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Potentially Harmful Organisms and Substances in Feedstuffs and Animal Faeces

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Deutsche Forschungsgemeinschaft (DFG)

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Report 5

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the ad hoc Working Group
"For the Evaluation of Potentially Harmful Organisms and Substances in Feedstuffs and Animal Faeces" of the Senate Commission for the Assessment of Chemicals Used in Agriculture

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1 Introduction

Jürgen Unshelm

Specialisation and the division of labour in agriculture have led not only to the establishment of certain forms of housing and management conditions for farm animals, but also to the presence of a particularly high density of animals in individual regions. The result of this has been that not only does feed growing dominate plant production in such regions, but also that large amounts of feed especially concentrates have to be imported to these areas. These concentration effects are combined with the fact that slaughtering and meat processing generally take place in the same regions, which leads to additional risks due to high volumes of waste water containing nutrients and pathogens and due to the necessary recycling of slaughter-house offal like gut and stomach contents to agriculture. In addition to other negative consequences, there is an increase in epidemiological risks by introducing and spreading harmful organisms and their metabolic products in the environment, which also has to be regarded as a consequence of measures associated with intensive animal husbandry. These measures include the increasing use of substances for preventive health care and to fully exploit the performance that is realisable on a genetic basis, as well as the use of agents to control storage pests, the use of preservatives, drugs,[1] purification agents, disinfectants, agents which improve feed conversion, and other organic and inorganic substances. The adverse effects of harmful organisms and their metabolic products, and also of substances for the prevention of health problems, are firstly the transfer of undesired substances into the human food chain, secondly the negative effect on animal health, and thirdly the harmful effects, usually time-delayed, of spreading these substances in the environment, especially via faeces, urine and animal production residues.

The ad hoc group "For the Evaluation of Potentially Harmful Organisms and Substances in Feedstuffs and Animal Faeces" was therefore given the task of investigating the importance of harmful

[1] A separate report published by the DFG dealing with drug residues in feedstuffs and excrements is planned.

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organisms and their metabolic products, substances for health care and antibacterial growth promoters, and also other organic and inorganic substances. Further investigations address the extent to which information concerning these facts and interrelations is lacking in this field, and whether, if we have sufficient knowledge, too little is being done to protect the environment and to avoid damage to the health of humans and animals.

In order to facilitate greater comprehensibility and clarity of the subject, which is virtually endless, the individual chapters have been organised uniformly wherever possible. After the respective substance has been characterised, its occurrence and in particular the reasons for its production and use are discussed, as well as epidemiological considerations of the causes of its entry into the chain of consequences and its persistence in the individual stages of that chain. Subsequently the consequences are discussed, firstly for feedstuffs, secondly for agricultural livestock, their excreta and their products, and thirdly, although only in general terms, for humans, since the aspects of foodstuffs and protection of the work force are the task of other committees and groups of the DFG (Deutsche Forschungsgemeinschaft). Nor will the consequences of the use of banned substances be discussed, although the group is aware of the problem of the incorrect or illegal use of such substances abroad and the possibility of illegal importation. Individual politically charged cases of current interest (e.g. dioxins in feedstuffs) were deliberately not addressed.

Furthermore, the consequences for the environment, for water, soil and air, are to be included in the status report, and the economic significance will be emphasised, which is particularly important in revealing the need for political action.

The next point to be discussed within the individual sections is aspects of legislation. Due to the increasing complexity of the situation caused by national, supranational and international legislation/directives, time limits had to be determined; for this reason the status report refers to the period up to 31st December 1998.

In addition, in each section the recommended research and necessary action will be dealt with, forming the most important part of the whole status report. Each section ends with a summary and a list of the most relevant literature.

The purpose of this status report is to provide information, on the basis of the data and facts determined by the ad hoc group, as

to what needs to be done in order to reduce, and as far as possible avoid, the risks from potentially harmful organisms and substances in feedstuffs and animal faeces. The measures which may be necessary can be divided into two categories. Firstly, measures are needed to clarify which facts and interrelations are not yet sufficiently well known so that the required research is provided for the relevant problem areas. This information is of particular importance to research institutes and agencies which promote research. Secondly, it has to be made clear that in many cases the causes and interrelations, and thus everything necessary to avoid or eliminate the danger, are already familiar without satisfactory measures having been taken. In these cases the recommended action will be emphasised, naturally taking account of legal, political and economic considerations.

The recommended research and need for action thus represent the core of the status report, and due to their importance they not only form the conclusion of each chapter, but also represent the focal point of the conclusion of the whole status report.

2 Harmful Organisms and Their Metabolites

2.1 Viruses and Unconventional Pathogens

Alfred Metzler

2.1.1 Introduction

All warm-blooded animals harbour viruses which are released into the outside world with infectious excretions. In addition to warm-blooded creatures, fish and other animal species and even bacteria and plants excrete a variety of viruses which often find their way into the environment, especially in the aquatic system. Fortunately, the animal viruses being prevalent in Europe are chiefly host-specific, i.e. they are pathogenic only in representatives of the same or of a closely related species and do not, therefore, trigger zoonosis (Krauss et al. 1997).

The fact that pathogenic viruses are introduced into stocks of animals through trade in animals and via fomites (i.e. any object that may be contaminated by a virus), especially foodstuffs containing abattoir and catering waste, has long since been recognised. Within animal populations virus infections are spread as a result of direct and indirect contact between excreting animals and susceptible recipients. Infected animals excrete the pathogens, often in large quantities, in nasal secretions and saliva, in faeces and urine as well as through abrasions of the skin and mucous membranes. Occasionally viral pathogens are excreted in milk. Virus transmission via plant-based feed or drinking-water has so far not been observed (Mahnel 1986; Strauch 1987, 1991 b,c,d; Strauch and Ballarini 1994). However, similar to numerous human viruses with a predilection for the digestive tract (enteric viruses), corresponding animal viruses may survive for varying periods in the environment, especially in water, spreading by passing through natural barriers (soil filters)

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and by proving resistant to disinfectants. At the present time, the risk of infection due to viruses in animal excretions can only be assessed with reference to relevant experiences with viruses which are pathogenic to man.

Human enteric viruses are known to be transmitted in water. In addition to direct contact between excreting individuals and susceptible recipients, the significance of water as a means of virus transmission should not be under-estimated (Metzler 1996; Metzler et al. 1996). Given this scenario, it is understandable that relevant guidelines produced both by the WHO and the EU (Anonymous 1975a, 1980a, 1990) stipulate that drinking and recreational water should be free of infectious pathogens and consequently free of pathogenic viruses.

2.1.2 Viral Pathogens of Animals, Including Prions

2.1.2.1 Epidemiological Aspects

Infectious diseases result in increasingly high economic losses in terms of animal production. The current situation with hog cholera (syn. swine fever, i.e. a notifiable virus infection) reflects the difficulties of implementing a control procedure even in those countries with comprehensive eradication guidelines. There are basically two approaches to the eradication of viral diseases which can be summed up under immune- and exposure prophylaxis. Since no satisfactory vaccine is available to combat numerous infectious diseases (this also applies to hog cholera), exposure prophylaxis must be ensured by adopting suitable procedures for the movement of animals (trade in animals) and the handling of contaminated fomites, especially carcasses and foodstuff of animal origin (Figure 2.1). In the case of a notifiable viral disease, the affected herds are invariably culled. Universally valid hygiene measures for the use of farm-specific or external fertilisers (animal manure from communal storage tanks or biogas plants, compost and sewage sludge) are aimed in the same direction. In this context the following definitions should be noted: i) (liquid) manure or slurry are meant to contain all animal excreta (faeces and urine) as well as any embedding material and

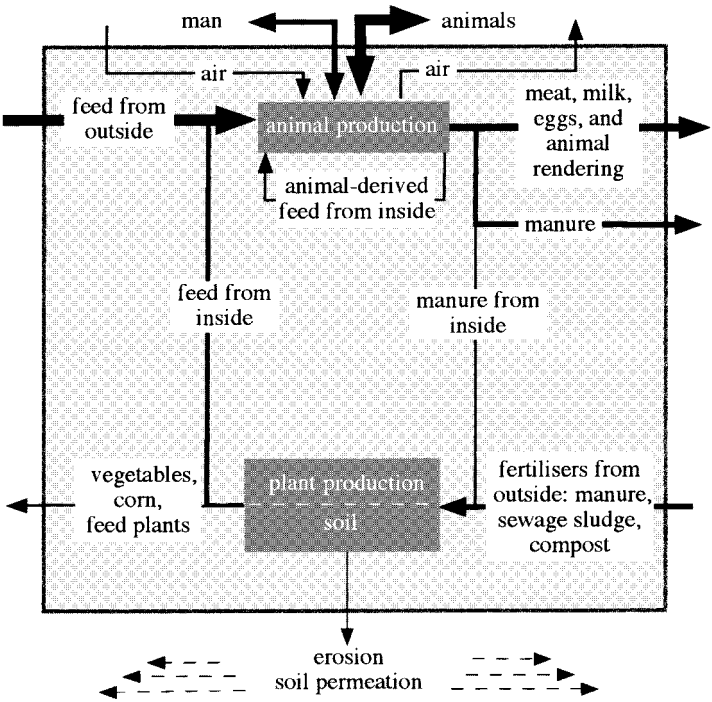

Figure 2.1: Epidemiology of infectious diseases in animal production.

(cleaning) water; ii) in some animal housing systems (especially with milking cows) urine and (cleaning) water are collected in pits, whereas faeces and embedding material (solid farmyard manure) are separately composted in stock piles. Manure and urine are generally stored under anaerobic and ambient temperature conditions.

Numerous edicts have been passed regarding the implementation of infectious hygiene requirements which are crucial to successful animal rearing. These include legislation governing the disposal of animal carcasses, foodstuffs, waste, animal-derived fertilisers and sewage sludge. Some of these regulations are region-specific whilst others have achieved international recognition beyond state borders.

2.1.2.2 The Dynamics of Virus Inactivation

Every attempt at exposure prophylaxis is aimed at breaking up chains of infection. This is achieved by controlled access to animal stocks for both trade animals and humans and by disinfecting inanimate surfaces and sterilising foodstuffs of animal origin (Strauch and Ballarini 1994). The success of any disinfection or sterilisation programme depends on four key factors: firstly, the number of pathogens initially present, secondly, the specific properties of the pathogen (tenacity), thirdly, the nature and extent of the inactivator (e.g. temperature or concentration of the disinfectant) and lastly, the duration of effect of this inactivator.

2.1.2.3 Viruses in Animal Excretions, Especially Manure, Urine and Farm Yard Manure

Like humans, livestock and domestic animals are hosts for a broad spectrum of mainly species-specific enteric viruses. On the one hand these include viruses, the clinical significance of which has only been partly elucidated and, on the other hand, notifiable infectious pathogens, such as foot and mouth disease virus, porcine encephalomyelitis virus, swine vesicular disease virus, or pseudorabies virus.

Apart from the behaviour in manure, relatively little is known of the environmental behaviour of animal-pathogenic viruses. This also applies without exception to the many live vaccines frequently used (Bryan et al. 1994; Kaashoek et al. 1994; Mettenleiter et al. 1994; Van Engelenburg et al. 1994; Young and Smith 1995). In addition, constant attention should be paid to newly recognised viral infections and changes in the manifestation of disease (Parwani et al. 1996; Pirtle and Beran 1996; Pongsuwanna et al. 1996; Smyth et al. 1996). The techniques needed to investigate the environmental behaviour of relevant viral pathogens (Mahnel 1986), i.e. sample processing and detection procedures, are often lacking. Lastly, experimental studies involving notifiable infectious pathogens are compounded by extensive containment requirements.

The tenacity of viral pathogens is of major interest for the reasons outlined above. Legislation aimed at the eradication and prevention of the transmission of infectious pathogens has been passed in the case of notifiable diseases (Anonymous 1980b, 1985, 1991). In

addition to the universally valid guidelines for the disinfection of stables and barns (Strauch et al. 1991), special instructions and recommendations apply to the chemical-biological processing of farm yard manure and manure (Böhm et al. 1992). In the case of hog cholera, an EU guideline (Anonymous 1980 b) stipulates that anaerobically stored manure may be disposed as fertiliser following storage lasting at least 42 days. Similar information is not available for other notifiable infectious agents.

An aerobically controlled, microbe-induced self-heating process occurs with a calibrated total solids content during the storage of farm yard manure. This mainly contributes to the well established sanitation process. Self-heating does not occur during the anaerobic storage of manure or urine, so that a reduction in the number of any pathogen or test organism present will be delayed (Rapp 1995). A field study (Pesaro et al. 1995) showed that viral tenacity is influenced to considerable extent by the type of stored liquids. It was further observed that the heat-resistant *Parvovirus* was effectively inactivated by the prevailing (bio)-chemical processes in both manure and urine. In contrast, and notably of practical significance, rotaviruses remained infectious for a long time, especially in manure (D_{90}-value over 6 months). In urine pits the corresponding value was less than 1 month. This was probably due to the efficient generation of ammonia, a known virucidal compound (Ward and Ashley 1977).

Overall, it would appear that better hygiene conditions can be expected with the conventional processing of animal excretions (farm yard manure and urine) than with the increasingly common practice of manure production. Current EU minimal hygiene recommendations for the treatment of livestock excretions should be mentioned in this context (Strauch 1991 c,d; Strauch and Ballarini 1994): manure kept under anaerobic conditions should be stored untouched for 60 days during the summer months and for 90 days during the winter months before being applied to pastures. This technical measure, which is not easily implemented, aims at reducing any contamination with infectious agents to an acceptable level via the natural processes of inactivation. In manure, however, the primary aim of hygiene safety during an outbreak, as observed with the rotaviruses, will not be achieved simply by storage. Specific aeration, combined with a temperature rise, is occasionally used to improve the hygiene of manure (Rückert et al. 1990; Fink et al. 1996).

A virological hygiene standard, similar to that sometimes applied to sewage sludge, would appear desirable. Technically straightforward and economically and ecologically appropriate processes for rendering manure hygienically safe would have to be developed before this requirement could be put into practice. Adequate sanitation cannot be guaranteed by the mesophilic (±35 °C) fermentation of manure occasionally practised in communal biogas plants (Wekerle et al. 1987; Bendixen 1994; Lund et al. 1995). Finally, alternative methods for disinfecting manure such as oligolysis (Müller 1985) or microwave processing (Schorpp and Böhm 1990) have not been implemented.

The viruses present in the excretions of infected animal stocks reach farmland either indirectly by land application of manure and sludge from communal sewage treatment plants or directly via pasture farming. Depending on the tenacity of any prevalent virus, this could lead to the long-term contamination of plants, soils and water conducts. As stated earlier, very little is known about virus transmission via these epidemiologically secondary routes. Widespread transmission in water to remote farms cannot be ruled out. However, the possible consequences of such an incident remain speculative for the time being.

2.1.2.4 Pathogenic Animal Viruses and Prions in Animal Feed

2.1.2.4.1 Potential External Risks

The health risk to animal husbandry as a result of viruses in feed produced externally, namely from abattoir, catering and kitchen waste, is well documented (Strauch 1991a; Schneidawind and Habit 1993; Strauch and Ballarini 1994; Cahenzli 1997). Foot-and-mouth disease and hog cholera are of current significance. Catering leftovers and waste obtained from the commercial production of foodstuffs (vegetables, eggs) are nowadays increasingly used in thermophilic-controlled (≥55 °C) biogas plants and subsequently applied as fertiliser. A field study conducted in a newly designed plant (retention time of 18 days) indicated effective inactivation of representative viral pathogens (Pesaro et al. 1999). A D_{90}-value of approximately 6 hours was recorded with a *Parvovirus*, known to be remarkably thermo-resistant.

Unconventional pathogens (prions), such as the one associated with bovine spongiform encephalopathy (BSE), must be considered a substantial risk given the existing zoonotic potential. Prions are characterised by their extreme tenacity (Brown et al. 1990; Brown and Gajdusek 1991; Taylor 1993; Groschup and Haas 1995; Collinge and Rossor 1996). There is no general consensus as regards the nature of "infectious" prions (Bastian 1993; Weissmann 1991). Nevertheless, serodiagnostic tests are now becoming available (Kascsak et al. 1993; Arya 1996; Van Keulen et al. 1996). The origin of the causative agent of BSE, which remains subject to confirmation (Straub 1996 a,b), is generally attributed to the use of animal meal containing inadequately treated confiscates of sheep with scrapie and, more recently, of cattle with BSE (Hörnlimann et al. 1994; Kaaden et al. 1994; Wilesmith 1994). Sterility is achieved with conventional pathogens by autoclaving (20 minutes, 121 °C). Inadequate sanitation must be assumed with unconventional pathogens under the same conditions. Based on previous experience, a D_{90}-value of 30 minutes is assumed for Creutzfeld-Jakob pathogens by autoclaving at 134 °C (Wallhäußer 1995). A $10^{5.3}$ reduction in infectivity titre was obtained within 30 minutes in a more recent study involving scrapie pathogens under the afore-mentioned conditions (Brown et al. 1990).

The incidence and tenacity of BSE pathogens in animal excretions has so far not been elucidated. The following questions which, for the time being, cannot be answered with any certainty, arise in endemic areas:

- Do exposed animals excrete some of the ingested prions in the faeces?
- Do diseased animals excrete prions in the faeces or in other excretions?
- Do prions pass into abattoir drainage systems following the slaughter of infected animals of healthy appearance?
- In the case of prion excretion, what is the concentration and tenacity of these agents in the contaminated outside world?

The latter becomes a topic of debate if potentially contaminated animal waste is used as fertiliser and if one considers that cattle ingest several hundred kilograms of soil every year with raw fodder. The scrapie agent is known to remain in soil for years without being to-

tally inactivated (Brown and Gajdusek 1991). Finally, this pathogen was also detected in hay mites (Wisniewski et al. 1996), a fact which is considered to have been responsible for the re-emergence of outbreaks amongst previously non-infected animals.

Whilst the use of most by-products from abattoirs is subject to legislation, no regulation has so far been achieved regarding the disposal of the contents from the rumen and intestines (Anonymous 1975 b; Strauch 1987, 1991 a, b, c; Strauch and Ballarini 1994; Böhm 1995). Rumen contents are occasionally fed to pigs following heat-sterilisation. Elsewhere they are mixed without pre-treatment with the content of manure storage tanks. As regards conventional pathogens, compacted rumen dung can be rendered hygienically safe by composting (Wulfert and Zimmermann 1994) or by other thermophilic processes.

2.1.2.4.2 Potential Risk Within a Farm

Once a virus has infiltrated a given animal stock, the pathogen spreads as a result of direct or indirect contact between excreting animals and susceptible recipients. As observed with caprine arthritis encephalitis, milk is the vehicle of viral transmission among goats. Attempts to clean up herds of IBR-infected beef cattle showed that farm management (processing of feed residues and avoidance of direct contact between seropositive reagents and uninfected animals) is a crucial factor for success (Ackermann et al. 1990). The significance of aerosols (Mack et al. 1986) and secondarily contaminated drinking-water ought not to be under-estimated (Mahnel 1986). At the present time it has not been confirmed whether rodents are of practical significance in the transmission of animal viruses, either as passive virus carriers or secondary hosts.

2.1.2.5 Virus Transmission in the Air

Aerial transmission of animal-pathogenic viruses to remote farms has been confirmed in the case of foot-and-mouth disease (Donaldson and Gibson 1987; Moutou and Durand 1994) and pseudorabies (Christensen et al. 1993).

2.1.2.6 Viruses in Domestic Animal Excretions

When excreted outside the dwelling area, viruses from domestic animals may enter communal drains or directly penetrate the soil and water systems. Infectious excretions from domestic animals can also infiltrate compost and biogas plants.

2.1.3 Conclusions

It has been established that diseased and sub-clinically infected animals excrete numerous pathogens, including viruses of high and low virulence. These pathogens can spread to remote locations following land application of manure and sludge from communal sewage treatment plants or during pasturing. This situation is classed as "not harmless" (Strauch and Ballarini 1994). However, animal excretions have yet to be recognised as a carrier for the remote transmission of viral pathogens, including notifiable disease agents. The trade in animals, contaminated fomites, including animal-derived feed, and aerosols are of paramount importance in terms of pathogen transmission between farms. Direct animal contact and management procedures are responsible for the transmission of pathogens in primary infected animal populations. It has yet to be established whether prevalent animal pathogens, such as prions or rotaviruses, may be transmitted to man (in Asia transmission of pandemic influenza-virus strains from animal to man is recognised). In this respect particular attention must be paid to direct contact with infected animals (domestic animal owners, farmers, veterinarians, and abattoir personnel) and indirect contact, e.g. with contaminated drinking and recreational water (general population).

2.1.4 Recommended Research

The following questions can only be partly answered at the present time:

- How frequently and to what extent are pathogenic animal viruses and prions introduced into the environment with animal excretions?
- What is the behaviour of these pathogens in the environment? Manure and urine storage tanks, abattoir drains and possible secondary hosts are of particular relevance.
- How do viruses behave under varying treatment regimens? What is the virucidal effect of communal manure storage tanks and biogas plants? How do new manure treatment techniques, such as the separation of solids from the liquid phase, influence viral tenacity?
- To what extent are human populations (certain professional categories, general population) exposed to viruses and unconventional agents originating from animal excretions? To what extent are drinking-water and recreational water affected?
- What are relevant, i.e. ecologically and economically appropriate virucidal procedures for manure?
- Can a virological hygiene standard be defined for manure?
- What measures (immune and exposure prophylaxis) can be implemented to prevent virus transmission onto and within farms? Ideal vaccines should not only protect against disease but also prevent infection and viral shedding.

In order to answer these questions, techniques for specific and quantitative virus detection have to be developed. Particular attention should be paid not only to the detection of viral components but also to viral infectivity. In order to elicit epidemiological clarification, it must be possible to trace viruses beyond the boundary of the species, right back to their origin, i.e. the pre-requisites of molecular epidemiological evaluations must be provided. This will help to design appropriate measures to prevent contamination with viral pathogens.

2.1.5 Recommended Action

Introduction of specific virus monitoring for sources of drinking-water.

2.1.6 Summary

The transmission of pathogenic viruses into animal stocks through the trade of animals and through fomites, including the use of abattoir and catering waste in animal feed, has long since been recognised. Viral infections are transmitted within infected livestock as a result of direct and indirect contact between excreting animals and susceptible recipients. Infected animals not only excrete infectious pathogens in nasal secretions and saliva, faeces and urine, but also through abrasions of the skin and mucous membranes. Some viral pathogens are preferentially transmitted in milk. Relatively little is known of the behavioural pattern of these viruses which are released into the environment during pasturing and following the use of contaminated fertilisers, such as manure and sewage sludge, on pastures. However, no transmission of viral pathogens has so far been observed with this type of livestock management. Nevertheless, animal viruses can, like human pathogens, contaminate soils and water supplies. Little light has, however, been shed on such viral contamination and its potential repercussions. Based on experience gleaned with notifiable virus diseases, the potential risk to animal stocks ought only to be slight. Since viruses are mostly host-specific, man is not immediately at risk from viruses contained in animal excretions. It remains to be seen whether potential zoonotic pathogens, including prions and rotaviruses, can be transmitted to man via contaminated plant- (or animal-) based foodstuffs or via water. The extent and duration of exposure to potentially zoonotic agents together with any changing susceptibility of exposed populations should be taken into account when assessing this possibility.

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2.2 Bacteria in Feedstuffs and Faeces

2.2.1 Bacteria in Feedstuffs

Johann Bauer and Stefan Hörmansdorfer

2.2.1.1 Introduction

Feedstuffs are always contaminated with microorganisms originating either from the primary flora of agricultural crops, from animals or from processing. In this way, certain species of bacteria or germ accumulation may adversely affect feed quality and thus lead to poor performance or even sickness in animals.

2.2.1.2 Relevant Bacteria, Their Characterisation, Incidence and Significance

2.2.1.2.1 Gram-positive Cocci

Gram-positive, pigmented, sometimes facultatively anaerobic cocci, arranged in irregular clusters and belonging to the genera *Micrococcus, Planococcus* and *Staphylococcus* are widely distributed. Certain pathogenic species are also capable of producing exotoxins in addition to their further virulence factors. They can cause feed spoilage due to their lipolytic and proteolytic properties and their ability to form acids from oligosaccharides and are indicators for poor hygienic conditions during storage (Götz 1978; Bucher 1979; Kocur 1986; Schleifer 1986; Pioch et al. 1988; Bräuning 1989; Peters and Schumacher-Perdreau 1992).

Recommended research: Clarification of the pathogenicity of staphylococci present in feedstuffs with particular emphasis on their toxin-producing capability.

Chain-forming cocci of the genera *Streptococcus, Enterococcus, Lactococcus, Aerococcus, Gemella* and *Leuconostoc* are Gram-positive, facultatively anaerobic bacteria with fermentative metabolism.

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They are rarely isolated from feedstuffs. The facultatively pathogenic *Enterococci,* of which some strains are capable of toxin production, serve to indicate faecal contamination (Bucher 1973; Hardie 1986; Bisping and Amtsberg 1988; Lütticken 1992; Holt et al. 1994).

Recommended research: Toxin production by enterococci.

2.2.1.2.2 Endospore-forming Gram-positive Rods

Genus: *Bacillus*

Bacillus spp. are Gram-positive or Gram-labile, catalase-positive, aerobic or facultatively anaerobic, widely distributed rods which form highly resistant endospores. They are regularly found in feedstuffs and cause spoilage due to their amylolytic, proteolytic and lipolytic properties. Some species are capable of toxin production (see Table 2.1) (Claus and Berkeley 1986; Dutkiewicz et al. 1989).

Recommended research: Differentiation of the *Bacillus* flora in various groups of feedstuffs; presence of toxin-producing *Bacillus* spp. in feedstuffs.

Genus: *Clostridium*

Table 2.1: Toxinogenic *Bacillus* species (Freer 1988)

Bacillus sp.	Toxin
B. alvei	Alveolysine
B. anthracis	Anthrax toxin
B. cereus	Cereolysine O, haemolysin II, lethal toxin, emetic toxin, diarrhoeic toxin
B. laterosporus	Laterosporolysine
B. pumilus	Cytotoxin
B. sphaericus	Larvicidal toxin
B. subtilis	Surfactin
B. thuringiensis	Crystal protoxin and active δ-toxin, thuringiolysine,
Subsp. kurstaki and berliner	α-toxin
Subsp. israelensis	Crystal protoxin and insecticidal toxin, cytolytic toxin
Subsp. tenebrionis	Crystal protoxin and insecticidal toxin

Table 2.2: Toxinogenic *Clostridium* species and their toxins (Freer 1988)

Species	Toxins
Cl. botulinum	Neurotoxins A, B, C_1, D, E, F, C_2-toxin (C_{2I} and C_{2II}), botulinosine
Cl. difficile	Toxin A and B
Cl. perfringens	β-Toxin, δ-toxin, ε-toxin, θ-toxin (perfringolysine), ι-toxin components A and B, enterotoxin
Cl. sordellii	Lethal toxin, haemorrhagic toxin
Cl. tetani	Tetanus neurotoxin (tetanospasmin), tetanolysine
Cl. septicum	α-Toxin (similar to *Cl. histolyticum* and *Cl. chauvoei*), septicolysine
Cl. chauvoei	α-Toxin, SH-activated δ-toxin, chauvoelysine
Cl. novyi	α-Toxin (oedema-inducing toxin), SH-activated δ-toxin (oedematolysine), ε-toxin

Clostridium spp. are Gram-positive, catalase-negative, obligatorily anaerobic, endospore-forming rods. They are distributed widely and can also be detected in feedstuffs.

Clostridium spp. produce organic acids and various hydrolases which cause spoilage. Various species cause intoxications and infectious diseases due to their ability to produce potent toxins (see Table 2.2) (Stenberg and Estola 1963; Cato et al. 1986; McLoughlin et al. 1988; Jonsson 1990; Kinde et al. 1991; Ruckdeschel 1992).

Recommended research: Presence and distribution of toxin-producing *Clostridium* spp. in feedstuffs.

2.2.1.2.3 Uniform, Asporogenic Gram-positive Rods

Genus: *Lactobacillus*

Lactobacilli are Gram-positive, asporogenic, facultatively anaerobic rods with fermentative metabolism. They mainly produce lactate. *Lactobacilli* are wide-spread and belong to the predominant flora of fermented feedstuffs. Their preservative effect is based on the production of antibacterially active compounds (see Table 2.3) (Kandler and Weiss 1986; ten Brink et al. 1994).

Table 2.3: Antibacterial substances produced by *Lactobacillus* spp. and other lactic acid bacteria (Kandler 1982; Falbe and Regitz 1989; Muriana and Klaenhammer 1991; Barefoot and Nettles 1993; Hammes and Tichazek 1994; ten Brink et al. 1994; Schillinger et al. 1995)

Classes	Substance	Range of action
Peroxides	Hydrogen peroxide	Broad, especially against Gram-negative bacteria (*E.coli*, *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp.)
Acids	Lactic acid	
	Acetic acid	
	Formic acid	
	Benzoic acid	
Bacteriocins	Lactocin S	Lactic acid bacteria
	Plantaricin A	
	Bavaricin	Lactic acid bacteria; *Listeria*
	Curvacin A	
	Sakacin A	
	Sakacin P	
	Lactacin F	*Lactobacillus* (Lb.) sp., *Enterococcus faecalis*
	Lactacin B	*Lb. helveticus, Lb. delbrückii*
	Curvaticin FS47	*Lb.* sp., *Listeria* sp., *Bacillus* sp.
	Caseicin 80	*Lb. casei*
	Helveticin J	*Lb. helveticus, Lb. delbrückii*
	Helveticin V-1829	
	Fermenticin	*Lb. fermentum*
	Lactocin 27	*Lb. helveticus, Lb. acidophilus*
	Plantaricin S	Lactic acid bacteria, *Micrococcus* sp., *Propionibacterium* sp., *Clostridium* sp.

