 1989). The metabolization of some non-essential amino acids (uptake of glutamic acid and glutamine, synthesis of alanine) and of some hexoses (partial transformation into glucose of the fructose issued from sucrose, and of the galactose issued from lactose) has been described (R  rat *et al.*, 1984). There is variable timing of the absorption of carbohydrate enzymic digestion products, e.g. high for cerelese and sucrose, medium for starch and slow for lactose. Large metabolic phenomena occur in the digestive wall, resulting in the absorption of high amounts of lactic acid (R  rat *et al.*, 1992). Modification of the availability of nutrients by the technological processes undergone by feeds has also been evidenced.

Microbial metabolism in the large intestine was also estimated by measurement of the production and absorption of the main products (volatile fatty acids) of the digestive flora in the large bowel, allowing a large recovery of energy from nutrients poorly broken down in the small intestine. The production and absorption of volatile fatty acids increased more or less with the crude fibre content of the feed, according to its nature, and with the presence of some indigestible carbohydrates such as lactose or sorbitol in the diet (R  rat *et al.*, 1993). In contrast to the early energy absorption issued from enzymic degradation, energy

absorption under the form of volatile fatty acids happened later, being thus able partially to bridge the energy gap during the interprandial period. Energy expenditure due to aerobic metabolism of the organs drained by the portal vein after meal intake (18% more than during fasting) was evaluated on the basis of measurements of oxygen consumption from porto-arterial differences (Rérat and Vaissade, 1993).

Large amounts of endogenous products derived from secretions, intestinal desquamations, mucus or even from blood (albumin, urea, free amino acids) are continuously discharged into the gut lumen. It is interesting to know the amounts secreted, their variations and sources, and the amounts reabsorbed, in order to determine their share in nitrogen requirements. From 1981, we combined the various techniques developed to study digestion, absorption and secretions, respectively, together with the availability of stable isotopes (^{15}N), in order to study endogenous nitrogen fluxes in the digestive tract. In pigs fed a casein diet, the reabsorption of endogenous nitrogen amounted to 79% up to the end of the small intestine and 88% over the whole tract (Souffrant *et al.*, 1993).

From 1985, an extension of Rérat's methodology allowed the study of the kinetics of the liver's disposal of nutrients, by simultaneously using cannulation of a hepatic vein (liver blood output) and an electromagnetic probe around the hepatic artery. This approach evidenced the influence of absorption kinetics on the capacities of organs such as the liver or the peripheral tissues to store and release nutrients. It also allowed dynamic analysis of the role played by the factor 'time' in protein tissue metabolism. This methodology enabled us to assess the digestion of some carbohydrates (Rérat *et al.*, 1992), proteins (Simoes-Nunes *et al.*, 1989) and medium-chain triglycerides (Guillot *et al.*, 1993), as well as the kinetics of their uptake and release by the liver and, by difference, by the peripheral tissues. The appearance of amino acids in the portal blood was greater, more rapid and more

homogeneous after duodenal infusion of an oligopeptide solution than after that of free amino acids, irrespective of the amount of the infusate. Hourly insulin and glucagon production were highly correlated with the appearance of amino acids in the portal vein. Peripheral uptake was appreciably less well balanced after infusion of free amino acids than after infusion of small peptides (Rérat *et al.*, 1992). Thus, due to absorption kinetics, large variations in the temporal distribution of free amino acids in the tissues may be at the origin of transitory imbalances in tissue amino acid uptake and, as a result, of lower nutritive value (Rérat, 1995).

Integrative Approaches: Neurohumoral Regulations and Adaptations

The regulation of digestive functions and nutrient metabolic utilization is of utmost importance. Two main aspects have to be considered: the regulation of the functions (food intake, food passage, luminal hydrolyses, absorption and metabolism) and the adaptation of the functions to the composition of the diet or to modifications of digestive ability. Our group participated in the growing knowledge of the integrated neurohumoral control of nutritional functions. We shall briefly develop here aspects concerning the role of afferences from the digestive tract in relation to the control of food intake in the pig, and the consequences of a small intestine resection or bypass.

Initial studies showed that the destruction of the ventromedial hypothalamus (supposed to be the 'satiety centre') induced severe hyperphagia, making animals obese (Auffray, 1969). Then the ontogenesis of the feeding behaviour of the pig was described, but a better knowledge of the pig's brain was necessary. This led the neurobiology group of J.P. Laplace to initiate from 1986 the development of a specific stereotaxic technique for the pig (Marcilloux *et al.*, 1989) and the preparation of a stereotaxic atlas of the whole brain of the pig (Félix *et al.*, 1999).

Considering that the brain probably participates in, and needs to be informed on, the functioning of the digestive tract, particular attention was paid to the role of the peripheral nervous system and mainly the vagus nerve. The functional importance of the vagal afferent firing from visceral receptors, considered as an essential input to the brain, was evaluated in conscious pigs owing to an original surgical technique (Laplace, 1980b) for total selective vagal deafferentation (i.e. interruption of the vagal afferent pathways from the digestive tract to the brain stem). It allowed us to find evidence for the role and general significance of sensory afferent information. The gastroduodenal area appeared to be the origin of numerous afferent messages which could contribute to the control of food intake, gastric emptying, pancreatic function, etc. For example, gastric emptying was strongly affected (Laplace and Cuber, 1984). The vagal deafferentation also induced strong changes in the pancreas, which exhibited significant reductions in the pancreatic tissue mass and in various enzyme activities, thus suggesting the importance of intestinal sensibility for the pancreas (Laplace and Simoes-Nunès, 1987).

The wide knowledge accumulated on digestive function in relation to pig production was further extended to biomedical research, with attention being turned to the question of intestinal adaptation after either small intestine resection or bypass, the consequences of which on somatic and visceral growth were studied under various conditions. The results led

us to hypothesize that some neurohumoral relationships might support the restoration of an adequate proportion between overall functional digestive ability, body size and its nutrient requirements (Laplace, 1975). In order to explore the possible humoral mechanisms involved in compensatory hypertrophy, we developed a long-term cross-circulation technique between conscious pigs paired for an identical blood group and histocompatibility (Laplace, 1972b). When continuously cross-circulated for 410 h, both the jejunectomized (30%) pigs and their intact partners showed significant hypertrophy of the small intestine, whether residual or intact, as compared with intact pigs cross-circulated between them. It was thus possible to demonstrate the involvement of a blood-borne factor responsible for compensatory hypertrophy (Laplace, 1980c). We hypothesized that compensatory hypertrophy of the residual small intestine after partial enterectomy might be mediated through a neurohumoral feedback whose efferent route was the evidenced blood-borne factor, and the afferent route might be the vagal sensory pathway. Further work confirmed that compensatory mucosal hypertrophy after small bowel resection was severely impaired by vagal deafferentation (Laplace, 1982). This suggested that, besides intrinsic control factors of epithelial renewal, the visceral sensitivity supported by vagal afferences might be a determinant of a true adaptation as a result of a type of homeostatic control of functional digestive ability.

References

- Abello, J., Laplace, J.P. and Corring, T. (1988) Biliary and pancreatic secretory component of the migrating myoelectric complex in the pig. Effect on intraduodenal pH. *Reproduction Nutrition Developpement* 28, 953–967.
- Arsac, M. and Rérat, A. (1962) Technique de fistulation de la veine porte chez le porc. *Annales de Biologie animale Biochimie Biophysique* 2, 335–343.
- Auffray, P. (1969) Effets des lésions des noyaux ventro-médians hypothalamiques sur la prise d'aliment chez le Porc. *Annales de Biologie animale Biochimie Biophysique* 9, 513–526.
- Auffray, P., Martinet, J. and Rérat, A. (1967) Quelques aspects du transit gastro-intestinal chez le Porc. *Annales de Biologie animale Biochimie Biophysique* 7, 261–279.
- Aumaître, A. (1971) Le développement des enzymes dans le tube digestif du jeune porcelet: importance pour le sevrage et signification nutritionnelle. *Annales de Zootechnie* 20, 551–575.

- Aumaitre, A., Jouandet, C. and Salmon-Legagneur, E. (1964) Etude des activités amylolytiques chez le porcelet au cours du sevrage. *Annales de Zootechnie* 13 (suppl.), 56–74.
- Aumaitre, A., Février, C., Rérat, A., Rigaud, J. and Thivend, P. (1969) Application de l'analyse en continu à l'étude des variations de la glycémie du sang porte au cours de la digestion chez le Porc. *Comptes Rendus de l'Académie des Sciences (Paris)* 268D, 717–720.
- Aumaitre, A., Peiniau, J. and Madec, F. (1995) Digestive adaptation after weaning and nutritional consequences in the piglet. *Pig News and Information* 16, 73N–80N.
- Corring, T. (1977) Possible role of hydrolysis products of the dietary components in the mechanisms of the exocrine pancreatic adaptation to the diet. *World Review of Nutrition and Dietetics* 27, 132–144.
- Corring, T. (1980) Endogenous secretions in the pig. In: Low, A.G. and Partridge, I.G. (eds) *Current Concepts of Digestion and Absorption in Pigs*. NIRD Press, Reading, UK, pp. 136–150.
- Corring, T., Aumaitre, A. and Rérat, A. (1972) Fistulation permanente du pancréas exocrine chez le porc. Application: réponse de la sécrétion pancréatique au repas. *Annales de Biologie animale Biochimie Biophysique* 12, 109–124.
- Cuber, J.C., Laplace, J.P. and Villiers, P.A. (1980) Fistulation de l'estomac et contenus gastriques résiduels après ingestion d'un régime semi-purifié à base d'amidon de maïs chez le porc. *Reproduction Nutrition Développement* 20, 1161–1172.
- Cuber, J.C., Laplace, J.P., Laredo, C., Levenez, F. and Chayvialle, J.A. (1988) Variations of plasma immunoreactive motilin, pancreatic polypeptide, gastrin and somatostatin along the duodenal motility cycle in the pig. *Regulatory Peptides* 23, 27–35.
- Darcy, B. and Laplace, J.P. (1980) Digestion dans l'intestin grêle chez le Porc. 1. Définition des conditions d'obtention des digesta. *Annales de Zootechnie* 29, 137–145.
- Darcy, B., Laplace, J.P. and Villiers, P.A. (1981) Digestion dans l'intestin grêle chez le porc. 4 – Cinétique de passage des digesta au niveau de la jonction iléo-caeco-colique et bilans de la digestion selon la nature de l'amidon et la source de protéines alimentaires. *Annales de Zootechnie* 30, 31–62.
- Darcy-Vrillon, B. and Laplace, J.P. (1984) Digestion des protéines dans l'intestin grêle chez le porc. 3 – Digestibilité des acides aminés et variations post-prandiales de la composition des digesta selon la source de protéines d'un régime à base d'amidon de blé purifié. *Annales de Zootechnie* 33, 467–488.
- Félix, B., Léger, M.E., Albe-Fessard, D., Marcilloux, J.C., Rampin, O. and Laplace, J.P. (1999) Stereotaxic atlas of the pig brain. *Brain Research Bulletin* 49, 1–138.
- Fuller, M.F., Darcy-Vrillon, B., Laplace, J.P., Picard, M., Cadenhead, A., Jung, J., Brown, D. and Franklin, M.F. (1994) The measurement of dietary amino acid digestibility in pigs, rats and chickens: a comparison of methodologies. *Animal Feed Science and Technology* 48, 305–324.
- Galibois, I., Simoes-Nunes, C., Rérat, A. and Savoie, L. (1989) Net appearance of amino acids in portal blood during the digestion of casein or rapeseed proteins in the pig. *Canadian Journal of Physiology and Pharmacology* 67, 1409–1417.
- Gueguen, L. and Rérat, A. (1967) Cinétique de l'absorption intestinale du phosphore chez le porc. *Annales de Biologie animale Biochimie Biophysique* 7, 39–46.
- Guillot, E., Vaugelade, P., Lemarchal, P. and Rérat, A. (1993) Intestinal absorption and liver uptake of medium chain fatty acids in non anaesthetized pigs. *British Journal of Nutrition* 69, 431–442.
- Juste, C., Corring, T. and Bréant, P. (1979) Bile excretion in the pig: magnitude and response to meal consumption. *Annales de Biologie animale Biochimie Biophysique* 19, 79–90.
- Juste, C., Demarne, Y. and Corring, T. (1983) Response of bile flow, biliary lipids and bile acid pool in the pig to quantitative variations in dietary fat. *Journal of Nutrition* 113, 1691–1701.
- Juste, C., Legrand-Defretin, V., Corring, T. and Rérat, A. (1988) Intestinal absorption of bile acids in the Pig. *Digestive Diseases and Sciences* 33, 67–73.
- Laplace, J.P. (1972a) Motricité gastro-intestinale chez le porc: étude descriptive par électromyographie et corrélations nutritionnelles. *Recueil de Médecine Vétérinaire* 148, 37–61.
- Laplace, J.P. (1972b) Circulation sanguine croisée, chronique et non contrôlée chez le porc éveillé: technique chirurgicale, entretien et validité. *Journal de Physiologie (Paris)* 64, 165–172.
- Laplace, J.P. (1975) D'une théorie générale des processus d'adaptation au cas particulier de l'entérectomie. Résultats élémentaires obtenus chez le porc. In: Inserm-Colloques (ed.) *Réanimation Entérale à Faible Débit Continu*, Vol. 53. I.N.S.E.R.M., Paris, pp. 75–88.

- Laplace, J.P. (1980a) Stomach and small intestine motility in the pig: electro-myography in nutritional studies. In: Low, A.G. and Partridge, I.G. (eds) *Current Concepts of Digestion and Absorption in Pigs*. NIRD Press, Reading, UK, pp. 24–51.
- Laplace, J.P. (1980b) Surgical deprivation of vagal afferences from the gastro intestinal tract of the pig; clinical and EMG studies. In: Christensen, J. (ed.) *Gastro Intestinal Motility*. Raven Press, New York, pp. 145–152.
- Laplace, J.P. (1980c) Compensatory hypertrophy of the residual small intestine after partial enterectomy. A neuro-humoral feedback? *Annales de Recherches Vétérinaires* 11, 165–177.
- Laplace, J.P. (1982) Impairment by vagal deafferentation of the compensatory hypertrophy after enterectomy, at high and low feeding levels. In: Robinson, J.W.L., Dowling, R.H. and Riecken, E.O. (eds) *Mechanisms of Intestinal Adaptation*. MTP Press, Lancaster, UK, pp. 321–331.
- Laplace, J.P. (1984) Intestinal motility; a review of myoelectric and motor migrating complexes. In: Batt, R.M. and Lawrence, T.L.J. (eds) *Function and Dysfunction of the Small Intestine*. Liverpool University Press, Liverpool, UK, pp. 1–20.
- Laplace, J.P. and Cuber, J.C. (1984) Déafférentation vagale totale et évacuation gastrique chez le porc. *Reproduction Nutrition Développement* 24, 655–670.
- Laplace, J.P. and Simoes-Nunes, C. (1987) Pancreatic size and enzyme contents after vagal deafferentation in jejunectomized pigs under free or restricted feeding. *Gut* 28 (S1), 169–173.
- Laplace, J.P. and Tomassone, R. (1970) Evacuation gastroduodénale chez le porc. Fistulation chronique par voie thoracique extrapleurale: recherche d'une technique d'analyse mathématique de l'évacuation. *Annales de Zootechnie* 19, 303–332.
- Laplace, J.P., Darcy-Vrillon, B. and Picard, M. (1985) Evaluation de la disponibilité des acides aminés: choix raisonné d'une méthode. In: INRA-ITP (ed.) *Journées de la Recherche Porcine en France*. ITP, Paris, pp. 353–370.
- Laplace, J.P., Darcy-Vrillon, B., Perez, J.M., Henry, Y., Giger, S. and Sauvant, D. (1989) Associative effects between two fibre sources on ileal and overall digestibilities of amino acids, energy and cell-wall components in growing pigs. *British Journal of Nutrition* 61, 75–87.
- Legrand-Defretin, V., Juste, C., Corring, T. and Rérat, A. (1986) Enterohepatic circulation of bile acids in pigs: diurnal pattern and effect of a reentrant biliary fistula. *American Journal of Physiology* 250, G295–G301.
- Marcilloux, J.C., Rampin, O., Félix, M.B., Laplace, J.P. and Albe-Fessard, D. (1989) A stereotaxic apparatus for the study of the central nervous structures in the pig. *Brain Research Bulletin* 22, 591–597.
- Pion, R. and Rérat, A. (1967) Influence d'une supplémentation en lysine sur l'évolution de l'aminocidémie porte du Porc en croissance, au cours de la digestion d'une ration à base de blé. *Comptes Rendus de l'Académie des Sciences (Paris)* 264, 632–635.
- Raulin, J., Lorette, C., Flanzky, J. and Rérat, A. (1966) Distribution des acides gras cis et trans dans les triglycérides de la lymphe de porc. *Biochimica Biophysica Acta* 116, 385–388.
- Rérat, A. (1971) Mesure du débit de sang dans la veine porte à l'aide d'un débitmètre électromagnétique chez le Porc. *Annales de Biologie animale Biochimie Biophysique* 11, 175–180.
- Rérat, A. (1988) Experimentelle Ergebnisse über physiologische Vorgänge der Verdauung und Absorption in Bezug zur Fütterung und dem Metabolismus der Aminosäuren. Tagungsbericht, ETH Zürich, Institut für Nutztierwissenschaften (Tagung zur Verleihung des Roche Research Prize for Animal Nutrition).
- Rérat, A. (1995) Nutritional value of protein hydrolysis products (oligopeptides and free amino acids) as a consequence of absorption and metabolism. *Archives of Animal Nutrition* 48, 23–36.
- Rérat, A. and Loughnon, J. (1963) Etudes sur le transit digestif chez le porc. *Annales de Biologie animale Biochimie Biophysique* 3 (suppl.), 21–30.
- Rérat, A. and Vaissade, P. (1993) Relations entre la prise alimentaire et la consommation d'oxygène des organes drainés par la veine porte chez le porc éveillé. *Reproduction Nutrition Développement* 33, 1–17.
- Rérat, A., Vaugelade, P. and Villiers, P. (1980) A new method for measuring the absorption of nutrients in the pig: critical examination. In: Low, A.G. and Partridge, I.G. (eds) *Current Concepts of Digestion and Absorption in Pigs*. NIRD Press, Reading, UK, pp. 177–216.
- Rérat, A., Vaissade, P. and Vaugelade, P. (1984) Absorption kinetics of some carbohydrates in conscious pigs. 2. Quantitative aspects. *British Journal of Nutrition* 51, 517–529.

- Rérat, A., Simoes-Nunes, C., Mendy, F., Vaissade, P. and Vaugelade, P. (1992) Splanchnic fluxes of aminoacids after duodenal infusion of carbohydrate solutions containing free aminoacids or oligopeptides in the non anaesthetized pigs. *British Journal of Nutrition* 68, 111–138.
- Rérat, A., Giusi-Périer, A. and Vaissade, P. (1993) Absorption balances and kinetics of nutrients and bacterial metabolites in conscious pigs after intake of maltose or maltitol rich diets. *Journal of Animal Science* 71, 2473–2488.
- Ruckebusch, Y. and Laplace, J.P. (1967) La motricité intestinale chez le mouton éveillé: phénomènes mécaniques et électriques. *Comptes Rendus des Séances de la Société de Biologie* 161, 2517–2523.
- Simoes-Nunes, C., Rérat, A., Galibois, I., Vaugelade, P. and Vaissade, P. (1989) Hepatic and gut balances of glucose, aminonitrogen, ammonia and urea in the pig after ingestion of casein or rapeseed proteins. *Nutrition Reports International* 40, 901–907.
- Souffrant, W.B., Rérat, A., Laplace, J.P., Darcy-Vrillon, B., Köhler, R., Corring, T. and Gebhardt, G. (1993) Exogenous and endogenous contributions to nitrogen fluxes in the digestive tract of pigs fed a casein diet. 3. Recycling of the endogenous nitrogen. *Reproduction Nutrition Développement* 33, 373–382.

