

Spirulina Platensis in Poultry Nutrition

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By

Hosna Hajati and Mojtaba Zaghari

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I dedicate this book to my wife Zari, my daughters Bahareh and Mina and my son Amin, the motives of my life.

—M. Zaghari

I dedicate this book to my two lovely angels, mom and dad, thank you for all your supports, you are my life style modeling.

—H. Hajati

CONTENTS

List of Figures.....	x
List of Tables.....	xii
Preface.....	xv
Key of Abbreviations	xvii
Chapter One.....	1
Organic Feed	
1.1 Introduction	
1.2 Herbal additives in poultry diet	
1.3 Algae	
1.4 Different variety of Algae	
Chapter Two	20
<i>Spirulina</i> Rearing Condition	
2.1 Introduction	
2.2 Morphology and taxonomy	
2.3 <i>Spirulina</i> species	
2.4 Effective factors on the growth of <i>Spirulina</i>	
2.5 Small scale production	
2.6 Commercial cultivation	
2.7 Universal <i>Spirulina</i> production	
2.8 <i>Spirulina</i> harvesting and processing	
Chapter Three	40
<i>Spirulina</i> Nutritional Importance	
3.1 Introduction	
3.2 Chemical analysis	
3.3 Toxicology	
3.4 <i>Spirulina</i> product quality and consistency	

Chapter Four	63
Antimicrobial Characteristics of <i>Spirulina</i>	
4.1 Introduction	
4.2 Antimicrobial activity	
4.3 Antibacterial activity	
4.4 Antifungal activity	
4.5 Antiviral activity	
Chapter Five	88
Antioxidant Characteristics of <i>Spirulina</i>	
5.1 Introduction	
5.2 Antioxidants	
5.3 Antioxidant activity of <i>Spirulina</i>	
Chapter Six	108
<i>Spirulina</i> in Broiler Nutrition	
6.1 Introduction	
6.2 <i>Spirulina</i> nutritional value	
6.3 Growth performance	
6.4 Carcass characteristics	
6.5 Gut morphology	
6.6 Ileal microflora	
6.7 Hematological parameters	
6.8 Immunity	
6.9 Antioxidant status	
Chapter Seven.....	130
<i>Spirulina</i> in Layer Nutrition	
7.1 Introduction	
7.2 Egg production	
7.3 Egg quality	
Chapter Eight.....	145
<i>Spirulina</i> in Breeder Nutrition	
8.1 Introduction	
8.2 Chick quality	
8.3 Breeder reproduction	
8.4 Female breeder reproduction	
8.5 Male breeder reproduction	

Chapter Nine.....	164
<i>Spirulina</i> in Quail Nutrition	
9.1 Introduction	
9.2 Growth performance	
9.3 Meat quality	
9.4 Immunity	
9.5 Egg production	
9.6 Egg quality	
9.7 Fertility, hatchability and chick quality	
Chapter Ten	185
<i>Spirulina</i> in Waterfowl and Pet Bird Nutrition	
10.1 Introduction	
10.2 <i>Spirulina</i> in waterfowl habitat	
10.3 Algae in domestic waterfowl	
10.4 Pet birds	
Glossary.....	197
Index	202

LIST OF FIGURES

Figure 2-1: Microscopic view of microalgae <i>Spirulina</i> (photograph by E. Koru 2014)	21
Figure 2-2: Life cycle of <i>Spirulina spp.</i> (Ciferri 1983)	21
Figure 2-3: SEM image of a trichome of axenic <i>S. platensis</i> . (Ciferri 1983, photograph by R. Locci)	22
Figure 2-4: SEM image of non-axenic trichomes of <i>S. maxima</i> . (Ciferri 1983, photograph by R. Locci)	23
Figure 2-5: Growth rates of <i>S. platensis</i> and <i>S. fusiformis</i> at different temperature. The values are means of three replicates (Rafiqul Islam et al. 2003)	25
Figure 2-6: The relation between light irradiance and photosynthetic rate (Carvalho et al. 2011).....	27
Figure 2-7: <i>Spirulina</i> production in small scale (Photograph by authors)	29
Figure 2-8: <i>Spirulina</i> production in India (Photograph by Borowitzka).....	31
Figure 2-9: <i>Spirulina</i> production in California (Photograph by Borowitzka).....	31
Figure 2-10: <i>Spirulina</i> production in Czech Republic (Photograph by Borowitzka)	32
Figure 2-11: <i>Spirulina</i> bag culture in Israel (Photograph by Borowitzka)	32
Figure 2-12: Using filter 0.45 µ for evaluating <i>Spirulina</i> biomass (Photograph by authors)	33
Figure 2-13: <i>Spirulina</i> production flow chart (Cysewski 2010).....	34
Figure 3-1: Comparing the cell wall structure of four microalgae (<i>Chlorella vulgaris</i> , <i>Nannochloropsis oculata</i> , <i>Arthorspira platensis</i> and <i>Scenedesmus rubescens</i>) (Kröger, Klemm, and Nelles 2018).....	47
Figure 3-2: Molecular structure of two forms of β-carotene (Moorhead, Capelli, and Cysewski 2011).....	50
Figure 4-1: Two kinds of disaccharide repeating units of calcium- <i>Spirulina</i> (Belay and Gershwin 2007).....	76
Figure 5-1: The structures of BHA and BHT	90
Figure 5-2: Classification of polyphenols (Han, Shen, and Lou 2007).....	92
Figure 5-3: Oxidation of meat pigments (Boles and Pegg 2010).....	93
Figure 5-4: Oxidative stressors, effect of oxidation and antioxidants (Adapted from Estévez 2015)	94
Figure 5-5: Chemical structure of chlorophyll (Asghari et al. 2016).....	95
Figure 5-6: Chemical structure of two forms of β-carotene (Asghari et al. 2016). ..	96
Figure 5-7: Chemical structure of C-phycocyanin (Asghari et al. 2016).....	97
Figure 6-1: Broiler chicks in a commercial farm (Photograph by authors.....	114
Figure 7-1: Chemical structure of microcystins (Cardozo et al. 2007).....	131
Figure 7-2: Biosynthetic pathway of some carotenoids. GGPP: geranylgeranyl diphosphate	134

Figure 7-3: Regression between BASF yolk color fan values and level of <i>Spirulina</i> in the diet (Zahroojian, Moravej, and Shivazad 2013)	135
Figure 7-4: Effect of treatments on egg yolk color score (Zahroojian, Moravej, and Shivazad 2013)	136
Figure 8-1: Factors affecting performance of commercial broiler breeders (Designed by authors)	148
Figure 8-2: Effect of vitamin E as a natural antioxidant on broiler breeder egg production (Zaghari et al. 2013).....	149
Figure 8-3: A healthy commercial broiler breeder flock at 50 weeks of age (Photograph by authors).....	149
Figure 8-4: Normal and abnormal avian sperm membrane	153
Figure 8-5: Mating behavior of broiler breeder male enhance by phytochemical (Durape 2007)	157
Figure 9-1: Experimental pens of <i>Japanese</i> quail chicks at 2 days post hatch fed with diet contained <i>Spirulina platensis</i> (Photograph by authors).....	166
Figure 9-2: Humoral and cell immunity systems (Belay and Gershwin 2007).....	169
Figure 9-3: Effects of <i>Spirulina</i> on body immune responses (Belay and Gershwin 2007)	170
Figure 9-4: Experimental house of <i>Japanese</i> laying quails fed with diet contained <i>Spirulina platensis</i> (Photograph by authors).....	172
Figure 9-5: Evaluating egg yolk color of <i>Japanese</i> layer quails fed with diet contained <i>Spirulina platensis</i> (Photograph by authors).....	174
Figure 10-1: Pink flamingos in an African lake fed on <i>Spirulina</i> (Henrikson 2010)	186
Figure 10-2: Rearing <i>Pekin</i> duck for meat production in a closed system (Photograph by authors).....	189
Figure 10-3: Pools of rearing rainbow trout fed with diet contained <i>Spirulina platensis</i> (Photograph by authors).....	190
Figure 10-4: Chemical structures and conversion pathways of four dietary carotenoids (lutein, zeaxanthin, β -cryptoxanthin, and β -carotene) into the metabolically altered carotenoids in canary feathers (canary xanthophylls A, B, and C, echinenone, canthaxanthin, and α -doradexanthin).....	191
Figure 10-5: A healthy <i>Malango</i> parrot feed on <i>Spirulina</i> as a feed additive (Photograph by authors).....	192

LIST OF TABLES

Table 2-1: Chemical composition of four different strains of <i>Spirulina</i> algae (Charpy and Larkum 1999).....	24
Table 2-2: Main pigments of <i>Spirulina</i> microalgae (Carvalho et al. 2011)	27
Table 3-1: Different reports of <i>Spirulina platensis</i> composition	41
Table 3-2: Nutritional profile of <i>Spirulina</i> Powder (composition by 100 g) (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015).....	42
Table 3-3: Amino acid composition of <i>Spirulina</i> (g/100 g DM) (Habib et al. 2008), Amino. Dat (Kyntäjä et al. 2014).....	44
Table 3-4: Energy and digestible amino acid values of <i>Spirulina</i> for poultry (Kyntäjä et al. 2014)	45
Table 3-5: Protein values of <i>Spirulina</i> compared with casein as a standard (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015).....	45
Table 3-6: The amount of standardized ileal digestible of some amino acids in <i>Spirulina</i> (Kyntäjä et al. 2014).....	46
Table 3-7: The amount of pigments in <i>Spirulina</i> (Zahroojian, Moravej, and Shivazad 2013)	46
Table 3-8: Fatty acid composition of <i>Spirulina platensis</i> powder (Ötles and Pire 2001)	49
Tabel 3-9: Vitamin content of 100 g <i>Spirulina</i> (Gershwin and Belay 2008).....	51
Table 3-10: Mineral content of <i>Spirulina</i> (Belay 1997, Kyntäjä et al. (2014)	52
Table 3-11: Natural sources of iron (Belay 1997, Earhrise farms; <i>Prevention Magazine</i> 1992; Henrikson 2010).....	53
Table 3-12: Heavy metal concentration in <i>Spirulina</i> and the international recommendation as ppm (Becker 1994; Belay and Gershwin 2007)	56
Table 3-13: Consistency and shelf life of <i>Spirulina</i> powder (Bealy and Gershwin 2007)	56
Table 4-1: Preliminary phyto-chemical analysis of <i>Spirulina platensis</i> and <i>Chlorella pyrenoidosa</i> . + Present, ND: Not detected (Ali and Doumandji 2017)	65
Table 4-2: Mechanism of action of <i>Spirulina</i> antimicrobial materials (Pradhan, Das, and Das 2014)	66
Table 4-3: Antibacterial substances from different algae and their target bacterial pathogens (Pradhan, Das, and Das 2014).....	67
Table 4-4: GC-MS analysis of different compounds in ethanol extract of <i>Spirulina</i> (Ramasamy 2014).....	69
Table 4-5: GC-MS analysis of different compounds in hexane extract of <i>Spirulina</i> (Ramasamy 2014).....	70
Table 4-6: GC-MS analysis of different compounds in chloroform extract of <i>Spirulina</i> (Ramasamy 2014)	71

Table 4-7: GC-MS analysis of different compounds in dichloromethane extract of <i>Spirulina</i> (Ramasamy 2014)	72
Table 4-8: Minimum inhibitory concentration of <i>Spirulina platensis</i> extract using different solvent (mg/ml) against bacteria (Usharani et al. 2015)	74
Table 4-9: Minimum inhibitory concentration of <i>Spirulina platensis</i> extract using different solvent (mg/ml) against fungi (Usharani et al. 2015)	75
Table 5-1: Content of phenolic compounds in <i>Spirulina platensis</i> (Goiris et al. 2014)	91
Table 5-2: The amount (mg: g–1 GAE) of total phenolic content of <i>Spirulina platensis</i> extracts by different solvents (Machu et al. 2015)	99
Table 6-1: Chemical and energy composition of <i>Spirulina platensis</i> and soybean meal, expressed on natural matter basis (Alvarenga et al. 2011)	109
Table 6-2: Comparing the amino acid content and ratio of amino acid/lysine in <i>Spirulina platensis</i> and SBM in broiler diets (Alvarenga et al. 2011)	110
Table 6-3: Coefficients of metabolizable energy and of some nutrients from <i>Spirulina platensis</i> obtained with broilers (Alvarenga et al. 2011)	111
Table 6-4: Effects of <i>Spirulina platensis</i> on growth performance of broiler chickens at different periods (Hajati and Zaghari, unpublished data)	113
Table 6-5: Effects of <i>Spirulina platensis</i> on carcass characteristics of broilers at 42 days (Hajati and Zaghari, unpublished data)	114
Table 6-6: Effects of <i>Spirulina platensis</i> on gut characteristics of broilers at 42 days (Hajati and Zaghari, unpublished data)	116
Table 6-7: Effects of <i>Spirulina platensis</i> on ileal microbial population (log CFU/g of digesta) of broilers at 42 days (Hajati and Zaghari, unpublished data)	117
Table 6-8: Effects of <i>Spirulina platensis</i> on hematological parameters in broiler chickens (Hajati and Zaghari, unpublished data)	119
Table 7-1: <i>Spirulina</i> on egg production, feed intake, feed conversion ratio, egg weight and yolk color (Zahroojian, Moravej, and Shivazad 2011)	132
Table 7-2: Effect of <i>Spirulina platensis</i> supplementation on layer hens egg quality traits (Selim, Hussein, and Abou-Elkhair 2018)	137
Table 7-3: The effect of dietary <i>Spirulina</i> on serum and yolk cholesterol levels (Narahari et al. 2005)	138
Table 8-1: Antioxidant distribution in the tissues of a newly hatched chick (Surai, Fisinin, and Karadas 2016)	146
Table 8-2: Effect of <i>Spirulina platensis</i> or <i>Curcuma</i> powder supplementation on fertility and hatchability percentages of golden montazah hens at 40 weeks of age (Mobarez et al. 2018)	151
Table 8-3: The effects of different levels of dietary <i>Spirulina</i> and local hen strains on egg weight, egg weight loss, fertility, hatchability and chicks' weight of Sinai and Gimmizah laying hens (Mariey, Samak, and Ibrahim 2002)	152
Table Error! No text of specified style in document.-4: Plasma membrane functionality, sperm abnormality, semen, MDA and blood testosterone concentrations (mean \pm SE) of roosters fed different levels of α -tocopherol acetate in both heavy- and standard weight roosters (Ajafar et al. 2018)	155
Table 9-1: The effects of <i>Spirulina platensis</i> on egg production of Japanese quails (Abouelezz 2017)	171

Table 9-2: Effects of <i>Spirulina platensis</i> on egg quantity and quality traits of layer quails (<i>Hajati and Zaghari</i> , unpublished data).....	172
Table 9-3: Effects of <i>Spirulina platensis</i> on egg quantity and quality traits of layer quails (<i>Hajati and Zaghari</i> , unpublished data).....	173

PREFACE

We are pleased to present our findings about organic feeds in poultry. In our primary study, we found that algae have valuable nutrients with high digestibility and possess the potential to be used in organic feeding of poultry. This text is the outcome of the authors' research about algae and their effective substances on poultry.

Our approach was first to define all aspects of *Spirulina* as a functional feed in different poultry species. However, there is limited scientific literature focusing on usage of algae, especially *Spirulina*, in poultry diets, and we faced some difficulties in conducting specialized trials.

Our second goal was introducing new feed sources in poultry nutrition to compensate for the land crop shortage and improve public health, especially in regions where infectious diseases and cancer incidence is high. The present book has 10 chapters as follows: organic feed, *Spirulina* rearing condition, nutritional importance of *Spirulina*, antimicrobial characteristics of *Spirulina*, antioxidant characteristics of *Spirulina*, *Spirulina* in broilers nutrition, *Spirulina* in layers nutrition, *Spirulina* in breeders nutrition, *Spirulina* in quails nutrition, and *Spirulina* in waterfowl and pet birds nutrition.

This book may be useful for poultry nutritionists and veterinarians for improving the state of poultry health as well as meat and egg quality.

We gratefully thank all of the researchers who cooperated with us in gathering information. In addition, we would like to show appreciation to our family members for their unwavering companionship.

We feel that we have achieved our goal of producing an outstanding book. Although the field of poultry nutrition progressing rapidly, we hope our discussion of these issues will be useful for a long time.

The authors dedicate the book to those who are concerned about public health and welfare.

Please feel free to contact us if you have any questions or recommendations.

M. Zaghari

H. Hajati

2018/08/04

KEY OF ABBREVIATIONS

ABC	ATP binding cassette
ABTS	2,2'-azino-bis 3-ethyl benzthiazoline-6-sulphonic acid
ADF	Acid detergent fiber
AF	Aflatoxin
AFDW	Ash free dry weight
AIDS	<i>Acquired immune deficiency syndrome</i>
ALA	Alpha-linolenic acid
ALT	Alanine amino transferase
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy corrected for nitrogen
AP	Allophycocyanin
APX	Ascorbate peroxidase
AR	Arachidonic
As	Arsenic
AST	Aspartate aminotransferase
BAU	Bangladesh Agricultural University
BHA	Butylated hydroxyanisole

BHT	Butylated hydroxyl toluene
BV	Biological value
BWG	Body weight gain
Ca	Calcium
Ca-Sp	Calcium <i>spirulan</i>
CAT	Catalase
Cd	Cadmium
CF	Crude fiber
COX-2	Cyclooxygenase-2
CP	Crude protein
Cu	Copper
DHA	Docosahexaenoic acid
DM	Dry matter
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-picrylhydrazyl
EAAI	Estimation of essential amino acid index
EC	Electrical conductivity
EE	Ether extract
EM	Egg mass
EPA	Eicosa-pentaenoic acid

EPEI	<i>European</i> production efficiency index
EPR	Egg production rate
ESA	<i>European</i> space agency
EW	Egg weight
FA	Fatty acid
FAO	Food and agriculture organization
FCR	Feed conversion ratio
FDA	Food and drug administration
Fe	Iron
FI	Feed intake
FOI	French Oil Institute
FRAP	Ferric ion reducing antioxidant power
GAE	Gallic acid equivalent
GALT	Gut-associated lymphoid tissues
GC-MS	<i>Gas</i> chromatography mass spectrometry
GGPP	Geranyl geranyl diphosphate
GLnA	Gamma-linolenic acid
GM	Genetically modified
GMPs	Good manufacturing practices
GPx	Glutathione peroxidase

GRAS	Generally recognized as safe
GSE	Grape seed extract
Hb	Hemoglobin
HCT	Hematocrit
HDL	High density lipoprotein
HepG2	Hepato cellular carcinoma cell line
Hg	Mercury
HIV	<i>Human immunodeficiency virus</i>
HMG-coA	3-hydroxyl-3-methylglutaryl coenzyme A
HPLC	High performance liquid chromatography
HSV	Herpes simplex virus
HU	Haugh unit
IADC	Ileal apparent digestibility coefficients
IgA	Immunoglobulin A
iNOS	Inducible nitric oxide synthase
IPGSR	Institute of Post-graduate Studies and Research laboratory
LDL	Low density lipoprotein
LPS	Lipo polysaccharides
MCs	Microcystins
MDA	<i>Malon dialdehyde</i>

MDR-1	Multidrug resistance-1
Mg	Magnesium
MIC	<i>Minimum inhibitory</i> concentration
Mn	Manganese
MOS	Mannan oligosaccharides
NADPH	Nicotinamide adenine dinucleotide phosphate
NASA	National aeronautics and space administration
NDF	Neutral detergent fiber
NDV	<i>Newcastle disease</i> virus
NF-κB	Nuclear factor kappa
NO	Nitric oxide
ORAC	Oxygen radical absorbance capacity
P	Phosphorus
P.P.M	Part per million
Pb	Lead
Pc	<i>Phycocyanin</i>
PE	Phycoerythrin
PG	<i>Prostaglandins</i>
PUFA	Polyunsaturated fatty acid
PVC	Packed cell volume

PV-C	Polyvinyl-chloride
PX	Peroxidase
RBC	Red blood cell
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT	Retention time
SA	<i>Schizochytrium sp.</i> algae
SAC	Siam Algae Company
SBM	Soybean meal
SCP	Single cell protein
SD	Standard deviation
Se	Selenium
SEM	Standard error of the mean
SID	Standardized ileal digestibility
SOD	Super oxide dismutase
SRBC	Sheep red blood cell
SRBP	Sterol regulatory element binding protein
TA	Tocopherol acetate
TBARS	Thio-barbituric acid reactive substances
TBHQ	Tert-butyl hydroquinone

TC	Total cholesterol
TDS	<i>Total dissolved solids</i>
TEAC	Trolox equivalence antioxidant capacity
TEC	Total erythrocyte count
TG	Triacylglycerol
THP	T helper peptides
TME	True metabolizable energy
TNF	<i>Tumor necrosis factor</i>
TPE	Total protein efficiency
VLDL	Very low density lipoprotein
WBC	White blood cell
WHO	World health organization

CHAPTER ONE

ORGANIC FEED

“When God created the Garden of Eden, She became the first permaculturalist.”
—Khang Kijarro Nguyen

1.1 Introduction

Nowadays, a major concern of organic feeding of poultry is to ensure the quality of the poultry products and improve the level of human public health. In general, organic feed is the product of a farming system which avoids the use of man-made fertilizers, pesticides, growth regulators, hormones and antibiotics. Irradiation and use of genetically modified (GM) crops and organisms are prohibited by organic legislations (Blair 2008; Adedeji 2013). From an economical perspective, organic feed is more expensive than conventional feed; thus, products of organic farming cost more (Blair 2008). The use of organic feed and feed additives such as herbal additives, certain probiotics and prebiotics in poultry diets has been scrutinized by poultry nutritionists to improve the birds' health and welfare (Gerzilov et al. 2011). It was reported that algae have high nutritional value and may be considered as a feed ingredient in organic feeding poultry (Gerrard et al. 2015). It should be noted that before choosing these edibles, diet formulators should be aware of some parameters such as birds' species, ages and health statuses, nutritional characteristics of feed ingredients, synergisms and antagonisms between nutrients, and the effect of each organic feed additive on birds' physiological systems. The aim of this chapter is to provide an overview of using algae as an organic functional feed ingredient.

1.2 Herbal additives in poultry diet

Using antibiotic growth promoters as feed additives was banned by the European Union in 2006 due to cross-resistance against pathogens and residues in tissues, so scientists are searching for organic, safe, functional alternatives to antibiotics (Anadón 2006; Klose et al. 2006; Kools,

Moltmann, and Knacker 2008; Hajati, Hassanabadi, and Waldroup 2011). The primary alternatives studies included acidification of the feed by organic acids (Taherpour et al. 2012; Rodríguez-Lecompte et al. 2012), feeding probiotic organisms (Taheri et al. 2009; Taheri et al. 2010; Momtazan et al. 2011; Zaghari et al. 2015) and feeding prebiotic compounds (Khaksar et al. 2008; Hajati and Rezaei 2010; Khalaji et al. 2011; Yalçinkaya et al. 2012). Also, medicinal plants and essential oils extracted from plants are interesting due to their special characteristics that stimulate digestive processes in animals (Ciftci et al. 2005; Ertas et al. 2005; Al-Kassie 2009). Useful antimicrobial phytochemicals can be divided into several categories: phenolics and polyphenols (simple phenols, phenolic acids, quinones, flavones, tannins and coumarins), terpenoids and essential oils, alkaloids, and lectins and polypeptides (Cowan 1999; Hernandez et al. 2004; Aengwanich et al. 2009; Pandey and Kumar 2013; Chandra et al. 2017). Beneficial effects of herbal additives in farm animals may be due to their positive effects on feed intake and digestive secretions, immune stimulation, or antibacterial, coccidiostatical, antihelminthical, antiviral or anti-inflammatory activity (Abbas et al. 2010; Fotea et al. 2010; Kumar et al. 2014; Giannenas et al. 2017; Duvvu et al. 2018).

In plant tissues, pH values are dependent on the presence of polycarboxylic acids, phosphate salts, fiber and proteins (Al-Dabbas et al. 2010). The active constituents in the leaves, stem, seeds, roots and barks of medicinal plants are highly effective in combating different diseases and improving the digestion, which in turn could improve the performance of the consumers (Ashayerizadeh et al. 2009). With respect to biological origin, formulation, chemical description and purity, phytobiotics comprise a wide range of substances, and four subgroups may be classified: 1) herbs (produced from flowering, non-woody and non-persistent plants), 2) botanicals (entire or processed parts of a plant, such as root, leaves or bark), 3) essential oils (hydro-distilled extracts of volatile plant compounds), and 4) oleoresins (extracts based on non-aqueous solvents) (Windisch and Kroismayr 2006). Compared with synthetic antibiotics or inorganic chemicals, plant-derived products have proven to be natural, less toxic, residue free, and are thought to be ideal feed additives in animal nutrition (Wang et al. 1998; Yang et al. 2009; Hashemi and Davoodi 2010). The active compounds of phytobiotics are secondary plant constituents (Yang et al. 2009; Al-Kassie 2010; Grashorn 2010).

Four factors may affect the effectiveness of phytobiotic additives: 1) plant parts (i.e., leaves, stem, root and seed) and their physical properties, 2)

source, 3) harvest time and 4) compatibility with the other ingredient(s) in the feed (Wang et al. 1998). In addition, the efficacy of dietary essential oil can be affected by intrinsic and extrinsic factors such as nutritional status of animals, infection, diet composition and environment (Giannenas et al. 2003; Lee et al. 2004).

Herbal additives can be used in organic poultry nutrition to improve birds' health and productivity, and farmers can also use certain herbal additives to enrich birds' products (i.e., meat, eggs) with natural antioxidants and antimicrobial compounds in order to combat human cancer and infectious diseases (Huffman 2001; Güllüce et al. 2003; Proestos et al. 2005; Ji et al. 2009; Doughari et al. 2009; Hajati et al. 2014a).

Our previous research revealed that using up to 6% citrus pulp in broiler chicken (Ross 308 strain) diets did not have any adverse effect on birds' performance; however, it can be used to reduce feed cost and environmental impact (Hajati, Hassanabadi, and Yansari 2012).

In another study, we found that grape seed extract (GSE) (150, 300, 450 ppm) had antimicrobial activity against *E. coli* and *S. typhi* under in vitro conditions. Nano-emulsification of GSE could not improve the antibacterial effect of GSE under in vitro conditions, but it improved GSE's antibacterial effect against *coliforms* and *E. coli* populations in the intestinal tract of broiler chickens (Hajati et al. 2014c). *In ovo* injections of 4.5 mg GSE/egg on 18th day of incubation increased hatchability of broiler chickens and it had no adverse effect on the broiler chickens' performance during starter period. It was documented that the antioxidant potential of grape seed is twenty and fiftyfold greater than that of vitamins E and C, respectively, arising from increased levels of polyphenols proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin (Shi et al. 2003). It seems that GSE can be used as an effective anti-stress additive during the incubation period to improve broilers' hatchability and performance (Hajati et al. 2014b). Hydro-alcoholic GSE supplementation in Cobb mail broilers decreased serum glucose in the birds before they suffered from heat stress condition; this may be beneficial for the animals and humans who suffer from diabetes. Grape seed supplementation at the level of 300 mg/kg could improve live weight and European production efficiency, and suppress the detrimental effect of heat stress on blood metabolites such as the levels of glucose, cholesterol and HSP70 gene expression in birds suffering from chronic heat stress condition (Hajati et al. 2015a). We concluded that GSE has the potential to be considered as an herbal additive to improve the health and

welfare of poultry, especially during periods of chronic heat stress (Hajati et al. 2015b). Also, GSE is preferred over synthetic antioxidants for the health and economic goals since it is a natural waste by-product (Hajati et al. 2018).

In ovo feeding of grapefruit seed extract decreased the *E. coli* population in the ileum content of birds at 10 d, but there was no difference in the *Lactobacillus* population of broilers at 10 days (Hajati 2016). *Echinacea purpurea* extract supplementation in quail diets improved feed conversion ratio; however, it decreased the carcass yield of the birds (Seifi et al. 2018). Khalaji et al. (2011) assessed the effects of dietary black cumin seeds, *Artemisia* leaves and *Camellia L.* plant extract in broiler chicks. They found that black cumin seeds alone or mixed with *Artemisia* leaves improved broiler health and performance, but *Camellia L.* plant extract negatively affected broiler body weight and feed intake. With regards to our previous evidence about the effects of herbal extracts on poultry, we concluded that certain amounts of some herbal extracts may promote poultry health and performance.

1.3 Algae

Algae are usually spread throughout the water. Algae are divided by dimensions into macroalgae (macroscopic algae) and microalgae (microscopic algae). Microalgae are unicellular organisms that have the ability grow under different environmental conditions, whereas macroalgae or seaweeds are complex multi-cellular organisms which grow in marine environments (Enzing et al. 2014).

The blue-green algae are microalgae; they are also called cyanobacteria because of their prokaryotic cell type (Kovac et al. 2013). Cyanobacteria are organisms that have some properties of bacteria and some of algae. Their size is similar to algae. Cyanobacteria contain blue-green and green pigments and have the ability to use sunlight to convert carbon dioxide into sugars and oxygen during the process of photosynthesis; they were given their name due to this phenomenon (WHO 2003). The Food and Agriculture Organization of the United Nations (FAO) (2014) estimated that humans consumed about 9.04 million tons of the 23.8 million tons of seaweeds in the global harvest of 2012. The global harvest of algae in 2013 was estimated to be worth about US\$6.7 billion, over 95% of which was produced in mariculture. It was reported that China and Indonesia were the top producers (FAO 2015).

Algae have high nutritional value (Fujiwara-Arasaki, Mino, and Kuroda 1984; Dillon, Phuc, and Dubacq 1995; Leng, Stambolie, and Bell 1995; Dawczynski, Schubert, and Jahreis 2007). Also, they are being considered as functional foods (Herrero, Cifuentes, and Ibáñez 2006; Holdt and Kraan 2011; Wells et al. 2017). Algae contain bioactive compounds, or phytochemicals, that may improve consumers' health in addition to their role in basic nutrition (Hafting et al. 2012). The path from algal research to producing new food and feed products or dietary supplements is highly affected by industrial, regulatory and nutritional considerations (Finley et al. 2014).

Algae cultivation can be independent of external conditions. They are efficient in converting solar energy. In comparison with higher plants, algae do not require fertile soil. Some algae species reproduce very quickly, so these organisms present a really remarkable source of biomass and certain substances (Kovac et al. 2013).

It is interesting to notice that even when algae are used in small amounts they may improve the immune system, lipid metabolism, gut function and stress resistance (Romay et al. 1998; Adarme-Vega et al. 2012; Shields and Lupatsch 2012; Cian et al. 2015; Norambuena et al. 2015), as well as increase appetite, weight and number of eggs, and reproductive performance, or reduce cholesterol levels (Svircev 2005). A large number of nutritional and toxicological evaluations reported that algae biomass is valuable feed supplement and can be considered a substitute for common protein sources (soybean meal, fish meal, etc.). Poultry are the main target in domestic animals because using algae in poultry diets is the most promising opportunity for algae commercial use in animal feeding (Becker 2007). In poultry, algae can be used as a partial replacement for common protein sources with the incorporation of 5–10% (Spolaore et al. 2006). Also, according to Gouveia et al. (2008), they may serve as almost the sole protein source in laying hens, and they are approved as chicken feed in many countries (Kovac et al. 2013; Van der Spiegel et al. 2013).

Barka and Blecker (2016) reported that protein extracted from pure or mixed cultures of algae, yeast, fungi or bacteria that are called single-cell protein (SCP) can be used as a substitute for the common protein sources used for humans and animals. The technical potential of microalgae for reduction of greenhouse gas has been recognized for many years (Taylor 2008; Campbell, Beer, and Batten 2011). Algae have the ability to use carbon dioxide (Packer 2009), and they have the capability to produce higher biomass than land crops (Walsh et al. 2016). Today, biofuel

production from the marine resources, by using the algae biomass or their oil extract, is an interesting issue (Pittman, Dean, and Osundeko 2011; Shurin et al. 2013; Rogers et al. 2014; Beal et al. 2015). The use of microalgae as an energy production system combined with waste water treatment and production of high-value products is important for efficient economic processes (Bowles 2007).

It is expected that algae and insects will each account for about 2% of the alternative protein market share by 2024. Also, new sources of proteins are estimated to make up 33% (i.e., 311 million metric tons—MMTs) of global protein consumption by 2054, with algae and insects accounting for about 18% and 11% of the alternative protein market, respectively. This indicates that about 56 MMT of algae will be consumed globally by 2054. Microalgal products also have a high value in marketing for astaxanthin that is expected to triple by 2017 (Enzing et al. 2014).

Algae consumption may prevent heart defects due to their considerable ω -3 fatty acid content and negligible cholesterol. This is very important for improving social health and combating diseases such as cardiovascular diseases and cancers (Enzing et al. 2014).

1.4 Different variety of Algae

Microalgae (unicellular organisms) and macroalgae (multicellular organisms) make up the large algae group that is considered photosynthetic organisms (Barka and Blecker 2016). They appeared on Earth about 3.5 billion years ago and they are regarded as the first form of life (Margulis 1981). Algae are autotrophic organisms, but, they lack root, stem, leaf, or flower.

Among the marine macroalgae, red and green algae such as *Porphyra spp.* (*laver*), *Pyropia spp.* (*nori*), *Palmaria palmata* (*dulse*) and *Ulva spp.* (*sea lettuce*) often contain high levels of protein in contrast to lower levels in most brown algae (Taboada et al. 2013; Angell et al. 2016).

In addition to macroalgae, some microalgae are cultivated for foods and food additives (FAO 2016). Some of the microalgae are eukaryotic, while others do not have membrane-bound nuclei (prokaryotes, cyanobacteria). Microalgae have a higher level of productivity than traditional agricultural crops and can be grown in climatic conditions, such as desert and coastal areas (Christaki, Florou-Paneri, and Bonos 2011). In addition to their high production yield, they are environmentally friendly (Barka and Blecker 2016). More than 30,000 microalgae species are recognized; however,

fewer than 10 are commercially produced (Gouveia et al. 2008). Today, domesticated *Spirulina* and *Chlorella* from several large producers have GRAS (generally recognized as safe) designations (FDA 2016).

Chlorella is one of the most ancient microalgae species used in human diet. Commercial utilization of *Chlorella* was introduced in 1961 by Nihon Chlorella Inc. in Japan (Iwamoto 2004). *Chlorella* contains β -1,3-glucan, which stimulates body immune responses. The name *Chlorella* is derived from the Greek *chloros* and Latin *ella*, which mean “green” and “small”, respectively (Andrade et al. 2018). The mass production mode for *Chlorella* is either heterotrophic or mixotrophic (Barka and Blecker 2016). In addition to carbon dioxide, acetic acid can be added as an organic carbon source in the mixotrophic production system; however, in the heterotrophic mode carbon is supplied by the sole organic carbon source. The *Chlorella* biomass obtained by heterotrophic mass production exhibits a superior quality for consumption as a health food (Iwamoto 2004). Rezvani, Zaghari, and Moravej (2012) arranged a trial to study the effects of *Chlorella* algae on male broiler (Ross 308) performance and immune system. They added different levels of *Chlorella* (0.07, 0.14 or 0.21%) to the basal diet. They concluded that dietary supplementation of *Chlorella* improved the broilers’ performance. Also, dietary *Chlorella* numerically increased the cell immunity response of the birds.

The name *Spirulina* is based on the biomass’s spiral shape. *Arthrospira platensis* and *Arthrospira Maxima* are cultivated worldwide (Andrade et al. 2018). *Arthrospira* (*Spirulina* sp.) is considered as an obligatory alkalophile, with the maximal growth rate being obtained at pH 9.5–9.8 (Hu 2004). Its ability to thrive in a high-pH environment limits the development of other microorganisms and favors its large-scale outdoor mass production. Mixotrophic mass production presents higher yields (Chen and Zhang 1997). The highest number of production facilities of *Arthrospira* sp. mass production are located in the Asia-Pacific region (Lee 1997).

Park, Lee, and Kim (2018) reported that using dietary *Spirulina* improved dry matter and nitrogen digestibility, cecal *Lactobacillus* population, excreta ammonia emission, antioxidant enzyme activity and drip loss of breast meat in broiler chickens. Microalgae have been identified as one of the most reliable sources of protein. Some microalgal sources have protein contents higher than those of conventional animal or plant sources. The protein content of *Spirulina platensis* (65%) is higher than that of dried

skimmed milk (36%), soy flour (37%), chicken (24%), fish (24%), beef (22%) and peanuts (26%) (Moorhead, Capelli, and Cysewski 2011).

Dunaliella sp. is the most halotolerant eukaryotic photosynthetic organism. It is able to adapt to different salt concentrations (Ben-Amotz 2004). These algae are one of the best natural sources of β -carotene. In a modern intensive production, an area of 50,000 m² can produce 3,650 kg per year. The optimal growth medium for *Dunaliella* should ideally contain around 1.5 M NaCl, more than 0.4 M MgSO₄, and 0.1 M CaCl₂ under pH control (Ben-Amotz 2004).

Aphanizomenon sp. is a freshwater cyanobacterium that was discovered in the 1980s at Klamath Lake in Oregon, USA (Carmichael, Drapeau, and Anderson 2000).

Nostoc sp. can develop under various climatic conditions including the polar regions, hot springs and deserts. The optimum temperature for the growth of this cyanobacterium is between 15°C and 25°C (Cui 1983). It has great adaptability to a wide range of temperatures. *Nostoc* was consumed by the Chinese to survive during times of famine 2,000 years ago (Danxiang, Yonghong, and Zhengyu 2004), but cultivation of this organism has never progressed beyond the experimental level.

Scenedesmus sp. is mostly cultivated for biofuel production because of its high lipid content (31.7%) and high biomass productivity compared to other microalgae sources (Xia et al. 2014). Investigations into the feasibility of growing the *Porphyridium* biomass outdoors were carried out by Vonshak, Cohen, and Richmond (1985) in a laboratory study. It was found that, although the optimum temperature for the growth of *Porphyridium* is known to be 25°C, it can keep its photosynthetic activity at up to 35°C. Its production rate is estimated at up to 22 g.m⁻².d⁻¹ (dry weight), which was obtained during outdoor cultivation (Barka and Blecker 2016).

Tetraselmis sp. is also a halotolerant form of microalgae that can be used either as food or feed, or for biofuel synthesis (Sommer, Potts, and Morrissy 1990; Reitan et al. 1994; Robert et al. 2001; Patil et al. 2007). Sing et al. (2014) showed that a peak productivity of 37.5±3.1g.m⁻².d⁻¹ ash free dry weight (AFDW) was reached in a recycled medium upon transition from 14% to 7% NaCl.

In conclusion, with regards to the valuable nutrient content of algae and the potential of producing algae in controlled systems, it seems that they can be considered as an organic feed in poultry nutrition.

References

- Abbas, R. Z., Z. Iqbal, M. N. Khan, M. A. Zafar, and M. A. Zia. 2010. "Anticoccidial activity of *Curcuma longa* L. in broilers." *Brazilian Archives of Biology and Technology* 53 (1): 63–67.
- Adarme-Vega, T. C., D. K. Lim, M. Timmins, F. Vernen, Y. Li, and P. M. Schenk. 2012. "Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production." *Microbial cell factories* 11 (1): 96.
- Adedeji, O. S. 2013. "Effect of different organic feed ingredients on growth performance, haematological characteristics and serum parameters of broiler chickens." *World Journal of Agricultural Sciences* 9 (9): 137–42.
- Aengwanich, W., M. Suttajit, T. Srikhun, and T. Boonsorn. 2009. "Antibiotic effect of polyphenolic compound extracted from tamarind (*Tamarindus indica* L.) seed coat on productive performance of broilers." *International Journal of Poultry Science* 8 (8): 749–51.
- Al-Dabbas, M. M., K. Al-Ismail, R. A. Taleb, and S. Ibrahim. 2010. "Acid-base buffering properties of five legumes and selected food in vitro." *American Journal of Agricultural and Biological Sciences* 5 (2): 154–60.
- Al-Kassie, G. A. 2009. "Influence of two plant extracts derived from thyme and cinnamon on broiler performance." *Pakistan Veterinary Journal* 29 (4): 169–73.
- Al-Kassie, G. A. M. 2010. "The effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks." *International Journal of Poultry Science* 9 (5): 495-98.
- Anadón, A. 2006. "WS14 – The EU ban of antibiotics as feed additives (2006): alternatives and consumer safety." *Journal of Veterinary Pharmacology and Therapeutics* 29:41–44.
- Andrade, L. M., C. J. Andrade, M. Dias, C. A. O. Nascimento, and M. A. Mendes. 2018. "Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; An Overview." *MOJ Food Processing & Technology* 6 (1): 45–58.
- Angell, A. R., M. Leonardo Mata, D. N. Rocky, and A. P. Nicholas. 2016. "The protein content of seaweeds: a universal nitrogen-to-protein

- conversion factor of five." *Journal of Applied Phycology* 28 (1): 511-524.
- Ashayerizadeh, O., B. Dastar, M. S. Shargh, A. Ashayerizadeh, E. Rahmatnejad, and S. M. R. Hossaini. 2009. "Use of garlic (*Allium sativum*), black cumin seeds (*Nigella sativa* L.) and wild mint (*Mentha longifolia*) in broiler chickens diets." *Journal of Animal and Veterinary Advances* 8 (9): 1860–63.
- Barka, A., and C. Blecker. 2016. "Microalgae as a potential source of single-cell proteins. A review." *Base*. 20(3).
- Beal, C. M., L. N. Gerber, D. L. Sills, M. E. Huntley, S. C. Machesky, M. J. Walsh, J. W. Tester, I. Archibald, J. Granados, and C. H. Greene. 2015. "Algal biofuel production for fuels and feed in a 100-ha facility: a comprehensive techno-economic analysis and life cycle assessment." *Algal Research* 10:266–79.
- Becker, E. W. 2007. "Micro algae as a source of protein." *Biotechnology Advances* 25 (2): 207–10.
- Ben-Amotz, A. 2004. "Industrial production of microalgal cell-mass and secondary products—major industrial species." In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, edited by A. Richmond. Oxford: Blackwell Science, 2004. p.273–280.
- Blair, R. 2008. *Nutrition and feeding of organic Poultry*. Abingdon: CABI.
- Bowles, D., ed. 2007. *Micro-and macro-algae: utility for industrial applications: outputs from the EPOBIO project*. Speen: CPL Press.
- Campbell, P. K., T. Beer, and D. Batten. 2011. "Life cycle assessment of biodiesel production from microalgae in ponds." *Bioresource Technology* 102 (1): 50–56.
- Carmichael, W. W., C. Drapeau, and D. M. Anderson. 2000. "Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. and Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use." *Journal of Applied Phycology* 12 (6): 585–95.
- Chandra, H., P. Bishnoi, A. Yadav, B. Patni, A. P. Mishra, and A. R. Nautiyal. 2017. "Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review." *Plants* 6 (2): 16.
- Chen, F., and Y. Zhang. 1997. "High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system." *Enzyme and Microbial Technology* 20 (3): 221–24.
- Christaki, E., P. Florou-Paneri, and E. Bonos. 2011. "Microalgae: a novel ingredient in nutrition." *International Journal of Food Sciences and nutrition* 62 (8): 794–99.