Recommended research: Production of metabolites with antibacterial activity by *Lactobacillus* spp. in feedstuffs and their effect on animal health.

Genus: *Listeria*

The genus *Listeria* with its 7 different species comprises short, facultatively anaerobic, catalase-positive, Gram-positive rods, motile at a temperature of 20–25 °C and producing acids from glucose. *L. monocytogenes* and *L. ivanovii* are facultatively pathogenic. *Listeria* spp. are widely found in the environment and frequently isolated

from silage (Welshimer 1981; Seeliger and Jones 1986; Albrecht 1992; Holt et al. 1994).

Recommended research: The health risk of *Listeria*-contaminated feedstuffs; strategies to reduce *Listeria*-contamination of silage.

Genus: *Erysipelothrix*

The only species of this genus is *Erysipelothrix rhusiopathiae* – a straight, slender, nonmotile, facultatively anaerobic, α-haemolysing, Gram-positive rod. The pathogen, which causes erysipelas in swine, other mammals and birds, occurs widespread in nature and can be found in food crops, water and fish (Jones 1986; Erler 1987).

Recommended research: The presence of *E. rhusiopathiae* in feedstuffs and particularly in pig feed.

2.2.1.2.4 Irregular, Asporogenic, Gram-positive Bacteria

These include the following genera: *Corynebacterium, Clavibacter, Brevibacterium, Propionibacterium, Arthrobacter, Curtobacterium, Microbacterium, Aureobacterium, Cellulomonas, Agromyces* and *Eubacterium*.

These are short, markedly pleomorphic, generally catalase-positive, asporogenic, Gram-positive rods. A distinction is made between obligatorily aerobic, facultatively anaerobic or aerotolerant genera. *Corynebacteria* are frequently associated with mucosal membranes, whilst other genera are found in the environment or in milk and milk products etc. They may participate in spoilage of feedstuffs (Casida 1986; Collins and Bradbury 1986; Collins and Cummins 1986; Collins and Keddie 1986; Cummins and Johnson 1986; Jones and Keddie 1986; Keddie et al. 1986; Komagata and Suzuki 1986; Moore and Holdeman Moore 1986; Stackebrandt and Keddie 1986; Holt et al. 1994).

Recommended research: Presence of *Propionibacterium* spp. in fermented feedstuffs and milk replacer; the significance of *Propionibacterium* spp. in feedstuffs.

2.2.1.2.5 Gram-negative, Aerobic Rods

Pseudomonadaceae

Pseudomonadaceae are straight or curved, obligatorily aerobic, partly pigmented or haemolysing, Gram-negative rods with polar flagellae. They are divided into four genera: *Pseudomonas*, *Xanthomonas, Frateuria* and *Zoogloea*.

Pseudomonadaceae are widely distributed in the environment, in water and also in feedstuffs. They belong to the primary flora of cereals and are taken as indicator flora for fresh, uncontaminated vegetable-based feedstuffs. Only a few of the *Pseudomonas* and *Xanthomonas* species are considered to be facultatively pathogenic (Palleroni 1984 a, b; Bisping and Amtsberg 1988; Brandis and Pulverer 1988; Kleinhempel et al. 1989; Burkhardt 1992; Gedek 1995).

Recommended research: Differentiation of *Pseudomonadaceae* found in feedstuffs. Evaluation of their pathogenic potential.

2.2.1.2.6 Facultatively Anaerobic, Gram-negative Rods

Enterobacteriaceae

Enterobacteriaceae are coccoid, facultatively anaerobic, Gram-negative rods with usually peritrichous flagellae, distributed ubiquitously as modest saprophytes. They are divided up into various genera such as *Escherichia, Salmonella, Enterobacter, Pantoea, Erwinia, Citrobacter, Klebsiella, Proteus* and *Serratia*.

Members of the genera *Pantoea* and *Erwinia* belong to the primary flora of vegetable-based feedstuffs and serve to indicate fresh, uncontaminated food.

The genera *Escherichia*, other coliform germs and the genera *Enterobacter, Citrobacter, Klebsiella* and *Proteus*, commonly found in human and animal intestinal tracts, occur less frequently in feedstuffs where they indicate faecal contamination. Various strains of *Escherichia coli* and *Enterobacter cloacae* are adhesive to mucosal membranes, produce toxins and are therefore classified as infectious pathogens.

Members of the genus *Salmonella* are detected time and time again mainly in feedstuffs of animal origin such as carcass meal or

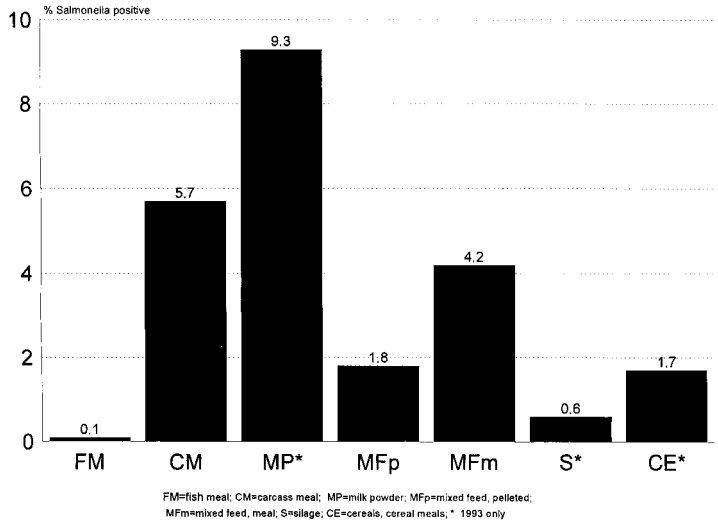

Figure 2.2: Occurrence of Salmonella in feedstuff. Mean values for 1991–1993 without imported feed (BGA 1992, 1993; BgVV 1994).

milk powder (see Figure 2.2). Imported feedstuffs pose specific problems in this respect. *Salmonella*-contaminated feedstuffs are nevertheless considered a source of pathogens for humans and animals alike, even when the *Salmonella* strains isolated from feedstuffs generally do not belong to those 50 of the more than 2300 serotypes which are highly prevalent in disease outbreaks (Le Minor 1984; Richard 1984; Spicher and Istford 1986; Bisping and Amtsberg 1988; Sixl et al. 1989; Beutin 1990; Pioch 1991; Bockemühl 1992; Burkhardt 1992; Krämer 1992; Clarke and Gyles 1993; Hartung 1993; Holt et al. 1994; Baljer 1995; Gedek 1995).

Recommended research: The presence and significance of pathogenic *Enterobacteriaceae* in feedstuffs; improved methods for the detection of *Salmonella* in feedstuffs in regard to sensitivity and investigation duration.

2.2.1.2.7 Mycobacteria

Genus: *Mycobacterium*

Mycobacterium spp. are slender, straight or slightly curved, non-motile, catalase-positive, obligatorily aerobic, acid- or acid-alcohol-positive, Gram-positive rods. The synthesis of waxy substances containing mycolic acid is a typical feature of this genus. *Mycobacteria* grow with brightly pigmented, coarse colonies after 2 to 60 days incubation. Apart from a few obligatorily pathogenic species (see Table 2.4), most *Mycobacterium* spp. are harmless, environmental saprophytes (see Table 2.5), which can invade feedstuffs through soil and water (see Table 2.6). The significance of the *Mycobacterium avium-Mycobacterium intracellulare* complex has increased in recent years, particularly in conjunction with AIDS (Beerwerth and

Table 2.4: *Mycobacterium* spp. as pathogens (Rolle and Mayr 1993; Goodfellow et al. 1986; Salfinger and Kafader 1992)

Species	Disease	Host
M. tuberculosis	Tuberculosis	Humans, mammals
M. africanum	Tuberculosis	Humans
M. bovis	Tuberculosis	Mammals, humans
M. avium	Tuberculosis	Birds, pigs, (humans)
M. microti	Tuberculosis	Vole
M. leprae	Leprosy	Humans
M. paratuberculosis	Paratuberculosis	Cattle, small ruminants
M. marinum	Fish tuberculosis	Fish, (humans)

Table 2.5: Environmental occurrence of *Mycobacterium* spp. (Beerwerth and Schürmann 1969)

Sample	Samples examined	Mycobacteria positive	%
Forest soil	198	78	39.4
Meadow soil	175	123	70.3
Soil of arable land	158	138	87.3
Soil of sewage fields	99	99	100.0
Raw sewage	100	93	93.0

Table 2.6: Occurrence of *Mycobacterium avium* in 58 porcine feedstuffs (Kauker and Rheinwald 1972)

Feedstuff	Number of samples	*M. avium* positive	%
Fish meal	12	6	50.0
Protein concentrate	17	7	41.2
Carcass meal	13	3	23.1
Tapioca meal	4	1	25.0
Soybean meal	6	1	16.7
Middlings	2	0	0.0
Wheat	1	0	0.0
Maize	1	0	0.0
Barley	1	1	/
Maize silage	1	0	0.0

Schürmann 1969; Kauker and Rheinwald 1972; Wayne and Kubica 1986; Dimov and Gonzalez 1986; Timoney et al. 1988 b; Morris 1991; Rolle and Mayr 1993; Holt et al. 1994).

Recommended research: The presence of *Mycobacterium* species, especially of pathogenic ones in feedstuffs.

2.2.1.2.8 *Actinomycetes*

Actinomycetes are filament-forming, mostly non-motile, aerobic, catalase-positive, partly pigmented, partly acid-fast, Gram-positive bacteria. They grow with a vegetative substrate mycelium, which fragments into cocci and rods, occasionally accompanied by aerial hyphae.

Conidia or spores may be produced, e.g. in sporangia, sclerotia or synnemata. *Actinomycetes* are divided into numerous suprageneric groups.

They occur widely in the environment and are isolated mainly from soil, plants and plant-based feedstuffs, especially hay and cereals (see Table 2.7). Because of their large arsenal of hydrolytic enzymes they can cause spoilage of feedstuff and thus serve as spoilage indicators, too.

Moreover, *Actinomycetes* may be capable of releasing numerous metabolites with antibacterial or antibiotic effects, even into feedstuffs (see Table 2.8). Some species of *Actinomycetes* are known

Table 2.7: Occurrence of *Actinomycetes* in feedstuffs

Feedstuff	Examined samples (n)	*Actinomycetes* (n)	positive %
Hay	95	49	51.58
Cereals	38	7	18.42
Coarse meal	7	4	57.14
Mixed feed (non-pelleted)	77	34	44.16
Mixed feed (pelleted)	33	3	9.09
Silage	173	37	21.39
Grass silage	99	21	21.21
Maize silage	60	13	21.67

to trigger disease or allergy in humans and animals (Lacey 1978; Williams 1978; Timoney et al. 1988a; Goodfellow 1989a, b; Goodfellow and Lechevalier 1989; Williams et al. 1989a, b; Lacey 1989; McCarthy 1989; Schaal 1992; Holt et al. 1994; Gedek 1995).

Recommended research: The development of methods to differentiate between *Actinomycetes* isolated from feedstuffs; the appearance of antimicrobial metabolites in feedstuffs; the interaction between *Actinomycetes* and other feedstuff microflora.

2.2.1.3 Legal Position

The laws governing feedstuffs, animal epidemics and the disposal of animal carcasses provide the basis for the legal assessment of the marketability of feedstuffs.

It can therefore be deduced that feedstuffs must also be free from pathogens or toxic metabolites of microbial origin. The permissible level of environmental microorganisms is not governed by limit values. So-called outline values for the microorganism content of commercial feedstuffs have only come to light in recent years based on the 2/3 value (see Table 2.9). This refers to the maximum microbial count which cannot be exceeded by 2/3 of all random samples of a product manufactured according to the principles of good manufacturing practice (Anonymous 1975; Anonymous 1995; Anonymous 1998; Kummer et al. 1977; Schmidt 1991; Gedek 1995).

Table 2.8: Antibiotic, antimycotic and antiparasitic metabolites of *Actinomycetes* (Hopwood and Merrick 1977; Bauer and Seeger 1979; Rothrock and Gottlieb 1981; Bruns 1983; Williams et al. 1989 b; Rosin 1992; Holt et al. 1994)

Species	Metabolite	Susceptible organisms
Actinomadura cremea	Rifamycin	Bacteria
Actinomadura kijaniata	Kijanimycin	Gram-positive bacteria, anaerobes, *Plasmodium* sp.
Actinoplanes teichomyceticus	Teicoplanin	Bacteria
Actinosynnema mirum	Nocardicin	n.s.
Actinosynnema pretiosum	Ansamitocin, tomaymycin, macbecin, dynacin, nocardicin	n.s.
Amycolata autotrophica	Nocardin	n.s.
Amycolatopsis mediterranei	Rifamycin	Bacteria
Amycolatopsis orientalis	Vancomycin	Bacteria
Amycolatopsis sulphurea	Vhelocardin	n.s.
Glycomyces harbinensis	Azaserin	n.s.
Kibdelosporangium aridum	Aricidine A, B, C (glycopeptide antibiotics)	n.s.
Kitasatosporia griseola	Setamycin	Bacteria, trichomonads
Kitasatosporia medicidica	Mediocidicin	n.s.
Kitasatosporia phosalacinea	Phosalacine	n.s.
Kitasatosporia setae	Setamycin	Bacteria, trichomonads
Micromonospora sp.	Gentamycin	Bacteria
Nocardia lurida	Ristocetin	n.s.
Nocardiopsis flava	Madumycin	n.s.
Nocardiopsis mutabilis	Polynitroxin	n.s.
Nocardiopsis syringae	Nocamycin	n.s.
Planomonospora parontospora ssp. *antibiotica*	Sporangiomycin	n.s.
Saccharomonospora spp.	Thermoviridin	n.s.
Saccharopolyspora spp.	Sporaricin, erythromycins A, B	Bacteria
Streptomyces achromogenes var. rubradiris	Rubradirin	n.s.
Streptomyces alanosinicus	Alanosin	Tumours, virus, fungi
Streptomyces albaduncus	Danomycin	Bacteria
Streptomyces albospinus	Spinamycin	Fungi
Streptomyces ambofaciens	Spiramycin	Bacteria

Table 2.8 (*continued*)

Species	Metabolite	Susceptible organisms
Streptomyces antibioticus	Oleandomycin, actinomycin	Bacteria
Streptomyces antimycoticus	Endomycins A,B,C,D	Fungi
Streptomyces atratus	Rufomycins A, B	Bacteria
Streptomyces aureofaciens	Chlortetracycline, tetracycline	Bacteria
Streptomyces bikiniensis var. zorbonensis	Zorbamycin, zorbonomycin	n.s.
Streptomyces bobili	Cinerubin-like antibiotic	n.s.
Streptomyces caeruleus	Caerulomycin	n.s.
Streptomyces celluloflavus	Aureothricin	n.s.
Streptomyces champavatii	Champamycins A, B, champavatin	Fungi
Streptomyces cinerochromogenes	Cineromycins A, B	Bacteria
Streptomyces cinnamonensis	Actithiazinic acid	Mycobacteria
Streptomyces clavuligerus	Cephamycins	n.s.
Streptomyces crystallinus	Hygromycin A	Bacteria
Streptomyces cuspidosporus	Sparsomycin, tubercidin	n.s.
Streptomyces djakartensis	Niddamycin (macrolide antibiotic)	Bacteria
Streptomyces ederensis	Moenomycins A, B_1, B_2, C	Bacteria
Streptomyces erumpens	Tetrins A, B and other polyenes	Fungi
Streptomyces erythreus	Erythromycin	Bacteria
Streptomyces erythrogriseus	Erygrisin	Bacteria
Streptomyces fimbriatus	Septacidin	Tumours, fungi
Streptomyces flavopersicus	Spectinomycin	Bacteria
Streptomyces floridae	Viomycin complex	n.s.
Streptomyces fradiae	Neomycin, tylosin	Bacteria
Streptomyces gancidicus	Gancidins A, W	Tumours, Gram-positive bacteria
Streptomyces geysiriensis	Moenomycins A, B_1, B_2, C	Bacteria
Streptomyces ghanaensis	Moenomycins A, B_1, B_2, C	Bacteria

Table 2.8 (*continued*)

Species	Metabolite	Susceptible organisms
Streptomyces griseoloalbus	Grisein-(albomycin)-complex	Bacteria
Streptomyces griseus	Streptomycin	Bacteria
	Cycloheximide	Fungi
Streptomyces halstedii	Carbomycin	Bacteria
Streptomyces hydroscopicus	Turimycin	n.s.
Streptomyces hygroscopicus var. geldanus	Geldanamycin	n.s.
Streptomyces inusitatus	Oxamicetin	n.s.
Streptomyces kanamyceticus	Kanamycin	Bacteria
Streptomyces kasugaensis	Aureothricin, kasugamycin	n.s.
Streptomyces kitazawensis	Antimycin	Fungi
Streptomyces laurentii	Thiostrepton	n.s.
Streptomyces lavendofoliae	Streptothricin	Bacteria
Streptomyces lavendulae	Framycetin	Bacteria
Streptomyces lincolnensis	Lincomycin	Bacteria
Streptomyces lipmannii	Cephamycine	n.s.
Streptomyces lomondensis	Lomofungin	Bacteria, fungi
Streptomyces mauvecolor	Peptimycin	Tumours
Streptomyces mediolani	Peptide antibiotics	n.s.
Streptomyces niveoruber	Cinerubins A, B	n.s.
Streptomyces niveus	Novobiocin	Bacteria
Streptomyces nodosus	Amphotericin B	Fungi
Streptomyces nojiriensis	Nojirimycin	Bacteria
Streptomyces noursei	Nystatin, cycloheximide	Fungi
Streptomyces orientalis	Vancomycin	Bacteria
Streptomyces parvulus	Actinomycin	n.s.
Streptomyces peucetius	Daunomycin,	Bacteria, tumours
	Polyene antimycotics	Fungi
Streptomyces pulveraceus	Neomycins E, F	Bacteria
	Paromomycin	Bacteria
	Zygomycin B	n.s.
	Naramycin B	n.s.
	Cycloheximide	Fungi
Streptomyces purpeofuscus	Negamycin	n.s.

Table 2.8 (*continued*)

Species	Metabolite	Susceptible organisms
Streptomyces rameus	Streptomycin	Bacteria
Streptomyces reticuli var. protomycicus	Protomycin	Fungi
Streptomyces rimosus	Oxytetracycline	Bacteria
Streptomyces roseoflavus	Flavomycin	Bacteria
	Mycelin	Fungi
Streptomyces sannanensis	Sannamycins	n.s.
Streptomyces sp.	Fosfomycin	Bacteria
Streptomyces spectabilis	Spectinomycin	Bacteria
Streptomyces sphaeroides	Novobiocin	Bacteria
Streptomyces sulfonofaciens	Carbapenem component (β-lactam antibiotic)	Bacteria
Streptomyces tanashiensis	Luteomycin	n.s.
Streptomyces violaceus (Streptomyces venezuelae)	Chloramphenicol	Bacteria
Streptomyces virginiae	Virginiamycin	Bacteria
Streptomyces vitaminophilus	Pyrrolomycin antibiotics	n.s.
Streptosporangium albidum	Sporoviridin-like antibiotics	n.s.
Streptosporangium viridogriseum ssp. *viridogriseum*	Sporoviridin	n.s.
Streptosporangium viridogriseum ssp. *kofuense*	Chloramphenicol	Bacteria
Thermoactinomyces thalophilus	Thermorubin	Gram-positive bacteria
Thermomonospora formosensis	Rifamycin O and S	n.s.

n.s. = no specification

Table 2.9: Reference values for bacteria content of feedstuffs (Schmidt 1991; Gedek 1995)

Feedstuff	Mesophilic bacteria Cfu/g (in millions)
Single feedstuff	
Dairy by-products, dried	0.1
Blood Meal	0.2
Carcass Meal, Fish Meal	1
Oil production residues	
– extracted oilseed meal	1
– oilseed cake	2
Cereals and cereal by-products	
– middlings	2
– bran (wheat, rye)	5
– cereal seed, wholemeal	
• maize	5
• wheat, rye	5
• barley	8
• oat	15
Mixed feedstuff (meal) for	
– young and fattening poultry	2
– laying hens	3
– piglets	5
– fattening pigs	5
– calves	2
– dairy cows and cattle	5
Mixed feedstuff (pressed) for	
– young and fattening poultry	0.5
– laying hens	0.5
– piglets	0.5
– fattening pigs	1
– calves	0.5
– dairy cows and cattle	1
Milk substitute	0.5
Protein concentrate	2

2.2.1.4 Recommended Research (Summary Report)

Essential research requirements can be summarised as follows:

- Tests to determine the composition of the bacterial flora of feedstuffs; clarification of the pathogenetic significance of dominant bacterial species.
- The drafting of criteria for objective assessment (standard values) to determine the hygienic status of feedstuffs.
- Investigation of the significance of feedstuffs in the spread of pathogenic or facultatively pathogenic bacteria to humans and/or animals.
- Development, improvement or evaluation of rapid, sensitive methods for detection of pathogenetically significant bacteria in feedstuffs.
- Research of the presence of bacterial metabolites with toxic and/or pharmacological (antibiotic) effects.

2.2.1.5 Recommended Action

Given the gaps in our knowledge there is no definite need to take action at the present time.

2.2.1.6 Summary

The spectrum of the various species of bacteria found in feedstuffs is remarkably broad. Much of these can be attributed to the following genera: *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Enterobacter.* The detection of bacteria alone does not confirm spoilage or a health risk right from the outset. In fact, the microbial content and the composition of the bacterial flora tend to shed light on the hygienic status of the respective feedstuffs. Thus the yellow pigmented germs of the genus *Erwinia* and *Pantoea agglomerans* together with *Pseudomonadaceae* indicate whether cereals, cereal products and cereal-enriched mixed feed are fresh and unspoiled. The detection of *Salmonellae* or toxinogenic *E. coli* must be assessed differently. This, however, calls for a distinction to be made between certain

species of bacteria in addition to the microbial count. Objective criteria for the evaluation (standard values) of the hygienic status are required.

It is particularly important to establish the extent to which infectious pathogens of bacterial origin are spread with the vector feedstuff. This is described over and over again, but concrete data has been acquired only in the case of *Salmonella* spp. and *Listeria* spp. Moreover, the bacteria found in feedstuffs can produce numerous substances with toxic and/or pharmacological (e.g. antibiotic) effects. The significance attached to the natural presence of such compounds in feedstuffs is far from negligible concerning preventive medicine and forensic science.

Rapid, selective and sensitive techniques to facilitate detection of both the bacteria and their metabolites in the complex feedstuff matrix are needed in an attempt to clarify such matters.

Given the gaps in our knowledge at the present time, action is not justified from an objective standpoint.

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2.2.2 Bacteria and Bacterial Metabolites in Faeces From Farm Animals

Reinhard Böhm and Andrea Pfirrmann

2.2.2.1 Introduction

Bacteria from faecal sources as slurry or solid manure are of environmental importance since the relevant materials are stored and handled in the farms and applied to agricultural soils. This means that pathogenic and non pathogenic bacteria, fungi, viruses and parasites are released, because treatment of the faeces, which will inactivate their infectious burden, will only be used in rare cases and storage alone is not enough for this purpose (Munch et al. 1987).

Table 2.10: Number of farm animals and amount of resulting faeces in the Federal Republic of Germany in December 1997 (Anonymous 1998)

Species	Number of farm animals in 1000	Amount of resulting faeces in 1000 t/a
Cattle	15,227.2	194,603.62
Swine	24,795.2	63,971.62
Poultry	102,731.3	5,136.87
Total	142,753.7	264,312.11

Bacteria from the gut of farm animals may be also spread via the air from the animal stalls to the environment, when using liquid or solid manure as a fertiliser, or from the slaughterhouse via waste water into surface-water. Depending on the circumstances and type of bacteria this may result in an introduction of pathogenic, or genetically engineered microorganisms or antibiotic-resistant bacteria, into the biocoenosis, the soil, the surface- and sometimes also the ground-water. Little attention has been paid in the past to bacterial metabolites and toxins in faecal media, which may be produced by propagation or lysis of bacterial or fungal cells during storage, aerobic or anaerobic treatment and will be released by the spreading of solid or liquid manure. Bacterial products such as endotoxins or exotoxins may also be propagated aerogenically, and risk has to be taken into account if these materials are taken up by humans or animals and via the pollution of water, air or soil. Table 2.10, which lists the annual "production" of faeces from animal husbandry in Germany, shows the extent of the possible propagation of those microorganisms via faecal material into the environment.

2.2.2.2 The Presence of Bacteria in Manure

The occurrence of bacteria in manure and soils is of epidemiological importance. This paper will report the type and amount of bacteria described in the literature as present in liquid or solid manure. In general it must be stated, that more information is available (or can be concluded from clinical cases) concerning the qualitative aspect, than data giving exact figures about the frequency of isolation from manure and the range of germ count determined in experimental

studies or during the analysis of epidemics. The availability of data is reported in the following order:

- gram-positive bacteria,
- gram-negative bacteria,
- *Mycobacteria*,
- *Actinomycetes*,
- genetically engineered bacteria and
- antibiotic-resistant bacteria.

2.2.2.2.1 Gram-positive Bacteria in Manure

Gram-positive bacteria in faecal media may occur as excreted pathogens, transient contaminants or saprophytic flora. Good information is available about qualitative and quantitative data from *Bacillus anthracis* and *Listeria monocytogenes*. Relatively good but not complete data may be found in literature concerning *Enterococci* (D-*Streptococci*), *Bacillus cereus*, especially toxigenic types, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium chauvoei* and *Erysipelothrix rhusiopathiae*. Only fragmentary data can be found concerning *Staphylococci* (e.g. *Staph. aureus*) and *Clostridium botulinum*. Table 2.11 gives an example concerning the sources of *Listeria monocytogenes* with regard to the excretion of this species into manure.

2.2.2.2.2 Gram-negative Bacteria

Due to the fact that *Enterobacteriaceae* have their special habitat in the gut of animals, more data are available concerning this group. Nevertheless except for *Salmonella* those data are mainly qualitative. Table 2.12 summarises some data concerning *Salmonella*. Good or sufficient but incomplete data are available for *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Pasteurella multocida*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Escherichia coli*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Yersinia frederiksenii*, *Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter coli*, *Treponema hyodysenteriae*, *Leptospira pomona*, *Leptospira interrogans* and *Leptospira icterohaemorrhagiae*. Almost no data could be found concerning *Pseudomonas aeruginosa* and the wide-spread *Proteus* spp.

Table 2.11: Occurrence of *Listeria monocytogenes* in the nasal secretion and in the faeces of clinical healthy domestic animals. Arranged by Weiß and Amtsberg (1995)

Species	Sample material	Number of animals (livestock)	Number of *L.m.*-findings (%)	Remarks
Sheep	Faeces	520 (11)	8 (1.5)	
Sheep	Nasal secretion	233 (6)	70 (30.0)	Corn silage, livestock with listeriosis
Sheep	Faeces	90 (6)	19 (21.1)	Corn silage, livestock with listeriosis
Sheep	Nasal secretion	125 (3)	1 (0.8)	Turnip leaf silage
Sheep	Nasal secretion	32 (1)	2 (6.3)	Grass silage
Cattle	Faeces	622	41 (6.6)	Grass silage, livestock with listeriosis
Cattle	Faeces	120	2 (1.7)	
Cattle	Faeces	80 (2)	12 (15.0)	Grass silage
Cattle	Faeces	62 (2)	1 (1.6)	Corn silage
Cattle	Faeces	45 (3)	0	Turnip leaf silage
Cattle	Nasal secretion	62 (2)	1 (1.6)	Corn silage
Cattle	Faeces	300	32 (10.7)	
Cattle	Faeces	3878 (2499)	258 (6.7)	
Cattle	Faeces	79 (9)	40 (51.0)	
Swine	Faeces	577	2 (0.3)	
Swine	Faeces	96	3 (3.1)	
Swine	Faeces	172	3 (1.7)	
Poultry	Faeces	465	14 (3.0)	
Broiler chicken	Faeces	2373 (146)	98 (4.1)	
Duck	Faeces	30	2 (6.7)	
Goose	Faeces	20	2 (10.0)	
Turkey	Faeces	40	3 (7.5)	
Horse	Faeces	400	49 (12.3)	
Dog	Faeces	300	4 (1.3)	
Cat	Faeces	275	1 (0.4)	

Table 2.12: Detection of different species of *Salmonella* in animal faeces

Bacteria species	Occurrence in		
	Faeces	Manure	Liquid manure
Salmonella spp.	+ Swine (Mafu et al. 1989)	+ Cattle (Philipp et al. 1990)	+ Swine (Rüprich 1994) + Mixed slurry (Rapp 1995) + (Philipp et al. 1990)
S. typhimurium	+ Calf/cattle/swine (Weber 1988)	(+)	(+)
S. enteritidis	+ Poultry (Schroeter et al. 1995) + Cattle (Weber 1988)	(+)	(+)
S. agona	+ Cattle (Weber and Enders 1990)	(+)	(+)
S. dublin	+ Cattle/calf (Weber 1988)	(+)	(+)
S. choleraesuis	(+)	(+)	(+)
S. abortusbovis	(+)	(+)	(+)
Other serovars	Swine:	(+)	(+)
S. brandenburg	+	(+)	(+)
S. bredeny	+	(+)	(+)
S. derby	+	(+)	(+)
S. hadar	+	(+)	1.5×10^3 CFU/ml
S. heidelberg	+	(+)	(Rüprich 1994)
S. infantis	+ (Mafu et al. 1989)	(+)	(+)
S. london	+	(+)	1.5×10^3 CFU/ml
S. saint paul	+	(+)	(+)

+ = detected, (+) = no specific qualitative or quantitative data available, but occurrence is to be expected

Table 2.13: Detection of different *Mycobacteria* in animal faeces

Bacteria species	Occurrence in		
	Faeces	Manure	Liquid manure
M. bovis / *M. avium*	(+)	(+)	(+)
M. paratuberculosis	Goats: + (Vihan et al. 1989) Cattle: + (Kim et al. 1989) Cattle: + (Ley 1992)	(+)	(+)

(+) = occurrence is to be expected, but no specific literature available, + = detected

2.2.2.2.3 *Mycobacteria* and *Actinomycetes* spp.