90 Effect of Phytase Addition on Amino Acid and Dry Matter Digestibilities and Growth in Pigs

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Decreasing the concentration of dietary Ca and P, the addition of 500 FTU phytase, or the combination of reducing dietary Ca and P plus the addition of 500 FTU phytase increased amino acid and dry matter digestibilities in pigs. The data suggest that phytase increases amino acid and energy availability, but the increase in energy availability may be more than the increase in amino acid availability.

Introduction

Phytase supplementation has been reported to increase total tract digestibility of dry matter (DM), crude protein and the ten essential amino acids (Mroz *et al.*, 1994). Yi *et al.* (1996), however, reported that digestibility of dry matter and the apparent absorption of N were decreased

linearly as the amount of available phosphorus (aP) increased. Similarly, previous research from our laboratory (Johnston and Southern, 1999) showed that either reduced dietary Ca and aP and/or the addition of phytase increased lysine availability in chicks. Therefore, the objective of our research was to determine the effect of dietary Ca and aP level and/or microbial

phytase on growth performance and digestibility of amino acids and dry matter in pigs.

Materials and Methods

These experiments were approved by the University Animal Care and Use Committee. Ileal digesta was collected from eight barrows (body weight = 56 kg) fitted with steered ileocaecal cannulas (Mroz *et al.*, 1996) and was lyophilized, ground and analysed for amino acids, DM and chromium concentrations to allow for the determination of apparent ileal digestibility. The experiment was a Latin rectangle with four treatments. Diet 1 (control) was based on maize and soybean and was adequate in Ca and P (0.5% Ca, 0.19% aP); Diet 2 was Diet 1 but with reduced Ca and P (0.4% Ca, 0.09% aP); Diet 3 was Diet 1 with 500 FTU of phytase; and Diet 4 was Diet 2 with 500 FTU phytase. Pigs were fed twice daily at 0700 and 1900 h to a total daily intake of 2.6 times the maintenance requirement of 106 kcal ME kg⁻¹ BW^{0.75} (NRC, 1998). Within each treatment period, ileal contents were collected and contents for the individual pig were combined for two 24 h periods. There was a 6-day period between each collection and after each diet change.

In a second experiment (106 days), 150 gilts (initial body weight = 20 kg) were used. Each treatment was replicated five times with six gilts each. Treatments were: (i) positive control (NRC, 1998; adequate in amino acids, ME, Ca and P); (ii) a diet with 85% of the amino acids of Diet 1, but adequate in Ca and P; (iii) 85% amino acids formulated with phytase expected to supply amino acids, ME, Ca and P with added phytase; (iv) Diet 3 but with no added phytase; and (v) Diet 4 but adequate in Ca and P. The nutrient matrix values that were used for the phytase addition were: Ca 144%, aP 144%, ME 15,246 kcal kg⁻¹, lysine 12%, methionine 5%, threonine 5% and tryptophan 2%. The phytase was provided at 0.083% of the diet, and therefore was expected to provide the following

nutrients: Ca 0.12%, aP 0.12%, ME 12.7 kcal kg⁻¹, lysine 0.01%, methionine 0.004%, threonine 0.004% and tryptophan 0.002%.

Results and Discussion

Decreasing the concentration of dietary Ca and P (Diet 2) increased ($P < 0.1$) amino acid digestibility in eight of the nine amino acids measured. Phytase addition to the Ca and P adequate diet (Diet 3) increased ($P < 0.1$) digestibility of four of the nine amino acids and tended ($P = 0.15$) to increase the digestibility of three others. Reducing dietary Ca and P along with phytase addition (Diet 4) increased ($P < 0.1$) digestibility of all nine amino acids compared with the control diet. This is in agreement with Yi *et al.* (1996) who reported improved amino acid digestibility with phytase addition and increased apparent digestibility of the ten essential amino acids when aP was lowered from 0.60 to 0.45%. Dry matter digestibility increased ($P < 0.04$) with Ca and P reduction, phytase addition, and the combination of the two factors. These data are in contrast to O'Quinn *et al.* (1997), who reported no increase in apparent ileal DM digestibility when phytase was added to sorghum-based diets for pigs.

In Experiment 2, pigs fed diets with reduced amino acid concentrations had lower daily gain ($P < 0.01$) than pigs fed the positive control diet adequate in amino acids and other nutrients (Diet 1). Pigs fed Diet 3 (added phytase) had gain:feed ratios equal to, or slightly greater than, those for pigs fed Diets 1 or 2, but they had greater gain:feed ratios than pigs fed Diet 4 ($P < 0.05$) or Diet 5 ($P = 0.15$). Phytase addition to a diet with reduced levels of amino acids, Ca, aP and ME produced gain:feed ratios slightly higher than those for pigs fed the positive control diet. However, the gain:feed ratio was reduced in the diet without phytase and formulated to be deficient in amino acids, Ca, P and ME (Diet 4). Feed efficiency was also decreased in pigs fed the diet without added phytase but

which had adequate Ca and P (Diet 5). These results suggest that not all of the increased feed efficiency from phytase was in response to increased Ca and P, but that amino acid and energy availability also was improved when phytase was added to the diet. In addition, pigs fed the diet containing phytase (Diet 3) had more muscle and fat than pigs fed the diet without phytase (Diet 2). Pigs fed Diet 3 with phytase also tended to have more muscle and fat than pigs fed Diet 4 (no phytase), but the effect was not significant. These data sug-

gest that dietary phytase increased amino acid and energy availability in these diets, but the increase in energy availability was more than the increase in amino acid availability. In addition, these data indicate that reducing dietary levels of Ca and P may increase the availability of energy and amino acids. In conclusion, Ca and P reduction and/or phytase addition increased amino acid digestibility, and phytase improved utilization of amino acids and ME, as well as Ca and P, in diets for pigs.

References

- Johnston, S.L. and Southern, L.L. (1999) The effect of phytase addition and(or) the reduction of dietary calcium and available phosphorus on lysine bioavailability in growing chicks. *Poultry Science* 78 (Supplement 1), 101.
- Mroz, Z., Jongbloed, A.W. and Kemme, P.A. (1994) Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. *Journal of Animal Science* 72, 126–132.
- Mroz, Z., Bakker, G.C.M., Jongbloed, A.W., Dekker, R.A., Jongbloed, R. and van Beers, A. (1996) Apparent digestibility of nutrients in diets with different energy density, as estimated by direct and marker methods for pigs with or without ileo-cecal cannulas. *Journal of Animal Science* 74, 403–412.
- NRC (1998) *Nutrient Requirements of Swine*, 10th revised edn. National Academy Press, Washington, DC.
- O'Quinn, P.R., Knabe, D.A. and Gregg, E.J. (1997) Efficacy of natuphos® in sorghum-based diets of finishing swine. *Journal of Animal Science* 75, 1299–1307.
- Yi, Z., Kornegay, E.T. and Denbow, D.M. (1996) Effect of microbial phytase on nitrogen retention of turkey poults fed corn–soybean meal diets. *Poultry Science* 75, 979–990.

91 Effect of Transportation Stress on Intramucosal pH and Intestinal Permeability

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Data from three experiments suggested that gastrointestinal blood flow and intestinal permeability were affected by transportation stress. In the first experiment, transportation resulted in a lower intestinal pH. In the second experiment, there was a tendency towards an inverse relationship between intestinal permeability and time of necropsy of pigs transported for 2 h. In the third experiment, intestinal permeability of pigs submitted to necropsy immediately and 1 h after transport was higher compared with necropsy 2 or 3 h after exposure to rest. In future, nutritional compounds may be used to avoid increased intestinal permeability upon transport.

Introduction

In Dutch practice, pigs are transported from breeding to finishing farms at the age of approximately 10 weeks and to the slaughterhouse at a weight of 100–110 kg. Vibration, noise, restraint and handling associated with transport may trigger physiological changes that may produce profound changes in heart rate and renal blood flow (Stephans and Rader, 1983) and increase translocation of endotoxin from the gut (Zucker and Krüger, 1998). Therefore, it can be suggested that transportation stress may affect blood flow to the gastrointestinal tract and subsequently increase intestinal permeability. This suggestion is supported by data from three experiments.

Material and Methods

In the first experiment six specific pathogen-free pigs (± 25 kg body weight) were fed, transported for half an hour and

thereafter anaesthetized and instrumented, while six littermates were not transported before instrumentation. Intramucosal pH of the distal jejunum was measured by tonometry once an hour for a period of 8 h, starting 30 min after finishing instrumentation.

In the second experiment, ten pigs of about 100 kg body weight (BW) were transported for 2 h and rested for 2–4 h before dissection was carried out. Ten other pigs were not transported but were tranquilized in the stable before dissection was conducted. Immediately after dissection, part of the distal jejunum was stripped of muscle layers and mounted in Ussing chambers. The macromolecular probe horseradish peroxidase (HRP; molecular mass 40 kDa) was added mucosally and serosal samples were taken every 30 min for 3 h to determine the intestinal permeability. In the third experiment the intestinal permeability of 15 pigs (± 25 kg BW) that had been transported for 2 h was determined by measuring the mucosal-to-serosal flux of HRP in Ussing chambers.

The pigs were dissected immediately after transport or after 1, 2 or 3 h exposure to rest.

Results

In the first experiment, the intramucosal pH averaged 7.02 ± 0.06 for the transported pigs and was significantly higher for the non-transported pigs (7.26 ± 0.04).

In the second experiment, the HRP flux of transported pigs was the same as the HRP flux of non-transported pigs, but in the transported group there was a tendency towards an inverse correlation between HRP flux and time of rest before dissection. In the third experiment, the HRP flux of pigs submitted to dissection immediately and 1 h after transport was higher than in pigs submitted to dissection 2 or 3 h after exposure to rest (Fig. 91.1).

Discussion

The reduced intramucosal pH in the first experiment suggests a reduction of blood flow to the gastrointestinal tract, since intramucosal pH is significantly reduced by a mechanical reduction of the flow in the

superior mesenteric artery (Pargger *et al.*, 1997; van der Meulen *et al.*, 1997). Although it has been shown before that a reduction of the blood flow in the superior mesenteric artery increases intestinal permeability to HRP (van der Meulen *et al.*, 1997), such an increase in HRP flux was not measured for transported pigs in the second experiment. This may be related to the time between transport and dissection, as was shown in the third experiment, while it is also known that indices of (transportation) stress (plasma cortisol and β -endorphin) are reduced during a 2–3 h rest in lairage before slaughter (Warris *et al.*, 1992). The increased intestinal permeability during and shortly after transportation may enable toxins, for example, to reach the circulation (Zucker and Krüger, 1998) or even bacterial translocation from the gastrointestinal tract (Berg, 1992) and may explain the disease problems often seen after transport (Berends *et al.*, 1996). At the present, ischaemia-reperfusion experiments in pigs are being carried out to investigate whether (nutritional) compounds can support the intestinal barrier in preventing an increase of intestinal permeability; in the future, nutritional compounds will probably be used to avoid increased intestinal permeability upon transport.

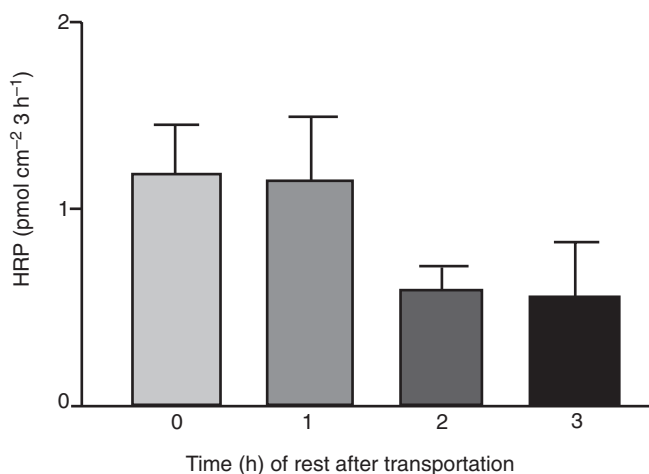


Fig. 91.1. Mucosal-to-serosal flux of HRP in jejunum of pigs following 2 h transport and subsequent 0 or 1–3 h rest.

References

- Berends, B.R., Urlings, H.A.P., Snijders, J.M.A. and van Knapen, F. (1996) Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. *International Journal of Food Microbiology* 30, 37–53.
- Berg, R.D. (1992) Bacterial translocation from the gastrointestinal tract. *Journal of Medicine* 23, 217–244.
- Pargger, H., Staender, S., Studer, W., Schellscheidt, O., Mihatsch, M.J., Scheidegger, D. and Skarvan, K. (1997) Occlusive mesenteric ischemia and its effects on jejunal intramucosal pH, mesenteric oxygen consumption and oxygen tensions from surfaces of the jejunum of anesthetized pig. *Intensive Care Medicine* 23, 91–99.
- Stephans, D.B. and Rader, R.D. (1983) Effects of vibration, noise and restraint on heart rate, blood pressure and renal blood flow in the pig. *Journal of the Royal Society of Medicine* 76, 841–847.
- van der Meulen, J., Graaf, G.J. and Nabuurs, M.J.A. (1997) Intestinal ischemia-reperfusion and macromolecular transport in the pig. *Proceedings ID-DLO Symposium Gastro-Intestinal Disorders in Juveniles*. ID-DLO, Lelystad, p. 20.
- Warriss, P.D., Brown, S.N., Edwards, J.E., Anil, M.H. and Fordham, D.P. (1992) Time in lairage needed by pigs to recover from the stress of transport. *Veterinary Record* 131, 194–196.
- Zucker, B.A. and Krüger, M. (1998) Auswirkungen von Transportbelastungen auf den Endotoxingehalt im Blut von Schlachtschweinen. *Berliner und Münchener Tierärztliche Wochenschrift* 111, 208–210.

92 Nutrient Intake Level Affects Histology and Permeability of the Small Intestine in Newly Weaned Piglets

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This experiment was conducted to evaluate the effect of feeding piglets milk at a low or high nutrient level on histology and the integrity of the small intestinal mucosa from 0 to 4 days postweaning. Tissue samples were taken in the proximal, mid and distal part of the jejunum for histology measurements. Nutrient transport through the mucosa from the mid-jejunum was evaluated *in vitro* by a 2 h absorption test using an Ussing chamber. Nutrient intake level significantly affected villus length at the proximal jejunum but not at the mid- or distal jejunum. The day of dissection post weaning affected villus length, crypt depth and the ratio of villus length to crypt depth in the proximal and mid-jejunum. High nutrient intake level (186 g dry matter l⁻¹) had lower paracellular transport (mannitol permeability coefficient) compared with low nutrient intake level (62 g dry matter l⁻¹). Transcellular transport (GlySar permeability coefficient) was not affected by nutrient intake level. Paracellular transport increased at days 1 and 2 after weaning but did not further increase at day 4.

Introduction

In pigs, weaning causes immediate (days 0–2) adverse effects, such as low feed intake, independently of diet composition (McCracken *et al.*, 1995). Low feed intake results in less nutrients within the gut lumen, which may impact negatively on the digestive and absorptive capacity of the small intestine. The integrity of the small intestinal epithelium may be compromised, increasing its permeability. Therefore, a study was performed to evaluate the effects of low nutrient intake on histology and *in vitro* permeability of the small intestine.

Material and methods

Forty-eight newly weaned Yorkshire × (Dutch Landrace × Finnish Landrace) barrows (age 25.8 ± 2.0 days) were used in the experiment. Creep feed was not provided during the suckling period. On the day of weaning, piglets were transported 10 km to our research facility, where they were individually housed in floor pens 50 × 90 cm² with transparent plastic walls. Each pen was equipped with a plastic trough. Water was supplied via the milk. On the day of weaning, gut tissue samples were taken from 12 animals as reference value.

Thirty-six piglets were divided into two

groups: high (H) and low (L) nutrient intake level. A liquid milk diet (milk protein 300 g kg⁻¹, lactose 300 g kg⁻¹ and butter oil 300 g kg⁻¹) was fed four times per day at 0900, 1230, 1700 and 2130 h. Feed intake was registered. The H group was offered milk (dry matter content: 186 g kg⁻¹) according to requirements for maintenance and growth based on body weight for digestible energy (NRC, 1998). The L group was offered the same volume of milk but with reduced dry matter content (62 g kg⁻¹). On the day of weaning and on days 1, 2 and 4 post weaning, gut tissue samples were taken under complete anaesthesia by inhalation of a mixture of N₂O/O₂ and isoflurane. A midline laparotomy was performed and samples were taken from three jejunal sites: 0.5 m and 3.5 m distal from the ligament of Treitz and 0.5 m proximal to the ileocaecal ligament. For macroscopic histology the tissue samples were treated according to previously described procedures (Kik *et al.*, 1990). At 3.5 m distal from the ligament of Treitz, tissue samples (5 cm) were also taken to measure transepithelial transport in TNO transport Ussing chambers. Permeability coefficients (P_{ms}) of ³H-mannitol (ICN) and ¹⁴C-GlySar (Zeneca Cambridge Research Biochemicals) were determined as described by van der Klis and Versantvoort (1999).

Results and Discussion

During day 1 postweaning, feed refusal in both groups increased the ratio of intake level high versus low to 4. From day 2 onwards the offered amount of milk was consumed. Low nutrient intake level resulted in more reduced villus length than high nutrient intake level, with the difference significant ($P < 0.05$) only in the proximal jejunum. The nutrient intake level did not affect crypt depth or the ratio of villus length to crypt depth. The number of days to dissection post weaning affected villus length and the ratio of villus length to crypt depth in the proximal, mid and distal parts of the jejunum. The number of goblet cells was not affected by nutrient intake level, diet composition or day of dissection. High nutrient intake level resulted in lower values for paracellular transport (Mannitol). Paracellular transport was increased at days 2 and 4 post weaning compared with day 1 (Table 92.1). The transcellular transport (apparent permeability coefficient; Papp GlySar) was not affected.

Based on these results, it was hypothesized that the integrity of the gut mucosa of piglets at the low nutrient intake level is worse than in piglets at the high nutrient intake level because of the presence of less

Table 92.1. Villus length (μm), crypt depth (μm) and transcellular (GlySar) and paracellular (Mann) transport rates (10⁻⁶ cm s⁻¹) in piglets fed at a high or low nutrient intake level at different time post weaning.

		Intake level (I)			Day post weaning (D)					P value ²	
		High	Low	SEM	0 ¹	1	2	4	SEM	I	D
Villus length	prox	465 ^a	375 ^b	25	502	443 ^a	355 ^b	409 ^{ab}	22	*	*
	mid	370	326	22	351	382 ^a	286 ^b	319 ^b	19	NS	**
	dist	227	212	13	255	228	207	242	11	NS	NS
Crypt depth	prox	193	182	7	178	169 ^a	172 ^a	196 ^b	6	NS	**
	mid	177	171	6	176	163 ^a	161 ^a	189 ^b	5	NS	***
	dist	156	152	8	157	143	137	163	7	NS	t
Mann	mid	8.2 ^a	12.1 ^b	0.9	6.6	7.8 ^a	11.4 ^b	11.1 ^b	0.8	**	***
GlySar	mid	13.0	16.3	1.5	16.6	14.3	16.7	17.8	1.3	NS	NS

¹Values of day 0 are not included in the statistical analysis.