- Cian, R. E., S. R. Drago, F. S. de Medina, and O. Martínez-Augustin. 2015. "Proteins and carbohydrates from red seaweeds: evidence for beneficial effects on gut function and microbiota." *Marine drugs* 13 (8): 5358–83.
- Ciftci, M., T. Guler, B. Dalkiliç, and O. N. Ertas. 2005. "The effect of anise oil (*Pimpinella anisum* L.) on broiler performance." *International Journal of Poultry Science* 4 (11): 851–55.
- Cowan, M. M. 1999. "Plant products as antimicrobial agents." *Clinical Microbiology reviews* 12 (4): 564–82.
- Cui, Z. 1983. "Culture trial of *Facai* in soil-soaked solution." *Science and Technology Letters. Inner Mongolia* 4:10–38.
- Danxiang H., B. Yonghong, and H. Zhengyu. 2004. "Industrial production of microalgal cell-mass and secondary products – major industrial species: *Nostoc*." In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, edited by A. Richmond, 304–31. Oxford, UK: Blackwell.
- Dawczynski, C., R. Schubert, and G. Jahreis. 2007. "Amino acids, fatty acids, and dietary fibre in edible seaweed products." *Food Chemistry* 103 (3): 891–99.
- Dillon, J. C., A. P. Phuc, and J. P. Dubacq. 1995. "Nutritional value of the alga *Spirulina*." In *Plants in Human Nutrition*, Vol. 77, 32–46. Karger Publishers.
- Doughari, J. H., I. S. Human, S. Bennade, and P. A. Ndakidemi 2009. "Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria." *Journal of Medicinal Plants Research* 3 (11): 839–48.
- Duvvu, M. V., K. A. Rao, C. V. Sessaiah, and D. S. Kumar. 2018. "Effect of Garlic Supplementation on the Growth Performance and Body Condition Score in Murrah Buffalo Calves." *International Journal of Current Microbiology and Applied Sciences* 7 (2): 2972–77.
- Enzing, C., M. Ploeg, M. Barbosa, and L. Sijtsma. 2014. Microalgae-based products for the food and feed sector: an outlook for Europe. " *JRC Scientific and policy reports*. 19-37.
- Ertas, O. N., T. Guler, M. Ciftci, B. Dalkiliç, and U. G. Simsek. 2005. "The effect of an essential oil mix derived from oregano, clove and anise on broiler performance." *International Journal of Poultry Science* 4 (11): 879–84.
- FAO (Food and Agriculture Organization of the United Nations) 2014. *The State of the World Fisheries and Aquaculture 2014*. Rome: FAO.

- FAO 2015. *FAO Global Aquaculture Production data base updated to 2013—Summary information*. Rome: FAO.
- FAO 2016. *The State of the World Fisheries and Aquaculture 2016. Contribution to Food Security and Nutrition for All*. Rome: FAO.
- FDA 2016. Summary: Substances Generally Regarded As Safe (Final Rule). U. S. Food and Drug Administration. <http://www.fda.gov/AboutFDA/ReportsManualsForms/Reports/EconomicAnalyses/ucm517103.htm>. Accessed October 24, 2016.
- Finley J. W., J. W. Finley, K. Ellwood, and J. Hoadley. 2014. “Launching a new food product or dietary supplement in the United States: industrial, regulatory, and nutritional considerations.” *Annual Review of Nutrition* 34:421–47.
- Fotea, L., E. Costăchescu, G. Hoha, and D. Leonte. 2010. “The effect of oregano essential oil (*Origanum vulgare* L) on broiler performance.” *Lucrari stiintifice* 53:491–94.
- Fujiwara-Arasaki, T., N. Mino, and M. Kuroda. 1984. “The protein value in human nutrition of edible marine algae in Japan.” *Hydrobiologia* 116 (1): 513–16.
- Gerrard, C. L., J. Smith, R. Nelder, A. Bright, M. Colley, R. Clements, and B. D. Pearce. 2015. “100% organic poultry feed: can algae replace soybean expeller in organic broiler diets?” *Organic farming* 1 (1): 38–45.
- Gerzilov, V., N. Bozakova, A. Bochukov, G. Penchev, M. Lyutskanov, S. Popova-Ralcheva, and V. Sredkova. 2011. “Influence of the prebiotic Salgard and a herb mixture on pekin ducklings in organic poultry production, I: Growth performance and blood biochemical parameters.” *Biotechnology in Animal Husbandry* 27 (1): 33–43.
- Giannenas, I., E. Bonos, E. Christaki, and P. Florou-Paneri. 2017. “Oregano: A Feed Additive With Functional Properties.” *Therapeutic Foods* 8:179.
- Giannenas, I., P. Florou-Paneri, M. Papazahariadou, E. Christaki, N. A. Botsoglou, and A. B. Spais. 2003. “Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*.” *Archives of Animal Nutrition* 57 (2): 99–106.
- Gouveia, L., A. P. Batista, I. Sousa, A. Raymundo, and N. M. Bandarra. 2008. “Microalgae in novel food products.” In *Food Chemistry Research Developments*, edited by K. Papadoupoulos, 75–111. Hauppauge, NY: Nova Science Publishers.

- Grashorn, M. A. 2010. "Use of phytobiotics in broiler nutrition—an alternative to infeed antibiotics." *Journal of Animal and Feed Sciences* 19 (3): 338–47.
- Güllüce, M., M. Sökmen, D. Daferera, G. Açar, H. Özkan, N. Kartal, M. Polissiou, A. Sökmen, and F. Şahin. 2003. "In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L." *Journal of Agricultural and Food Chemistry* 51 (14): 3958–65.
- Hafting, J. T., A. T. Critchley, M. L. Cornish, S. A. Hubley, and A. F. Archibald. 2012. "On-land cultivation of functional seaweed products for human usage." *Journal of Applied Phycology* 24 (3): 385–92.
- Hajati, H. 2016. "Effects of in ovo feeding of grapefruit seed extract on hatchability and ileal microbial population of broiler chickens." The Proceedings of the XXV World's Poultry Congress, 5-9 September 2016, Beijing China, 54.
- Hajati, H., and M. Rezaei. 2010. "The application of prebiotics in poultry production." *International Journal of Poultry Science* 9 (3): 298–304.
- Hajati, H., A. Hasanabadi, and F. Ahmadian. 2014a. Application of Medicinal Plants in Poultry Nutrition. *Journal of Medicinal Plants and By-Products*. 3 (1): 1–12.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. Nassiri. 2014b. "The effect of in ovo injection of grape seed extract and vitamin C on hatchability, antioxidant activity, yolk sac weight, performance and ileal micro flora of broiler chickens." *Research Opinions in Animal and Veterinary Sciences*. 4 (12): 633-38.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. R. Nassiri. 2015a. "The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and HSP70 gene expression of broiler chickens suffering from chronic heat stress." *Italian Journal of Animal Science* 14 (3): 3273.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. R. Nassiri. 2015b. "The effect of grape seed extract and vitamin C feed supplements carcass characteristics, gut morphology and ileal microflora in broiler chickens exposed to chronic heat stress." *Iranian Journal of Applied Animal Science*. 5 (1): 155-165.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. R. Nassiri. 2018. "The Effect of Grape Seed Extract Supplementation on Performance, Antioxidant Enzyme Activity, and Immune Responses in Broiler Chickens Exposed to Chronic Heat Stress." *Iranian Journal of Applied Animal Science*. 8 (1): 109-117.

- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, M. R. Nassiri, and R. Safari. 2014c. "Antibacterial characteristics of grape seed extract and nano-grape seed extract in in vitro and in vivo assays." *Journal of Animal and Poultry Sciences* 3 (4): 117–25.
- Hajati, H., A. Hassanabadi, and P. W. Waldroup. 2011. "Effects of dietary supplementation with pumpkin oil (*Cucurbita pepo*) on performance and blood fat of broiler chickens during finisher period." *American Journal of Animal and Veterinary Sciences* 6 (1): 40–44.
- Hajati, H., A. Hassanabadi, and A. T. Yansari. 2012. "Effect of citrus pulp on performance and some blood parameters of broiler chickens." 1st International and The 4th National Congress on Recycling of Organic Waste in Agriculture, Isfahan, Iran, April 26–27, 2012.
- Hashemi, S. R., and H. Davoodi. 2010. "Phytogenics as new class of feed additive in poultry industry." *Journal of Animal and Veterinary Advances* 9 (17): 2295–304.
- Hernandez, F., J. Madrid, V. Garcia, J. Orengo, and M. D. Megias. 2004. "Influence of two plant extracts on broilers performance, digestibility, and digestive organ size." *Poultry Science* 83 (2): 169–74.
- Hernández-Corona, A., I. Nieves, M. Meckes, G. Chamorro, and B. L. Barron. 2002. Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. *Antiviral Research* 56(3): 279–285.
- Herrero, M., A. Cifuentes, and E. Ibáñez. 2006. "Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review." *Food chemistry* 98 (1): 136–48.
- Holdt, S. L., and S. Kraan. 2011. "Bioactive compounds in seaweed: functional food applications and legislation." *Journal of Applied Phycology* 23 (3): 543–97.
- Hu, Q. 2004. "Industrial Production of Microalgal Cell-Mass and Secondary Products - Major Industrial Species: *Arthrospira* (*Spirulina*) *Platensis*". *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, Editor: Amos Richmond, Blackwell Publishing Ltd, Chapter 12: 264–272.
- Huffman, M. A. 2001. "Self-Medicative Behavior in the African Great Apes: An Evolutionary Perspective into the Origins of Human Traditional Medicine: In addition to giving us a deeper understanding of our closest living relatives, the study of great ape self-medication provides a window into the origins of herbal medicine use by humans and promises to provide new insights into ways of treating parasite infections and other serious diseases." *AIBS Bulletin* 51 (8): 651–61.

- Iwamoto, H. 2004. "Industrial production of microalgal cell-mass and secondary products-major industrial species: *Chlorella*". *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, Editor: Amos Richmond, Blackwell Publishing Ltd, Chapter 11: 253-263.
- Ji, H. F., X. J. Li, and H. Y. Zhang. 2009. "Natural products and drug discovery: can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia?" *EMBO Reports* 10 (3): 194-200.
- Khaksar, V., A. Golian, H. Kermanshahi, A. R. Movasseghe, and A. Jamshidi. 2008. "Effect of prebiotic ferment on gut development and performance of broiler chickens fed diet low in digestible amino acids." *Journal of Animal and Veterinary Advances* 7:251-57.
- Khalaji, S., M. Zaghari, K. H. Hatami, S. Hedari-Dastjerdi, L. Lotfi, and H. Nazarian. 2011. "Black cumin seeds, *Artemisia leaves* (*Artemisia sieberi*), and *Camellia L.* plant extract as phytogetic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population." *Poultry Science* 90 (11): 2500-10.
- Khalaji, S., M. Zaghari, and S. Nezafati. 2011. "The effects of mannan-oligosaccharides on cecal microbial populations, blood parameters, immune response and performance of broiler chicks under controlled condition." *African Journal of Biochemistry Research* 5 (5): 160-64.
- Klose, V., M. Mohnl, R. Plail, G. Schatzmayr, and A. P. Loibner. 2006. "Development of a competitive exclusion product for poultry meeting the regulatory requirements for registration in the European Union." *Molecular nutrition and food research* 50 (6): 563-71.
- Kools, S. A., J. F. Moltmann, and T. Knacker. 2008. "Estimating the use of veterinary medicines in the European union." *Regulatory Toxicology and Pharmacology* 50 (1): 59-65.
- Kovac, D. J., J. B. Simeunovic, O. B. Babic, A. C. Mišan, and I. L. Milovanovic. 2013. "Algae in food and feed." *Food Feed Research* 40 (1): 21-31.
- Kumar, M., V. Kumar, D. Roy, R. Kushwaha, and S. Vaiswani. 2014. "Application of herbal feed additives in animal nutrition—A review." *International Journal of Livestock Research* 4 (9): 1-8.
- Lee, Y. K. 1997. "Commercial production of microalgae in the Asia-Pacific rim." *Journal of Applied Phycology* 9 (5): 403-411.
- Lee, K. W., H. Everts, H. J. Kappert, J. Van Der Kuilen, A. G. Lemmens, M. Frehner, A. C. Beynen. 2004. "Growth performance, intestinal viscosity, fat digestibility and plasma cholesterol in broiler chickens fed a rye-containing diet without or with essential oil components." *International Journal of Poultry Science* 3 (9): 613-18.

- Leng, R. A., J. H. Stambolie, and R. Bell. 1995. "Duckweed—A potential high-protein feed resource for domestic animals and fish." *Livestock Research for Rural Development* 7 (1): 36.
- Margulis, L. 1981. "Symbiosis in cell evolution: Life and its environment on the early earth." Research supported by NASA, Boston University, and California Institute of Technology. San Francisco, W. H. Freeman and Co. 438 p.
- Momtazan, R., H. Moravej, M. Zaghari, and H. R. Taheri. 2011. "A note on the effects of a combination of an enzyme complex and probiotic in the diet on performance of broiler chickens." *Irish Journal of Agricultural and Food Research*. 50(2): 249–54.
- Moorhead, K., B. Capelli, and G. R. Cysewski. 2011. *Spirulina: Nature's Superfood*. Kailua Kona, HI: Cyanotech Corporation.
- Norambuena, F., K. Hermon, V. Skrzypczyk, J. A. Emery, Y. Sharon, A. Beard, and G. M. Turchini. 2015. "Algae in fish feed: performances and fatty acid metabolism in juvenile Atlantic salmon." *PloS one* 10 (4): e0124042.
- Packer, M. 2009. "Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy." *Energy Policy* 37 (9): 3428–37.
- Pandey, A. K., and S. Kumar. 2013. "Perspective on plant products as antimicrobial agents: A review." *Pharmacologia* 4 (7): 469–80.
- Park, J. H., S. I. Lee, and I. H. Kim. 2018. "Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens." *Poultry Science* 97 (7): 2451–59.
- Patil, V., T. Källqvist, E. Olsen, G. Vogt, and H. R. Gislerød. 2007. "Fatty acid composition of 12 microalgae for possible use in aquaculture feed." *Aquaculture International* 15 (1): 1–9.
- Pittman, J. K., A. P. Dean, and O. Osundeko. 2011. "The potential of sustainable algal biofuel production using wastewater resources." *Bioresource Technology* 102 (1): 17–25.
- Proestos, C., N. Chorianopoulos, G. J. Nychas, and M. Komaitis. 2005. "RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity." *Journal of Agricultural and Food Chemistry* 53 (4): 1190–95.
- Reitan, K. I., J. R. Rainuzzo, and Y. Olsen. 1994. "Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae." *Aquaculture International* 2 (1): 33–48.

- Rezvani, M., M. Zaghari, and H. Moravej. 2012. "A survey on *Chlorella vulgaris* effect's on performance and cellular immunity in broilers." *International Journal of Agricultural Science and Research* 3:9–15.
- Robert, R., G. Parisi, L. Rodolfi, B. M. Poli, and M. R. Tredici. 2001. "Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostrea gigas* larvae." *Aquaculture* 192 (2–4): 333–46.
- Rodríguez-Lecompte, J. C., A. Yitbarek, J. Brady, S. Sharif, M. D. Cavanagh, G. Crow, W. Guenter, J. D. House, and G. Camelo-Jaimes. 2012. "The effect of microbial-nutrient interaction on the immune system of young chicks after early probiotic and organic acid administration." *Journal of Animal Science* 90 (7): 2246–54.
- Rogers, J. N., J. N. Rosenberg, B. J. Guzman, V. H. Oh, L. E. Mimbela, A. Ghassemi, M. J. Betenbaugh, G. A. Oyler, and M. D. Donohue. 2014. "A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales." *Algal research* 4:76–88.
- Romay, C. H., J. Armesto, D. Ramirez, R. González, N. Ledon, and I. Garcia. 1998. "Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae." *Inflammation Research* 47 (1): 36–41.
- Seifi, S., R. Khoshbakht, H. Hajati, and A. Gilani. 2018. Appraisal of purple coneflower (*Echinacea purpurea*) extract on production performance, internal organs, and gut microflora of japanese quail. *Acta Scientiarum. Animal Sciences*, 40. e37230
- Shi, J., J. Yu, J. E. Pohorly, and Y. Kakuda. 2003. "Polyphenolics in grape seeds—biochemistry and functionality." *Journal of Medicinal Food* 6 (4): 291–99.
- Shields, R. J., and I. Lupatsch. 2012. "Algae for Aquaculture and Animal Feeds." *Technikfolgenabschätzung—Theorie und Praxis* 21:23–37.
- Shurin, J. B., R. L. Abbott, M. S. Deal, G. T. Kwan, E Litchman, R. C. McBride, S. Mandal, and V. H. Smith. 2013. "Industrial-strength ecology: trade-offs and opportunities in algal biofuel production." *Ecology letters* 16 (11): 1393–404.
- Sing, S. F., A. Isdepsky, M. A. Borowitzka, and D. M. Lewis. 2014. "Pilot-scale continuous recycling of growth medium for the mass culture of a halotolerant *Tetraselmis* sp. in raceway ponds under increasing salinity: A novel protocol for commercial microalgal biomass production." *Bioresource Technology* 161:47–54.
- Sommer, T. R., W. T. Potts, and N. M. Morriissy. 1990. "Recent progress in the use of processed microalgae in aquaculture." *Hydrobiologia* 204 (1): 435–43.

- Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert. 2006. "Commercial applications of microalgae." *Journal of Bioscience and Bioengineering* 101 (2): 87–96.
- Svircev, Z. 2005. *Mikroalge i cijanobakterije u biotehnologiji*. Novi Sad: Prirodno matematički fakultet.
- Taboada, M. C., R. Millán, and M. I. Miguez. 2013. Nutritional value of the marine algae wakame (*Undaria pinnatifida*) and nori (*Porphyra purpurea*) as food supplements. *Journal of applied phycology* 25(5): 1271–76.
- Taheri, H. R., H. Moravej, A. Malakzadegan, F. Tabandeh, M. Zaghari, M. Shivazad, and M. Adibmoradi. 2010. "Efficacy of *Pediococcus acidilactici*-based probiotic on intestinal Coliforms and villus height, serum cholesterol level and performance of broiler chickens." *African Journal of Biotechnology* 9 (44): 7564–67.
- Taheri, H., F. Tabandeh, H. Moravej, M. Zaghari, M. Shivazad, and P. Shariati. 2009. "Potential probiotic of *Lactobacillus johnsonii* LT171 for chicken nutrition." *African Journal of Biotechnology* 8(21): 5833–37.
- Taherpour, K., H. Moravej, H. R. Taheri, and M. Shivazad. 2012. "Effect of dietary inclusion of probiotic, prebiotic and butyric acid glycerides on resistance against coccidiosis in broiler chickens." *The Journal of Poultry Science* 49 (1): 57–61.
- Taylor, G. 2008. "Biofuels and the biorefinery concept." *Energy policy* 36 (12): 4406–9.
- Van der Spiegel, M., M. Y. Noordam, and H. J. Van der Fels-Klerx. 2013. "Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production." *Comprehensive Reviews in Food Science and Food Safety* 12 (6): 662–78.
- Vonshak, A., Z. Cohen, and A. Richmond. 1985. "The feasibility of mass cultivation of *Porphyridium*." *Biomass* 8 (1): 13–25.
- Walsh, M. J., L. G. Van Doren, D. L. Sills, I. Archibald, C. M. Beal, X. G. Lei, M. E. Huntley, Z. Johnson, and C. H. Greene. 2016. "Algal food and fuel coproduction can mitigate greenhouse gas emissions while improving land and water-use efficiency." *Environmental Research Letters* 11 (11): 114006.
- Wang, R., D. Li, and S. Bourne. 1998. "Can 2000 years of herbal medicine history help us solve problems in the year 2000." *ALLTECHS ANNUAL SYMPOSIUM* 14:168–84.
- Wells, M. L., P. Potin, J. S. Craigie, J. A. Raven, S. S. Merchant, K. E. Helliwell, A. G. Smith, M. E. Camire, and S. H. Brawley. 2017.

- “Algae as nutritional and functional food sources: Revisiting our understanding.” *Journal of Applied Phycology* 29 (2): 949–82.
- Windisch, W., and A. Kroismayr. 2006. “The effects of phytobiotics on performance and gut function in monogastrics.” In *World nutrition forum: The future of animal nutrition*, September 07th-8th, 2006; Vienna, 85–90.
- WHO (World Health Organization). 2003. *Guidelines for safe recreational water environments: Coastal and fresh waters*, Vol. 1. World Health Organization. Printed in Malta.
- Xia, L., S. Song, Q. He, H. Yang, and C. Hu. 2014. “Selection of microalgae for biodiesel production in a scalable outdoor photobioreactor in north China.” *Bioresource Technology* 174:274–80.
- Yalçinkaya, I., M. Çinar, E. Yildirim, S. Erat, M. Başalan, and T. Güngör. 2012. “The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters.” *Italian Journal of Animal Science* 11 (3): e55.
- Yang, Y., P. A. Iji, M. Choct. 2009. “Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics.” *World’s Poultry Science Journal* 65 (1): 97–114.
- Zaghari, M., N. Zahroojian, M. Riahi, and S. Parhizkar. 2015. “Effect of *Bacillus subtilis* spore (GalliPro®) nutrients equivalency value on broiler chicken performance.” *Italian Journal of Animal Science* 14 (1): 3555.
- Zheng, W., and S. Y. Wang. 2001. “Antioxidant activity and phenolic compounds in selected herbs.” *Journal of agricultural and food chemistry* 49 (11): 5165–70.

CHAPTER TWO

SPIRULINA REARING CONDITION

“Patience is the secret to good food”

—Gail Simmons

2.1 Introduction

There are more than 25,000 species of algae in the world. *Spirulina* is an aquatic organism that has existed for the past 3.5 billion years (Henrikson 2010; Christaki 2014; Usmani et al. 2015). It consumes carbon dioxide dissolved in the water as a nutrient source for its propagation. In fact, *Spirulina* is a photosynthesizing cyanophyte that grows in intense sunshine under high temperatures and alkaline conditions. *Spirulina* has been consumed by different societies as food for centuries and only rediscovered in recent years (Henrikson 2010). It is well documented that *Spirulina* was used as food for the Aztecs and other Mesoamericans during the 16th century (Affan et al. 2015). Today, large farms of *Spirulina* are located in Hawaii, California, China, Thailand, Mexico, India, and at a smaller level in France, Australia, Cuba, Peru, Brazil, Washington (Henrikson 2010) and Iran. The aim of this chapter is to provide an overview of *Spirulina* algae growth and proliferation requirements.

2.2 Morphology and taxonomy

Spirulina was first isolated by P. J. Turpin from freshwater in 1827 (Ciferri 1983). It is a symbiotic, autotrophic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from the air. Figure 2-1 illustrates the microscopic view of the microalgae *Spirulina*.

Spirulina is rod- or disk-shaped. It contains photosynthetic pigments such as phycocyanin, which is blue in color, chlorophyll and carotenoids. Some *Spirulina* contain the pigment phycoerythrin (PE), giving them a red or pink color (Madhyastha and Vatsala 2007; Kamble et al. 2013). *Spirulina*

reproduce by binary fraction as asexual reproduction (Habib et al. 2008). Figure 2-2 shows the life cycle of *Spirulina* spp.

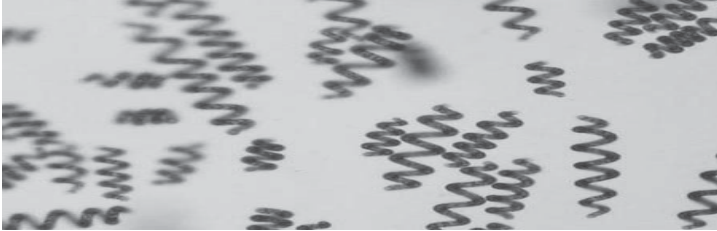


Figure 2-1: Microscopic view of the microalgae *Spirulina*. (Photograph by E. Koru 2014)

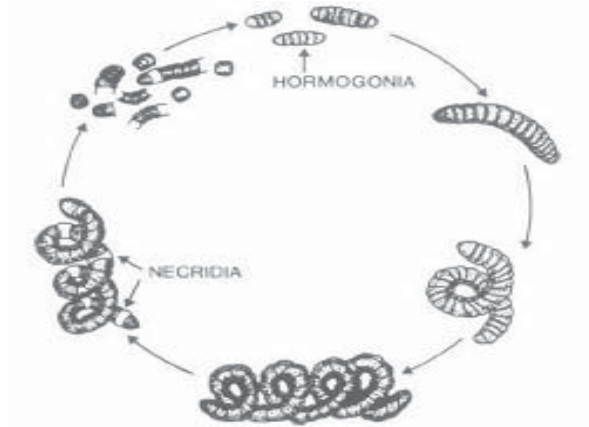


Figure 2-2: Life cycle of *Spirulina* spp. (Ciferri 1983)

Spirulina cells have vacuoles filled with gas that help them to float. Their trichome length is about 50 to 500 μm with a width of 3 to 4 μm . *Spirulina* has a cell wall similar to that of gram-negative bacteria. The cell wall contains peptidoglycan—a heteropolymer resistant to lysozyme treatment (Drews 1973). *Spirulina* also possess lipopolysaccharides (LPS) (Weise et al. 1970; Weckesser et al. 1974; Stanier and Bazine 1977; Weckesser, Drews, and Mayer 1979)—a property of most gram-negative bacteria (Tornabene et al. 1985). *Spirulina* has a flat body surface without cover so it can easily be digested by enzymatic systems (Habib et al. 2008). Stizenberger (1852) reported the first taxonomic manuscript. These microalgae were classified into two genera: *Spirulina* and *Arthrospira*

(Castenholz and Waterbury 1989). It was found that *Spirulina* and *Arthrospira* had different helix types, pores distribution in the cell walls, septos under light microscopy, diameters and fragmentation types of trichomes (filaments) (Gugliemi, Rippka, and Tandeau De Marsac 1993; Vonshak and Tomaselli 2000).

Arthrospira maxima and *Arthrospira platensis* are the most important species in the *Arthrospira* genus, and taxonomic differences in filaments, vacuoles and external cover or capsule regularity of each filament may be seen in these microalgae (Tomaselli 1997). The genus *Arthrospira* is used as food with excellent effects on the health state; however, in some cases it is called *Spirulina* (Sánchez et al. 2003), and it is difficult to recognize the exact effects of these two algae genera in researchers' findings. The worldwide investigation on these microalgae has therefore been carried out under the name of "*Spirulina*" (Habib et al. 2008).

The *Arthrospira* (*Spirulina*) species show great plasticity in morphology, which is attributed to environmental factors such as temperature and some physical and chemical factors and genetic change (Koru 2012). The non-symmetrical presence of a capsule around the filaments in *S. platensis* is a differentiating morphological characteristic compared with *S. maxima* (Ciferri 1983; Fox 1996; Belay 1997; Tomaselli 2002). Figures 2-3 and 2-4 show a scanning electron micrograph (SEM) image of a trichome of axenic *S. platensis* and a SEM image of non-axenic trichomes of *S. maxima*, respectively.

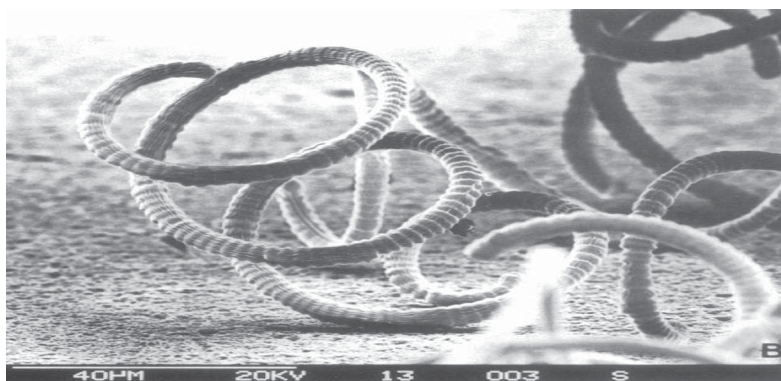


Figure 2-3: SEM image of a trichome of axenic *S. platensis* (Ciferri 1983, photograph by R. Locci).

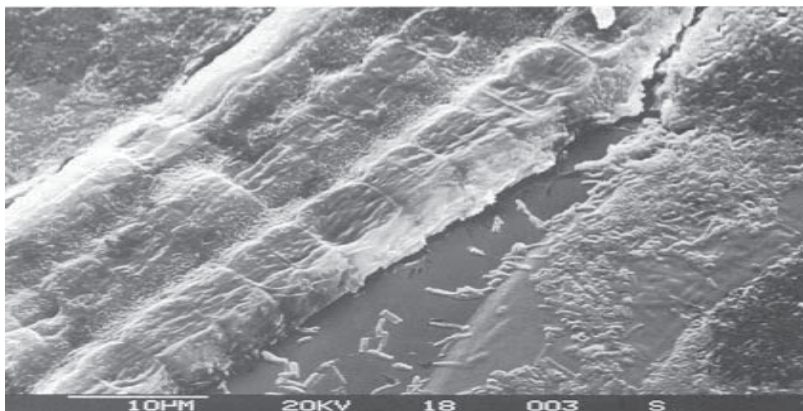


Figure 2-4: SEM image of non-axenic trichomes of *S. maxima* (Ciferri 1983, photograph by R. Locci).

Spirulina spp. belongs to the kingdom Monera (Chu 2012), class Cyanophyceae. This species is included in the Nostocales order and Oscillatoriaceae family (Moreira 2009). It seems *S. platensis*, which is primarily present in Africa, is a species that is more widely distributed. It is also found in Asia and South America. *S. maxima*, however, is mainly present in Central America. It is the main component of the phytoplankton of Lake Texcoco, which could be regarded as the original habitat of *S. maxima* (Durand-Chastel 1980). *S. platensis* mainly present in the alkaline saline lakes of the semidesert Sudan-Sahel zone, with its epicenter in Lake Chad, and the Rift Valley, which are considered the starting points of this species (Iltis 1970, 1971, 1980; Marty and Busson 1970).

2.3 *Spirulina* species

It is well documented that *Spirulina* algae includes the following species:

- 1) *Spirulina platensis* (Gomont) (*Arthrospira fusiformis*) (Voronichin)
- 2) *S. platensis* NIES-39
- 3) *S. platensis* Geitler
- 4) *S. platensis* (Nordstedt) Geitler
- 5) *S. subsalsa* fo. *versicolour* (Cohn) Koster
- 6) *S. subsalsa* Oersted
- 7) *S. maxima* (as *S. geitleri*) (Setch. et Gardner)
- 8) *S. subsalsa* Oersted ex Gomont
- 9) *S. major* Kützing

- 10) *S. texcoco*
- 11) *S. crater*
- 12) *Arthrospira fusiformis* (Voronichin)
- 13) *A. maxima*
- 14) *A. jenneri* (Kützing) Stitz
- 15) *S. labyrinthiformis*
- 16) *S. laxissima*
- 17) *S. lonar*
- 18) *S. nodosa*
- 19) *S. princeps* West and West
- 20) *S. laxa* G.M. Smith (Habib et al. 2008).

Table 2-1 shows the results of comparing the chemical composition of four different strains of *Spirulina* algae.

Table 2-1: Chemical composition of four different strains of *Spirulina* algae (Charpy and Larkum 1999).

<i>Spirulina</i> strain	<i>S. platensis</i>	<i>S. maxima</i>	<i>S. texcoco</i>	<i>S. crater</i>
Crude proteins	55-60	49-54	53-58	56-58
Total	15-18	13.5-15	10-12	11-13
Carbohydrates				
Total lipids	7.5-12	7.5-12	7.3-9.5	7.2-9.7
Ash	7-9	7-8.5	8-10	7-8.5
Moisture	7-8	7-8	7-8	7-8
Carotenoids	0.20-0.29	0.30-0.35	0.15-0.21	0.15-0.20
Chlorophyll α	0.7-0.8	0.67-0.73	0.70-0.78	0.70-0.80

2.4 Effective factors on the growth of *Spirulina*

2.4.1 Growth medium

The medium utilized in several centers of *Spirulina* production is the medium first developed by Zarrouk for *Spirulina* culture with little modification. *Spirulina* need a medium of high alkalinity at pH 10–12 (Capelli and Cysewski 2010) and a steady supply of bicarbonates ions (Ciferri 1983). Stanca and Popovici (1996) found that the use of urea as a nitrogen source in *Spirulina platensis* media increased both the total algae biomass and the biomass chlorophyll content. Tri-Panji and Suharyanto (2001) reported that *Spirulina* had the best growth (0.350 g biomass/L) in media containing C: N: P: Mg at the ratio of 1:3:0.3:0.2, respectively.

2.4.2 Carbon source

It was reported that organic compounds as the sole carbon source could not support the growth of *Spirulina* (Ogawa and Teuri 1970). Gupta and Bajaj (1983) found that media without sodium chloride fertilized with extracts of different organic manures caused a 5% to 30% increase in dry matter production of *Spirulina subsalsa* and *Spirulina platensis*. Costa, Colla, and Duarte Filho (2003) reported that *S. platensis* production in pond water was 0.78 ± 0.01 g/l (dry weight basis); however, supplementing it with 2.88 g sodium bicarbonate/L resulted in 0.82 ± 0.01 g/L after 40 hours.

2.4.3 Temperature

The temperature range of 30–35°C is optimal for maximum growth of *Spirulina platensis*. Rafiqul Islam et al. (2003) calculated the growth rate of *Spirulina platensis* and *Spirulina fusiformis* in different temperatures by using the following formula: $\text{growth rate} = \ln x_2 - \ln x_1 / t_2 - t_1$, in which x_1 and x_2 were algae biomass concentrations and t_1 and t_2 were time intervals. They reported that *Spirulina platensis* has a maximum specific growth rate (0.141) at 32°C and the maximum growth rate of *Spirulina fusiformis* (0.144) was seen at 37°C. The effect of temperature on *Spirulina platensis* and *Spirulina fusiformis* growth rates is shown in Figure 2-5. Colla, Bertolin, and Costa (2004) reported that temperature was the most important factor in production yield, and the greatest amount of gamma-linolenic acid (GLA) was obtained at 30°C.

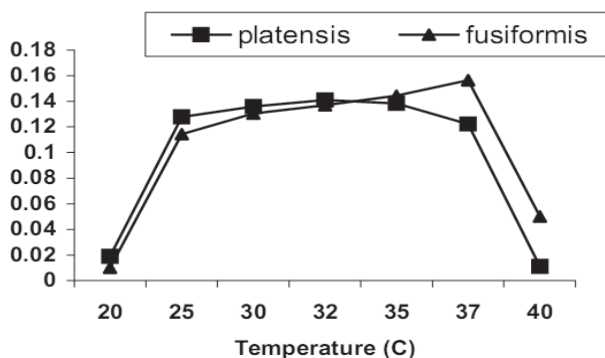


Figure 2-5: Growth rates of *S. platensis* and *S. fusiformis* at different temperatures. The values are means of three replicates. (Rafiqul Islam et al. 2003)

2.4.4 Water quality

Water quality refers to its salinity, pH, temperature, *total dissolved solids (TDS)*, hardness, heavy metals, microorganisms and electrical conductivity (EC). Argaman and Spivak (1974) reported that water quality may affect the solubility of nutrients added in the medium and also selective accumulation of certain heavy metals by algae during the growth phase. It should be noted that excessive algal biomass will cause problems such as oxygen depletion, high ammonia levels, release of toxins in the water column and the production of odorous compounds (Charpy and Larkum 1999).

2.4.5 Light

Subramaniyan and Jeeji Bai (1992) found that blue light yielded highest protein content followed by yellow, white, red and green lights. Also, white fluorescent light with a red-orange component provided higher energy for better protein and pigment synthesis. For selection of an artificial light source in order to produce microalgae, the producer should note some parameters such as light wave length, light intensity and frequency, electrical efficiency, heat increment, longevity and cost (Carvalho et al. 2011). A wave length of about 400–700 nm is appropriate for microalgal growth, and wavelengths within 600–700 nm are the most efficient for photosynthesis (Carvalho et al. 2011). Table 2-2 shows the main pigments of *Spirulina* and their light absorption wavelengths.

Spirulina platensis reached maximum growth with urea in the light intensity of $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, at 27°C . The maximum biomass was 1648 mg L^{-1} . The microalgae minimum growth occurred with the use of KNO_3 in the light intensity of $15 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and a temperature of 30°C . The minimum biomass was 486 mg L^{-1} (Danesi et al. 2011). The relation between light irradiance and photosynthetic rate is shown in Figure 2-6. There are three important points in Figure 2-6: I_c : compensation light intensity, which shows that the photosynthesis and therefore algae growth increase with increasing light intensity; I_s : light saturation, which shows the algae growth reach plateau; and I_h : light inhibition, which shows a negative effect of light intensity on algae growth rate. It is therefore clear that light irradiance higher than a certain amount needed to reach the plateau of algae growth can lead to reduction in algae growth and may even cause cell death (Carvalho et al. 2011).

Table 2-2: Main pigments of *Spirulina* microalgae. (Carvalho et al. 2011)

Pigment	color	range of absorption wavelength (nm)	nature	pigments
Chlorophylls	green	450-475 630-675	hydrophobic	Chlorophyll a Chlorophyll b Chlorophyll c ₁ , c ₂ , d
Phycobilins	blue, red	500-650	hydrophilic	phycocyanin phycoerythrin allophycocyanin
Carotenoids	yellow, orange	400-550	hydrophobic	β -carotene a-carotene lutein violaxanthin fucoxanthin

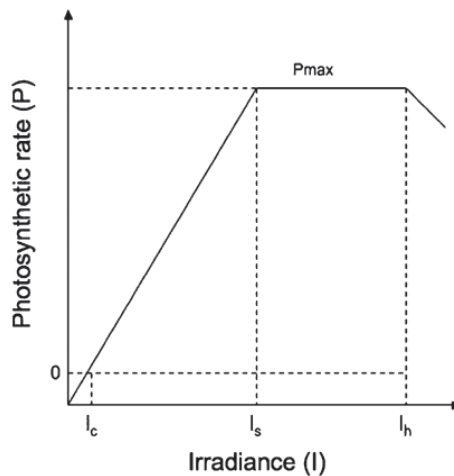


Figure 2-6: The relation between light irradiance and photosynthetic rate. (Carvalho et al. 2011)

2.4.6 pH

Vincent and Silvester (1979) reported that the pH of water had a direct effect on the physiological properties of algae. It grew well at pH values of 9–11. Pandey et al. (2010) found that at pH 9 the dry weight of *Spirulina platensis* was 0.91 g/500 ml, and protein and chlorophyll contents were 64.3% and 13.2 mg/gm, respectively.

2.4.7 Agitation

Agitation of algal cultures had the advantages of uniform distribution of CO₂ and prevention of thermal stratification. Aeration by rotators provides agitation of growing cells to maintain the cells in suspension. In fact, agitation is an optimum way to get good quality and better yields of *Spirulina* species (Dubey 2006).