Only limited data are available concerning mycobacteria although there should be more information from clinical cases. Table 2.13 summarises some data from the literature. No data are available about *Actinomyces* spp. except fragmentary data for *Actinomyces pyogenes*.

2.2.2.2.4 Genetically Engineered and Antibiotic-resistant Bacteria

As soon as genetically engineered bacteria are used in animal husbandry as oral live vaccines or probiotics, they will occur in manure also.

Concerning antibiotic-resistant bacteria, there is no doubt that the resistance patterns of several species isolated from manure or waste are the result of prophylactic or therapeutic treatment of the animals concerned. Antibiotic-resistant bacteria will be present in particular if the selection pressure continues due to persistence of antibiotics in manure. Table 2.14 gives some information concerning the behaviour of some antibiotics and sulphonamides in faecal matter.

2.2.2.3 Survival in Manure and Soil

Long-term survival in manure and the environment increases the epidemiological risk of transmission of a pathogen. Transmission cir-

Table 2.14: Data on biotransformation, elimination and stability of antimicrobial effective veterinary drugs as a function of the type of administration (by Kroker 1983)

Type of antibiotic	Way of elimination	Biotransformation	MTC-Value	Stability
Aminoglycosides (parenteral)	Urine (concentration: –0.2 mg/ml)	Scant, up to 70% of the applied dose are excreted in an effective form	Gram-positive bacteria: 1–4 µg/ml	pH 2.2–10: little loss of effectiveness. Thermoresistant at 100 °C
Aminoglycosides (oral)	Faeces (concentration up to 1 mg/g)	–	Gram-negative and gram-positive bacteria: 0.1–4 µg/ml	pH 2.2–10: little loss of effectiveness. Thermoresistant at 100 °C
Tetracyclines (parenteral)	Faeces, urine (concentration: 0–24 h: –0.3 mg/ml)	Scant, 15–20% in faeces in active form, remainder in urine, also in active form	Gram-negative and gram-positive bacteria: 0.1–4 µg/ml	Stable only in acid pH-range. At pH 7–8 and 37 °C inactivated to a large extent in 24 h. Inactivated by metal ions
Tetracyclines (oral)	Up to 80% with faeces (concentration up to 1 mg/g)	–	Gram-negative and gram-positive bacteria: 0.1–4 µg/ml	Stable only in acid pH-range. At pH 7–8 and 37 °C inactivated to a large extent in 24 h. Inactivated by metal ions
β-Lactame Benzyl-Isoxazolyl-penicillins (oral)	Urine	Scant 40–70% active metabolites	Mainly gram-positive bacteria: 0.1–4 µg/ml	Unstable at pH 4. Inactivation 50% after 4 h. Inactivated by metal ions

Table 2.14 (*continued*)

Type of antibiotic	Way of elimination	Biotransformation	MTC-Value	Stability
β-Lactame Ampicillin, Amoxycillin (oral)	Faeces (about 30%) urine (about 50% in 24 h), urine (concentration: –2.5 µg/ml)	Relatively little (about 50% of the applied dose in active form in the urine)	Gram-positive bacteria: 0.01–1 µg/ml Gram-negative bacteria: 1–5 µg/ml	Unstable in acids 81% inactivated in 7 days at 20 °C and pH 7
Chloramphenicol (parenteral)	Urine	Biotransformation to inactive metabolites to a great extent	Gram-negative and gram-positive bacteria: 0.5–10 µg/ml	pH>9.5 unstable pH 1–9.6 and 24 °C about 24 h
Chloramphenicol (oral)	Urine (resorption >90 %)	Biotransformation to inactive metabolites to a great extent	Gram-negative and gram-positive bacteria: 0.5–10 µg/ml	pH>9.5 unstable pH 1–9.6 and 24 °C about 24 h
Sulphonamides (parenteral)	Mostly urine	10–40% of the dose are eliminated as antimicrobially active	Gram-negative and gram-positive bacteria: 20–50 µg/ml	Poorly water-soluble, precipitation at decreasing pH. Relatively stable only under anaerobic conditions.
Sulphonamides (except oral sulphonamides with poor resorption)	Mostly urine	10–40% of the dose are eliminated as antimicrobially active	Gram-negative and gram-positive bacteria 20–50 µg/ml	Poorly water-soluble, precipitation at decreasing pH. Relatively stable only under anaerobic conditions
Oral sulphonamides with poor resorption	Faeces (80–90%)	–	–	Poorly water-soluble

cles may be closed directly via crops used as feedstuff for farm animals or indirectly via vectors such as birds or rodents. The risk that bacteria will be transported through the soil into groundwater is low, since most bacteria spread on agricultural soils may be found in the upper 50 cm of soil. Nevertheless it cannot be totally excluded that in certain types of soil and due to the presence of soil lesions, faecal bacteria may reach superficial ground-water layers under certain conditions. The accidental pollution of surface-water by liquid manure, or via waste water from slaughterhouses, is known to cause problems if the water is used as drinking-water for humans or animals.

2.2.2.3.1 Gram-positive Bacteria

Most information concerning survival in manure and soil are available for *Bacillus anthracis* and *Listeria monocytogenes* (Böhm 1995; Weiß and Amtsberg 1995). Spores of *Bacillus anthracis* may survive for more than one year in liquid or solid manure and for more than 100 years in soil. *Listeria* is frequently present as a saprozoonotic agent in soil and soil-related materials, which are their natural habitat.

Incomplete data concerning survival in manure and soil are available for *Enterococci*, *Bacillus cereus*, *Clostridium perfringens*, *Clostridium tetani*, *Clostridium chauvoei* and *Erysipelothrix rhusiopathiae*. Very incomplete information can be found in the literature concerning the survival of *Staphylococci*, *Bacillus alvei*, *Clostridium botulinum* and *Clostridium difficile*.

2.2.2.3.2 Gram-negative Bacteria

Salmonella has been intensively studied in this connection and a lot of data are available. Tables 2.15 and 2.16 give some information but do not cover all literature data. The data concerning *Escherichia coli* are less complete, especially with respect to toxigenic strains. Limited information is available concerning the survival of *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, *Pasteurella multocida*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Yersinia frederiksenii, Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter coli*, *Treponema hyodysenteriae*, *Leptospira pomona*, *Leptospira interrogans* and *Leptospira icterohaemorrhagiae* in manure and soil. Almost no data could be found for *Pseudomonas aeruginosa* and *Proteus* spp.

Table 2.15: Tenacity of *Salmonella* in liquid manure and soil

Bacteria species	Tenacity in	
	Animal faeces	Soil
Salmonella spp.	Manure of cattle: 5.5 months Manure of swine: 2.5 months Manure of poultry: 4 months (Philipp 1991)	Up to 60 d (Glathe et al. in Müller 1973)
S. typhimurium	Dried faeces: 930 d (Lerche in Müller 1973) poultry faeces pH 8.9, 5–8.9 °C: <6 d untreated cattle faeces: 166 d Slurry: 4 °C: 18 d Slurry: 20 °C: >63 d (Mitscherlich and Marth 1984)	251 d (Mair and Ross in Müller 1973) 110–159 d (Stewart in Müller 1973)
S. enteritidis	Alluvial manure: 8 °C: 321–188 d (Hahn in Müller 1973) Poultry faeces 20 °C, pH 6.2–7.4: 5–16 d Untreated cattle faeces: 117 d (Mitscherlich and Marth 1984)	Soil surface in summer: 72 d; at a depth of 20 cm in summer: 104 d (Gubkin in Müller 1973) loess loam, podzol, shale, chernozem: 15, 25, 15, 40 d (Glathe et al. in Müller 1973)
S. dublin	Cattle slurry naturally stored: 27 d (Best in Müller 1973) Swine slurry 7 °C: 87 d (Motz in Müller 1973) Slurry: >77 d (Mitscherlich and Marth 1984)	After the output of contaminated cattle slurry on the soil: 300 d, in a depth of 15 cm (Mitscherlich and Marth 1984)
S. choleraesuis	Swine slurry 7–20 °C: 72–36 d (Motz in Müller 1973)	n.i.
S. abortusbovis	n.i.	382 d (Delage in Müller 1973)

n.i. = no information found

Table 2.16: Survival times of *Salmonella* in Danube reed soils under different experimental conditions (Wagner 1993)

Variant (setup)	Storage temperature (°C)	Soil	Survival time (weeks) in
Slurry + Salmonella suspension (1)	20	P	up to 86
		G	up to 86
		H	up to 86
		N	70 but not 86
	4	P	54 but not 70
		G	70 but not 86
		H	up to 86
		N	70 but not 86
Slurry + Salmonella suspension (2)	20	P	up to 86
		G	up to 86
		H	up to 86
		N	up to 86
	4	P	54 but not 70
		G	54 but not 70
		H	up to 86
		N	54 but not 70
Salmonella suspension (1) Alone	20	P	up to 86
		G	up to 86
		H	up to 86
		N	70 but not 86
	4	P	54 but not 70
		G	70 but not 86
		H	up to 86
		N	38 but not 54
Salmonella suspension (2) Alone	20	P	54 but not 70
		G	up to 86
		H	up to 86
		N	up to 86
	4	P	54 but not 70
		G	70 but not 86
		H	up to 86
		N	70 but not 86

poor clay loam (humous) (P), poor clay loam (very humous) (G), loamy clay (H), low moorland – clay (N)

Table 2.17: Tenacity of *Mycobacteria* in liquid manure and soil

Bacteria species	Tenacity in	
	Animal faeces	Soil
M. bovis	Cattle slurry in sunlight: 60 d Cattle slurry in shadow: 73 d (Mitscherlich and Marth 1984)	At least 61 d (Mitscherlich and Marth 1984)
M. avium	In dry poultry faeces: 10 months In wet faeces: 13 months In litter: 12 months (Rolle and Mayr 1987)	2 yrs (Rolle and Mayr 1987; Mitscherlich and Marth 1984)
M. paratuberculosis	In faeces >12 months (Rolle and Mayr 1987)	Approximately 1 yr (Mitscherlich and Marth 1984)

2.2.2.3.3 *Mycobacteria* and *Actinomyces* spp.

Some data concerning the survival of several *Mycobacteria* species are given in Table 2.17. Almost no data are available for *Actinomyces* spp.

2.2.2.3.4 Genetically Engineered and Antibiotic-resistant Bacteria

Until now very limited information is available concerning the survival of genetically engineered bacteria in the environment. Their tenacity mainly depends on the construct and the properties of the receiving strain. A general statement from Tebbe et al. (1994) is that the fitness of such microorganisms is less than that of the natural microflora but that their propagation is possible in some parts of the soil fauna. Faecal media have a certain importance in the distribution and spreading of antibiotic-resistant bacteria as Tschäpe (1996) was able to demonstrate. Table 2.18 gives some information as to how this could happen in the course of time.

2.2.2.4 Recommended Research

A differentiation must be made between some general needs concerning future research in the field and special research tasks.

Table 2.18: The clonal spread of two streptothricin plasmids [pIE636 (W_3) and pIE660 (X)] in bacteria of different origin (Tschäpe 1996)

Origin	1982[1]	1983	1984	1985	1986	1987	1989[2]	1995
Bacterial flora in the gut of swine	–	W_3	W_3, X	W_3, X	W_3, X	W_3, X	W_3, X	W_3, X
Slurry	–	W_3	W_3, X	W_3, X	W_3, X	W_3, X	W_3, X	W_3, X
Sick animals	–	–	–	W_3, X	–	W_3, X	W_3, X	W_3, X
Bacterial flora in the gut of employees	–	–	X	X, W_3	nt	nt	X	X
Bacterial flora in the gut of relatives	–	–	X	nt	nt	nt	X	X
Bacterial flora in the gut of healthy adults	–	–	–	X, W_3	X	X, W_3	W_3, X	W_3, X
Food	–	–	–	X	X, W_3	X, W_3	nt	nt
Infection of the urinary tract	–	–	–	–	X	X	X	X
Salmonellosis	–	–	–	–	X	–	W_3	W_3
Shigellosis	–	–	–	–	–	X	–	–

[1] beginning of use; [2] end of use; nt=not tested; Streptothricin F was used from 1982 to 1989 in agriculture in the former GDR for nutritional purposes in swine production. Except on IncW_3 and X the sat-determinants could be identified in the following plasmid groups: IncB, C, FII, H1, H2, I_1, I_2, M, N, Q, U, Z

There is a general need to elaborate the data about bacterial metabolites in manure, although some data are available about airborne endotoxins from and in animal houses, no information could be found in the literature concerning exotoxins in the air, and especially endotoxins and exotoxins in solid or liquid manure generally and with respect to aerobic or anaerobic biotechnological treatment. The microbiology of the storage and treatment of slurry is widely unexplored. Another general research requirement is to complete the data about the occurrence and tenacity of bacteria in manure and soil. Most data are old and only qualitative. The data have to be analysed. Two general environmental aspects should be investi-

gated in the future. One research task should concern the influence of the steady introduction of certain bacteria from faeces into the ecosystem soil, especially from the point of view of the horizontal gene transfer of certain bacteria. The other task should concern the importance of the introduction of bacterial pathogens into the rural environment and the consequences of their introduction, as well as their propagation from the point of view of epidemiology. Methodical needs do exist to develop and improve techniques for the sensitive environmental monitoring of soil and other environmental compartments for bacteria of animal origin. This task is connected with the need to detect and confirm transmission routes for bacterial pathogens via the environment with modern methods of molecular epidemiology. A special need exists to elaborate representative data concerning the occurrence and distribution of toxigenic *E. coli*, *Campylobacter* and *Yersinia enterocolitica* from animal husbandry by molecular typing techniques.

Concerning genetically engineered organisms, the particular aspect of the survival of carrier live vaccines in liquid and solid manure must be investigated. Furthermore, if integrated plasmid-encoded parts of virulence factors, or those in the genomic DNA, may be transmitted to populations of related soil bacteria which are class II pathogens, this problem must be investigated. Strategies must be developed for self-limiting safety systems for carrier live vaccines, in order to minimise such risk. In spite of the fact that antibiotic-resistant bacteria from the gut of farm animals reflect the effect of administered drugs, some questions are still open concerning the resulting risks to humans, especially with respect to the transmission routes. Since there are other sources for such bacteria, it is necessary to trace the route of spread of antibiotic-resistant bacteria by molecular epidemiology (e.g. RAPD).

The environmental aspect of the constant introduction of resistant bacterial populations from animal husbandry into the biocoenosis, and the effects of the horizontal gene transfer of resistance patterns into soil and water organisms require some more intensive attention. This includes the risk assessment of transmission via the environment to human populations.

The indirect introduction of undesired bacteria from the gut of farm animals into the biocoenosis and into surface- as well as ground-water, needs more attention. In particular the spread of bacteria via waste water from slaughterhouses and the meat processing

industry must be traced by molecular typing methods. The risks must be compared with those resulting from treatment of municipal waste water.

2.2.2.5 Recommended Action

Since much data is lacking concerning the survival and occurrence of bacterial pathogens in solid and liquid manure, as well as in the environment, these gaps have to be filled by scientific research using modern methods. This means that research must be funded into standardised qualitative and quantitative methods, including molecular techniques, to investigate faecal media and environmental samples for most target species. In order to put knowledge concerning transmission via the environment onto a solid scientific footing, DNA-fingerprint techniques should be applied in environmental monitoring, which must include possible living vectors. The same applies to the investigation of epidemiological pathways for genetically engineered organisms, especially for living carrier vaccines and antibiotic-resistant bacteria. Almost no information is available concerning the microbiology during storage of solid and liquid manure, especially with respect to the possible formation of endo- and exotoxins in such an environment; this still requires funding for scientific research. At the moment results indicate that if legal and/or administrative action is necessary it must be taken.

In order to minimise the introduction of bacterial pathogens, especially of zoonotic agents into animal husbandry, concerted action must be initiated concerning hygiene requirements for the housing, feeding and slaughtering of animals and the treatment of effluent from slaughterhouses, including vector control. Safe hygienic treatment of waste-water from slaughtering, as well as solid and liquid manure, may be a further aim for the legal protection of surface- and ground-water.

Common general rules of good agricultural practice for using solid and liquid manure must be defined for Europe within the next year or two, in order to bring agriculture out of the discussion concerning environmental pollution.

2.2.2.6 Summary

Bacteria and bacterial toxins in effluent from animal husbandry

The actual knowledge concerning the occurrence of bacterial pathogens in faecal media from farm animals, their number and survival in solid and liquid manure as well as their fate in the environment, is summarised in this contribution. There are only a few groups of bacterial pathogens such as *Salmonella* for which the knowledge is good and the publications are relatively new. For most of the bacterial pathogens incomplete and/or only old data are available. Not enough research had been carried out concerning the fate and transmission of genetically engineered live vaccines and antibiotic-resistant bacteria. The same applies to environmental monitoring of zoonotic bacteria with DNA-fingerprint techniques, in order to identify the sources and routes of transmission. Recommendations are given for research and administrative activities.

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Further reference must be taken from the full German version.

2.3 Fungi in Feedstuffs and Faeces

2.3.1 Fungi and Their Metabolites in Feedstuffs

Johann Bauer, Isabell Schneweis
and Stefan Hörmansdorfer

2.3.1.1 Introduction

True fungi (*Eumycota)* form their own kingdom (Fungi) with the divisions *Ascomycota, Basidiomycota, Chytridiomycota* and *Zygomycota* (Hawksworth et al. 1995). The division *Deuteromycotina* (*Fungi imperfecti*) is no longer included in the new system, but must still be used here for technical reasons.

Feedstuffs, especially those of vegetable origin, are subject to constant contamination by fungi (see Figure 2.3), whose importance to feed hygiene is based in particular on the fact that they produce a number of metabolites with antibiotic and/or toxic effects, and also cause infectious diseases or allergies. Furthermore, as xerotolerant organisms they may initiate spoilage.

2.3.1.2 Relevant Fungi, Their Characteristics, Incidence and Importance

2.3.1.2.1 Division: *Zygomycota*

Zygomycetes grow with nonseptate mycelium and are capable of fermentation under anaerobic conditions. They generally reproduce via asexual sporangiospores, more rarely via sexually produced zygospores (Zycha and Siepmann 1969; Gedek 1980).

Class: *Zygomycetes*

Family: *Mucoraceae*

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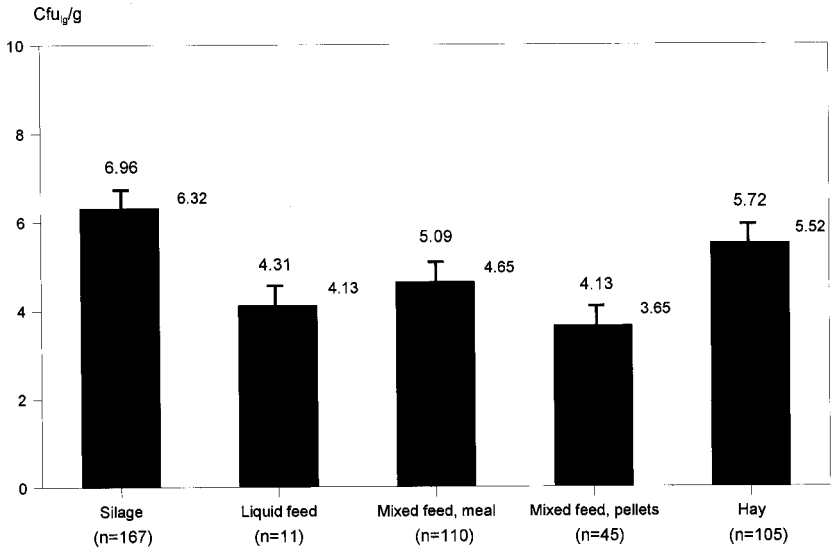

Figure 2.3: Fungal counts in feed.

Genus: *Mucor*
Members of the genus *Mucor* are ubiquitous saprophytes with aerial mycelium like cotton wool. They grow optimally at 25 °C and pH 8.0 and occur frequently in feedstuffs, especially in hay, maize silage and floury mixed feeds (see Table 2.19). Because of their lipolytic, proteolytic and saccharolytic activities they are counted among the spoilage flora; most *Mucor* species are apathogenic (Zycha and Siepmann 1969; Gedek 1980; Kamimura 1986; Reiß 1986; Weber 1993; Watanabe 1994; Samson and van Reenen-Hoekstra 1995).

Genus: *Rhizopus*
This genus contains 6 species, which form dark pigmented sporangia on their aerial mycelium. *Rhizopus* species can be found as putrefactive agents on plants, especially maize, sugar beet, cereals and fruits. They maintain a spoilage process due to the formation of a multiplicity of degrading enzymes. Some species produce mycotoxins (rhizonin or isofumigaclavins [=roquefortine A]); furthermore they can transform zearalenone to zearalenone-4-*β*-D-glucopyranoside. They may occasionally cause systemic mycoses or allergic dis-

Table 2.19: Occurrence of dominant families or genera of fungi in feedstuffs

Feedstuff	Sample (*n*)	*Aspergillus* sp.		*Dematiaceae*		*Fusarium*		*Mucoraceae*		*Penicillium*		*Scopulariopsis*		*Wallemia*	
		(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Mixed feed	167	126	75.4	77	46.1	24	14.4	54	32.2	75	44.8	7	4.2	19	11.4
Meal	110	87	79.1	74	67.3	24	21.8	43	39.1	61	55.5	5	4.5	15	13.6
Pellet	57	39	68.4	3	5.3	0	0.0	11	19.2	14	24.6	2	3.5	4	7.0
Cereals	48	29	60.4	37	77.1	24	50.0	12	25.0	20	41.7	0	0.0	1	2.1
Oats	15	9	60.0	14	93.3	9	60.0	6	40.0	5	33.3	0	0.0	0	0.0
Barley	19	11	57.9	13	68.4	8	42.1	3	15.8	8	42.1	0	0.0	1	5.2
Wheat	14	9	64.3	10	71.4	7	50.0	3	21.4	7	50.0	0	0.0	0	0.0
Roughage	126	106	84.1	77	61.1	11	8.7	85	67.5	11	8.7	7	5.5	59	46.8
Hay	105	98	93.3	59	56.2	6	5.7	78	74.3	9	8.6	7	6.7	52	49.5
Straw	21	8	38.1	18	85.7	5	23.8	7	33.2	2	9.5	0	0.0	7	33.3
Ensiled feed	177	59	33.3	36	20.3	10	5.6	56	31.6	45	25.4	3	1.7	2	1.1
Grass silage	111	36	32.4	32	28.8	2	1.8	31	27.9	17	15.3	1	0.9	2	1.8
Maize silage	66	23	24.8	4	6.1	8	12.1	25	37.9	28	42.2	2	3.0	0	0.0

eases (Gedek 1980; Seviour et al. 1982; El-Sharkawy and Abul-Hajj 1987; Roth et al. 1990; Smith and Henderson 1991; Jong and McManus 1993; Smith et al. 1994; Samson and van Reenen-Hoekstra 1995).

Genus: *Absidia*
The 21 species of the genus *Absidia* are grouped in the sections *Cylindrospora, Repens* and *Glauca*. As soil fungi they also occur on cereals, maize, hay, growing pasture vegetation and also in dung and manure. To date no production of mycotoxins is known, but they can occasionally cause mycoses (Zycha and Siepmann 1969; Samson and van Reenen-Hoekstra 1995; Younan et al. 1995).

Recommended research: Taxonomy of *Mucoraceae* in feeds. Incidence of toxin-producing species. Relevance of the formation of mycotoxin pyranosides in feedstuffs during their storage period.

2.3.1.2.2 Division: *Ascomycota*

These fungi grow with septated mycelium and multiply chiefly via asexual conidia. In sexual reproduction ascospores are formed.

Class: *Eurotiales*

Family: *Monascaceae*

Genus: *Monascus*
The genus *Monascus* contains the species *M. pilosus* and *M. ruber* (= *M. purpureus*). They grow with brown to reddish-brown colonies and form conidia and ascospores in cleistothecia. These mesophilic and xerophilic moulds produce a number of reddish pigments, monacolins with antimycetic and antibacterial activity, and the nephrotoxic citrinin. *Monascus* species are found frequently in silage, especially in grass silage, also in cereals, maize and sorghum millet. They must be regarded as feed spoilers, since they form substances with adverse effects (Ciegler et al. 1971; Hawksworth 1983; Frevel et al. 1985; Engel 1986; Ober and Kunz 1987; Schuh and Baumgartner 1988; Spicher and Isfort 1988; Zöllner et al. 1992; Blanc et al. 1994; Blanc et al. 1995; Samson and van Reenen-Hoekstra 1995).

Recommended research: Incidence and importance of monacolins and citrinin in silage.

Family: *Trichocomaceae*

Genus: *Eurotium*
The genus *Eurotium* is divided into the categories *Aculeate, Tuberculate, Lobate-reticulate* and *Microtuberculate*. Cleistothecia are formed as fruit-bodies, the anamorph belongs to the *Aspergillus glaucus* group. *Eurotia* are spread worldwide and occur especially on cereals, mixed feedstuffs and roughage (see Table 2.20). Because of their extreme xerophilia they are considered among the most important spoilage-inducing microorganisms. Furthermore they produce various toxic metabolites, including sterigmatocystin (Blaser 1975; Cole and Cox 1981; Kozakiewicz 1989; Sauer et al. 1992; Samson and van Reenen-Hoekstra 1995).

Recommended research: Sterigmatocystin production in feeds.

Class: *Hypocreales*

Family: *Clavicipitaceae*

Genus: *Claviceps*
The ubiquitous species *C. purpurea* is the most important of the 36 species of the genus *Claviceps*. These fungi are parasites of graminaceous plants (especially rye, wheat, barley, oats and maize). A greyish-black sclerotium, called ergot, develops from the mycelium on the ears. In addition to other constituents this contains biogenic amines, clavine alkaloids and in particular the lysergic acid derivatives known as ergot alkaloids. The alkaloid content depends on several factors and is highest immediately after harvesting. Ergot alkaloids produce gangrene by contraction of the smooth musculature in blood vessels and agalactia by inhibition of prolactin secretion (Floss et al. 1973; Müller and Löffler 1982; Klug 1986; Hapke 1988; Wolff 1994).

Recommended research: Investigation of the toxicity of ergot alkaloids and alkaloid mixtures for farm animals.

Table 2.20: Occurrence of *Aspergillus* spp. in feedstuffs.

Feedstuff	Sample (*n*)	*A. candidus*		*A. flavus*		*A. fumigatus*		*A. glaucus*		*A. niger*		*A.* sp.*		Σ**	
		(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Mixed feed	167	40	24.0	22	13.2	15	9.0	108	64.7	11	6.6	19	11.4	126	75.4
Meal	110	31	28.2	22	20.0	9	8.2	76	69.1	11	10.0	15	13.6	87	79.1
Pellet	57	9	15.8	0	0.0	6	10.5	32	56.1	0	0.0	4	7.0	39	68.4
Cereals	48	6	12.5	5	10.4	2	4.2	26	54.2	1	2.0	4	8.3	29	60.4
Oats	15	3	20.0	3	20.0	0	0.0	9	60.0	1	6.7	1	6.7	9	60.0
Barley	19	1	5.3	1	5.3	2	10.5	9	47.4	0	0.0	3	15.8	11	57.9
Wheat	14	2	14.3	1	7.1	0	0.0	8	57.1	0	0.0	0	0.0	9	64.3
Roughage	126	1	0.8	2	1.6	24	19.0	100	79.4	27	21.4	14	11.1	106	84.1
Hay	105	1	1.0	1	1.0	22	21.0	95	90.5	26	24.8	12	11.4	98	93.3
Straw	21	0	0.0	1	4.8	2	9.5	5	23.8	1	4.8	2	9.5	8	38.1
Ensiled feed	177	1	0.6	9	5.1	37	20.9	18	10.2	5	2.8	1	0.6	59	33.3
Grass silage	111	1	0.9	4	3.6	20	18.0	13	11.7	3	2.7	1	0.9	36	32.4
Maize silage	66	0	0.0	5	7.6	17	25.8	5	7.6	2	3.0	0	0.0	23	24.8

* not further differentiated
** samples contaminated with *Aspergillus* spp.

Saccharomycetales

Family: *Saccharomycetaceae*

Genus: *Saccharomyces*
The genus *Saccharomyces* has 10 species and belongs to the hemiascomycetes with naked asci. These yeasts, which are osmotolerant and sometimes thermophilic, occur especially in feeds with a high sugar content, but also in maize silage, on cereals and in soil. Some strains produce glycoproteins, called "killer toxins", which are particularly toxic to closely related species. *Saccharomyces* species may be the cause of mycosis; no mycotoxins are produced (Gedek 1980; Pfeiffer et al. 1988; Middelhoven et al. 1990; Polonelli et al. 1991; Barnett 1992; Müller 1992; Martini and Vaughan-Martini 1993; Pettoello-Mantovani et al. 1995).