²Level of significance: NS = not significant; t = $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^{abc} Least square means in the same row without a common character in the superscript differ significantly ($P < 0.05$).

mature mucosa cells. These coincide with a higher permeability of the tight junctions (paracellular transport route) between cells, while the permeability of the mucosa cells as such is not affected (Madara, 1994). This may explain why the permeability coefficient for mannitol was increased, while the coefficient for GlySar was not affected.

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References

- Kik, M.J.L., Huisman, J., van der Poel, A.F.B. and Mouwen, J.M.V.M. (1990) Pathologic changes of the small intestinal mucosa of piglets after feeding of *Phaseolus vulgaris* beans. *Veterinary Pathology* 27, 329–334.
- Madara, J.L. and Trier, J.S. (1994) The functional morphology of the mucosa of the small intestine. In: Johnson, L.R. (ed.) *Physiology of the Gastrointestinal Tract*. Raven Press, New York, pp. 1577–1624.
- McCracken, B.A., Gaskins, H.R., Ruwe-Kaiser, P.J., Klasing, K.C. and Jewell, D.E. (1995) Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *Journal of Nutrition* 125, 2838–2845.
- National Research Council (1998) *Nutrient Requirements of Swine*, 10th edn. National Academy Press, Washington, DC, 190 pp.
- van der Klis, J.D. and Versantvoort, C.H.M. (1999) On the relationship between intestinal morphology and absorptive capacity in broilers. *Proceedings 12th European Symposium on Poultry Nutrition*. WPSA, Veldhoven, The Netherlands, pp. 163–165.

93 Effect of PVTC Cannulation on Ileal and Faecal Digestibility in Growing Pigs

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The effect of caecectomy as part of the PVTC cannulation of pigs (65–100 kg) was evaluated on the apparent ileal and faecal digestibility of nutrients and contents of (volatile) fatty acids in ileal digesta using three experimental diets. The results suggest that presence or absence of the caecum in PVTC-cannulated pigs affects the composition of ileal digesta with regard to the content of propionic acid and also slightly affects apparent ileal digestibility data with regard to the non-protein fraction. This may be the result of reflux of caecal content during digesta collection or to a difference in microbial colonization of the ileum between PVTC-cannulated pigs either with or without an intact caecum.

Introduction

In the post-valve T-caecum (PVTC) technique for cannulating the ileum in pigs, the caecum is normally removed and replaced by a T-cannula (van Leeuwen *et al.*, 1991). The removal of the caecum is supposed to have no effect on the ileal digestibility values determined with this technique. However, PVTC-cannulation and caecum removal may affect faecal digestibility values determined in PVTC-cannulated pigs. Therefore, a study was performed to evaluate the effect of PVTC cannulation, with or without sparing the caecum, on the ileal and faecal digestibility of nutrients and content of lactic acid, formic acid and volatile fatty acids (VFA) in ileal digesta.

Materials and Methods

Eighteen barrows were used in the experiment with three experimental periods (P1,

P2 and P3) and housed in metabolism cages with automatic feeders.

Three experimental diets were evaluated. Diet I was based on maize, barley and soybean meal (157 g kg⁻¹ crude protein). High-protein diet II (249 g kg⁻¹ crude protein) was based on maize, barley, soybean meal and potato protein, while high-fibre diet III (88 g kg⁻¹ crude fibre) was based on maize, barley, soybean meal, sunflower meal and sugarbeet pulp.

In P1, faecal digestibility was determined with intact pigs (66 kg). In P2, the ileal digestibility was determined with PVTC-cannulated pigs with or without caecum (nine pigs each; 78 kg) (van Leeuwen *et al.*, 1991). In P3, faecal digestibility with PVTC-cannulated pigs (93 kg) with or without caecum was established.

Data were analysed statistically using analysis of variance, followed by a Student's *t*-test for comparison of treatment means.

Results and Discussion

Table 93.1 shows the contents of (volatile) fatty acids in ileal digesta for diets I and II. PVTC cannulation with an intact caecum increased the level of propionic acid ($P < 0.05$) and tended to decrease the level of lactic acid ($P < 0.10$). Comparison between diets I and III (results not shown) did not reveal differences for either the content of (volatile) fatty acids or the effect of caecectomy.

The apparent ileal digestibility of nutrients in diets I and II is given in Table 93.2. Digestibility of diet II was higher than that of diet I. Leaving the caecum intact during PVTC surgery resulted in a slight increase in apparent ileal digestibility of the non-

protein organic matter (OM) fraction (carbohydrate + fat fraction) ($P < 0.05$) and a tendency towards a slightly lower digestibility of dry matter and organic matter ($P < 0.10$). Caecum removal did not affect the apparent ileal digestibility of crude protein and amino acids.

The data suggest that the presence or absence of the caecum in PVTC-cannulated pigs may affect composition of ileal digesta with regard to the content of (volatile) fatty acids resulting from fermentation. Caecectomy in PVTC-cannulated pigs might slightly influence the apparent ileal digestibility of the non-protein fraction of the diet. This may be the result of a slight reflux of caecal content during digesta collection or to a differ-

Table 93.1. Effect of the diet and of caecectomy as part of PVTC cannulation on the content of lactic acid, formic acid, acetic acid, propionic acid and butyric acid in ileal digesta (g kg⁻¹) in pigs fed a normal (I) or high-protein diet (II).

	Lactic acid	Acetic acid	Propionic acid	Butyric acid
Diet I	0.44	0.30	0.08	0.05
Diet II	0.43	0.44	0.13	0.06
Caecum removal				
Caecum –	0.56	0.33	0.04 ^a	0.03
Caecum +	0.31	0.40	0.17 ^b	0.07
<i>P</i> -values				
Group (G)	0.97	0.11	0.06	0.78
Caecum (C)	0.09	0.37	< 0.01	0.19
G × C	0.60	0.33	< 0.01	0.40

^{ab}Values with a different superscript between diets or –/+ caecum differ significantly at $P < 0.05$.

Table 93.2. Effect of the diet and of caecectomy as part of the PVTC cannulation of pigs on apparent ileal digestibility (%) of dry matter, ash, crude protein, carbohydrates + fats (N-free OM), and amino acids of a normal (I) or high-protein diet (II).

	Dry matter	Ash	Organic matter	Crude protein (N × 6.25)	N-free OM ¹	Lys	Met
Diet I	73.1 ^a	32.1	75.9 ^a	77.0 ^a	75.7 ^a	83.0 ^a	88.3 ^a
Diet II	75.0 ^b	35.0	77.5 ^b	82.4 ^b	75.5 ^a	88.5 ^b	89.9 ^b
Caecum removal							
Caecum –	73.3	32.6	76.0	79.0	74.8 ^a	85.3	89.0
Caecum +	74.8	34.5	77.5	80.3	76.4 ^b	86.2	89.2
<i>P</i> -values							
Group (G)	0.02	0.14	0.05	< 0.01	0.76	< 0.01	0.02
Caecum (C)	0.06	0.32	0.06	0.19	0.04	0.27	0.67
G × C	0.74	0.29	0.59	0.20	0.89	0.31	0.92

¹N-free OM = carbohydrates + fat.

^{ab}Values with a different superscript between diets or –/+ caecum differ significantly at $P < 0.05$.

ence in microbial colonization of the ileum between PVTC-cannulated pigs either with or without an intact caecum.

The apparent faecal digestibility of nutrients was significantly affected by diet composition. Digestibility for dry matter, ash, OM and N-free OM was significantly lower for diet III with sugarbeet pulp ($P < 0.05$). On the other hand, apparent crude protein digestibility was highest for the high-protein diet II ($P < 0.05$). Caecum removal significantly decreased faecal digestibility of the ash fraction (50.4 vs. 56.6; $P < 0.05$). This effect was apparent for all three diets, but most pronounced for diets I and II.

Different studies have shown that caecectomy in pigs does not affect faecal digestibility (Gargallo and Zimmerman, 1981; Pérez-Lanzac *et al.*, 1990) but could increase overall retention time of digesta (Pérez-Lanzac *et al.*, 1990). Our data suggest that the caecum could play a role in mineral absorption or release of the minerals from the ingredient matrix by the fermentative action in the caecum.

The caecum has been shown to be an active site of fermentation in pigs (Gargallo and Zimmerman, 1981; Glitso *et al.*, 1998). The colon in pigs, however, seems to have an adequate compensatory fermentative capacity when the caecum is removed.

References

- Gargallo, J. and Zimmerman, D.R. (1981) Effects of dietary cellulose levels on intact and cecectomised pigs. *Journal of Animal Science* 53, 395–402.
- Glitso, L.V., Brunsgaard, G., Hojsgaard, S., Sandstrom, B. and Bach Knudsen, K.E. (1998) Intestinal degradation in pigs of rye dietary fibre with different structural characteristics. *British Journal of Nutrition* 80, 457–468.
- Pérez-Lanzac, J., Thielmans, M.F and Bodart, C. (1990) A note on the effect of caecectomy in pigs fed different amounts of dietary fibre. *Annales Zootechnique* 39, 221–231.
- van Leeuwen, P., van Kleef, D.J., van Kempen, G.J.M., Huisman, J. and Verstegen, M.W.A. (1991) The post-valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acids in maize, groundnut and sunflower meal. *Journal of Animal Physiology and Animal Nutrition* 65, 183–193.

94 Effects of Dietary Supplementation of Exogenous Fibre on the Faecal Excretion of Major Odour-causing Volatile Compounds in Pigs

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A study was conducted to examine effects of dietary supplementation of exogenous fibre in maize and soybean meal-based diets on the faecal concentrations of various odour-causing volatile compounds in pigs. Ammonia (NH₃) and hydrogen sulphide (H₂S) were the major target compounds. Other detected volatile compounds were volatile fatty acids (VFA), their derivatives (AA: acetic acid; BA: butyric acid; PA: propionic acid; 2-MPA: 2-methyl propionic acid; 3-MBA: 3-methyl butyric acid; 2-MBA: 2-methyl butyric acid; and PTA: pentanoic acid), phenol (*p*-cresol) and indoles (skatole). Dietary inclusion of cellulose and pectin at the levels of 4.5 and 9.0% appeared to increase the concentrations of various VFA and their derivatives in faeces, suggesting increased microbial fermentation activity in the hindgut. However, dietary inclusion of cellulose and pectin at the tested levels did not reduce ($P > 0.05$) the concentrations of four major volatile odour compounds including NH₃, H₂S, *p*-cresol and skatole. Because of their relatively high content, NH₃ and H₂S are the major odour-causing compounds in swine manure.

Introduction

Odour associated with swine manure has become a serious environmental issue facing the swine industry (e.g. Sutton *et al.*, 1999). Various volatile organic compounds are known to be largely responsible for the characteristic swine odour (Mackie *et al.*, 1998). Dietary strategies have been shown to be effective in controlling the production and emission of odour-causing compounds (e.g. Sutton *et al.*, 1999). This study was conducted to examine effects of sources and levels of fibre on the excretion

of various odour-causing volatile compounds in pigs.

Materials and Methods

Animals and diets

Five Yorkshire barrows (40–100 kg) were individually housed in stainless-steel metabolism crates and fed experimental diets (Table 94.1) according to a 5 × 5 Latin square design. Each experimental period lasted for 14 days with 10 days for

adaptation and 4 days for collection of faecal samples.

Sample collection and analysis

Faecal samples were frozen, pulverized to be homogeneous under liquid nitrogen and stored at -75°C for the analysis of ammonia (NH_3), hydrogen sulphide (H_2S) and other volatile odour-causing compounds. NH_3 and H_2S were analysed by spectrophotometric-based procedures. Pulverized frozen faecal samples (2 g) were extracted with 100% methanol (6 ml); the extract was analysed for other volatile odour compounds by gas chromatography–mass spectrometry with decanol ($40\text{ }\mu\text{g ml}^{-1}$) as an internal standard.

Statistical analysis

Because of missing values for some of the endpoint analysis, data were subjected to least square analyses of variance as well as normal analysis of variance. Where appropriate, means and least square means between each treatment and the control diet were compared by Dunnett's test with SAS procedures.

Results and Discussion

It is not very clear whether volatile fatty acids (VFA) and their derivatives contribute to the odour of swine manure. There is no evidence that these VFA can be potentially harmful to the environment.

Table 94.1. Formulation ($\text{g } 100\text{ g}^{-1}$, on as-fed basis) of experimental diets.

Ingredients	Experimental diets				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Soybean meal	29.0	29.0	29.0	29.0	29.0
Maize	51.0	51.0	51.0	51.0	51.0
Maize starch	16.7	8.6	0.2	8.6	0.2
Solkafloc (cellulose)	0.0	4.5	9.0	0.0	0.0
Pectin				4.5	9.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Other	2.8	6.4	10.3	6.4	10.3
Calculated nutritive values:					
Digestible energy (MJ kg^{-1})	14.51	14.47	14.20	14.20	14.21
Crude protein (%)	17.0	17.0	17.0	17.0	17.0

Table 94.2. Faecal contents (mg g^{-1} dry matter faeces, mean $\pm$ SE; $n = 4$ or 5) of volatile fatty acids and their derivatives in response to exogenous fibre.

Items	Experimental diets				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
AA, acetic acid	21.94 ± 3.69	27.09 ± 4.12	21.92 ± 3.69	19.26 ± 3.69	30.49 ± 3.69
BA, butyric acid	5.90 ± 2.12	7.29 ± 2.37	9.97 ± 2.12	8.66 ± 2.12	10.52 ± 2.12
2-MPA, 2-methyl-propionic acid	1.16 ± 0.19	0.91 ± 0.20	1.26 ± 0.19	1.12 ± 0.19	1.03 ± 0.19
2-MBA, 2-methyl butyric acid	0.77 ± 0.10	0.54 ± 0.12	0.71 ± 0.10	0.68 ± 0.10	0.83 ± 0.10
3-MBA, 3-methyl butyric acid	0.93 ± 0.14	0.61 ± 0.15	0.81 ± 0.14	0.81 ± 0.14	0.83 ± 0.14
PA, propionic acid	6.73 ± 1.22	8.29 ± 1.36	7.71 ± 1.22	7.53 ± 1.22	12.08 ± 1.22
PTA, pentanoic acid	1.35 ± 0.46	1.12 ± 0.52	1.78 ± 0.46	1.26 ± 0.52	2.31 ± 0.46

The faecal excretion of VFA and their derivatives is presented in Table 94.2. Dietary inclusion of 9% pectin increased ($P < 0.05$) propionic acid content. Although dietary inclusion of 4.5 and 9.0% cellulose and apple pectin appeared to increase most VFA contents, the increases were not significant in most cases ($P > 0.05$). NH_3 and H_2S are not only responsible for typical swine odour but also potentially cause damage to the environment, contributing to acid rain and the pollution of freshwater

resources (e.g. Mackie *et al.*, 1998). Supplementation of exogenous fibre did not decrease ($P > 0.05$) the faecal content of NH_3 and H_2S (Fig. 94.1). Furthermore, inclusion of extra fibre did not affect ($P > 0.05$) the faecal content of two other key odour-causing compounds, *p*-cresol and skatole (Fig. 94.2). It is important to note that NH_3 and H_2S are the leading air pollutants due to their relatively high concentrations. Further research should target these compounds.

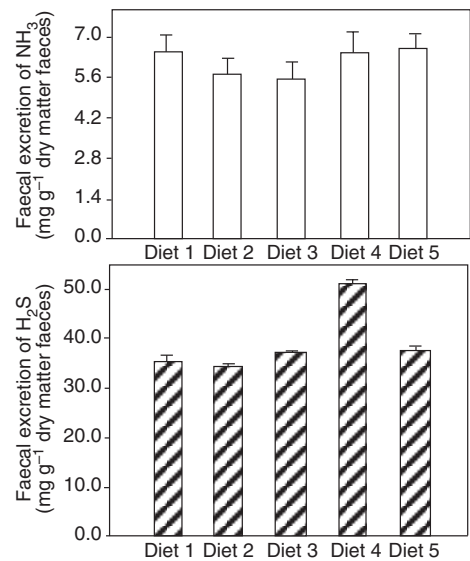


Fig. 94.1. Faecal NH_3 and H_2S contents response to exogenous fibre feeding.

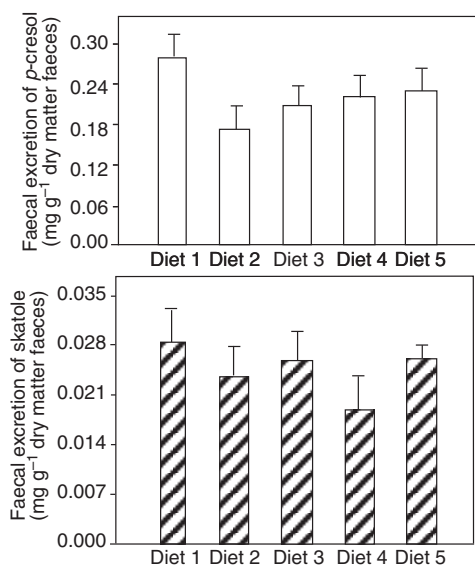


Fig. 94.2. Faecal *p*-cresol and skatole contents response to exogenous fibre.

References

- Mackie, R.I., Stroot, P.G. and Varel, V.H. (1998) Biochemical identification and biological origin of key odor components in livestock waste. *Journal of Animal Science* 76, 1331–1342.
- Sutton, A.L., Kepharat, K.B., Verstegen, M.W.A., Canh, T.T. and Hobbs, P.J. (1999) Potential for reduction of odorous compounds in swine manure through diet modification. *Journal of Animal Science* 77, 430–439.

95 Reduced Faecal Excretion of Calcium, Phosphorus and Nitrogen by Young Pigs Fed Low Phytic Acid Barley

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Diets containing low phytic acid mutant barley (MB) compared with normal barley (NB) reduced faecal excretion of phosphorus (P), calcium (Ca) and nitrogen (N) by 56, 33 and 38%, respectively, and increased absorption by 24, 19 and 7%, respectively, when barley was the only source of phytic acid in the diet. When soybean meal was the protein source, faecal P excretion was reduced 15% by MB compared with NB. In conclusion, MB significantly reduced the faecal excretion of P, Ca and N, and increased the absorption of P and Ca compared to NB.

Introduction

Most of the phosphorus (P) in cereal grains and oilseed meals (60 to 80%) is bound in the form of phytate (myo-inositol hexaphosphate) (Maga, 1982) and is poorly available to swine (NRC, 1998). Because of the poor availability of phytate P, about 200,000 tons of P are excreted annually in swine manure in the USA (Cromwell and Coffey, 1991). Large pork production farms usually apply manure to the land at maximum allowable amounts. In this situation the P applied exceeds the requirement for plant growth. Excess P is bound to soil particles and becomes a potential environmental problem when it contaminates lakes and streams as a result of soil erosion (Sorenson, 1988). Therefore, it is prudent to reduce the amount of P excreted in animal waste to minimize environmental P pollution.