2.4.8 Contamination

Insects may be a potential source of *Spirulina platensis* media contamination (Venkataraman and Sindhukanya 1981). It was reported that the mosquito larvae fed on the algal decreased the yield of algae production up to 10%. Mahadevaswamy and Venkataraman (1987) reported the presence of bacterial contaminants in outdoor cultivation of *Spirulina platensis*. Rainfall may cause the contamination of outdoor open ponds. It was reported that contamination by green algae and bacteria usually occur in these ponds (Ang 2004).

2.4.9 Nutritional culture

Previous researchers studied the effect of the nutritional sources on the growth rate of cyanobacteria (Faintuch et al. 1991). They found that mixtures of KNO₃, urea and ammonia-N had significant effect on the growth of *Spirulina maxima*. The highest growth rate of *S. platensis* was seen in the presence of 2.57 g KNO₃/liter. Its growth rate was about 0.3–0.4/day. Chang et al. (1999) stated the possibility of using nitrifying bacteria for providing nitrogen in *Spirulina* mass culture. It is interesting to notice that *Spirulina* may growth in different agro-industrial wastes such as sugar mill waste effluent, poultry industry waste, fertilizer factory waste, urban waste, and organic matter (Habib et al. 2008).

2.5 Small scale production

Spirulina production in small quantities is considered as a potential income-generating activity for families or small farms (Figure 2-7). *Spirulina* can be used as a valuable supplement in human and animal diets (Habib et al. 2008). The growth media of *Spirulina* are the cause of the major cost of small-scale production. Raoof, Kaushik, and Prasanna (2006) studied the use of a new medium formulated for mass production of *Spirulina sp.* by using Zarrouk's standard medium and other cost-effective substituted chemicals. This medium contained single super phosphate (1.25 g/liter), sodium nitrate (2.50 g/liter), muriate of potash (0.98 g/liter), sodium chloride (0.50 g/liter), magnesium sulfate (0.15 g/liter), calcium chloride (0.04 g/liter) and sodium bicarbonate (commercial grade—8 g/liter). Economic evaluation revealed the cost of the preparation of 1,000 liters of Zarrouk's medium (US\$79.50) compared to Rs. 736 (US\$16.00) for the revised medium. *Spirulina* production cost involves some factors such as growth rate of the algae, rate of culture contamination, harvest efficiency and farm management (Ang 2004).



Figure 2-7: *Spirulina* production in small scale. (Photograph by authors)

2.6 Commercial cultivation

Today, there are three species of algae that are considered in commercial production: *Chlorella*, *Spirulina* and *Dunaliella*. *Spirulina* may be the species that is most widely cultivated commercially (Jiménez et al. 2003). There has been over six decades of intensive ecological and physiological research in large-scale production of *Spirulina*. Nowadays, commercial

production of this algae is based almost exclusively on open ponds of the raceway type (Tredici 2004); however, some companies use closed tubular bioreactors (Spektorova et al. 1997). Japan was the first country that cultured *Chlorella* in large-scale media in the early 1960s; this was followed by *Spirulina* in the early 1970s at Lake Texcoco, Mexico (Habib et al. 2008). Large-scale cultivation of *Spirulina* is usually carried out in shallow ponds equipped with paddle wheels for mixing the culture. Two types of open raceway ponds are typically used: the first is lined by concrete and is therefore expensive, the second is a shallow earthen tunnel lined with polyvinyl-chloride (PV-C) or other alternative durable plastic material (Habib et al. 2008). Mata et al. (2010) reported that the highest productivity obtained for *Chlorella* and *Spirulina* was 7.70 g/L per day and 130 g/ m² per day for *Chlorella*, and 4.3 g/m² per day and 51 g/L per day for *Spirulina*. It is necessary to consider the production cost in large-scale production of *Spirulina* algae.

2.7 Universal *Spirulina* production

Lakes containing *Spirulina* are found in Peru, Chile, Myanmar, Australia and across the Sahara and East Africa (Henrikson 2010). The United States of America has a number of the largest intensive farms in the world, mainly based in Hawaii and California (Habib et al. 2008). *Spirulina* has been approved for consumption as a human food by many countries, states and territories such as Argentina, Australia, Bahrain, the Bahamas, Bangladesh, Belarus, Belgium, Brazil, Bulgaria, Canada, Chad, Chile, China, Colombia, Costa Rica, Croatia, the Czech Republic, Denmark, Egypt, Ethiopia, Finland, France, Germany, Greece, Guam, Haiti, Hong Kong, Hungary, India, Iceland, Indonesia, Ireland, Israel, Italy, Jamaica, Japan, Kenya, South Korea, Kuwait, Liechtenstein, Luxembourg, Macedonia, Malaysia, Mexico, Myanmar, Monaco, the Netherlands, New Zealand, Nigeria, Norway, Peru, the Philippines, Poland, Portugal, Romania, Russia, Saudi Arabia, Singapore, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Togo, Turkey, Ukraine, the United Kingdom, the USA, Venezuela, Vietnam and Zimbabwe (Henrikson 2010). *Spirulina* is an interesting edible substance because of its activity against *acquired immune deficiency syndrome* (AIDS), lipidemia, obesity and diabetes, and for its high nutritive value that can help human and animal health states, for its use as biofuel, its usage as bio-fertilizer and other uses (Henrikson 2010). Figures 2-8, 2-9, 2-10 and 2-11 show *Spirulina* production in India, California, the Czech Republic and Israel, respectively.



Figure 2-8: *Spirulina* production in India. (Photograph by Borowitzka)

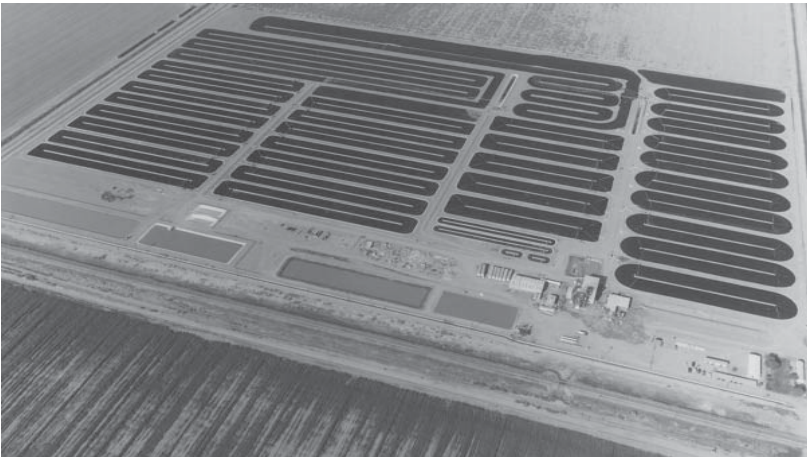


Figure 2-9: *Spirulina* production in California. (Photograph by Borowitzka)

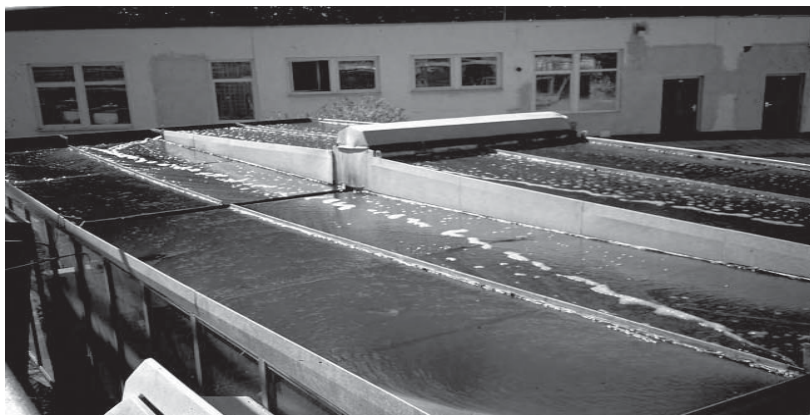


Figure 2-10: *Spirulina* production in the Czech Republic. (Photograph by Borowitzka)

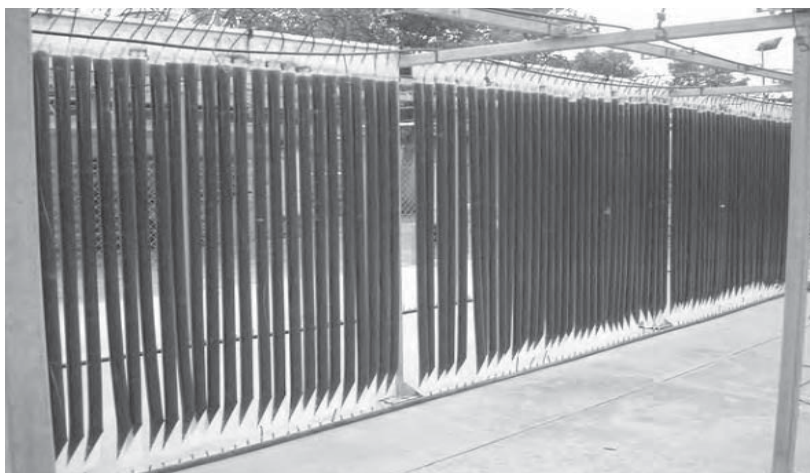


Figure 2-11: *Spirulina* bag culture in Israel. (Photograph by Borowitzka)

2.8 *Spirulina* harvesting and processing

Spirulina harvesting involves the following stages:

- a. Filtration and cleaning: A filter is necessary at the entrance of the water pond (Figure 2-12);

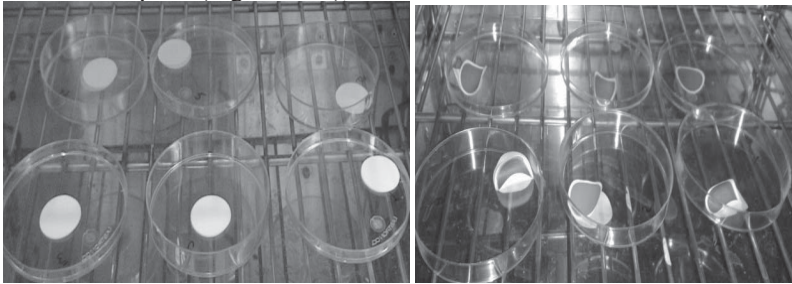


Figure 2-12: Using filter 0.45 μ for evaluating *Spirulina* biomass. (Photograph by authors)

- b. Washing: This stage is necessary to reduce salts content;
- c. Neutralization: for neutralizing the biomass with the addition of an acid solution;
- d. Disintegration: to break down trichomes with a grinder;
- e. Dehydration by spray-drying: This step has high economic importance because it involves about 20–30% of the production cost;
- f. Packing: It is usually packed in sealed plastic bags to avoid hygroscopic action on the dry *Spirulina*;
- g. Storage: It is stored in fresh, dry, unlit, pest-free and hygienic storerooms to prevent pigments deteriorating (Ayala 1998).

Figure 2-13 shows the *Spirulina* production pathway:

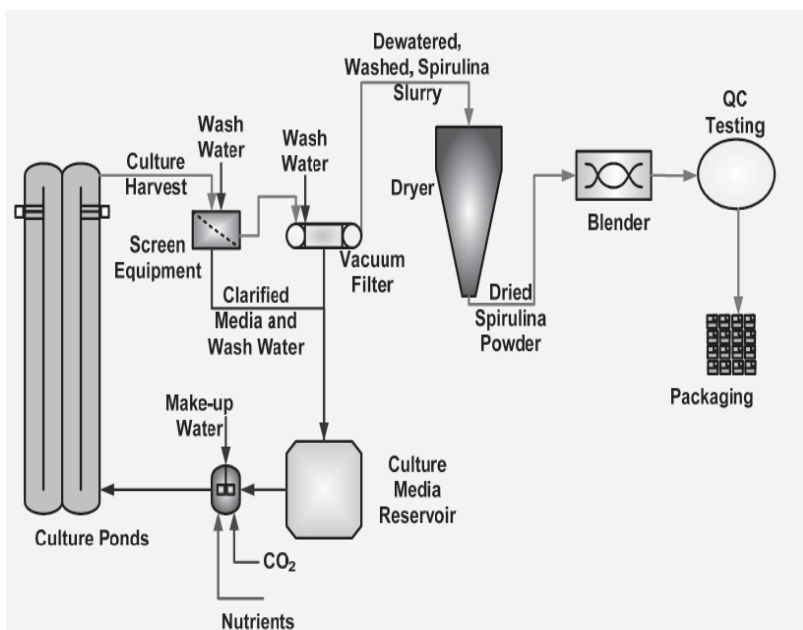


Figure 2-13: *Spirulina* production flow chart. (Cysewski 2010)

References

- Affan, M. A., D. W. Lee, S. M. Al-Harbi, H. J. Kim, N. I. Abdulwassi, S. J. Heo, C. Oh, H. S. Park, C. W. Ma, H. Y. Lee, and D. H. Kang. 2015. "Variation of *Spirulina maxima* biomass production in different depths of urea-used culture medium." *Brazilian Journal of Microbiology* 46 (4): 991–1000.
- Ang, P. O. 2004. Asian Pacific Phycology in the 21st century: Prospects and challenges. Proceedings of The Second Asian Pacific Phycological Forum, held in Hong Kong, China, 21-25 June 1999. Reprinted from *Hydrobiologia*. 1st ed, 2004. Springer Science+Business Media Dordrecht. ISBN 978-94-007-0944-7.
- Argaman, Y. and E. Spivak. 1974. "Wastewater as source of algae." *Water Resources* 8:317.
- Ayala, F. 1998. "Guía sobre el cultivo de *Spirulina*." *Biotechnología de Microorganismos Fotoautótrofos* 3–20.
- Belay, A. M. H. A. 1997. "Mass culture of *Spirulina* outdoors—the Earthrise Farms experience." *Spirulina platensis* (Arthrospira):

- physiology, cell-biology and biotechnology. Edited by Avigad Vonshak. Taylor and Francis Ltd. Chapter 8, pp 131–58. ISBN 0-7484-0674-3.
- Borowitzka, M. A. Commercialising slime commercial Production of fine chemicals and biofuels from microalgae (cont'd). PPT Pamphlet by Algae R and D Center, Murdoch University. Muradel Pty Ltd.
- Capelli, B., and G. R. Cysewski. 2010. "Potential health benefits of spirulina microalgae." *Nutrafoods* 9 (2): 19–26.
- Carvalho, A. P., S. O. Silva, J. M. Baptista, and F. X. Malcata. 2011. "Light requirements in microalgal photobioreactors: an overview of biophotonic aspects." *Applied Microbiology and Biotechnology* 89 (5): 1275–88.
- Castenholz, R. W., and J. B. Waterbury. 1989. "Oxygenic photosynthetic bacteria. Section 19." In *Bergey's Manual of Systematic Bacteriology*, Vol. 3, edited by J. T. Staley, M. P. Bryant, N. Pfennig, and J. G. Holt, 1710–806. Baltimore, MD: Williams and Wilkins Co.
- Chang, Z. Z., W. B. Zhu, M. X. Ye, Y. Fang, and J. Y. Zhang. 1999. "The possibility of nitrifying bacteria inoculation in Spirulina mass culture." *Jiangsu Journal of Agricultural Sciences* 15:191–92.
- Charpy, L., and A. W. D. Larkum. 1999. Marine cyanobacteria. *Ô Musée océanographique, Monaco* (numéro spécial 19). Pp 640. ISBN 2-7260-0210-2.
- Christaki, E. 2014. "Microalgae as a Potential New Generation of Material for Various Innovative Products." *Oceanography* 1:e106.
- Chu, W. L. 2012. "Biotechnological applications of microalgae." *IeJSME* 6 (1): S24–S37.
- Ciferri, O. 1983. "Spirulina, the edible microorganism." *Microbiological Reviews* 47 (4): 551.
- Colla, L. M., T. E. Bertolin, and J. A. V. Costa. 2004. "Fatty Acids Profile of Spirulina platensis Grown Under Different Temperatures and Nitrogen Concentrations." *Zeitschrift für Naturforschung C* 59 (1–2): 55–59.
- Costa, J. A. V., L. M. Colla, and P. Duarte Filho. 2003. "Spirulina platensis Growth in Open Raceway Ponds Using Fresh Water Supplemented with Carbon, Nitrogen and Metal Ions." *Zeitschrift für Naturforschung C* 58 (1–2): 76–80.
- Cysewski, G. R. 2010. Commercial Production of Spirulina. PPT pamphlet by Cyanotech. Pp 24.
- Danesi, E. D. G., C. O. Rangel-Yagui, S. Sato, and J. C. M. D. Carvalho. 2011. "Growth and content of Spirulina platensis biomass chlorophyll cultivated at different values of light intensity and temperature using

- different nitrogen sources.” *Brazilian Journal of Microbiology* 42 (1): 362–73.
- Drews, G. 1973. “Fine structure and chemical composition of the cell envelopes.” In Carr, . G. & Whitton, B. A. (Eds.). *The Biology of Blue-Green Algae. Botanical monographs*. Vol 9, Univ. of Calif. Press, Berkeley, pp. 99-116.
- Dubey, R. C. 2006. *A textbook of Biotechnology*. 4th ed., S. Chand & Co P Ltd, New Delhi, p. 732. ISBN 81-219-2608-4.
- Durand-Chastel, H. 1980. “Production and use of *Spirulina* in Mexico.” In *Algae Biomass*, edited by G. Shelef and C. J. Soeder, 39. Amsterdam: Elsevier/North-Holland Biomedical Press.
- Faintuch, B. L., S. Sato, and E. Aquarone. 1991. “Influence of the nutritional sources on the growth-rate of cyanobacteria.” *Arquivos de Biologia e Tecnologia* 34 (1): 13–30.
- Fox, R. D. 1996. *Spirulina: Production and Potential*. Edisud. Axis-en-Provence, France, 232 pp.
- Gugliemi, G., R. Rippka, and N. Tandeau De Marsac. 1993. “Main properties that justify the different taxonomic position of *Spirulina* spp. and *Arthrospira* spp. among cyanobacteria.” *Bulletin de l'Institut océanographique* 13–23.
- Gupta, R. S. and A. Bajaj. 1983. “Halo-tolerant Blue-green algae and their role as fertilizer.” *Advanced Applied Phycology* 22:309–21.
- Habib, M. A. B., M. Parvin, T. C. Huntington, and M. R. Hasan. 2008. “A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish.” FAO Fisheries and Aquaculture Circular no. 1034. Rome: Food and Agriculture Organization of the United Nations.
- Henrikson, R. 2010. *Spirulina World Food: How this micro algae can transform your health and our planet*. Ronore Enterprises, Incorporated. Ronore Enterprises, Inc., Hana, Maui, Hawaii.
- Iltis, A. 1970. Phytoplankton des eaux natronées du Kanem (Tchad): 4. Note sur les espèces du genre *Oscillatoria*, sous-genre *Spirulina* (Cyanophyta). *Cahiers ORSTOM. Série Hydrobiologie*, 4(3-4), 129-134.
- Iltis, A. 1971. Note sur *Oscillatoria* (sous-genre *Spirulina*) *platensis* (Nordst.) Bourrelly (Cyanophyta) au Tchad. *Cahiers ORSTOM. Série Hydrobiologie*, 5(1): 53-72.
- Iltis, A. 1980. “Ecologie de *Spirulina platensis* dans les milieux natronés d’Afrique sahélienne.” In *Prospettive della Coltura di Spirulina in Italia*, edited by R. Materassi, Rome: CNR. Pp 41-48.

- Jiménez, C., B. R. Cossío, D. Labella, and F. X. Niell. 2003. "The feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain." *Aquaculture* 217 (1–4): 179–90.
- Kamble, S. P., R. B. Gaikar, R. B. Padalia, and K. D. Shinde. 2013. "Extraction and purification of C-phycocyanin from dry *Spirulina* powder and evaluating its antioxidant, anticoagulation and prevention of DNA damage activity." *Journal of Applied Pharmaceutical Science* 3 (8): 149.
- Koru, E. 2012. "Earth food *Spirulina* (*Arthrospira*): production and quality standards." *Food Additive*. Edited by Yehia El-Samragy. InTech. ISBN: 978-953-51-0067-6. Pp:191-202.
- Madhyastha, H. K., and T. M. Vatsala. 2007. "Pigment production in *Spirulina fusciformis* in different photophysical conditions." *Biomolecular Engineering* 24 (3): 301–5.
- Mahadevaswamy, M. and L. V. Venkataraman. 1987. "Bacterial contaminants in blue green alga *Spirulina* produced for use as biomass protein." *Archiv fuer Hydrobiologie* 110 (4): 623–30.
- Marty, F. and F. Busson. 1970. Données cytologiques et systématiques sur *Spirulina platensis* (Gom.) Geitler et *Spirulina Geitleri* J.De Toni. *Schweizerische Zeitschrift für Hydrologie*. 32 (2): 559-65.
- Mata, T. M., A. A. Martins, and N. S. Caetano. 2010. "Microalgae for biodiesel production and other applications: A review." *Renewable and sustainable energy reviews* 14 (1): 217–32.
- Moreira, S. L. 2009. Reactor design for a family production of *Spirulina* spp. and parameters determination for a *Spirulina* spp. culture. Master thesis. Universidade do Porto. Department of Chemical Engineering. 65 pp.
- Ogawa, T and G. Teuri. 1970. Blue green algae *Spirulina*. *Journal of Fermentation Technology*, 48:361.
- Pandey, J. P., N. Pathak, and A. Tiwari. 2010. "Standardization of pH and Light Intensity for the Biomass Production of *Spirulina platensis*." *Journal of Algal Biomass Utilization* 1 (2): 93–102.
- Rafiqul Islam, M., A. Hassan, G. Sulebele, C. Orosco, and P. Roustaian. 2003. "Influence of Temperature on Growth and Biochemical Composition of *Spirulina platensis* and *Spirulina fusiformis*." *Iranian International Journal of Sciences* 4 (2): 97–106.
- Raoof, B., B. D. Kaushik, and R. Prasanna. 2006. "Formulation of a low-cost medium for mass production of *Spirulina*." *Biomass and Bioenergy* 30 (6): 537–42.

- Sánchez, M., J. Bernal-Castillo, C. Rozo, and I. Rodríguez. 2003. "Spirulina (Arthrospira): an edible microorganism: a review." *Universitas Scientiarum* 8 (1): 7–24.
- Spektorova, L., R. L. Creswell, and D. Vaughn. 1997. "Closed tubular cultivators: an innovative system for commercial culture of macroalgae." *World Aquaculture baton rouge*. 28:39–44.
- Stanca, D and E. Popovici. 1996. "Urea as nitrogen source in modified Zarrouk medium." *Reviews in Biology* 41:25–31.
- Stanier, R. Y., and G. C. Bazine. 1977. "Phototrophic Prokaryotes: The Cyanobacteria." *Annual Review of Microbiology* 31 (1): 225–74.
- Stizenberger, E. 1852. *Spirulina und Arthrospira* (nov. gen.). *Hedwigia*, 1, 32-34.
- Subramaniam, S. K. and N. J. Bai. 1992. "Effect of different nitrogen levels and light quality on growth, protein and synthesis in *Spirulina fusiformis*." In Proceedings of the *Spirulina* ETTA National Symposium, MCRC, Madras [Chennai], 97–99.
- Supramaniam, S. K., & Bai, N. J. (1992). Effect of different nitrogen levels and light quality on growth, protein and synthesis in *Spirulina fusiformis*. In *Proc. Spirulina ETTA National Symposium, MCRC, Madras* (pp. 97-99).
- Tomaselli, L. 1997. "Morphology, ultrastructure and taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*." *Spirulina platensis (Arthrospira): physiology, cell-biology and Biotechnology*, 1–16.
- Tomaselli, L. 2002. "Morphology, Ultrastructure and Taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*." In *Spirulina platensis (Arthrospira) Physiology, Cell Biology and Biotechnology*, edited by A. Vonshak, 1–17. London: Taylor and Francis.
- Tornabene, T. G., T. F. Bourne, S. Raziuddin, and A. Ben-Amotz. 1985. "Lipid and lipopolysaccharide constituents of cyanobacterium *Spirulina platensis* (Cyanophyceae, Nostocales)." *Marine Ecology Progress Series* 121–25.
- Tredici, M. R. 2004. "Mass production of microalgae: photobioreactors." *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*. Edited by A. Richmond. Blackwell Publishing. 1:178–214.
- Tri-Panji and Suharyanto. 2001. "Optimization media from low-cost nutrient sources for growing *Spirulina platensis* and carotenoid production." *Menara Perkebunan* 69 (1): 18–28.
- Usmani, M. A., K. Toppo, S. Nayaka, M. R. Suseela, and S. Sheikh. 2015. Role of algae in sustainable Food, Health and Nutritional Security: An

- Overview. International day conference for biological diversity for sustainable development. 22 May 2015. 83–87.
- Vincent, W. F. and W. B. Silvester. 1979. "Growth of blue-green algae in the Manukau (New Zealand) oxidation ponds—II. Experimental studies on algal interaction." *Water research* .13(8): 717-723.
- Vonshak, A. 1997. Appendices in *Spirulina platensis (Arthrospira): Physiology, Cell Biology and Biotechnology*, edited by A. Vonshak, 213–26. London: Taylor and Francis.
- Vonshak, A. and L. Tomaselli. 2000. Arthrospira (Spirulina): systematics and ecophysiology. In *The ecology of cyanobacteria*. Springer, Dordrecht, pp. 505-522.
- Weckesser, J., G. Drews, H. Mayer. 1979. "Lipopolysaccharides of photosynthetic prokaryotes." *Annual Reviews in Microbiology* 33 (1): 215–39.
- Weckesser, J., A. Katz, G. Drews, H. Mayer, and I. Fromme. 1974. "Lipopolysaccharide containing L-acofriose in the filamentous blue-green alga *Anabaena variabilis*." *Journal of bacteriology* 120 (2): 672–78.
- Weise, G., G. Drews, B. Jann, and K. Jann. 1970. "Identification and analysis of a lipopolysaccharide in cell walls of the blue-green alga *Anacystis nidulans*." *Archiv für Mikrobiologie* 71 (1): 89–98.

CHAPTER THREE

SPIRULINA'S NUTRITIONAL IMPORTANCE

“What you see depends on how you view the world. To most people this is just dirt. To a farmer, this is POTENTIAL.”
Doe Zantamata

3.1 Introduction

Microalgae have the potential to synthesize different bioactive substances which have different effects on physiological processes. These bioactive materials include proteins, carbohydrates, fatty acids, vitamins, minerals and enzymes. In general, *Spirulina* has a higher protein content than other plant protein sources. It digests well in human and animal digestive systems because of the special structure of the *Spirulina* cell wall due to its lack of cellulose. *Spirulina* has high amounts of vitamin ω -3 fatty acids, vitamin B₁₂ (cyanocobalamin), β -carotene, iron, calcium and phosphorous. These microalgae possess acceptable organoleptic characteristics without toxicities (Milledge 2011; Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015). In this chapter, we discuss the chemical composition of *Spirulina*.

3.2 Chemical analysis

The chemical composition of *Spirulina* depends on its growth culture nutrients, rearing condition, harvest time, and drying and extraction method. The usual composition of *Spirulina* is considered as protein: 50–70%; carbohydrates: 15–25%; lipids: 6–13%; nucleic acids: 4.2–6%; and minerals: 2.2–4.8% (Hoseini, Khosravi-Darani, and Mozafari 2013). The values of appearance metabolizable energy (AME) and appearance metabolizable energy corrected for nitrogen (AMEn) for Cobb 500 broiler chickens were reported around 2500 kcal/kg DM and 2900 kcal/kg DM, respectively (Alvarenga et al. 2011). These values are close to the energy levels derived from wheat and barley for broiler, and much more than that from soybean meal (SBM). We evaluated the chemical composition of *Spirulina platensis* in the University of Tehran's nutrition laboratories by routine standard methods (AOAC 1990), as shown in Table 3-1.

Table 3-1: Different reports of *Spirulina platensis* composition.

	Crude protein (%)	Crude fat (%)	Calcium (%)	Phosphorus (%)	Moisture (%)	Ash (%)
Zaghari and Hajati (unpublished data)	64.86±0.31	1.73±0.11	1.02±0.08	1.41±0.09	3.7 ± 0.12	12.51±0.6
Evans, Smith, and Moritz (2015)	76.0	4.95	1.20	1.36	5.10	-
FOI, France	65	4	-	-	-	3
SAC, Thailand	55-70	5-7	-	-	4-6	3-6
IPGSR, Malaysia	61	6	-	-	6	9
BAU, Bangladesh	60	7	-	-	9	11
Kyntäjä et al. (2014)	70.4	7.5	0.18	0.96	6.4	7.5

FOI = French Oil Institute; SAC = Siam Algae Co. Ltd; IPGSR = Institute of Post-graduate Studies and Research laboratory, University of Malaya; BAU = Bangladesh Agricultural University.

Spirulina composition may vary according to the culturing conditions and the methods of analysis. Table 3-2 shows the different nutrients analyses of *Spirulina* reported by Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos (2015).

Table 3-2: Nutritional profile of *Spirulina* Powder (composition by 100 g). (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015)

		Vitamins	
Calories	373	Vitamin A (as β -carotene)	352.000 IU
Total fat (g)	4.3	Vitamin K	1090 mcg
Saturated fat	1.95	Thiamin HCL (Vitamin B ₁)	0.5 mg
Polyunsaturated fat	1.93	Riboflavin (Vitamin B ₂)	4.53 mg
Monounsaturated fat	0.26	Niacin (Vitamin B ₃)	14.9 mg
Cholesterol	<0.1	Vitamin B ₆ (Pyridox. HCL)	0.96 mg
Total carbohydrate (g)	17.8	Vitamin B ₁₂	162 mcg
Dietary fiber	7.7	Minerals	
Sugars	1.3	Calcium	468 mg
Lactose	<0.1	Iron	87.4 mg
Protein B	63	Phosphorus	961 mg
Essential amino acids (mg)		Iodine	142 mcg
Histidine	1000	Magnesium	319 mg
Isoleucine	3500	Zinc	1.45 mg
Leucine	5380	Selenium	25.5 mcg
Lysine	2960	Cooper	0.47 mg
Methionine	1170	Manganese	3.26 mg
Phenylalanine	2750	Chromium	<400 mcg
Threonine	2860	Potassium	1660 mg
Tryptophan	1090	Sodium	641 mg
Valine	3940		
Non-essential amino acids (mg)		Phytonutrients	
Alanine	4590	Phycocyanin	17.2%
Arginine	4310	Chlorophyll	1.2%
Aspartic acid	5990	Super oxide dismutase (SOD)	531.000 IU
Cystine	590	Gamma linolenic acid (GLnA)	1080 mg
Glutamic acid	9130	Total carotenoids	504 mg
Glycine	3130	β -carotene	211 mg
Proline	2380	Zeaxanthin	101 mg
Serine	2760		
Tyrosine	2500		

3.2.1 Proteins

It is well documented that *Spirulina* contain high amount of crude protein (50–70% dry mater basis). The crude protein content of *Spirulina* is quite unique among microorganisms. The amount of amino acids like methionine, cystine and lysine in *Spirulina* is lower than that in animal proteins such as meat, eggs or milk; however, it is superior to all standard plant proteins (Habib et al. 2008). Its protein content is more than that of animal and fish flesh (15–25%), soybeans (35%), dried milk (35%), peanuts (25%), eggs (12%), grains (8–14%) or whole milk (3%). Mucopolysaccharides are the main components of the cell walls of *Spirulina*. It has high digestibility and absorbs well in the body, and so it can be considered for improving the health status of sick people with gut malabsorption or malnutrition diseases such as kwashiorkor, and also for children and older people (Henrikson 2010).

Marrez et al. (2014) reported that different sources of nitrogen like nitrite, nitrate, ammonium and urea affect the growth and amino acids contents of *Spirulina platensis*. The amino acid profile of *Spirulina* is shown in Table 3-3. Urea caused the highest amino acids levels in algae after one month cultivation. It was reported that the net protein utilization and protein efficiency ratio of *Spirulina* are about 53-92% and 1.8–2.6, respectively. However, the protein efficiency ratio of pure casein, maize, rice and wheat are 2.5, 1.23, 2.2 and 1.15, respectively (Hoseini, Khosravi-Darani, and Mozafari 2013). The biological value of *Spirulina* is comparable with those of other sources of animal and plant proteins (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015).

Table 3-3: Amino acid composition of *Spirulina* (g/100 g DM) (Habib et al. 2008), Amino. Dat (Kynttjä et al. 2014).

Source	Methion- ine	Lysine	Leucine	Tyrosine	Phenyla- lanine	Glutamic Acid	Aspartic Acid	Trypto- phan	Cystine
Siam Algae Co. Ltd., Thailand	1.3-2.0	2.6-3.3	5.9-6.5	2.6-3.3	2.6-3.3	7.3-9.5	5.2-6.0	1.0-1.6	0.5-0.7
IPGSR, Malaysia	2.75±0.0 5	4.63±0.0 7	8.37±0.1 3	3.42±0.1	4.1±0.08	7.04±0.1 4	5.37±0.1 1	1.98±0.05	0.6±0.0 3
AMINODAT ® 5.0	1.14	2.69	4.32	-	2.49	6.14	4.68	0.91	0.51
(EVONIK COMPANY) Kynttjä et al. (2014)	2.4	4.4	8.1	4.2	4.1	12.8	9.5	-	1.2
Source	Serine	Arginine	Histidine	Threonine	Proline	Valine	Isoleucine	Alanine	Glycine
IPGSR, Malaysia	3.84±0.0 6	4.94±0.0 7	2.81±0.0 6	3.35±0.0 6	4.02±0.0 6	4.02±0.0 6	3.85±0.1	10.81±0.1 4	6.66±0. 1
AMINODAT ® 5.0	2.21	3.24	0.88	2.39	2.07	3.04	2.38	3.91	2.74
(EVONIK COMPANY) Kynttjä et al. (2014)	4.6	6.7	1.5	4.6	3.5	5.9	5.2	7.0	4.6

Poultry feed formulators can consider the energy and digestible amino acid values of *Spirulina* shown in Table 3-4 as a guide.

Table 3-4: Energy and digestible amino acid values of *Spirulina* for poultry (Kyntäjä et al. 2014).

	Broiler	Laying hen	Poultry	g/kg DM			
	MJ/kg DM			Dig. Lys	Dig. Met	Dig. Cys	Reference feed ingredient
Algae <i>Spirulina</i> UK 2012	14.0	15.8	16.2	28.0	15.4	7.4	Fish meal, CP>680 g/kg

The protein content of *Spirulina* is superior to that of vegetable sources such as soybean (Ciferri 1983; De León, Bourges, and Camacho 2005).

So it seems that considering *Spirulina* as a protein supplement for both humans and animals is a good strategy to improve protein efficiency (Dillon and Phan 1993). As shown in Table 3-5, the protein values of *Spirulina* are close to the casein as an animal protein standard.

Table 3-5: Protein values of *Spirulina* compared with the casein as a standard. (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015)

characteristics	<i>Spirulina</i>	Reference protein (casein)	% of the reference
Biologic value	75	87	86.20
Net protein utilization	62	83	74.69
Digestibility	85	95	89.47
Protein efficiency	1.9	2.5	76.00

Table 3-6 shows the standardized ileal digestible amount of some amino acids of *Spirulina*. These data are important for poultry feed formulation.

Table 3-6: The standardized ileal digestible amounts of some amino acids in *Spirulina* (Kyntäjä et al. 2014).

		g/kg DM					Reference ingredient	feed
		SID Lys	SID Met	SID Cys	SID Thr	SID Val		
Algae	<i>Spirulina</i>	27.5	14.	6.2	28.	37.	Fish meal, CP>680	
UK 2012			8		8	3		

3.2.2 Nucleic acids

Nucleic acids of *Spirulina* refer to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) fractions (Gutiérrez-Salmeán, Fabila-Castillo, and Chamorro-Cevallos 2015). The nucleic acid content of *Spirulina* is less than 6% of the algae DM (Culleton et al. 1999). The high nucleic acids content of feed ingredients due to catabolism to uric acid can cause gout and kidney stones in poultry species.

3.2.3 Pigments

Spirulina contain different pigments such as chlorophyll, β -carotene, astaxanthin, xanthophylls and phycocyanin (Dillon et al. 1995; Sánchez et al. 2003; Begum et al. 2016). These pigments may be used for food, feed, cosmetic, nutraceutical and pharmaceutical industries (Anderson et al. 1991; Zahroojian et al. 2011; Ali and Saleh 2012; Begum et al. 2016;). Phycocyanin has the potential to be a food colorant (Yoshida et al. 1996), an emulsifier, and a thickening and gelling substance in the food industry (Belay and Gershwin 2007). The amounts of the main pigments in *Spirulina* algae are shown in Table 3-7.

Table 3-7: Pigment amounts in *Spirulina* (Zahroojian, Moravej, and Shivazad 2013)

Pigments	mg 100 g ⁻¹
Total carotenoids	400-500
Carotene	160-260
Xanthophyll	170-240
Chlorophyll	1300-1700
Phycocyanin	15000-19000

Phycocyanobilin can decrease nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity by being reduced to phycocyanorubin. Thus, this issue should be considered in the treatment of some diseases such as cardiovascular disease, diabetes, metabolic syndrome, allergic reactions, cancer, Parkinson's disease, and Alzheimer's disease (Hoseini, Khosravi-Darani, and Mozafari 2013; McCarty 2007).

3.2.4 Carbohydrates

The carbohydrate content of *Spirulina* is about 15–25% dry matter (Sotiroudīs and Sotiroudīs 2013). *Spirulina platensis* contains a branched polysaccharide that is structurally similar to glycogen (Hoseini et al. 2013). The cell walls of *Spirulina* are similar to those of gram-positive bacteria, since they consist of glucosamines and muramic acid associated with peptides (Falquet and Hurni 2006). Figure 3-1 shows the schematic structure of cell walls of different microalgae that contain complex sugars and protein.

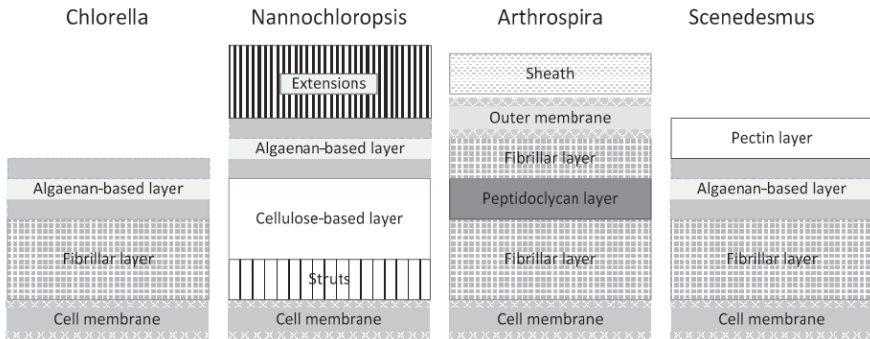


Figure 3-1: Comparing the cell wall structures of four microalgae (*Chlorella vulgaris*, *Nannochloropsis oculata*, *Arthorspira platensis* and *Scenedesmus rubescens*). (Kröger, Klemm, and Nelles 2018)

From a nutritional viewpoint, the only carbohydrate present in sufficient quantities is mesoinositol phosphate, which is an excellent source of organic phosphorus and inositol (350–850 mg/kg dry matter) (Challem, Passwater, and Mindell 1981). The inositol concentration is about eight times that of beef and several hundred times that of the vegetables with the highest levels. It is interesting to note that *Spirulina* polysaccharides have a stimulating effect on DNA repair mechanisms (Pang, Guo, and Ruan 1988), which might explain the radio-protective effect mentioned several

times in relation to *Spirulina* (Qishen, Baojiang, and Kolman 1989). Calcium spirulina, a sulphated polysaccharide fraction with antiviral properties, has been extensively purified and shown to be composed of rhamnose, 3-O-methylrhamnose (acofriose), 2,3-di-O-methylrhamnose, 3-O-methylxylose, uronic acids and sulfate (Lee et al. 1998; Hayashi et al. 1996).

3.2.5 Lipids

Lipids of *Spirulina* are categorized into two parts: saponifiable fraction (83%) and non-saponifiable fraction (17%), containing essential pigments, paraffin, sterols and terpene alcohol. Fatty acids, especially w_6 , make up more than 50% of the total lipid content of *Spirulina* (Parages et al. 2012; Gershwin and Belay 2008). The gamma-linolenic acid (GLnA) content of *Spirulina maxima* and *Spirulina platensis* is 10–20% and 49% of their fatty acids, respectively. So, *Spirulina* is a good source of GLnA. Also, *Spirulina maxima* contains unsaturated oleic and linoleic acids and saturated palmitic acid, which comprise more than 60% of its lipids. It was reported that monogalactosyl and sulfoquinovosyl diacylglycerol as well as phosphatidylglycerol are the major *Spirulina* lipids (Petkov and Furnadzieva 1988). Each 10 g of *Spirulina* provides over 100 mg of GLnA (Sotiroudis and Sotiroudis 2013). It is interesting to note that sulfolipids from cyanobacteria are active against the AIDS virus (Henrikson 2010). Roessler (1990) found that altering the culture condition can enhance the lipid content of microalgae. Roessler (1990) also reported that some microalgae have the potential to store lipids (triglycerides) up to 70% dry weight under nitrogen-starvation. Amongst the microalgae, *Chlorella* has the potential for use in biodiesel production. *Chlorella protothecoides* produces a crude lipid content of 55.2% dry weight when grown under heterotrophic conditions on glucose (Xu, Miao, and Wu 2006). Previous research showed that the biodiesel produced by *Chlorella protothecoides* was of high quality, with high heating value and viscosity (Xu, Miao, and Wu 2006). Table 3-8 shows the fatty acid composition of *Spirulina platensis* reported by Ötles and Pire (2001).