Recommended research: Taxonomy of *Saccharomyces* species in feedstuffs. Effects of "killer toxins" and enzymes (proteases) in mammals.

2.3.1.2.3 Form-division: *Deuteromycotina*

The "form-division *Deuteromycotina*" (*Fungi imperfecti*) includes fungi of which only the asexual reproductive form is known (mitosporic fungi). The mycelium of these fungi is always septated; reproduction happens by continuous formation of conidia from short cells (sterigmata) (Strasburger 1983; Schlegel 1985).

Family: *Moniliaceae*

Genus: *Aspergillus*
The genus *Aspergillus* includes 185 species. These are modest, mesophilic fungi, which develop conidiophores on the apical ends of a vesicle with metulae and phialides or with phialides only, from which intensively coloured conidia grow; sclerotia are occasionally formed. *Aspergillus* species are found particularly in roughage and mixed feedstuffs, on cereals and in fermented feeds (see Tables 2.19 and 2.20). As well as acids, vitamins and degrading enzymes, they produce a multiplicity of substances with antibiotic, antimycetic and/or toxic effects (see Tables 2.21 and 2.22). They are therefore

Table 2.21: Metabolites of *Aspergillus* spp. (Smith and Henderson 1991; Smith et al. 1994; Samson and van Reenen-Hoekstra 1995)

Species	Metabolite
A. alliaceus	Ochratoxin A
A. alutaceus	Ochratoxin A, penicillic acid, secalonic acid A, viomellein, xanthomegnin, emodin, kojic acid, neoaspergillic acid
A. caespitosus	Fumitremorgin, verruculogen
A. candidus	Xanthoascin, candidulin, terphenyllin
A. clavatus	Cytochalasin E, patulin, tryptiquivalin, ascladiol, clavatol, kojic acid, xanthocillin
A. flavus	Aflatoxin B_1+B_2, aflatrem, cyclopiazonic acid, aspergillic acid, aflavinin, paspalinin
A. fumigatus	Fumigaclavins, fumitoxin, fumitremorgins A+C, gliotoxin, verruculogen, fumigallin, fumigatin, kojic acid, spinulosin
A. niger	Malformin, naphthoquinone, nigragillin
A. nomius	Aflatoxin B_1, B_2, G_1, G_2
A. ochraceus	Ochratoxin A, penicillic acid, secalonic acid A
A. parasiticus	Aflatoxin B_1, B_2, G_1, G_2, aspergillic acid, kojic acid
A. sydowii	Sterigmatocystin, griseofulvin, nidulotoxin
A. tamarii	Cyclopiazonic acid, fumigaclavin A
A. terreus	Citreoviridin, citrinin, gliotoxin, patulin, territrem, tremorgin, terrein, terreinic acid, terretonin, terredionol
A. ustus	Austamide, austdiol, austins, austocystins, sterigmatocystin, kojic acid
A. versicolor	Stergimatocystin, cyclopiazonic acid, nidulotoxin
A. wentii	Emodin, kojic acid, wentilactone

regarded as one of the main causes of mycogenic intoxication and residue formation in foodstuffs (e.g. *A. flavus*: aflatoxin). They can also cause systemic mycosis and allergic diseases (Walser and Kleinschrot 1979; Gedek 1980; Cole and Cox 1981; Bauer and Gareis 1987; Kozakiewicz 1989; Roth et al. 1990; Weber 1993; Samson and van Reenen-Hoekstra 1995).

Recommended research: Monitoring of the metabolites of dominant *Aspergillus* species, toxicity and carry-over studies.

Table 2.22: *Aspergillus* toxins in feedstuffs (Cole and Cox 1981; Schuh 1989; Roth et al. 1990; Smith and Henderson 1991; Abel et al. 1995)

Mycotoxin	Total formula	Occurrence	Animal species, administration	LD_{50}	Effect
Aflatoxin B_1	$C_{17}H_{12}O_6$	Peanuts, cereals, maize, oil seed remainders	Rabbit, PO Mouse, PO Monkey, PO	0.3 mg/kg 9.0 mg/kg 7.8 mg/kg	Hepatotoxic, nephrotoxic, mutagenic, teratogenic, carcinogenic
Aflatoxin B_2	$C_{17}H_{14}O_6$		Duck (1 d, PO)	84.8 µg/50g	*Symptoms:* depression of productivity, ataxia, jaundice, liver cirrhosis
Aflatoxin G_1	$C_{17}H_{12}O_7$		Duck (1 d, PO)	39.2 µg/50g	
Aflatoxin G_2	$C_{17}H_{14}O_7$		Duck (1 d, PO)	172.5 µg/50g	
Aflatoxin M_1	$C_{17}H_{12}O_7$	Milk, dairy products	Duck (1 d, PO)	0.8 mg/kg	
Aflatoxin M_2	$C_{17}H_{14}O_7$	Milk, dairy products	Duck (1 d, PO)	3.1 mg/kg	
Citrinin	$C_{13}H_{14}O_5$	Cereals, mixed feedstuff	Rat, PO	43.0 mg/kg	Nephrotoxic, hepatotoxic
Fumitremorgin A	$C_{32}H_{41}O_7N_3$				Neurotoxic, tremorgenic
Fumitremorgin B	$C_{27}H_{33}O_5N_3$	(Silage)			Neurotoxic, tremorgenic
Fumitremorgin C	$C_{22}H_{25}O_3N_3$				Neurotoxic, tremorgenic
Gliotoxin	$C_{13}H_{14}N_2O_4$	Hay	Rabbit, IV, IP	45.0 mg/kg	Antibiotic, antiviral, antimycotic; strongly cytotoxic
Ochratoxin A	$C_{26}H_{18}ClNO_6$	Maize, cereals, mixed feedstuff, beans	Rat, PO Pig, PO	20–22 mg/kg 6.0 mg/kg	Nephrotoxic, hepatotoxic, carcinogenic, teratogenic
Sterigmatocystin	$C_{18}H_{12}O_6$	Barley, wheat, maize	Rat, PO Monkey, IP Mouse, PO	166.0 mg/kg 32.0 mg/kg 820.0 mg/kg	Hepatotoxic, carcinogenic
TR-2 toxin	$C_{22}H_{27}O_6N_3$		Mouse, PO	126.7 mg/kg	Neurotoxic, tremorgenic
Verruculogen	$C_{27}H_{33}O_7N_3$	(Silage)	Mouse, IP	2.4 mg/kg	Neurotoxic, tremorgenic

Genus: *Cephalosporium*
Fungi of the genus *Cephalosporium* grow in velvety, olive-black colonies. They are ubiquitous saprophytes and occur especially in insufficiently dried green feed and also on rye, wheat and triticales. They are also capable of producing antibiotics (e.g. cephalosporin C, fusidic acid) and mycotoxins, in particular trichothecenes. They are regarded as feed spoilers and occur rarely as infectious pathogens (Gedek 1980; McKemy and Morgan-Jones 1991; Weber 1993; Olvang et al. 1995; Perdiguero et al. 1995; Samson and von Reenen-Hoekstra 1995; Zaitz et al. 1995; Tan and Sim 1996).

Recommended research: Testing of the ability of *Cephalosporium* species to produce toxins.

Genus: *Paecilomyces*
Paecilomyces species, the imperfect form of the genus *Byssochlamys*, is capable of growing under low oxygen partial pressure, high carbon dioxide content, at saline concentrations of 7–10% and at temperatures up to 45 °C. They occur world-wide as saprophytes and are occasionally isolated from feedstuffs, especially from fermented feeds. Due to their lipolytic properties and the production of mycotoxins (patulin, byssochlaminic acid, byssotoxin and/or malformin) they are counted among the feed spoilers (Gedek 1980; Reiß 1986; Castro et al. 1990; Roth et al. 1990; Adler and Lew 1993; Samson and van Reenen-Hoekstra 1995).

Recommended research: Incidence of patulin in fermented feedstuffs, effect of patulin on farm animals.

Genus: *Penicillium*
Penicillium species form brush-shaped, branching conidiophores. As well as degrading enzymes they produce a number of toxic and/or antibiotic metabolites (see Tables 2.23 and 2.24). *Penicillium* species are an important component of the soil flora and the "storage flora" of cereals. They are found especially in floury mixed feeds, types of grain and also in maize silage (see Table 2.19). They are among the most important spoilage germs. Due to the production of mycotoxins such as ochratoxin A or citrinin, for example, they may lead to illness, reduced performance and residue formation in foodstuffs (Mislivec and Tuite 1970; Gedek 1980; Bauer and Gareis 1987).

Table 2.23: Metabolites of *Penicillium* spp. (Smith and Henderson 1991; Smith et al. 1994; Samson and van Reenen-Hoekstra 1995)

Species	Metabolite
P. aurantiogriseum	Penicillic acid, xanthomegnin, viomellein, penitrem A, viridicatin
P. brasilianum	Penicillic acid, verruculogen, viridicatumtoxin, verruculotoxin, fumitremorgin, paraherquamide
P. canescens	Penitrem A, griseofulvin, rugulosin
P. chrysogenum	Roquefortine C, PR-toxin, penicillin, ochratoxin A
P. citreonigrum	Citreoviridin, citrinin
P. citreoviride	Citreoviridin
P. citrinum	Citrinin, penitrem A
P. coprophilum	Griseofulvin, roquefortine C
P. crustosum	Penitrem A, roquefortine C, isofumigaclavine, viridicatin
P. expansum	Patulin, citrinin, roquefortine C
P. griseofulvum	Patulin, cyclopiazonic acid, roquefortine C, griseofulvin
P. islandicum	Cyclochlorotine, luteoskyrin, islanditoxin, rugulosin, emodin, erythroskyrin
P. oxalicum	Oxalin, secalonic acid D, roquefortine C
P. purpurogenum	Rubratoxins, purpurogenones
P. roqueforti	Chemotype I: roquefortine C, PR-toxin, isofumigaclavines A+B Chemotype II: patulin, penicillic acid, roquefortine C
P. simplicissimum	Fumitremorgin B, penicillic acid, verruculogen, viridicatumtoxin
P. verrucosum	Ochratoxin A, citrinin, tremorgin, viomellein
P. viridicatum	Xanthomegnin, viomellein, penicillic acid, griseofulvin, penitrem A, brevianamide, viridicatin

Recommended research: Incidence and effect of certain *Penicillium* toxins in feeds.

Genus: *Scopulariopsis*

The 19 species of the genus *Scopulariopsis* comprise the anamorph of *Microascus*. As soil fungi they are occasionally isolated from feedstuffs (see Table 2.19), where they may contribute to spoilage. They may also occasionally cause systemic mycoses (Morton and Smith 1963; Reiß 1986; Appel and Hummel 1994; Samson and van Reenen-Hoekstra 1995).

Table 2.24: *Penicillium* toxins in feedstuffs (Cole and Cox 1981; Schuh 1989; Roth et al. 1990; Smith and Henderson 1991; Abel et al. 1995)

Mycotoxin	Total formula	Occurrence	Animal species, administration	LD_{50}	Effect
Citreoviridin	$C_{23}H_{30}O_6$	Maize, cereals	Rat, SC	3.6 mg/kg	Neurotoxic, paralytic
Citrinin	$C_{13}H_{14}O_5$	Cereals	Mouse, IP, Rat, PO	35.7 mg/kg 43.0 mg/kg	Nephrotoxic, carcinogenic
Ochratoxin A	$C_{26}H_{18}ClNO_6$	Maize, cereals, mixed feedstuff, beans	Rat, PO Pig, PO	20–22 mg/kg 6.0 mg/kg	Nephrotoxic, hepatotoxic, carcinogenic, teratogenic
Patulin	$C_7H_6O_4$	Silage (?)	Mouse, IV	20.0 mg/kg	Antibiotic, hepatotoxic, haemorrhagic
Penicillic acid	$C_6H_{10}O_4$	Maize, feedstuff	Mouse, PO	35.0 mg/kg	Antibiotic, carcinogenic
Penitrem A	$C_{37}H_{44}O_6NCl$		Mouse, IP	1.1 mg/kg	Neurotoxic, tremorgenic
Penitrem B	$C_{37}H_{45}O_5N$		Mouse, IP	5.8 mg/kg	Neurotoxic, tremorgenic
PR-toxin	$C_{17}H_{20}O_6$		Mouse, IP	7.0 mg/kg	Hepatotoxic
Roquefortine C	$C_{22}H_{23}N_5O_{11}$	Silage	Mouse, IP	184.0 mg/kg	Neurotoxic (?)
Rubratoxin	$C_{26}H_{30}O_{11}$	Cereals, maize, soya bean	Mouse, IPF Fowl, PO	3.0 mg/kg 83.0 mg/kg	Hepatotoxic, nephrotoxic

Recommended research: None

Genus: *Trichoderma*
The genus *Trichoderma* is divided into 5 sections (*Trichoderma, Longibrachiatum, Saturnisporum, Pachybasium* and *Hypocreanum*). *Trichoderma* species grow at up to 38 °C and form greenish-yellow pigmented colonies. As soil fungi they also occur in feedstuffs such as cereals, maize, mixed feeds and silage, and due to the production of degrading enzymes, they are regarded as feed spoilers. As well as peptides with antibiotic effect (trichovorin) they are also suspected of producing trichothecenes. Occasionally they also cause mycoses (Bissett 1983; Bissett 1991; Adler and Lew 1993; Nout et al. 1993; Naar and Kecskes 1995; Ritieni et al. 1995; Seguin and Degeilh 1995; Wada et al. 1995; Kurzatkowski et al. 1996; Neethling and Nevalainen 1996).

Recommended research: Incidence of *Trichoderma* species in feeds; clarification of the capability to produce toxins.

Family: *Tuberculariaceae*

Genus: *Fusarium*
Fusaria grow with bright red or orange pigmented colonies with luxuriant, aerial mycelium-like cotton wool. They form chlamydospores, microconidia and banana-shaped macroconidia. They grow all over the world as soil fungi, either saprophytic or invading living plants as parasites. Particularly affected are legumes, potatoes, and especially various types of cereals as well as maize. They are found relatively frequently in feedstuffs, such as cereals, straw and floury mixed feeds (see Table 2.19). As well as enzymes (e.g. hemicellulase) and substances with antibiotic activity, they produce a number of highly toxic mycotoxins (e.g. trichothecenes, fumonisins and zearalenone) (see Tables 2.25 and 2.26). Consumption of feedstuffs containing *Fusaria* toxins leads in particular to loss of performance, refusal to feed or disturbed reproduction (see Table 2.26) (Burgess 1981; Reiß 1986; Roth et al. 1990).

Recommended research: Strategies to prevent the infection of plants by *Fusaria*. Detoxification of feedstuffs containing *Fusaria* toxins or reduction of the toxic effects. Incidence of certain *Fusaria* toxins

Table 2.25: Metabolites of *Fusarium* spp. (Smith and Henderson 1991; Smith et al. 1994; Samson and van Reenen-Hoekstra 1995)

Species	Metabolite
F. acuminatum	T-2 toxin, moniliformin
F. avenaceum	Antibiotic Y, moniliformin, trichothecenes, fusarin C, zearalenone
F. culmorum	Zearalenone, deoxynivalenol, fusarin C, culmorin, butenolides
F. equiseti	Zearalenone, T-2 toxin, nivalenol, moniliformin, equisetin, methyltetraminic acid
F. graminearum	Zearalenone, deoxynivalenol, T-2 toxin, nivalenol, diacetoxyscirpenol, fusarin C, culmorin, butenolides
F. moniliforme	T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol, zearalenone, moniliformin, fumonisins B1+B2
F. oxysporum	Moniliformin, fusaric acid, enniatin, naphthoquinone
F. pallidoroseum	Zearalenone, trichothecenes type A, moniliformin
F. poae	T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol, fusarin C, butenolides
F. sambucinum	Zearalenone, trichothecenes type A, fusarin C, enniatin
F. solani	Fusaric acid, naphthoquinone
F. sporotrichoides	T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol, zearalenone, fusarin C, butenolides
F. tricinctum	Trichothecenes type A, fusarin C, cyclodepsipeptide complex
F. verticilloides	Moniliformin, fusarin C, fumonisins, gibberellins, fusaric acid, naphthoquinone

(e.g. moniliformin) in feeds; investigation of their toxicity for farm animals.

Family: *Cryptococcaceae*

Genus: *Candida*
Candida species grow with white colonies, optimally at 25–37 °C and pH 2–8. They form blastospores, pseudomycelium and/or septated hyphae; *C. albicans* also forms chlamydospores. *Candida* species are ubiquitous saprophytes and occur not infrequently in starch-rich, fermented feedstuffs; many species are found on the mucosa of humans and animals. Due to their hemicellulolytic and li-

Table 2.26: *Fusarium* toxins in feedstuffs (Cole and Cox 1981; Chelkowski 1989; Schuh 1989; Roth et al. 1990; Smith and Henderson 1991; Abel et al. 1995)

Mycotoxin	Total formula	Occurrence	Animal species, administration	LD_{50}	Effect
Deoxynivalenol	$C_{15}H_{20}O_6$	Maize, cereals	Mouse, IP	70.0 mg/kg	Refusal of feed, vomitus
Diacetoxyscirpenol	$C_{19}H_{26}O_7$	Maize, cereals	Mouse, IP	23.0 mg/kg	Cytotoxic, dermotoxic
Fumonisin B_1	$C_{34}H_{59}NO_{15}$	Maize			Hepatotoxic, carcinogenic *Horse:* leukoencephalomalacia *Pig:* lung oedema
Fumonisin B_2	$C_{34}H_{59}NO_{14}$	Maize			
Fumonisin B_3	$C_{34}H_{59}NO_{14}$	Maize (?)			
Fumonisin B_4	$C_{34}H_{59}NO_{14}$	Maize (?)			
Fumonisin A_1	$C_{36}H_{61}NO_{16}$	Maize (?)			
Fumonisin A_2	$C_{35}H_{61}NO_{15}$	Maize (?)			
Fusarenone X	$C_{17}H_{22}O_8$		Mouse, IP	3–4 mg/kg	Cytotoxic, dermotoxic
HT-2 toxin	$C_{22}H_{32}O_8$	Maize, cereals	Mouse, IP	9.0 mg/kg	Cytotoxic, dermotoxic, enterotoxic
Moniliformin	C_4HO_3Na	Maize	Mouse, PO	20–29 mg/kg	Gastroenteritic, haemorrhagic, cardiotoxic
15-Monoacetoxyscirpenol	$C_{17}H_{24}O_6$	Cereals	Mouse, IP	0.8 mg/kg	Cytotoxic, dermotoxic
Neosolaniol	$C_{19}H_{26}O_8$		Mouse, IP	15.5 mg/kg	Cytotoxic
Nivalenol	$C_{15}H_{20}O_7$	Maize, cereals	Mouse, IP	40.0 mg/kg	Cytotoxic, enterotoxic
Scirpentriol	$C_{15}H_{22}O_7$		Rat, IP	0.8 mg/kg (?)	Cytotoxic, dermotoxic
T-2 tetraol	$C_{15}H_{22}O_6$		Fowl, 1 d, PO	>10.0 mg/kg	Cytotoxic
T-2 toxin	$C_{24}H_{30}O_9$	Maize, cereals	Mouse, IP	5.2 mg/kg	Cytotoxic, enterotoxic, dermotoxic
T-2 triol	$C_{20}H_{34}O_7$		Mouse, IP	108.0 mg/kg	Cytotoxic
Zearalenone	$C_{18}H_{22}O_5$	Maize, cereals	Mouse, PO	>20,000 mg/kg	Oestrogen-like

polytic properties they are feed contaminants, certain species cause systemic mycoses (Gedek 1980; Middelhoven et al. 1990; Weber 1993; Seeliger and Schütt-Gerowitt 1994).

Recommended research: Differentiation of the *Candida* species occurring in feeds.

Genus: *Trichosporon*
The 19 species of this genus form blastospores, pseudomycelium, arthrospores and true mycelium. The genus includes psychrophilic, mesophilic and thermophilic fungi. They are ubiquitous and modest saprophytes and occur frequently in feeds, especially silage. They produce degrading enzymes and are therefore counted among the feed contaminants; several species are opportunistic disease pathogens (King and Jong 1977; Gedek 1980; Middelhoven et al. 1990; Gueho et al. 1992; Costa et al. 1993; Weber 1993; Seeliger and Schütt-Gerowitt 1994; Samson and van Reenen-Hoekstra 1995; Assaf and Weil 1996).

Recommended research: None

Family: *Dematiaceae*

Genus: *Alternaria*
Fungi of the genus *Alternaria* are characterised by darkly pigmented colonies and ellipsoid macroconidia. As environmental fungi they are among the primary flora of fresh agricultural plants. *Alternariae* have been detected particularly in cereals, maize, hay, straw, fresh feed, grass, legumes and mixed feedstuffs. As producers of degrading enzymes and of antibiotic and toxic compounds (alternariol, alternariol monomethyl ether, altuene, altuenisol, altertoxin I, II and III, tenuazonic acid and fumonisin B1, B2 and B3) they are classified as feed spoilers. They may occasionally cause intoxication, allergies and mycoses (Cole and Cox 1981; Reiß 1986; Stack and Prival 1986; Roth et al. 1990; Müller 1991; Mirocha et al. 1996).

Recommended research: Toxin-producing capacity of the *Alternaria* species occurring in this country. Natural occurrence of *Alternaria* toxins in feeds.

Genus: *Cladosporium*
The 50 species of the genus *Cladosporium* grow in velvety, olive-green colonies optimally at 25 °C and pH 3.1–7.7. *Cladosporia* are environmental fungi and are frequently detected in oats, barley, wheat, straw and floury feedstuffs. They count as feed contaminants, especially because of their lipolytic properties; furthermore they can cause allergies (Ellis 1971; Ellis 1976; Gedek 1980; Reiß 1986; Roth et al. 1990; Koivikko et al. 1991; Potter et al. 1991; Hawksworth et al. 1995; Samson and van Reenen-Hoekstra 1995).

Recommended research: Differentiation of *Cladosporium* species occurring in feeds, and also testing of their capacity to produce toxins.

Genus: *Stachybotrys*
Colonies of *Stachybotrys* are blackish green and powdery at the front, and colourless behind. They occur worldwide in soil, plant seeds, cereals, straw and hay. As well as saccharidases and proteases they produce chiefly antibiotic and antimycetic substances and also highly toxic trichothecenes (e.g. verrucarol, verrucarin J and B, roridin E, satratoxin H, G and F, trichoverin A and B) and are thus the cause of stachybotryotoxicosis (Cole and Cox 1981; Turner and Aldridge 1983; Liukkonnen 1986; McKenzie 1991; Hawksworth et al. 1995; Samson and van Reenen-Hoekstra 1995).

Recommended research: Incidence of *Stachybotrys* species and *Stachybotrys* toxins in feeds, especially in straw.

Genus: *Wallemia*
Wallemia species grow in brown to orange colonies at an optimal temperature of 24–30 °C. As xerophilic storage fungi they still develop at an a_w level below 0.75. They are isolated especially from hay, straw, cereals, dry feed, milk powder, soya beans and litter (see Table 2.19). *Wallemia sebi* produces the toxins wallemia A and B, walleminol and azasteroids with antibiotic, antimycetic and tumour-inhibiting properties. *Wallemia* species are regarded as putrefying fungi and feed spoilers (Reiß 1986; Sakamoto et al. 1989; Wood et al. 1990; Takahashi et al. 1993; Smith et al. 1994; Samson and van Reenen-Hoekstra 1995; Hanhela et al. 1995).

Recommended research: Incidence and toxin production of *Wallemia* species in feeds.

2.3.1.3 Legal Position

There are no special regulations for the content of fungal spores in feedstuffs; however, guidelines for the content of fungal spores in commercial feedstuffs are being drawn up (see Table 2.27). Legal limits have been set for the content of aflatoxins and ergot (Anonymous 1997).

2.3.1.4 Recommended Research (Summary)

- Differentiation of the various species of fungi occurring in feeds.
- Drawing up objectifiable criteria for determining the hygienic status.
- Investigation of mycotoxins of fungi other than *Aspergillus, Penicillium* and *Fusarium*.
- Intensive research of the fungal flora and their metabolites in basic feeds (silage and hay).
- Investigation of the substances with pharmacological effects produced by fungi in feeds.
- Toxicity studies in farm animals with practice-relevant amounts of toxin and toxin combinations.
- Improvement in the strategies for avoiding fungal infections of feed plants and feeds, development of practicable detoxification measures.

2.3.1.5 Recommended Action

In view of the level of knowledge regarding the toxicity and incidence of ochratoxin A in the food chain and in humans, we should aim for regulations which limit their presence in feedstuffs. This must be carried out in correlation with corresponding regulations in the field of foodstuffs.

Table 2.27: Reference values for the contents of moulds and *Dematiaceae* in feedstuffs (Schmidt 1991; Gedek 1995)

Feedstuff	Moulds and *Dematiaceae* Cfu/g (×1000)
Single feedstuff	
Dairy byproducts, dried	1
Blood meals	1
Carcass meals, fish meals	5
Remainders of oil production	
– extracted oilseed meal	10
– oilseed cake	2
Cereals and cereal byproducts	
– middlings	20
– bran (wheat, rye)	50
– cereal seed, wholemeal	
• maize	40
• wheat, rye	40
• barley	50
• oats	70
Mixed feedstuff (meal) for	
– young and fattening poultry	50
– laying hens	50
– piglets	50
– fattening pigs	50
– calves	20
– dairy cows and cattle	50
Mixed feedstuff (pressed) for	
– young and fattening poultry	5
– laying hens	5
– piglets	5
– fattening pigs	10
– calves	5
– dairy cows and cattle	10
Milk replacer	5
Protein concentrate	20

2.3.1.6 Summary

Fungi are almost always detected in feedstuffs. These are generally moulds of the genera *Aspergillus, Penicillium* and *Fusarium*. But species of the genera *Mucor, Rhizopus*, *Monascus, Eurotium, Cephalosporium, Paecilomyces, Scopulariopsis, Trichoderma, Stachybotrys* and *Wallemia* are also found. Among the yeast species *Candida, Saccharomyces, Torulopsis* and *Geotrichum* are frequently isolated. Furthermore, the presence of *Claviceps purpurea* must be considered in cereals. The highest contents of fungi can be expected in silage and roughage. Since certain species of moulds, e.g. those of the *A. glaucus* group, can grow even at relatively low a_w levels, these fungi initiate spoilage of feeds.

It is true that feedstuffs affected by fungi may be the cause of mycosis, but the probability of this can be judged as rather low. Of far more importance are toxic fungal metabolites (mycotoxins) and allergising cell components, which can lead to loss of performance and diseases. While there is already considerable knowledge concerning the ability of species of *Aspergillus, Penicillium* and *Fusarium* to produce toxins, this knowledge is scant with regard to other genera of fungi. There are a number of investigations which document the natural occurrence of aflatoxins, deoxynivalenol, ochratoxin A and zearalenone in single and mixed feedstuffs; on the other hand information is scarce concerning the incidence of other mycotoxins or the situation in the basic feeds hay and silage. There are also deficiencies with regard to the effects of practice-relevant amounts of mycotoxin on farm animals.

To minimise the problems caused by the presence of mycotoxins in feeds, strategies are needed to avoid fungal infection of feed plants and contamination of feedstuffs. Furthermore, procedures should also be available for the detoxification of feeds containing mycotoxins.

It should also be mentioned that fungi produce metabolites with pharmacological (e.g. antibiotic) effect. Since they can definitely affect the performance of the animals and possibly also lead to legal consequences for the farmer, knowledge of their natural occurrence is necessary.

Further development of specific and sensitive analysis methods is needed to clarify all these questions.

In view of our knowledge about the toxicity and incidence of ochratoxin A in the food chain, we should be aiming at regulations for a limit in feedstuffs.

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2.3.2 Fungi and Fungal Metabolites in Animal Excrements

Johann Bauer and Isabell Schneweis

2.3.2.1 Introduction

As well as bacteria and viruses, animal faeces can also contain different species of fungi. On one hand, these are mere passengers, having been ingested with feed, survived the gastrointestinal passage undamaged and being eliminated with the faeces. But there are also fungi with a primary location in the gastrointestinal tract, which live within the host as symbionts, commensals or parasites. So called "coprophilic fungi" prefer faeces as optimal growth substrate; they thrive as saprophytes and degrade cellulose, lignin, keratin, chitin, etc. enzymatically (Klare 1970, Klare 1971; Singh 1984).

Fungal metabolites found in faeces are produced either by proliferation and growth of fungi within the host or they are ingested with feed and eliminated either unchanged or metabolised.

2.3.2.2 Relevant Fungi, Their Occurrence and Importance

Faeces from farm animals are almost always contaminated with a broad range of different fungi (Klare 1970; Schreiber 1980; see Table 2.28).

In no way does the detection of fungi in faeces suggest disease or a health risk for animals or humans. As already mentioned, they may be harmless passengers of the gastrointestinal tract. There is a different situation, if pathogens from dermal or systemic mycoses (e.g. *Candida albicans*, *Cryptococcus neoformans*, *Microsporum* sp., *Trichophyton* sp. or *A. fumigatus*) are eliminated via faeces. This does of course constitute a real infection risk for both the animal and the herd, and also for the humans caring for the animals. An allergy risk should be assumed especially if particles from dried faeces have been aerosolized by animal movement or air circulation.