Methods

Crossbred barrows ($n = 35$) averaging 13.5 kg were used to evaluate a low phytic acid mutant barley (MB) containing the 1pa1-1 allele compared with a near-isogenic normal hybrid barley (NB) in a 35-day experiment. The MB and NB, respectively, contained 0.35 and 0.35% total phosphorus (tP) and 0.21 and 0.11% phytate-free (estimated available) P (aP). Of the five treatments, T1 was an NB diet containing 0.14% aP and 0.32% tP; T2 was an MB diet containing 0.22% aP and 0.32% tP; T3 was T1 with monosodium phosphate added to increase aP to 0.22% to equal the aP in diet 2; T4 was an NB diet containing 0.57% tP, 0.30% aP and 0.65% Ca; and T5 was an MB diet containing 0.50% tP, 0.30% aP and 0.65% Ca. T1 to T3 were supplemented with whey protein concentrate, blood cells and ground limestone to bring

Ca to 0.50%. T4 and T5 were practical diets supplemented with soybean meal, ground limestone and dicalcium phosphate to meet NRC (1998) requirements. Pigs were housed in individual metabolism pens and fed to appetite. Diets contained 0.05% chromic oxide as a non-digestible indicator to determine nutrient digestibilities. Data were analysed by analysis of variance using GLM procedures of SAS. Predetermined single df treatment comparisons were T1 vs. T2, T1 vs. T3, T2 vs. T3, and T4 vs. T5.

Results and Discussion

Mutant barley significantly reduced faecal excretion and increased absorption of P, Ca

and N compared with NB (Table 95.1). The reduction in faecal excretion was greatest for T2 vs. T3 when barley was the only source of phytate in the diet. When soybean meal replaced whey protein concentrate and blood cells as the protein supplement, the reduction in faecal excretion of nutrients was not as dramatic (T4 vs. T5). These results with low phytic barley are in agreement with our earlier work with low phytic acid maize (Veum *et al.*, 1998). Reducing the phytic acid content of the cereal grain without lowering the total P content results in an increase in the available P, which is directly available to the animal, as shown by the results of our experiments.

The reduction in phytic acid in this low phytic acid barley did not produce any

Table 95.1. Intake, excretion and absorption (g day⁻¹) of phosphorus, calcium and nitrogen.

Item	Treatments ^a				
	T1 NB+WPC	T2 MB+WPC	T3 NB+iP+WPC	T4 NB+SBM	T5 MB+iP
Phosphorus					
Intake ^b	5.02	5.22	6.81	9.59	7.80
Faeces ^c	2.95	1.78	4.03	4.37	3.70
Absorbed ^d	2.07	3.44	2.78	5.22	4.10
Urine	0.02	0.06	0.09	0.16	0.11
Calcium					
Intake	7.49	8.13	7.71	9.74	9.89
Faeces ^e	2.14	1.33	1.99	3.19	3.05
Absorbed ^f	5.35	6.80	5.72	6.55	6.84
Urine ^g	2.90	2.42	1.79	1.06	1.34
Nitrogen					
Intake ^h	34.16	36.85	39.77	41.78	42.86
Faeces ⁱ	8.26	8.00	12.89	9.51	11.57
Absorbed	25.91	28.84	26.88	32.27	31.29
Urine ^j	1.79	1.28	1.32	2.06	1.86

^aNB, normal barley; MB, mutant barley; WPC, whey protein concentrate; iP, inorganic P; SBM, soybean meal.

^bT1 and T2 vs. T3, and T4 vs. T5 ($P < 0.01$).

^cT1 vs. T2 and T3 ($P < 0.03$), T2 vs. T3 ($P < 0.0001$).

^dT2 vs. T1 ($P < 0.001$) and T3 ($P < 0.03$).

^eT2 vs. T1 ($P < 0.05$) and T3 ($P < 0.10$).

^fT2 vs. T1 and T3 ($P < 0.01$).

^gT3 vs. T1 ($P < 0.01$) and T2 ($P < 0.09$).

^hT1 vs. T3 ($P < 0.08$).

ⁱT3 vs. T1 and T2 ($P < 0.03$).

^jT1 vs. T2 and T3 ($P < 0.15$).

detrimental nutritional effects, as indicated by the fact that average daily gain, average daily feed intake and feed efficiency were similar for T2 vs. T3 and T4 vs. T5 (data not shown). The forces required to break the third metacarpal and the radius bone from the right front foot were also similar for T2 vs. T3 and T4 vs. T5 (data not shown).

There were few differences between T4 and T5 in the criteria measured because these were our practical diets that were formulated to contain equal amounts of Ca and aP. However, the practical diet containing NB (T4) required more inorganic P supplementation to adjust for the higher concentration of phytic acid.

Conclusions

Mutant low phytic acid barley significantly increased P, Ca and N absorption and reduced P, Ca and N excretion compared with normal barley when barley was the only source of phytic acid.

Reducing the phytic acid content in the mutant barley did not produce any detrimental nutritional effects, based on pig performance and bone breaking strength.

Pig performance and bone breaking strength confirmed our estimate of 0.21% available P in MB and 0.11% available P in NB. Therefore, this mutant barley contained about twice the available P of the normal barley.

References

- Cromwell, G.L. and Coffey, R.D. (1991) Phosphorus – a key essential nutrient, yet a possible major pollutant – its central role in animal nutrition. In: Lyons, T.P. (ed.) *Biotechnology in the Feed Industry*. Alltech Technical Publications, Nicholasville, Kentucky.
- Maga, J.A. (1982) Phytate: its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *Journal of Agricultural and Food Chemistry* 30, 1–9.
- NRC (1998) *Nutrient Requirements of Swine*, 10th revised edn. National Academy Press, Washington, DC.
- Sorenson, A. (1988) Reconciling agriculture's need with water quality. *Proceedings New Mexico Water Resources Research Institute*, Sante Fe, New Mexico (Oct. 28).
- Veum, T.L., Raboy, V., Ertl, D. and Ledoux, D. (1998) Low phytic acid corn improves calcium and phosphorus utilization for growing pigs. *Journal of Animal Science* 76, Supplement 1, 177 (Abstract).

96 *In vivo* and *in vitro* Ileal Digestibility of Protein and Amino Acids in Barleys

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The aim of the study was the determination of *in vivo* and *in vitro* protein and amino acid ileal digestibility of seven barleys and comparison of both methodologies. The *in vivo* ileal apparent digestibility of protein and amino acids was determined using six ileorectal anastomosed pigs, which were fed the different barleys according to a seven-period design. The *in vitro* protein and amino acid digestibility of the seven barleys was determined using the two-step enzymatic method described by Boisen and Fernández (1995). The relationship between *in vivo* and *in vitro* determinations was compared and it appears to be in good agreement.

Introduction

Several *in vitro* methods for feed evaluation have been developed as alternatives to traditional expensive and time-consuming *in vivo* trials. However, there is a dearth of adequately conducted validation studies for *in vitro* digestibility assays. It appears that the three-step (pepsin, pancreatin, Viscozyme) multi-enzyme closed system proposed by Boisen and Fernández (1997) to allow prediction of organic matter and gross energy digestibility in the pig has particular promise for practical feed evaluation. The situation for the *in vitro* prediction of protein and amino acid digestibility is not so clearly defined. The two-step multi-enzyme system (Boisen and Fernández, 1995) appears to be the most popular approach for practical application but has led to variable results. The objective of this study was to evaluate the relationships between the obtained digestibilities of crude protein (CP) and amino acids (AA) with the *in vitro* method proposed by

Boisen and Fernández (1995) and the corresponding *in vivo* values.

Material and Methods

Samples

Seven varieties of Spanish barley were used in this study for the determination of their *in vivo* and *in vitro* CP and AA digestibility. Diets used in the *in vivo* trial were based on the barleys as the sole source of energy and protein (94.5% barley, 5.5% vitamin/mineral complex).

In vivo trial

Six male pigs (Landrace × Large White) were surgically modified with an ileorectal anastomosis (IRA) and housed individually in metabolism cages. Pigs were fed twice a day, being offered about 90 g feed kg^{0.75} day⁻¹ and with water continuously avail-

able. Pigs were fed the seven diets according to a seven-period design. Each period consisted of two parts: an adaptation period of 5 days followed by a 2-day collection period. Ileal digesta was homogenized and aliquots were taken for CP and AA analysis.

In vitro technique

Samples of about 0.5 g were treated using a two-step enzymatic digestion (pepsin + pancreatin), basically according to the methodology described by Boisen and Fernández (1995). The undigested residues were obtained by filtration, their CP and AA content was analysed and the *in vitro* digestibility was calculated.

Results and Discussion

According to Boisen and Fernández (1995), endogenous protein losses (EPL) can be predicted from *in vitro* undigested dry matter (UDM) from the equation:

$$\text{EPL (g kg}^{-1} \text{ DM intake)} = 13.2 + (0.066 \times \text{UDM (g kg}^{-1} \text{ DM)}).$$

So the prediction of apparent ileal digestibility of protein ($\text{pdN}_{\text{ileal}}$) can be made by using the equation:

$$\text{pdN}_{\text{ileal}}(\%) = \frac{\text{dN}_{\text{in vitro}}(\%) - 100 \times 13.2 + (0.66 \times \text{UDM})(\text{g kg}^{-1} \text{ DM})}{\text{protein feed (g kg}^{-1} \text{ DM)}}$$

Table 96.1. *In vitro* vs. *in vivo* apparent protein digestibility in barleys.

Barley	<i>In vitro</i> N digestibility (real) (%)	Undigested dry matter (g kg ⁻¹ DM)	Apparent protein digestibility (%)	
			<i>In vitro</i>	<i>In vivo</i>
Klaxon	86.5	198.8	62.3	68.6
Trait Union	89.4	185.8	68.4	66.9
Beka	88.1	180.5	66.9	68.4
Pallas	88.2	203.4	67.2	67.9
Patty	87.6	192.4	66.3	64.5
Barbarrosa	88.9	198.5	69.8	74.1
H. Grinyon	88.5	213.4	67.9	68.7

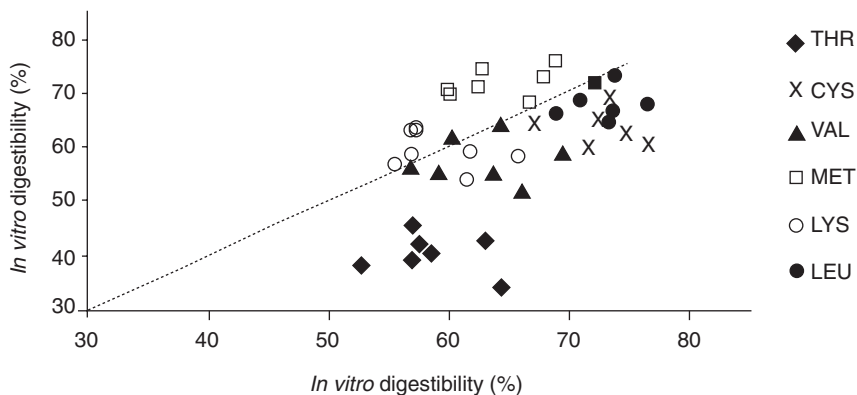


Fig. 96.1. Relationship between *in vitro* and *in vivo* apparent digestibility of amino acids in barleys.

Comparison between *in vitro* and *in vivo* apparent protein digestibility is shown in Table 96.1. A very good agreement can be seen for five of the seven barley samples, the exceptions being Klaxon and Barbarrosa with a low *in vitro* value and a high *in vivo* value, respectively. However, the relationship between these values, given by the equation $dN_{in\ vivo} = (0.48 \times pdN_{in\ vitro}) + 36.1$, has a very low regression coefficient ($r^2 = 0.15$). This low correlation is mainly due to the small number of samples and the narrow variability range in their chemical composition. Under these conditions just one sample can have a significant effect, as can be appreciated by the correlation obtained without including the Klaxon barley in the regression ($r^2 = 0.72$). Alternatively, when *in vivo* or *in vitro* data from three other samples, including barley, soybean meal and rapeseed meal (used as standards – not shown) are added to the data on the seven barleys, the regression coefficient is clearly improved ($r^2 = 0.83$).

Similarly, the relationships between individual amino acids result in low correlation values. However, the *in vitro* method appears to be able to detect differences between the amino acids (Fig. 96.1), with the exception of threonine, which results in unusually low values for the *in vitro* method.

Better and more accurate prediction equations could be obtained by combining the *in vitro* digestibility values with some particular information on the chemical composition of the barley.

It can be concluded that prediction equations give reasonable digestibility values for individual samples.

Acknowledgements

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References

- Boisen, S. and Fernández, J.A. (1995) Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analysis. *Animal Feed Science and Technology* 51, 29–43.
- Boisen, S. and Fernández, J.A. (1997) Prediction of the total tract digestibility of energy in feedstuffs and pigs diets by *in vitro* analyses. *Animal Feed Science and Technology* 68, 277–286.

97 Effect of Protein Source and Feed Intake Level on Histology of the Small Intestine in Newly Weaned Piglets

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This experiment was conducted to evaluate the effect of feeding weaned piglets a diet containing milk protein or feather meal protein as the main protein source at restricted or *ad libitum* level on the histology of the small intestinal mucosa from 0 to 14 days post weaning. Tissue samples were taken in the proximal, mid and distal part of the jejunum. Protein source, feed intake level and time postweaning significantly affected villus–crypt structure in the small intestine. At the *ad libitum* feed intake level, milk protein had higher villus length and crypt depth.

Introduction

In pigs, weaning is associated with adverse effects such as low and variable feed intake, growth stasis and compromised integrity of the epithelium of the small intestine. These adverse effects can be both diet dependent and diet independent (McCracken *et al.*, 1995). One diet-dependent factor is composition, which, together with digestive capacity of the animal and feed intake level, will determine the amount and type of nutrients available in the small intestine for digestion or fermentation. Low intake and/or high microbial activity in the gut lumen may cause an inflammatory response and villus atrophy in the small intestine and hence affect nutrient digestion and absorption. This experiment was performed to evaluate the effect of feed intake level and protein source on histology of the small intestinal tissue.

Material and Methods

Two experimental diets were used. The two diets had a crude protein content of 195 g kg⁻¹ of which 80 g kg⁻¹ was based on either highly digestible protein from milk or poorly digestible protein from hydrolysed feather meal. Both diets were fed either *ad libitum* or at a restricted level. In total 156 newly weaned York × (Dutch Landrace × Finnish Landrace) barrows (age ± 27 days) were used. Creep feed was not provided during the suckling period. On the day of weaning, piglets were transported 10 km to our research facility, where they were weighed and individually housed in floor pens 50 × 90 cm² with transparent plastic walls. Each pen was equipped with a plastic feeder with water supplied via a nipple drinker.

On the day of weaning, gut tissue samples were taken from 12 animals as reference value. The remaining 144 piglets were

weighed and divided into four groups. Two groups of 54 piglets each were fed one of the experimental diets *ad libitum*. At day 3 postweaning (PW), 18 piglets with high voluntary feed intake (VFI) in each of the *ad libitum* fed groups were selected; the remaining 36 piglets from each of those groups were excluded from the trial. Two groups of 18 piglets each were fed one of the experimental diets at a restricted level. The restricted feeding level was 40 g day⁻¹ during the first 3 days PW; from day 4 onwards the restricted feeding level was 33% that of the previous-day VFI of the *ad libitum* fed piglets. Feed was given four times per day at 0800, 1200, 1600 and 2000 h.

At the day of weaning and at 4, 7 and 14 days PW a midline laparotomy was performed under complete anaesthesia by inhalation of a mixture of N₂O/O₂ and isoflurane. Tissue samples were taken from three jejunal sites: 0.5 m and 3.5 m distal from the ligament of Treitz and 0.5 m proximal to the ileocaecal ligament. For macroscopic histology the tissue samples were treated according to previously described procedures (Kik *et al.*, 1990).

Results and Discussion

Milk protein resulted in higher villus length compared with feather meal protein, but this was significant ($P < 0.05$) only in the mid-jejunum. *Ad libitum* feed intake resulted in significant ($P < 0.01$) higher villus length and crypt depth than restricted feed intake at all three sites in the jejunum. *Ad libitum* feed intake resulted in lower villus length to crypt depth ratio at the mid-jejunum (Table 97.1). Pluske *et al.* (1996) and Makkink *et al.* (1994) also found a relationship between voluntary feed intake and mucosal architecture in weaned piglets with a compromised villus-crypt structure due to low feed intake reported by McCracken *et al.* (1995, 1999) and Beers-Schreurs (1996). Milk protein at the *ad libitum* feed intake level resulted in higher villus length and crypt depth compared with the feather meal protein. This was in contrast with McCracken *et al.* (1999), Beers-Schreurs (1996) and Makkink *et al.* (1994), who did not find an effect of diet (protein) composition on mucosal architecture. This difference may be due to the higher cumulative

Table 97.1. The effect of protein source (PS) (milk, feather) and feed intake level (L) on histological parameters (µm) at the proximal (Prox), mid (Mid) and distal (Dist) jejunum of weaned piglets.

	Intake level				Level of significance		
	Restricted		<i>Ad libitum</i>				
	Milk	Feather	Milk	Feather	PS	L	PS × L
Villous							
Prox	251 ^a	241 ^a	330 ^b	298 ^b	NS	***	NS
Mid	249 ^a	242 ^a	320 ^b	261 ^a	*	**	t
Dist	169 ^a	184 ^a	266 ^c	224 ^b	NS	***	*
Crypt							
Prox	188 ^a	211 ^a	281 ^c	245 ^b	NS	***	**
Mid	180 ^a	184 ^a	254 ^c	231 ^b	NS	***	t
Dist	148 ^a	160 ^a	194 ^b	180 ^b	NS	***	t
V:C							
Prox	1.4	1.2	1.2	1.2	NS	NS	NS
Mid	1.4 ^a	1.3 ^{ab}	1.3 ^{ab}	1.1 ^b	NS	*	NS
Dist	1.2	1.2	1.4	1.3	NS	t	NS

^{abc} Least square means in the same row without a common character in the superscript differ significantly ($P < 0.05$).

NS, not significant; t, $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

voluntary feed intake at day 7 in this trial in combination with bioactive compounds in milk and/or the negative effects associated with feather meal protein. Time post weaning significantly affected villus length and crypt depth. Villus length was reduced at day 4 (37%) and day 7 (32%) PW, com-

pared with villus length at the day of weaning. Villus length at day 14 PW was increased back to 94% of the length at the day of weaning. Crypt depth was increased at day 4 (13%), day 7 (30%) and day 14 (39%) PW, compared with crypt depth at the day of weaning.

Reference

- Beers-Schreurs, H.M.G. van (1996) Changes in the function of the large intestine of weaned pigs. PhD thesis, University of Utrecht, The Netherlands.
- Kik, M.J.L., Huisman, J., van der Poel, A.F.B. and Mouwen, J.M.V.M. (1990) Pathologic changes of the small intestinal mucosa of piglets after feeding of *Phaseolus vulgaris* beans. *Veterinary Pathology* 27, 329–334.
- Makkink, C.A., Negulescu, G.P., Guixin, Q. and Verstegen, M.W.A. (1994) Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. *British Journal of Nutrition* 72, 353–368.
- McCracken, B.A., Gaskins, H.R., Ruwe-Kaiser, P.J., Klasing, K.C. and Jewell, D.E. (1995) Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *Journal of Nutrition* 125, 2838–2845.
- McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A. and Gaskins, H.R. (1999) Weaning anorexia may contribute to local inflammation in the piglet small intestine. *Journal of Nutrition* 129, 613–619.
- Pluske, J.R., Williams, I.H. and Aherne, F.X. (1996) Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. *Animal Science* 62, 145–158.