Table 3-8: Fatty acid composition of *Spirulina platensis* powder. (Ötles and Pire 2001)

Fatty acid	Content (%)
(C ₁₄) Myristic acid	0.23
(C ₁₆) Palmitic acid	46.07
(C _{16:1}) Δ^9 Palmitoleic acid	1.26
(C _{18:1}) Δ^9 Oleic acid	5.26
(C _{18:2}) $\Delta^{9,12}$ Linoleic acid	17.43
(C _{18:3}) $\Delta^{9,12,15}$ γ -Linolenic acid	8.87
Others	20.88

3.2.6 Vitamins

Vitamins are natural organic compounds that are essential in only small amounts in human and animal diets. They have several functions that are as follows: They may act as enzyme cofactors, such as vitamins A, K, and C, thiamin, niacin, riboflavin, vitamin B₆, biotin, pantothenic acid, folate, and vitamin B₁₂. Some vitamins function as cofactors in metabolic oxidation–reduction reactions (i.e. vitamins E, K, and C, niacin, riboflavin, and pantothenic acid). Additionally, some vitamins are biological antioxidants, such as vitamins E and C. It is well documented that two vitamins (vitamins A and D) function as hormones. Also, vitamin A acts as a photoreceptive cofactor in vision (Combs and McClung 2016). Vitamins and minerals in foods are bound to natural food complexes with proteins, carbohydrates and lipids (Henrikson 2010). *Spirulina* contain vitamins that have positive effects on metabolism of proteins, carbohydrate, fats and the body physiology processes. There are large amounts of natural β -carotene in *Spirulina*. The β -carotene is a precursor of vitamin A. Figure 3-2 shows the molecular structure of two forms of β -carotene. The National Cancer Institute in the United States of America reported that an intake of 6.0 mg of β -carotene per day may help in reducing the risk of cancer. If consuming 4.0 g *Spirulina* per day, the need for 6.0 mg β -carotene and sufficient amounts of B-group vitamins, iron and calcium will be met (Habib et al. 2008).

Wang et al. (2008) reported that the conversion ratio of *Spirulina* β -carotene to vitamin A was estimated to be 4.5 to 1 (by weight) in adults. Palan et al. (1992) found that β -carotene has a protective effect against the development and progression of cervical cancer. Also, Kornhauser et al.

(1986) reported that β -carotene prevents skin damage due to sunlight; also, it protects the skin against cancers.

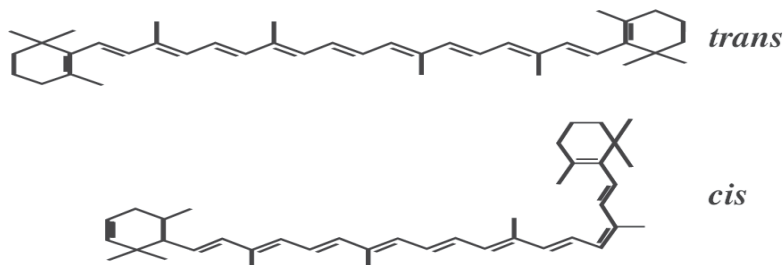


Figure 3-2: Molecular structure of two forms of β -carotene. (Moorhead et al. 2011)

It was reported that β -carotene works in combination with vitamin C to reduce the energy of free radicals. In the absence of vitamin C, β -carotene can actually form a pro-oxidant, leading to accelerated tissue cellular damage (Moorhead, Capelli, and Cysewski 2011). It was shown that the absorbance of natural β -carotene in chickens and rats was 10 times more than that of the synthetic variety (Moorhead, Capelli, and Cysewski 2011). It was documented that *Spirulina* is not only rich in β -carotene but also contains other very important carotenoids like zeaxanthin and β -cryptoxanthin as well as lesser known carotenoids such as myxoxanthophyll and echinenone (Choubert 1979; Moorhead et al. 2011; Fazilati et al. 2016). No toxicity or side effects have been found, even with very large doses of β -carotene.

Excess β -carotene circulates in the blood and is stored in fat tissue; an orange tint to the skin, especially in the palms of the hands, indicates reserves of β -carotene (Moorhead et al. 2011). It was reported that algal vitamin B₁₂ is a bioavailable source for mammals. However, previous studies showed that pseudo vitamin B₁₂ (an inactive corrinoid), which predominated in the *Spirulina* tablets, is not suitable for use as a vitamin B₁₂ source (Watanabe et al. 2006). *Spirulina* has an exceptionally high content of vitamin B₁₂; thus, this algae might be considered as a good source for breeder poultry flocks since, due to biosecurity legislation, they do not consume any animal-origin feed.

It is well known that β -carotene also promotes many aspects of immune function (Bendich 1988). It is interesting to note that β -carotene stimulates immunocompetence in healthy individuals and enhances immune function in people who have tested HIV positive (Garewal et al. 1992). Moorhead,

Capelli, and Cysewski (2011) reported that *Spirulina* is an excellent source of vitamin B₁₂. This vitamin is essential for normal growth and neurological function. Vitamin B₁₂ deficiency causes fatigue and moodiness, and eventually neurological damage. In addition, *Spirulina* is a good natural source of vitamins K₁ and K₂. Vitamin K₁, also called “phylloquinone”, is related to blood health because about 50% of the 16 known proteins that depend on this vitamin are necessary for blood coagulation. Also, Moorhead, Capelli, and Cysewski (2011) reported that vitamin K₁ has benefits for bone health, and it has beneficial effects on Alzheimer's disease patients. Vitamin K₂ is also known as “menaquinone”. This vitamin is important for the proper metabolism of calcium, promoting bone and liver health, alleviating osteoporosis, and preventing a variety of cancers and cardiovascular diseases. Vitamin K₂ can activate a critical protein required to bind calcium, thus strengthening the skeleton. The vitamin content of *Spirulina* is shown in Table 3-9.

Table 3-9: Vitamin content of 100 g *Spirulina*. (Gershwin and Belay 2008)

Vitamin A	352000 IU
Total carotenoids	504 mg
β-carotene	211 mg
Zeaxanthin	101 mg
Vitamin K	1090 µg
Vitamin B ₁	0.5 mg
Vitamin B ₂	4.5 mg
Niacin	14.9 mg
Vitamin B ₆	0.96 mg
Vitamin B ₁₂	162 µg

3.2.7 Minerals

This microalga is rich in potassium, but it also contains calcium, chlorine, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc (Habib et al. 2008). The mineral concentration of *Spirulina* depends on its rearing condition and the growth culture of the algae. The mineral content of *Spirulina* is shown in Table 3-10.

Table 3-10: Mineral content of *Spirulina* (Belay 1997, Kyntäjä et al. 2014).

Reference	Belay (1997, Earthrise farms)	Kyntäjä et al. (2014)
Minerals		
Calcium	70 mg/10 g	1.8 g/kg DM
Iron	10 mg/10 g	920 mg/kg DM
Phosphorus	80 mg/10 g	9.6 g/kg DM
Magnesium	40 mg/10 g	2.6 g/kg DM
Zinc	300 mcg/10 g	16.6 mg/kg DM
Selenium	10 mcg/10 g	0.15 mg/kg DM
Copper	120 mcg/10 g	3.4 mg/kg DM
Manganese	500 mcg/10 g	26 mg/kg DM
Chromium	25 mcg/10 g	-
Sodium	90 mg/10 g	5.7 g/kg DM
Potassium	140 mg/10 g	13.8 g/kg DM
Germanium	60 mcg/10 g	-
Sulfur	-	6.7 g/kg DM

The iron content of *Spirulina spp.* is considerable and about 550–6000 mg/kg. Falquet and Hurni (2006) reported that *Spirulina* can be used as a source of iron to prevent or treat anemia. Also, Henrikson (2010) reported that iron is essential for strong red blood cells and a healthy immune system. The iron content of *Spirulina* can be absorbed in the digestive tract easily. The blue pigment of *Spirulina*, phycocyanin, forms soluble complexes with iron and other minerals during digestion that make this mineral more bioavailable. Jassby (1988) reported that *Spirulina* iron is two times more absorbable than iron found in vegetables and most of meat products. Previous studies showed that *Spirulina* iron is about 60% more absorbable than iron supplements such as iron sulfate (Henrikson 2010). Table 3-11 shows the natural sources of iron.

Table 3-11: Natural sources of iron. (Belay 1997, Earthrise farms); Prevention Magazine 1992; Henrikson 2010)

source	amount	mg iron
<i>Spirulina</i>	10 g	10
<i>Chlorella</i>	10 g	10
Chicken liver, cooked	3 ounces	7.2
Crab, pieces, steamed	½ cup	6.0
Beef liver, fried	½ cup	5.3
Soybeans, boiled	½ cup	4.4
Blackstrap molasses	10 g	3.2
Spinach, cooked	½ cup	3.2
Beef, sirloin, broiled	3 ounces	2.9
Potato, baked	one	2.8
Scallops, steamed	3 ounces	2.5
Pistachios, dried	1/4 cup	2.2
Broccoli, cooked	1 spear	2.1
Cashews, dry-roasted	1/4 cup	2.1
Turkey, dark meat	3 ounces	2.0
Spinach, raw chopped	½ cup	0.8

Spirulina contains a considerable amount of calcium that is higher than the amount in milk. It is well documented that calcium is critical for bones and muscles. Calcium deficiencies may lead to osteoporosis in adult women. Also, *Spirulina* contains magnesium, which helps with the absorption of calcium and regulating blood pressure. There are small amounts of iodine and sodium in *Spirulina*. Humans and animals need trace minerals for the high efficiency of enzyme systems and physiological functions; thus, consuming adequate amount of trace minerals is important to maintain one's health. Consuming 10 g of *Spirulina* supplies manganese (25% DV), chromium (21% DV), selenium (14% DV), copper (6% DV) and zinc (2% DV) (Henrikson 2010).

Sharma and Azeez (1988) conducted an experiment about the bioaccumulation of copper and cobalt by *Spirulina* at different temperatures. They reported a high accumulation capacity. They also observed a negative correlation between metal accumulated and the survival ratio of the algae. Gabbey-Azaria and Tel-Or (1993) studied the mechanism of salt tolerance in cyanobacteria, especially on *Spirulina subsalsa*. They observed that *S. subsalsa* cells in a fresh seawater medium increased their sodium and chloride intracellular content and developed

the capability to initiate sodium and chloride efflux in the light. In the dark, this process stops but the cells remain viable or active. In this situation, enhanced respiration may be driven by salt influx and may have triggered the events leading to salt adaptation. The biosynthesis and accumulation of organic compatible solutes in the cyanobacterium cell is a slow and secondary response at a steady-state osmotic stability. It was reported that *Spirulina platensis* has a relatively high tolerance to salinity. It is able to grow in a medium containing up to 70 g NaCl/liter. There is no marked inhibition of the growth rate or changes in the biomass composition usually observed in the range of 1–30 g NaCl/litre in a medium (Habib et al. 2008).

Bolsunovskii and Kosinenko (2000) assessed the status of intracellular phosphorus (P) pools using radioactive and non-radioactive P. They found that the stage of replenishment of the intracellular P pool may affect the P turnover estimation in aquatic environments during a short-term measurement of P uptake. Hernández and Olguín (2002) studied the mineral absorption capacity of cells in four species of *Spirulina*. It was found that two species contained high percentages of protein (68.95%) as a result of being cultivated in a Zarrouk medium at two light intensities (66 $\mu\text{mol photon/m}^2/\text{second}$ and 144 $\mu\text{mol photon/m}^2/\text{second}$) in batch culture. A third species of *Spirulina*, cultivated in a “Complex” medium and exposed to 66 $\mu\text{mol photon/m}^2/\text{second}$, contained a high percentage of lipids (30%). The fourth species of *Spirulina* contained a high percentage of polysaccharides that was about 25.54% when cultured in a “Complex” medium and exposed to 144 $\mu\text{mol photon/m}^2/\text{second}$. It was reported that the chemical composition of *Spirulina sp.* cells did have a strong influence on their adsorption capacities (q_{max}) for Pb and Cd, which were highest (172.41 and 45 mg/g of cells at pH 5 and 4.5, respectively) when cells exhibited the higher polysaccharide content. For Cr VI, the highest q_{max} was shown by cells cultivated in a Zarrouk medium and showing the higher protein content (at pH 2.0). The pH did not affect the adsorption of Pb II in the range of 3 to 5.5, nor Cd in the range of 4 to 7. For Cr II, adsorption was observed only at a pH equal to 2.0 or lower (Habib et al. 2008).

Previous studies showed that *Spirulina* has a unique ability to detoxify or to chelate toxic minerals—a characteristic that is not yet confirmed in any other microalgae (Maeda and Sakaguchi 1990; Okamura and Aoyama 1994).

Habib et al. (2008) reported that *Spirulina* can be used for reducing arsenic from water and food. In addition, it may be used to chelate or detoxify the poisonous effect of heavy metals from water, food and the environment. Beijing University has extracted bioactive molecules from *Spirulina* which could neutralize or detoxify the toxic and poisonous effect of heavy metals, and which showed anti-tumor activity (Habib et al. 2008). Several institutions in China are focusing on biomolecules which show anti-tumor, anti-age and anti-radiation properties (Liu et al. 1991; Li and Qi 1997). The mineral content of *Spirulina* has high bioavailability and can be a good choice for women during pregnancy and lactation. It is also beneficial for malnourished children (Seshadri 1993). The World Health Organization (WHO) has considered *Spirulina* as one of the most valuable foods on Earth, and the USA's National Aeronautics and Space Administration (NASA) has noted it as an excellent compact food for space travel, as small amounts can provide a wide range of nutrients (Khan, Bhadouria, and Bisen 2005).

In conclusion, supplementation of animal diets with *Spirulina* can decrease the need for adding trace minerals and vitamins in the form of feed supplements. Regardless of economic viewpoint, using organic sources of feed ingredients is parallel to the principle of life cycle assessment and decreases the impact of animal production on environment emissions (FAO 2016; Riber et al. 2017).

3.3 Toxicology

Spirulina has no aflatoxin, ochratoxin A, sterigmatocystin, citrinin, patulin, penicillinacid, zearalenone, diacetoxyscirpenol or trichothecene (Belay and Gershwini 2007), and no toxicity was reported from different animal studies (Becker and Venkataraman 1984). As mentioned above, the nucleic acid content of *Spirulina* can increase uric acid levels in the body and cause diseases such as gout; thus, plasma uric acid level should be considered. *Spirulina* does not produce heavy metals but it has the ability to absorb heavy metals from water (Rangsayatorn et al. 2002; Chen and Pan 2005; Gong et al. 2005; Jagiełło et al. 2006); thus, the level of heavy metals in water and the algae should be considered (Belay and Gershwini 2007). Table 3-12 shows the heavy metal concentrations in *Spirulina* from different regions and the international allowance of the heavy metals in single-cell proteins.

Table 3-12: Heavy metal concentration in *Spirulina* and the international recommendation as ppm. (Becker 1994; Belay and Gershwini 2007)

	<i>As</i>	<i>Pb</i>	<i>Hg</i>	<i>Cd</i>	<i>reference</i>
Maximum allowance	2.0	5.0	0.1	1.0	IUPAC 1974
<i>Spirulina</i> (India)	0.97	3.95	0.07	0.62	Becker and Venkataraman 1982
<i>Spirulina</i> (Mexico)	2.9	5.1	0.5	0.5	Boudene et al. 1975
<i>Spirulina</i> (Chad)	1.8	3.7	0.5	-	Boudene et al. 1975
<i>Spirulina</i> (Chile)	<0.002	<0.002	<0.0005	-	AEH report

3.4 *Spirulina* product quality and consistency

Considering the quality of *Spirulina*, culture and good manufacturing practices (GMPs) are necessary for *Spirulina* product quality and its safety (Belay 1997). The consistency and shelf life of *Spirulina* powder is shown in Table 3-13. It is interesting to note that the loss percentage of *Spirulina* nutrients is in the range of 5–25% after keeping the product for up to 4.5 years.

Table 3-13: Consistency and shelf life of *Spirulina* powder. (Belay and Gershwini 2007)

Land	Initial level of carotenoids (mg/100 g)	Final level of carotenoids (mg/100 g)	Loss (%)	Time elapsed when final level was measured (years)
1	479	380	21	4.5
2	495	374	25	4.5
3	452	397	22	4.5
4	498	411	17	4.4
5	520	470	10	4.3
6	471	449	5	4.3
7	477	424	12	4.3
8	486	463	5	4.3
9	464	444	4	4.2
10	493	425	14	4.2
11	487	417	15	4.2
12	521	493	5	4.1
Mean	487	429	13	4.3
STD	21	36	7	0.1
N	12	12	12	12

Source: Data from Earthrise.

References

- Ali, S. K., and A. M. Saleh. 2012. "Spirulina—an overview." *International Journal of Pharmacy and Pharmaceutical Sciences* 4 (3): 9–15.
- AOAC. (1990). Official method of analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Alvarenga, R. R., P. B. Rodrigues, V. D. S. Cantarelli, M. G. Zangeronimo, J. W. D. Silva Júnior, L. R. D. Silva, L. M. D. Santos, and L. J. Pereira. 2011. "Energy values and chemical composition of spirulina (*Spirulina platensis*) evaluated with broilers." *Revista Brasileira de Zootecnia* 40 (5): 992–96.
- Anderson, D. W., C. S. Tang, E. and Ross. 1991. "The xanthophylls of *Spirulina* and their effect on egg yolk pigmentation." *Poultry Science* 70 (1): 115–19.
- Becker E. W. 1994. Microalgae: Biotechnology and microbiology. In: Baddiley J, et al., editors. New York: Cambridge University Press. 178 Pp.
- Becker, E. W., and L. V. Venkataraman. 1982. *Biotechnology and Exploration of Algae: The Indian Approach*. Eschborn: German Agency for Technical Cooperation. Eschborn
- Becker, E. W., and L. V. Venkataraman. 1984. "Production and utilization of the blue-green alga *Spirulina* in India." *Biomass* 4 (2): 105–25.
- Begum, H., F. M. Yusoff, S. Banerjee, H. Khatoon, and M. Shariff. 2016. "Availability and utilization of pigments from microalgae." *Critical Reviews in Food Science and Nutrition* 56 (13): 2209–22.
- Belay, A. M. H. A. 1997. "Mass culture of *Spirulina* outdoors—the Earthrise Farms experience." *Spirulina platensis*, 131–58.
- Bendich, A. 1988. "A role for carotenoids in immune function." *Clinical Nutrition* 7 (3): 113.
- Bolsunovskii, A. Y., and S. V. Kosinenko. 2000. "Intracellular phosphorus pool of the cyanobacterium *Spirulina platensis*." *Microbiology* 69 (1): 116–18.
- Boudene, C., E. Collas, and C. Jenkins. 1975. "Recherche et dosage de divers toxiques minéraux dans les algues spirulines de different origins et evaluation de la toxicité a long terme chez le rat d'un lot d'algues spirulines de provenance mexicaine." *Annales de la nutrition et de l'alimentation* 29:577.
- Challem, J. J., R. A. Passwater, and E. M. Mindell. 1981. *Spirulina*. New Canaan, CT: Keats Publishing Inc.

- Chen, H., and S. S. Pan. 2005. "Bioremediation potential of spirulina: toxicity and biosorption studies of lead." *Journal of Zhejiang University. Science. B* 6 (3): 171.
- Choubert Jr., G. 1979. "Tentative utilization of spirulin algae as a source of carotenoid pigments for rainbow trout." *Aquaculture* 18 (2): 135–43.
- Ciferri, O. 1983. "Spirulina, the edible microorganism." *Microbiological Reviews* 47 (4): 551.
- Combs Jr., G. F. and J. P. McClung. 2016. "The vitamins: fundamental aspects in nutrition and health." *Academic press*.
- Culleton, B. F., M. G. Larson, W. B. Kannel, and D. Levy. 1999. "Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study." *Annals of Internal Medicine* 131 (1): 7–13.
- De León, J. M., H. Bourges, and M. E. Camacho. 2005. "Amino acid composition of some Mexican foods." *Archivos Latinoamericanos de Nutrición* 55:172–86.
- Dillon, J. C., and P. A. Phan. 1993. "Spirulina as a source of proteins in human nutrition." *Bulletin de l'Institut océanographique*, 103–107.
- Dillon, J. C., A. P. Phuc, and J. P. Dubacq. 1995. "Nutritional value of the alga Spirulina." In *Plants in Human Nutrition*, Vol. 77, 32–46. Karger Publishers.
- Evans, A. M., D. L. Smith, and J. S. Moritz. 2015. "Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance." *Journal of Applied Poultry Research* 24 (2): 206–14.
- Falquet, J., and J. P. Hurni. 2006. *Spiruline: aspects nutritionnels*. Antenna Technologies Novembre 2006
- Falquet, J., and J. P. Hurni. 1997. "The nutritional aspects of Spirulina." Antenna Foundation. Accessed July 25, 2017. https://www.antenna.ch/wp-content/uploads/2017/03/AspectNut_UK.pdf.
- FAO (Food and Agriculture Organization of the United Nations). 2016. *Environmental performance of animal feeds supply chains: Guidelines for assessment*. Livestock Environmental Assessment and Performance Partnership. Rome: FAO.
- Fazilati, M., A. M. Latifi, H. Salavati, and A. Choopani. 2016. "Antioxidant Properties of Spirulina." *Journal of Applied Biotechnology Reports* 3 (1): 345–51.
- Gabbey-Azaria, R., and E. Tel-Or. 1993. "Mechanisms of salt tolerance in cyanobacteria." In *Plant Responses to the Environment*, edited by P. M. Gresshoff, 692–98. Boca Raton, FL: CRC Press.

- Garewal, H. S., N. M. Ampel, R. R. Watson, R. H. Prabhala, and C. L. Dols. 1992. "A preliminary trial of beta-carotene in subjects infected with the human immunodeficiency virus." *The Journal of Nutrition* 122 (suppl_3): 728–32.
- Belay, A., and M. E. Gershwin. 2007. "Spirulina (Arthrospira)." In *Spirulina in Human Nutrition and Health*, pp. 11-35. CRC Press, 2007.
- Gershwin, M. E., and A. Belay. 2008. *Spirulina in Human Nutrition and Health*. CRC Press, Taylor and Francis Group, Boca Raton: London, New York. CRC Press.
- Gong, R., Y. Ding, H. Liu, Q. Chen, and Z. Liu. 2005. "Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass." *Chemosphere* 58 (1): 125–30.
- Gutiérrez-Salmeán, G., L. Fabila-Castillo, and G. Chamorro-Cevallos. 2015. "Aspectos nutricionales y toxicológicos de *Spirulina* (arthrospira)." *Nutricion hospitalaria* 32 (1): 34–40.
- Habib, M. A. B., M. Parvin, T. C. Huntington, and M. R. Hasan. 2008. "A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish." FAO Fisheries and Aquaculture Circular no. 1034. Rome: FAO.
- Hayashi, T., K. Hayashi, M. Maeda, and I. Kojima. 1996. "Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*." *Journal of Natural Products* 59 (1): 83–87.
- Henrikson, R. 2010. *Spirulina World Food: How this micro algae can transform your health and our planet*. Ronore Enterprises, Incorporated. Hawaii, USA.
- Hernández, E., and E. J. Olguín. 2002. "Biosorption of heavy metals influenced by the chemical composition of *Spirulina* sp. (Arthrospira) biomass." *Environmental Technology* 23 (12): 1369–77.
- Hoseini, S. M., K. Khosravi-Darani, and M. R. Mozafari. 2013. "Nutritional and medical applications of spirulina microalgae." *Mini Reviews in Medicinal Chemistry* 13 (8): 1231–37.
- IUPAC (International Union of Pure and Applied Chemistry). 1974. *Proposed guidelines for testing of single cell protein destined as major protein source for animal feed*. Information Bulletin, IUPAC. Technical Reports, No.12. Oxford: IUPAC Secretariat.
- Jagiello, M., E. Minta, K. Chojnacka, and P. Kafarski. 2006. "Mode of biosorption of chromium (III) by *Spirulina* species cells from aqueous solutions." *Water Environment Research* 78 (7): 740–43.

- Jassby, A. 1988. "Spirulina: a model for microalgae as human food." *Algae and Human Affairs* (C. A. Lembi and J. R. Waaland, eds.), pp.149–79.
- Khan, Z., P. Bhadouria, and P. S. Bisen. 2005. "Nutritional and therapeutic potential of Spirulina." *Current Pharmaceutical Biotechnology* 6 (5): 373–79.
- Kornhauser, A., W. Wamer, and A. Giles. 1986. "Protective effects of beta-carotene against psoralen phototoxicity: relevance to protection against carcinogenesis." In *Antimutagenesis and Anticarcinogenesis Mechanisms*, D. M. Shankel et al. (eds.), 465–81. Boston, MA: Springer.
- Kröger, M., M. Klemm, and M. Nelles. 2018. "Hydrothermal Disintegration and Extraction of Different Microalgae Species." *Energies* 11 (2): 450.
- Kyntäjä, S., K. Partanen, H. Siljander-Rasi, and T. Jalava. 2014. Tables of composition and nutritional values of organically produced feed materials for pigs and poultry. MTT report 164. ISSN: 1798-6419 (Verkkojulkaisu)
- Lee, J. B., T. Hayashi, K. Hayashi, U. Sankawa, M. Maeda, T. Nemoto, and H. Nakanishi. 1998. "Further purification and structural analysis of calcium spirulan from *Spirulina platensis*." *Journal of Natural Products* 61 (9): 1101–4.
- Li, D. M., and Y. Z. Qi. 1997. "Spirulina industry in China: present status and future prospects." *Journal of Applied Phycology* 9 (1): 25–28.
- Liu, L. C., B. J. Guo, and J. S. Ruan. 1991. "Antitumour activity of polysaccharides extracted from *Spirulina*." *Oceanogr* 5:33–37.
- Maeda, S. H. I. G. E. R. U., and T. A. K. A. S. H. I. Sakaguchi. 1990. "Accumulation and detoxification of toxic metal elements by algae." *Introduction to Applied Phycology*. 109–36.
- Marrez, D., M. Naguib, Y. Sultan, Z. Daw, and A. Higazy. 2014. "Evaluation of chemical composition for *Spirulina platensis* in different culture media." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5:1161–71.
- McCarty, M. F. 2007. "Perspective Clinical Potential of *Spirulina* as a Source of Phycocyanobilin." *Journal of Medicinal Food* 10 (4): 566–70.
- Milledge, J. J. 2011. "Commercial application of microalgae other than as biofuels: a brief review." *Reviews in Environmental Science and Bio/Technology* 10 (1): 31–41.
- Moorhead, K., B. Capelli, and G. R. Cysewski. 2011. *Spirulina: Nature's Superfood*. Kailua Kona, HI: Cyanotech Corporation.

- Okamura, H., and I. Aoyama. 1994. "Interactive toxic effect and distribution of heavy metals in phytoplankton." *Environmental Toxicology* 9 (1): 7–15.
- Ötles, S., and R. Pire. 2001. "Fatty acid composition of *Chlorella* and *Spirulina* microalgae species." *Journal of AOAC International* 84 (6): 1708–14.
- Palan, P. R., M. S. Mikhail, J. Basu, and S. L. Romney. 1992. "β-Carotene levels in exfoliated cervicovaginal epithelial cells in cervical intraepithelial neoplasia and cervical cancer." *American Journal of Obstetrics and Gynecology* 167 (6): 1899–903.
- Pang, Q. S., B. J. Guo, and J. H. Ruan. 1988. "Enhancement of endonuclease activity and repair DNA synthesis by polysaccharide of *Spirulina platensis*." *Yi chuan xue bao = Acta genetica Sinica* 15 (5): 374.
- Parages, M. L., R. M. Rico, R. T. Abdala-Díaz, M. Chabrillón, T. G. Sotiroudis, and C. Jiménez. 2012. "Acidic polysaccharides of *Arthrospira* (*Spirulina*) *platensis* induce the synthesis of TNF-α in RAW macrophages." *Journal of Applied Phycology* 24 (6): 1537–46.
- Petkov, G. D., and S. T. Furnadzieva. 1988. "Fatty acid composition of acylolipids from *Spirulina Platensis*." *Dokladi na bolgarskata akademija na naukite* 41 (1): 103–4.
- Prevention Magazine. 1992. *The Complete Book of Vitamins and Minerals for Health*. Random House Value Publishing, ISBN-13: 978-0517080221.
- Qishen, P., G. Baojiang, and A. Kolman. 1989. "Radioprotective effect of extract from *Spirulina platensis* in mouse bone marrow cells studied by using the micronucleus test." *Toxicology Letters* 48 (2): 165–69.
- Rangsayatorn, N., E. S. Upatham, M. Kruatrachue, P. Pokethitiyook, and G. R. Lanza. 2002. "Phytoremediation potential of *Spirulina* (*Arthrospira*) *platensis*: biosorption and toxicity studies of cadmium." *Environmental Pollution* 119 (1): 45–53.
- Riber, A. B., H. A. Van de Weerd, I. C. De Jong, and S. Steenfeldt. 2017. "Review of environmental enrichment for broiler chickens." *Poultry Science* 97 (2): 378–96.
- Roessler, P. G. 1990. "Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions." *Journal of Phycology* 26 (3): 393–99.
- Sánchez, M., J. Bernal-Castillo, C. Roza, and I. Rodríguez. 2003. "*Spirulina* (*Arthrospira*): an edible microorganism: a review." *Universitas Scientiarum* 8 (1): 7–24.

- Seshadri, C. V. 1993. "Large scale nutritional supplementation with spirulina alga." All India Coordinated Project on Spirulina. Shri Amm Murugappa Chettiar Research Center (MCRC), Madras, India.
- Sharma, R. M., and P. A. Azeez. 1988. "Accumulation of copper and cobalt by blue-green algae at different temperatures." *International Journal of Environmental Analytical Chemistry* 32 (2): 87–95.
- Sotiroudis, T. G., and G. T. Sotiroudis. 2013. "Health aspects of Spirulina (Arthrospira) microalga food supplement." *Journal of the Serbian Chemical Society* 78 (3): 395–405.
- Wang, J., Y. Wang, Z. Wang, L. Li, J. Qin, W. Lai, Y. Fu, P. M. Suter, R. M. Russell, M. A. Grusak. and G. Tang. 2008. "Vitamin A equivalence of spirulina β -carotene in Chinese adults as assessed by using a stable-isotope reference method." *The American Journal of Clinical Nutrition* 87 (6): 1730–37.
- Fu, Y., Suter, P.M., Russell, R.M., Grusak, M.A. and Tang, G., 2008. Vitamin A equivalence of spirulina β -carotene in Chinese adults as assessed by using a stable-isotope reference method-. *The American journal of clinical nutrition*, 87(6), pp.1730-1737.
- Watanabe, F., E. Miyamoto, T. Fujita, Y. Tanioka, and Y. Nakano. 2006. "Characterization of a corrinoid compound in the edible (blue-green) alga, Suizenji-nori." *Bioscience, Biotechnology, and Biochemistry* 70 (12): 3066–68.
- Xu, H., X. Miao, and Q. Wu. 2006. "High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters." *Journal of Biotechnology* 126 (4): 499–507.
- Yoshida, A., Y. Takagaki, and T. Nishimune. 1996. "Enzyme immunoassay for phycocyanin as the main component of Spirulina colour in foods." *Bioscience, Biotechnology, and Biochemistry* 60 (1): 57–60.
- Zahroojian, N., H. Moravej, and M. Shivazad. 2013. "Effects of dietary marine algae (*Spirulina platensis*) on egg quality and production performance of laying hens." *Journal of Agricultural Science and Technology* 15:1353–60.
- Zahroojian, N., Moravej, H., & Shivazad, M. (2011). Comparison of marine algae (*Spirulina platensis*) and synthetic pigment in enhancing egg yolk colour of laying hens. *British poultry science*, 52(5), 584-588.

CHAPTER FOUR

ANTIMICROBIAL CHARACTERISTICS OF *SPIRULINA*

“The doctor of the future will no longer treat the human frame with drugs but rather will cure and prevent disease with NUTRITION .”

Thomas Edison

4.1 Introduction

Nowadays, studying the pharmacological usage of cyanobacteria is an attractive field. Regarding bacterial resistance to antibiotics, natural antimicrobial substances play an important role in improving public health. *Spirulina* has some components that seem to be effective against microbes. This has high value, especially in organic feeding of birds. With the usage of natural antimicrobial substances, the birds' health and the quality of their products will improve much more. The goal of this chapter is to review the antimicrobial characteristics of *Spirulina*.

4.2 Antimicrobial activity

Natural products were used in ancient China, India and North African for the treatment of several diseases (Kumar et al. 2013). Previous researchers found that cyanobacteria can produce intracellular and extracellular bioactive substances with antialgal, antibacterial, antifungal and antiviral activities (Noaman et al. 2004; El-sheekh et al. 2006; El-sheekh et al. 2008; Kumar et al. 2013). It was shown that *Spirulina platensis* has antiviral (Hernández-Corona et al. 2002), antibacterial (Ozdemir et al. 2004), antiplatelet (Hsiao et al. 2005), anticardiotoxic (Khan et al. 2005), hypocholesterolemic (Nagaoka et al. 2005), antinephratic (Khan et al. 2006) and anti-hepatotoxic effects (Mohan et al. 2006). The antimicrobial activity found in *S. platensis* extract may be due to γ -linolenic acid (Demule, Decaire, and Decano 1996), active fatty acids (Xue et al. 2002), the synergetic effect of lauric and palmitoleic acid (Mendiola et al. 2007), phycocyanin, phycocyanobilin and allophycocyanin (Nuhu 2013), amides,

alkaloids (Ghasemi, et al. 2004; Usharani et al. 2015), heptadecane and tetradecane (Ozdemir et al. 2004), tetramine, spermine and piperazine (Prashantkumar, Angadi, and Vidyasagar 2013; Shanab 2007). It was claimed that lipids kill microorganisms by disrupting the cellular membrane (Gudmundur 2005) of bacteria, fungi and yeasts because they may penetrate the microorganism's cell wall (Mala et al. 2009). Calcium-*Spirulan* (Ca-SP) is a sulfated polysaccharide in *Spirulina platensis*. It consists of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid and calcium sulfate. Ca-SP acts against HIV, the herpes simplex virus, the human cytomegalovirus, the influenza A virus, the mumps virus and the measles virus. The calcium molecule of Ca-SP is essential for the inhibition of the viral infection (Lee et al. 1998).

In addition, the cell extract of *Spirulina maxima* has shown antimicrobial activity against *Bacillus subtilis*, *Streptococcus aureus*, *Saccharomyces cerevisiae* and *Candida albicans* (Saranraj and Sivasakthi 2014). Several studies were conducted to study the effect of different solvents on extracting antimicrobial substances from algae. Shanmughapriya et al. (2008) reported that methanol-to-toluene (3:1) was the best solvent for extracting antimicrobial compounds from fresh algae. Abedin and Taha (2008) found that acetone and diethyl ether extracts (EE) of *Spirulina platensis* gave the highest antimicrobial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Santoyo et al. (2006) studied the effects of three different parameters (temperature, solvent and extraction time) on the antioxidant and antimicrobial compounds of *Spirulina platensis* liquid extract. They reported that hexane and petroleum ether were more effective than ethanol for *Spirulina platensis* extraction. Furthermore, they tried to optimize the temperature and found that 115°C (hexane) and 170°C (petroleum ether) at 9 minutes were the best extraction temperatures and time at which to extract higher amounts of antimicrobial compounds from *Spirulina platensis* (Santoyo et al. 2006).

Ansari et al. (2013) reported that *Spirulina platensis* is the source of phenolic compounds such as caffeic, chlorogenic, salicylic, synaptic and trans-cinnamic acids. Phenolic compounds are natural antimicrobial and antioxidant materials whose benzenic rings were substituted by one or more hydroxyl groups (Manach et al. 2004). Phlorotannins are tannin compounds which have been detected only in marine algae. Phlorotannins are formed by the polymerization of phloroglucinol (1, 3, 5-

trihydroxybenzene) monomer units in the acetate-malonate pathway (Ragan 1986; Waterman and Mole 1994; Arnold and Targett 1998). Pagnussatt et al. (2014) studied the effect of phenolic extract from *Spirulina sp.* on the growth of *Fusarium graminearum* and mycotoxin levels. They found that the phenolic extract from *Spirulina sp.* reduced the growth of fungal colonies. Ali and Doumandji (2017) reported that the content of total phenol and total flavonoid, phycocyanin and chlorophyll in *Spirulina* was 33.57 ± 1.11 , 15.35 ± 0.54 , 55.7 ± 1.23 and 8.12 ± 1.24 , respectively. The phyto-chemical analysis of *Spirulina platensis* and *Chlorella pyrenoidosa* is shown in Table 4-1.

Table 4-1: Preliminary phyto-chemical analysis of *Spirulina platensis* and *Chlorella pyrenoidosa*. + Present, ND: Not detected. (Ali and Doumandji 2017)

Chemical compounds wanted	Organic extracts				
	Ether	Hexane	Dichloromethane	Acetone	Methanol
Phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannin	-	-	-	-	-
Sterols and Terpenoids	-	-	-	-	-
Quinonic substances	-	-	-	-	-
Alkaloids	-	-	-	+	+
Cardiac Glycosides	-	-	-	-	+

Sun et al. (2016) reported that *Spirulina platensis* has an antibacterial peptide with enzymatic hydrolysis using alkaline protease and papain enzymes. They also found that the minimum inhibitory concentration of this peptide from *Spirulina platensis* was 8 mg/mL for *E. coli* and 16 mg/mL for *Staphylococcus aureus*. The mechanism of action of *Spirulina* antimicrobial materials is shown in Table 4-2, briefly.

Table 4-2: Mechanism of action of *Spirulina* antimicrobial materials. (Pradhan, Das, and Das 2014)

Antimicrobial agent	Mechanism of action	References
Carotenoids	Digestion of cell wall by lysozyme enzymes	Cucco et al. (2007)
Flavonoids	Increase in permeability of the inner bacterial membrane and a dissipation of the membrane potential	Siripatrawan, Vitchayakitti, and Sanguandeekul (2013)
Polyphenols	Binds to adhesions, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption	Sahasrabudhe and Deodhar, (2010)
Polysaccharide	Inhibition of hyaluronidase	Sahasrabudhe and Deodhar, (2010)
Fatty acids and Lipids	Disruption of the cellular membrane	Lampe et al. (1998) and Desbois, Mearns-Spragg, and Smith (2009)

4.3 Antibacterial activity

Ozdemir et al. (2001) reported that various extracts of *Spirulina* showed antimicrobial activity on both gram-positive and gram-negative bacteria. However, it was reported that *Spirulina platensis* is more effective on gram-positive bacteria than gram-negative bacteria (El-Sheekh et al. 2014). This may be due to the fact that the cell wall in gram-positive bacteria consists of a single layer, whereas gram-negative bacterial have multilayered cell walls (Ergene et al. 2006). The antibacterial activity of the algae extract could be due to the presence of different chemicals such as 1-Octadecene, 1-Heptadecane (Lee et al. 2007, Mishra and Sree 2007), flavonoids, triterpenoids, phenolic compounds, fatty acids (Kellam et al. 1988, Demule et al. 1996, Lampe et al. 1998), acrylic acid (Pradhan, Das, and Das 2014) or free hydroxyl group (Yu, Jia, and Dai 2009). Unsaturated and saturated long-chain fatty acids (more than 10 carbons) can lyse protoplasts of the bacteria (Pradhan, Das, and Das 2014).

Antibacterial substances from different algae and their target bacterial pathogens are shown in Table 4-3.