Table 2.28: Occurrence of fungi in the faeces of domestic animals (Uden et al. 1957; Uden et al. 1958; Klare 1970; Forstenaicher 1980; Schreiber 1980; Singh 1984; Lowe et al. 1987; Nooruddin and Singh 1987; Teunissen et al. 1991)

Division	Genus	Species
Zygomycota	*Absidia*	*A. spinosa*
		A. ramosa
		A. corymbifera
	Mucor	*M. pusillus*
		M. circinelloides
		M. hiemalis
		M. racemosus
		M. mucedo
	Rhizopus	*R. arrhizus*
	Pilobolus	
	Thamnidium	
Chytridiomycota	*Neocallimastix*	*N. patriciarum*
		N. frontalis
		N. joyonni
	Piromyces	*P. communis*
	Caecomyces	*C. communis*
	Orpinomyces	*O. bovis*
	Anaeromyces	
Ascomycota	*Saccharomyces*	*S. cerevisiae*
		S. chevalieri
		S. exiguus
		S. fragilis
		S. drosophilarum
	Pichia	*P. bovis*
		P. farinosa
		P. fermentans
	Trichosporon	*T. cutaneum*
Deuteromycotina	*Aspergillus*	*A. repens*
		A. ruber
		A. amstelodami
		A. chevalieri
		A. mangini
		A. fumigatus
		A. flavus
		A. parasiticus
		A. versicolor
		A. sydowii

Table 2.28 (*continued*)

Division	Genus	Species
Deuteromycotina	*Aspergillus*	*A. candidus*
		A. niger
		A. terreus
		A. clavatus
		A. nidulans
	Candida	*C. albicans*
		C. slooffii
		C. tropicalis
		C. krusei
		C. parapsilosis
		C. guilliermondii
		C. macedoniensis
		C. utilis
		C. bovina
	Cladosporium	*C. herbarum*
	Cryptococcus	*C. neoformans*
	Fusarium	*F. semitectum*
		F. oxysporum
		F. moniliforme
	Geotrichum	*G. candidum*
	Microsporum	*M. gypseum*
	Monilia	
	Paecilomyces	
	Penicillium	*P. brevicompactum*
		P. chrysogenum
		P. decumbens
		P. expansum
		P. frequentans
		P. funiculosum
		P. griseofulvum
		P. nigricans
		P. notatum
		P. paraherquei
		P. roqueforti
		P. verrucosum
	Scopulariopsis	*S. brevicaulis*
	Stachybotrys	

2.3.2.3 Occurrence and Importance of Fungal Metabolites

Fungal metabolites enter animals primarily by feed. These include e.g. aflatoxins, trichothecenes (deoxynivalenol, nivalenol, T-2 toxin, diacetoxyscirpenol), ochratoxin A, zearalenone and fumonisins. However, metabolites of mycotic origin may also be produced in the animal itself, for example enzymes of anaerobic rumen fungi or gliotoxin released during infection with *A. fumigatus* (Bauer et al. 1989a). These substances are subject to various metabolic reactions within the organism (e.g. oxidation, reduction, hydrolysis, conjugation to glucuronic acid or sulphate), which are accomplished either by the macroorganism itself or by microorganisms present in the gastrointestinal tract (Goldmann 1982). These reactions mostly lead to compounds with lower toxicity. They may also, however, result in the formation of metabolites with higher toxicity, e.g. carcinogens and mutagens (Kujawa et al. 1987). Knowledge of these metabolic reactions is necessary in order to be able to estimate the metabolites expected in faeces and urine (Table 2.29).

A risk to the health of animals or humans due to fungal metabolites present in faecal matter can hardly be expected since the amounts are small.

2.3.2.4 Legal Position

At present there is no legal requirement for the appraisal of fungi in animal faeces.

2.3.2.5 Recommended Research

A survey of the literature has shown that only little is known about the occurrence of fungi, either qualitative or quantitative, in animal faeces. This is necessary, however, in order to facilitate estimation of the associated risk of mycogenic infection and allergy to humans and animals. There is also the question of how phytopathogenic fungi in particular behave in slurry and whether the spreading of slurry contaminated with fungi onto agricultural land might possibly lead to fungal diseases in plants.

Table 2.29: Metabolites of mycotoxins in excreta from animals (Engel and Hagemeister 1978; Enders 1984; Kiessling et al. 1984; Visconti and Mirocha 1985; Cote et al. 1986; Bauer et al. 1989b; Bauer 1993)

Mycotoxin	Metabolites
Aflatoxin B_1	Aflatoxin B_1
	Aflatoxin M_1
	Aflatoxin P_1
	Aflatoxin Q_1
	Aflatoxicol
Deoxynivalenol	Deoxynivalenol
	De-epoxy-deoxynivalenol
Diacetoxyscirpenol	15-Monoacetoxyscirpenol
	De-epoxy-15-monoacetoxyscirpenol
	Scirpentriol
	De-epoxy-scirpentriol
Ochratoxin	Ochratoxin A
	4-Hydroxy-ochratoxin A
T-2-toxin	T-2-toxin
	3′-Hydroxy-T-2-toxin
	3′-Hydroxy-3-acetoxy-T-2-toxin
	HT-2-toxin
	3′-Hydroxy-HT-2-toxin
	T-2-triol
	4-Deacetylneosolaniol
	T-2-tetraol
	4-Acetoxy-T-2-tetraol
	8-Acetoxy-T-2-tetraol
	15-Acetoxy-T-2-tetraol
	De-epoxy-3′-hydroxy-HT-2-toxin
	De-epoxy-T-2-tetraol
Zearalenone	α-Zearalenol
	Zearalenone

General investigations are necessary concerning the obligatorily anaerobic fungi and their metabolic products in animal excrements. With regard to the occurrence of fungal metabolites in excrements, mycotoxins and their transformation products are of particular interest for reasons of animal health and food hygiene. The extent to which examination of excrements can be used for the diagnosis of mycotoxicoses should be investigated. Therefore both sensi-

tive analysis procedures and knowledge of the metabolism of mycotoxins in animals are necessary.

2.3.2.6 Recommended Action

The present situation does not give rise to any need for action.

2.3.2.7 Summary

Faeces contain different species of fungi or fungal metabolites. These are chiefly moulds of the genera *Aspergillus, Penicillium, Absidia, Mucor, Rhizopus, Pilobolus* and *Thamnidium* and also yeasts of the genera *Saccharomyces, Candida, Pichia, Torulopsis, Cryptococcus* and *Trichosporon.* Occasionally dermatophytes (*Trichophyton* sp. and *Microsporum* sp.) and anaerobic fungi of the family *Neocallimasticaceae* have also been isolated.

Fungal metabolic products and their metabolites can have distinctly different properties of action in excrements. Mycotoxins of importance in regards to animal health and food hygiene are aflatoxins, ochratoxin A, deoxynivalenol, diacetoxyscirpenol, T-2-toxin and zearalenone or their metabolites, which usually show lower toxicity.

Generally, little is known about the occurrence of fungi and fungal metabolites in animal excrements. This knowledge, however, is important to improve the assessment of infection and allergy risks for man and animal due to faecal fungi. There are considerable gaps in our knowledge regarding culture, growth and production of metabolites of or due to obligatorily anaerobic fungi. The extent to which mycotoxins, eliminated in excrements, or their metabolites can be used for diagnosis of mycotoxin-induced problems in herds, should also be investigated.

Because of the gaps in our knowledge a factually grounded need for action cannot be deduced.

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2.4 A Review of the Parasites in Feedstuffs and Animal Faeces

Dietrich Barth

2.4.1 Introduction

This survey concentrates on the importance of parasites in the feedstuffs and faeces of agricultural farm animals in German-speaking areas. Attention is drawn to three special cases of parasitic zoonosis, which are not transmitted via the feed or faeces of agricultural farm animals.

2.4.2 Description

Developmental stages of many endoparasites, in the form of cysts or preliminary stages, are eliminated in faeces in large numbers. In the case of protozoa these are oocysts or sporocysts, in the case of helminths they are eggs or the first-stage larvae developed from these. The contamination of feedstuffs with developmental stages of animal parasites is a necessary phase in the life cycle of many endoparasites. Some 2–20% of animal parasites are of significance either economically or with regard to veterinary medicine (Eckert et al. 1992). Most of the parasites which occur in the faeces and feedstuffs of our agricultural farm animals are host-specific and thus not pathogenic to humans. They also include pathogens, however, which can become dangerous to humans, such as the common liver fluke, *Fasciola hepatica,* or the protozoa *Balantidium coli, Giardia* spp. and *Cryptosporidium parvum* (Arambulo and Moran 1980; Straub et al. 1993; Strauch and Ballarini 1994). The faeces of pets (dogs and cats), or waste water or feedstuffs, can also be infectious for humans if they contain developmental stages of pet parasites

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such as roundworms and hookworms, the protozoa *Toxoplasma* and *Sarcocystis* or the tapeworms *Echinococcus* or *Taenia*. Feedstuffs can also be contaminated with developmental stages of parasites which have humans as their regular definitive host, and farm animals such as cattle as an intermediate host, as in the case of *Taenia saginata* or *Sarcocystis hominis* (Rommel 1992).

The life span of parasitic cysts in the environment depends to a great extent on the microclimate and the composition of the substrate, in which they occur. In general the thin-shelled eggs of strongyles are most easily destroyed by adverse environmental influences, while the third-stage strongyle larvae, liver fluke eggs, coccidia oocysts, round worm eggs, whipworm eggs and tapeworm eggs are least susceptible. Temperatures above 55 °C kill parasitic cysts within a few minutes by coagulation of the encapsulating proteins, whereas lower temperatures, down to approximately –18 °C or below, are survived for a relatively long period. They are susceptible to dehydration. Gamma and electron irradiation are tolerated better than UV rays. Embryonated helminth eggs are generally more resistant than unembryonated and sporulated oocysts are more resistant than non-sporulated ones. In practice cleaning and decontamination of stalls is best achieved through the use of hot water or steam. Disinfectants active against bacteria and viruses are generally ineffective against parasitic cysts. Antiparasitic acting disinfectants need at least 2 hours to have an effect.

2.4.3 Incidence

2.4.3.1 Parasites in Animal Faeces

In view of the ubiquitous presence of endoparasites in farm animals, it can be safely assumed that any solid dung, liquid manure, or even waste water from slaughterhouses, contain developmental stages of parasites. Although the high temperatures of 40–60 °C required for swift destruction of the developmental stages of the parasites is generated during storage of manure, this does not apply to storage of slurry. For this reason, slurry from cattle should be stored in summer for 2 months and in winter for 4–5 months, and slurry from pigs

should be stored all the year round for at least 6 months (Enigk 1980). The addition of disinfectants (formalin) or lime wash is not appropriate for routine use due to lack of efficacy and ecological considerations (Enigk 1980; Literature in Eckert 1992). Aeration, heat putrefaction, drying, composting or aerobic heat treatment are not practicable due to the financial costs (Scherb and Forstner 1971; Lüttig 1972; Splisteser and Frick 1973; Enigk et al. 1975; Hahne et al. 1996). The provisions of the fertilising regulations (Anonymus 1996) permit the application of fertilisers to cultivated arable land, only as the need arises, and state the periods related to crop farming during which slurry should not be applied i.e., between 15th November and 15th January, and also when the soil is either saturated with water, frozen or heavily covered with snow. In spite of these limitations it is still possible that slurry which has only been stored for a short time is spread on areas used for agriculture.

2.4.3.2 Parasites in Feed

Green forage from grazed or slurried areas is by far the feedstuff with the highest parasitic contamination. Pasture grass can be contaminated with infectious stages of gastrointestinal strongyles (from ruminants, horses, pigs), lungworms (from ruminants), liver flukes (from ruminants), ascarids (from horses, pigs), whipworms (from ruminants, pigs), tapeworms (from ruminants, horses, humans) and coccidia (from ruminants, horses, pigs, humans). If the green forage originates from meadows which have not been fertilised with slurry or with solid dung, there is only a residual risk from parasites originating from game (Literature in Kutzer 1988; Rehbein and Haupt 1994). Grass silage and conventionally obtained hay are only free of the developmental stages of parasites after several months of storage. Straw, grains and finished feeds can be classified as safe (Bürger 1985).

2.4.3.3 Special Cases

Toxoplasma gondii

In Germany 40–60% of the population are infected with the protozoa *Toxoplasma gondii* via raw or poorly cooked meat or through ac-

cidental ingestion of oocysts derived from cat faeces (Zuber and Jacquier 1995). Currently there are no effective measures that can break the infection chain. Development of a vaccine for cats or the most important intermediate hosts, pigs and sheeps should be a high research priority.

Taenia saginata

The infection of humans with the tapeworm *Taenia saginata* occurs via raw beef. The present measures of meat inspection are not sufficient to control this, well-practised detection methods are needed to identify "measles" in carcasses, as well as an economically justifiable treatment of cattle. The initial results available suggest the possibility of developing a vaccine against the larvae of *T. saginata* (Lightowlers 1994; Eckert and Deplazes 1995).

Echinococcus multilocularis

Foxes contaminate the environment with the eggs of *Echinococcus multilocularis*. Larval stages of this parasite can cause a severe disease, alveolar echinococcosis, in humans. New techniques using molecular biology (polymerase chain reaction) permit definitive identification of the eggs of *Echinococcus multilocularis* (Mathis et al. 1996; Monnier et al. 1996) and thus investigation of the risk potential for humans.

2.4.4 Consequences

None of the parasitic cysts with which feeds can be contaminated, multiplies in the feed or has any effect on this. Parasitic infection of our agricultural farm animals leads to performance losses which are of economic importance (Table 2.30).

Effective control of parasites is only possible if epidemiological data are constantly kept up to date. Unfortunately it has been ascertained that data concerning the presence of parasites is being gathered more and more rarely. The danger for humans, of becoming infected with helminths via feedstuffs and/or faeces from agricultural farm animals, is slight. The risk of infection by protozoa of animal

Table 2.30: The veterinary pharmaceutical market (Source: I & G Veterinär-report 1994)

Product group	Share (%) of total drug turnover in	
	Germany	World
Antibiotics	25.3	33.3
Vaccine	14.6	22.3
Antiparasitics	14.8	18.8

origin is greater. *Cryptosporidium parvum* is disseminated worldwide in humans and many species of animals (Tzipori 1988; O'Donoghue 1995). The most important source of infection with this parasite are calves which contaminate surface water and drinking water (Smith and Rose 1990; Gornik and Exner 1991). The extent to which other animal species are a reservoir for these pathogens, and the course of the individual infection chains, are unknown. Recent investigations showed a regional seroprevalence of *C. parvum* in 1.3–14.7% of cats in the United States (Mc Reynolds et al. 1998). *Cryptosporidium* oocysts are extremely resistant to disinfectants (Current 1986), and causal treatment of cryptosporidiosis is currently not possible (O'Donoghue 1995).

The sources of infection and the route of transmission of human microsporidioses are also to a great extent unknown (Weber et al. 1996). Agricultural farm animals must be considered as a possible pathogen reservoir of these parasites of humans.

The trend towards what is called "species-related" animal husbandry will probably lead to increased levels of parasitosis (Hoy and Stehmann 1994; Scharf et al. 1995). The extent to which modern animal husbandry conditions affect the development and survival of parasites, and the extent of the relevance of parasitosis to economics and animal welfare, must be investigated.

2.4.5 Legal Position

Under animal epizootic disease law no form of transmissible parasitosis of agricultural farm animals is subject to a requirement for notification or control. Environmental protection law and the laws on medicinal products or animal protection do not deal with the subject of parasitic contamination of feeds or faeces. Under § 3 of the feedstuffs act (FMG, Anonymous 1998) it is forbidden to manufacture, sell or feed animals with feedstuffs which are capable of damaging the health of animals. Under § 7 para 3 of FMG the seller of feedstuffs must guarantee that the feed is of trade standard purity and unpolluted quality. If animal faeces, which are subject to the waste product laws, are disposed of in agricultural areas, under § 10 of the regional agricultural and waste product act (KrW-/AbfG, Anonymous 1994) among other things the health of humans must not be affected, and animals and plants must not be endangered. If animal faeces, sewage sludge, biological waste and other similar substances which are subject to the waste product laws, are used as secondary raw fertilisers, they must comply with the provisions arising from § 8 of the KrW-/AbfG, as well as the provisions of the fertiliser law and the fertiliser regulations concerning the sale of fertilisers. Sewage sludge can not be spread on pastures (Anonymous 1992). Under § 1 para 2 of the fertiliser regulations (Anonymous 1991) fertilisers and substances according to § 1 no. 3–5 of the fertiliser law (Anonymous 1977), which contain organic constituents, may only be sold commercially if they are harmless as regards causing diseases in humans and animals by transmission of disease pathogens and damage to plants, plant products or the soil by transmission of harmful organisms. These provisions also apply to farm manure (slurry, liquid manure, stall dung, straw and similar by-products of agricultural production, which are used for fertilisation purposes), if these are sold for profit. This provision is based on § 5 para 1 of the fertiliser law. At present there is no authority in the fertiliser law to enable limitation from a health and disease hygiene point of view of the application of farm manure of animal origin to ones own land on a farm (Oswald, personal communication 1998). The new edition of the biowaste, compost and fertiliser regulations contains requirements for the health safety of organic waste (Däschner, personal communication 1997).

2.4.6 Recommended Research

- Continuous updating of epidemiological data to prevent the incidence of parasites in animals and their environment
- Epidemiology of parasitosis to be included in the conditions of what is called "species-related" animal husbandry
- Incidence and pathogenicity of *Giardia* in ruminants
- Incidence and treatment of cryptosporidiosis and microsporidiosis in agricultural farm animals
- Mechanism of action of disinfectants against parasites with the aim to develop disinfectants with an antiparasitic action
- Development of inexpensive procedures for the disinfection of slurry
- Development of practicable methods for the detection of parasites in ready-to-use feeds
- Basic research into the development of a vaccine against *Toxoplasma gondii* in cats
- Development of a vaccine against *Taenia saginata* in cattle and of detection methods for low-measles in beef
- Clarification of the routes of infection of humans with the eggs of *Echinococcus multilocularis*.

2.4.7 Recommended Action

It is recommended that cryptosporidiosis in calves be made notifiable to facilitate important epidemiological research projects.

2.4.8 Summary

No new knowledge can be added to the evaluation of parasitic contamination of feedstuffs formulated by Bürger in 1985: "Insofar as they are not contaminated at a later stage, all feedstuffs are harmless from the point of view of parasites, if high temperatures are used in their manufacture or processing. Cereals are harmless. All

feeds which are stored dry and for a long time have a much reduced infection risk. Silage which is free from defects is less harmful as regards parasites after sufficient storage. Fresh feeds such as pasture grass, mown green forage for summer stall feeding from directly or indirectly contaminated areas, fresh meat, fresh slaughterhouse waste and wet feeds for fish represent a more or less high risk of infection with various types of parasite. These risks are for the most part documented, although incompletely quantified. The potential risks from dry and ready-to-use feeds and also imported goods have barely been investigated."

Although the faeces or feeds of agricultural farm animals do not represent a source of infection for humans, attention is drawn to the zoonoses toxoplasmosis, taeniosis (*Taenia saginata*) and alveolar echinococcosis (*Echinococcus multilocularis*).

There is a need for research in the form of epidemiological investigations into the zoonotically relevant parasites *Giardia*, *Microsporida* and especially *Cryptosporidium*, into the animal-specific parasites in solid dung or slurry and into the connection with forms of animal husbandry known as species-related. There are no practicable methods for the detection of parasites in ready-to-use feeds and no disinfectants with high antiparasitic effectiveness. There is also a requirement for fundamental principles for the development of vaccines (*Toxoplasma gondii, Taenia saginata*) and for epidemiological investigations (*Echinococcus multilocularis*) or diagnostic investigations (*Cysticercus bovis*) for the forms of zoonosis described as special cases.

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2.5 Pests in Stored Feedstuffs

Josef Kamphues and Christoph Reichmuth

2.5.1 Introduction

Because of the high nutrient content in most feedstuffs, as in human foods, feedstuffs provide a suitable substrate for various groups of pests; these include vertebrates such as rodents (e.g. rats, mice) and birds (e.g. sparrows) and also invertebrates such as molluscs (snails), insects (e.g. beetles, moths) and arachnids (mites).

Feedstuffs may be attacked by these harmful organisms even before they are harvested, as in the case of an attack on plants by snails. Of more importance in practice, however, is an infestation between harvesting and the actual use of the feedstuffs, which are generally harvested and then stored for future use, so the term feedstock pests typifies perfectly the preferred objective of these harmful organisms.

A differentiation must also be made in the infestation of feedstuffs, between organisms which occur only temporarily, rarely only on the periphery of the stock of feed (e.g. Diptera on the surface of the silo gate), those which need the feedstock more or less continuously as a feed basis and a living area (e.g. many types of arthropods) and those which need the stock not just as a source of nutrients, but also for their reproduction and development (e.g. grain weevils which deposit their eggs and develop in grain).

2.5.2 Incidence (Conditions, Frequency and Intensity of Infestation)

Where there is generally favourable availability of the substrate or nutrient of the feedstuff (especially after it has lost its original integrity

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due to processes of mechanical chopping) the climatic conditions like relative humidity and temperature as well as the water content of the feedstuffs together with the time available will determine the final extent of damage after an initial infestation with pests.

For most feedstock pests the optimal water content in the feed varies between 14% (optimal for many beetles) and a maximum of 20% (optimal for some species of mites). If a certain infestation of pests is present, in the end – relatively regardless of the original moisture content in the stock – there will be a higher water content in the feed, because of the metabolism of the insects and mites, and warming will also occur (vicious circle).

In spite of the high level of tolerance to extremely high and low temperatures of many arthropods which damage feedstuffs, as has been confirmed for example by the short-term survival of flour mites at temperatures of –15 to +40 °C and of the granary weevil at –7 to +40 °C, the speed of development of a massive infestation very probably depends on the surrounding temperature during the storage of the feed.

Table 2.31, which follows, gives an impression of the extent of infestation of feedstock pests (observed in field studies).

Table 2.31: Details on the frequency of infestation of feedstock by pests

Incidence	Authors
• 50% of all milling companies and feedstuff stores are continually infested with feedstock pests	Hefner et al. 1988
• 12% of all cereal batches submitted by farmers to the agricultural trade: heavily infested with grain weevils	Gall et al. 1988
• 90% of all cereal stocks on farms in Great Britain: infested with mites	Griffiths et al. 1976
• 10% of all mixed feed submitted[1] for pigs: massive infestation with mites	Kamphues 1988
Field study of horse feedstuffs: massive infestation with mites in • 10% of all oat and straw samples • 19% of all hay samples	Küstermann 1989
Private households (stocks of pet food and muesli) almost 50% are faced with the dried fruit moth	Madel 1993

[1] for quality control

2.5.3 Consequences of an Infestation by Feedstock Pests

Under the present production conditions, besides the loss of feed mass and nutritive value, the most important risk of infestation by feedstock pests are direct or indirect adverse effects on hygienic quality (promotion of biotic deterioration). The chief health risks of infestation by feedstock pests are the encouragement of microbial deterioration (by fungi and bacteria, especially by those species which are able to produce toxins) and the possible contamination of the feed with infectious pathogens, with components of the pests which have a potentially allergising capacity (e.g. excrements from mites, wing scales of dried fruit moths) and the improper use of pesticides, with which the feed can then become contaminated.

The following Tables 2.32 and 2.33 describe, in a way which is easy to understand, the consequences of a feed infestation by feedstock pests for the feed in itself, for the animals to which the feed is offered and for humans who have to handle the contaminated feed.

2.5.4 Legal Position

§ 3 para 4 of the feedstuffs law provides for direct action against the use of badly contaminated feedstuffs as feed (Anonymous 1998 a).

A severe infestation of feeds by feedstock pests (which to date has nowhere been precisely and bindingly defined) would indisputably represent an infringement of trade regulations, and thus requires a declaration (which would considerably reduce its market value).

Paragraphs 2 and 3 of § 3 of the feedstuffs law, under which it is forbidden "to put feedstuffs on sale" or "to use feedstuffs which are capable" ... "of *impairing animals' health*", are also helpful in this connection. It might, however, be very difficult to attribute proof forensically of a direct harmful effect solely to the infestation of feedstuffs by certain feedstock pests. There have been no suitable experimental investigations of this possible effect (feed infested with moths or beetles in animal experiments) nor can more recent results

Table 2.32: The effects of an infestation of feedstock pests on the feed

Feedstock pests	Chief effects on the feed (type, particulars)	Secondary effects (type, particulars)
Birds	Contamination with excrements (path. microorganisms such as salmonellae, chlamydiae, mycobacteria)	Contamination of the feed store, possible introduction of parasites?
Harmful rodents (rats, mice)	Massive loss of feed; contamination of the feed with salmonellae, leptospirae, possibly Aujeszky virus, European swine fever virus; tendency to microbial spoilage of feed ↑ (urine ⇒ moisture in the feed)	Destruction of packaging materials; use of rodenticides (secondary intoxication[1]); dead rodents → hay → botulism
Moths	Cocoon formation leads to loss of fluidity → leads to "dead zones", tendency to localised spoilage, consumption of substrate (grain moth with development in grain), moth components: some are potent allergens	Disturbance of the function of conveyor systems for mixed feed, also affects route to the animal; contamination with infectious pathogens?
Beetles	Consumption of substrate (some development of larvae in the grain); tendency to microbial spoilage due to destruction of the integrity of the grain, moisture and heat; allergens?	Also some damage caused by gnawing at feed packaging, possible vector function of beetles (gumboro virus in lesser meal worms)
Mites	Susceptibility to microbial spoilage ↑, symbiotic relationship with moulds; contamination with infectious pathogens; mite excrement: potent allergens	Loss of viability due to damage to the construction of the germ; via "dust" from feed → allergies in humans (cross-reactions with house dust allergies)

[1] due to contamination of the feed where there is improper use or as the result of ingestion of poisoned rodents by dogs, cats or wild animals (e.g. owls)

Table 2.33: Consequences for animals and humans of an infestation of feed by feedstock pests

Object	Effects
Animals	• reduced feed intake and less effective utilisation of feed • reduced supply of amino acids and vitamins • intake of contaminants (parasite parts or products; infectious pathogens and microorganisms of the spoilage) • clinical disorders (e.g. diarrhoea) • alterations of organs (e.g. dystrophic liver changes) • skin infection (only limited, transient) • exposure of the respiratory tract (aspiration of the feed "dust") • induction of antibody formation/sensitisation
Humans	• economic losses (loss of image!) • functional disturbance in the area of feed demand • tainting of foodstuffs with the same spectrum of feedstock pests • exposure while handling infested feedstuffs (skin, respiratory tract) • allergisation (cross reactions in humans with "house dust allergies") • dermatitis • consumption of toxic metabolites of feedstock pests?

(for example, isolated administration of mites to piglets; Schulze-Becking 1993) serve as proof. According to published case reports, the adverse effects of infested feedstuffs are in practice generally not isolated effects of an infestation of pests, but – after critical evaluation – are the results of overall defective hygienic conditions of the feed, since in practice an isolated infestation with certain feedstock pests is more often the exception (Kamphues and Schulze-Becking 1992).

With the knowledge at present available it might also be difficult to prevent the use of feedstuffs which are affected by feedstock pests on the basis of *animal protection law*, not least in the situation where a "certain amount" of infestation is widely spread through many feedstuffs, and for this reason is seen, or has to be seen, as "natural" or "unavoidable".

Nor is the infestation of feedstock by pests taken into particular account in the regulations of *animal disease legislation*, which could change, however, if for example the role of certain feedstock pests as vectors (e.g. for salmonellae) were of particular epidemiological importance.

2.5.5 Substances for Preventive Health Protection and Agents for the Control of Feedstock Pests and Organisms Which Damage Feedstock

Insofar as the sale of plant protection agents is concerned, for the control of pests in infested agricultural products of plant origin, including cereals, flour, bran and other mill products as well as feedstuffs made from these raw materials, to which the plant protection law applies, only those preparations are permitted which are included in the current register of agents (Anonymous 1998 b) of the BBA (Federal Biological Research Centre for Agriculture and Forestry), Part V, Stored Product Protection.

The Dangerous Substances Order (Anonymous 1993) applies to control measures using chemical substances not within the purview of the plant protection law; under this the handling of these substances is regulated by labour protection law. Lists of agents which have been tested and recognised by the Federal Institute of Health and Consumer Protection (BGVV) as disinfestation agents and processes for the control of animals pests (arthropods) (Anonymous 1998 c; Hoffmann 1989) and for deratisation under § 10 c of the Federal disease legislation (Anonymous 1998 d), are published in the Federal Health Gazette ("Bundesgesundheitsblatt").

2.5.5.1 Insecticides

The tables, which for reasons of space are only included in the complete German version, provide a survey of the insecticides registered under the plant protection law (phosphine, carbon dioxide, nitrogen, pirimiphos-methyl, pyrethrum and diatomaceous earth) which are used for the protection of stored products. When using

different agents a differentiation must be made between the treatment of empty spaces before storage, of spaces with stored feeds and of infested stocks.

The preparations used in the protection of stocks can be divided by active substances (see above), by the condition of the aggregate (solid, liquid, gaseous), by the different dosages (depending on the species, type of storage and the different materials in or on which the stocks are stored) and by the time needed for them to take effect. The prescribed withholding time (between administration and possible utilisation of the treated goods) is an important factor in practice (possible risks of residue formation).