98 Effect of a CCK-A Receptor Blocker, Tarazepide, on Pig Feeding Behaviour

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The CCK-A receptor blocker Tarazepide (Solvay Pharmaceuticals GmbH, Hannover, Germany) was orally administered, in three different doses, to growing pigs with a small pre-feed. Feeding behaviour changed for a few hours after treatment. Pigs were lazy and calm. There was a slight tendency towards reduced food consumption; however, lowered feed consumption did not affect production results.

Introduction

Cholecystokinin (CCK) is recognized as a regulator of satiety (Moran *et al.*, 1993; Rose *et al.*, 1996) and nociception (Baber *et al.*, 1989). It is probable that these processes are mainly mediated by CCK-A receptors located in the brain but peripheral CCK-A receptors may also participate.

The aim of this investigation was to find whether blockade of the CCK-A receptor by Tarazepide (TA) affected appetite, feeding behaviour, feed consumption and body weight gain in the growing pig.

Material and Methods

Investigations were conducted under production conditions directly after moving gilt-weaners from nursery to fattening quarters. Animals were weighed every week and studies started when pigs reached an average of about 30 kg body weight (BW). TA was given 15 min before the morning meal at 0800 h; the evening meal was given at 1500 h.

Two experiments were performed. The first was performed on ten gilts kept in individual pens, the second on 20 gilts of which half were kept in individual pens and half in group pens. TA was given every 3 days in increasing doses: 0.1 mg, 1 mg and 10 mg kg⁻¹ BW, while the highest dose (10 mg kg⁻¹ BW) was also given over 6 successive days. In the first experiment, animals were given their daily feed portion (c.2% BW) for 1 h and after this time the remainder was taken out and weighed. During the second experiment, gilts were given the same amount of feed but it stayed in the feeders until the next meal, at which time the feed refusal was weighed.

Results

There were no special behavioural effects of TA at 0.1 and 1 mg. However, compared

with other days, it was observed that those pigs receiving 1 mg TA ate the whole meal portion within 30 min and they were calmer. After the meal they lay down immediately, but they stood up if somebody was near the feeder. When pigs received 10 mg TA they ate more slowly: it took them 1 h to eat the whole morning portion. It was also observed that pigs took small breaks during feed consumption, lying down for some minutes before standing up to finish the meal. They exhibited signs of aggression when the rest of the feed was removed after 1 h. Pigs were more aggressive and more nervous at the morning meal. There were no statistically significant changes in daily feed intake in pigs receiving Tarazepide at any dose of TA in experiment 1 or 2.

Discussion

In summary, there was no confirmed statistical influence of TA on pig production results. Differences between experimental groups were rather more consistent with differences in animal maintenance. Distinct influences of TA were observed on animal behaviour. After TA consumption pigs became lazy and calm; they tended to eat more slowly and less than usual. It is interesting that this influence was short-lived and there was no difference if TA was given once or successively for 6 days. TA was effective for about 5–6 h. It is very puzzling that lowered feed consumption by TA had no negative effects on production results when comparing the control group with the group receiving TA.

Acknowledgements

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References

- Baber, N.S., Dourish, C.T. and Hill, D.R. (1989) The role of CCK, cerulein, and CCK antagonists in nociception. *Pain* 39, 307–328.
- Moran, T.H., Ameglio, P.J., Jackson Peyton, H., Schwartz, G.J. and McHugh (1993) Blockade of type A, but not type B, CCK receptors postpones satiety in rhesus monkeys. *American Journal Physiology* 265, R620–R624.
- Rose, Ch., Vargas, F., Facchinetti, P., Bourgeat, P., Bambal, P.B., Bishop, P.B., Chan, S.M.T., Moore, A.N.J., Ganellin, C.R. and Schwartz, J.-Ch. (1996) Characterization and inhibition of a cholecystokinin-inactivating serine peptidase. *Nature* 380, 403–409.
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99 The Survival of Potentially Pathogenic *E. coli* in Fermented Liquid Feed

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Fermentation by lactic acid bacteria can be used as a means of eliminating *Escherichia coli* from liquid feed for pigs. The survival rate of six strains of *E. coli* in fermented liquid feed (FLF) maintained at 20°C or 37°C was determined. At 20°C it took between 3 h and 9 h for *E. coli* to be eliminated from FLF, whereas at 37°C no *E. coli* were detectable after 2.5 h. This has important implications in the management of liquid feed systems.

Introduction

Escherichia coli is responsible for a number of transmissible and non-transmissible pig diseases in the postweaning period. A common route for *E. coli* to enter the piglet is through the feeding of contaminated feedstuffs. This has implications for the dissemination of enteric disease in pigs and the subsequent spread of these organisms through the food chain to humans.

Liquid feed provides an ideal medium for the proliferation of enteric pathogens. Therefore, unless it is sterilized, liquid feed has the potential to be a vector for

such organisms. Fermenting liquid feed with lactic acid bacteria results in a feed with a pH of *c.* 3.8 containing 132–244 mM lactic acid. The combination of low pH and lactic acid has an antimicrobial effect which enables fermented liquid feed (FLF) systems to resist contamination by pathogenic bacteria (Jensen and Mikkelsen, 1998). In an on-farm process, FLF may be maintained as a continuous fermentation by retaining a proportion of FLF to act as an inoculum for new feed. However, on many farms the temperature of FLF is not regulated and is subject to fluctuations. Many bacteria respond to environmental

stress by initiating a ‘shock’ response, involving the synthesis of shock proteins that reduce membrane permeability and aid DNA repair (Rowbury, 1995; Phadtare *et al.*, 1999; White, 2000). Furthermore, specific shock proteins synthesized in response to one environmental stress may confer enhanced survivability to other environmental stresses. *E. coli* produce cold shock proteins in response to a reduction in temperature from its optimum growth temperature of 37°C (Phadtare *et al.*, 1999).

The objective of this study was to examine the effect of temperature on the survival of *E. coli* in FLF.

Materials and Methods

A study was conducted according to a factorial design in which factor 1 was the temperature of the fermentation (20°C or 37°C), factor 2 was the duration of fermentation (48 h, 72 h or 96 h) and factor 3 was the strain of *E. coli* (K88(99), K88(100), K88(101), O157:H7, K99(185) or K99(230)).

Fermented liquid feed was prepared by

inoculating 800 ml liquid feed (1 part γ (Co⁶⁰) irradiated feed, 3 parts sterile water) with *c.* 10⁶ CFU ml⁻¹ *Lactobacillus plantarum* (Alltech Inc., Nicholasville, Kentucky, USA). Feeds were fermented at 20°C or 37°C for 48 h, 72 h or 96 h. Triplicate samples of each FLF were inoculated with *c.* 10⁷ CFU ml⁻¹ of each strain of *E. coli*. The number of viable *E. coli* remaining in the feed was enumerated at 15 min intervals for 3 h after inoculation and again 9 h after inoculation. Viable *E. coli* were enumerated by serially diluting 1 ml of FLF in maximal recovery diluent (MRD) and spread-plating on to violet red bile agar (VRBA). The decimal reduction time (DRT) was calculated for each strain of *E. coli* for each fermentation time/temperature regime. The concentration of lactic acid in the supernatant of centrifuged samples of FLF was determined by HPLC using an Aminex HPX-87H cation exchange column (Biorad, Hemel Hempstead, UK). Samples were eluted with 0.5 mM sulphuric acid, and lactic acid content was measured using a Varian 9050 UV detector (Varian Assoc., Walton-on-Thames, UK). Statistical analyses of DRT

Table 99.1. Decimal reduction time of six strains of *E. coli* in FLF fermented for 48 h, 72 h or 96 h.

Time	<i>E. coli</i> strain						SED
	K88(99)	K88(100)	K88(101)	O157:H7	K99(185)	K99(230)	
48 h	25.2 ^a	26.1 ^a	22.3 ^b	12.16	22.0 ^b	22.2 ^b	0.96
72 h	23.7 ^a	23.6 ^a	24.2 ^a	9.3	16.5 ^b	14.6 ^b	0.96
96 h	22.9 ^a	17.4 ^b	24.3 ^a	10.3	15.8 ^b	14.0 ^b	0.96

^{ab} Means are not significantly different (*P* > 0.05).

Table 99.2. Lactic acid concentration (mM) of liquid feed fermented for different time periods at 20°C or 37°C.

Fermentation time (h)	Temperature	
	20°C	37°C
0	1.0 ^c	1.0 ^c
48	133 ^a	254 ^b
72	192 ^a	239 ^b
96	229 ^b	245 ^b

^{a,b,c} means are not significantly different (*P* > 0.05).

and lactic acid concentration were carried out by analysis of variance using Minitab (10.2).

Results

There was no significant reduction in the numbers of any strain of *E. coli* over the 3 h test period in FLF samples challenged with *E. coli* at 20°C. Numbers of *E. coli* remained at 10^7 – 10^8 CFU ml⁻¹ FLF throughout. However, 9 h after inoculation no viable *E. coli* were detected. As no DRT could be calculated for *E. coli* in FLF at 20°C, the data for 37°C FLF were analysed according to a two-factor design (fermentation time vs. *E. coli* strain). These data for DRT are presented in Table 99.1. Factorial analysis of the data showed that the main effect of fermentation time was to reduce the DRT from 21.7 min to 18.7 and 17.5 min (SED 0.39) in FLF fermented for 48 h, 72 h and 96 h, respectively. The interaction between fermentation time and strain was also highly significant ($P < 0.001$).

The results of the lactic acid analyses for FLF are presented in Table 99.2. In FLF fermented at 20°C, lactic acid production was slower than in FLF fermented at 37°C.

Discussion and Conclusions

This study demonstrated that fermentation is an effective mechanism for eliminating potential pathogens such as *E. coli* from liquid feed. However, the rate at which these pathogens die off is dependent upon the temperature at which the liquid feed is maintained. A reduction in temperature from 37°C to 20°C appeared to enable *E. coli* to withstand the antimicrobial effects of lactic acid present in FLF. There was no significant difference in lactic acid levels in FLF fermented at 20°C for 96 h and 37°C for 48 h, 72 h or 96 h. This indicated that lactic acid concentration alone was not responsible for the difference in survival rate between *E. coli* in FLF at 20°C compared with FLF at 37°C. This was possibly due to the expression of cold-shock proteins that enabled *E. coli* to withstand the antimicrobial effects of lactic acid. There were marked differences between the ability of different strains of *E. coli* to withstand the antimicrobial effect of FLF, with O157:H7 being the most susceptible and strains of K88 being the least susceptible. The effect of temperature on the ability of *E. coli* to survive in FLF has implications for the management of liquid feed systems.

References

- Jensen, B.B. and Mikkelsen, L.L. (1998) Feeding liquid diets to pigs. In: Garnsworthy, P.C. and Wiseman, J. (eds) *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, UK, pp. 107–126.
- Phadtare, S., Alsina, J. and Inouye, M. (1999) Cold-shock response and cold-shock proteins. *Current Opinion in Microbiology* 2, 175–180.
- Rowbury, R. (1995) An assessment of environmental factors influencing acid tolerance and sensitivity of *Escherichia coli*, *Salmonella* spp. and other enterobacteria. *Letters in Applied Microbiology* 20, 333–337.
- White, D. (2000) *The Physiology and Biochemistry of Prokaryotes*, 2nd edn. Oxford University Press, Oxford, UK, 565 pp.

100 *In vivo* and *in vitro* Protein and Amino Acid Digestibility of Soybean and Rapeseed Products in Pig Diets

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The apparent ileal protein and amino acid digestibility was determined in soybean meal and rapeseed products by *in vivo* and *in vitro* procedures. The relationship between *in vitro* predicted and *in vivo* values of protein digestibility was closer for soybean ($r^2 = 0.59$) than for rapeseed products ($r^2 = 0.33$). The highest correlation was found for lysine ($r^2 = 0.74$) in mixtures containing soybean meals. It was not possible to predict with satisfactory precision the apparent ileal digestibility of protein and amino acid in rapeseed products using *in vitro* technique. Studies on factors affecting the *in vitro* values of protein digestibility continue.

Introduction

Double-low rapeseed meal (RSM) is commonly used in pig diets as a protein feed to replace soybean meal (SBM). Nutritional value and nutrient availability of RSM and rapeseed cake are affected by technological parameters applied during processing (Grala *et al.*, 1994). In recent years several different *in vitro* methods have been developed for evaluating protein digestibility in pig feeds. Validation of the methods has been attempted by relationship between *in vitro* results and apparent ileal digestibility (e.g. Graham *et al.*, 1989; Babinszky *et al.*, 1990; Cone and van der Poel, 1993; Boisen and Fernández, 1995).

The purpose of this study was to compare *in vivo* (apparent ileal digestibility) and *in vitro* (predicted apparent ileal digestibility calculated from *in vitro* results) values of protein and lysine (Lys), methionine (Met), cystine (Cys) and threonine (Thr) digestibilities in soybean and

rapeseed meals and diets containing soybean meal and differently treated rapeseed cakes.

Materials and Methods

Feeds

Two soybean meals (SBM 1 and SBM 2), two rapeseed meals (RSM 1 and RSM 2) and eight diets were used in the study. Diets 1–5 contained soybean meal (SBM 2), but only in diet 1 as a single source of protein. Diets 6–8 contained rapeseed cakes as the only source of protein. The main components of the diets are given in Table 100.1. SBM and RSM were diluted with maize starch to obtain similar protein levels as in the diets (154–219 g kg⁻¹ dry matter). Samples of diets were representative aliquots from experimental diets used in previous trials with ileofistulated pigs. The samples were stored deep-frozen from

preparation until *in vitro* assays were performed.

Methods

The *in vivo* digestibility trials were performed with pigs within a body weight range of 30–60 kg, as described by Buraczewska *et al.* (1999). The *in vitro* method developed by Boisen and Fernández (1995) was used for prediction of digestibility of protein and amino acids at the ileal level. Precaecal digestion was simulated by two consecutive incubations corresponding to digestion in the stomach and in the small intestine: with pepsin at pH 2.0 for 6 h and with pancreatin at pH 6.8 for 18 h at 39°C. The predicted apparent ileal digestibilities of protein (pdN) and amino acids (pdLys, pdMet, pdCys and pdThr) were calculated from *in vitro* values after correction for endogenous losses using regression equations. All *in vitro* data are mean values of two measurements made in two different series.

Results and Discussion

Protein digestibilities determined by *in vitro* assay (dN *in vitro*) were in all cases higher than the corresponding *in vivo* values (dN). These data are in agreement with values presented elsewhere (Graham *et al.*, 1989; Babinszky *et al.*, 1990; Boisen and

Fernández, 1995). As shown in Table 100.2, calculated values of predicted apparent ileal digestibility of protein from *in vitro* results (pdN) were in general higher than *in vivo* values (dN). The exceptions were RSM 1 and diet 2, which had a little higher dN than pdN. The relationship between pdN and dN was low ($r^2 = 0.46$) for all samples and was closer for soybean ($r^2 = 0.59$) than for rapeseed products ($r^2 = 0.33$). Boisen and Fernández (1995) obtained poor correlation between pdN and dN ($r^2 = 0.57$) for 48 feed mixtures, but high ($r^2 = 0.92$) for 15 feedstuffs. Low relationship between *in vitro* and *in vivo* data ($r^2 = 0.23$) was also found by Cone and van der Poel (1993) for 48 feed mixtures using a similar *in vitro* technique.

Correlations between predicted and *in vivo* estimated digestibility of amino acids (pdAA and dAA) were higher for soybean than rapeseed products for all four amino acids. The closest linear relationship between pdAA and dAA was found for lysine ($r^2 = 0.74$); it was very poor for threonine ($r^2 = 0.02$) in soybean meals and diets. The relationship between pdAA and dAA in rapeseed products was very poor for all amino acids.

Studies continue in order to improve the *in vitro* method for better prediction of amino acid availability from rapeseed products; their high fibre content may induce greater losses of ileal endogenous nitrogen (Grala, 1997).

Table 100.1. The main components of the diets (g kg⁻¹).

	Diets							
	1	2	3	4	5	6	7	8
Soybean meal (SBM 2)	393	140	260	200	118			
Rapeseed meal (RSM 2)					335			
Wheat		830						
Maize			708					
Barley				760				
Rapeseed cake, dehulled toasted						370		
Rapeseed cake, toasted							458	
Rapeseed cake, non-toasted								390

Table 100.2. *In vivo* (dN and dAA) and predicted digestibility (pdN and pdAA) of protein and amino acids calculated from *in vitro* values (%).

	N		Lys		Met		Cys		Thr	
	d	pd	d	pd	d	pd	d	pd	d	pd
SBM1	82.7	84.4	87.2	89.5	87.8	90.4	77.7	82.1	78.2	83.1
SBM2	77.0	83.2	80.2	88.7	81.9	88.7	68.8	80.6	72.2	82.0
Diet 1	77.0	82.6	80.2	88.1	81.9	88.1	68.8	80.3	72.2	83.0
Diet 2	79.4	79.2	75.6	81.5	82.8	86.6	79.7	80.9	74.2	75.1
Diet 3	74.7	77.0	77.8	81.8	80.4	90.2	72.5	79.5	77.0	76.7
Diet 4	73.0	74.9	74.6	80.7	78.4	83.6	70.9	77.0	72.1	72.5
RSM1	72.4	66.6	72.3	77.6	86.1	78.8	71.7	71.8	70.2	70.6
RSM2	59.5	71.9	61.8	80.7	75.3	82.0	60.6	76.8	57.2	74.9
Diet 5	64.8	77.8	68.8	84.8	76.8	80.8	60.6	70.1	63.7	66.8
Diet 6	82.7	84.4	78.6	74.9	87.7	80.5	83.4	68.6	72.3	62.3
Diet 7	69.3	73.3	69.8	68.2	83.9	72.3	71.8	60.7	63.4	55.4
Diet 8	76.2	81.8	81.0	78.5	87.6	84.3	82.0	71.6	72.9	66.7

SBM1, SBM2, RSM1 and RSM2 diluted with maize starch.

References

- Babinszky, L., van der Meer, J.M., Boer, H. and den Hartog, L.A. (1990) An *in vitro* method for prediction of the digestible crude protein content in pig feeds. *Journal of the Science of Food and Agriculture* 50, 173–178.
- Boisen, S. and Fernández, J.A. (1995) Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. *Animal Feed Science and Technology* 51, 23–43.
- Buraczewska, L., Wasilewko, J., Fandrejowski, H., Zebrowska, T. and Han, I.K. (1999) Formulation of pig diets according to ileal digestible amino acid content. *Livestock Production Science* 59, 13–24.
- Cone, J.W. and van der Poel, A.F.B. (1993) Prediction of apparent ileal digestibility in pigs with a two-step *in-vitro* method. *Journal of Food Science* 62, 393–400.
- Graham, H., Löwgren, W. and Åman, P. (1989) An *in vitro* method for studying digestion in the pigs. 2. Comparison with *in vivo* ileal and faecal digestibilities. *British Journal of Science* 61, 689–698.
- Grala, W., Buraczewska, L., Gdala, J. and Pastuszewska, B. (1994) Effect of thermal processing on the protein value of double-low rapeseed products. 1. Effect of toasting temperature on protein value of rapeseed meal for pigs. *Journal of Animal and Feed Sciences* 3, 33–42.
- Grala, W. (1997) Nitrogen utilization in pigs as affected by dietary induced losses of ileal endogenous nitrogen. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

101 Cluster Analysis for Meat and Bone Meals (MBM) from Brazil and USA

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Sixty-one samples of meat and bone meal (MBM) from Brazil and USA were grouped according to chemical, physical and amino acid digestibility values. Data were analysed for principal components, cluster analysis and variance analysis. Two main groups with five subgroups were established with the samples of MBM.