Table 4-3: Antibacterial substances from different algae and their target bacterial pathogens. (Pradhan, Das, and Das 2014)

Antibacterial compound	Microalgae	Target bacterial pathogens	References
pigments	<i>Anabaena cylindrical</i> <i>Chlorococcum humicola</i> , <i>Spirulina platensis</i> , <i>Nostoc</i>	<i>E. coli</i> , <i>S. Typhimurium</i> , <i>K. pneumoniae</i> , v. <i>Cholera</i> , <i>S. Aureus</i> , <i>B. Subtilis</i> , <i>Streptococcus sp.</i> , <i>Pseudomonas sp.</i> , <i>Bacillus sp.</i> , <i>Staphylococcus sp.</i> , <i>E. coli</i> , <i>Enterobacteria aerogens</i>	Goud, Seshikala, and Charya (2007), Bhagavathy, Sumathi, and Madhushree (2011), Muthulakshmi et al. (2012), Fan et al. (2013)
Fatty acids and lipids	<i>Dunaliella salina</i> , <i>Haematococcus pluvialis</i> , <i>Phaeodactylum tricornutum</i> , <i>Chaetoceros muelleri</i> , <i>Spirulina platensis</i>	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>MRSA</i> , <i>Listonella anguillarum</i> , <i>Lactococcus garvieae</i> , <i>Vibrio spp.</i>	Xue et al. (2002), Herrero et al. (2006), Santoyo et al. (2009)
Carbohydrates	<i>Anabaena sphaerica</i> , <i>Chroococcus turgidus</i> , <i>Oscillatoria limnetica</i> , <i>S. Platensis</i> , <i>Porphyridium cruentum</i>	<i>E. coli</i> , <i>S. Typhimurium</i> , <i>S. Faecalis</i>	O'Doherty et al. (2010)
Polyphenols	<i>Anabaena sphaerica</i> , <i>Chroococcus turgidus</i> , <i>Oscillatoria limnetica</i> <i>Spirulina platensis</i>	<i>Salmonella typhi</i> , <i>Streptococcus</i> , <i>E. coli</i> , <i>Staphylococcus aureus</i>	Gao and Zhang (2010), Klejdu et al. (2010), Shu et al. (2011), Hetta et al. (2014)

Microalgal cultures of *A. platensis* showed significant antibacterial activity against six *Vibrio* strains: *Vibrio parahaemolyticus*, *Vibrio anguillarum*, *Vibrio splendidus*, *Vibrio scophthalmi*, *Vibrio alginolyticus*, and *Vibrio lentus* (Kokou et al. 2012). Medina-Jaritz et al. (2011) reported that aqueous extracts of *Spirulina maxima* exhibited antibacterial activity against all tested organisms, except *Bacillus subtilis*, while methanol extract showed antimicrobial activity against all microorganisms, even *Staphylococcus aureus*.

Kumar et al. (2011) studied the methanol and acetone extracts of *Spirulina platensis*. They determined the hexadecane, heptadecane, Eicosane, octadecane, phytol and pentadecane levels by gas chromatography mass spectrometry (GC-MS) and reported that the mentioned compounds had antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium*.

El-Baz et al. (2013) studied the antimicrobial activities of *Spirulina platensis* ethanolic extract. They found that there was no inhibition zone with *Escherichia coli* and *Salmonella typhi* (gram-negative bacteria, of the family Enterobacteriaceae) and *Staphylococcus aureus* (gram-positive bacteria, of the family Firmicutes); however, they reported the significant inhibition zones for *Enterococcus faecalis* and *Candida albicans*. Antibacterial activity against *Streptococcus pyogenes* or *S. aureus* was proven for the phycobiliproteins, water-soluble pigments and isolated from *Spirulina fusiformis* (Najdenski et al. 2013). Purified C-phycocyanin from *S. platensis* markedly inhibited the growth of some drug-resistant bacteria: *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *S. aureus* (Sarada, Kumar, and Rengasamy 2011, Muthulakshmi et al. 2012, Murugan 2012).

El-Monem et al. (2018) studied the effect of pH and various solvents (70% acetone, 70% methanol or 70% ethanol) on antibacterial activities of *Spirulina platensis* extract. The maximum inhibition of *Staphylococcus aureus* NCTC-7447 growth was observed in acetone extract at pH 8.0 and 10, while it was seen at pH 8.0 for *E. coli* NCTC-10418. Elbagowry (2014) reported the stronger inhibitory effect on some tested microorganisms induced by 70% acetone, followed by 70% methanol and then finally by 70% ethanol. Salamatullah (2014) examined total phenolic and flavonoid contents of different solvent *Spirulina* extracts (acetone, methanol and ethanol). The highest phenolic content was seen in acetone extract while the highest flavonoid content was found in methanol. However, Indumathi (2016) reported that acetone extract of *Spirulina* had

no effect on most bacteria except *Enterobacter sp.* and *K. pneumoniae*. Chakraborty, Jayaswal, and Pankaj (2015) studied the antibacterial efficacy of *Spirulina platensis* extract against gram-positive and gram-negative bacteria. *Spirulina* water extract showed maximum bacterial inhibition zone followed by methanol, acetone and ethanol extracts. Elshouny et al. (2017) studied the effect of *Spirulina platensis*, *Chlorella vulgaris*, *Saragassum wightii* and *Saragassum latifolium* extracts by methanol, ethanol, ethyl acetate and chloroform solvents as antimicrobial agents against five bacterial pathogens: *S. aureus*, *E. coli*, *P. aeruginosa*, *Salmonella sp.* and *Shigella sp.* They showed that methanol extracts of cyanobacteria showed highest antibacterial activity against the selected bacterial pathogens. The GC-MS analysis of different compounds in ethanol, hexane, chloroform and dichloromethane extracts of *Spirulina* are shown in Tables 4-4, 4-5, 4-6 and 4-7, respectively.

Table 4-4: GC-MS analysis of different compounds in ethanol extract of *Spirulina*. (Ramasamy 2014)

peak	RT	compounds	%
1	9.351	Pentadecane	0.18
2	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.19
3	11.1	Hexadecane	0.18
4	13.22	Heptadecane	4.23
5	16.66	1,9-Nonanediol, methanesulfonate	0.16
6	19.88	n-hexadecanoic acid	2.5
7	23.86	Phytol	2.12
8	27.51	Docosane	1.07
9	28.57	3-Octadecane	2.43
10	29.11	Heneicosane	45.85
11	30.3	17-Pentatriacontane	0.49
12	31.26	Dodecane, 5 methyl	1.39
13	31.6	11,15- dimethylhepatatriacontane	0.83
14	33.33	Z-14-Nonacosane	1.31
15	33.74	Octadecane	27.04
16	39.61	Triacontane	10.01

Table 4-5: GC-MS analysis of different compounds in hexane extract of *Spirulina*. (Ramasamy 2014)

peak	RT	compounds	%
1	7.96	Tetradecane	0.19
2	9.33	Pentadecane	0.11
3	9.6	Phenol, 2,5-bis(1,1-dimethylethyl)	1.01
4	9.73	Butylatedhydroxytoluene	0.07
5	11.1	Hexadecane	0.19
6	13.21	Hepatadecane	0.31
7	13.5	Hepacosane	0.07
8	15.63	Octadecane	0.14
9	18.93	Dodecane, 2,6,11 trimethy	0.08
10	19.99	1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester	0.11
11	20.97	Eicosane	0.11
12	23.53	Hepacosane	0.09
13	25.44	Decosane	0.72
14	26.02	Octacosane	0.07
15	27.02	Tricosane	1.63
16	28.38	Tetracosane	7.22
17	29.13	Heneicosane	24.41
18	29.59	Pentacosane	10.75
19	30.35	Pentacosane	0.23
20	30.86	Hexacosane	10.63
21	31.6	Tetracosane, 1- bromo	0.38
22	31.76	Tetracosane, 9-octyl	1.13
23	32.35	Heptacosane	10.74
24	33.43	Octacosane	0.82
25	33.77	Dotriacontane	14.72
26	34.16	Octacosane	6.3
27	36.143	Oxalic acid, isobutyl octadecyl ester	0.46
28	36.392	Heptacosane	4.03
29	39.188	Triacontane	1.49

Table 4-6: GC-MS analysis of different compounds in chloroform extract of *Spirulina*. (Ramasamy 2014)

peak	RT	compounds	%
1	7.96	Tetradecane	0.81
2	9.34	Decane 2,3,5 - trimethy	1.05
3	9.6	Phenol, 2,5-bis(1,1-dimethylethyl)	1.75
4	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.6
5	11.1	Hexadecane	0.79
6	13.22	Heptadecane	12.3
7	16.66	8-Azabicyclo[3.2.1] octane	0.9
8	19.85	n-Hexadecanoic acid	3.45
9	22.45	Pentafluoropropionic acid, undecyl ester	0.54
10	23.82	Phytol	1.53
11	25.17	Dichloroacetic acid, tri decyl ester	1.75
12	26.56	Sulfurous acid, butyl tetra decyl ester	0.99
13	27.51	Octadecane, 1 - iodo	1.93
14	27.86	1-Hentetracontanol	0.63
15	28.57	Tetrapentacontane	3.1
16	28.95	Pentacosane	15.8
17	29.65	Nonahexacontanoic acid	1.25
18	30.52	1-(2-methylpropenyl) aziridine	11.28
19	32.36	Undecane	2.1
20	33.08	Hepatafluorobutanoic acid, heptadecyl ester	0.94
21	33.65	Octacosane	28.66
22	35.86	1-Decanol, 2-hexyl	3.25
23	36.49	Tetrapentacontane, 1,54 -dibromo	1.86
24	37.16	Tetrapentacontane, 1,54 -dibromo	1.65
25	39.25	1-Hentetracontanol	1.09

Table 4-7: GC-MS analysis of different compounds in dichloromethane extract of *Spirulina*. (Ramasamy 2014)

peak	RT	compounds	%
1	6.72	Cinnamaldehyde	0.2
2	7.09	2-Butenoic acid, 2-propenylidene ester	0.59
3	7.86	1-Tetradecane	0.33
4	9.35	Pentadecane	0.44
5	9.6	Phenol, 2,5-bis(1,1-dimethylethyl)	1.78
6	10.22	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	0.4
7	10.97	1-Hexadecane	0.38
8	11.11	Hexadecane	0.2
9	11.96	Iso-valeraldehydepropyleneglycol acetyl	0.12
10	12.63	Ar-tumerone	0.43
11	12.23	Heptadecane	6.66
12	13.46	Curlone	0.15
13	14.99	2-(2- xycyclohexyloxy)pyridine-n-oxide	0.1
14	15.46	1-Octadecane	0.57
15	16.66	Bicyclo[3.1.1]heptan, 2,6,6-trimethy-, (1.alpha., 2.beta.,5.alpha)	0.54
16	17.32	Dodeca-1,6-dien-12-ol, 6,10-dimethyl	0.14
17	17.8	7-Octadecyne, 2-methyl	0.11
18	18.96	Pentadecanoic acid, 14-methyl ester	0.47
19	19.93	n-hexadecanoic acid	5.24
20	20.79	Cycloeicosane	0.58
21	21.7	n-hexadecanoic acid	0.12
22	23.04	cis,cis,cis-7,10,13 - hexadecatriena	0.13
23	23.41	9,11-Octadecadienoic acid methyl ester	1.83
24	23.83	Phytol	0.69
25	24.21	1-pentadecyne	3.13
26	24.64	Acetic acid isopropylidene-hydrazine	0.51
27	25.44	Docosane	2.14
28	25.79	1,19-Eicosadiene	0.1
29	26.39	3-methylhexyl isothiocyanate	0.11
30	27.02	Heptadecane, 3-methyl	4.83
31	27.88	Tetrapentacontane	0.08
32	28.38	Tetracosane	9.57
33	29.59	Pentacosane	14.09
34	30.35	Pentacosane	0.85
35	30.5	Tetracosane, 3-ethyl	0.36

36	30.86	Hexacosane	12.36
37	31.75	Tetracosane	1.5
38	32.35	Heptacosane	9.61
39	33.43	Octacosane	1.38
40	33.64	Hexacosane	0.74
41	33.93	1-Docosene	0.26
42	34.16	Heneicosane	6.88
43	34.82	Dichloroaceticacid, tridecyl ester	0.27
44	35.49	Hexacosane	1.21
45	35.75	Heptacosane	0.72
46	36.14	1-Hexacosane	0.34
47	36.39	Nonacosane	4.51
48	37.24	17-Pentatriacontane	0.49
49	38.06	Eicosane	0.7
50	38.4	Eicosane	0.63
51	38.39	Cyclopentane, 1,1,3-trimethyl	0.38

El-Baky, El Baz, and El-Baroty (2008) reported that *Spirulina maxima* extracts showed antibacterial activities against six bacteria species (*B. subtilis*, *B. cereus*, *S. aureus*, *M. luteus*, *K. pneumoniae*, *S. marcescens*) with *minimum inhibitory* concentrations (MICs) ranged from 30–40 µg/mL. Table 4-8 shows the MIC of *Spirulina platensis* extracts using different solvents against bacteria. It seems that the difference in the results of the studies may due to the difference in algae growth media, algae species, kind of solvent and method of extraction.

Spirulina has been shown to enhance the chicken defense system via increasing microbial killing activity (Qureshi, Garlich, and Kidd 1996). It seems that the antibacterial effect of *Spirulina platensis* from killing pathogenic bacteria had a potential for alleviating dysbacteriosis in the chicken gut and decreasing clinical signs of diarrhea (Gružauskas et al. 2004).

Table 4-8: MIC of *Spirulina platensis* extract using different solvents (mg/ml) against bacteria. (Usharani et al. 2015)

	Hexane	Methanol	Acetone	Ethanol	Petroleum ether	Positive control*
<i>Staphylococcus aureus</i>	10	1.25	1.25	5	2.50	5
<i>Streptococcus pyogenes</i>	10	1.25	2.50	10	5	10
<i>Streptococcus epidermidis</i>	20	1.25	2.50	10	5	10
<i>Proteus mirabilis</i>	5	1.25	1.25	5	2.50	10
<i>Bacillus cereus</i>	10	1.25	1.25	5	2.50	10
<i>Escherichia coli</i>	10	2.50	2.50	10	5	5
<i>Pseudomonas aeruginosa</i>	20	2.50	5	10	10	5
<i>Vibrio cholerae</i>	80	5	10	40	20	20
<i>Salmonella typhi</i>	40	5	5	20	10	20
<i>Klebsiella pneumoniae</i>	10	1.25	2.50	5	2.50	10
<i>Shigella flexneri</i>	5	1.25	1.25	5	2.50	10

*Ampicillin (5 µg)

4.4 Antifungal activity

It was reported that diethyl ether and acetone extract of *Spirulina platensis* had the highest antibacterial and antifungal activity (Abedin and Taha 2008; Ozdemir, et al. 2004). Previous studies showed that *Spirulina* phenolic extracts had antifungal activity by inhibiting ergosterol, which is a component of the fungal cell membrane (Peeler et al. 1989), glucosamine, a growth factor present in the fungal cells of some genera (Sparringa and Owens 1999) and some other proteins. The activity of ATP binding cassette (ABC) transporters, which are critical in cell homeostasis, inhibited by phenolic compounds like phenolic acids, flavonoids, catechins, chalcones, xanthenes, stilbenes, anthocyanins, tannins, anthraquinones, and naphthoquinones which have a lipophilic nature (You et al. 1995; Meschini et al. 2003). It was found that polyphenols can also bind directly to the proteins and disturb the tertiary structure of proteins, thus effectively inhibiting the function of ABC transporters (Ma and Wink 2008; Wink and Schimmer 2010). Table 4-9 shows the MIC of *Spirulina platensis* extract by different solvents on some *Aspergillus* and *Candida* species.

Table 4-9: MIC of *Spirulina platensis* extract using different solvents (mg/ml) against fungi. (Usharani et al. 2015)

	Hexane	Methanol	Acetone	Ethanol	Petroleum ether	Positive control*
<i>Aspergillus flavus</i>	32	8	16	32	16	8
<i>Aspergillus niger</i>	16	4	8	16	8	8
<i>Aspergillus fumigatus</i>	32	8	8	16	16	8
<i>Candida tropicalis</i>	64	16	32	32	64	32
<i>Candida albicans</i>	16	2	4	8	8	4
<i>Candida glabrata</i>	32	4	8	16	8	4

*Flucanazole (100 units/disc)

4.5 Antiviral activity

Spirulina platensis contains a wide range of bioactive molecules and, thus, can be a rich source of different types of medicine substances (Blinkova, Gorobets, and Baturu 2001; Rechter et al. 2006; Singh et al. 2011; Challouf et al. 2011; Arun, Gupta, and Singh 2012; Priyadarshani and Rath 2012; Kumar et al. 2013; Abd El Baky and El-Baroty 2013). *Spirulina platensis* natural materials exhibited a potent activity against several enveloped viruses by blocking viral absorption penetration and some replication stages of progeny viruses after penetration into cells (Yakoot and Salem 2012). Administration of a low level of *Spirulina* can reduce viral replication, whereas at higher levels it can block replication. It was shown that water-soluble extracts of *Spirulina* could inhibit viral cell penetration and replication of the herpes simplex virus type 1 (HSV-1) (Daoud and Soliman 2015). The *Spirulina* extract inhibits viral protein synthesis without adverse effects on host cell functions. The antiviral activity is attributed to Ca-SP, which has been shown to inhibit replication of many viruses by inhibiting viral penetration into target cells without host toxicity (Kulshreshtha et al. 2008; Lee et al. 2008; Deng and Chow 2010; Karkos et al. 2011). The antiviral activity of water-soluble extracts of *Spirulina* may related to the combined action of Ca-SP and Immulina (Belay and Gershwin 2007). Two kinds of disaccharide repeating units of calcium-*Spirulan* are shown in Figure 4-1.

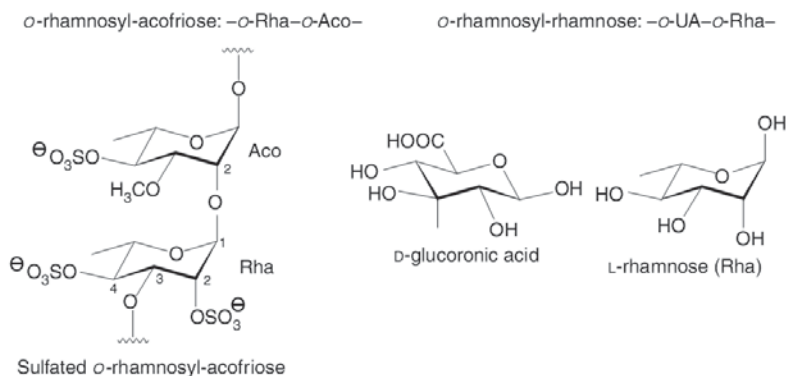


Figure 4-1: Two kinds of disaccharide repeating units of calcium-*Spirulan*. (Belay and Gershwin 2007)

In general, viral growth is divided into three stages, and antiviral action may take place during one or more stages: Stage I, which consists of adsorption and invasion of cells; Stage II, or the eclipse phase, during which the cell is forced to synthesize multiple copies of said virus; and Stage III, or maturity and release of virus particles (Amaro, Guedes, and Malcata 2011). Hetta et al. (2014) reported that the methanol extract of *Spirulina* (70% v/v) showed antiviral activity with 56.7%, 60%, 53.3%, and 50% inhibition against rotavirus Wa strain, adenovirus type 7, adenovirus type 40 and Coxsackievirus B4, respectively. *Spirulina* n-hexane fraction was also active with all tested viruses with a percent inhibition of 66.7%, 63.3%, 50%, and 50%, respectively. The ethyl acetate fraction had antiviral activity against only the rotavirus Wa strain, with 53.3% inhibition (Hetta et al. 2014).

Ozcelik et al. (2005) reported that the fatty acids exhibit antiviral activity. The gas chromatography (GC) analysis showed the presence of α -pinene and α -terpineol, which were reported for their antiviral activity (Astani, Reichling, and Schnitzler 2010). Also, it was shown that the phenolic components such as tannins had antiviral activity (Ayehunie et al. 1998; Nuhu 2013). El-Baky, El Baz, and El-Baroty (2008) reported that sulfated polysaccharide extracted with hot water from *S. platensis* (reared in a medium containing 45 ppm nitrogen) had the highest antiviral activity when the concentration of 20 μg was used. This may be due to the high molecular weight of sulfated polysaccharides. The antiviral activities of sulfated polysaccharides increased with increasing degrees of sulfating and molecular weight (El-Baky, El Baz, and El-Baroty 2008). Sulphated

exopolysaccharides from marine microalgae have been claimed to interfere with Stage I of some enveloped viruses (Batinic and Robey 1992); they offer competitive advantages because of their broad spectrum of antiviral properties against viruses such as HSV and HIV-1 (Damonte, Pujol, and Coto 2004). Chirasuwan et al. (2007) examined the role of sulfate groups in the polysaccharides extracted from *S. platensis* by eliminating sulfate groups before testing for anti HSV-1. In the absence of sulfate groups in polysaccharide, no significant anti HSV-1 activity was seen; thus, it seems that sulfate groups play an important role in the antiviral activity of *Spirulina* extracts. The antiviral activity of *Spirulina* may also relate to three groups of substances: sulfated polysaccharides, sulfoglycolipids and a protein-bound pigment—the allo-phycoecyanin (Belay and Gershwint 2007). Few research projects have investigated the antiviral effect of *Spirulina platensis* on avian Newcastle and influenza (Mobarez et al. 2018). Results showed that *Spirulina platensis* had a role in the mitigation of infections; nevertheless, none of them scrutinized the precise mode of actions. This area therefore needs more investigation. McWhinney et al. (1989) reported that β -carotene supplementation to the diet of cockerels significantly increased antibody production against Newcastle disease. Different algae are rich sources of carotenoids; therefore, using *Spirulina* or another algae as a feed additive might improve the immune response to vaccines used for protecting the birds from diseases.

References

- Abd El Baky, H. H., and G. S. El-Baroty. 2013. "Healthy benefit of microalgal bioactive substances." *Journal of Aquatic Science* 1 (1): 11–23.
- Abedin, R. M., H. M. and Taha. 2008. "Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*." *Global Journal of Biotechnology and Biochemistry* 3 (1): 22–31.
- Al-ghanayem, A. A. 2017. "Antimicrobial activity of *Spirulina platensis* extracts against certain pathogenic bacteria and fungi." *Advances in Bioresearch* 8 (6): 96-101.
- Ali, I. H., and A. Doumandji. 2017. "Comparative phytochemical analysis and in vitro antimicrobial activities of the cyanobacterium *Spirulina platensis* and the green alga *Chlorella pyrenoidosa*: potential

- application of bioactive components as an alternative to infectious diseases.” *Bulletin de l’Institut Scientifique, Rabat*, no. 39, 41–49.
- Amaro, H. M., A. C. Guedes, and F. X. Malcata. 2011. “Antimicrobial activities of microalgae: an invited review.” *Science against microbial pathogens: communicating current research and technological advances* 3:1272–84.
- Ansari, M. A., A. Anurag, Z. Fatima, and S. Hameed. 2013. “Natural phenolic compounds: a potential antifungal agent.” *Microbiology* 1189–95.
- Arnold, T. M., and N. M. Targett. 1998. “Quantifying in situ rates of phlorotannin synthesis and polymerization in marine brown algae.” *Journal of Chemical Ecology* 24 (3): 577–95.
- Arun, N., S. Gupta, and D. P. Singh. 2012. “Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi.” *International Journal of Pharmaceutical Sciences and Research* 3 (12): 4866.
- Astani, A., J. Reichling, and P. Schnitzler. 2010. “Comparative study on the antiviral activity of selected monoterpenes derived from essential oils.” *Phytotherapy Research* 24 (5): 673–79.
- Ayehunie, S., A. Belay, T. W. Baba, and R. M. Ruprecht. 1998. “Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*).” *Journal of acquired immune deficiency syndromes and human retrovirology: official publication of the International Retrovirology Association* 18 (1): 7–12.
- Batinic, D., and F. A. Robey. 1992. “The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides.” *Journal of Biological Chemistry* 267 (10): 6664–71.
- Belay, A., and Gershwin, M. E. 2007. *Spirulina (Arthrospira)*. In *Spirulina in Human Nutrition and Health* (pp. 11-35). CRC Press.
- Bhagavathy, S., P. Sumathi, and M. Madhushree. 2011. “Antimutagenic assay of carotenoids from green algae *Chlorococcum humicola* using *Salmonella typhimurium* TA98, TA100 and TA102.” *Asian Pacific Journal of Tropical Disease* 1 (4): 308–16.
- Blinkova, L. P., O. B. Gorobets, and A. P. Baturo. 2001. “Biological activity of *Spirulina*.” *Zhurnal mikrobiologii, epidemiologii, i immunobiologii*, no. 2, 114–18.
- Chakraborty, B., R. P. Jayaswal, and P. P. Pankaj. 2015. “Antimicrobial Activity of *Spirulina platensis* Extract Against Gram Positive and Gram Negative Bacteria—A Comparative Study.” *International*

- Journal of Current Pharmaceutical Review and Research* 6 (4): 212–14.
- Challouf, R., L. Trabelsi, R. Ben Dhieb, O. El Abed, A. Yahia, K. Ghazzi, J. Ben Ammar, H. Omran, and H. Ben Ouada. 2011. "Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*." *Brazilian Archives of Biology and Technology* 54 (4): 831–38.
- Chirasuwan, N., R. Chaiklahan, M. Ruengjitchatchawalya, B. Bunnag, and M. Tanticharoen. 2007. "Anti HSV-1 activity of *Spirulina platensis* polysaccharide." *Kasetsart Journal (Natural Science)* 41:311–18.
- Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2007. "Effects of β -carotene on adult immune condition and antibacterial activity in the eggs of the Grey Partridge, *Perdix perdix*." *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 147 (4): 1038–46.
- Damonte, E. B., C. A. Pujol, and C. E. Coto. 2004. "Prospects for the therapy and prevention of dengue virus infections." *Advances in Virus Research* 63:239–86.
- Daoud, H. M., and E. M. Soliman. 2015. "Evaluation of *Spirulina platensis* extract as natural antiviral against foot and mouth disease virus strains (A, O, SAT2)." *Veterinary World* 8 (10):1260.
- Demule, M. C. Z., G. Z. Decaire, and M. S. Decano. 1996. "Bioactive substances from *Spirulina platensis* (Cyanobacteria)." *International Journal of Experimental Botany* 58:93–96.
- Deng, R., and T. J. Chow. 2010. "Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*." *Cardiovascular Therapeutics* 28 (4): e33–e45.
- Desbois, A. P., A. Mearns-Spragg, and V. J. Smith. 2009. "A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA)." *Marine Biotechnology* 11 (1): 45–52.
- Elbagowry, H. M. A. 2014. "Antimicrobial activities of some selected fresh water algae isolated from gharblah governorate." Master's Thesis, Faculty of Science, Tanta University.
- El-Baky, H. H. A., F. K. El Baz, and G. S. El-Baroty. 2008. "Characterization of nutraceutical compounds in blue green alga *Spirulina maxima*." *Journal of Medicinal Plants Research* 2 (10): 292–300.
- El-Baz, F. K., W. M. El-Senousy, A. B. El-Sayed, and M. M. Kamel. 2013. "In vitro antiviral and antimicrobial activities of *Spirulina*

- platensis extract.” *Journal of Applied Pharmaceutical Science* 3 (12): 52–56.
- El-Monem, A. M. A., M. M. Gharieb, A. E. M. Hussian, and K. M. Doman. 2018. “Effect of pH on phytochemical and antibacterial activities of *Spirulina platensis*.” *International Journal of Applied Environmental Sciences* 13 (4): 339–51.
- El-Sheekh, M. M., S. M. Daboor, M. A. Swelim, and S. Mohamed. 2014. “Production and characterization of antimicrobial active substance from *Spirulina platensis*.” *Iranian Journal of Microbiology* 6 (2): 112–19.
- El-Sheekh, M. M., A. M. Dawah, A. M. A. El-Rahman, H. M. El-Adel, and R. A. A. El-Hay. 2008. “Antimicrobial activity of the cyanobacteria *Anabaena wisconsinense* and *Oscillatoria curvipes* against pathogens of fish in aquaculture.” *Annals of Microbiology* 58 (3): 527.
- El-Sheekh, M. M., M. E. Osman, M. A. Dyab, and M. S. Amer. 2006. “Production and characterization of antimicrobial active substance from the cyanobacterium *Nostoc muscorum*.” *Environmental Toxicology and Pharmacology* 21 (1): 42–50.
- Elshouny, W. A. E. F., M. M. El-Sheekh, S. Z. Sabae, M. A. Khalil, and H. M. Badr. 2017. “Antimicrobial Activity of *Spirulina platensis* Against Aquatic Bacterial Isolates.” *The Journal of Microbiology, Biotechnology and Food Sciences* 6 (5): 1203.
- Ergene, A., P. Guler, S. Tan, E. Hamzaoglu, and A. Duran. 2006. “Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. *artvinense*.” *African Journal of Biotechnology* 5 (11): 1087.
- Fan, M., Z. Liao, R. xin Wang, and N. Xu. 2013. “Isolation and antibacterial activity of *Anabaena phycocyanin*.” *African Journal of Biotechnology* 12 (15): 1869–73.
- Gao, D., and Y. Zhang. 2010. “Comparative antibacterial activities of crude polysaccharides and flavonoids from *Zingiber officinale* and their extraction.” *Asian Journal of Traditional Medicines* 5 (6): 235–38.
- Ghasemi, Y., M. T. Yazdi, A. Shafiee, M. Amini, S. Shokravi, and G. Zarrini. 2004. “Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*.” *Pharmaceutical Biology* 42 (4–5): 318–22.
- Goud, M. J. P., D. Seshikala, and M. S. Charya. 2007. “Antibacterial activity and biomolecular composition of certain fresh water microalgae collected from River Godavari (India).” *International Journal on Algae* 9 (4): 350–58.

- Gružauskas, R., R. Lekavičius, A. Racevičiūtė-Stupelienė, V. Šašytė, V. Tėvelis, and G. J. Švirmickas. 2004. "Viščiukų broilerių virškinimo procesų optimizavimas simbiotiniais preparatais." *Veterinarija ir zootechnika* 28 (50): 51–56.
- Gudmundur, B. 2005. Antimicrobial polypeptides and lipids as a part of innate defense mechanism of fish and human fetus. Institutionen för medicinsk biokemi och biofysik (MBB)/Department of Medical Biochemistry and Biophysics. ISBN: 91-7140-546-1.
- Haq, A. U., C. A. Bailey, and A. Chinnah. 1996. "Effect of β -carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets." *Poultry Science* 75 (9): 1092–97.
- Hernández-Corona, A., I. Nieves, M. Meckes, G. Chamorro, and B. L. Barron. 2002. "Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2." *Antiviral Research* 56 (3): 279–85.
- Herrero, M., L. Jaime, P. J. Martín-Álvarez, A. Cifuentes, and E. Ibáñez. 2006. "Optimization of the extraction of antioxidants from *Dunaliella salina* microalga by pressurized liquids." *Journal of Agricultural and Food Chemistry* 54 (15): 5597–603.
- Hetta, M., R. Mahmoud, W. El-Senousy, M. Ibrahim, G. El-Taweel, and G. Ali. 2014. "Antiviral and antimicrobial activities of *Spirulina platensis*." *World Journal of Pharmacy and Pharmaceutical Science* 3 (6): 31–39.
- Hsiao, G., P. H. Chou, M. Y. Shen, D. S. Chou, C. H. Lin, and J. R. Sheu. 2005. "C-phycocyanin, a very potent and novel platelet aggregation inhibitor from *Spirulina platensis*." *Journal of Agricultural and Food Chemistry* 53 (20): 7734–40.
- Indumathi, A. 2016. "Comparative Study on the Antibacterial Activity of *S. platensis* and *Oscillatoria* sp. Grown *In vitro*." *International Journal of Current Microbiology and Applied Sciences* 3, 14–18.
- Karkos, P. D., S. C. Leong, C. D. Karkos, N. Sivaji, and D. A. Assimakopoulos. 2011. "Spirulina in clinical practice: evidence-based human applications." *Evidence-Based Complementary and Alternative Medicine* 2011, Article ID 531053.
- Kellam, S. J., R. J. P. Cannell, A. M. Owsianka, and J. M. Walker. 1988. "Results of a large scale screening programmed to detect antifungal activity from marine and freshwater micro algae in laboratory culture." *British Phycological Journal* 23:45–47.
- Khan, M., J. C. Shobha, I. K. Mohan, M. U. R. Naidu, C. Sundaram, S. Singh, P. Kuppasamy, and V. K. Kutala. 2005. "Protective effect of

- Spirulina against doxorubicin-induced cardiotoxicity.” *Phytotherapy Research* 19 (12): 1030–37.
- Khan, M., J. C. Shobha, I. K. Mohan, I. K., M. U. Rao Naidu, A. Prayag, and V. K. Kutala. 2006. “Spirulina attenuates cyclosporine-induced nephrotoxicity in rats.” *Journal of Applied Toxicology* 26 (5): 444–51.
- Klejdus, B., L. Lojková, M. Plaza, M. Šnóblová, and D. Štěrbová. 2010. “Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry.” *Journal of Chromatography A* 1217 (51): 7956–65.
- Kokou, F., P. Makridis, M. Kentouri, and P. Divanach. 2012. “Antibacterial activity in microalgae cultures.” *Aquaculture Research* 43 (10): 1520–27.
- Kulshreshtha, A., U. Jarouliya, P. Bhadauriya, G. B. K. S. Prasad, and P. S. Bisen. 2008. “Spirulina in health care management.” *Current Pharmaceutical Biotechnology* 9 (5): 400–5.
- Kumar, V., A. K. Bhatnagar, and J. B. Srivastava. 2011. “Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound.” *Journal of Medicinal Plants Research* 5 (32): 7043–48.
- Kumar, V., P. S. Tirumalai, A. Singh, A. K. Bhatnagar, and J. N. Shrivastava. 2013. “Natural compounds from algae and *Spirulina platensis* and its antimicrobial activity.” *Indo Global Journal of Pharmaceutical Science* 3 (3): 212–23.
- Lampe, M. F., L. M. Ballweber, C. E. Isaacs, D. L. Patton, and W. E. Stamm. 1998. “Killing of *Chlamydia trachomatis* by novel antimicrobial lipids adapted from compounds in human breast milk.” *Antimicrobial Agents and Chemotherapy* 42 (5): 1239–44.
- Lee, E. H., J. E. Park, Y. J. Choi, K. B. Huh, and W. Y. Kim. 2008. “A randomized study to establish the effects of spirulina in type 2 diabetes mellitus patients.” *Nutrition Research and Practice* 2 (4): 295–300.
- Lee, J. B., T. Hayashi, K. Hayashi, U. Sankawa, M. Maeda, T. Nemoto, and H. Nakanishi. 1998. “Further purification and structural analysis of calcium spirulan from *Spirulina platensis*.” *Journal of Natural Products* 61 (9): 1101–4.
- Lee, S. H., K. S. Chang, M. S. Su, Y. S. Huang, Y. S., and H. D. Jang. 2007. “Effects of some Chinese medicinal plant extracts on five different fungi.” *Food Control* 18 (12): 1547–54.
- Ma, Y., and M. Wink. 2008. “Lobeline, a piperidine alkaloid from *Lobelia* can reverse P-gp dependent multidrug resistance in tumor cells.” *Phytomedicine* 15 (9): 754–58.

- Mala, R., M. Sarojini, S. Saravanababu, and G. Umadevi. 2009. "Screening for antimicrobial activity of crude extracts of *Spirulina platensis*." *Journal of Cell and Tissue Research* 9 (3): 1951–55.
- Manach, C., A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez. 2004. "Polyphenols: food sources and bioavailability." *The American Journal of Clinical Nutrition* 79 (5): 727–47.
- McWhinney, S. L. R., C. A. Bailey, and B. Panigrahy. 1989. "Immunoenhancing effect of B-carotene in chicks." *Poultry Science* 68 (Suppl 1): 94.
- Medina-Jaritz, N. B., D. R. Perez-Solis, S. L., Ruiloba de Leon, and R. Olvera-Ramírez. 2011. "Antimicrobial activity of aqueous and methanolic extracts from *Arthrospira maxima*." In *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*, edited by A. Méndez-Vilas, 1267–71.
- Mendiola, J. A., L. Jaime, S. Santoyo, G. Reglero, A. Cifuentes, E. Ibáñez, and F. J. Señoráns. 2007. "Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*." *Food Chemistry* 102 (4): 1357–67.
- Meschini, S., M. Marra, A. Calcabrini, E. Federici, C. Galeffi, and G. Arancia. 2003. "Voacamine, a bisindolic alkaloid from *Peschiera fuchsiaefolia*, enhances the cytotoxic effect of doxorubicin on multidrug-resistant tumor cells." *International Journal of Oncology* 23 (6): 1505–13.
- Mishra, P. M., and A. Sree. 2007. "Antibacterial activity and GCMS analysis of the extract of leaves of *Finlaysonia obovata* (a mangrove plant)." *Asian Journal of Plant Sciences* 6 (1): 168–72.
- Mobarez S. M., A. M. Rizk, A. M. Abdel latif, and Osama A. El-Sayed. 2018. "Effect of supplementing diet with *Spirulina platensis* algae or turmeric on productive and reproductive performance of golden montazah layers." *Egyptian Poultry Science Journal* 38 (1): 109–25.
- Mohan, I. K., M. Khan, J. C. Shobha, M. U. R. Naidu, A. Prayag, P. Kuppusamy, and V. K. Kutala. 2006. "Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats." *Cancer Chemotherapy and Pharmacology* 58 (6): 802.
- Murugan, T. 2012. "Antibacterial activity of C-phycocyanin against clinical isolates by disc diffusion method." *Journal of Pharmacy Research* 5 (6): 3020–21.
- Muthulakshmi, M., A. Saranya, M. Sudha, and G. Selvakumar. 2012. "Extraction, partial purification, and antibacterial activity of phycocyanin from *Spirulina* isolated from fresh water body against

- various human pathogens.” *Journal of Algal Biomass Utilization* 3 (3): 7–11.
- Nagaoka, S., K. Shimizu, H. Kaneko, F. Shibayama, K. Morikawa, Y. Kanamaru, A. Otsuka., T. Hirahashi, and T. Kato. 2005. “A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats.” *The Journal of Nutrition* 135 (10): 2425–30.
- Najdenski, H. M., L. G. Gigova, I. I. Iliev, P. S. Pilarski, J. Lukavský, I. V. Tsvetkova, M. S. Ninova, and V. K. Kussovski. 2013. “Antibacterial and antifungal activities of selected microalgae and cyanobacteria.” *International Journal of Food Science and Technology* 48 (7): 1533–40.
- Noaman, N. H., A. Fattah, M. Khaleafa, S. H. Zaky. 2004. “Factors affecting antimicrobial activity of *Synechococcus leopoliensis*.” *Microbiological Research* 159 (4): 395–402.
- Nuhu, A. A. 2013. “*Spirulina* (Arthrospira): An important source of nutritional and medicinal compounds.” *Journal of Marine Biology* 84: 1–8.
- O’Doherty, J. V., S. Dillon, S. Figat, J. J. Callan, and T. Sweeney. 2010. “The effects of lactose inclusion and seaweed extract derived from *Laminaria* spp. on performance, digestibility of diet components and microbial populations in newly weaned pigs.” *Animal Feed Science and Technology* 157 (3–4): 173–80.
- Özçelik, B., M. Aslan, I. Orhan, and T. Karaoglu. 2005. “Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of *Pistacia vera*.” *Microbiological Research* 160 (2): 159–64.
- Ozdemir, G., M. C. Dalay, K. Kuçukakyuz, B. Pazarbaşı, and M. Yilmaz. 2001. “Determining the Antimicrobial Activity Capacity of Various Extracts of *Spirulina platensis* Produced in Turkey’s Conditions.” *Ege Journal of Fisheries and Aquatic Sciences* 18 (1): 161–166.
- Ozdemir, G., N. Ulku Karabay, M. C. Dalay, and B. Pazarbaşı. 2004. “Antibacterial activity of volatile component and various extracts of *Spirulina platensis*.” *Phytotherapy Research* 18 (9): 754–57.
- Pagnussatt, F. A., E. M. Del Ponte, J. Garda-Buffon, and E. Badiale-Furlong. 2014. “Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp.” *Pesticide Biochemistry and Physiology* 108:21–26.
- Peeler, T. C., M. B. Stephenson, K. J. Einspahr, and G. A. Thompson. 1989. “Lipid characterization of an enriched plasma membrane fraction of *Dunaliella salina* grown in media of varying salinity.” *Plant Physiology* 89 (3): 970–76.

- Pradhan, J., S. Das, and B. K. Das. 2014. "Antibacterial activity of freshwater microalgae: A review." *African Journal of Pharmacy and Pharmacology* 8 (32): 809–18.
- Prashantkumar, P., S. B. Angadi, and G. M. Vidyasagar. 2013. Antimicrobial activity of blue-green and green algae. *Indian Journal of Pharmaceutical Sciences*. Pp 647-648.
- Priyadarshani, I., and B. Rath. 2012. "Commercial and industrial applications of micro algae—A review." *Journal of Algal Biomass Utilization* 3 (4): 89–100.
- Qureshi, M. A., J. D. Garlich, and M. T. Kidd. 1996. "Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens." *Immunopharmacology and Immunotoxicology* 18 (3): 465–76.
- Ragan, M. A. 1986. "Phlorotannins, brown algal polyphenols." *Progress in Phycological Research* 4:177–241.
- Ramasamy, V. 2014. "Chemical composition of *Spirulina* by gas chromatography coupled with mass spectrophotometer (GC-MS)." *International Journal of Pharmaceutical and Phytopharmacological Research* 3 (3).
- Rechter, S., T. König, S. Auerchs, S. Thulke, H. Walter, H. Dörnenburg, C. Walter, and M. Marschall. 2006. "Antiviral activity of *Arthrospira*-derived spirulan-like substances." *Antiviral Research* 72 (3): 197–206.
- Salamatullah, A. 2014. Characterization of extraction methods to recover phenolic-rich antioxidants from blue green algae (*spirulina*) using response surface approaches. MSc thesis in food science and technology. Lincoln, Nebraska. May 2014. 82 pages.
- Santoyo, S., M. Herrero, F. J. Señorans, A. Cifuentes, E. Ibáñez, and L. Jaime. 2006. "Functional characterization of pressurized liquid extracts of *Spirulina platensis*." *European Food Research and Technology* 224 (1): 75.
- Santoyo, S., I. Rodríguez-Meizoso, A. Cifuentes, L. Jaime, G. G. B. Reina, F. J. Señorans, and E. Ibáñez. 2009. "Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae." *LWT-Food Science and Technology* 42 (7): 1213–18.
- Sarada, D. V., C. S. Kumar, and R. Rengasamy. 2011. "Purified C-phycocyanin from *Spirulina platensis* (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria." *World Journal of Microbiology and Biotechnology* 27 (4): 779–83.