In this connection we must also discuss the extent to which the permits of preparations for the disinfestation of agricultural products which are intended for later use as food, – as is the case generally at present – should be transferred directly to feedstuffs. The tolerance limits for surviving insects and mites given for the disinfestation of feed, need to be considered. This would lead to reduced dosages and thus to use of less of the agent. In comparison under the LMBG (foodstuffs protection law) (Anonymous 1997) there is nil tolerance of live pests in foodstuffs, when the goods are put on sale.

2.5.5.2 Rodenticides

Anticoagulants are chiefly used as rodenticides, and a growing resistance to these has been observed. If vitamin K (a coagulation factor) is used in animal feed which rodents can also ingest, this produces an increased resistance in these rodents to anticoagulants. The choice of active substances for the control of rodents is therefore undertaken depending on the situation and in accordance with the requirements of the authorities (Pelz 1996).

As well as anticoagulants there are also gases registered for the control of harmful rodents (active substance: hydrogen cyanide) and also products containing zinc phosphide (active substance: phosphine, evolved with water from the phosphide), which can be used in enclosed areas or put outside. With anticoagulants too, a differentiation should be made between products which can only be used in enclosed areas, and those which can be used in enclosed areas and also in open country (generally as bait). More precise data

(dosage, time to take effect etc.) can also be found in the relevant tables in the full German version.

The control of rats and mice in Germany falls within the area of responsibility of various Federal Ministries, depending on whether there is a risk of the transmission of disease under Federal disease legislation (Anonymous 1979), whether stored products are to be protected from rats under the plant protection law and also for rats outside both the areas already mentioned. The lists of the Federal Institute of Health and Consumer Protection (BGVV) (Anonymous 1998d) and the list of plant protection agents, Part 5 Stored Products Protection (Anonymous 1998b), in the current version, comprise recommendations for rodent control. The relevant tables in the full German version contain the rodenticides registered under the plant protection law (Anonymous 1998e).

2.5.6 Recommended Research

The adverse effects of an infestation by feedstock pests on the feed itself are widely known (loss of palatability, nutrients and fluidity, the introduction and spread of infectious pathogens, an increased susceptibility to microbial spoilage and the production of toxins); there are, however, greater uncertainties and unanswered questions regarding the evaluation of potential adverse effects on the animal (feed intake and utilisation, the induction of disease or resistance reactions) or on humans who handle these contaminated feedstuffs (sensitisation, allergies). There is therefore also a need for research in this respect. As well as this aspect, the questions of control (including biocides which are not permitted as stored product protection agents) and resistance development, and also possible residues in the feed (due to the use of the relevant agent) also require intensive experimental investigation. Furthermore, the possible vector function of feedstock pests in connection with different animal diseases (e.g. swine fever) needs further research work (the most recent example: transmission of the scrapie pathogen via hay mites). Finally, potential effects of infested feedstuffs on the health of humans who are exposed to loaded feed (by direct skin contact, aspiration of feedstuff dust) should be examined in more detail.

2.5.7 Recommended Action

An urgent need for action is seen, with better and more intensive information for those practising agriculture, concerning the conditions which lead to an infestation of feedstock pests and the recognition of infested feed batches. Only early detection of an infestation of feedstock pests (with regard to the actual feed and the store) will protect from the risk, probably even more serious than the infestation, of forced microbial spoilage of the feed. Setting of a maximum tolerable extent of infestation of feedstuffs (e.g. in the feedstuffs legislation) cannot be recommended as long as there are no suitable animal experiments (including dose-effect relationship), from which such limits can be inferred on the basis of the negative effects on health and/or performance. Finally, a need for action is also seen with regard to the registration of certain preparations of insecticides for use in infested feedstuffs. For example there is no registration for contact insecticides for use in all feedstuffs and ingredients which are not cereals or cereal by-products (i.e. mixed feed and complete diets, which contain other ingredients besides cereals and cereal by-products).

2.5.8 Summary

Infestation of feedstuffs and their stores by harmful rodents, moths, beetles and mites was, is and remains a permanent problem for all those who produce, store, sell and use feed (formerly only in agriculture and the milling industry, today also private households storing pet food). In the past, measures to prevent or minimise the infestation of feedstuffs by pests were essentially on economic grounds (to avoid the loss of feed), today they are indispensable as an essential part of efforts for better hygienic conditions of the feed from the point of view of the animal (prevention of health risks) and of the consumer (introduction of toxins into the food chain of humans), and not least in the interest of the reputation and image of food of animal or plant origin. As well as efforts for the registration of contact insecticides for use in infested feedstuffs, questions which need to

be dealt with in future are the development of resistance in the control of rodents (anticoagulants) and in other pests (resistance in moths, beetles and mites to phosphine products). These matters are of increasing interest and therefore require experimental research. Finally the vector function of feedstock pests related to various animal diseases has suddenly achieved unexpected urgency (see the note on the suspected transmission of the scrapie pathogen by hay mites), so that here too, there is an undisputed need for further work. The microbial spoilage of feed, which is clearly encouraged by feedstock pests (as the result of which mycotoxins may occur in feed) has consequences in the end for the quality of foods (e.g. ochratoxin in the carcasses of slaughtered pigs), so that all efforts should be made to minimise infestation by feedstock pests as a preventive protection of the consumer.

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3 Substances for the Prevention of Health Problems and Agents to Improve Feed Conversion

3.1 Cleansing Agents

Andrea Schänzler and Reinhard Böhm

3.1.1 Cleansing Agents Used in Agriculture

Several types of cleansing agents are used in agriculture including the cleansing of milking equipment on farms. These substances can generally be divided into three groups: alkaline, acid and neutral cleansing agents. Table 3.1 gives some examples of some common substances used here.

3.1.1.1 Alkaline Substances

Alkaline surfactants used in cleaning are generally applied at such low concentrations and in such small amounts that the pH value of slurry remains unaffected. If phosphates are used it can be demonstrated that not more than an additional 2.9% are released into the slurry compared to its normal phosphate content. In certain cases this may contribute to the release of phosphates into soil and ground-water (Wildbrett and Böhner 1990).

3.1.1.2 Acid Substances

In the case of mineral acids such as hydrochloric, nitric, phosphoric or sulphuric acids, the pH-effects are generally too low to cause any

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Table 3.1: Cleansing agents used in agriculture

Alkaline substances	Acid substances	Neutral substances
Sodium hydroxide	Hydrochloric acid	Non ionic surfactants:
Sodium carbonate	Nitric acid	• Alkylphenol ethoxylates (APE)
Sodium hydrogen carbonate	Sulphuric acid	• Fatty alcohol ethoxylates (FAE)
Sodium silicate	Phosphoric acid	Betaine
Trisodium phosphate	Sodium dihydrogen phosphate	Aminoacylamino acids
Tetrasodium diphosphate	Citric acid	
Pentasodium triphosphate	Tartaric acid	
Ammonium hydroxide	Gluconic acid	
Alkyl carboxylates	Sulphamic acid	
Fatty alcohol sulphates (FAS)	Quaternary ammonium compounds (QAC)	
Linear alkylbenzene sulphonates (LAS)		

environmental risk, if they are released into the environment via slurry. But phosphorus and nitrogen may be increased in the slurry by about 0.1% (Wildbrett 1988). Organic acids are rapidly metabolised in slurry by microbial activity. Under legal requirements in Germany and in most European countries, surfactants used in this field in acid solution must be more than 90% biologically degradable. The biodegradation may take place in the slurry during storage or in the soil. The degradation in soil depends on the type of surfactant, as has been shown for sewage sludge application (Ward and Larson 1989; Liu et al. 1992; Comellas et al. 1993).

3.1.1.3 Neutral Substances

As mentioned above most surfactants used in animal husbandry pass first into the slurry and then, if residual concentrations remain, into the soil. The amount of surfactants released into the air is negligible. Most surfactants are biodegradable due to legal requirements but this information generally refers to waste water treatment plants

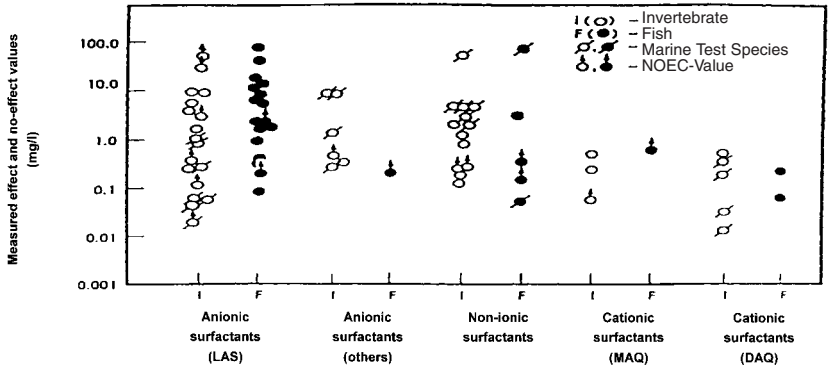

Figure 3.1: No-effect levels of selected surfactants in several ecotoxicological tests (Lewis 1991). MAQ=monoalkyl quaternary ammonium salts; DAQ= dialkyl quaternary ammonium salts

and is determined by OECD-Tests (Gledhill et al. 1991; Gode 1990). Due to rapid degradation in surface-water, in general no enrichment would be expected in such biotopes (Mix-Spagl 1990; Guckert et al. 1996). Of all the types of surfactant the linear alkylbenzene sulphonates (LAS) have best been investigated. Figure 3.1 gives some information concerning the non-effect-level for several types of aquatic organisms (Lewis 1991).

3.1.2 Special Aspects of Ecotoxicology

From research concerning sewage sludge utilisation it is known that such biodegradable surfactants are also rapidly metabolised in soil (Kunt 1993; Haugh and Rennie 1994; Ang and Abdul 1992). Anionic surfactants such as LAS will be degraded over a wide concentration range (0.1 to 10times the calculated concentration in sludge and slurry). Half of the concentration is mineralised within 18–28 days (Ward and Larson 1989). The velocity of degradation depends on moisture and temperature (Scheunert et al. 1990). Application of slurry containing ten times more LAS than expected would not disturb the function of the soil microflora (dehydrogenase-test and soil respiration) or that of the macroflora (Callahan et al. 1994; Scham-

Table 3.2: pH values and dry matter content of the soil samples investigated (data from Schamper and Zbil 1996)

Experimental set up	Soil depth	Sample taken at									
		Day 1		Day 9		Day 14		Day 29		Day 185	
		pH	DM	pH	DM	pH	DM	pH	DM	pH	DM
Reference*	A	6.02	53.70	5.78	69.10	5.40	74.10	5.51	74.51	5.76	72.85
	B	6.08	74.22	5.33	77.14	5.40	77.28	5.52	78.75	5.59	78.37
Slurry (S)	A	5.82	62.52	5.57	63.87	5.78	64.56	5.88	68.15	6.02	63.71
20 m^3/ha	B	5.94	75.43	5.71	73.72	5.79	74.05	5.78	76.52	5.82	71.73
S + Formalin	A	5.83	58.10	5.75	60.03	5.88	67.53	5.77	70.05	5.73	62.95
16 kg/m^3	B	5.71	74.86	5.66	73.12	5.58	76.14	5.66	78.19	5.75	73.69
S + Formalin	A	5.37	63.25	5.67	68.92	5.73	63.51	5.77	69.06	5.28	72.66
50 kg/m^3	B	5.48	76.88	5.45	71.99	5.45	75.72	5.48	76.15	5.29	73.69
S + NaOH (50%)	A	6.49	60.73	6.21	62.31	6.45	64.72	6.32	70.72	5.47	65.83
12 kg/m^3	B	5.62	82.20	5.64	72.65	5.52	72.63	5.64	78.39	5.51	79.41
S + PES (15%)	A	5.54	62.14	5.53	63.04	5.49	65.03	5.50	69.95	5.18	68.46
40 kg/m^3	B	5.58	76.19	5.53	75.60	5.53	77.20	5.54	79.50	5.33	77.61
S + $Ca(OH)_2$ (40%)	A	6.15	58.93	6.89	59.65	6.45	62.65	6.57	66.30	5.71	65.02
40 kg/m^3	B	5.69	78.15	5.62	74.97	5.58	76.00	5.59	80.67	5.64	77.61
S + Surfactant	A	5.80	63.12	5.76	62.66	5.63	66.93	5.68	71.13	5.56	66.22
10 kg/m^3	B	5.67	77.91	5.59	76.06	5.62	78.04	5.54	81.62	5.58	78.22

DM = Dry matter of soil sample in %
Soil layer A = 0–2 cm
Soil layer B = 8–10 cm
* = unfertilised meadow

Table 3.3: Results from measuring the dehydrogenase activity of soils fertilised with disinfected slurry. The data are given as mg TPF determined in 1 g soil and 24 h incubation (data from Schamper and Zbil 1996)

Experimental set up	Soil depth	Sample taken at									
		Day 1		Day 9		Day 14		Day 29		Day 185	
		TPF	s*	TPF	s*	TPF	s*	TPF	s*	TPF	s*
Reference*	A	4.90	0.54	2.43	0.28	2.42	0.57	1.76	0.53	2.34	0.16
	B	1.49	0.02	0.95	0.20	1.07	0.15	0.94	0.15	0.88	0.11
Slurry (S)	A	2.68	0.09	2.50	0.20	3.67	0.02	3.84	0.53	3.06	0.11
20 m^3/ha	B	1.10	0.07	1.08	0.04	1.66	0.08	1.40	0.11	1.28	0.08
S +Formalin	A	4.46	0.17	4.35	1.77	2.46	0.50	3.55	0.08	2.63	0.04
16 kg/m^3	B	1.43	0.21	1.26	0.02	1.37	0.10	1.07	0.00	1.00	0.07
S +Formalin	A	1.40	0.30	2.10	0.05	2.60	0.90	1.79	0.35	1.12	0.23
50 kg/m^3	B	0.88	0.06	1.37	0.27	1.08	0.06	1.10	0.04	0.87	0.11
S +NaOH (50%)	A	5.00	1.39	4.72	0.77	4.73	0.09	4.24	0.62	2.85	0.74
12 kg/m^3	B	0.88	0.02	1.13	0.12	1.32	0.03	1.02	0.07	0.72	0.25
S +PES (15%)	A	3.02	0.26	2.99	1.36	2.27	0.27	2.56	0.47	1.79	0.31
40 kg/m^3	B	0.87	0.06	1.15	0.17	0.88	0.01	0.96	0.11	0.73	0.41
S +$Ca(OH)_2$ (40%)	A	4.33	0.91	3.88	2.18	4.32	0.34	4.12	1.48	3.30	0.48
40 kg/m^3	B	0.89	0.09	1.32	0.14	1.50	0.10	0.99	0.31	0.73	0.02
S + Surfactant	A	2.83	0.19	3.89	1.06	2.67	0.17	3.19	0.62	2.45	0.09
10 kg/m^3	B	1.04	0.16	1.35	0.11	0.91	0.07	1.20	0.04	0.75	0.37

TPF=Results given in formation of Triphenylformazan (in µg)
s*=Standard deviation
Soil layer A=0–2 cm
Soil layer B=8–10 cm

per 1998). This is shown in Tables 3.2, 3.3 and 3.13 (see Section 3.2). Nothing is known about synergistic effects between surfactants and pathogens from the manure as regards transport in the soil from the surface to the ground-water.

3.1.3 Recommended Research

Since experience concerning soil systems and the ecotoxicological behaviour of surfactants is very limited it seems useful to develop new methods using terrestrial systems in laboratory and field-tests, which will give more and better information than the methods used in testing herbicides and insecticides. Even if we could expect that the use of biodegradable surfactants will result in an induction of microbial degradation in slurry, as is the case with disinfectants, more detailed research is necessary to identify the pathways.

If surfactants from slurry were to influence the migration of viruses, parasites and bacteria through the natural soil, this would be a matter of concern. For this reason, whether such synergisms do exist must be investigated.

3.1.4 Recommended Action

The only required action is to initiate and finance research in the above mentioned fields. There are many legal regulations as can be seen from the full German version of this contribution.

3.1.5 Summary

Cleansing agents in agriculture

The types of cleansing agent are named and their chemical nature as well as ecotoxicological behaviour is briefly described. The organic and inorganic chemicals applied are generally released via

slurry into the environment. This results in dilution which avoids negative effects to water, soil and air so long as biodegradable surfactants are used. Ecotoxicological testing procedures using terrestrial systems must be improved. The possibility that surfactants will accelerate the movement of pathogens from soil surface to the ground-water must also be investigated.

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Further references must be taken from the full German version.

3.2 Disinfectants

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3.2.1 Introduction

Disinfection is an important measure in the field of preventive animal health care and in order to interrupt epidemiological pathways in the case of notifiable diseases. In particular the disinfection of liquid manure is regarded as critical from the point of view of the farmer who has to bring it out to his meadows or arable land and who fears negative effects to his crop. There is also the point of view of the water-protection authorities who fear leaching of chemicals into the ground-water.

3.2.2 Disinfectants Used in Animal Husbandry

The following types of disinfectant are used alone or in combination for disinfection of animal houses, slurry and milking equipment:

- Aldehydes
- Alcohols
- Chlorine (inorganic and organic compounds)
- Iodophors
- Oxidising agents
- Lyes and acids
- Phenols
- Surfactants (cationic and amphoteric).

The most important aldehydes are formaldehyde and glutaraldehyde, which are used for surface disinfection and for disinfecting slurry. Chlorines are not very effective on rough surfaces in animal

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houses but very common for the disinfection of the milking equipment on dairy farms. Phenols are only used in a few European countries as surface disinfectants due to their limited efficacy and undesired use properties.

Alkalines are used as sodium hydroxide in veterinary disinfection measures and as lime for certain purposes. Sodium hydroxide may be used for surface disinfection as well as for disinfecting slurry. Lime as burned or slacked lime may be used for disinfection of solid or liquid manure and within limitations also for certain surfaces.

Acids are mainly used as preservatives but formic acid is a very effective disinfectant for veterinary purposes. Peroxides are used more and more in this field due to their low toxicity. Peracetic acid is the most common one of these, but several other compounds are also used, generally for surface disinfection. All the other chemicals are either used for special purposes (alcohols, iodophors) or are part of commercially prepared disinfectants (surfactants).

3.2.3 The Application of Disinfectants in Animal Husbandry

From the point of view of the environmental effects caused there are two basically different concentration levels. A low level if disinfection is carried out in the animal house itself. Table 3.4 gives an example for calculating the dilution in slurry, additional dilution must be expected under practical conditions due to the water from cleansing procedures (Winterhalder 1985). If the average volume in a slurry storage tank is between 200 m^3 and 500 m^3 at the time of disinfection and the surface of an animal house of 700 m^2 has to be disinfected, this results in spreading approximately 300 l of disinfectant at the dilution used. This means that at least a dilution of 1 : 600 to 1 : 1600 could be expected. In the case of notifiable diseases not only the animal house itself but also the manure has to be disinfected. The latter will result in relatively high concentrations of selected disinfectants as listed in Table 3.5. If milking equipment is cleaned and disinfected, dilutions from 1 : 50 up to 1 : 1000 have to be taken into account (Heinz et al. 1987).

Table 3.4: Example of the calculation of the final concentrations of disinfectant in the slurry after surface disinfection in a pig fattening unit (Winterhalder 1985)

Basis: Pig fattening unit (700 places) run in the "all out all in"-method		
Space requirement	0.97 m^2/animal	
	0.97 m^2×700 animals	679 m^2 of area
Amount of slurry/day	10% of live weight	
	(100 kg×10%)×700 animals	7000 l/d
Amount of disinfectant (use dilution)	0.4 l/m^2×679 m^2 at a concentration of 2%	271.6 l use solution
		= 5.5 l concentrate
	= 5.5 l concentrate in 7000 l slurry	= 0.0785%

3.2.4 The Toxicological and Environmental Effects of Disinfectants

Toxicological data exist with respect to mice, rats and humans for most pure substances used as disinfectants or in mixtures (Gerhartz and Elvers 1985). Table 3.6 shows the classification according to German requirements with respect to toxicity and water hazard. Table 3.7 summarises the maximum allowable concentrations at the place of work (MAC values) for some of the chemicals involved.

Those disinfectants which will leave the farm via municipal waste water, which is the exception and generally limited to those chemicals used for milking devices and storage tanks, have to be considered from the point of view of disturbing the waste water treatment plant. Table 3.8 shows the results of the relevant OECD testing procedure for some disinfectants, indicating that most of the disinfectants will cause no problem due to dilution and/or decomposition (OECD 1971, 1981, 1984, 1992a, 1992b).

If the disinfectants are released via slurry they will be diluted, neutralised, decomposed or possibly degraded by microbiological activity. Such activity may be induced in slurry, as was shown by Ishaque et al. (1985) for phenolic compounds (Figure 3.2). Several

Table 3.5: Recommendations for the disinfection of liquid manure

Disinfectant	Vegetative bacteria	Naked viruses	Enveloped viruses	Mycobacteria	Spores
40% Lime wash	60 kg/m^3	60 kg/m^3	40 kg/m^3	–	–
Exposure time	4 d	4 d	4 d		
Soda lye	30 l/m^3 (1.5% NaOH)	30 l/m^3	20 l/m^3 (0.8% NaOH)	–	–
Exposure time	4 d	4 d	4 d		
Formalin (37% formaldehyde)	15 l (kg)/m^3	15 l/m^3	10 l (kg)/m^3 (1.0% formalin)	25 l (kg)/m^3 (2.5% formalin)	up to 5% dry matter 50 kg/m^3 5–10% dry matter 100 kg/m^3
Exposure time	4 d	4 d	4 d	14 d	4 d
Peracetic acid PES 15 (15% peracetic acid)	25 l/m^3 (0.375% peracetic acid)	–	40 l/m^3 (0.6% peracetic acid)	–	–
Exposure time	1 h		4 d		–

Table 3.6: Toxicity and water pollution classification according to German regulations

Compound	LD_{50} oral / rat	Water pollution class
Formic acid	1250 mg/kg	1
Benzalkonium chloride	234–1170 mg/kg	3
Acetic acid	3530 mg/kg	1
Formaldehyde	800 mg/kg	2
Glutardialdehyde	600–2380 mg/kg	2
Lime $Ca(OH)_2$	7340 mg/kg	1
Soda lye	–	1
o-Cresol	530–1300 mg/kg	2
Peracetic acid	263 mg/kg	2
Citric acid	11500 mg/kg	0

Table 3.7: Maximal allowable concentration at the place of work (MAC value) of selected disinfectants (Anonymous 1996)

Compound	MAC ml/m^3 (ppm)	MAC mg/m^3
Formic acid	5	9
Calcium oxide	50	35
Chlorine (Cl_2)	0.5	1.5
Chlorinated biphenyls	0.05–0.1	0.5–1
Ethanol	1000	1900
Acetic acid	10	25
Formaldehyde	0.5	0.6
Glutaraldehyde	0.2	0.8
Iodine	0.1	1
Cresol	5	22
Methanol	200	260
Sodium hydroxide		2
Phenol	5	19
Propanol	400	900
Nitric acid	2	5
Hydrogen peroxide	1	1.4

Table 3.8: Results of testing the biodegradability for sewage treatment plants of some selected disinfectants (Gode and Hachmann 1992)

Disinfectant	Tested concentration	OECD-Test	Result
Formaldehyde	5 ppm	301 D/E	easily degradable
Glyoxal	5 ppm	301 D/E	easily degradable
Glutaraldehyde	5 ppm	301 D/E	easily degradable
Ethanol	5 ppm	301 D/E	easily degradable
Isopropanol	5 ppm	301 D/E	easily degradable
n-Propanol	50 ppm	301 D/E	easily degradable
o-Phenylphenol	2 ppm	301 D/E	easily degradable
p-Cl-*m*-Cresol	2 ppm	301 D/E	easily degradable
Peracetic acid	5 ppm	301 D/E	easily degradable
Benzalkonium chloride	5 ppm	301 D/E	not easily degradable
Benzalkonium chloride	5 ppm	303 A	can be eliminated or degraded

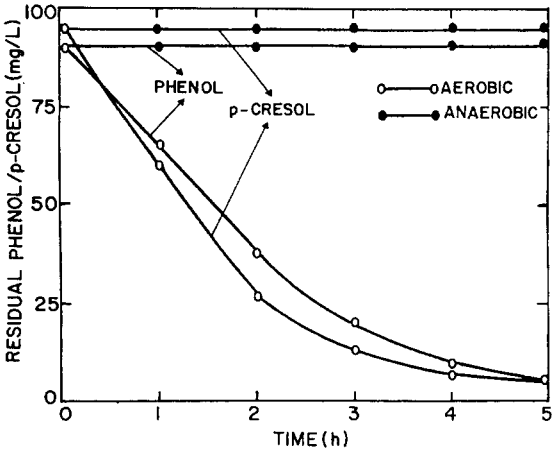

Figure 3.2: Quantitative utilisation of phenol and *p*-cresol by mixed resting-cell suspensions prepared from liquid swine waste (Isahaque et al. 1985).

Table 3.9: Degradation rates of several disinfectants reported as half-life times (Howard 1993)

	Soil	Air	Surface-water	Ground-water
Chloroform	4 w – 6 months	4 w – 8 months	4 w – 6 months	8 w – 5 a
Formic acid	1–7 d	5–50 d	1–7 d	2–14 d
Formaldehyde	1–7 d	1–6 h	1–7 d	2–14 d
o-Cresol	1–7 d	2–6 h	1–7 d	2–14 d
m-Cresol	2–29 d	1–11 h	2–29 d	4–49 d
p-Cresol	1–16 h	1–15 h	1–16 h	2 h–28 d
Phenol	1–10 d	2–22 h	5–53 h	0.5–7 d
2,4-Dimethylphenol	1–7 d	1–11 h	1–7 d	2–14 d
Ethanol	2–24 h	0.5–5 d	6–26 h	0.5–2 d
Isopropanol	1–7 d	6–72 h	1–7 d	2–14 d
Methanol	1–7 d	3–30 d	1–7 d	1–7 d
Peracetic acid	4–48 h	0.5–6 d	4 h–7 d	2–14 d

authors report on the elimination of formaldehyde and chlorines from slurry (Motz 1979; Kurzweg et al. 1988; Böhner and Guthy 1991 a,b). Alkaline and acid substances are rapidly neutralised, peroxides decompose under such circumstances and at the low concentrations expected. Even if residuals are spread with the slurry on the soil, the persistence of such substances in the environment is short, as can be seen from Table 3.9.

Of special interest are the results obtained from laboratory and field tests concerning the environmental effect of disinfected slurry. If such high concentrations of disinfectants are applied their effect on plants, soil and ground-water must be carefully studied. If the effect is low or negligible, it can definitely be stated with high probability that low concentrations will not harm the environment. Concerning the effects on plants, the results of laboratory tests can be seen in Table 3.10 and those of field tests in Table 3.11. They may be summarised as follows:

The results concerning the effect of different disinfectants in slurry on garden cress in a standardised test are listed in Table 3.10. Formaldehyde has the strongest influence on germination and growth height, at higher concentrations lime and peracetic acid have no negative influences on any parameter measured here. This does not fully

Table 3.10: Results of the testing of disinfected slurry in the cress test according to Britzius and Böhm (1981); Markert (1990); Ley (1992)

Disinfectant/ concentration in slurry	Slurry	Germination time			Time of growth			Growth height		
		L	M	S	L	M	S	L	M	S
Calcium cyanamide										
10 kg/m^3	C		X			X			X	
15 kg/m^3	C		X			X			X	
20 kg/m^3	C		X			X			X	
Formalin (37% formaldehyde)										
1.0%	C	X			X			X		
1.0%	S		X		X			X		
1.5%	C	X			X			X		
1.5%	S		X		X			X		
2.0%	C	X			X			X		
2.0%	S			X					X	
2.5%	C		X			X			X	
3.0%	C		X		X					X
4.0%	C			NG						
40% Lime wash										
40 kg/m^3	C/S	X			X			X		
60 kg/m^3	C/S	X			X			X		
100 kg/m^3	C/S	X			X			X		
Peracetic acid										
0.3%	S	X			X			X		
0.6%	S	X			X			X		
Sodium hydroxide										
0.5%	C/S	X			X			X		
1.0%	C/S	X			X			X		
1.5%	C	X				X		X		
1.5%	S	X				X			X	
2.0%	C	X				X		X		
2.0%	S	X				X			X	

L = no or low influence compared with the control
M = moderate influence compared with the control
S = strong influence compared with the control
NG = no germination
C = cattle slurry
S = pig slurry
Germination time: L = 2–5 d M = 5–10 d S = more than 10 d
Time of growth: L = more than 10 d M = 8–10 d S = less than 8 d
Growth height: L = more than 5 cm M = 3–5 cm S = less than 3 cm

Table 3.11: Results of the field experiments with pig slurry containing disinfectants spread on grass land according to Britzius and Böhm (1981); Markert (1990)

Disinfectant/ concentration in slurry	m^3 slurry per ha	Harvest untreated control	Harvest control fertilised with slurry	Harvest field slurry + disinfectant
Formalin (37% formaldehyde)				
1.0%	31 m^3/ha	11 t/ha	21 t/ha	23 t/ha
4.5%	31 m^3/ha	11 t/ha	21 t/ha	20 t/ha
40% Lime wash				
40 kg/m^3	31 m^3/ha	11 t/ha	21 t/ha	13 t/ha
60 kg/m^3	31 m^3/ha	11 t/ha	21 t/ha	14 t/ha
Peracetic acid				
0.3%	30 m^3/ha	n.d.	0.27 kg dry matter/m^2	0.38 kg dm/m^2
0.6%	30 m^3/ha	n.d.		0.42 kg dm/m^2
Sodium hydroxide				
1.0%	31 m^3/ha	11 t/ha	21 t/ha	14 t/ha
1.5%	31 m^3/ha	11 t/ha	21 t/ha	16 t/ha

n.d. = not done

correspond with the results of the field tests, listed in Table 3.11. The effect on plant communities is different; in all cases the harvested crop was better than in the untreated control fields and in some cases was higher or equal to the fertilised control fields.