Introduction

Meat and bone meal (MBM) is an important ingredient in feed formulation all over the world, due to the economics of feeding with slaughterhouse residuals and the need to decrease N and P excretion in the environment. In general, MBM composition is considered primarily on the basis of protein (NRC, 1998) but should instead be considered in terms of multiple variables. In this research, samples from different rendering plants and slaughterhouses from USA and Brazil were analysed for principal components to classify samples hierarchically within homogeneous groups.

procedures and the percentages of amino acid digestibilities were obtained using ileal digesta collection according to Bellaver (1998). The variables were percentages of dry matter (DM), crude protein (CP) into DM (CPDM), fat (FAT), ash (ASH), Ca, P, CP digested in HCl and pepsin (CPH-CLP), pH (INPH), particle size (DGM, μm), and digestibilities of tryptophan (TRYD), lysine (LYSD), threonine (THRD), and methionine + cysteine (MCDIG). Data were analysed for principal components (2) and cluster hierarchy classification using the SPAD (1998) program and averages were analysed by variance on the SAS (1996) program.

Material and Methods

Samples of MBM from Brazil were obtained from local swine slaughterhouses as a combination of offals, animals dead on arrival and residuals from meat processing, excluding blood. North American samples, obtained from rendering plants and slaughterhouses, were a mixture of swine and bovine residuals and dead animals. Triplicates from each of 61 MBM samples were analysed according to AOAC (1995)

Results and Discussion

Table 101.1 presents a simple correlation matrix using all samples. Data from the table can be quickly interpreted through Fig. 101.1, where opposing arrows indicate a strong relationship (e.g. DM and the all digestibility variables). The values can be synthesized in this figure where all variables (13) are resumed in the two principal components (coordinates), with 64.15% of all variation being explained by the

averages of these groups are presented in Table 101.2. MBM from USA has lower LYSD, which may be due to higher DM (more processing heat) and larger DGM than Brazilian samples.

Table 101.2. Cluster analysis that minimizes the distance within groups with percentage averages and standard deviations (SD) for dry matter (DM), crude protein (CP), ether extract (EE), ash, particle size (DGM, μm) and lysine digestibility (LYSD).

MBM	Group	<i>n</i>	DM	SD	CP	SD	EE	SD	Ash	SD	DGM	SD	LYSD	SD
All	1	29	93.9	0.2	47.9	1.2	12.3	0.6	34.8	1.3	774.8	24.3	82.5	1.3
	2	32	96.0	0.2	54.6	1.1	16.6	0.6	27.4	1.2	821.1	23.1	70.0	1.2
Brazil	1a	6	93.3	0.9	60.3	7.4	13.8	1.9	21.1	5.0	861.7	23.3	84.0	4.1
	1b	8	94.2	1.0	46.8	4.7	14.2	3.5	32.7	2.7	843.3	112.4	83.5	5.0
	1c	15	94.0	1.0	43.5	2.5	10.7	1.2	41.4	3.3	703.6	121.9	81.5	6.1
USA	2a	11	96.6	0.9	55.3	6.1	17.2	2.9	24.2	3.6	870.9	161.7	62.1	6.4
	2b	21	95.6	1.4	54.2	4.1	16.4	3.8	29.1	3.4	795.1	110.8	74.2	4.9

Averages between groups 1 and 2 using all samples are significantly different by *t*-test at various levels of significance ($P < 0.02$ to $P < 0.0001$) except for DGM ($P < 0.17$). Subgroups to 1a–1c and 2a–2b were also tested but not reported in this paper.

References

- Association of Official Analytical Chemists (AOAC) (1995) Animal feed. In: Cunniff, P. (ed.) *Official Methods of Analysis of AOAC International*, 16th edn. AOAC, Washington, DC, pp. 4:1–30.
- Bellaver, C. and Easter, R.A. (1998) *Pesquisa Agropecuária Brasileira* 33, 737–744.
- NRC (1998) *Nutrient Requirement of Swine*, 10th edn. National Academy Press, Washington, DC, 189 pp.
- SAS (1996) *SAS Release 6.12 CDROM*. SAS Institute, Cary, North Carolina, USA.
- SPAD (1998) Windows. Logiciel d'Analyse des Données. 1 CDROM v. 3.5. CISIA-CERESTA, St Mandé.

102 Surgical Stomach Reduction in Swine as a Model for Human Obesity

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An experiment was carried out with the objective of evaluating weight gain, feed intake, laboratory findings and histology of the excluded stomach of swine subjected to Roux en Y gastric bypasses. Sixteen growing barrows from a commercial lean-type crossbred line of pigs were used. The initial weight was 52.84 ± 1.03 kg and the pigs were 98 days of age on the day of surgery. Final body weight for the non-surgery group was estimated to be 140.42 kg (using the non-surgery weight equation) and the observed body weight for the surgery group was 71.19 kg, both at day 90 of the experiment.

Introduction

Obesity is a multifactorial disease in which genetics, physiology and environmental factors are involved and it happens as a result of an inappropriate balance between energy intake and expenditure (Loh *et al.*, 1998; Carek and Dickerson, 1999). Physicians established overweight and obesity parameters in humans as an index of body mass (IBM). The formulae index is given by weight (kg) divided by height (cm). Normal IBM limits are between 20 and 25; obesity ranges from 30 to 40 and over 40 it is considered a pathological obesity or morbid condition that requires surgery (Brenner, 1999). Obesity is increasing at alarming rates, reaching more than 30% during the 1990s in the USA (Poston *et al.*, 1998). Medical expenditure and programmes to reduce excess weight in the USA reached around US\$100 billion per year (Brenner, 1999). To conduct this research with humans it would be possible to collect only some of the variables measured in this experiment. On the other hand, pigs are considered the best models for the study of nutrition issues in the

omnivorous human, as discussed by Miller and Ulley (1987), because the morphology and physiology of the gastrointestinal systems are very similar. For this reason, the objective of this experiment was to evaluate performance and biochemical and histological parameters of pigs subjected to Roux en Y gastric bypasses.

Material and Methods

Sixteen growing barrows from a commercial lean-type crossbred line of pigs were used. Eight of them were subjected to Roux en Y gastric bypasses according to the technique of Capella and Capella (1996) so that the stomach was excluded (Fig. 102.1). The initial weight was 52.84 ± 1.03 kg and the pigs were 98 days of age on the day of surgery. Final body weight for the non-surgery (NS) group was estimated to be 140.42 kg, using the NS weight equation (NSWt), and the observed body weight for the surgery (S) group was 71.19 kg, both at day 90 of the experiment. For the first 11 days after surgery all the pigs were fed restrictively; thereafter, feed was delivered

ad libitum according to a diet calculated to furnish the NRC (1998) requirements. Fresh water was provided *ad libitum* at all times, using wall drinkers. The analysed variables were: total body weight (Wt) during the period of 90 days after surgery; average daily feed intake (ADFI) during the experiment; mucosal thickness at fundic and pyloric regions of the stomach at day 90 and blood plasma variables at the end of the experiment. The SAS (1996) system was used to calculate polynomial equations for Wt and ADFI, in which the coefficients of regression were linear (d), quadratic (d^2), cubic (d^3) and fourth (d^4) degree.

Results and Discussion

Weight gain and average daily feed intake for the NS and S groups are presented in the equations of Table 102.1. The parameter estimates for the intercepts were significant (at least $P < 0.06$). The other regression coefficients were significant (at

least $P < 0.001$). Blood variables and gastric wall thickness at the end of experiment are presented in Table 102.2. Pigs with surgery presented lower volumes of red blood cells and a lower haemoglobin and protein concentration in the plasma than the control group, indicating an anaemic status of the animals. Values for total protein, haemoglobin and haematocrit (packed cell volume) of the NS group were close to the data presented by Miller and Ullrey (1987). Surgery pigs presented a significant decrease in stomach wall thickness, due to the reduced function of the excluded segment (Fig. 102.2 and Table 102.2). There were no other alterations of blood parameters. It was concluded that the surgical procedure employed was quite effective in controlling feed intake and weight gain and therefore is an effective animal model for human obesity and for use in modelling feed intake and gain in pig production. However, it must also be noted that the surgery promotes an anaemic condition that may require further treatment.

Table 102.1. Equations for prediction of weight gain (Wt) and average daily feed intake (ADFI) of non-surgery (NS) and surgery (S) groups.

Variable	Intercept	d	d^2	d^3	d^4	r^2
NSWt	53.647846	-0.887245	0.071505	-0.000983	0.000004634	0.99
SWt	53.723394	-1.577280	0.065240	-0.000837	0.000003677	0.99
NSADFI	-0.915797	0.134085	-0.001026			0.92
SADFI	-0.615912	0.078461	-0.000555			0.89

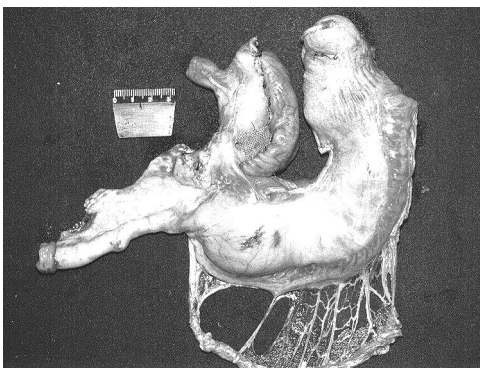


Fig. 102.1. Excluded stomach (Roux en Y) according to the Capella procedure.

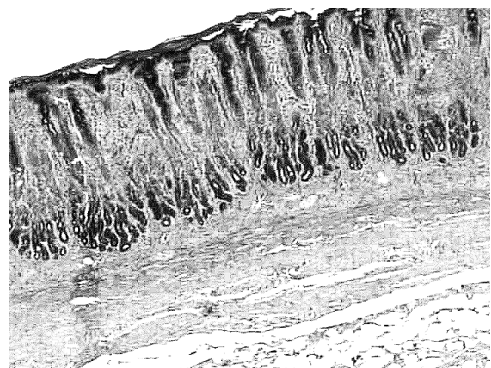


Fig. 102.2. Pyloric mucosal surface of the surgery group.

Table 102.2. Blood variables and gastric wall thickness at the end of the experiment.

Variables	NS	S	P NS = S
Cholesterol (mg dl ⁻¹)	107.53	108.41	0.8863
Glucose (mg dl ⁻¹)	80.72	83.40	0.7053
Protein (g dl ⁻¹)	7.49	6.47	0.0162
Triglycerides (mg dl ⁻¹)	41.64	44.93	0.4844
Haemoglobin (g dl ⁻¹)	13.04	9.13	0.0001
Haematocrit (packed cell volume) (%)	41.00	30.16	0.0001
Fundic mucosae (mm)	0.73	0.59	0.1081
Pyloric mucosae (mm)	1.08	0.65	0.0369

NS, non-surgery animals; S, surgery animals.

References

- Brenner, A.S. (1999) Gastroplastia vertical restritiva com derivação gastrointestinal em Y de Roux em suínos. DSc thesis, The Federal University of Paraná, Curitiba, Brazil.
- Capella, J.F. and Capella, R.F. (1996) The weight reduction operation of choice: vertical banded gastroplasty or gastric bypass. *American Journal of Surgery* 171, 74–79.
- Carek, P.J. and Dickerson, L.M. (1999) Current concepts in the pharmacological management of obesity. *Drugs* 57, 883–904.
- Loh, M.Y., Flatt, W.P., Martin, R.J. and Hausman, D.B. (1998) Dietary fat type and level influence adiposity development in obese but not lean Zucker rats. *Proceedings of the Society of Experimental Biological Medicine* 218, 38–44.
- NRC (1998) *Nutrient Requirement of Swine*, 10th edn. National Academy Press, Washington, DC, 189 pp.
- Miller, E.R. and Ullrey, D.E. (1987) The pig as a model for human nutrition. *Annual Review of Nutrition* 7, 361–382.
- Poston, W.S. 2nd, Foreyt, J.P., Borrell, L. and Haddock, C.K. (1998) Challenges in obesity management. *South Med Journal* 91, 710–720.
- SAS (1996) *SAS Release 6.12 CD-ROM*. SAS Institute, Cary, North Carolina.

103 Dietary Vitamins C and E for Growing Pigs

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The objective of this study was to determine whether the supplementation of a pig diet with vitamin E (α -tocopherol) or vitamin C (ascorbate) influenced, beside other parameters, the amount of tissue α -tocopherol concentration. Dietary vitamin E supplementation increased the α -tocopherol content in liver, longissimus dorsi muscle and backfat. On the other hand, the average liver vitamin E concentration of the vitamin C-supplemented group was higher than that of the control group, although the vitamin E contents of the control and vitamin C diets were identical.

Introduction

Vitamins E and C have antioxidative properties. In animal nutrition, and especially in diets for pigs, the supplementation of vitamin E is widely used. Dietary α -tocopherol has an overall impact on the quality of animal products and maintains consumer acceptability (Hoving-Bolink *et al.*, 1998). We presume that a part of dietary vitamin E is already used in the gut (protection of nutrients that are susceptible to oxidation). Therefore, it does not reach the primary place of action located in the tissues. Several studies have suggested that vitamins E and C act synergistically (e.g. Mitsumoto *et al.*, 1991). Vitamin E acts as the primary antioxidant by quenching lipid peroxy radicals. The resulting vitamin E radical is then regenerated by vitamin C into the active form. However, vitamin C supplementation of animal feed proved to be an effective way to save vitamin E. As a consequence, the vitamin E content in animal products is enhanced and probably less dietary vitamin E is necessary.

Materials and Methods

Twelve Large White pigs (barrows), weighing approximately 21 kg (Table 103.1), were randomly divided into three groups of four animals each. The pigs were housed individually and fattened until they reached 103 kg body weight (BW). They were given water and feed *ad libitum*. BW gain and feed intake were recorded weekly. Pigs were fed a control diet (CON), or the same diet supplemented with either 225 mg ascorbate (C) or 200 mg α -tocopherol acetate (E) kg^{-1} feed. The feed was composed mainly of barley (35%), maize (30%), extruded soybean meal (10%) and extruded sunflower meal (10%). The calculated amount of digestible energy was 13.2 MJ, crude protein 180 g and lysine 10.6 g kg^{-1} feed. The analysed vitamin E content was 60, 63 and 246 mg kg^{-1} feed for treatments CON, C and E, respectively. The vitamin C concentration was 51, 195 and 34 mg kg^{-1} feed, respectively. The animals were slaughtered and dissected in a local abattoir according to standard methods.

Table 103.1. Treatment effects on growth performance, carcass characteristics and vitamin E content of longissimus dorsi muscle, liver and backfat. Values represent means (standard deviation).

	Treatment		
	CON	C	E
Body weight start (kg)	21.5 (2.3)	21.7 (2.1)	20.5 (2.0)
Body weight end (kg)	104.3 (2.7)	103.1 (3.6)	103.0 (2.3)
Daily feed intake (kg)	2.36 (0.22)	2.29 (0.15)	2.13 (0.29)
Daily body weight gain (g)	849 (79)	797 (100)	709 (77)
Feed conversion ratio (kg kg ⁻¹)	2.78 (0.01)	2.89 (0.20)	2.99 (0.15)
Slaughter yield (%)	82.1 (1.9)	83.3 (1.3)	82.0 (1.0)
Percentage of carcass (%)			
Lean cuts	51.4 (2.1)	51.4 (2.3)	52.2 (3.6)
Total fat tissue	18.9 (2.1)	18.8 (2.2)	18.5 (1.6)
Longissimus dorsi muscle			
pH value (pH 1) ¹	6.14 (0.11)	6.01 (0.12)	6.10 (0.13)
pH value (pH 2) ²	5.44 (0.03)	5.44 (0.01)	5.48 (0.06)
Meat colour brightness ² (%)	31.83 (2.89)	32.00 (1.32)	30.67 (8.01)
α -Tocopherol (mg 100 g ⁻¹ dry weight)			
Liver	3.98 ^a (0.52)	7.18 ^b (2.87)	10.04 ^b (1.68)
Longissimus dorsi muscle	1.39 (0.20)	1.30 (0.19)	1.69 (0.19)
Backfat outer layer	1.83 ^a (0.04)	1.64 ^a (0.6)	3.06 ^b (0.7)

^{ab}Mean values with different superscripts in a row are significantly different ($P < 0.05$).

Lean cuts = total weight of back, shoulder and ham.

Total fat tissue = total weight of kidney-, back-, ham- and shoulder-fat.

¹45 min post-mortem; ²24 h post-mortem.

Results and Discussion

Neither vitamin C nor vitamin E supplementation of the diets had a significant effect on growth performance (Table 103.1). Nevertheless, a slight reduction in daily feed intake could be observed in treatment E compared with treatment CON, with consequences on daily BW gain and feed conversion ratio. Similar results have been reported: that no performance responses to dietary vitamin C (Mahan *et al.*, 1994) or vitamin E (Gebert, 1998) fed to grower-finisher pigs could be observed. Slaughter yield, percentage of lean cuts and total fat tissue, as well as selected meat quality parameters such as pH values and meat colour brightness, were not influenced by dietary treatment (Table 103.1) and are consistent with previous results (Cannon *et al.*, 1996; Gebert, 1998).

The liver α -tocopherol content in treatments C and E was higher than that of treatment CON ($P < 0.05$). Although the

vitamin E content of the control and vitamin C diets was the same, the average liver α -tocopherol content of the vitamin C-supplemented group was higher than that of the control group. Many studies have suggested that vitamins E and C act synergistically. We assumed that vitamin E acts as the primary antioxidant by quenching lipid peroxid radicals. The resulting vitamin E radical is then regenerated by vitamin C (Schaefer *et al.*, 1995). On the other hand, research investigating the benefits of vitamin C supplementation in pigs on vitamin E deposition in tissues has been inconsistent. The α -tocopherol content of the muscle samples (longissimus dorsi, 10th rib) tended ($P = 0.104$) to increase as the dietary level of the vitamin E increased, but there was no effect of supplemental vitamin C on vitamin E concentration in longissimus dorsi muscle. In contrast, the backfat α -tocopherol content was strongly influenced by dietary vitamin E supplementation ($P < 0.01$). The results of other investigations by

Cannon *et al.* (1996) and Gebert (1998) concerning the α -tocopherol deposition, with comparable vitamin E supplementation of pig diets, confirm our findings.

Conclusions

Our experiment suggests that there was no beneficial performance response to added vitamin C or vitamin E in diets for growing-finishing pigs. It has been reported that adverse environmental conditions, a disease insult or other stressful situations such as weaning may increase the vitamin C requirement. Therefore it can be

speculated that a growth and feed efficiency response can be achieved when vitamin C supplemented the diet during the initial period postweaning, but not thereafter under good health conditions. The vitamin E supplementation of the feed increased the α -tocopherol content in the examined tissues and also provided a natural dietary source of vitamin E for humans. On the other hand, dietary vitamin C supplementation proved to be an effective way to enhance the liver α -tocopherol content. It might also be of interest if a vitamin C supplementation of pig diets affects the α -tocopherol content of other animal products as well.