- Saranraj, P., and S. Sivasakthi. 2014. "Spirulina platensis—food for future: a review." *Asian Journal of Pharmaceutical Science and Technology* 4 (1): 26–33.
- Shanab, S. M. M. 2007. "Bioactive allelo-chemical compounds from *Oscillatoria* species (Egyptian isolates)." *International Journal of Agriculture and Biology* 9 (4): 617–21.
- Shanmughapriya, S., A. Manilal, S. Sujith, J. Selvin, G. S. Kiran, and K. Natarajaseenivasan. 2008. "Antimicrobial activity of seaweeds extracts against multiresistant pathogens." *Annals of Microbiology* 58 (3): 535–41.
- Shu, Y., Y. Liu, L. Li, J. Feng, B. Lou, X. Zhou, and H. Wu. 2011. "Antibacterial activity of quercetin on oral infectious pathogens." *African Journal of Microbiology Research* 5 (30): 5358–61.
- Sahasrabudhe, A., and M. Deodhar. 2010. "Anti- hyaluronidase, Anti-elastase Activity of *Garcinia indica*." *International Journal of Botany* 6:1–10.
- Singh, R. K., S. P. Tiwari, A. K. Rai, and T. M. Mohapatra. 2011. "Cyanobacteria: an emerging source for drug discovery." *The Journal of Antibiotics* 64 (6): 401.
- Siripatrawan, U., W. Vitchayakitti, and R. Sanguandeeikul. 2013. "Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution." *International Journal of Food Science and Technology* 48 (1): 22–27.
- Sparringa, R. A., and J. D. Owens. 1999. "Glucosamine content of tempe mould, *Rhizopus oligosporus*." *International Journal of Food Microbiology* 47 (1–2): 153–57.
- Sun, Y., R. Chang, Q. Li, and B. Li. 2016. "Isolation and characterization of an antibacterial peptide from protein hydrolysates of *Spirulina platensis*." *European Food Research and Technology* 242 (5): 685–92.
- Usharani, G., G. Srinivasan, S. Sivasakthi, and P. Saranraj. 2015. "Antimicrobial Activity of *Spirulina platensis* Solvent Extracts Against Pathogenic Bacteria and Fungi." *Advances in Biological Research* 9 (5): 292–98.
- Waterman, P. G., and S. Mole. 1994. *Analysis of phenolic plant metabolites*. Oxford, UK: Blackwell Scientific Publications.
- Wink, M. 2012. "Secondary metabolites from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents." *Frontiers in Microbiology* 3:130.
- Wink, M., and O. Schimmer. 2010. "Molecular modes of action of defensive secondary metabolites." *Functions and Biotechnology of Plant Secondary Metabolites* 39:21–161.

- Xue, C., Y. Hu, H. Saito, Z. Zhang, Z. Li, Y. Cai, C. Ou, H. Lin, and A. B. Imbs. 2002. "Molecular species composition of glycolipids from *Spirulina platensis*." *Food Chemistry* 77 (1): 9–13.
- Yakoot, M., and A. Salem. 2012. "Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial." *BMC Gastroenterology* 12 (1): 32.
- You, M., D. M. Wickramaratne, G. L. Silva, H. Chai, T. E. Chagwedera, N. R. Farnsworth, G. A. Cordell A. D. Kinghorn and J. M. Pezzuto. 1995. "(-)-Roemerine, an aporphine alkaloid from *Annona senegalensis* that reverses the multidrug-resistance phenotype with cultured cells." *Journal of Natural Products* 58 (4): 598–604.
- Yu, H., S. Jia, and Y. Dai. 2009. "Growth characteristics of the cyanobacterium *Nostoc flagelliforme* in photoautotrophic, mixotrophic and heterotrophic cultivation." *Journal of Applied Phycology* 21 (1): 127.
- Yücepete, A., and B. Özcelik. 2016. "Bioactive Peptides Isolated from Microalgae *Spirulina platensis* and their Biofunctional Activities." *Academic Food Journal/Akademik GIDA* 14 (4): 412-17.

CHAPTER FIVE

ANTIOXIDANT CHARACTERISTICS OF *SPIRULINA*

“Let your food be your medicine.”
—Hippocrates

5.1 Introduction

To overcome the harmful effects of oxidative stress in the poultry industry, using new organic ingredients in birds diets as a natural antioxidant is a valuable strategy. There are different kinds of oxidative stress such as photo-oxidative stress (due to exposure to light or radiation), drug-dependent stress, metabolic stress, pathological stress and environmental stress (due to critical environmental conditions such as high or low temperature, high altitude, etc.) (Lin et al. 2006; Lara and Rostagno 2013; Sikiru 2018). Oxidative stress has negative effect on poultry's health state, performance and reproductive traits (Eid, Ebeid, and Younis 2006; Lin et al. 2006; Khan et al. 2012; Hajati et al. 2018). It was well documented that algal species have antioxidant capacity. This characteristic is of high significance for improving the poultry products' quality and consumers health. In this chapter, we will focus on *Spirulina platensis* antioxidant substances and their functions.

5.2 Antioxidants

It was predicted that up to 2020 cancer disease would be the cause of seven out of every 10 deaths in developing countries (Boutayeb 2006; Ferlay et al. 2015; Agnihotri, Aruoma, and Bahorun 2014). Regarding the high cost of cancer treatment, using natural antioxidants is a valuable strategy to prevent this fatal disease (Asghari et al. 2016). Oxygen is the essential gas for aerobic respiration; also, it may act as a reactive atom that has the potential of being a part of damaging molecules like hydroperoxyl radicals, superoxide anions, singlet oxygen, hydrogen peroxide, organic

peroxides, nitric oxide, peroxy nitrite and triplet oxygen (Khan and Wilson 1995). Free radicals have the ability to donate or accept an electron from other molecules (Cheeseman and Slater 1993). This ability would help to stabilize the free radical at the beginning but soon starts to produce other substances. So a chain reaction may begin and thousands of free-radical reactions can occur within a few seconds after the primary reaction (Kumar 2011). These reactive species are capable of damaging the vital biological molecules like DNA, proteins, carbohydrates and lipids (Young and Woodside 2001), and they cause a homeostatic disruption (Anbudhasan et al. 2014).

In addition to cancer, free radicals may cause degenerative diseases like aging and age-related macular degeneration (Devasagayam et al. 2004). Oxidative stress will occur when there is an imbalance between the production of reactive species and protection activity of antioxidants (White et al. 2014). Oxidative stress at the cellular level may occur as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation or strenuous physical activity (Percival 1996). One of the common diseases in chickens is coccidiosis. Its incidence is higher in humid climates. The coccidian parasites promote lipid peroxidation and imbalance in the antioxidant status in infected birds (Georgieva et al. 2011a, 2011b). In addition, vaccination, mycotoxins, heavy metals and excess of vitamin A also caused oxidative stress in poultry (Surai 2007). Using antioxidants can protect cells against oxidative stress and reduce infections (Allen, Danforth, and Augustine 1998). Antioxidants are materials that can delay or inhibit the oxidation of foods. Usually, these substances are present in foods, but at very low levels. Supplementary quantities can aid in preventing oxidation, increasing shelf life and improving overall quality (Haworth 2003). Antioxidants are the first line in protecting the body against free-radical damage; thus, they play a critical role in maintaining optimum health and well-being for humans and animals (Percival 1996). Antioxidant substances are categorized into two groups: synthetic and natural (Gupta and Sharma 2006). Some of the synthetic antioxidants are: propyl- and dodecyl gallate, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butyl hydroquinone (TBHQ) (Yanishlieva-Maslarova and Heinonen 2001). The chemical structures of BHA and BHT are shown in Figure 5-1.

It was reported that BHT and BHA are the antioxidants used most frequently (Lalas and Tsaknis 2002; Gunstone 2004). But there are some reports that have shown the carcinogenic characteristics of these synthetic

antioxidants (Kahl and Kappus 1993; Gohari Ardabili, Farhoosh, and Haddad Khodaparast 2010). Therefore, the search for bio-effective, non-toxic natural antioxidants is considered a valuable endeavor for improving

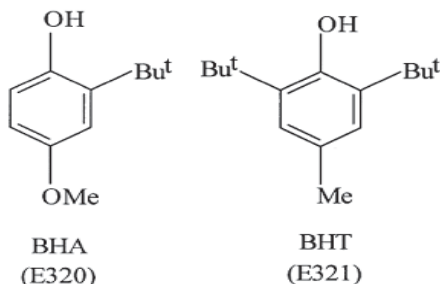


Figure 5-1: The structures of BHA and BHT.

public health (Gupta and Sharma 2006). The natural antioxidant substances can be considered water-soluble like vitamin C and phenolic compounds; lipid-soluble like vitamin E and carotenoids; exogenous enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx); exogenous proteins like albumin, ceruloplasmin, transferrin and haptoglobin; and some minerals like Se, Mn, Cu, Zn (Gupta and Sharma 2006). These compounds protect cells from oxidative damage and may therefore prevent chronic diseases such as cancer, cardiovascular disease and diabetes (Podsędek 2007). There are some methods for evaluating the antioxidant capacities of natural substances, such as: trolox equivalence antioxidant capacity (TEAC) assay, ferric ion reducing antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC) assay, inhibiting the oxidation of low-density lipoprotein (LDL) assay, cellular antioxidant activity assay and DPPH (2,2-diphenyl-picrylhydrazyl) assay (Xu et al. 2017). Phenolic compounds can present in all parts of plants (Asif 2015), like fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson 1990). The antibacterial, antioxidant and anti-inflammatory activity of herbal-extract polyphenols have been reported previously (Hajati et al. 2015a, 2015b; Gessner et al. 2013; Liu et al. 2016). It was reported that phenolic substances perform antioxidant roles by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Alhamdani 2006). Phenolic compounds have a benzenic ring replaced by at least one hydroxyl group (Manach et al. 2004). The phenolic compound contents in *Spirulina platensis* are shown in Table 5-1.

Table 5-1: Phenolic compound contents in *Spirulina platensis*. (Goiris et al. 2014)

Phenolic compound	<i>Spirulina</i> (ng/g)
Phloroglucinol	51000
p-Coumaric acid	920
Ferulic acid	0.97
Apigenin	6

The classification of phenolic compounds is shown in Figure 5-2.

Natural antioxidants have the potential to improve the shelf life of meat products by delaying the onset of oxidation (Haworth 2003). The red color of meat after cutting is due to its surface oxygenating. In the blooming process, the meat becomes fully oxygenated and the production of oxymyoglobin occurs. Also, oxymyoglobin reacts with oxygen and further oxidizes to produce metmyoglobin. When about 70% of the myoglobin becomes oxidized and forms metmyoglobin, the meat surface becomes discolored or brown as shown in Figure 5-3.

Previous studies on natural products derived from algae over the past 40 years have led to isolation of over 15,000 novel compounds, many of which have bioactive functions (Cardozo et al. 2007; Blunt et al. 2013). Microalgae and seaweeds produce a wide range of antioxidant compounds, including pigments like β -carotene, astaxanthin, phycocyanin and phycoerythrin, and sulfated polysaccharides such as fucoidans and heterofucans (Miranda et al. 1998; Chu 2011; Klein and Buchholz 2013). These substances scavenge harmful free radicals, which are involved in the most common cancers and other degenerative diseases including poor brain function (Dillard and German 2000). Oxidative stressors, effect of oxidation and antioxidants are shown in Figure 5-4, briefly.

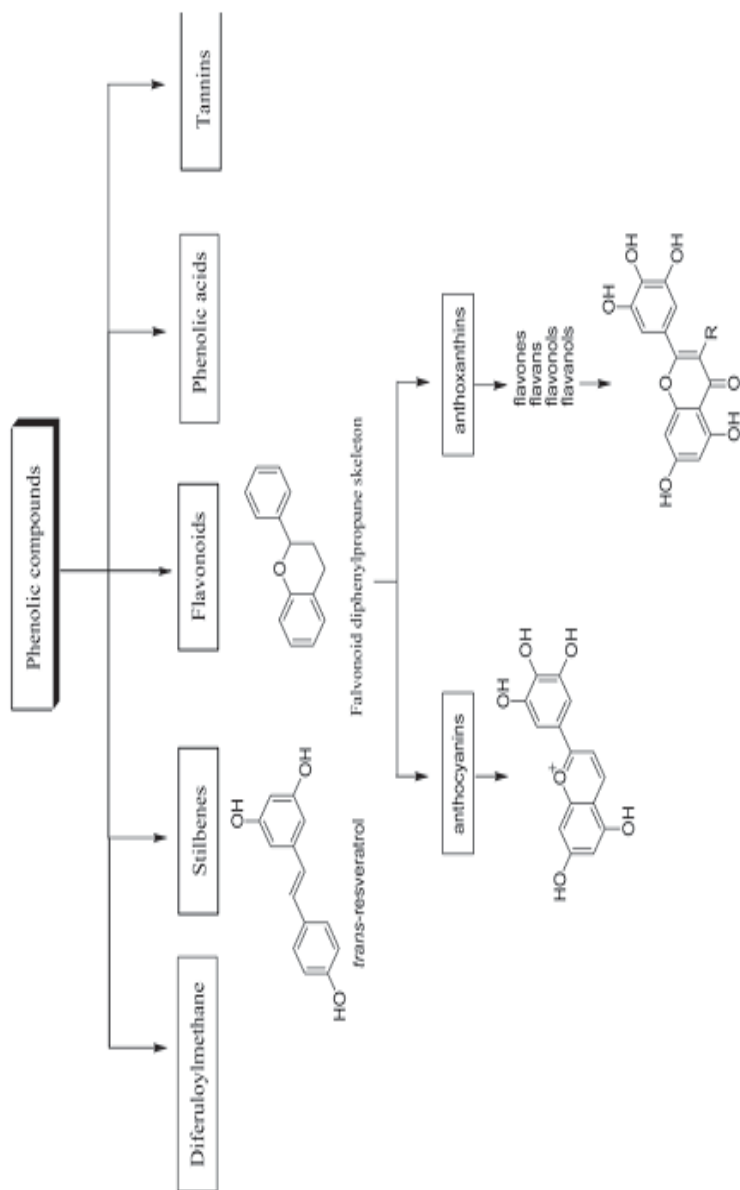


Figure 5-2: Classification of polyphenols. (Han, Shen, and Lou 2007)

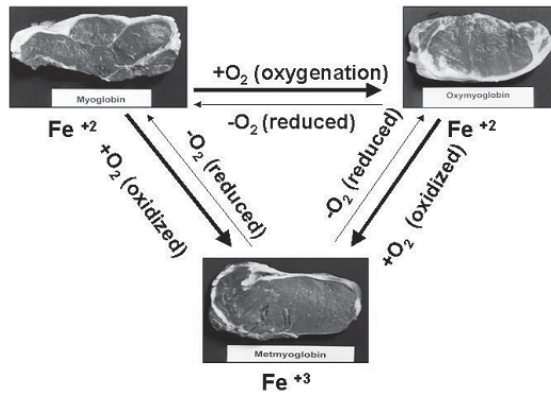


Figure 5-3: Oxidation of meat pigments. (Boles and Pegg 2010)

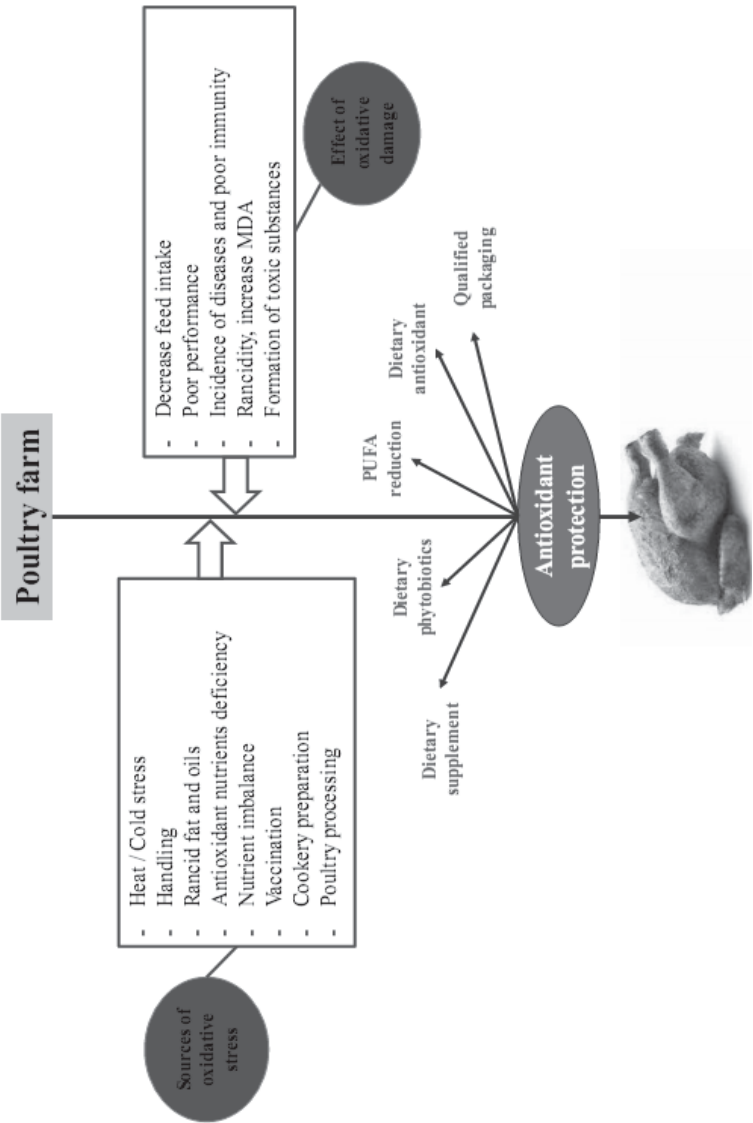


Figure 5-4: Oxidative stressors, effect of oxidation and antioxidants. (Adapted from Estévez 2015)

5.3 Antioxidant activity of *Spirulina*

There are a large number of *Spirulina* species; however, three species of *Spirulina*, including *Spirulina platensis* (*Arthrospira platensis*), *Spirulina maxima* (*Arthrospira maxima*) and *Spirulina fusiformis* (*Arthrospira fusiformis*), are considered to have high nutritional and therapeutic values (Deng and Chow 2010). It was reported that *Spirulina* had a positive effect on the body immune state, so it can be orally administered to patients suffering from cancers and viral diseases (Asghari et al. 2016). Miranda et al. (1998) evaluated the antioxidant activity of carotenoids, phenolics and tocopherols extracted from *Spirulina maxima*. They found that the phenolic compounds responsible for the algae antioxidant characteristics were organic acids (caffeic, chlorogenic, quinic, salicylic, synaptic and trans-cinnamic). They also mentioned that these compounds may act individually or synergistically. *Spirulina platensis* has free-radical scavenging characteristics and antioxidant activity due to a number of natural pigments such as zeaxanthin, β -cryptoxanthin, xanthophylls, chlorophyll, β -carotene, phycoerythrin and phycocyanin, myxoxanthophyll, and echinenone (Gad et al. 2011; Zaid et al. 2015; Asghari et al. 2016). The chemical structure of chlorophyll is similar to that of hemoglobin. The chlorophyll may stimulate tissue growth through the facilitation of a rapid carbon dioxide and oxygen interchange (Asghari et al. 2016). The derivatives of chlorophyll such as pheophorbide b and pheophytin b are strong antioxidants (Asghari et al. 2016). The chemical structure of chlorophyll is shown in Figure 5-5.

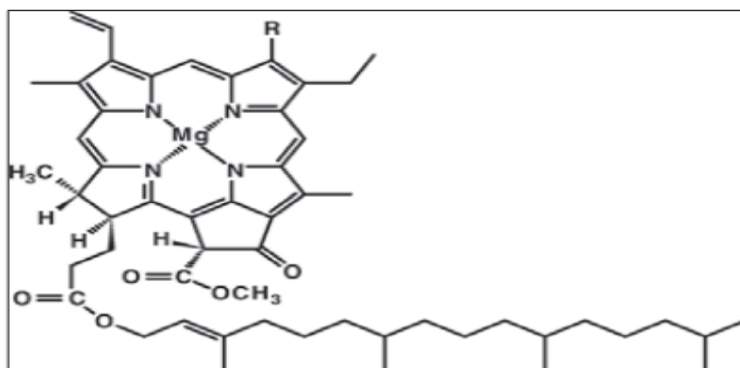


Figure 5-5: Chemical structure of chlorophyll (Asghari et al. 2016).

Mishima et al. (1998) reported that the calcium in *Spirulina* may inhibit tumor invasion and metastasis in lung cancer. *Spirulina* has no side effects and is non-toxic in nature (Desai and Sivakami 2004; Vonshak 1997; Parages et al. 2012). Previous studies on preschool children in India revealed that *Spirulina fusiformis* is an effective source of dietary vitamin A. However, dietary *Spirulina* supplementation did not increase serum concentrations of β -carotene (Bodri 2004). It was demonstrated that phycocyanin exhibits pro-oxidant activity when exposed to light, but it does not demonstrate antioxidant activity in the dark (Zhou et al. 2005). The only two carotenoid antioxidants that never become pro-oxidants are astaxanthin and zeaxanthin (Moorhead, Capelli, and Cysewski 2011). Previous studies showed that β -carotene may act synergistically with vitamin E (Jacob 1995; Sies and Stahl 1995). It was also reported that β -carotene accounts for 80% of the carotenoids present in *Spirulina platensis* (Vaidyaratnam 1994). *Spirulina* contains up to 2,000 IU β -carotene/g dry weight (Krinsky and Johnson 2005; Mohan et al. 2014; Rang Rao et al. 2010). Beta-carotene can help to protect skin against the damaging effects of sunlight, so it helps to prevent skin cancers (Krinsky and Johnson 2005). It may help to decrease the incidence of lung cancer, prevent chemically induced tumors in animals, prevent precancerous pre-chromosome damage and enhance immunological resistance (Devasagayam et al. 2004; Krinsky and Johnson. 2005; Asghari et al. 2016). The chemical structures of two forms of β -carotene are shown in Figure 5-6.

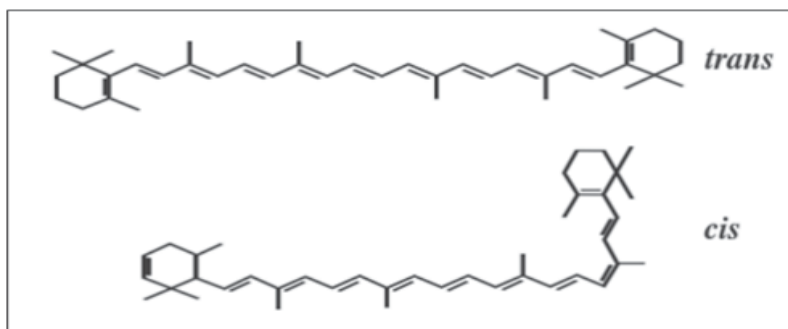


Figure 5-6: Chemical structures of two forms of β -carotene. (Asghari et al. 2016)

Spirulina can decrease the amount of creatine kinase, which is an indicator of muscular breakdown. This may be explained by the antioxidant potential of *Spirulina* (Asghari et al. 2016). Ishii et al. (1999) demonstrated the influence of *Spirulina* on IgA levels in human saliva.

They reported that *Spirulina* enhanced IgA production, suggesting the important role of microalga in mucosal immunity.

Spirulina platensis contains phycobilisomes as protein pigment complexes (Bermejo-Bescós, Piñero-Estrada, and del Fresno 2008). Phycobilisomes are mainly composed of polypeptides called “phycobiliproteins”. The two additional important phycobiliproteins which occur in this microalgae are phycocyanin and allophycocyanin; both of them have the same chromophore group. Phycocyanin is a powerful water-soluble antioxidant (Mohan et al. 2014; Akao et al. 2009). Phycocyanin can scavenge free radicals, including alkoxyl, hydroxyl and peroxy radicals. It decreases nitrite production, down regulates inducible nitric oxide synthase (iNOS) expression and inhibits liver microsomal lipid peroxidation (Asghari et al. 2016). Phycocyanin in *Spirulina* inhibits the growth of human leukemia K562 cells (Subhashini et al. 2004). The chemical structure of C-phycocyanin is shown in Figure 5-7.

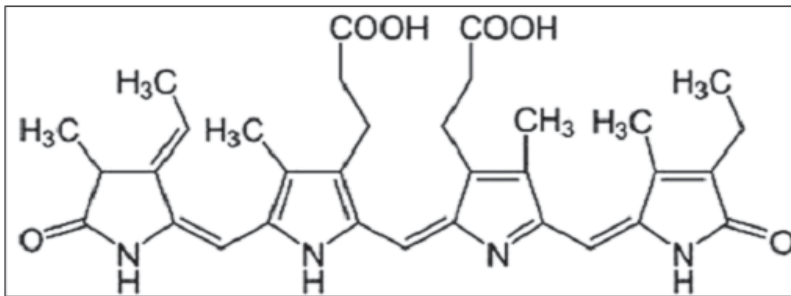


Figure 5-7: Chemical structure of C-phycocyanin. (Asghari et al. 2016)

NASA and the European Space Agency (ESA) recommended *Spirulina* as one of the primary foods during long-term space missions (Asghari et al. 2016). Alyasiri et al. (2018) studied the in vitro and in vivo antioxidant effect of *Spirulina platensis* against lead induced toxicity in rats. They reported that *Spirulina* had the potential to decrease the poisonous action of lead via its scavenging activity against free radicals. *Spirulina* has a high amount of superoxide dismutase (SOD) enzyme, which is an important free-radical scavenging enzyme. This enzyme can be used therapeutically for the treatment of various diseases related to oxidative stress or as a component in anti-wrinkle skin lotions and face masks because it is believed that aging is a consequence of oxidative stress (Capelli and Cysewski 2010; Desai and Sivakami 2014). El-Baky, El Baz, and El-Baroty (2009) reported that increasing H₂O₂ concentrations in the

algae medium increased activities of antioxidant enzymes—i.e., catalase (CAT), peroxidase (PX), ascorbate peroxidase (APX) and superoxide dismutase (SOD). Bashandy et al. (2016) studied the antioxidant potential of *Spirulina platensis* in male rats. They reported that *Spirulina platensis* improved antioxidant status in rats under oxidative stress induced by sodium arsenite. They concluded that *Spirulina platensis* decreased the arsenic burden in testicular tissue and restored the depleted zinc. Several researchers conclude that β -carotene, vitamin C, vitamin E, selenium, manganese and phycocyanin are responsible for the antioxidant potential of *Spirulina platensis* (Bhat and Madyastha 2001; Mazo, Gmoshinskiĭ and Zilova 2004; Tang and Suter 2011; Stivala et al. 1996; Mueller and Boehm 2011; El-Demerdash 2001; Plazinski 2013; Yüce tepe and Özcelik 2016). *Spirulina platensis* may control pro-inflammatory cytokine expression and secretion through repression of nuclear factor kappa (NF- κ B). It was reported that activation of the NF- κ B pathway creates a major pathway for the development of inflammatory diseases (Ku et al. 2013). There are some reports on non-polar antioxidant substances of *Spirulina*, including carotenoids (β -carotene, astaxanthin, and zeaxanthin), chlorophylls and fatty acids, stating that they were significantly enhanced by salinity stress and were reported to have higher antioxidant activities (Endo, Usuki, and Kaneda 1985; Murthy et al. 2005). Wu et al. (2005) reported that the total phenolic contents of aqueous extract of *Spirulina* are five times greater, and *Spirulina* has much higher antioxidant activity, than *Chlorella* extract. Chu et al. (2010) stated that aqueous extract of *Spirulina* has a protective effect against apoptotic cell death induced by the free radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis 3-ethyl benzthiazoline-6-sulphonic acid (ABTS).

Herrero et al. (2005) reported that ethanol is better than other solvents for the extraction of antioxidants from *Spirulina* because it is considered GRAS. Lu et al. (2006) demonstrated that consuming dietary *Spirulina* has preventive effects on human skeletal muscle damage under oxidative stress induced by exercise; thus, it can enhance the activity of blood superoxide dismutase (SOD) and reduce the level of malondialdehyde (MDA). Mohan et al. (2006) reported that *Spirulina* had protective effects against nephrotoxicity induced by cisplatin in rats. It reduced the levels of MDA, SOD, catalase and glutathione peroxidase. Gad et al. (2011) stated that aqueous extract of *Spirulina* has free-radical scavenging properties and protective effects against liver damage induced by carbon tetrachloride (CCl₄) in rats. Chu et al. (2002) demonstrated that brightness and inoculum density of the algae culture may affect the phycocyanin content of *Spirulina*, which can vary from 0.11 to 12.7% of dry weight

(Chu et al. 2002). Phycocyanin is almost 16 times more effective than trolox (vitamin E analog) and 20 times more efficient than vitamin C as an antioxidant in protecting human erythrocytes against lysis induced by peroxy radicals (Romay and González 2000). Nishanth et al. (2010) reported that phycocyanin down-regulated the expression of the multidrug resistance-1 (MDR-1) protein in the human hepatocellular carcinoma cell line (HepG2), and this increased its sensitivity to doxorubicin. The inhibitory mechanism of phycocyanin is activated through the down-regulation of ROS and cyclooxygenase-2 (COX-2) pathways.

Machu et al. (2015) studied the phenolic contents and antioxidant capacities in algal food products. They used different solvents for preparing *Spirulina platensis* extract. The extraction processes were as follows: 1) extraction by distilled water (80°C for 10 minutes in a water bath with constant shaking); 2) extraction by methanol-water-acetic acid (30:69:1, v/v/v) (70°C for 50 minutes in a water bath with constant shaking); 3) extraction by 80% methanol (70°C for 60 minutes in a water bath with constant shaking); 4) extraction by 70% acetone (30°C for 30 minutes in a water bath with constant shaking); 5) extraction by 100% methanol (lab temperature \approx 23°C for 24 hours with constant shaking). The total phenolic content of *Spirulina platensis* is shown in Table 5-2. The results are shown as mean \pm SD (n = 4).

Table 5-2: Total phenolic content (mg·g⁻¹ GAE) of *Spirulina platensis* extracts using different solvents. (Machu et al. 2015)

algae	1	2	3	4	5
<i>Spirulina platensis</i>	43.2 \pm 1.0 ^a	17.0 \pm 0.5 ^b	23.9 \pm 0.1 ^c	18.4 \pm 0.1 ^d	24.4 \pm 0.2 ^c

a–e values in the same line sharing a common letter are not significantly different at P<0.05.

Using dietary algae may improve antioxidant enzyme activity and increase lipid and protein oxidative stability of broiler meat (Delles et al. 2014). It was documented that adding dietary microalgal astaxanthin resulted in high bioavailability to both layer hens and broiler chicks and improved redox status and antioxidant defense in the birds' plasma, tissues and eggs (Lei et al. 2017). For further information about the function of antioxidant substances of *Spirulina* in poultry species, see the following chapters.

References

- Agnihotri, A. K., O. I. Aruoma, and T. Bahorun. 2014. "Cancer: Global health perspectives." *Archives of Medical and Biomedical Research* 1 (1): 1–9.
- Akao, Y., T. Ebihara, H. Masuda, Y. Saeki, T. Akazawa, K. Hazeki, O. Hazeki, M. Matsumoto, T. and Seya. 2009. "Enhancement of antitumor natural killer cell activation by orally administered *Spirulina* extract in mice." *Cancer Science* 100 (8): 1494–501.
- Al-Azzawie, H. F., and M. S. S. Alhamdani. 2006. "Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits." *Life Sciences* 78 (12): 1371–77.
- Alyasiri, T., S. Alchalabi, and I. AlMayaly. 2018. "In vitro and in vivo antioxidant effect of *Spirulina platensis* against lead induced toxicity in rats." *Asian Journal of Agriculture and Biology* 6 (1): 66–77.
- Anbudhasan, P., A. Surendraraj, S. Karkuzhali, and S. Sathishkumaran. 2014. "Natural antioxidants and its benefits." *International Journal of Food and Nutritional Sciences* 3 (6): 225–32.
- Asghari, A., M. Fazilati, A. M. Latifi, H. Salavati, and A. Choopani. 2016. "A Review on Antioxidant Properties of *Spirulina*." *Journal of Applied Biotechnology Reports* 3 (1): 345–51.
- Asif, M. 2015. "Chemistry and antioxidant activity of plants containing some phenolic compounds." *Chemistry International* 1 (1): 35–52.
- Bashandy, S. A., S. A. El Awdan, H. Ebaid, and I. M. Alhazza. 2016. "Antioxidant potential of *Spirulina platensis* mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats." *Oxidative Medicine and Cellular Longevity* 2016, Article ID 7174351.
- Bermejo-Bescós, P., E. Piñero-Estrada, and Á. M. V. del Fresno. 2008. "Neuroprotection by *Spirulina platensis* protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells." *Toxicology in Vitro* 22 (6): 1496–502.
- Bhat, V. B., and K. M. Madyastha. 2001. "Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA." *Biochemical and Biophysical Research Communications* 285 (2): 262–66.
- Blunt, J. W., B. R. Copp, R. A. Keyzers, M. H. Munro, and M. R. Prinsep. 2013. "Marine natural products." *Natural Product Reports* 30 (2): 237–323.
- Bodri, B. 2004. *How to Help Support the Body's Healing after Intense Radioactive or Radiation Exposure*. Reno, NV: Top Shape Publishing, LLC.

- Boles, J. A., and R. Pegg. 2010. *Meat Color*. Montana State University and Saskatchewan Food Product Innovation Program, Department of Applied Microbiology and Food Sciences, University of Saskatchewan. 4 pages pamphlet.
- Boutayeb, A. 2006. "The double burden of communicable and non-communicable diseases in developing countries." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100 (3): 191–99.
- Capelli, B., and G. R. Cysewski. 2010. "Potential health benefits of spirulina microalgae." *Nutrafoods* 9 (2): 19–26.
- Cardozo, K. H., T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, N. P. Lopes, S. Campos, and E. Pinto. 2007. "Metabolites from algae with economical impact." *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 146 (1–2): 60–78.
- Cheeseman, K. H., and T. F. Slater. 1993. "An introduction to free radical biochemistry." *British Medical Bulletin* 49 (3): 481–93.
- Chu, W. L. 2011. "Potential applications of antioxidant compounds derived from algae." *Current Topics in Nutraceuticals Research* 9 (3): 83.
- Chu, W. L., Y. W. Lim, A. K. Radhakrishnan, and P. E. Lim. 2010. "Protective effect of aqueous extract from *Spirulina platensis* against cell death induced by free radicals." *BMC Complementary and Alternative Medicine* 10 (1): 53.
- Chu, W. L., Phang, S. M., Miyakawa, K., & Tosu, K. (2002). Influence of irradiance and inoculum density on the pigmentation of *Spirulina platensis*. *Asia Pacific Journal of Molecular Biology & Biotechnology*, 10(2), 109–117.
- Deng, R., and T. J. Chow. 2010. "Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*." *Cardiovascular Therapeutics* 28 (4): e33–e45.
- Desai, K., and S. Sivakami. 2004. "Spirulina: the wonder food of the 21st Century." *Asia-Pacific Biotech News* 8 (23): 1298–302.
- Devasagayam, T. P. A., J. C. Tilak, K. K. Bloor, K. S. Sane, S. S. Ghaskadbi, and R. D. Lele. 2004. "Free radicals and antioxidants in human health: current status and future prospects." *The Journal of the Association of Physicians of India* 52 (794804): 4.
- Dillard C. J., and J. B. German. 2000. Phytochemicals: nutraceuticals and human health. *Journal of the Science of food and agriculture* 80(12):1744–1756.
- El-Baky, H. H. A., F. K. El Baz, and G. S. El-Baroty. 2009. "Enhancement of antioxidant production in *Spirulina platensis* under oxidative stress." *Acta Physiologiae Plantarum* 31 (3): 623.

- El-Demerdash, F. M. 2001. "Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver, and blood of rats." *Journal of Environmental Science and Health, Part B* 36 (4): 489–99.
- Endo, Y., R. Usuki, and T. Kaneda. 1985. "Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. II. The mechanism of antioxidative action of chlorophyll." *Journal of the American Oil Chemists' Society* 62 (9): 1387–90.
- Faulkner, D. J. 2001. "Marine natural products." *Natural Product Reports* 18 (1): 1R–49R.
- Ferlay, J., I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. Maxwell Parkin, D. Forman, and F. Bray. 2015. "Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012." *International Journal of Cancer* 136 (5): E359–E386.
- Allen, P. C., H. D. Danforth, and P. C. Augustine. 1998. "Dietary modulation of avian coccidiosis." *International Journal for Parasitology* 28 (7): 1131–40.
- Delles, R. M., Y. L. Xiong, A. D. True, T. Ao, and K. A. Dawson. 2014. "Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity." *Poultry Science* 93 (6): 1561–70.
- Eid, Y., T. Ebeid, and H. Younis. 2006. "Vitamin E supplementation reduces dexamethasone-induced oxidative stress in chicken semen." *British Poultry Science* 47 (3): 350–56.
- Estévez, M. 2015. "Oxidative damage to poultry: from farm to fork." *Poultry Science* 94 (6): 1368–78.
- Gad, A. S., Y. A. Khadrawy, A. A. El-Nekeety, S. R. Mohamed, N. S. Hassan, and M. A. Abdel-Wahhab. 2011. "Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats." *Nutrition* 27 (5): 582–89.
- Georgieva, N. V., M. Gabrashanska, V. Koinarski, and S. Ermidou-Pollet. 2011a. "Antioxidant status in Eimeria acervulina infected chickens after dietary selenium treatment." *Trace elements and electrolytes* 28 (1): 42.
- Georgieva, N. V., M. Gabrashanska, V. Koinarski, and Z. Yaneva. 2011b. "Zinc supplementation against Eimeria acervulina-induced oxidative damage in broiler chickens." *Veterinary Medicine International* 2011, Article ID 647124.
- Gessner, D. K., A. Fiesel, E. Most, J. Dinges, G. Wen, R. Ringseis, and K. Eder. 2013. "Supplementation of a grape seed and grape marc meal

- extract decreases activities of the oxidative stress-responsive transcription factors NF- κ B and Nrf2 in the duodenal mucosa of pigs.” *Acta Veterinaria Scandinavica* 55 (1): 18.
- Gohari Ardabili, A., R. Farhoosh, and M. H. Haddad Khodaparast. 2010. “Frying stability of canola oil in presence of pumpkin seed and olive oils.” *European Journal of Lipid Science and Technology* 112 (8): 871–77.
- Goiris, K., K. Muylaert, S. Voorspoels, B. Noten, D. De Paepe, G. J. E. Baart, and L. De Cooman. 2014. “Detection of flavonoids in microalgae from different evolutionary lineages.” *Journal of Phycology* 50 (3): 483–92.
- Gunstone, F. D. 2004. *The Chemistry of Oils and Fats: Sources, Composition, Properties and Uses*. Oxford, UK: Blackwell Publishing.
- Gupta, V. K. and S. K. Sharma. 2006. “Plants as natural antioxidants.” *Natural Product Radiance* 5 (4): 326–34.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. Nassiri. 2015a. “The effect of grape seed extract and vitamin C feed supplements carcass characteristics, gut morphology and ileal microflora in broiler chickens exposed to chronic heat stress.” *Iranian Journal of Applied Animal Science* 5 (1): 155–65.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. R. Nassiri. 2015b. “The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and *HSP70* gene expression of broiler chickens suffering from chronic heat stress.” *Italian Journal of Animal Science* 14 (3): 3273.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam, and M. Nassiri. 2018. “The Effect of Grape Seed Extract Supplementation on Performance, Antioxidant Enzyme Activity, and Immune Responses in Broiler Chickens Exposed to Chronic Heat Stress.” *Iranian Journal of Applied Animal Science* 8 (1): 109–17.
- Han, X., T. Shen, and H. Lou. 2007. “Dietary polyphenols and Their Biological Significance.” *International Journal of Molecular Sciences* 8:950–88.
- Haworth, J. E. 2003. “Natural antioxidants review.” In *Proceedings of the 56th Reciprocal Meats Conference*, Columbia, June 2003, 95–98.
- Herrero, M., P. J. Martín-Álvarez, F. J. Señoráns, A. Cifuentes, and E. Ibáñez. 2005. “Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga.” *Food Chemistry* 93 (3): 417–23.