The effects on the microflora and macrofauna of agricultural soils fertilised with freshly disinfected slurry had been studied in laboratory and field trials by Schamper and Zbil (1996) and Schamper (1998). From their results it can be concluded that the laboratory tests applied here (Lumistox® and OECD 207) were not suitable for obtaining the relevant information. Only the field tests gave reliable and substantial results, within limitations especially for monitoring the earthworms in such situations. The two parameters which gave the most useful results are the determination of the dehydrogenase activity of such soils and of the soil respiration. No residual negative effects could be measured with formaldehyde, sodium hydroxide, lime-wash and peracetic acid as summarised in Tables 3.2, 3.3 (see

Table 3.12: Soil respiration. The percentage of deviation for the meadow fertilised with slurry is calculated as the base for the respiration of the unfertilised meadow, all other percentages are given in relation to the meadow fertilised only with slurry (data from Schamper and Zbil 1996)

Soil layer A	Day 1	Day 2	Day 6	Day 8	Day 21	Day 142
Basal respiration 0–24 h						
Slurry (S) 20 m^3/ha	201.78	117.14	129.77	102.25	101.11	151.58
Formalin 16 kg/m^3 S	85.73	90.92	168.25	118.33	66.80	68.64
NaOH (50%) 12 kg/m^3 S	93.81	78.64	108.81	199.42	68.09	103.84
PES (15%) 40 kg/m^3 S	72.22	187.40	177.93	297.91	110.36	70.31
$Ca(OH)_2$ (40%) 40 kg/m^3 S	78.79	122.93	131.85	139.68	61.99	56.80
C mineralisation day 1–day 8						
Slurry (S) 20m^3/ha	116.17	78.57	104.82	83.20	100.92	90.99
Formalin 16 kg/m^3 S	88.03	119.83	117.22	92.37	58.00	152.90
NaOH (50%) 12 kg/m^3 S	68.33	134.25	88.96	105.29	70.47	167.39
PES (15%) 40 kg/m^3 S	85.18	143.47	87.86	70.57	103.64	187.72
$Ca(OH)_2$ (40%) 40 kg/m^3 S	73.83	83.34	101.07	86.01	94.91	106.95
C mineralisation day 9–day 22						
Slurry (S) 20 m^3/ha	98.61	91.46	85.31	96.74	122.87	87.10
Formalin 16 kg/m^3 S	86.36	118.16	125.11	84.12	92.12	88.25
NaOH (50%) 12 kg/m^3 S	81.02	208.93	99.53	86.59	52.60	90.41
PES (15%) 40 kg/m^3 S	87.02	226.36	121.69	122.65	76.54	133.21
$Ca(OH)_2$ (40%) 40 kg/m^3 S	65.11	147.17	106.60	77.10	94.09	106.56
C mineralisation day 23–day 43						
Slurry (S) 20 m^3/ha	72.19	81.92	111.28	60.97	103.64	94.90
Formalin 16 kg/m^3 S	91.12	121.51	123.88	79.41	47.21	111.48
NaOH (50%) 12 kg/m^3 S	75.07	100.75	117.00	100.43	65.29	123.28
PES (15%) 40 kg/m^3 S	88.18	89.68	86.90	102.43	77.62	103.17
$Ca(OH)_2$ (40%) 40 kg/m^3 S	78.57	127.10	132.64	55.11	81.61	113.46

Table 3.12 (*continued)*

Soil layer B	Day 2	Day 6	Day 8	Day 21	Day 142
Basal respiration 0–24 h					
Slurry (S) 20 m^3/ha	68.61	97.18	62.50	50.41	107.28
Formalin 16 kg/m^3 S	31.38	168.46	221.94	169.16	196.98
NaOH (50%) 12 kg/m^3 S	19.68	162.03	326.45	120.26	139.95
PES (15%) 40 kg/m^3 S	253.90	260.58	465.16	228.78	192.96
$Ca(OH)_2$ (40%) 40 kg/m^3 S	158.16	79.05	234.84	138.47	82.16
C mineralisation day 1–day 8					
Slurry (S) 20 m^3/ha	46.11	74.00	59.80	90.62	70.99
Formalin 16 kg/m^3 S	214.80	135.81	129.18	120.80	302.42
NaOH (50%) 12 kg/m^3 S	306.20	129.15	125.09	102.15	166.38
PES (15%) 40 kg/m^3 S	212.05	136.78	77.47	106.74	201.09
$Ca(OH)_2$ (40%) 40 kg/m^3 S	99.31	210.42	123.38	161.52	106.29
C mineralisation day 9–day 22					
Slurry (S) 20 m^3/ha	29.34	10.19	156.24	346.24	70.82
Formalin 16 kg/m^3 S	872.81	1365.26	62.01	137.31	81.58
NaOH (50%) 12 kg/m^3 S	1223.25	607.89	58.16	82.22	159.84
PES (15%) 40 kg/m^3 S	1190.79	1085.26	95.08	43.91	190.86
$Ca(OH)_2$ (40%) 40 kg/m^3 S	1111.84	763.68	109.01	112.56	119.16
C mineralisation day 23–day 43					
Slurry (S) 20 m^3/ha	111.66	94.94	30.48	53.21	92.23
Formalin 16 kg/m^3 S	109.36	136.31	123.80	236.74	97.78
NaOH (50%) 12 kg/m^3 S	109.43	177.32	174.16	124.27	164.58
PES (15%) 40 kg/m^3 S	43.93	43.78	296.33	129.76	100.93
$Ca(OH)_2$ (40%) 40 kg/m^3 S	136.34	122.96	186.39	281.63	124.91

A = Soil layer 0–2 cm
B = Soil layer 8–10 cm
PES = Peracetic acid (15%)

Section 3.1) and Table 3.12. Only in a few experimental set-ups are the values below those of the reference meadow, which was fertilised only with slurry; in most cases the values are within the same range or better.

Concerning the effects on native earthworms the results obtained from field trials are given in Table 3.13. This example shows the variations due to the biological properties and mobility of these animals. No significant negative effect could be observed under these circumstances.

Concerning the influence on the atmosphere the disinfectants themselves seem to be negligible due to their physical properties and the amounts applied. The only negative effect to be taken into account are the nitrogen losses (ammonia) if the slurry is disinfected with alkalines such as lime or soda-lye, as can be seen in Table 3.14 (Markert 1990).

Information concerning the legal requirements in Germany must be taken from the full German version.

3.2.5 Recommended Research

Since little information is available on the way disinfectants are metabolised in slurry and the circumstances under which this occurs, more detailed research should be carried out in this field. This should include the investigation of bacterial populations which are constantly exposed to low concentrations of disinfectants in slurry, and which may change their surface properties in relation to other undesired phenomena, such as the selection of antibiotic resistance. Although some research has been carried out concerning the effect of high concentrations of certain disinfectants on soil, much information is still missing concerning other compounds. Composed commercially available disinfectants may not behave in the same way as pure substances either in slurry or in soil. The possible negative effects of long-time application of such combined chemicals must be investigated with more precise techniques than those applied until now. The synergistic effects, especially those between surfactants, soil and microorganisms with respect to their migration through the soil, must be studied.

Table 3.13: Amount of earthworms found after fertilisation of meadows with slurry and disinfected slurry (data from Schamper and Zbil 1996)

A: Biomass (g/m^2) endogeal or epigeal and vertical burrowers (aneciques) earthworms

Biomass g/m^2	Endogeal				Epigeal and aneciques			
	Spring		Autumn		Spring		Autumn	
Experimental conditions	g/m^2	s*	g/m^2	s*	g/m^2	s*	g/m^2	s*
Reference*	15	7	76	22	80	53	144	57
Slurry (S) 20 m^3/ha S	9	8	50	10	109	51	79	34
Formalin 16 kg/m^3 S	4	2	48	18	144	14	106	38
Formalin 50 kg/m^3 S	9	3	52	19	148	111	115	49
NaOH (50%) 12 kg/m^3 S	3	5	90	27	103	43	143	47
PES (15%) 40 kg/m^3 S	6	2	65	19	63	53	107	22
$Ca(OH)_2$ (40%) 40 kg/m^3 S	3	5	35	25	132	86	140	72
Surfactant 10 kg/m^3 S	1	1	22	17	74	14	126	54

B: Abundance (Individuals/m^2) endogeal or epigeal and vertical burrowers (aneciques) earthworms

Abundance Ind/m^2	Endogeal				Epigeal and aneciques			
	Spring		Autumn		Spring		Autumn	
Experimental conditions	Ind/m^2	s*	Ind/m^2	s*	Ind/m^2	s*	Ind/m^2	s*
Reference*	70	39	492	174	122	10	130	51
Slurry 20 m^3/ha	64	45	308	179	96	20	84	23
Formalin 16 kg/m^3 S	24	11	336	101	94	20	110	16
Formalin 50 kg/m^3 S	58	24	306	119	130	68	132	21
NaOH (50%) 12 kg/m^3 S	6	12	542	209	78	25	128	33
PES (15%) 40 kg/m^3 S	20	5	302	93	74	48	130	50
$Ca(OH)_2$ (40%) 40 kg/m^3 S	14	23	192	108	100	67	134	104
Surfactant 10 kg/m^3 S	4	5	176	113	86	29	96	36

s* = Standard deviation
Ind/m^2 = Individuals/m^2
* = Unfertilised meadow

Table 3.14: Nitrogen content and nitrogen losses in disinfected swine slurry (Markert 1990)

Experimental conditions	Temperature °C	Nitrogen kg/m^3	Percentage of control
Slurry	+ 20	6.08	100%
(control)	+ 4	6.09	100%
Slurry +	+ 20	6.24	103%
1% Formalin	+ 4	6.19	102%
Slurry +	+ 20	6.47	106%
1.5% Formalin	+ 4	6.43	106%
Slurry +	+ 20	6.59	108%
2% Formalin	+ 4	6.03	99%
Slurry +	+ 20	5.59	92%
0.5% NaOH	+ 4	5.78	95%
Slurry +	+ 20	4.73	78%
1% NaOH	+ 4	5.25	86%
Slurry +	+ 20	4.22	69%
1.5% NaOH	+ 4	5.67	93%
Slurry + 40% Lime wash			
40 kg/m^3	+ 20	5.7	94%
	+ 4	5.7	94%
60 kg/m^3	+ 20	5.26	87%
	+ 4	5.58	92%
100 kg/m^3	+ 20	3.65	60%
	+ 4	4.11	67%

No information is available as to how metabolites of complex disinfectants such as quaternary ammonia compounds or biguanides may behave in soil; this must also be investigated. Based on the EU "biocides directive", methods suitable for the ecotoxicological testing of disinfectants have to be developed, the tests applied to aquatic systems or terrestrial systems for insecticides or herbicides are not suitable for this special purpose. Although there has been no indication until now that disinfectants will migrate from the soil surface to the ground-water, this has to be verified with more sensitive methods than have been applied until now.

3.2.6 Recommended Action

The most urgent action is to initiate the above-mentioned research by appropriate funding. With respect to the "biocides directive" obligatory implementation of the test methods given there for disinfectants, for those compounds used in animal husbandry, must be avoided as they are insufficient.

Those disinfectants used in the case of notifiable diseases, especially zoonoses, should generally be removed from the scope of the EU biocides directive. They should follow those regulations which are relevant for medical products, otherwise two different tasks concerning public health will be in conflict.

3.2.7 Summary

Disinfectants in Agriculture

The environmental effects of several types of disinfectant which are used in animal husbandry have been considered. These chemicals are generally released into the environment via slurry; only a negligible amount of disinfectants used in animal husbandry will go into municipal wastewater. It has been stated that two different concentration levels in slurry have to be taken into account, a low level if disinfectants are used in animal houses and a high level if disinfectants are used for the inactivation of pathogens in the slurry itself, mainly in the case of notifiable diseases.

The slurry normally acts as a buffer system in which the disinfectants may be diluted, metabolised, neutralised, decomposed and absorbed. Thus in the low concentration range (dilution rates 1 : 600 to 1 : 1600 of the concentrations used) no severe environmental effects would be assumed. The high concentration range for some disinfectants has been investigated much more intensively. No negative effects, or only those negative effects which disappeared within a relatively short period of time, were found for the compounds investigated in laboratory and field tests (formalin, NaOH, lime wash and peracetic acid). It was also recognised that most methods for

ecotoxicological testing, described for aquatic and/or terrestrial systems, are not appropriate for such disinfectants. With respect to the "EU biocides directive" more sensitive and relevant test methods have to be developed and disinfectants used in human and/or veterinary medicine against pathogenic and zoonotic agents should generally be excluded from the scope of this directive. Research is required to understand the behaviour (e.g. metabolisation) of disinfectants in slurry and in the soil. Synergistic effects between disinfectants, surfactants, soil, microorganisms and viruses have to be studied more intensively with respect to the occurrence of resistance phenomena and leaching into ground-water.

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Further references must be taken from the full German version.

3.3 Substances to Improve Feed Utilisation

Ernst Pfeffer and Karen Güthler

3.3.1 Performance Promoters

3.3.1.1 Description

According to Rosin (1992) antibiotics are traditionally natural substances with an antibacterial action which are obtained by biosynthesis and chemotherapeutic agents are substances with an antimicrobial action which are manufactured by chemical synthesis. In Germany eight antibiotics and two substances of chemosynthetic origin were registered as performance promoters by 1998. The pharmacological properties of these substances are listed in Table 3.15.

3.3.1.2 Occurrence

3.3.1.2.1 Reasons for the Use of Performance Promoters

Administration of these substances produces general improvements in performance traits such as an increase in live weight gain and the feed conversion efficiency per kg of gain.

Apart from this the suppression of harmful intestinal bacteria leads to a reduction in the frequency of diarrhoea and thus to stabilisation of the animal's health (Richter and Ranft 1991).

3.3.1.2.2 Epidemiology

A number of studies have been carried out into the precise mechanisms of action and the results of these have been summarised in several reviews (among others Greife and Berschauer 1988; O'Connor 1980; Menke and Krampitz 1973).

According to these, the site of action of performance promoters is the gastrointestinal tract, where they selectively affect the compo-

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Table 3.15: Properties of performance enhancers registered at present (according to Richter et al. 1996)

Performance enhancer	Enteric absorption[1]	Spectrum of action	Inhibition	Use in human or veterinary medicine	Cross-resistance to therapeutically significant antibiotics
Oligosaccharides (avilamycin)		Gram+	Formation of the aminoacyl-t RNA on the 30S subunit of the ribosome	No use	Not known
Phosphoglycolipids (flavophospholipol)	–	Gram+	Cell wall synthesis inhibitor	No use	Not known
Ionophores (monensin, salinomycin)	–/+	Gram+	Cell wall synthesis inhibitors	No use	Not known
Macrolides (tylosin, spiramycin)	–/+	Gram+	Protein synthesis inhibitors	Use	Macrolides/lincosamides (human, veterinary medicine)
Peptolides (virginiamycin)	–	Gram+	Protein synthesis inhibitor	No use	Macrolides/lincosamides (human, veterinary medicine)
Polypeptides (zinc bacitracin)	–	Gram+	Cell wall synthesis inhibitor	Use of bacitracin	Not known
Quinoxaline (carbadox, olaquindox)	+	Gram+ and –	DNA synthesis inhibitors	No use	Not known

[1] – no absorption; –/+ slight absorption; + high absorption quota

sition of the microflora which colonise this in a manner which is beneficial to the host, with the following effects:

Reductions in the degradation of peptides and amino acids, ureolysis and microbial protein synthesis. According to O'Connor (1980) this leads to both protein-sparing in the feed and improved availability of essential amino acids. Accordingly reductions in the excretion of nitrogen in the faeces or increases in the apparent digestibility of the crude protein have been detected in fattening pigs (Roth et al. 1994). The ammonia production in the intestine is reduced directly by inhibition of ammonia producing bacterial strains and indirectly by the reduced dissimilation of nitrogen (Nagaraja 1996).

The weight of intestine is reduced and the proportion of lymphatic connective tissue in the intestinal wall is reduced, the length of microvilli and depth of crypts is increased (Nousiainen 1991). O'Connor (1980) points out that inhibition of toxigenic bacterial strains caused by the antibacterial action delays the cell-renewal rate of the mucosa and reduces the strength of the intestinal wall. This causes an increase in the absorptive capacity for glucose, fatty acids, vitamins and trace elements (Greife and Berschauer 1988).

In ruminants ionophore antibiotics cause a reduction in the accumulation of formaldehyde and elementary hydrogen and thus, since these are methane precursors, the elimination of methane (Nagaraja 1996).

Furthermore, a shift of the propionate/acetate ratio in the ruminal fluid takes place favoring the energetically more valuable propionate by inhibiting Gram-negative and lactate-producing bacteria.

As regards the efficacy of performance promoters in ruminants, there are differences between the ionophores monensin and salinomycin, and lasalocid which is not licensed in Germany on the one hand, and on the other hand flavophospholipol and avoparcin, the latter of which is no longer licensed (Meixner and Flachowsky 1990).

Effects may occur on the intermediate metabolism, if there is partial absorption after marginal limits of concentration are reached in the chyme of the small intestine. Thus Schole et al. (1985) have detected metabolic effects similar to those of anabolic hormones in carbadox, olaquindox, flavophospholipol and aureomycin, which is not licensed anywhere in the EU.

Disease prophylaxis and the therapeutic effects include prevention respectively a less severe course of the disease – especially

in diseases which cause diarrhoea in young animals (Richter and Ranft 1991; Windisch et al. 1994). Thus the mortality is reduced and the health of animals is strengthened. This in turn leads to better use of the genetically based performance potential.

3.3.1.3 Consequences

3.3.1.3.1 Consequences for the Feed

The homogeneity of feeds depends to a certain extent on adjusting the density and the particle size distribution between the feed components and the additives. A higher proportion of fine particles in the feed leads to an increase in the risk of contamination of subsequent batches of feed and a safety risk for the staff responsible for storage. In connection with this Heidenreich et al. (1992) ascertained, in a trial of zinc bacitracin, that generics exhibited a wider particle size distribution and higher dust emission levels than branded products.

As the result of this knowledge the particle density and particle size of mixed feed components have now been adjusted (Eckstein 1996).

3.3.1.3.2 Consequences for the Agricultural Farm Animal

The extent of an improvement in performance due to the addition of antibiotics depends on the following influencing factors (according to Greife and Berschauer 1988):

Substance:	Type, method of action, duration of administration, dose size
Animal:	Species age or body weight, breed or specialised breed, sex, type of production, performance level, condition of health
Feed:	Composition of diet, feeding intensity, provision of individual nutrients
Environment:	Climate, housing and hygiene conditions, stress factors.

According to Greife and Berschauer (1988) there is stimulation of the feed intake by 2 to 5%, and increase in gain by 2 to 10%, im-

provements in the digestibility of crude nutrients and utilisation of proteins by up to 6%, an increase in the metabolisable energy due to reduction in the fermentation losses by approximately 3% and approximately 10% reduction in losses during rearing.

In general the relative increases in performance are the less pronounced the better the genetically based performance potential can be utilised by optimal feeding and environmental conditions (Blaha 1996; Flachowsky et al. 1992).

According to Kamphues (1994) toxicological problems and damage to the livestock herds, due to performance promoters, have several causes. First of all there are the toxic or allergic properties of individual substances. According to Spierenburg et al. (1988 a) quinoxalines lead to a change in the adrenal cortex even at the permitted dosage. Furthermore, carbadox has a carcinogenic action. The toxic effect of ionophores is potentiated by simultaneous use of the therapeutic agent tiamulin.

Finally, improper use of performance promoters (deliberate or accidental overdosage, errors concerning the feed), carry over or false declarations may also cause damages (Kamphues 1994).

3.3.1.3.3 Consequences as Regards Exposure of Humans

Olaquindox can trigger photo-allergies and chronic photosensitive dermatitides (Schauder 1989).

The development of bacterial resistance to an antibiotic may in principle take place in two ways. Chromosomal resistance is caused by spontaneous mutation in the genome and is aimed at a certain active substance or similar active substances. It can only be transmitted by heritage. Extrachromosomal resistance, on the other hand, is achieved between all types of enterobacteria by the exchange of R-plasmids (circular, double-stranged DNA). In this process, up to ten different resistance properties can be transmitted at the same time, so that the recipient develops multiple resistance to various groups of antibiotics (Prescott and Baggot 1993). In particular the quinoxalines olaquindox and carbadox are suspected of producing such multiple resistance, since they exhibit a broad spectrum of action against both Gram-positive and Gram-negative bacteria (Linton 1985). Under field conditions it is true that as yet no multiresistance-producing action of quinoxaline has been ascertained, but in-vitro studies have shown that in some bacterial strains a multiple resistance to strepto-

mycin, spectinomycin and ampicillin was transmitted at the same time as the plasmid-bound resistance to carbadox. In no case was there sole transmission of carbadox resistance (Ohmae 1985).

In general any addition of antibiotic, even at what is called the "nutritive" dose, may induce resistance (Trolldenier 1995). The ability of resistant bacteria to permeate depends both on the bacterial strain and on the duration and size of the dosage of the antibiotic administered (Prescott and Baggot 1993). In connection with this, Spierenburg et al. (1988 b) ascertained that therapeutically effective concentrations may result in the intestinal content of pigs, if the feed contains quinoxaline at the permitted concentration.

Of the ten performance promoters licensed in Germany up to 1998, tylosin (a macrolide) has found therapeutic or prophylactic use in veterinary medicine, spiramycin (a macrolide) and zinc bacitracin (a polypeptide) have found use in veterinary and human medicine (see Table 3.15). According to Rosin (1992) macrolides and lincosamides are to a great extent mutually cross-resistant. Cross-resistance of tylosin has been proven with erythromycin (a macrolide) and lincomycin. The polypeptide virginiamycin can also trigger cross-resistance with the macrolide/lincosamide group (Linton 1981; Threlfall 1985). For similar reasons the glycopeptide avoparcin was banned as a performance promoter by emergency order in January 1996 after results of investigations at the Robert-Koch Institute, since to date it has not been possible to rule out that it might trigger cross-resistance to other glycopeptide antibiotics (vancomycin), which are needed in human medicine as a reserve against resistant enterococcus and staphylococcus strains (Richter et al. 1996; Rosin 1992).

3.3.1.3.4 Consequences for the Environment

No reliable data are available concerning the amounts of specifically performance promoters eliminated with faeces. For antibiotics used therapeutically, this proportion is not less than 70–90% of the administered dose (Kroker 1983). For quinoxalines a lower proportion may be appropriate, since they may be absorbed more completely than normal performance promoters. Furthermore, administered antibiotics are subject to biotransformation even in the intestinal tract, i.e. only part of the amount eliminated is still biologically active; for the substances described by Kroker (1983) the proportions are between 15 and 90%.

Some consequences of antibiotic residues are the accumulation of resistant pathogens in slurry and also an effect on the production of methane in biogas plants. Since antibiotics are generally substances which are degraded swiftly (Böhm 1996) and thus selection pressure is cancelled before resistance can spread, a health risk from resistant bacteria via this route cannot be ruled out. To date there are few definite research results which answer the other important ecological questions (e.g. a change in the soil microflora) (Böhm 1996).

The effects of flavophospholipol and zinc bacitracin have lead to disturbances of the aerobic and thermophilic treatment of slurry. Only for monensin at concentrations above 8 mg/l complete breakdown of methane production was detected in biogas plants which had not been adapted (Winterhalder 1985). Anaerobic-mesophilic treatment of slurry may also be affected, but not to the same extent (Al-Wakeel 1977).

3.3.1.4 Legal Position

Since 1987 the manufacturer has to submit proof of the safety and efficacy of the substances in the form of a dossier, in order to register performance promoters, enzymes, probiotics and preservatives as feed additives. For those substances already licensed before 1987, this proof must be furnished subsequently by the manufacturer before the year 2000. Since 1st October 1999 registration has been binding upon the manufacturer. For tylosin phosphate, spiramycin, virginiamycin and zinc bacitracin registration was temporarily withdrawn on 1st June 1999 because of the resistance problems. A list of registrations of the feed additives described in chapters 3.3 and 4 can be found in the German full version. This contains not only the chemical structural formulae of the registered performance promoters, but also the registered free amino acids.

Under Order No. 256/1999 the antibiotics listed in the Appendix to the Order must be licensed (Anonymous 1999).

3.3.1.5 Recommended Research

Data on the ecotoxicological effects of performance promoters on the environment, in particular in soil and plants, are also needed.

The direct entry of antibiotics into the aquatic system should be regarded as negative, since this should not occur at all with correct spreading of slurry. For this reason no need is seen for ecotoxicological experiments with aquatic organisms (Böhm 1996).

The effects of alternatives which will be used in order to dispense with performance promoters, such as an increased use of zinc, copper or acids, should be critically examined.

3.3.1.6 Recommended Action

In the context described in Section 3.3.1.4 there is no need for political or legal action.

3.3.1.7 Summary

By 1998 ten performance promoters were licensed in Germany for use in animal production as feed additives; eight of these are antibiotics and two are of chemosynthetic origin. They are mixed into industrially manufactured mixed feeds by premix companies or mixed feed manufacturers. Via various actions on digestive processes in the intestinal tract, which have not yet been fully explained, they improve the utilisation of nutrients and energy and reduce losses during rearing, which leads to a general improvement in the performance of the animals. However, under certain conditions, in particular improper use, toxicological damage cannot be ruled out.

Since administration of certain antibiotics is associated with the developing of plasmids with resistance genes, the question arises as to whether and to what extent this represents a risk for the efficacy of antibiotics required therapeutically. To date potential cross-resistance has been detected for tylosin and virginiamycin to antibiotics of the macrolide/lincosamide group. However, all antibiotics not yet included in directive 87/153/EEC for safety testing are subject to renewed evaluation by the year 2000; registration until the year 2003 will depend on the results of this evaluation. For this reason there is no need for political or legal action.

To date very little is known concerning the behaviour of antibiotic residues which pass into the environment via slurry. The process of methane production in biogas plants can be disturbed by an-

tibiotics in slurry. For the remaining relevant ecosystems (soil, atmosphere, vegetation) there are to date no concrete results. For this reason the main requirement for research is seen to be in this field.

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3.3.2 Enzymes

3.3.2.1 Description

Enzymes are proteins which catalyse chemical reactions in biological systems.

Enzymes are highly specific to reaction type and substrate, and accelerate reactions by at least one million fold (Stryer 1990).

3.3.2.1.1 Carbohydrases (Non-starch Polysaccharide-cleaving Enzymes)

Non-starch polysaccharides (NSP) are plant structural substances and occur as chief constituents of the cell walls of cereals and legumes. These multiple compounds of various sugars are characterised by their polysaccharide structure with chiefly β-glycoside bonds (cellulose, β-glucane, pentosane, pectin), and unlike starches cannot be hydrolysed by enzymes natural to the animal (Müller 1993). Enzymes which bring about the hydrolysis of these NSPs (cellulase, β-glucanases, xylanases, arabino-xylanases) are produced today by fungal and bacterial cultures (Broz 1993).

3.3.2.1.2 Phytase

The enzyme phytase catalyses the hydrolysis of phytic acid to phosphate and inositol. This is of importance, since most of the phosphorus in vegetable seeds is present in the form of phytin, and enzymes endogenous to the animal's body are not available to cleave this. It occurs in plant seeds chiefly in the aleurone layer and in parts of the pericarp. The proportion of phytin phosphorus to total phosphorus varies according to the type, species and external environmental factors such as climate, position and application of fertiliser, in cereals it is approximately two thirds. Wheat, triticales, rye and barley contain appreciable amounts of natural phytase (Houseman and De Bruyne 1989).

It is possible to produce microbial phytase using *Aspergillus niger* and genetic technology (Bolduan 1995).

3.3.2.2 Occurrence

3.3.2.2.1 Reasons for Use

NSPs cause the following nutritional and physiological phenomena:

- The NSP proportion of cereals is 9–20% (Müller 1993). Since NSPs are virtually indigestible, they reduce the energy density in feed.
- The NSP fractions which do not dissolve easily in water enclose nutrients which would otherwise be digestible ("cage effect").
- The soluble forms swell in the intestine thus causing higher viscosity of the gastro-intestinal contents. This leads to a longer retention time of the feed in the digestive tract, reduced absorption of nutrients and increased bacterial colonisation in the small intestine as well as moist and sticky consistency of excreta. This affects in the first instance the quality of litter in poultry farming and thus the chances for proliferation of disease pathogens.

The addition of carbohydrases achieves positive changes, in particular in the last two effects mentioned. On the other hand the splitting of NSPs into monosaccharides, and thus the increase in digestibility, is of almost no importance.

Phytase is added to feed, in order to make a larger proportion of the phytin phosphorus available to the animal. In this way the need to supplement phosphate in the feed is reduced. The efficiency of using phytin-phosphorus with the aid of phytase depends on the phytase activity in the feed. Depending on the feed, the digestibility of the phosphorus after addition of 500–1000 U/kg increases by up to 50 percentile points (GfE 1997).

3.3.2.2.2 Epidemiology

The ruminal flora exhibits such high enzyme activity that ruminants are able to break down both phytin and the NSPs including pectin.