References

- Cannon, J.E., Morgan, J.B., Schmidt, G.R., Tatum, J.D., Sofos, J.N., Smith, G.C., Delmore, R.J. and Williams, S.N. (1996) Growth and fresh meat quality characteristics of pigs supplemented with vitamin E. *Journal of Animal Science* 74, 98–105.
- Gebert, S. (1998) Effects of microbial phytase and vitamin E in fat supplemented diets for growing pigs and laying hens on performance, nutrient utilization and product stability against oxidative alterations. Diss. ETH Nr. 12563, ETH Zürich, Switzerland.
- Hoving-Bolink, A.H., Eikelenboom, G., van Diepen, J.Th.M., Jongbloed, A.W. and Houben, J.H. (1998) Effect of dietary vitamin E supplementation on pork quality. *Meat Science* 49, 205–212.
- Mahan, D.C., Lepine, A.J. and Dabrowski, K. (1994) Efficacy of magnesium-L-ascorbyl-2-phosphate as a vitamin C source for weanling and growing-finishing swine. *Journal of Animal Science* 72, 2354–2361.
- Mitsumoto, M., Faustman, C., Cassens, R.G., Arnold, R.N., Schaefer, D. and Scheller, K.K. (1991) Vitamin E and C improve pigment and lipid stability in ground beef. *Journal of Food Science* 56, 194–197.
- Schaefer, D.M., Liu, Q., Faustman, C. and Yin, M.-C. (1995) Supranutritional administration of vitamins E and C improves oxidative stability of beef. *Journal of Nutrition* 125, 1792S–1798S.

104 High Available Phosphorus Maize and Phytase in the Diets of Pigs

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Growth performance and utilization of phosphorus (P) were compared in pigs fed high available P (HAP) or normal (NOR) maize with or without phytase. Weight gain and feed efficiency were expectedly depressed with the phytase-unsupplemented NOR maize diet; supplementation with phytase improved these performance criteria. The addition of phytase to HAP maize diets produced a numerical improvement in weight gain. Supplementing the NOR maize diet with phytase improved P digestibility and retention. Adding phytase to HAP maize gave a numerical increase in phosphorus digestibility and retention. Phosphorus digestibility in HAP maize was increased by 28% over the NOR maize diet.

Introduction

The biological availability of phosphorus (P) for young pigs in maize grain is less than 15%. About 70% of the P in maize grain is bound to phytic acid, which is unavailable to pigs. The efficacy of phytase for making available the phytate P present in plant-derived feed ingredients has been characterized in numerous studies (Lei *et al.*, 1993; Mroz *et al.*, 1994; Cromwell *et al.*, 1995). The development of HAP maize provides an additional strategy for further increasing the availability of phytate P and reducing the environmental impact of P excretion. The nutrient composition of HAP maize is similar to that of NOR maize but the level of phytate phosphorus in the kernel is significantly lower in HAP maize (Ertl *et al.*, 1998). The objectives of the following experiments were to evaluate growth performance and P utilization in young pigs fed HAP or NOR maize-soybean diets and phytase.

Materials and Methods

The growth performance of 48 pigs offered each of four diets arranged in a 2×2 factorial of HAP or NOR maize and phytase at 0 or 600 units kg^{-1} diet were examined in the first experiment. The diets were formulated to contain 190 g kg^{-1} crude protein, 3.5 kcal digestible energy g^{-1} , 11.8 g lysine kg^{-1} , 7.2 g Ca kg^{-1} and 4.1 g P kg^{-1} and contained no added inorganic P. The average initial weight was 9.2 kg and the pigs had unrestricted access to feed and water for 28 days. In the second experiment, six pigs, individually housed in stainless-steel metabolism crates, received each of the four diets used in the first experiment to evaluate nutrient digestibility. Pigs were fed in two equal feedings daily for a 5-day adjustment period, followed by a 5-day period of total but separate collection of faeces and urine. Faecal and urinary P were analysed spectrophotometrically and plasma P by colorimetry.

Results and Discussion

Weight gain and feed efficiency were expectedly depressed with the phytase-unsupplemented NOR maize diet; and supplementation with 600 phytase units (PU) kg^{-1} improved ($P < 0.05$) these performance criteria (Table 104.1). Weight gain and feed efficiency were higher ($P < 0.05$) with HAP compared with NOR maize diets. The addition of phytase to HAP maize diets produced a numerical improvement in weight gain and feed efficiency. These results are consistent with observed responses in phytase-supplemented pigs fed maize-soybean meal diets (Lei *et al.*, 1993; Cromwell *et al.*, 1995; Kornegay and Qian, 1996). Plasma P was

lowest when a NOR maize phytase-unsupplemented diet was fed. Adding phytase at 600 PU kg^{-1} to NOR maize diets led to a significant improvement ($P < 0.05$) in plasma P. Phosphorus levels in plasma of pigs fed HAP maize were higher ($P < 0.05$) than in those fed NOR maize, an indication that the P from HAP maize is more bioavailable. As seen in studies with phytase, consuming a HAP maize-soybean meal diet improved ($P < 0.05$) weight gain, feed efficiency and plasma P compared with NOR maize without phytase supplementation for pigs.

Supplementing the NOR maize diet with 600 phytase units kg^{-1} improved ($P < 0.05$) P digestibility and retention from 39 and 36%, respectively, to 55 and

Table 104.1. Growth performance of pigs fed high available phosphorus (HAP) or normal maize with or without phytase supplementation in experiment 1. Initial levels of P (90.5 mg l^{-1}) were similar for all treatment groups.

	Phytase (PU kg ⁻¹)				SD
	HAP maize		Normal maize		
	0	600	0	600	
Weight gain (kg day ⁻¹) ^{ab}	0.491 ^c	0.517 ^c	0.430 ^d	0.482 ^c	0.057
Feed intake (kg day ⁻¹)	0.918	0.896	0.854	0.923	0.149
Gain:feed (kg kg ⁻¹) ^a	0.546 ^{cd}	0.581 ^c	0.508 ^d	0.530 ^{cd}	0.062
Plasma P (mg l ⁻¹) ^{ab}	75.81 ^{cd}	80.58 ^c	51.58 ^e	68.20 ^d	11.96
<i>n</i>	11	12	12	12	

^a Main effect of maize at $P < 0.05$.

^b Main effect of phytase at $P < 0.05$.

^{cdef} Least square means of treatment effects with different superscripts significantly different at $P < 0.05$.

Table 104.2. Phosphorus balance of pigs fed high available phosphorus (HAP) or normal (NOR) maize with or without phytase in experiment 2.

	Phytase (PU kg ⁻¹)				SD
	HAP maize		Normal maize		
	0	600	0	600	
Intake (g day ⁻¹)	2.19 ^a	1.98 ^c	2.15 ^{ab}	2.12 ^{ab}	0.07
Faecal (g day ⁻¹)	1.02 ^b	0.84 ^b	1.32 ^a	0.95 ^b	0.21
Urinary (g day ⁻¹)	0.26 ^a	0.15 ^b	0.24 ^{ab}	0.21 ^{ab}	0.07
Absorbed (g day ⁻¹)	1.17 ^a	1.14 ^a	0.82 ^b	1.16 ^a	0.19
Retained (g day ⁻¹)	0.92 ^a	1.00 ^a	0.60 ^b	0.96 ^a	0.21
Digested (%)	53.49 ^a	58.13 ^a	38.65 ^b	55.10 ^a	9.31
Retained (%)	41.75 ^a	50.63 ^a	27.65 ^b	45.18 ^a	10.07
<i>n</i>	6	6	6	6	

^{abc} Least square means of treatment effects with different superscripts differ significantly at $P < 0.05$.

53%, respectively (Table 104.2). Phosphorus digestibility (53%) and retention (50%) in the HAP maize diet were higher ($P < 0.05$) than those of the NOR maize diet (39 and 36%, respectively). Adding phytase to HAP maize gave a numerical increase in phosphorus digestibility (58%) and retention (51%), compared with HAP without phytase (53.49 and 41.75%, respectively). The amount of P absorbed from the HAP maize diet was 30% higher than the amount absorbed from the NOR maize diet. Faecal excretion of P was highest ($P < 0.05$) with the consumption of a NOR maize phytase-unsupplemented diet.

Addition of phytase to either HAP or NOR maize diets resulted in numerically lower faecal P output. Urinary P output was reduced with consumption of the HAP maize and phytase diet, compared with a HAP maize phytase-unsupplemented diet. These results demonstrate that the support of optimal growth performance, improvement in bioavailability of P and reduction of faecal and urinary P excretion with the addition of phytase can also be attained by the use of HAP maize. Synergism between HAP maize and phytase supplementation was evident in these experiments.

References

- Cromwell, G.L., Coffey, R.D., Parker, G.R., Monegue, H.J. and Randolph, J.H. (1995) Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. *Journal of Animal Science* 73, 2000–2008.
- Ertl, D.S., Young, K.A. and Rayboy, V. (1998) Plant genetic approaches to phosphorus management in agricultural production. *Journal of Environmental Quality* 27, 299–304.
- Kornegay, E.T. and Qian, H. (1996) Replacement of inorganic phosphorus by microbial phytase for young pigs fed on a maize-soybean meal diet. *British Journal of Nutrition* 76, 563–578.
- Lei, X.G., Ku, P.K., Miller, E.R. and Yokayama, M.T. (1993) Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. *Journal of Animal Science* 71, 3359–3367.
- Mroz, Z., Jongbloed, A.W. and Kemme, P.A. (1994) Apparent digestibility and retention of nutrients bound to phytate complexes as influenced by microbial phytase and feeding regimen in pigs. *Journal of Animal Science* 72, 126–132.

105 Estimation of Fat Content of Animal Bodies from their C and N Content

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On the basis of analytic determinations of the fat, carbon and nitrogen content of animal body samples of rats ($n = 98$), pigs ($n = 73$) and mice ($n = 98$), equations for predicting the fat content of animal bodies were derived. The different animal species equations based on C and N showed coefficients of variation of 5.3, 4.4 and 2.7% for rat, pig and mouse, respectively. The use of nitrogen and carbon analysis offers the possibility of reducing the expense of determining the nitrogen and fat content of animal bodies.

Introduction

Extraction by petrol ether with acid pretreatment is prescribed generally for the analysis of the crude fat content of animal bodies as a standard method. This method is very time consuming and labour intensive. Therefore the fat content (XL) was often calculated as the difference between dry matter (DM) and ash (XA) + protein (XP). Because in this procedure the residual fraction ($DM - XA - XP - XL$) is not taken into consideration, the fat content may be overestimated. The aim of the present investigation was to find out whether the C and N content can be used to estimate the fat content of animal bodies. Considering the mean C and N contents of body nutrients, one general valid equation for predicting the fat content of all animal bodies should be tested and, in addition, on the basis of animal body samples of rats, pigs and mice with widely varying nutrient composition, different animal species equations should be derived.

Material and Methods

The whole bodies of killed rats and pigs were softened in an autoclave prior to homogenization. Aliquots of the homogenate were used for determination of body protein (Kjeldahl method), or were freeze-dried for subsequent determination of ash, fat and carbon. The carbon content was calculated by gravimetric determination of the carbon dioxide from the calorimetric bomb (Hoffmann *et al.*, 1977; Jentsch *et al.*, 1983; Klein *et al.*, 2000).

The whole bodies of mice were freeze-dried and finely ground in a freezer mill under liquid nitrogen. Samples were analysed for C and N after combustion (LECO, CNS-2000), where C was measured by infrared absorption while N was determined by thermal conductivity.

The body fat content was determined by petrol ether extraction after treatment with hydrochloric acid.

The ranges of nutrient composition of all 269 animal bodies were wide and

amounted to 31.5–82.2% XP, 4.0–63.0% XL as well as –2–9.9% residual fraction.

Regression analysis and *t*-test were performed using the EXCEL® computer program. The level of significance was set at $P < 0.05$.

Results and Discussions

The C content of the body is the sum of the C values obtained for fat, protein and glycogen (Gly). Considering the mean C and N contents of body nutrients (Brouwer, 1965), the following equation was derived:

$$XL = 1.304\ C - 0.678\ XP - 0.58\ Gly.$$

The Gly content of the whole body is low and may be neglected. Therefore, the fat content (%) can be predicted from C and N values (%) according to the following equation:

$$XL = 1.304\ C - 4.237\ N.$$

This equation was tested and the results are presented in Table 105.1.

The significant differences between predicted values and measured values may be explained by the low glycogen content of bodies and by the use of the mean C and N contents of nutrients.

In pigs and rats these differences depend on the absolute fat content; however, the relationships are different. Whereas in rats the differences become larger with increasing fat content, in pigs they become smaller. For this reason the general application of this equation to estimate the fat content of all animal bodies cannot be recommended. Therefore, different equations depending on the animal species for predicting the fat content based on C and N content were derived by means of dual linear regression analysis ($y = XL\ [\%DM]$, $x_1 = C\ [\%DM]$, $x_2 = N\ [\%DM]$). The regression equations are given in Table 105.2.

The different animal species equations show prediction errors of 5.3, 4.4 and 2.7% for rat, pig and mouse, respectively. These results show that the prediction error could be reduced by application of the regressively derived equations, compared with the equation based on mean C and N contents of body nutrients. The species equations are obviously reliable for estimating the fat content.

Elemental analysis offers a fast and efficient way to determine C and N simultaneously and to reduce the expense of determining the nitrogen and fat content of animal bodies.

Table 105.1. Validation of the prediction equation $XL = 1.304\ C - 4.237\ N$ for animal bodies.

		XL [% DM]				Standard error of prediction (%)
	<i>n</i>	Measured value mean	Predicted value mean	Difference ± SD		
All animals	269	33.18	33.77	0.59	1.72	5.3
Rat	98	29.50	30.27	0.78	1.62	6.1
Pig	73	37.37	38.91	1.54	2.09	7.0
Mouse	98	33.73	33.44	−0.29	0.92	2.9

All means within a line are significantly different ($P < 0.05$).

Table 105.2. Equations for predicting the fat content in animal bodies.

	<i>n</i>	Equation	r^2	RSD	% RSD
Rat	98	$XL = 1.2484\ C - 4.001\ N$	0.974	1.55	5.3
Pig	73	$XL = 1.3535\ C - 4.776\ N$	0.991	1.63	4.4
Mouse	98	$XL = 1.3183\ C - 4.292\ N$	0.992	0.92	2.7

References

- Brouwer, E. (1965) Report of sub-committee on constants and factors. In: Blaxter, K.L. (ed.) *Energy Metabolism*. EAAP Publication No. 11, Academic Press, London, pp. 441–443.
- Hoffmann, L., Jentsch, W., Klein, M. and Schiemann, R. (1977) Die Verwertung der Futterenergie durch wachsende Schweine. 1. Mitt.: Energie- und Stickstoffumsatz im frühen Wachstumsstadium. *Archiv für Tierernährung* 27, 421–438.
- Jentsch, W., Schiemann, R. and Hoffmann, L. (1983) Gemeinschaftsuntersuchungen zur Präzisierung des Energie- und Proteinbedarfs für Mastschweine. 2. Mitt.: Energie- und Stickstoffumsatz von Masthybriden im Mastabschnitt von 30–120 kg. *Archiv für Tierernährung* 33, 552–570.
- Klein, M., Schadereit, R. and Küchenmeister, U. (2000) Energy metabolism and thyroid hormone levels of growing rats in response to different dietary proteins – soy protein or casein. *Archives of Animal Nutrition* 53, 99–125.

106 Digestible Lysine Requirement of Growing Finnish Landrace × Yorkshire Pigs Determined by N-balance Technique

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Digestible lysine requirements of growing pigs was determined using the N-balance technique. The basal diet consisted of barley and soybean meal. Other diets were formulated by substituting basal diet with wheat starch or with synthetic amino acids and casein.

Both castrated males and gilts improved N retention linearly by 2.5 g day⁻¹ with 1 g increase in digestible lysine content, indicating a higher requirement than 10.6 g fu⁻¹ (fu = feed unit, 9.3 MJ net energy; NE) in animals of 25–50 kg live weight. Retention of N also improved up to the highest level for animals between 50 and 105 kg live weight, but only by 1.7 g day⁻¹ per g digestible lysine fu⁻¹.

Introduction

Protein requirements of the growing pig are influenced by many factors, including

genetic growing potential and age/weight of the pig. The requirement has been expressed as g of digestible crude protein fu⁻¹ (fu = feed unit, 9.3 MJ NE) and of ileal

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digestible amino acids (lysine, threonine and the sum of methionine and cystine) in Finnish feeding tables for growing pigs since 1995 (Tuori *et al.*, 1995). Genetic growing potential and lean/fat ratio have been enhanced by breeding. Therefore it was decided to evaluate dietary ileal digestible lysine requirements to maximize nitrogen (N) retention in Finnish commercial crossbred pigs in two growing stages (25–50 kg and above 50 kg live weight), using the N balance technique.

Material and Methods

The study was conducted with six individually penned castrated male and six female Finnish Landrace $\times$ Yorkshire pigs. The experiment was designed as a 6×4 and 6×6 cyclic changeover design with two blocks (castrated males and gilts) over two stages of growth. The experiment for the second growth stage was conducted with the same pigs as in the first growth stage, starting immediately after experiment 1. The initial and final live weights of castrated males ($\pm$ SD) were 24.7 ($\pm$ 2.1), 52.6 ($\pm$ 3.2) and 105.5 ($\pm$ 3.6) kg. Weights of gilts were 28.6 ($\pm$ 3.2), 53.9 ($\pm$ 3.6) and 102.0 ($\pm$ 3.0) kg, respectively. The basal diet consisted of barley and soybean meal (765 and 211 g kg⁻¹). Other diets were formulated by substituting the basal feed for wheat starch or for synthetic amino acids and casein. The level of digestible essential amino acids in relation to lysine was kept at least the same as those in the ideal protein of Wang and Fuller (1989). Ileal digestibility of amino acids in basal diet

and in casein were according to Partanen *et al.* (1998) and Chung and Baker (1992), respectively.

Results and Discussion

Both castrated males and gilts between 25 and 50 kg live weight improved N retention linearly by 2.5 g day⁻¹ per 1 g increase in digestible lysine content up to the highest lysine level (Table 106.1). These results suggested that digestible lysine requirement was higher than 10.6 g fu⁻¹. Castrated males also improved N retention with increasing digestible lysine content up to highest lysine content between 50 and 105 kg. However, improvement was only 1.7 g day⁻¹ per 1 g increase in digestible lysine content. The results of gilts above 50 kg live weight were excluded, because of reduced feed intake caused by heat stress due to housing ventilation problems. Data suggested that the 50–105 kg live weight range was too wide to have only one lysine requirement and that the response was more dependent on the protein deposition capacity of the individual pig than dietary lysine content. Therefore no lysine requirement for growing pigs of that live weight range was proposed.

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References

- Chung, T.K. and Baker, D.H. (1992) Apparent and true amino acid digestibility of a crystalline amino acid mixture and of casein: comparison of values obtained with ileal-cannulated pigs and cecectomized cockerels. *Journal of Animal Science* 70, 3781–3790.
- Partanen, K., Valaja, J., Siljander-Rasi, H., Jalava, T. and Panula, S. (1998) Effects of carbadox or formic acid and diet type on ileal digestion of amino acids by pigs. *Journal of Animal and Feed Science* 7, 199–203.
- Tuori, M., Kaustell, K., Valaja, J., Aimonen, E., Saarisalo, E. and Huhtanen, P. (1995) *Rehutaulukot ja ruokintasuositukset*. Helsingin yliopisto, kotieläintieteen laitos; Kasvintuotannon tarkastuskeskus, maatalouskemia osasto; Maatalouden tutkimuskeskus, kotieläintuotannon tutkimus-

Table 106.1. Effect of dietary ileal digestible lysine content (g fu⁻¹; fu = feed unit, 9.3 MJ NE) on N-balance in growing pigs.