- Ishii, K., T. Katoh, Y. Okuwaki, and O. Hayashi. 1999. "Influence of dietary *Spirulina platensis* on IgA level in human saliva." *Journal of Kagawa Nutritio University* 30:27–33.
- Jacob, R. A. 1995. "The integrated antioxidant system." *Nutrition Research* 15 (5): 755–66.
- Kahl, R., and H. Kappus. 1993. "Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E." *Zeitschrift fur Lebensmittel-untersuchung und-forschung* 196 (4): 329–38.
- Khan, A. U., and T. Wilson. 1995. "Reactive oxygen species as cellular messengers." *Chemistry and Biology* 2 (7): 437–45.
- Khan, R. U., S. Naz, Z. Nikousefat, M. Selvaggi, V. Laudadio, and V. Tufarelli. 2012. "Effect of ascorbic acid in heat-stressed poultry." *World's Poultry Science Journal* 68 (3): 477–90.
- Klein, B., and R. Buchholz. 2013. "Microalgae as sources of food ingredients and nutraceuticals." In *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*, McNeil, B, D. Archer, I. Giavasis, L. Harvey, eds. 1st ed. Philadelphia, PA, USA, Woodhead publishing, 2013, 559–570.
- Krinsky, N. I., and E. J. Johnson. 2005. "Carotenoid actions and their relation to health and disease." *Molecular Aspects of Medicine* 26 (6): 459–516.
- Ku, C. S., T. X. Pham, Y. Park, B. Kim, M. S. Shin, I. Kang, and J. Lee. 2013. "Edible blue-green algae reduce the production of pro-inflammatory cytokines by inhibiting NF- κ B pathway in macrophages and splenocytes." *Biochimica et Biophysica Acta (BBA)-General Subjects* 1830 (4): 2981–88.
- Kumar, S. 2011. "Free radicals and antioxidants: human and food system." *Advances in Applied Science Research* 2 (1): 129–35.
- Lalas, S. and J. Tsaknis. 2002. "Extraction and identification of natural antioxidant from the Seeds of the *Moringa oleifera* tree variety of Malawi." *Journal of the American Oil Chemists' Society* 79 (7): 677–83.
- Lara, L. J., and M. H. Rostagno. 2013. "Impact of heat stress on poultry production." *Animals* 3 (2): 356–69.
- Lei, X. G., A. D. Magnuson, T. Sun, S. Tolba, R. Yin, and G. C. Liu. 2017. Novel Functions of Microalgal Phytochemicals in Feeding Broiler Chicks and Laying Hens. Department of Animal Science Cornell University. 4 pages.
- Lin, H., E. Decuypere, and J. Buyse. 2006. "Acute heat stress induces oxidative stress in broiler chickens." *Comparative Biochemistry and*

- Physiology Part A: Molecular and Integrative Physiology* 144 (1): 11–17.
- Lin, H., H. C. Jiao, J. Buyse, and E. Decuypere, E. 2006. “Strategies for preventing heat stress in poultry.” *World’s Poultry Science Journal* 62 (1): 71–86.
- Liu, L., C. Fu, M. Yan, H. Xie, S. Li, Q. Yu, S. He, and J. He. 2016. “Resveratrol modulates intestinal morphology and HSP70/90, NF- κ B and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress.” *Food and Function* 7 (3): 1329–38.
- Lu, H. K., C. C. Hsieh, J. J. Hsu, Y. K. Yang, and H. N. Chou. 2006. “Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress.” *European Journal of Applied Physiology* 98 (2): 220.
- Machu, L., L. Misurcova, J. Vavra Ambrozova, J. Orsavova, J. Mlcek, J. Sochor, and T. Jurikova. 2015. “Phenolic content and antioxidant capacity in algal food products.” *Molecules* 20 (1): 1118–33.
- Manach, C., A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez. 2004. “Polyphenols: food sources and bioavailability.” *The American Journal of Clinical Nutrition* 79 (5): 727–47.
- Mazo, V. K., I. V. Gmoshinskii, and I. S. Zilova. 2004. “Microalgae *Spirulina* in human nutrition.” *Voprosy pitaniia* 73 (1): 45–53.
- Miranda, M. S., R. G. Cintra, S. B. M. Barros, and J. Mancini-Filho. 1998. “Antioxidant activity of the microalga *Spirulina maxima*.” *Brazilian Journal of Medical and Biological Research* 31 (8): 1075–79.
- Mishima, T., J. Murata, M. Toyoshima, H. Fujii, M. Nakajima, T. Hayashi, T. Kato, and I. Saiki. 1998. “Inhibition of tumor invasion and metastasis by calciumspirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Spirulina platensis*.” *Clinical and Experimental Metastasis* 16 (6): 541–50.
- Mohan, A., N. Misra, D. Srivastav, D. Umapathy, and S. Kumar. 2014. “*Spirulina*, the nature’s wonder: A review.” *Lipids* 5:7–10.
- Mohan, I. K., M. Khan, J. C. Shobha, M. U. R. Naidu, A. Prayag, P. Kuppusamy, and V. K. Kutala. 2006. “Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats.” *Cancer Chemotherapy and Pharmacology* 58 (6): 802.
- Moorhead, K., B. Capelli, and G. R. Cysewski. 2011. *Spirulina: Nature’s Superfood*. Kailua Kona, HI: Cyanotech Corporation.
- Mueller, L., and V. Boehm. 2011. “Antioxidant activity of β -carotene compounds in different in vitro assays.” *Molecules* 16 (2): 1055–69.
- Murthy, K. C., A. Vanitha, J. Rajesha, M. M. Swamy, P. R. Sowmya, and G. A. Ravishankar. 2005. “In vivo antioxidant activity of carotenoids

- from *Dunaliella salina*—a green microalga.” *Life Sciences* 76 (12): 1381–90.
- Nishanth, R. P., B. S. Ramakrishna, R. G. Jyotsna, K. R. Roy, G. V. Reddy, P. K. Reddy, and P. Reddanna. 2010. “C-Phycocyanin inhibits MDR1 through reactive oxygen species and cyclooxygenase-2 mediated pathways in human hepatocellular carcinoma cell line.” *European Journal of Pharmacology* 649 (1–3): 74–83.
- Parages, M. L., R. M. Rico, R. T. Abdala-Díaz, M. Chabrilón, T. G. Sotiroidis, and C. Jiménez. 2012. “Acidic polysaccharides of *Arthrospira* (Spirulina) platensis induce the synthesis of TNF- α in RAW macrophages.” *Journal of Applied Phycology* 24 (6): 1537–46.
- Percival, M. 1996. Antioxidants. *Clinical Nutrition Insights* 1:1–6.
- Plazinski, W. 2013. “Binding of heavy metals by algal biosorbents. Theoretical models of kinetics, equilibria and thermodynamics.” *Advances in Colloid and Interface Science* 197:58–67.
- Podsedek, A. 2007. “Natural antioxidants and antioxidant capacity of Brassica vegetables: A review.” *LWT-Food Science and Technology* 40 (1): 1–11.
- Pratt, D. E. and B. J. F. Hudson. 1990. “Natural antioxidants not exploited commercially.” In *Food Antioxidants*, edited by B. J. F. Hudson, 171–92. Springer, Dordrecht
- Ranga Rao, A., R. L. Raghunath Reddy, V. Baskaran, R. Sarada, and G. A. Ravishankar. 2010. “Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model.” *Journal of Agricultural and Food Chemistry* 58 (15): 8553–59.
- Romay, C., and R. González. 2000. “Phycocyanin is an antioxidant protector of human erythrocytes against lysis by peroxyl radicals.” *Journal of Pharmacy and Pharmacology* 52 (4): 367–68.
- Sies, H., and W. Stahl. 1995. “Vitamins E and C, beta-carotene, and other carotenoids as antioxidants.” *The American Journal of Clinical Nutrition* 62 (6): 1315S–1321S.
- Sikiru, A. B. 2018. “Oxidative Stress and Reproductive Inefficiencies: The Science, Evidences, and Solutions.” *Agricultural Extension Journal (AEXTJ)* 2 (01): 17–26.
- Stivala, L. A., M. Savio, O. Cazzalini, R. Pizzala, L. Rehak, L. Bianchi, V. Vannini, and E. Prosperi. 1996. “Effect of β -carotene on cell cycle progression of human fibroblasts.” *Carcinogenesis* 17 (11): 2395–401.
- Subhashini, J., S. V. Mahipal, M. C. Reddy, M. M. Reddy, A. Rachamalla, and P. Reddanna. 2004. “Molecular mechanisms in C-Phycocyanin

- induced apoptosis in human chronic myeloid leukemia cell line-K562.” *Biochemical Pharmacology* 68 (3): 453–62.
- Surai, P. F. 2007. “Natural antioxidants in poultry nutrition: new developments.” In *Proceedings of the 16th European Symposium on Poultry Nutrition*, World Poultry Science Association. August 26 - 30, 2007 Strasbourg, France.
- Tang, G., and P. M. Suter. 2011. “Vitamin A, nutrition, and health values of algae: *Spirulina*, *Chlorella*, and *Dunaliella*.” *Journal of Pharmacy and Nutrition Sciences* 1 (2): 111-18.
- Vaidyaratnam, P. V. S. 1994. *Indian Medicinal Plants: A Compendium of 500 Species*, Vol. 4. Madras: Orient Longman Ltd.
- Vonshak, A., ed. 1997. *Spirulina platensis arthrospira: physiology, cell-biology and Biotechnology*. Taylor and Francis Ltd. CRC Press. 233 pages.
- White, P. A., R. Oliveira, A. P. Oliveira, M. R. Serafini, A. A. Araújo, D. P. Gelain, J. C. F. Moreira and M. R. Santos. 2014. “Antioxidant activity and mechanisms of action of natural compounds isolated from lichens: a systematic review.” *Molecules* 19 (9): 14496–527.
- Wu, L. C., J. A. A. Ho, M. C. Shieh, and I. W. Lu. 2005. “Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts.” *Journal of Agricultural and Food Chemistry* 53 (10): 4207–12.
- Xu, D. P., Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, J. J. Zhang, and H. B. Li. 2017. “Natural antioxidants in foods and medicinal plants: extraction, assessment and resources.” *International Journal of Molecular Sciences* 18 (1): 96.
- Yanishlieva-Maslarova, N. V., and I. M. Heinonen. 2001. “Sources of natural antioxidants: vegetables, fruits, herbs, spices and teas.” In *Antioxidants in food, practical applications*, edited by J. Pokorny, N. Yanishlieva, and M. Gordon, 210–66. England: Woodhead Publishing.
- Young, I. S., and J. V. Woodside. 2001. “Antioxidants in health and disease.” *Journal of Clinical Pathology* 54 (3): 176–86.
- Yüçetepe, A., and B. Özçelik. 2016. “Bioactive Peptides Isolated from Microalgae *Spirulina platensis* and their Biofunctional Activities.” *Academic Food Journal/Akademik GIDA* 14 (4): 412-417.
- Zaid, A. A. A., Hammad, D. M., & Sharaf, E. M. (2015). Antioxidant and anticancer activity of *Spirulina platensis* water extracts. *International Journal of Pharmacology*, 11(7): 846-851.
- Zhou, Z. P., L. N. Liu, X. L. Chen, J. X. Wang, M. Chen, Y. Z. Zhang, and B. C. Zhou. 2005. “Factors that [a]ffect antioxidant activity of C-phyocyanins from *Spirulina platensis*.” *Journal of Food Biochemistry* 29 (3): 313–22.

CHAPTER SIX

SPIRULINA IN BROILER NUTRITION

“When diet is wrong, medicine is of no use, when diet is correct, medicine is of no need.”
Ancient Ayurvedic Proverb

6.1 Introduction

Today, microalgae such as *Spirulina*, *Chlorella* and *Schizochytrium* have attracted considerable interest among poultry nutritionists due to their high nutritional and functional properties, which may be beneficial for broiler chickens (Jamil et al. 2015; Sugiharto and Lauridsen 2016; Park, Lee, and Kim 2018). It was well documented that using antibiotics in feed resulted in development of drug-resistant bacteria (Sørum and Sunde 2001), drug residues in the body of the birds (Burgat 1999) and disturbance in the balance of normal micro flora in the gut (Andremont 2000). So, nutritionists are searching for alternative strategies to overcome pathogenic bacteria infection in the body (Kaoud 2015). This effort has an important role in improving meat quality and sustainable broiler production (Kaoud 2015). On the other hand, due to the high reproduction rate of *Spirulina*, the area available for their growth can produce 125 times more protein than the same area of corn (Furst 1978). *Spirulina* is a cyanobacterium that has valuable nutrients, such as protein, amino acids, vitamins, minerals, essential fatty acid and pigments (Vonshak 2007; Beheshtipour et al. 2013; Holman and Malau-Aduli 2013). Indeed, *Spirulina platensis* is rich in polysaccharides, which may function as prebiotics (Beheshtipour et al. 2013; de Jesus Raposo, de Moraes, and de Moraes 2016). *Spirulina* can be considered a nutritional supplement that has various health benefits for humans, and a feed supplement for animals that has economic benefits (Kaoud 2015). There is a limited amount of data about using *Spirulina* as a feed ingredient in broiler diets. The aim of this chapter is to provide the overview of *Spirulina* microalgae usage as functional feed in broiler diets.

6.2 *Spirulina* nutritional value

Poultry nutritionists consider the blue-green algae *Spirulina platensis* and *Hermetia illucens* (black soldier fly) larvae as efficient ingredients in poultry diets due to their high protein contents and additional supply of vitamins and minerals (Gongnet et al. 2001; Finke 2002; Finke 2004; Finke 2013; Van Huis 2013; Bellof and Carrasco Alarcon 2013; Makkar et al. 2014; Neumann, Velten, and Liebert 2018). Microalgae can be used for biodiesel production due to its large amount of EE. The end product after the extraction process is a defatted microalgae residue rich in proteins and carbohydrates that may be used for animal nutrition (Tavernari et al. 2016a). Microalgae can be a good alternative source of proteins in the diet (Rogatto et al. 2004). Several studies reported the palatability, lack of toxicity, easy digestion, antioxidant activity, and hypocholesterolemic, anticancer, immunostimulatory, anti-inflammatory, antifungal and antiviral effects of *Spirulina* (Rodriguez-Hernández et al. 2001; Colla, Furlong, and Costa 2007; Uyisenga et al. 2010; Shokri, Khosravi, and Taghavi 2014). The result of evaluating the chemical and energy composition of *Spirulina platensis* and SBM is shown in Table 6-1.

Table 6-1: Chemical and energy composition of *Spirulina platensis* and SBM, expressed on natural matter basis. (Alvarenga et al. 2011)

Composition	<i>Spirulina</i>	SBM
Dry matter (%)	88.08	89.01
Crude protein (%)	58.20	46.47
Gross energy (kcal/kg)	4,286	3,952
Ethereal extract (%)	2.60	1.70
Crude fiber (%)	0.78	4.91
ADF (%)	0.79	5.36
NDF (%)	10.61	11.42
Mineral matter (%)	8.44	5.97
Calcium (%)	0.48	0.24
Total phosphorus (%)	1.06	0.46
AME (kcal/kg)	2,560	2,355
AMEn (kcal/kg)	2,204	2,083

Compared to SBM, *Spirulina* has greater values of dry matter, gross energy (9.60%), crude protein (26.56%), EE (54.45%), mineral content (42.77%), calcium (100%) and total phosphorus (130.77%); however, it has a lower content of crude fiber (83.95%), acid detergent fiber (85.12%)

and neutral detergent fiber (6.15%, Alvarenga et al. 2011). Compared with SBM, *Spirulina* has higher gross energy (9.6%), higher AME (9.8%) and higher AMEn (6.9%, Alvarenga et al. 2011). Previous studies reported that the metabolizable energy of *Spirulina* can vary from 2,500 to 3,290 kcal/kg and its phosphorus content may reach 41% (Yoshida and Hoshi 1980). Table 6-2 shows the amino acid content and ratio of amino acid/lysine in *Spirulina platensis* and SBM in broiler diets.

Table 6-2: Comparing the amino acid content and ratio of amino acid/lysine in *Spirulina platensis* and SBM in broiler diets. (Alvarenga et al. 2011)

Amino acid	Amino acid content (%)		Amino acid value/lysine	
	<i>Spirulina</i>	SBM	<i>Spirulina</i>	SBM
Aspartate	5.34	5.29	196	189
Glutamate	8.15	8.65	300	309
Serine	2.92	2.42	107	86
Glycine	3.00	2.01	110	72
Histidine	1.00	1.38	37	49
Arginine	3.96	3.55	146	127
Threonine	2.84	1.85	104	66
Alanine	4.54	2.02	167	72
Proline	2.15	2.36	79	84
Tyrosine	2.58	1.74	95	62
Valine	3.34	2.03	123	73
Methionine	1.98	0.79	73	28
Cystine	0.72	0.59	26	21
Isoleucine	3.06	2.04	113	73
Leucine	4.84	3.40	178	121
Phenylalanine	2.50	2.29	92	82
Lysine	2.72	2.80	100	100
Total	55.65	45.20	-	-

It was reported that dried full-fat *Spirulina* had energy value equal to 90% of corn energy—i.e., 2839 kcal TMEn/kg (Evans, Smith, and Moritz 2015 et al. 2015). The result of analyzing *Spirulina maxima* was as follows: 88.8% DM, 51.5% CP, 0.99% EE, 1.1% CF, 9.4% Ash, 0.34% Ca and 1.08% P (Tavernari et al. 2016a). The apparent metabolizable energy of *Spirulina maxima* is 2,865.9 kcal/kg as fed (3,219.4 kcal/kg, on DM basis) and 2,492.9 kcal/kg as fed (2,800.4 kcal/kg, on DM basis) when corrected

for nitrogen (Tavernari et al. 2016a). Also, the ileal apparent digestibility coefficients (IADC) of Ca and P from the *Spirulina maxima* were 84.1% and 94.78%, respectively (Tavernari et al. 2016b). It was reported that non-protein nitrogen in *Spirulina* can reach 11.5%. The non-protein nitrogen substances in *Spirulina* are nucleic acids, glucosamine, nitrogenous substances in the cell wall and other amines (Becker 2007). So, considering *Spirulina* crude protein can usually be an overestimation. *Spirulina* has a higher amount of sulfur amino acids compared to SBM (Alvarenga et al. 2011), which presents 1.39% of methionine and 2.46% of methionine and cystine (Rostagno 2005). With regards to ideal protein concept in the diet formulation, the use of *Spirulina* as a substitute for SBM can help to lessen inclusion of methionine in its synthetic form (Alvarenga et al. 2011). This has high economic importance. Coefficients of metabolizable energy and some nutrients of *Spirulina platensis* are shown in Table 6-3. These coefficients should be noted when formulating broiler diets using *Spirulina*.

Table 6-3: Coefficients of metabolizable energy and of some nutrients from *Spirulina platensis* obtained with broilers. (Alvarenga et al. 2011)

Component	Coefficient of metabolizable (%)	Deviation
Dry matter	62.97	± 2.96
Crude protein	52.86	± 4.58
Gross energy	56.11	± 2.04
Phosphorus	74.32	± 5.53
Calcium	64.07	± 7.87

6.3 Growth performance

Nowadays, there is increasing interest in the use of novel functional organic feed ingredients. It was reported that supplementing 1 g dietary *Spirulina platensis*/kg diet increased body weight about 6% and improved FCR about 6.3% (Kaoud 2015). Also, feeding 2, 4, or 8 g of *Spirulina platensis*/kg feed increased body weight and decreased FCR of broiler chickens (Jamil et al. 2015). In a recent study, Park, Lee, and Kim (2018) used different levels of *Spirulina* (0.0, 0.25, 0.5 or 1.0%) in broiler diets. They found the BWG, FCR and European production efficiency index (EPEI) improved in broilers fed with *Spirulina* compared with a control group (fed on a diet without *Spirulina*). Similar results were reported by Shanmugapriya et al. (2015b) when feeding 1% of *Spirulina platensis* to broiler chickens. They found that *Spirulina platensis* increased villi height

and therefore improved absorption capacity of broiler intestines. However, feeding 1.5% of *Spirulina* resulted in lower final bodyweight compared to feeding 0.5 or 1% of *Spirulina platensis* (Shanmugapriya et al. 2015b). It may be that the excessive intake of *Spirulina platensis* resulted in metabolic disturbances and affected the liver function leading to retarded growth rate in broilers (Shanmugapriya et al. 2015b).

High inclusion rates (20 +%) of *Spirulina* in chicken diets may decrease feed acceptance and growth rate (Ross and Dominy 1990; Evans, Smith, and Moritz 2015). The existence of high variability in nutrient content and amino acid composition of *Spirulina* is one reason for varying results (Austic et al. 2013). Evans, Smith, and Moritz (2015) observed no significant effects on broilers' body weight (21 days of age) with microalgae meal used at a rate between 6% and 16% in chicken diets. Venkataraman et al. (1994) reported similar conclusions with 14% and 17% *Spirulina platensis* inclusion rate (without additional minerals and vitamins) in chicken diets, but they found that body weight was significantly decreased with the inclusion of 21% *Spirulina* in the diet. We studied the effects of dietary supplementation of *Spirulina platensis* on feed intake, body weight gain, FCR, mortality and European production efficiency of broiler chickens. The results are shown in Table 6-4. In starter (0–10 d), grower (11–28 d) and finisher (29–42 d) periods, feed intake and body weight gain of broiler chickens fed a diet supplemented with 2 g *Spirulina*/kg was higher than those of the control group ($P<0.05$). In the whole period of rearing, broiler chickens fed with 1.5 or 2 g *Spirulina*/kg had higher feed intake and body weight gain ($P<0.05$). Broiler chickens fed with 1.5 or 2 g *Spirulina*/kg diet had higher European production efficiency factors in the whole period of rearing.

In our study, supplemental algae improved feed intake and body weight gain of broilers. This is in contrast with the findings of Toyomizu et al. (2001). They did not find any significant effect of adding dietary *Spirulina* in the performance of broilers. However, Shanmugapriya et al. (2015b) reported improvement of body weight gain and FCR in broilers that consumed *Spirulina* algae. Also, Mariey et al. (2014) reported that a low dietary level of *Spirulina* biomass (0.02 or 0.03%) improved performance of broiler chickens. Nutrient composition and physiological function of *Spirulina* may have positive effects in metabolism systems related to broilers' growth performance (Park, Lee, and Kim 2018). Figure 6-1 shows broiler chicks in a commercial farm.

Table 6-4: Effects of *Spirulina platensis* on growth performance of broiler chickens at different periods (Hajati and Zaghari, unpublished data).

<i>Spirulina platensis</i> (g/kg diet)						
	Control	1	1.5	2	SEM	Pr>F
1 to 10 d						
FI (g)	218.35 ^b	220.57 ^{ab}	224.11 ^{ab}	240.21 ^a	7.71	0.02
BWG (g)	200.03 ^d	202.91 ^c	203.20 ^c	209.63 ^b	0.964	0.038
FCR (g/g)	1.09 ^b	1.08 ^b	1.10 ^{ab}	1.14 ^a	0.01	0.0002
11 to 28 d						
FI (g)	1837.28 ^b	1834.38 ^b	1840.06 ^b	1878.75 ^a	12.37	0.02
BWG (g)	1151.5 ^d	1167.75 ^b	1171.5 ^b	1238.25 ^a	11.64	<0.0001
FCR (g/g)	1.59 ^a	1.57 ^a	1.57 ^a	1.51 ^b	0.011	0.016
29 to 42 d						
FI (g)	2345.53 ^b	2379.56 ^b	2467.31 ^a	2479.62 ^a	16.00	0.001
BWG (g)	1363.90 ^b	1375.76 ^b	1396.40 ^b	1431.05 ^a	10.60	0.043
FCR (g/g)	1.71	1.72	1.76	1.73	0.016	0.068
1 to 42 d						
FI (g)	4401.16 ^d	4434.51 ^c	4531.48 ^b	4608.58 ^a	16.00	0.001
BWG (g)	2715.43 ^d	2746.42 ^c	2771.1 ^b	2878.93 ^a	20.8	0.0011
FCR (g/g)	1.62	1.61	1.63	1.60	0.01	0.079
EPEF *	272.4 ^c	274.1 ^c	292.4 ^b	315.7 ^a	4.01	0.002

Means within the same row with uncommon superscripts differ significantly (P<0.05).

* European production efficiency factor



Figure 6-1: Broiler chicks in a commercial farm. (Photograph by authors)

6.4 Carcass characteristics

Spirulina platensis increased carcass percentage of broiler chicks (Raju et al. 2004; Kaoud 2012; Holman and Malau-Aduli 2013; Mariey et al. 2014). Sugiharto et al. (2018) reported that using *Spirulina platensis* at the level of 1% had no significant effect on broilers' carcass characteristics. We assessed the effects of dietary supplementation of *Spirulina platensis* on carcass traits of broilers. The results are shown in Table 6-5. Supplementation of diets with different levels of *Spirulina platensis* caused higher carcass yield in broiler chickens at 42 days ($P<0.05$); however, the broilers' breast yield (%), drumstick + thigh (%) and abdominal fat pad (%) were not different ($P>0.05$). Bellof and Carrasco Alarcon (2010) reported that adding dietary *Spirulina* improved carcass parameters of broilers in organic farming. Raach-Moujahed et al. (2011) reported that *Spirulina* improved the carcass yield of broilers (Arbor Acres strain) at a rate of 2.5% of incorporation.

Table 6-5: Effects of *Spirulina platensis* on carcass characteristics of broilers at 42 d. (Hajati and Zaghari, unpublished data)

	Control	1	1.5	2	SEM	Pr>F
Carcass (%)	63.43 ^c	64.06 ^b	66.14 ^a	67.21 ^a	0.38	0.03
Breast (%)	25.12	25.17	25.24	25.45	0.024	0.73
Drumstick+Thigh (%)	15.85	15.89	15.91	15.93	0.023	0.63
Abdominal fat (%)	1.37	1.26	1.34	1.31	0.008	0.31

Means within the same row with uncommon superscripts differ significantly ($P<0.05$).

6.5 Gut morphology

The critical role of villi height in the absorption of intestinal nutrients has been reported by Mekbungwan, Yamauchi, and Thongwittaya (2002). Furbeyre et al. (2017) reported that *Spirulina* increased villus height of the jejunum in weaned piglets. Also, Shanmugapriya et al. (2015a) reported that dietary *Spirulina* increased body weight gain and villus length in addition to fatty acid modification in broiler meat. We studied the effects of dietary supplementation of *Spirulina platensis* on gut morphology of broiler chickens and the results are shown in Table 6-6. The villus height of the duodenum in broiler chickens fed with 2 g *Spirulina*/kg diet was higher than that of the control group ($P<0.05$). The duodenum crypt depth and duodenum villus-to-crypt ratio in broilers fed with 1.5 or 2 g *Spirulina*/kg diet was lower and higher than those of the control group, respectively ($P<0.05$). The villus height of the jejunum in broiler chickens fed with 1.5 and 2 g *Spirulina*/kg diet was higher than that of the control group ($P<0.05$). The jejunum crypt depth and villus: crypt of broilers consumed different levels of *Spirulina* was lower and higher, respectively ($P<0.05$). The ileum villus height and villus-to-crypt ratio in broiler chickens fed with different levels of *Spirulina* were higher than those of the control group ($P<0.05$). These results revealed that dietary supplementation of *Spirulina* increased villus height in all segments of the small intestine (duodenum, jejunum, ileum), which can increase nutrient uptake and cause higher digestibility of nutrients. Higher body weight gain in broilers that consumed *Spirulina* may therefore be due to its positive effect on gut morphology.

Using *Spirulina* in broiler chicken diets may improve dry matter and nitrogen digestibility (Park, Lee, and Kim 2018), glutamic acid, proline, glycine, alanine, methionine, leucine and lysine (Evans et al. 2015). That may be due to algae proteolytic enzymes like pepsin, pancreatin and pronase (Mabeau and Fleurence 1993), or high-quality protein in the algae (Park, Lee, and Kim 2018), or the increased absorptive surface area of the small intestine that was seen in our study.

Table 6-6: Effects of *Spirulina platensis* on gut characteristics of broilers at 42 d. (Hajati and Zaghari, unpublished data)

		<i>Spirulina platensis</i> (g/kg diet)				
	Control	1	1.5	2	SEM	Pr>F
42 d						
Duodenum						
Morphology (μm)						
Villus height	1859.51 ^b	1865.5 ^b	1871.5 ^b	1992.35 ^a	21.72	0.00081
Crypt depth	200.17 ^a	192.7 ^{ab}	184.32 ^{bc}	179.6 ^c	12.07	0.023
Villus-to-crypt	9.28 ^c	9.68 ^{bc}	10.15 ^b	11.09 ^a	0.18	<0.0001
Jejunum						
Morphology (μm)						
Villus height	1731.15 ^b	1738.03 ^b	1859.25 ^a	1868.5 ^a	15.54	0.0001
Crypt depth	195.15 ^a	181.35 ^b	173.5 ^c	155.00 ^d	5.95	<0.0001
Villus-to-crypt	8.87 ^d	9.58 ^c	10.71 ^b	12.05 ^a	0.17	<0.0001
Ileum						
Morphology (μm)						
Villus height	983.93 ^c	1053.41 ^b	1097.97 ^a	1093.05 ^a	10.21	0.0086
Crypt depth	151.67	143.04	149.34	141.26	7.34	0.0769
Villus-to-crypt	6.48 ^c	7.36 ^b	7.35 ^b	7.73 ^a	0.199	0.0017

Means within the same row with uncommon superscripts differ significantly (P<0.05).

6.6 Ileal microflora

Gut microflora, especially harmful bacteria, have effect on noxious gas emission by excreta (Ferket et al. 2002). *Spirulina* is one of the most important microalgae showing antimicrobial activity against many pathogenic bacteria and fungi (Kumar et al. 2013). The bacterial clearance capacities of *Spirulina platensis* in chicks injected with *E. coli* or *Staphylococcus aureus* was reported by Nuhu (2013). It was found that *Spirulina platensis* extract was able to inhibit the growth of *Klebsiella pneumoniae*, *Shigella shigae*, *E. coli*, *S. aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *S. typhi* (Mala et al. 2009; Sivakumar and

Santhanam 2011; Pradhan et al. 2012). Also, Kaushik and Chauhan (2008) reported the antibacterial activities of *Spirulina platensis* against *S. aureus* and *E. coli*, while El-Baz et al. (2013) mentioned the antibacterial activities of *S. platensis* extract against *E. coli*, *S. aureus*, *S. typhi*, and *Enterococcus faecalis*. As mentioned in Chapter Four, several compounds such as tocopherols, C-phycocyanin, extracellular polysaccharides, γ -linolenic acid, active fatty acid lauric and palmitoleic acid, have been attributed to the antimicrobial activities of *Spirulina platensis* (Sarada, Kumar, and Rengasamy 2011; Challouf et al. 2011; El-Sheekh et al. 2014). *Spirulina* has prebiotic properties (Beheshtipour et al. 2013; de Jesus Raposo, de Moraes, and de Moraes 2016), so it may have a stimulating effect on the growth of LAB (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Streptococcus thermophilus*) (Bhowmik, Dubey, and Mehra 2009; Shanmugapriya et al. 2015a). Feeding *Spirulina*-containing diets may increase the *Lactobacillus* population and enhance the absorbability of dietary vitamins (Tokai 1987; Mariey, Samak, and Ibrahim 2012). As shown in Table 6-7, dietary supplementation of different levels of *Spirulina platensis* decreased *Coliforms* count in broilers' ileum contents ($P<0.05$); however, the count of ileal *Lactobacillus* increased in broilers fed with different levels of *Spirulina platensis* ($P<0.05$).

Table 6-7: Effects of *Spirulina platensis* on ileal microbial population (log CFU/g of digesta) of broilers at 42 d. (Hajati and Zaghari, unpublished data)

		<i>Spirulina platensis</i> (g/kg diet)				
	Control	1	1.5	2	SEM	Pr>F
42 d						
<i>Coliforms</i>	8.15 ^a	7.73 ^b	7.68 ^b	7.65 ^b	0.14	<0.0001
<i>Lactobacillus</i>	6.14 ^c	6.55 ^b	6.62 ^b	6.67 ^b	0.16	<0.0001

Means within the same row with uncommon superscripts differ significantly ($P<0.05$)

It is interesting to note that decrease in excreta noxious gas emission was seen in broilers fed with *Chlorella* (Yan, Lim, and Kim 2012) and *Spirulina* (Park, Lee, and Kim 2018). This may be a valuable strategy for managing the farm litter quality.

6.7 Hematological parameters

Zhang et al. (2001) found that the *Spirulina* polysaccharides increased the level of red blood cells (RBC), white blood cells (WBC) and hemoglobin in blood, and also increased nucleated cells in bone marrow of dogs. They reported that: “Polysaccharide extract of *Spirulina platensis* has chemo-protective and radio-protective capability, and may be a potential adjunct to cancer therapy” (Zhang et al. 2001). It was documented that *Spirulina* decreased the level of serum glucose (Takai, Hossayamada, and Kato 1991). Huang et al. (2005) reported that *Spirulina* polysaccharides decreased blood glucose and could protect the vascular systems of alloxan-induced diabetic rats.

The mechanism by which *Spirulina* may decrease blood glucose level may involve potentiating pancreatic secretion of insulin from B-cells or by increasing glucose transportation to the peripheral tissues (Layam and Reddy 2006). Zeweil et al. (2016) reported a reduction in serum cholesterol and increment in IgG titer of chickens. The antioxidant materials such as phycocyanin, phenolic compounds and polyunsaturated fatty acids in the *Spirulina* may decrease serum lipids levels (Colla, Muccillo-Baisch, and Costa 2008). Nagaoka et al. (2005) reported that *Spirulina platensis* concentrates or phycocyanin, a pigment extracted from *Spirulina*, had hypocholesterolemic activity in rats. The polyunsaturated fatty acids in *Spirulina platensis* may help to reduce serum lipid profiles (Iwata, Inayama, and Kato 1990; Kato et al. 1984; Colla, Muccillo-Baisch, and Costa 2008).

We conducted a study to evaluate the effects of dietary supplementation of *Spirulina platensis* on hematological parameters of broiler chickens, and the results are shown in Table 6-8. Adding different levels of *Spirulina* to broilers' diets increased hematocrit (HCT) and phosphorus levels ($P<0.05$), but it decreased the levels of total cholesterol ($P<0.05$). The concentration of blood calcium increased in broilers fed with 1.5 and 2 g *Spirulina*/kg diet ($P<0.05$). Broilers that consumed the 2 g *Spirulina*/kg diet had higher levels of total protein in blood ($P<0.05$).

Table 6-8: Effects of *Spirulina platensis* on hematological parameters in broiler chickens. (Hajati and Zaghari, unpublished data)

	<i>Spirulina platensis</i> (g/kg diet)				SEM	Pr>F
	Control	1	1.5	2		
Hematology, day 42						
WBC (10 ³ /ml)	148.25	151.3	153.22	154.13	0.64	0.15
RBC (10 ⁶ /ml)	2.17	2.32	2.30	2.35	0.04	0.23
HCT (%)	27.3 ^d	30.34 ^c	33.54 ^b	36.12 ^a	0.23	<0.0001
Total protein	37.64 ^b	38.46 ^b	38.71 ^b	45.52 ^a	0.58	0.0011
Cholesterol	163 ^a	142.5 ^b	129.65 ^b	116.3 ^b	4.65	<0.0001
Glucose	215.24	208.02	200.86	198.56	7.24	0.74
Calcium	2.60 ^c	2.58 ^c	2.92 ^b	3.14 ^a	0.03	<0.0001
Phosphorus	2.53 ^d	2.61 ^c	2.72 ^b	3.41 ^a	0.04	<0.0001

Means within the same row with uncommon superscripts differ significantly (P<0.05).

6.8 Immunity

Immulina, a polysaccharide with high molecular weight extracted from the microalgae *Arthrospira platensis*, can enhance adaptive immunity (Lobner et al. 2008). *Spirulina platensis* may promote functions of the phagocytic system, thus increasing the disease resistance in chickens (Al-Batshan et al. 2001). Qureshi, Garlich, and Kidd (1996) and Raju et al. (2004) reported that feeding chicks *Spirulina platensis* increased their humoral and cellular immune responses and improved their lymphoid organ development. Recently, Lokapirnasari, Yulianto, and Legowo (2016) showed that using dietary *Spirulina platensis* increased the number of leukocytes and decreased the mortality rate of broiler chicks. Farag et al. (2016) stated that the antimicrobial, anti-inflammatory, immunomodulatory and antioxidant potential capacities of *Spirulina platensis* might be responsible for promoting the poultry health state.

Zeweil et al. (2016) studied the effect of dietary *Spirulina platensis* (0.5 and 1 g/kg diet) and vitamin E (75 mg/kg diet) on the performance of

local-strain chickens suffering from heat stress. They found that addition of *Spirulina platensis* improved the growth performance and immunity state of chickens under heat stress conditions. Dietary supplementation of *Spirulina platensis* has been reported to increase the number of erythrocytes and amount of hemoglobin in broiler chicks (Jamil et al. 2015). Jamil et al. (2015) and Lokapirnasari, Yulianto, and Legowo (2016) documented that feeding *Spirulina platensis* to broiler chicks increased leukocyte numbers.

However, there is some evidence showing that feeding algae during the whole rearing period resulted in lower values of erythrocytes, hemoglobin and hematocrit of broilers (Sugiharto et al. 2018). This may be due to the presence of hepatotoxin (microcystin) in *Spirulina*, which may cause liver disruptions (Iwasa et al. 2002), or cyanotoxins that may induce liver problems (Roy-Lachapelle et al. 2017). Moreover, Bonos et al. (2016) showed that *Spirulina* supplementation (5 g/kg) could improve the meat quality of broilers by increasing the contents of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in the thigh muscles of broiler chickens.

6.9 Antioxidant status

Protein extracts of *Spirulina* scavenged hydroxyl radicals (Estrada, Bescós, and Del Fresno 2001). *Spirulina* supplementation resulted in significantly higher activities of superoxide dismutase (SOD) and catalase in erythrocytes along with an increase in reduced tripeptide glutathione content in broiler chickens (Reddy et al. 2004). Using *Spirulina* in broiler chicken diets increased serum SOD and GPx activity (Park et al. 2018). The antioxidative effects of *Spirulina* may relate to several active pigments, such as phycocyanin, polysaccharides, α -tocopherol and β -carotene, that have potent antioxidant activities, which act on free radicals individually or synergistically (Estrada, Bescós, and Del Fresno 2001; Riss et al. 2007). Also, phenolic substances of *Spirulina* such as salicylic, trans-cinnamic, synaptic, chlorogenic, quinic and caffeic acids cause the antioxidant characteristics of algae (Miranda et al. 1998). It is interesting to note that the antioxidant activity of *Spirulina* is greater than that of *Chlorella* (Wu et al. 2005). That may be due to the higher amount of phenolic content in *Spirulina* (23.87 vs. 15.25 mg tannic acid equivalent/g of algae aqueous extract). Gershwin and Belay (2008) reported that the antioxidant activity of phycocyanin is about 20 times more efficient than vitamin C. In addition, *Spirulina* contains SOD, which decreases the rate

of oxygen radical-generating reactions (Belay 2002). It was reported that using phycocyanin after oxalate treatment significantly increased catalase and glucose-6-phosphate dehydrogenase activity ($P < 0.001$) in RBC lysate, suggesting phycocyanin as a free-radical quencher (Farooq et al. 2006).