There are differences between pigs and poultry as regards the worthiness of using enzymes: pigs have a flora in the large intestine with a similar function to that in the forestomach of ruminants which ferments NSP to short-chain fatty acids that can be absorbed. The added carbohydrases would possibly hydrolyse the NSPs in the small intestine to monosaccharides which are absorbed there, which would bring about a slightly better energy supply for the pigs.

The antinutritive effects of NSPs play a small part in pigs, since they make better use of NSPs than poultry, due to the longer gastrointestinal tract and the presence of a colon. On the contrary, undigested carbohydrates are an essential dietary factor for pigs, as they exercise a positive influence on the large intestine and counteract diseases which cause diarrhoea. Lactoflora in the crop, and thus the ability to split β-glucanes, develops in poultry with increasing age, while this is present in full even in piglets (Bolduan 1994; Bolduan 1995).

Various cations are bound in phytin, and these are released by degradation via phytase and are available to the animal as trace elements (Kirchgeßner et al. 1994; Windisch et al. 1994).

Without the addition of phytase, monogastric animals must be given technical feed phosphates (monosodium phosphate and/or dicalcium phosphate) to cover their requirement for phosphorus. These substances are basic and thus in piglets bind the gastric acids, which may favour harmful coli bacteria (Bolduan 1995).

3.3.2.3 Consequences

3.3.2.3.1 Consequences for the Feed

Enzyme stabilisation may be achieved either in liquid form or by the addition of salts and sugars or by drying with binding to a substrate carrier. Dry enzymes retain their activity during storage for 50 weeks, thus clearly longer than liquid enzymes. On the other hand, normal temperatures during feed expansion destroy part of the activity of dry enzymes. Conditioning temperatures of 85 °C over 15 minutes are survived without considerable loss of activity. Another possibility is to spray liquid enzymes onto the feed after pelleting, although care must be taken to achieve high precision of distribution (Nissinen et al. 1993).

3.3.2.3.2 Consequences for the Agricultural Farm Animal

Enzymes act solely in the digestive tract, i.e. they do not pass through the intestinal wall and do not affect the intermediate metabolism (Flachowsky 1996).

Rimbach et al. (1996) found about one and a half times the cadmium accumulation in the liver and kidneys of pigs compared to a control group, when phytase was added. On the other hand, in chickens the use of phytase reduced the accumulation of cadmium in the liver and kidneys by up to 60% (Rambeck and Guillot 1996).

3.3.2.3.3 Consequences as Regards Exposure of Humans

A risk to the staff of manufacturing companies due to contamination with aerosols cannot be ruled out (Concoby 1995).

3.3.2.3.4 Consequences for the Environment

Added enzymes do not form residues, since they are proteins which are digested by the proteases in the animal to amino acids, or broken down microbially. The possibility of NSP utilisation by the addition of carbohydrases reduces or removes the restrictions existing to date on the use of various plant products in the diet, which may lead to more variable crop rotations. The addition of phytase alone does not affect the environment, but it does produce conditions for a reduction of phosphate additions, or even dispensing with these,

which can lead to considerable reductions in the level of phosphorus excretion from animals. A special low-phosphorus diet would reduce the phosphorus excretion of pigs by up to 35%, of poultry by up to 50% without loss of performance (Simons et al. 1990). Flachowsky and Schulz (1997) quoted a phosphorus reduction in faeces of 15–25% when adding phytase and minimisation of the nitrogen and phosphorus proportion by 0–4% when adding carbohydrases. These facts can mean a marked reduction in the burden on the soil, in particular in regions with a high cattle population.

3.3.2.4 Legal Position

Enzymes and probiotics have been included in the normal registration process for feed additives since 1993. Since 1st July 1999 the registration of enzymes and microorganisms has been harmonised throughout the EU. National registration of enzymes and microorganisms by special licence in the individual member states remains. An EU-wide registration already exists for phytase. Under Order No. 2690/1999 the enzyme preparations listed in the Appendix to the Order must be licensed (Anonymous 1999).

3.3.2.5 Recommended Research

Research is needed into the analysis of enzymes in feeds:

There are no uniform analytical methods for substances of different origin. Some processes can be harmonised, but for precise analysis knowledge is needed concerning the origin of each enzyme, since the various manufacturers modify the chemical properties of enzymes (including temperature stability, pH profile) depending on their purposes. In addition, the analysis results may be affected by the composition of the feed (Grassmann 1996).

3.3.2.6 Recommended Action

There is no need for political or legal action.

3.3.2.7 Summary

The use of enzymes in animal nutrition to improve the chemical degradation of otherwise indigestible nutrients is being discussed at present. Their use is only sensible if the enzyme is not available to the animal naturally, in this case these are carbohydrases and phytase for monogastric animals.

Carbohydrases break down the NSPs (non-starch polysaccharides) contained in the plant cell wall, which achieves on one hand release of the enclosed nutrients and on the other hand an improvement in the consistency of the gastro-intestinal contents.

Phytase permits utilisation of the phosphorus compound phytin contained in the plant seeds. With inclusion of this source of phosphorus in the animal's diet, the addition of technical feed phosphates can be reduced or even discontinued.

No risk to the environment is seen, since the enzymes are broken down in the intestines to amino acids and utilised by the animal.

For the analysis of enzymes in feedstuffs, a uniform method still needs to be developed for the preparations of the various manufacturers.

There is no need for action.

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4 Other Organic and Inorganic Substances

Ernst Pfeffer and Karen Güthler

4.1 Free Amino Acids in Feedstuffs

4.1.1 Description

According to Stryer (1990) amino acids are the elementary structural units of proteins. In the case of α-amino acids these are a carboxylic group, an amino group, a hydrogen atom and a side chain bound to a common carbon atom (the α-carbon atom). The tetrahedral arrangement of the four bond arms of the α-carbon atom permits two axially symmetrical forms, the L-isomer and the D-isomer. Almost all amino acids present in nature belong to the L-form, while synthetic production leads to racemic mixtures of equal parts of the L- and D-forms. For the protein synthesis of all agricultural farm animals only L-amino acids are possible, although D-isomers and hydroxy analogues in the animal's body can also be utilised via conversion to a symmetrical oxo-form and subsequent transamination. The efficiency of utilisation depends on the amino acids in question and also on the animal species. For D-methionine this is 90% in poultry and 100% in pigs, for D-tryptophane 20 and 80% respectively. D-lysine and D-threonine are not utilisable in this way (Bergovici and Fuller 1996).

The essential amino acid with the lowest proportion of feed protein in relation to the requirements of the animal determines the limit of protein utilisation. In most cases lysine determines this limit in pig farming and methionine in poultry farming (Limper 1998).

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4.1.2 Occurrence

4.1.2.1 Reasons for Use

With the addition of free amino acids the protein quality of the feed can be improved by balancing the individual amino acids, so that as the performance of the animals remains constant the protein content in the feed can be reduced.

4.1.2.2 Epidemiology

Non-ruminants have no mechanisms for synthesising essential amino acids (lysine, threonine, methionine, tryptophane, leucine, isoleucine, valine and phenylalanine, in birds also arginine) in the body. In young animals the synthesis of histidine, proline, glycine and glutamine is not nearly sufficient to cover requirements. They must, therefore, take up the missing amounts of the relevant amino acids with their feed. The quantitative amino acid composition of many plant feedstuffs does not correspond to the optimum for farm animals. In the normal cereal feedstuffs there are deficiencies in lysine, threonine, tryptophane and methionine, while other amino acids far exceed the required amount. Since different amino acids compete for the same enzyme and/or transport mechanisms, excessive presence of one amino acid affects the utilisation of the others; reduced feed uptake may result. Of particular importance here are the interactions between lysine and arginine in poultry (D'Mello 1994).

Ruminants are provided with all essential amino acids by the microorganisms of the rumen. "Rumen stable" amino acids available for sale for milking cows do not, according to evaluations by Jochmann et al. (1996), contribute to a significantly higher performance. The greatest efficacy seemed to be based on insufficient energy and crude protein supply, in particular in the first third of the lactation period.

4.1.3 Consequences

4.1.3.1 Consequences for the Feed

The composition of the feed may be designed with more bias upon economic and organisational criteria.

Free amino acids can partially replace feeds rich in limiting amino acids (especially soya), so that farm-produced feeds (in particular cereals) increase in importance.

4.1.3.2 Consequences for the Agricultural Farm Animal

If there is reduced crude protein content in the feed due to the addition of free amino acids and also phased feeding, the animal uses less energy for the conversion of excess crude protein into urea, which is then available for other metabolic processes (Limper 1998).

4.1.3.3 Consequences as Regards Exposure of Humans

Consequences for humans are not to be expected.

4.1.3.4 Consequences for the Environment

A drop in the protein content in the feed may result in a reduction in the elimination of nitrogen by 20–30% (Flachowsky and Schulz 1997). For this reason supplementation of free amino acids seems sensible in regions with high densities of livestock.

4.1.5 Recommended Research

There is no need for research concerning quantification of the physiological nutritional context.

4.1.6 Recommended Action

No action is needed.

4.1.7 Summary

Among those substances which non-ruminants need for protein synthesis are nine amino acids which they cannot synthesise themselves; these are called essential amino acids. The better the amino acids contained in the feed protein correspond to the requirements of the animal, the higher the protein uptake or the lower the elimination of nitrogen. In cereal diets in the first instance the lysine proportion limits performance, but threonine and tryptophane may also be limiting factors if the lysine content is compensated. In order to achieve optimum protein deposition the protein content of the feed must be elevated to such an extent that the requirement for the primary limiting amino acids is covered. This leads to an excess in the total protein in the feed, which is reflected in excretions.

If there is targeted supplementation of industrially produced free amino acids, the protein quality can be increased and thus the protein content in the feed can be reduced, whereby the elimination of nitrogen can be reduced by 20–30 percentile points (Flachowsky 1996). Negative effects on animal health are not to be expected.

Under German law amino acids are regarded as single feedstuffs, and can thus be added to the feed in the desired amounts, which, above a certain level, however, does not give farmers any economic advantage. There is no need for research except for quantification of the physiological nutritional context. No need for action is seen.

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4.2 Probiotics as Feed Additives in Agricultural Farm Animals

4.2.1 Description

Probiotics in the broad sense are microorganism preparations, which are used in the animal's diet in place of antibiotics as a feed additive to increase the performance and improve the health of the animals.

4.2.2 Occurrence

4.2.2.1 Reasons for Use

The normal gastro-intestinal tract of domestic animals 5–6 days after birth contains 400 to 500 different microorganism species with a total count of 10^{14} bacteria. This microflora may be subdivided in various groups: 1) the dominant main group, 2) the accompanying subdominant flora and 3) the residual flora.

1. The dominant main group (more than 90% strictly anaerobic) is composed chiefly of bifidus bacteria, Gram-positive lactobacilli and Gram-negative bacteroidaceae.
2. The accompanying subdominant microflora (less than 1%, facultative anaerobic) consists of Gram-negative *E. coli* and Gram-positive enterococci.
3. The residual flora (less than 0.001%) are chiefly *Clostridia*, staphylococci, *Pseudomonas*, *Proteus*, apathogenic and facultative pathogenic bacteria and *Candida* species. There is a balance between the different bacterial species, which varies, however, between the duodenum, the ileum, the colon and the caecum (Vanbelle et al. 1990).

The stable main flora developing in the intestine represents protection against infection by enteropathogenic bacteria from the accom-

panying or residual flora. The protective action of the stable main flora can be disturbed in particular by stress, antibiotic therapy or excessive hygiene. In such a case the administration of probiotics to animals restores the protective action of the eubiotic main flora (Fuller 1989).

4.2.2.2 Epidemiology

The action of probiotic additives with live bacteria at levels of 10^9 to 10^{10} colony-forming units per gram, is produced in the gastro-intestinal tract, which itself is already colonised by approximately 10^{14} microorganisms. Vanbelle et al. (1990) attempted to summarise the possible mechanisms of action as regards an improvement in animal performance and health aspects, as follows:

- Probiotics inhibit the growth of pathogenic bacteria by production of organic acids (lactic acid or short-chain fatty acids), antibiotic substances, bactericidal H_2O_2 and reduction of the pH level.
- They prevent the attachment of pathogenic bacteria. The adhesion, penetration and multiplication of pathogenic bacteria in the enterocytes is prevented by means of a biofilm of many-layered adhesive lactobacilli on the enterocytes of the intestinal epithelium (Gedek 1986).
- They produce metabolites, which can neutralise bacterial toxins "in situ" or inhibit their production.
- The enzymes contained in the probiotic increase the enzymatic digestibility of the feed or detoxify dangerous toxins of the intestinal flora.
- They stimulate the development of the immune system and vitamin production, and increase the activities of lactase, sucrose and maltase in the cells of the ciliated border.
- They multiply in the digestive tract and compete with pathogenic bacteria.
- They reduce microbial degradation, lead to a better balance between lactobacilli and coli bacteria with the result that they spare nutrients (amino acids, carbohydrates), reduce the absorption of toxic compounds (including ammonia, amines, indole, skatole, mercaptane and sulphides) and better protect the

bile acids and fatty acids from conversion into poisonous or harmful substances.

4.2.3 Consequences

4.2.3.1 Consequences for the Feed

No consequences for feeds are to be expected.

4.2.3.2 Consequences for the Agricultural Farm Animal

To clarify the question of the extent of performance improvement due to probiotics the *Nutrition Abstracts* of the Commonwealth Agricultural Bureaux from 1985 to 1995 were examined. In 21 experiments on various animal species, bacterial probiotics only four times exhibited significantly higher gains (at least $P \leq 0.05$) and seven times a certain improvement in feed consumption compared to the non-supplemented control group. Additions of yeast probiotics increased the FCM amount significantly in four cases in sixteen lactation experiments, increased the milk fat percent in one case, increased the daily fat content in two cases and increased the milk protein percentage and the daily milk protein content in just one case. In ten experiments with growing ruminants, yeast probiotic additives improved the gain or feed utilisation significantly in one case. In each of three experiments on pigs and poultry, yeast-based probiotics improved the gain of broilers in only one case, feed utilisation was not improved.

4.2.3.3 Consequences with Regard to Exposure of Humans

No consequences for humans are to be expected.

4.2.3.4 Consequences for the Environment

Effects on the environment are not to be expected.

4.2.5 Recommended Research

The effects of probiotics on the composition and metabolism of the intestinal flora are not yet sufficiently known. What is more, interactions between the intestinal flora and metabolism in the mucosa require further research.

4.2.6 Recommended Action

There is no need for political or legal action.

4.2.7 Summary

Probiotics are preparations of one or more strains of living microorganisms, which are thought to cause an increase in performance by stabilising the health of agricultural farm animals when used as feed additives. According to the scientific concept the efficacy is based on an effect on the microbial balance in the intestinal tract. By adding probiotics the power of the beneficial intestinal flora to compete against potential enteropathogens is thought to be strengthened, giving support to the positive metabolic processes. In practical use, however, the scientific theories of the efficacy of probiotics could only partly be confirmed.

No effects on humans or on the environment are expected.

The effects of probiotics on the composition and metabolism of the intestinal flora are not yet sufficiently known. What is more, interactions between the intestinal flora and metabolism in the mucosa require further research. There is no need for political or legal action.

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4.3 Preservatives in Feedstuffs

4.3.1 Description

Preservatives against microbial spoilage in feeds, consist of organic and three inorganic acids (ortho-phosphoric acid, hydrochloric acid and sulphuric acid) also some of their salts. The acid concentration in the feed is 0.5–4.0% depending on the strength of the acid.[1]

The site of action of organic acids are the feed, the digestive tract of the animal and the intermediate metabolism.

4.3.2 Occurrences

4.3.2.1 Reasons for Use

Feed preservatives have a bacteriostatic action and in this way prevent microbial degeneration of the feed.

Some of these substances may cause an increase in performance in agricultural farm animals, and are therefore also used for nutritive purposes (in particular methanoic acid (formic acid), propanoic acid (propionic acid), fumaric acid and citric acid as well as the calcium and sodium salts of methanoic acid (Ca-, Na-methanoate) and the calcium salt of propionic acid (Ca-propionate)).

4.3.2.2 Epidemiology

The efficacy as regards preservative performance depends on the strength of the acid (pK_s level), the corresponding base (acid anion) and the concentration in the feed.

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[1] For further information see the German publication "Potenzielle Schadorganismen und Stoffe in Futtermitteln sowie in tierischen Fäkalien", Wiley-VCH Verlag, Weinheim, 2000.

Preservatives reduce the buffer capacity of the feedstuff and thus the pH level in the stomach. The corresponding base then serves as a complexing agent for basic-acting cations and in this way cancels the buffer substances in the feedstuff (Meixner and Flachowsky 1990).

The acid environment in the stomach determines the conversion of pepsinogen into pepsin, which occurs from pH 3 (Eckel 1997). Pepsin itself has two activity maxima at pH 3.5 and pH 2, and a chiefly sufficient proteolysis takes place from pH 4 (Taylor 1959). Piglets do not achieve a normal amount of hydrochloric acid production in the stomach until 7–10 weeks. Up to this time the pH level in the stomach may rise sharply after feeding (up to pH 5–6). In this case organic acids may lead to a drop in the pH of the stomach and thus contribute to better protein utilisation. On the other hand, a high acidity of the stomach content decimates bacterial reproductions and colonisation in the duodenum (Kirchgeßner and Roth 1991).

4.3.3 Consequences

4.3.3.1 Consequences for the Feed

The corresponding base in each case serves as a complexing agent for base-acting cations and in this way cancels the buffer substances in the feed (Meixner and Flachowsky 1990).

Preservatives provide an improvement in the storability of the feed and reduce the bacterial count, due to their antimicrobial properties. This leads to fewer problems in the animals from disease pathogens (Singh-Verma 1973; Meixner and Flachowsky 1990; Kirchgeßner and Roth 1991). The aggregate state plays a part in the technical processing, since crystalline forms, unlike fluids, do not have a corrosive action (Kirchgeßner and Roth 1991).

4.3.3.2 Consequences for the Agricultural Farm Animal

Due to the reduction in the bacterial count in the feed the animal has fewer problems from disease pathogens (Meixner and Flachowsky 1990; Kirchgeßner and Roth 1991).

The metabolic actions of organic acids include effects caused by changes in the microflora. These include a reduction in the microbial degradation of nutrients and reduced intestinal formation of ammonia, amines and toxins (Roth and Kirchgeßner 1989).

According to evaluations by Meixner and Flachowsky (1990) organic acids in the feed increase feed intake by 0.8% and daily gain by 5.7% and reduce the feed conversion efficiency per unit of gain by 4.9%. In combination with performance promoters they usually have an additive effect. No increases in performance occurred in poultry, with the exception of fumaric acid in fattening broilers. Here improvements in the live weight gains and feed efficiency were detected by 2% points in each case.

4.3.3.3 Consequences with Regard to Exposure of Humans

In direct contact with the skin and airways, preservatives in concentrated form represent a potential danger to humans. If applied properly there is no danger to humans from preserved feed.

4.3.3.4 Consequences for the Environment

Effects on the environment are not to be expected, since all registered preservatives for feedstuffs occur in nature and are therefore well broken down.

4.3.5 Recommended Research

In view of the even distribution of the preservative in the feed to be preserved, research work is necessary.

4.3.6 Recommended Action

No need for action is seen.

4.3.7 Summary

Feedstuff preservatives against microbial spoilage consist of low-molecular organic or inorganic acids. Once added to the feedstuff their bacteriostatic properties reduce the bacterial count. Furthermore, they cause a drop in the buffer capacity of the feeds, which may have nutritional physiological advantages for the animal. The addition of some acids may, due to changes in the microflora in the intestinal tract and enteral absorption, lead to metabolic effects on the animal. This is sometimes observed in improvements in the daily gain and in feed consumption.

Properly preserved feedstuffs do not represent a health risk to humans. No effects on the environment are to be expected.

In view of the even distribution of the preservatives in the feed being preserved, further research is necessary. No need for action is seen.

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5 Concluding Remarks and Outlook

Jürgen Unshelm and Sibylle Rehmann

This status report demonstrates the extent of problems due to potentially harmful organisms and damaging substances in feedstuffs and/or animal faeces. This problem has been caused by the now customary housing and management conditions of farm animals, and the increase in the local density of animal production which has taken place in this field, due to the need to increase performance and other developments, and has become apparent not least because of the considerable improvement in detection methods.

This concluding summary will once more present the most important points, to emphasise the catalogue of recommended research drawn up by the ad hoc group, and furthermore to state which measures are urgently required in the interests of both agriculture and the consumer, and in some cases also of environmental protection. Unlike the previous chapters, in which both the research and the action recommended were dealt with in detail, the following pages will mainly discuss the predominant themes.

One area which was mentioned directly or indirectly in almost all chapters of the report, is the field of epidemiology, which in Germany receives too little attention and support. In this field there are still gaps in our knowledge, which include the following in particular:

- Risks to the health of humans and animals in relation to pathogens,
- the known, and in particular the as yet unknown possible routes of infection,
- the extent to which residues are capable of causing illnesses and hormonal or metabolic imbalances,
- the geographical and occupational circumstances associated with this,
- the detectable negative consequences of the condition of drinking- and bathing-water,
- the epidemiological consequences of various housing and management systems, including those ecologically oriented processes established recently,

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- the consequences of feedstuff imports and their effect on the fauna of feedstock pests,
- the effect of drug residues, insecticides and other substances in the environment.

It should therefore be urgently recommended that lasting support be given to the research projects planned in this field. Political measures are also essential, however, since the importance of epidemiology is underestimated in Germany and the constant reductions in staff and resources, especially in the faculties of agricultural and veterinary science, have left too little scope to devote to the intensive additional research needed for these tasks. In the field of molecular epidemiology in particular, the use of modern DNA fingerprinting techniques makes it possible to trace actual infection chains. This would have to be promoted alongside traditional epidemiology, since the use of such techniques can explain the transmission routes of disease pathogens via the environment.

It is noticeable, however, that scarcely any relevant documentation is available even in the field of human medicine. The reasons for this, apart from misunderstanding of the importance of epidemiology, and the resulting overemphasis on individual cases, include the data protection laws as well as financial aspects.

A second larger subject field which should be discussed here, and which is linked to epidemiology in many respects, is the definition of hygiene standards, not only in feedstuffs and in faecal matter such as slurry and solid dung, but also in all other areas of animal husbandry. Quantitative and qualitative criteria must be worked out for these, so that in each case the infection risk can be evaluated, taking into account the wide range of factors which have a positive or negative influence. Epidemiological aspects play a part here, in that determining quantitative assessment criteria for hygiene standards should be based less on the detection methods which are constantly being improved, than on the epidemiological knowledge regarding the point at which a risk to humans and animals occurs due to unfavourable circumstances, or populations of wild animals act as vectors due to latent infections.

Another aspect, which plays a large part in the definition of hygiene standards, including the corresponding quantitative assessment criteria, is the obvious necessity for targeted research into the toxic, pharmacological, and possibly also the antibiotic effects of the

metabolic products of bacteria and fungi, especially in feedstuffs, upon agricultural farm animals and humans, both in the fields of nutrition and of working occupational medicine. Research is also needed into the metabolisation and persistence of cleansing agents and disinfectants. Equally important is an improvement in the data for estimating the environmental risks of the substance groups named, as well as for older drugs which predate toxicological studies. This leads to a number of questions as to methods, which have been discussed many times in the previous chapters, and will here only be summarised briefly.

Included in particular are the development, improvement and standardisation of detection procedures for many pathogens such as viruses, bacteria, fungi and parasites, and also of storage pests, the vector function of which is frequently underestimated; particular attention should be given to zoonotic pathogens. A relatively recent example, which explains the importance of the vector function of storage pests, is the discussion concerning transmission of the scrapie pathogen by mites in hay. The effects which can be described more as indirect, such as inhalable feed dust containing feedstuff pests and fungi, are without doubt underestimated. To be investigated here also are the health risks for agricultural farm animals and for humans who have the tasks of storage, preparing mixed feeds and cleansing the accommodation and equipment. Here too, detection procedures play an important part, especially in connection with epidemiological investigations to determine quantitative and qualitative evaluation criteria. Much pioneer work still has to be done on basic research into the composition and differentiation of fungal flora, also as regards the expansion of mycotoxin research into fungal species in various feedstuffs, which to date has attracted little attention and investigation, especially in the case of basic feeds which have until now been disregarded. A suitable test system must also be developed for the terrestrial and plant spheres, to determine the ecotoxicological effects of antimicrobial active additives.

Another much discussed subject area is that of reasonably priced and environmentally safe cleansing and disinfection procedures. With regard to the disinfection of the slurry itself, the general question naturally arises as to how chemical disinfection methods, which might disturb the ecosystem, can be replaced by physical processes which would be free from residues. The alternatives known to date are generally associated with high expenditure and

energy use but new technologies or processes, which are at present being tested in the field of pet animals, might be of ground-breaking importance. It is true that the cleansing and disinfection of surfaces in the stalls does not cause any ecotoxicological problems according to present scientific knowledge. However, the question of whether suitable building materials and the use of modern cleansing techniques could not minimise the use of biocidal chemicals, should be investigated. Neither is anything known as yet about interaction of the cleansing and disinfection agents used, with other constituents of the slurry, and with microorganisms.

Preventative health measures for agricultural farm animals were also critically examined by the ad hoc group. On the one hand the use of probiotics, or substances said to improve the utilisation of the feed and the performance of the animals, led to many critical questions, which to a great extent remain unanswered. Thus the development of resistance due to antibacterially active substances cannot be ruled out, ecotoxicological effects on soil and plants have not been explained, and furthermore some active substances may disturb biogas systems. On the other hand there is a need for further research into the safe use of vaccines, as recent examples have shown. The ideal vaccine should not only protect from a disease, but also prevent transmission, and thus the further spread of the pathogen. In addition the vaccine strain should not spread uncontrolled in the environment. Modern methods of genetic technology, such as the development of carrier vaccines, and/or the inclusion of constructs which inactivate the vaccine strain in the environment, may prevent this. The development of such vaccines is not being sufficiently promoted in Germany.

Another matter for discussion are procedures which are best included under the term treatment of animals and substances. Firstly we must clarify whether it would not be possible to tackle in the animal, in a more targeted and systematic fashion than so far, those disease pathogens of bacterial, viral, or parasitic origin which are transmissible to humans, although in some cases there are still gaps in the research of possible transmission routes. Another example is treatment by means of the detoxification of feedstuffs which contain mycotoxins, especially as our knowledge of all the possible consequences, in particular also of the toxic effects on agricultural farm animals, is still inadequate, despite intensive research. There is also a very great general need for research in the field of older

drugs, which predate toxicological studies; there is not sufficient data available about the ecotoxicology of these. First of all, the active substance groups which are relevant to the environment must be identified, and the situation with regard to residues of these in animal faeces must be ascertained in order to permit estimation of the exposure and develop the relevant detection methods. There is also a lack of knowledge concerning the additive and synergetic effects of drug residues in soil and water, the effect on the decomposition processes in animal faeces and about the spread of resistance genes in the environment via animal excretions. In this sector there is also a clear need for legislative action.

The examples mentioned so far dealt predominantly, but in no way exclusively, with research recommendations, and in many cases the related recommendations for action have been briefly discussed. If the numerous gaps in our knowledge already mentioned are to be closed, this will inevitably lead to additional recommendations for action. Thus for example threshold limits or recommended values supported by epidemiological research are of little benefit, in particular in preventing zoonotic diseases, if they are not accompanied by improved hygiene measures in all areas of animal husbandry. With regard to the protection of feedstocks, there are no agents or procedures for effective pesticide control in some important indication fields, which so far as possible do not form residues and are not toxic. Possibly fumigation with inert gases would be applicable here, a further recommendation for research and action. Another typical example of a need for action is to prevent unsuitable ecotoxicological test procedures for cleansing and disinfection agents from becoming established, as has already been pointed out. It will also be of importance to obtain uniform and effective regulations for the transport and spreading of slurry in the European Union, which are subject to fines or punishment and are monitored regularly. In particular we must avoid coming to agreement on an inadequate and small common denominator.

During the discussion within the group it was frequently pointed out that the problem of harmful substances in feedstuffs and animal faeces naturally also requires a corresponding level of education in those involved and those responsible; among the many negative examples is the failure to recognise the presence of storage pests such as mites, insufficient knowledge about effective, and the same time environmentally friendly cleansing and disinfection pro-

cedures, resulting in unnecessary overdosage, too little understanding of the transmission routes of disease pathogens, which are sometimes complicated, of the injurious effects of mycotoxins from feedstuffs on humans and animals, and also much more.

The group also discussed on several occasions the proposal that the promoting institutions, and in particular their experts, should promote the taking of higher risks in scientific institutions. This is urgently necessary to facilitate innovative scientific approaches, and in many cases even to make this possible. There is a danger of keeping to preferred tried and tested, and sometimes well-trodden paths, with results that may be relatively interesting, but are not ground-breaking.

Very much the same also applies to the need for political action. Thus almost insoluble problems arise if a new, important research facility is instigated, which may at first sight not be particularly spectacular or of interest to day-to-day politics. For most scientific institutions in Germany, especially in the university sector, this will not be possible for the reasons mentioned.

Certainly the research results already achieved will lead to a greater need for action in the legal, economic and political fields, in order to convert the knowledge newly obtained to sensible uses. At present, however, our first task is to initiate and promote these research directions.

The ad hoc group "For the Evaluation of Potentially Harmful Organisms and Substances in Feedstuffs and Animal Faeces" has drawn up this status report in many years of working in an honorary capacity. We must hope that the suggestions and proposals of the group, as regards the recommendations for research and also political action, will fall upon fertile ground.

Appendix

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