								P-values					
Digestible lysine content (g fu ⁻¹)								SEM ^a	Sex	Lysine	1	2	3
Pigs 25–50 kg LW													
Digestible lysine (g fu ⁻¹)	5.9	6.8	7.7	8.7	9.8	10.6							
Energy intake (fu day ⁻¹)	1.69	1.65	1.65	1.65	1.64	1.62	0.00	***	***	***			***
Digestible lysine intake (g day ⁻¹)	10.0	11.3	12.7	14.3	16.0	17.3	0.23		***	***			
N intake (g day ⁻¹)	34.4	38.9	39.9	41.5	42.7	46.8	0.38	*	***	***			***
Faecal N excretion (g day ⁻¹)	8.6	9.3	9.2	9.4	8.6	9.1	0.26						
Urinary N excretion (g day ⁻¹)	9.5	10.7	10.1	9.0	7.5	9.8	0.46		**	t			***
N retained (g day ⁻¹)	16.4	18.9	20.6	23.0	26.6	27.9	0.53		***	***			
Castrates, > 50 kg LW													
Digestible lysine (g fu ⁻¹)	5.3	6.2	7.2	8.1	9.1	10.1							
Energy intake (fu day ⁻¹)	2.82	2.80	2.73	2.72	2.75	2.71	0.01	–	***	***	***	**	
Digestible lysine intake (g day ⁻¹)	15.0	17.3	19.4	21.9	24.7	27.1	0.27	–	***	***	***		
N intake (g day ⁻¹)	57.8	66.6	74.7	76.5	79.3	80.9	0.61	–	***	***	***	***	*
Faecal N excretion (g day ⁻¹)	8.6	10.4	11.9	11.0	11.8	11.0	0.46	–	***	**	**		
Urinary N excretion (g day ⁻¹)	23.2	27.5	33.3	35.0	34.4	35.6	1.59	–	***	***	*		
N retained (g day ⁻¹)	25.9	28.8	29.5	30.5	33.2	34.3	1.15	–	***	***			

Lysine content and intake calculated according to amino acid analysis of barley, soybean and casein; ileal digestibility of amino acids in barley–soybean mixture according to Partanen *et al.* (1998); those of casein according to Chung and Baker (1992); synthetic amino acids assumed to be completely digestible.

^a Due to a missing observation, SEM for digestible lysine level 9.8 g fu⁻¹ in 25–50 kg pigs is 1.12 × tabulated value.

P-values 1, 2, 3: Linear, quadratic and cubic effect of lysine level.

Statistical significance: t, $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

laitos (Department of Animal Science, University of Helsinki; Department of Agricultural Chemistry, Plant Production Inspection Centre; Animal Production Research, Agricultural Research Centre of Finland), Helsinki, 99 pp.

Wang, T.C. and Fuller, M.F. (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *British Journal of Nutrition* 62, 77–89.

107 Efficacy of a New Phytase Preparation

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The efficacy of a new phytase preparation was evaluated in three experiments. Phytase supplementation of a negative control diet gave a linear improvement in growth performance and yielded similar performance to a positive control diet. *In vitro* release of phosphorus (P) in feeds with added phytase gave optimum phytase activities of 340 and 170 units kg⁻¹ of maize and soybean meal, respectively. Phytase supplementation of a maize–soybean meal diet significantly improved the digestibility of phytic acid and increased the release and absorption of P. The results of these three studies show that the new phytase preparation is efficacious in hydrolysing phytic acid and supporting growth in pigs.

Introduction

A significant portion of the phosphorus (P) in mature cereal grains and oilseeds is present as phytic acid P (Cheeke and Shull, 1985). Phytic acid P is poorly available to pigs, due to an inadequacy of intestinal phytase (Cromwell *et al.*, 1995). Consequently, diets formulated to supply adequate P lead to excretion of large quantities of P into the environment. Feed supplementation with phytase is a demonstrated way of improving phytic acid P utilization and minimizing P excretion (Lei *et al.*, 1993; Young *et al.*, 1993;

Adeola *et al.*, 1998). Any phytase preparation requires rigorous evaluation of its efficacy in hydrolysing phytic acid. The objective of the three studies in this report was to examine the effect of phytase addition to diets on pig growth performance and phytic acid hydrolysis.

Materials and Methods

Three experiments were conducted to evaluate the efficacy of a new phytase preparation. In the first experiment, a negative control (NC) maize–soybean meal-based

diet with no inorganic P was formulated to contain 20.7% crude protein (CP), 3.665 kcal digestible energy (DE) g⁻¹, 1.22% lysine, 0.51% Ca and 0.43% P and supplemented with 0, 250, 500, 750, 1000 or 1250 phytase units kg⁻¹. A positive control (PC) diet with inorganic P contained 0.87% Ca and 0.72% P. The seven diets were fed to 56 individually penned 10 kg pigs for 4 weeks.

The second experiment involved examining the *in vitro* release of P in feed ingredients or complete feed containing added levels of the new phytase preparation to give 0, 125, 250, 500, 750, 1000, 2000 or 4000 units kg⁻¹ sample. The enzyme was added last to initiate the reaction. Samples were incubated in a shaker maintained at 40°C for 1 h and HCl was added to give a concentration of 1 M HCl to stop the reaction.

In the third experiment, 36 barrows with an average initial weight of 19 kg were assigned to six diets and housed in stainless-steel metabolism crates. The NC diet contained 16.5% CP, 3.531 kcal DE g⁻¹, 0.8% lysine, 0.65% Ca and 0.33% P; the PC diet contained 0.65% Ca and 0.5% P. The NC diet was supplemented with 0, 250, 500, 750 or 1000 phytase units kg⁻¹ diet. Pigs were fed in a study consisting of a 5-day adjustment period followed by a 5-day period of total but separate collection of faeces and urine.

Results and Discussion

Phosphorus has posed challenges due to the inefficiency of P utilization in cereal-

based diets, expense of P supplementation, and pollution effects of P in animal wastes (Cheeke and Shull, 1985; Honeyman, 1993; Young *et al.*, 1993; Adeola *et al.*, 1998). The advent of stable phytase preparations has presented an effective tool to improve P digestibility and utilization, minimize P excretion, and allow prudent use of P supplements in diet formulation. In the present experiments, phytase supplementation gave a linear improvement ($P < 0.05$) in growth performance and yielded similar performance to the PC diet (Table 107.1). The data indicate that the phytase exerted a positive effect on P availability, which led to an improvement in growth performance of pigs.

In the *in vitro* experiment (Table 107.2), the response in P release to phytase concentration was curvilinear, and non-linear regression was used for data analyses. The optimum phytase activity was 340 units kg⁻¹ with a maximal release of 0.9 mg P g⁻¹ maize. For soybean meal, optimum phytase activity was 170 units kg⁻¹ with a maximal release of 1.8 mg P g⁻¹ sample. Optimum phytase activity was 240 units kg⁻¹ with a maximal release of 1.12 mg P g⁻¹ NC diet. For wheat and barley, optimum phytase activities were 60 and 70 units kg⁻¹, respectively, with a maximal release of 2.97 mg P g⁻¹ sample. When canola and sunflower meals were assayed, optimum phytase activities were 140 and 280 units kg⁻¹, respectively, with maximal release of 3.265 and 3.174 mg P g⁻¹ sample, respectively (Table 107.2).

Higher faecal phosphorus output

Table 107.1. Performance of pigs fed diets with graded levels of phytase in experiment 1.

Item	Phytase activity (units kg ⁻¹)							SE
	PC ^a	0 ^b	250	500	750	1000	1250	
Weight gain (g day ⁻¹) ^{cd}	570	484	535	565	551	569	599	20.1
Feed intake (g day ⁻¹) ^{cd}	1005	1125	1081	1174	1055	1043	1131	68.7
Gain/feed (g kg ⁻¹) ^{cd}	571	443	509	506	535	548	536	27.1
<i>n</i>	6	6	6	6	6	6	6	

^aPositive control diet.

^bNegative control diet.

^cPC vs. 0 at $P < 0.05$.

^dLinear at $P < 0.05$.

($P < 0.05$) in pigs fed the negative control diet than in those fed the phytase-supplemented diets led to both lower ($P < 0.05$) daily absorbed P and percentage P digestibility (Table 107.3). Phytase supplementation of maize–soybean meal diet significantly improved the digestibility of phytate and increased the release and absorption of phosphorus. Non-linear

broken-line regression analysis revealed that the optimum level of phytase in the maize–soybean meal diet used in the current experiment was 298 units kg^{-1} for digested P and 326 for percentage P digestibility. The results of these three studies show that the phytase preparation is efficacious in hydrolysing phytic acid and supporting growth in pigs.

Table 107.2. Effect of phytase activity on the hydrolysis of phytic acid in feed ingredients (expressed as μg of P released g^{-1} sample).

Item	Phytase activity (units kg^{-1})								SE
	0	125	250	500	750	1000	2000	4000	
Maize	286	436	514	684	813	900	1040	1219	24
Soybean meal	702	1123	1338	1468	1735	1833	1990	2171	36
NC diet	324	571	819	933	1024	1070	1207	1388	25
Wheat	2417	2846	2832	2888	2950	3015	3084	3017	40
Barley	2075	2208	2228	2342	2314	2526	2525	2560	48
Canola meal	1687	2123	2555	3055	3153	3270	3612	4177	39
Sunflower meal	1272	1581	1987	2630	2757	2931	3423	3710	45
PC diet	1971	3598	2588	2776	2838	2953	3115	3332	299
<i>n</i>	6	6	6	6	6	6	6	6	

Samples were incubated for 1 h in specified units of phytase at pH 5.5. Analysed P of the samples were (mg g^{-1}): maize 2.6; soybean meal 6.9; negative control (NC) diet 3.4; wheat 4.5; barley 3.6; canola meal 11.5; sunflower meal 15.6; and positive control (PC) diet 5.1.

Table 107.3. Phosphorus balance of pigs fed diets with graded levels of phytase in experiment 3.

Item	Phytase activity (units kg^{-1})							SE
	PC ^a	0 ^b	250	500	750	1000		
Intake (g day^{-1}) ^c	2.91	2.46	2.53	2.51	2.51	2.53	0.04	
Faecal (g day^{-1}) ^{cde}	1.34	1.41	1.22	1.17	1.06	1.14	0.06	
Urine (g day^{-1}) ^c	0.07	0.03	0.03	0.04	0.04	0.04	0.01	
Digested (g day^{-1}) ^{cde}	1.57	1.05	1.31	1.34	1.45	1.39	0.06	
Retained (g day^{-1}) ^{cde}	1.49	1.02	1.28	1.30	1.41	1.35	0.07	
Digested (%) ^{cde}	53.82	42.76	51.65	53.53	57.95	54.79	2.44	
Retained (%) ^{cde}	52.97	40.88	54.80	53.02	62.39	59.25	2.57	
<i>n</i>	6	6	6	6	6	6		

^aPositive control diet.

^bNegative control diet.

^cPC vs. 0 at $P < 0.05$.

^dLinear response at $P < 0.05$.

^eQuadratic response at $P < 0.05$.

References

Adeola, O., Orban, J.L., Ragland, D., Cline, T.R. and Sutton, A.L. (1998) Phytase and cholecalciferol supplementation of low-calcium and low-phosphorus diets for pigs. *Canadian Journal of Animal Science* 78, 307–313.

-
- Cheeke, P.R. and Shull, L.R. (1985) *Natural Toxicants in Feeds and Poisonous Plants*. AVI Publishing, Westport, Connecticut.
- Cromwell, G.L., Coffey, R.D., Parker, G.R., Monegue, H.J. and Randolph, J.H. (1995) Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. *Journal of Animal Science* 73, 2000–2008.
- Honeyman, M.S. (1993) Environment-friendly swine feed formulation to reduce nitrogen and phosphorus excretion. *American Journal of Alternative Agriculture* 8, 128–132.
- Lei, X.G., Ku, P.K., Miller, E.R. and Yokoyama, M.T. (1993) Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. *Journal of Animal Science* 71, 3359–3367.
- Young, L.G., Leunissen, M. and Atkinson, J.L. (1993) Addition of microbial phytase to diets of young pigs. *Journal of Animal Science* 71, 2147–2150.

Part VII

Concluding Remarks

Concluding Remarks

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I feel it as an honour to have been asked to conclude this 8th Symposium on Digestive Physiology in Pigs. Thus, I have to give some personal comments on the content of the coming Symposium and to identify important new areas of research. This might be a difficult academic exercise by one of the last 'survivors' of the first Symposium¹ ... yes, I am! However, I prefer to make it friendly and spontaneous. First of all, in your name and in my own, as a scientist involved for 35 years in pig research, may I say to Prof. Jan Erik Lindberg and all the Organizers that we have had an excellent Symposium.

I also personally enjoyed it because there is a real evolution in the state of the art from Symposium to Symposium. This evolution has to be continued in preparing

the 9th venue. When looking to choose what area of research should be privileged, three main cases can be distinguished:

1. The question of the quantification of endogenous nitrogen losses and of the estimation of a true precaecal digestibility of the various feed constituents had a growing impact through the different meetings we had over the past 15 years. I think that we reached at this Uppsala Symposium the end of the story, or close to it. Enough research work has been performed to improve the accuracy and convenience of the methods used to measure digestibility of various substrates and to estimate availability of amino acids. The current evaluation of feedstuffs might be directly considered within the annual meetings of

¹ The seven former Symposia were organized in Shinfield, UK (July 1979), Versailles, France (October 1982), Copenhagen, Denmark (May 1985), Jablonna, Poland (June 1988), Wageningen, The Netherlands (April 1991), Rostock, Germany (October 1994) and Saint Malo, France (May 1997).

the European Association of Animal Production, as it does not require much additional physiological experimentation.

2. Opposite to that, the questions related to the immune system, to the role of gut-associated lymphoid tissue, to the participation of the enteric nervous system in its function, and to the role of nutritional factors to improve immunological function, are of growing interest. This is of the highest importance, to understand the interactions between the gut and both the normal and the pathological flora. We had a first attempt in Rostock to look at that question. Then I invited Prof. Stokes to St Malo. But few people really understood his excellent talk. We listened to him again on Tuesday² and he received a lot of questions. This is the sign of the growing interest we have for the interaction between nutrition and immunity.

3. A third case is given by subjects which were explored a long time ago, then disappeared from the literature, and now come under the spotlight again. This is the case of the concept of luminal nutrition, which emerged during the 1960s from studies on intestinal hypertrophy after partial resection (Booth *et al.*, 1959; Dowling and Booth, 1967). Its role was then established for both maintenance of gut mass (Levine *et al.*, 1974) and intestinal adaptation (Dowling *et al.*, 1974). This was an important debate at the time of the development of clinical emergency techniques such as total parenteral nutrition and enteral nutrition in the 1970s. The question now recurs in relation to development of the gut, and with epithelial function and integrity. Therefore, every research subject has his own life expectancy, more or less cyclical.

Our role in organizing these Symposia is, by mixing people and facilitating dialogue, to harmonize our techniques and methods in order not to debate on differences of data that cannot be compared. Then, we have to identify new frontiers for knowledge, new ways leading to real quali-

tative breakthrough and, when possible, new techniques for further progress.

So our job is physiology – that is, true physiology or integrative physiology. But there are two meanings to these words:

- The first one means to relate a gene to a function, in order to identify a mechanism. This should receive much greater attention in future Symposia.
- The second one is to relate one function with another within the complex system of the whole organism, including hormonal and nervous regulations.

These two ways could both improve our knowledge and allow us to cross new frontiers.

Let us briefly mention some possible ways for the next few years:

1. We might further extend our work to various subjects such as nutrition and immunity, role of the enteric nervous system in digestive physiology and pathology, dietary manipulation of pathologies, cellular mechanisms of absorption and metabolism within the intestinal wall, cellular communication between the microflora and the epithelium.

2. We might also further explore relationships between organs and functions, such as intestinal motility pattern and kinetics of absorption, metabolic relationships between liver and intestine, or between liver, intestine and kidney. On the basis of my own experience and some very exciting data (Laplace, 1982; Laplace and Cuber, 1984; Laplace and Simoes-Nunes, 1987), may I also suggest the careful study of the reciprocal information of brain and gut, despite the difficulties? This might produce a lot of interesting surprises.

3. The role we may have concerning methodologies has been mentioned. I would like to show you what kind of progress we could expect from new non-invasive techniques, mainly from imaging or functional imaging in intact animals, for better welfare of laboratory-animals, and probably also for a more physiological approach. For exam-

² Lecture by C. Stokes: Development and function of the pig gastrointestinal immune system.

ple, in comparison with a classical stereotaxic atlas of the brain, magnetic nuclear resonance can provide very accurate images (Marcilloux *et al.*, 1993). As concerns the digestive tract, various fistulations have been used over the past decades. But, as regards food passage, the time of fistulations might come at the end with the development of internal scintigraphy (Malbert *et al.*, 1997), the regular use of external scintigraphy in pigs in the laboratory of C.H. Malbert

at INRA Saint-Gilles, and possibly in the future the application of positron emission tomography.

Thus, as a conclusion, my take-home message for all our young colleagues will be as follows: be imaginative and be excellent; be happy and make Physiology.

Looking forward to seeing you in Alberta (Canada) for the 9th Symposium on Digestive Physiology in Pigs.

References

- Booth, C.C., Evans, K.T., Menzies, T. and Street, D.F. (1959) Intestinal hypertrophy following partial resection of the small bowel in the rat. *British Journal of Surgery* 46, 403–410.
- Dowling, R.H. and Booth, C.C. (1967) Structural and functional changes following small intestinal resection in the rat. *Clinical Science* 32, 139–149.
- Dowling, R.H., Feldman, E.J., McNaughton, J. and Peters, T.J. (1974) Importance of luminal nutrition for intestinal adaptation following small bowel resection. *Digestion* 10, 216.
- Laplace, J.P. (1982) Impairment by vagal deafferentation of the compensatory hypertrophy after enterectomy, at high and low feeding levels. In: Robinson, J.W.L., Dowling, R.H. and Riecken, E.O. (eds) *Mechanisms of Intestinal Adaptation*. MTP Press, Lancaster, UK, pp. 321–331.
- Laplace, J.P. and Cuber, J.C. (1984) Déafférentation vagale totale et évacuation gastrique chez le porc. *Reproduction Nutrition Développement* 24, 655–670.
- Laplace, J.P. and Simoes-Nunes, C. (1987) Pancreatic size and enzyme contents after vagal deafferentation in jejunectomized pigs under free or restricted feeding. *Gut* 28 (S1), 169–173.
- Levine, G.M., Deren, J.J., Steiger, E. and Zinno, R. (1974) Role of oral intake in maintenance of gut mass and disaccharide activity. *Gastroenterology* 67, 975.
- Malbert, C.H., Mathis, C., Bobillier, E., Laplace, J.P. and Horowitz, M. (1997) Measurement of gastric emptying by intragastric gamma scintigraphy. *Neurogastroenterology and Motility* 9, 157–165.
- Marcilloux, J.C., Félix, M.B., Rampin, O., Stoffels, C., Ibazizen, M.T., Cabanis, E.A., Laplace, J.P. and Albe-Fessard, D. (1993) Preliminary results of a magnetic resonance imaging (MRI) study of the pig brain placed in stereotaxic conditions. *Neuroscience Letters* 156, 113–116.

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