References

- Al-Batshan, H. A., S. I. Al-Mufarrej, A. A. Al-Homaidan, and M. A. Qureshi. 2001. "Enhancement of chicken macrophage phagocytic function and nitrite production by dietary *Spirulina platensis*." *Immunopharmacology and Immunotoxicology* 23 (2): 281–89.
- Alvarenga, R. R., P. B. Rodrigues, V. D. S. Cantarelli, M. G. Zangeronimo, J. W. D. Silva Júnior, L. R. D. Silva, L. M. D. Santos, and L. J. Pereira. 2011. "Energy values and chemical composition of spirulina (*Spirulina platensis*) evaluated with broilers." *Revista Brasileira de Zootecnia* 40 (5): 992–96.
- Andreumont A. 2000. "Consequences of antibiotic therapy to the intestinal ecosystem." *Annales françaises d'anesthésie et de réanimation* 19:395–402.
- Austic, R. E., A. Mustafa, B. Jung, S. Gatrell, and X. G. Lei. 2013. "Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens." *Journal of Agricultural Food Chemistry* 61:7341–48.
- Becker, E. W. 2007. "Micro-algae as a source of protein." *Biotechnology Advances* 25 (2): 207–10.
- Beheshtipour, H., A. M. Mortazavian, R. Mohammadi, S. Sohrabvandi, and K. Khosravi-Darani. 2013. "Supplementation of *Spirulina platensis* and *Chlorella vulgaris* algae into probiotic fermented milks." *Comprehensive Reviews in Food Science and Food Safety* 12 (2): 144–54.
- Belay, A. 2002. "The potential application of *Spirulina* (Arthrospira) as a nutritional and therapeutic supplement in health management, Review." *Journal of American Nutraceutical Association* 5:27–48.
- Bellof, G., and L. S. Carrasco Alarcon. 2010. Einsatz der Mikroalge *Spirulina platensis* in der ökologischen Broilermast. [Effects of dietary *Spirulina platensis* on performance and economy of organic chicken production.] Hochschule Weihenstephan-Triesdorf, D-Freising-Weihenstephan, Fakultät Land- und Ernährungswirtschaft. FKZ: 08OE098 15 pages.
- Bellof, G., and L. S. Carrasco Alarcon. 2013. "Einsatz der Mikroalge *Spirulina platensis* in der ökologischen Broilermast: Effect of *Spirulina*

- platensis in Organic Broiler Production.” *Archiv für Geflügelkunde* 77:73–80.
- Bhowmik, D., J. Dubey, and S. Mehra. 2009. “Probiotic efficiency of *Spirulina platensis*-stimulating growth of lactic acid bacteria.” *World Journal of Dairy and Food Sciences* 4 (2): 160–63.
- Bonos, E., E. Kasapidou, A. Kargopoulos, A. Karampampas, E. Christaki, P. Florou-Paneri, and I. Nikolakakis. 2016. “*Spirulina* as a functional ingredient in broiler chicken diets.” *South African Journal of Animal Science* 46 (1): 94–102.
- Burgat V. 1999. “Residues of drugs of veterinary use in food.” *La Revue du praticien*. 41:985–90.
- Challouf, R., L. Trabelsi, R. Ben Dhieb, O. El Abed, A. Yahia, K. Ghazzi, J. Ben Ammar, H. Omran, and H. Ben Ouada. 2011. “Evaluation of cytotoxicity and biological activities in extracellular polysaccharides released by cyanobacterium *Arthrospira platensis*.” *Brazilian Archives of Biology and Technology* 54 (4): 831–38.
- Colla, L. M., E. B. Furlong, and J. A. V. Costa. 2007. “Antioxidant properties of *Spirulina* (*Arthrospira*) *platensis* cultivated under different temperatures and nitrogen regimes.” *Brazilian Archives of Biology and Technology* 50 (1): 161–67.
- Colla, L. M., A. L. Muccillo-Baisch, and J. A. V. Costa. 2008. “*Spirulina platensis* effects on the levels of total cholesterol, HDL and triacylglycerols in rabbits fed with a hypercholesterolemic diet.” *Brazilian Archives of Biology and Technology* 51 (2): 405–11.
- De Jesus Raposo, M. F., A. M. M. B. de Moraes, and R. M. S. C. de Moraes. 2016. “Emergent sources of prebiotics: seaweeds and microalgae.” *Marine Drugs* 14 (2): 27.
- Dermer, R. B., S. Ohse, M. Villela, S. M. D. Carvalho, and R. Fett. 2006. “Microalgae, products and applications.” *Ciência Rural* 36 (6): 1959–67.
- El-Baz, F. K., W. M. El-Senousy, A. B. El-Sayed, and M. M. Kamel. 2013. “In vitro antiviral and antimicrobial activities of *Spirulina platensis* extract.” *Journal of Applied Pharmaceutical Science* 3 (12): 52–56.
- El-Sheekh, M. M., S. M. Daboor, M. A. Swelim, and S. Mohamed. 2014. “Production and characterization of antimicrobial active substance from *Spirulina platensis*.” *Iranian Journal of Microbiology* 6 (2): 112–19.
- Estrada, J. P., P. B. Bescós, and A. V. Del Fresno. 2001. “Antioxidant activity of different fractions of *Spirulina platensis* protean extract.” *Il farmaco* 56 (5–7): 497–500.

- Evans, A. M., D. L. Smith, and J. S. Moritz. 2015. "Effects of algae incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance." *Journal of Applied Poultry Research* 24 (2): 206–14.
- Farag, M. R., M. Alagawany, M. A. El-Hack, K. and Dhama. 2016. "Nutritional and healthical aspects of Spirulina (Arthrospira) for poultry, animals and human." *International Journal of Pharmacology* 12 (12): 36–51.
- Farooq, S. M., A. S. Ebrahim, K. H. Subramhanya, R. Sakthivel, N. G. Rajesh, and P. Varalakshmi. 2006. Oxalate mediated nephronal impairment and its inhibition by c-phycocyanin: a study on urolithic rats. *Molecular and cellular biochemistry*, 284(1-2): 95-101.
- Ferket, P. R., E. Van Heugten, T. A. T. G. Van Kempen, and R. Angel. 2002. "Nutritional strategies to reduce environmental emissions from nonruminants." *Journal of Animal Science* 80 (E-suppl_2): E168–E182.
- Finke, M. D. 2002. "Complete nutrient composition of commercially raised invertebrates used as food for insectivores." *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association* 21 (3): 269–85.
- Finke, M.D. 2004. "Nutrient Content of Insects-Organic Value Recovery Solution Studies." In *Encyclopedia of Entomology* No. 10.1007/0-306-48380-7_2920. Berlin: Springer Verlag.
- Finke, M. D. 2013. "Complete Nutrient Content of Four Species of Feeder Insects." *Zoo Biology* 32:27–36.
- Furbeyre, H., J. Van Milgen, T. Mener, M. Gloaguen, and E. Labussière. 2017. "Effects of dietary supplementation with freshwater microalgae on growth performance, nutrient digestibility and gut health in weaned piglets." *Animal* 11 (2): 183–92.
- Furst, P. T. 1978. "Spirulina." *Human Nature*. 1 (3): 60-65.
- Gershwin, M. E., and A. Belay, eds. 2008. *Spirulina in Human Nutrition and Health*. Boca Raton, FL: CRC Press.
- Gongnet, G. P., E. Niess, M. Rodehutsord, and E. Pfeffer. 2001. "Algae-Meal (Spirulina platensis) from Lake Chad Replacing Soybean-Meal in Broiler Diets." *Archiv für Geflügelkunde* 65:265–68.
- Holman, B. W. B., and A. E. O. Malau-Aduli. 2013. "Spirulina as a livestock supplement and animal feed." *Journal of Animal Physiology and Animal Nutrition* 97 (4): 615–23.
- Huang, Z. X., X. T. Mei, D. H. Xu, S. B. Xu, and J. Y. Lv. 2005. "Protective effects of polysaccharide of Spirulina platensis and Sargassum thunbergii on vascular of alloxan induced diabetic rats."

- Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China Journal of Chinese materia medica* 30 (3): 211–15.
- Iwasa, M., M. Yamamoto, Y. Tanaka, M. Kaito, and Y. Adachi. 2002. "Spirulina-associated hepatotoxicity." *The American Journal of Gastroenterology* 97 (12): 3212.
- Iwata, K., T. Inayama, and T. Kato. 1990. "Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats." *Journal of Nutritional Science and Vitaminology* 36 (2): 165–71.
- Jamil, A. R., M. R. Akanda, M. M. Rahman, M. A. Hossain, and M. S. Islam. 2015. "Prebiotic competence of spirulina on the production performance of broiler chickens." *Journal of Advanced Veterinary and Animal Research* 2 (3): 304–9.
- Kaoud, H. A. 2012. "Effect of *Spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics." *Scientific Journal of Applied Research* 1:44–48.
- Kaoud, H. A. 2015. "Effect of *Spirulina platensis* as a dietary supplement on broiler performance in comparison with prebiotics." *Science Arena Publications Specialty Journal of Biological Sciences* 1 (2): 1–6.
- Kaoud, H. A., K. M. Mahran, A. Rezk, and M. A. Khalf. 2012. "Bioremediation the toxic effect of mercury on liver histopathology, some hematological parameters and enzymatic activity in Nile tilapia, *Oreochromis niloticus*." *Researcher* 4 (1): 60–70.
- Kato, K., K. Takemoto, H. Katayama, and Y. Kuwabara. 1984. "Effects of *Spirulina* (*Spirulina platensis*) on dietary hypercholesterolemia in rats." *Journal of Japanese Society of Nutrition and Food Science (Japan)*. 37: 323–332.
- Kaushik, P., and A. Chauhan. 2008. "In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis*." *Indian Journal of Microbiology* 48 (3): 348–52.
- Kumar, V., P. S. Tirumalai, A. Singh, A. K. Bhatnagar, and J. N. Shrivastavaet. 2013. "Natural compounds from algae and *Spirulina platensis* and its antimicrobial activity." *Indo Global Journal of Pharmaceutical Sciences* 3 (3): 212–23.
- Layam, A., and C. L. K. Reddy. 2006. "Antidiabetic property of spirulina." *Diabetologia croatica* 35 (2): 29–33.
- Lobner, M., A. Walsted, R. Larsen, K. Bendtzen, and C. H. Nielsen. 2008. "Enhancement of human adaptive immune responses by administration of a high-molecular-weight polysaccharide extract from the cyanobacterium *Arthrospira platensis*." *Journal of Medicinal Food* 11 (2): 313–22.

- Lokapirnasari, W. P., A. B. Yulianto, and D. Legowo. 2016. "The effect of Spirulina as feed additive to myocardial necrosis and leukocyte of chicken with avian influenza (H5N1) virus infection." *Procedia Chemistry* 18:213–17.
- Mabeau, S., and J. Fleurence. 1993. "Seaweed in food products: biochemical and nutritional aspects." *Trends in Food Science and Technology* 4 (4): 103–7.
- Makkar, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. "State-of-the-Art on Use of Insects as Animal Feed." *Animal Feed Science and Technology* 197:1–33.
- Mala, R., M. Sarojini, S. Saravanababu, and G. Umadevi. 2009. "Screening for antimicrobial activity of crude extracts of *Spirulina platensis*." *Journal of Cell and Tissue Research* 9 (3): 1951–55.
- Mariey, Y. A., H. R. Samak, and M. A. Ibrahim. 2012. "Effect of using *Spirulina platensis* algae as a feed additive for poultry diets: 1–productive and reproductive performances of local laying hens." *Egyptian Poultry Science* 32 (1): 201–15.
- Mariey, Y., H. Samak, H. Abou-Khashba, M. Sayed, and A. Abou-Zeid. 2014. "Effect of using *Spirulina platensis* algae as a feed additives for poultry diets: 2 productive performance of broiler." *Egyptian Poultry Science Journal* 34 (1): 245–58.
- Mekbungwan, A., K. E. Yamauchi, and N. Thongwittaya. 2002. "Intestinal morphology and enteral nutrient absorption of pigeon pea seed meal in piglets." *Animal Science Journal* 73 (6): 509–16.
- Miranda, M. S., R. G. Cintra, S. B. M. Barros, and J. Mancini-Filho. 1998. "Antioxidant activity of the microalga *Spirulina maxima*." *Brazilian Journal of Medical and Biological Research* 31 (8): 1075–79.
- Nagaoka, S., K. Shimizu, H. Kaneko, F. Shibayama, K. Morikawa, Y. Kanamaru, A. Otsuka, T. Hirahashi, and T. Kato. 2005. "A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats." *The Journal of Nutrition* 135 (10): 2425–30.
- Neumann, C., S. Velten, and F. Liebert. 2018. "The Graded Inclusion of Algae (*Spirulina platensis*) or Insect (*Hermetia illucens*) Meal as a Soybean Meal Substitute in Meat Type Chicken Diets Impacts on Growth, Nutrient Deposition and Dietary Protein Quality Depending on the Extent of Amino Acid Supplementation." *Open Journal of Animal Sciences* 8 (02): 163.
- Nuhu, A. A. 2013. "Spirulina (Arthrospira): An important source of nutritional and medicinal compounds." *Journal of Marine Biology*, 84: 1-7.

- Park, J. H., S. I. Lee, and I. H. Kim. 2018. "Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens." *Poultry Science* 97 (7): 2451–59.
- Pradhan, J., B. K. Das, S. Sahu, N. P. Marhual, A. K. Swain, B. K. Mishra, and A. E. Eknath. 2012. "Traditional antibacterial activity of freshwater microalga *Spirulina platensis* to aquatic pathogens." *Aquaculture Research* 43 (9): 1287–95.
- Qureshi, M. A., J. D. Garlich, and M. T. Kidd. 1996. "Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens." *Immunopharmacology and Immunotoxicology* 18 (3): 465–76.
- Raach-Moujahed, A., S. Hassani, S. Zairi, M. Bouallegue, C. Darej, B. Haddad, and C. Damergi. 2011. "Effect of dehydrated *Spirulina platensis* on performance and meat quality of broilers." *Research Opinions in Animal and Veterinary Sciences* 1 (8): 505–9.
- Raju, M. V. L. N., S. V. Rama Rao, K. Radhika, and M. M. Chawak. 2004. "Effects of *Spirulina platensis* or furazolidone on the performance and immune response of broiler chickens fed with aflatoxin contaminated diet." *Indian Journal of Animal Nutrition* 21 (1): 40–44.
- Reddy, B. S., N. Yuvaraj, V. Babitha, V. Ramnath, P. T. Philomina, and M. C. Sabu. 2004. "Antioxidant and hypolipidemic effects of *Spirulina* and natural carotenoids in broiler chicken." *Indian Veterinary Journal* 81:383–86.
- Riss, J., K. Décordé, T. Sutra, M. Delage, J. C. Baccou, N. Jouy, J. P. Brune, H. Oréal, J. P. Cristol, and J. M. Rouanet. 2007. "Phycobiliprotein C-phycoerythrin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters." *Journal of Agricultural and Food Chemistry* 55 (19): 7962–67.
- Rodriguez-Hernández, A., J. L. Ble-Castillo, M. A. Juárez-Oropeza, and J. C. Diaz-Zagoya. 2001. "*Spirulina maxima* prevents fatty liver formation in CD-1 male and female mice with experimental diabetes." *Life Sciences* 69 (9): 1029–37.
- Rogatto, G. P., C. D. Oliveira, J. D. Santos, F. D. B. Machado, F. Y. Nakamura, C. D. Moraes, A. D. M. Zagatto, M. C. Faria, M. Afonso, and M. D. Mello. 2004. "Influência da ingestão de espirulina sobre o metabolismo de ratos exercitados." *Revista Brasileira de Medicina do Esportiva* 10 (4): 258–63.

- Ross, E. and W. Dominy. 1990. "The Nutritional Value of Dehydrated, Blue-Green Algae (*Spirulina platensis*) for Poultry." *Poultry Science* 69 (5): 794–800. <https://doi.org/10.3382/ps.0690794>.
- Rostagno, H. S. 2005. *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais*. 2 ed. Universidade Federal de Viçosa. MG: UFV, 2005. 186p.
- Roy-Lachapelle, A., M. Sollicec, M. F. Bouchard, and S. Sauvé. 2017. "Detection of cyanotoxins in algae dietary supplements." *Toxins* 9 (3): 76.
- Sarada, D. V., C. S. Kumar, and R. Rengasamy. 2011. "Purified C-phycocyanin from *Spirulina platensis* (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria." *World Journal of Microbiology and Biotechnology* 27 (4): 779–83.
- Shanmugapriya, B., S. S. Babu, T. Hariharan, S. Sivaneshwaran, and M. B. Anusha. 2015a. "Dietary administration of *Spirulina platensis* as probiotics on health and histopathology in broiler chicks." *International Journal of Recent Scientific Research* 6:2650–53.
- Shanmugapriya, B., S. S. Babu, T. Hariharan, S. Sivaneshwaran, M. B. Anusha, and P. U. Raja. 2015b. Synergistic effect of "*Spirulina platensis* on performance and gut microbial load of broiler chicks." *Indo-Asian Journal of Multidisciplinary Research* 1:149–55.
- Shokri, H., A. R. Khosravi, and M. Taghavi. 2014. "Efficacy of *Spirulina platensis* on immune functions in cancer mice with systemic candidiasis." *Journal of Mycology Research* 1 (1): 7–13.
- Sivakumar, J., and P. Santhanam. 2011. "Antipathogenic activity of *Spirulina* powder." *Recent Research in Science and Technology* 3 (4): 158-161.
- Sørum, H., and M. Sunde. 2001. "Resistance to antibiotics in the normal flora of animals." *Veterinary Research* 32 (3–4): 227–41.
- Sugiharto, S., and C. Lauridsen. 2016. "Dietary *Chlorella* supplementation effect on immune responses and growth performances of broiler chickens exposed to post hatch holding time." *Livestock Research of Rural Development* 28 (7): 1-6.
- Sugiharto, S., T. Yudiarti, I. Isroli, and E. Widiastuti. 2018. "Effect of feeding duration of *Spirulina platensis* on growth performance, haematological parameters, intestinal microbial population and carcass traits of broiler chicks." *South African Journal of Animal Science* 48 (1): 98–107.
- Takai, Y., Y. Hossayamada, and T. Kato. 1991. "Effect of water soluble and water insoluble fractions of *Spirulina* over serum lipids and

- glucose resistance of rats.” *Nippon Eiyo Shokuryo Gakkaishi (Journal of Japan Society of Nutrition and Food Science)* 44:273–77.
- Tavernari, F. D. C., L. Roza, D. Surek, and M. L. B. da Silva. 2016a. “Apparent metabolizable energy from microalgae spirulina maxima for broiler chickens.” In *Embrapa Suínos e Aves-Resumo em anais de congresso (ALICE)*. Abstract submitted to the International Poultry Scientific Forum 2016, Georgia. Southern Poultry Science Society, 95.
- Tavernari, F. D. C., D. Surek, L. Roza, M. L. B. da Silva, and L. Albino. 2016b. “Broiler chicken ileal apparent digestibility of calcium and phosphorus from microalgae spirulina maxima.” In *Embrapa Suínos e Aves-Resumo em anais de congresso (ALICE)*. Abstract submitted to the International Poultry Scientific Forum 2016, Georgia. Southern Poultry Science Society, 95.
- Tokai, Y. 1987. “Effects of Spirulina on caecum content in rats.” *Chiba Hyg Coll Bull* 5 (2).
- Toyomizu, M., K. Suzuki, Y. Kawata, H. Kojima, and Y. Akiba. 2001. “Effective transformation of the cyanobacterium *Spirulina platensis* using electroporation.” *Journal of Applied Phycology* 13 (3): 209–14.
- Uyisenga, J. P., P. Nzayino, R. Seneza, L. Hishamunda, K. Uwantege, N. Gasana, and E. S. Bajyana. 2010. “In vitro study of antibacterial and antifungal activity of *Spirulina platensis*.” *International Journal of Ecology and Development* 16 (S10): 80–88.
- Van Huis, A. 2013. “Potential of Insects as Food and Feed in Assuring Food Security.” *Annual Review of Entomology* 58:563–83.
- Venkataraman, L. V., T. Somasekaran, and E. W. Becker. 1994. “Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chicks.” *British Poultry Science* 35 (3): 373–81.
- Vonshak, A. 2007. “Appendices: *Spirulina platensis* (Arthrospira): Physiology cell-biology and laboratory conditions.” *Journal of Aquaculture* 271 (1): 4439–448.
- Wu, L. C., J. A. A. Ho, M. C. Shieh, I. W. and Lu. 2005. “Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts.” *Journal of Agricultural and Food Chemistry* 53 (10): 4207–12.
- Yan, L., S. U. Lim, and I. H. Kim. 2012. “Effect of fermented chlorella supplementation on growth performance, nutrient digestibility, blood characteristics, fecal microbial and fecal noxious gas content in growing pigs.” *Asian-Australasian Journal of animal Sciences* 25 (12): 1742.
- Yoshida, M., and H. Hoshii, H. 1980. “Nutritive value of spirulina, green algae, for poultry feed.” *Japanese Poultry Science* 17 (1): 27–30.

- Zeweil, H., I. M. Abaza, S. M. Zahran, M. H. Ahmed, M. Haiam, and A. S. Asmaa. 2016. "Effect of *Spirulina platensis* as dietary supplement on some biological traits for chickens under heat stress condition." *Asian Journal of Biomedical and Pharmaceutical Sciences* 6 (56): 8-12.
- Zhang, H., A. Lin, Y. Sun, and Y. Deng. 2001. "Chemo-and radio-protective effects of polysaccharide of *Spirulina platensis* on hemopoietic system of mice and dogs." *Acta Pharmacologica Sinica* 22 (12): 1121–24.

CHAPTER SEVEN

SPIRULINA IN LAYER NUTRITION

“To eat is a necessity, but to eat intelligently is an art.”
—La Rochefoucauld

7.1 Introduction

There has been a rapid increase in organic egg production in most countries recently. This development is a response to an increased consumer demand for food that is perceived to be fresh, healthy and tasty, free of hormones, antibiotics and harmful chemicals, and produced in a way that is sustainable environmentally and without the use of genetically modified (GM) ingredients (Blair 2008). On the other hand, the losses due to diseases and mortality are higher in organic rearing. A Danish study of large organic layer flocks reported high mortality rates (15–20%) that are two to three times higher than in layers in battery cages (Kristensen 1998). Other researchers reported that 76–84% of organic layer farms in Finland tested positive for *Campylobacter* contamination, based on examination of droppings samples (Sulonen et al. 2007). *Spirulina* has the potential to be used as a natural feed source for layer hens because of its medicinal and nutritive values as discussed in previous chapters. This chapter chiefly discusses *Spirulina* usage in layer hens.

7.2 Egg production

Spirulina can potentially help to stimulate the immune system of layer hens. Qureshi et al. (1996) fed hens and broilers with different levels of *Spirulina* (0, 10, 100, 1000 and 10000 ppm). They documented that *Spirulina* supplementation strengthened multiple immunological functions such as macrophage function, antibody response and phagocytosis. They reported that using 10000 ppm *Spirulina* might also enhance potential resistance to pathogens.

It was documented that using *Spirulina* in hen diets improved egg production, egg weight and daily egg mass (Nikodémusz et al. 2010; Mariey et al. 2012). However, the inclusion of marine algae in hens' diets at the level of 4.8% decreased egg production (Herber and Van Elwyk 1996). With increasing levels of *Spirulina* powder extract, the average egg weight decreased, but egg yolk color and egg shape index increased (Peipei and Sumin 2018). The negative effect of higher dosage of *Spirulina* may relate to cyanotoxins. It should be noted that the environment of *Spirulina* culture is also suitable for the growth of some toxic cyanobacteria species, such as *Anabaena*, *Microcystis*, *Oscillatoria*, and *Nostoc* (do Carmo Bittencourt-Oliveira et al. 2005; Babica, Blaha, and Marsalek 2006). These cyanobacteria can produce microcystins (MCs), a group of monocyclic hepta-peptides that have more than 60 structural variables generally differing in the nature of the two L-amino acids and in the degree of methyl substitution (Soares et al. 2004). MCs are named according to their variable L-amino acids. MCs mainly cause morphological and functional changes in hepatocytes (Gulledge et al. 2002), inhibiting the activity of protein phosphatases, especially types 1 and 2A, both in vivo and in vitro (Runnegar et al. 1995; Barford 1996; Codd, Morrison, and Metcalf 2005), resulting in cell proliferation and cancer or an apoptotic process and cell death (Chen et al. 2005). So, quality control of *Spirulina* products must be considered. The chemical structure of MC is shown in Figure 7-1.

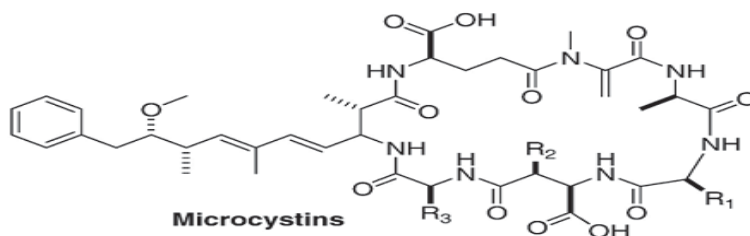


Figure 7-1: Chemical structure of microcystins. (Cardozo et al. 2007)

Some researchers reported that *Spirulina*-containing diets had a beneficial effect on FCR of laying hens (Nikodémusz et al. 2010; Mariey, Samak, and Ibrahim 2012). The significant improvement in FCR may be due to the fact that *Spirulina* improves absorption of minerals, protects from diarrhea and optimizes nutrient digestion processes (Gružauskas et al. 2004). Additionally, *Spirulina* has an excellent nutritional profile. It has a high amount of carotenoids and high-quality protein content and is rich in

minerals and vitamins (Ross and Dominy 1990). *Spirulina*-containing diets may enhance the absorbability of dietary vitamins (Mariey, Samak, and Ibrahim 2012). Zahroojian, Moravej, and Shivazad (2013) fed Hy-line W-36 hens with three levels of *Spirulina* (1.5, 2.0 and 2.5%). They reported that egg production, feed intake (FI), FCR and egg weight (EW) were not significantly affected by the dietary treatments. They claimed that these results may be due to the use of low levels of algae in their studies. Effects of *Spirulina* on egg production, FI, FCR, EW and yolk color are shown in Table 7-1.

Table 7-1: *Spirulina* on egg production, feed intake, FCR, egg weight and yolk color. (Zahroojian, Moravej, and Shivazad 2011)

Treatments	Egg production eggs produced/ hen d	Feed intake g/hen d	FCR g feed/g egg	Egg weight (g)	Yolk color
0 (control group)	0.76 ^a ±0.028	100.275 ^a ±1.356	1.562 ^a ±0.029	63.923 ^a ±0.801	1.55 ^d ±0.111
1.5% <i>Spirulina</i>	0.81 ^a ±0.028	97.803 ^a ±1.356	1.550 ^a ±0.029	63.198 ^a ±0.801	10.55 ^c ±0.111
2.0% <i>Spirulina</i>	0.85 ^a ±0.028	96.495 ^a ±1.356	1.520 ^a ±0.029	63.598 ^a ±0.801	11.43 ^b ±0.111
2.5% <i>Spirulina</i>	0.76 ^a ±0.028	97.393 ^a ±1.356	1.532 ^a ±0.029	63.473 ^a ±0.801	11.66 ^{ab} ±0.111
Synthetic pigment	0.81 ^a ±0.028	98.148 ^a ±1.356	1.567 ^a ±0.029	62.663 ^a ±0.801	11.93 ^a ±0.111
P-value	NS	NS	NS	NS	<0.001
CV	7.06	2.76	3.77	2.53	2.35

^{abc} Means in a column with different superscripts differ significantly (P<0.05).

Saxena et al. (1983) replaced the peanut cake with *Spirulina maxima* (5.6 to 16.6%) in laying hens' diets. The body weight gains of the control group and the group fed with 5.6% *Spirulina* were not significantly different, while at higher levels of algae a much higher weight gain was recorded. No toxic effects of *Spirulina* were detected during the experiment period. They concluded that *Spirulina* can fully replace peanut cake as a protein source.

In a recent study, dietary supplementation of *Spirulina platensis* at the levels of 0.1, 0.2 or 0.3% in diets of laying hens increased birds' BW, FI egg production rate (EPR), EW and egg mass (EM) (Selim, Hussein, and Abou-Elkhair 2018). Several authors reported that laying hens fed *Spirulina*-containing diets reached the best means of egg production and

FCR compared with the control group (Nikodémusz et al. 2010; Mariey, Samak, and Ibrahim 2012). In these experiments, the increase in EW for hens fed with the *Spirulina* diets may be related to heavier egg yolks. It was stated that feeding microalgae (*Chlorella vulgaris*) to layers increased the beneficial microbial diversity in the crop and ceca (Janczyk, Halle, and Souffrant 2009). It seems that the valuable nutrient contents of microalgae and changes in the intestinal microbial community are involved in some of the metabolism processes related to egg production (Park, Upadhaya, and Kim 2015).

7.3 Egg quality

The preference for well-pigmented poultry products is still evident in several markets; thus, poultry producers add colorants to layer diets as a means of improving the attractiveness of these products as a marketing strategy (Klaui and Bauernfeind 1981; Hencken 1992; Franchini and Padoa 1996; LiuFa, XuFang and Cheng 1997). Baking operations and the food processing industry prefer darker colored yolks more than the addition of artificial coloring agents (Zahroojian, Moravej, and Shivazad 2013). The microalgae carotenoids are in competition with the synthetic pigments (Spolaore et al. 2006). Although the synthetic forms are much less expensive than the natural ones, microalgae carotenoids have the advantage of supplying natural isomers in their natural ratio (Olaizola 2003).

Animals, including poultry, absorb carotenoids from the diet (Sirri et al. 2007). The fed xanthophylls may be found in the blood, muscle, liver, fat, skin and feathers of birds (Zahroojian, Moravej, and Shivazad 2011). In laying hens, the muscle and skin xanthophylls stores are transferred to the ovaries with the onset of sexual maturity, and parts of them are excreted in the egg yolk. Breed, strain and housing conditions may influence a hen's ability to deposit pigments in the yolk (Karunajeewa et al. 1984; Ponsano et al. 2004). Biosynthetic pathways of some carotenoids are shown in Figure 7-2.

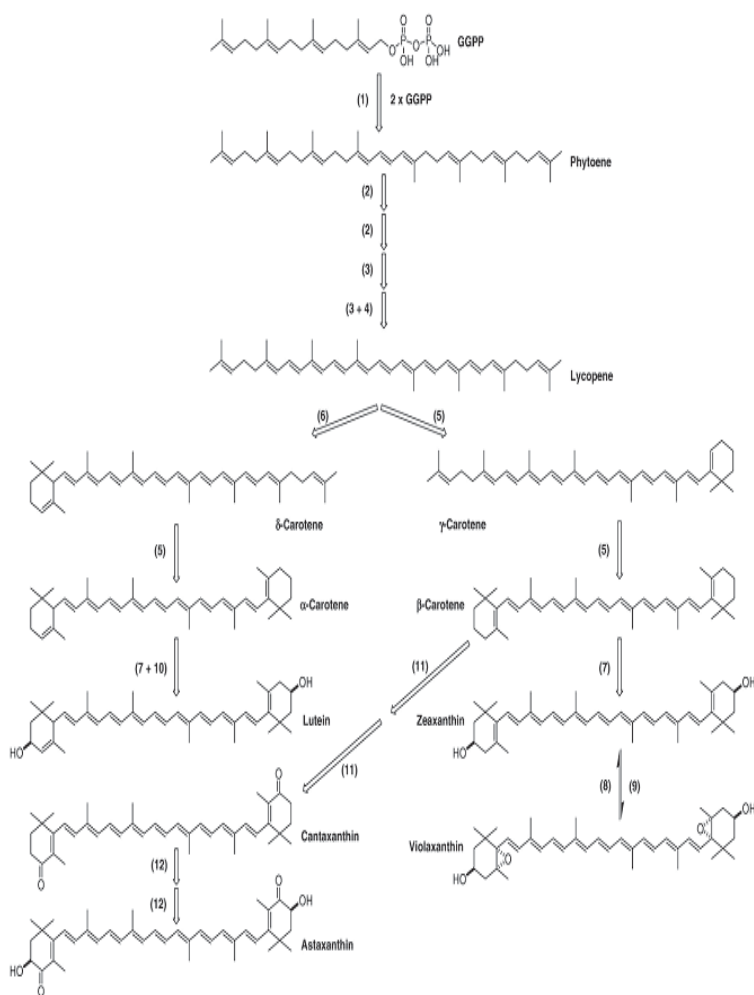


Figure 7-2: Biosynthetic pathway of some carotenoids. GGPP: geranylgeranyl diphosphate. Enzymes: (1) phytoenesynthase, (2) phytoenedesaturase, (3) ζ-carotene desaturase, (4) carotene isomerase, (5, 6) lycopene α- or β-cyclase, (7) β-ring hydroxylase, (8) zeaxanthine poxidase, (9) violaxanthinde-epoxidase, (10) ε-ring hydroxylase, (11) β-carotene ketolase, (12) β-carotene3'3'-hydroxylase. (Cardozo et al. 2007)

As mentioned in Chapters One and Three, *Spirulina* is an excellent source of valuable nutrients (protein, vitamins, minerals, pigments, etc.) and provides a natural source of carotenoids that are extremely effective in coloring egg yolks. Using *Spirulina* at the rate of 0.5–1.5% in layer hens' diets helps to enhance pigmentation benefits, but it is important to utilize *Spirulina* that contains a certain amount of carotenoids—at least 3.5 g of total carotenoids per kg (Lorenz 1999). There is a linear relationship between dietary *Spirulina* and egg yolk coloration (Avila and Cuca 1974). There is some evidence that using astaxanthin in layer hens' diets causes a linear increase in egg yolk color (Hammershøj 1995; Yang et al. 2006). Astaxanthin derived from algae meal provides excellent egg yolk pigmentation at concentrations as low as 2 ppm (Lorenz 1999). Even using low levels of astaxanthin from algae meal increases the yellow pigmentation of skin, feet and beaks (Lorenz 1999; Zahroojian, Moravej, and Shivazad 2013). There are lots of studies that showed using *Spirulina* in layer hens' diets enhanced yolk color and flesh of the birds (Blum and Calet 1975; Venkataraman, Somasekaran, and Becker 1994; Ross and Dominy 1990; Anderson, Tang, and Ross 1991, Toyomizu et al. 2001; Takashi 2003). In an Iranian study at the University of Tehran, yolk color scores of the control group and groups fed with different levels of *Spirulina* (1.5, 2.0, 2.5%) were 1.5, 10.5, 11.4 and 11.6 in the BASF color fan, respectively (Zahroojian, Moravej, and Shivazad 2013). The effect of *Spirulina* concentration on yolk color is better explained by a polynomial effect response curve, as shown in Figure 7-3.

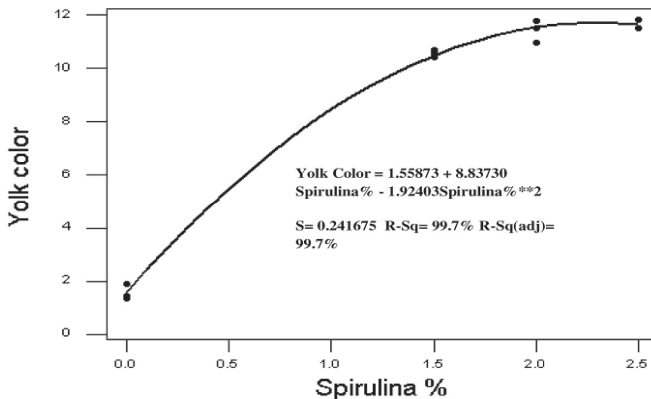


Figure 7-3: Regression between BASF yolk color fan values and level of *Spirulina* in the diet. (Zahroojian, Moravej, and Shivazad 2013)

Regarding Figure 7-4, the optimal level of yolk color (between 11.4 and 11.6 on the BASF yolk color fan) was achieved with 2.0–2.5% *Spirulina* in diet after ~ 7 days. The color levels of the egg yolks remained stable as long as the supplementation continued. The effect of treatments on egg yolk color score is shown in Figure 7-4.

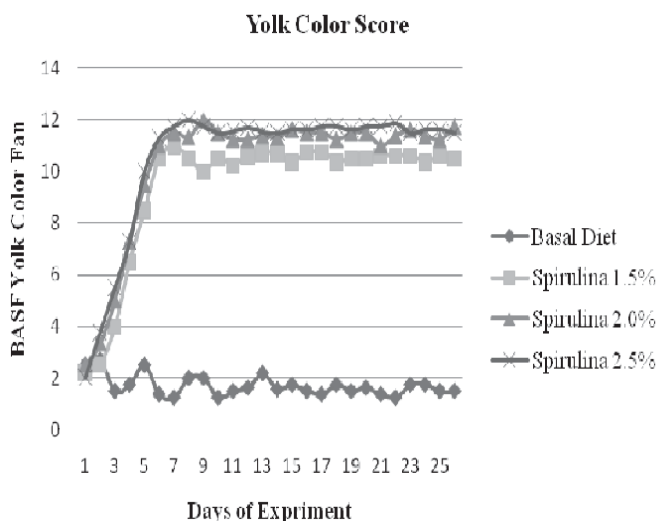


Figure 7-4: Effect of treatments on egg yolk color score. (Zahroojian, Moravej, and Shivazad 2013)

Dogan et al. (2016) reported that with an increase of supplementation level of *Spirulina* from 5 to 20 g/kg diet, the egg shell weight increased. This increase may relate to the high calcium content in *Spirulina* (Tokusoglu and Unal 2003). It was documented that supplementation of marine microalgae (*Schizochytrium*) powder at the levels of 0.5–1% had a positive effect on the egg shell thickness (Park, Upadhaya, and Kim 2015). Marine algae contain a good profile of minerals, so this may be the cause of their positive effect on the egg shell thickness; however, the mechanisms of this effect still remain unclear and further investigations are needed to know the relation between *Spirulina platensis* and mineral metabolism of laying hens (Selim, Hussein, and Abou-Elkhair 2018). Dietary *Spirulina* has no effect on egg shell percentage, yolk index, albumen percentage and haugh unit (HU) in layer hens (Mariey, Samak, and Ibrahim 2012; Zahroojian, Moravej, and Shivazad 2013). The effect

of *Spirulina platensis* supplementation on egg quality traits of layer hens after eight weeks feeding of the algae is shown in Table 7-2.

Table 7-2: Effect of *Spirulina platensis* supplementation on layer hens' egg quality traits. (Selim, Hussein, and Abou-Elkhair 2018)

	Treatments ¹					P-value		
parameters	control	T1	T2	T3	SEM ²	P-value		
Egg Shape Index	74.1	74.2	74.7	76.7	2.27	0.663	0.286	0.593
Shell,%	8.89	8.39	9.12	9.40	0.959	0.941	0.688	0.875
Albumen,%	52.2	53.4	54.9	53.0	1.25	0.253	0.361	0.117
Albumen Index,%	14.7	13.3	12.9	13.1	1.29	0.555	0.241	0.451
Yolk weight,%	38.9	37.2	35.9	37.6	1.16	0.172	0.204	0.078
Yolk shape index,%	44.5	44.8	43.7	44.7	2.31	0.962	0.930	0.846
Yolk color score	4.78 ^c	6.11 ^b	6.89 ^{ab}	7.33 ^a	0.272	<0.001	<0.001	0.050
HU	90.8	88.3	92.1	89.7	2.32	0.465	0.945	0.985
Shell thickness, mm	0.314 ^c	0.356 ^{bc}	0.401 ^{ab}	0.423 ^a	0.019	0.002	0.001	0.043

^{a,b} Values in the same row with a different superscript differ significantly at $P<0.05$.¹ T1 = 0.1% SP (1 kg/ton), T2 = 0.2% SP (2 kg/ton) and T3 = 0.3% SP (3 kg/ton).² Pooled standard error of the means.

Mariey, Samak, and Ibrahim (2012) reported that there were significant ($P<0.05$) reductions in yolk cholesterol and total lipids as the level of dietary *Spirulina* was increased. This reduction in yolk total lipids and cholesterol contents may be related to the lower levels in blood plasma of hens fed with the *Spirulina*-containing diets (Mariey, Samak, and Ibrahim 2012). Also, other researchers found that addition of brown marine algae in laying hen diets significantly decreased egg yolk cholesterol, and this effect may be due to the antioxidant properties of marine algae (Al-Harthi and El-Deek 2012).

Selim, Hussein, and Abou-Elkhair (2018) reported that serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in laying hens fed with *Spirulina platensis* decreased. They demonstrated that this reduction may be due to antioxidant and anti-inflammatory factors of *Spirulina platensis* content. Chen et al. (2011) reported that DHA from a microalgal source can inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, which reduces cholesterol synthesis, thus decreasing plasma cholesterol level. The HMG-CoA reductase is a rate-limiting enzyme in cholesterol biosynthesis and can cause the reduction in

plasma cholesterol. Also, decreased levels of blood triglyceride and cholesterol may be associated with polysaccharides in microalgae. Marine algae contains large quantities of soluble and insoluble dietary fibers (Lahaye and Jegou 1993), which can reduce blood lipids (Ara et al. 2002; Werman, Sukenik, and Mokady 2003). Serum and yolk cholesterol levels in layer hens fed with *Spirulina* are shown in Table 7-3.

Table 7-3: The effect of dietary *Spirulina* on serum and yolk cholesterol levels. (Narahari et al. 2005)

Parameters	0.1% <i>Spirulina</i>
Serum TG (mg/dl)	715 ^{ab} ±3.9
Serum TC (mg/dl)	138 ^b ±1.3
Serum LDL (mg/dl)	23.8 ^b ±0.16
Serum VLDL (mg/dl)	79.5 ^b ±1.1
Serum HDL (mg/dl)	34.7 ^b ±1.03
Yolk cholesterol (mg/g)	9.90 ^c ±0.22

TG: triglyceride, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high density lipoprotein, VLDL: very low density lipoprotein.

Microalgae are a hopeful source of W₃ and W₆ (Andrade et al. 2018). Briefly, alpha-linolenic acid (ALA), EPA and DHA are the main fatty acids in the ω-3 family (Chadha et al. 2015). GLA and arachidonic acid (AA) are the main fatty acids in the ω-6 fatty acids family. AA presents in the membrane of body cells and is the precursor of eicosanoid production (Andrade et al. 2018). Humans have a limited ability to convert dietary ALA to EPA and DHA, and the efficiency of the conversion is very low (less than 1%). Thus, dietary intake of EPA and especially DHA is necessary to maintain sufficient amounts in the human body. DHA is most commonly found in wild fish like salmon and mackerel, which feed on marine algae. The active forms of essential ω (n)-3 fatty acids, particularly DHA, in human diets play important roles during pregnancy and early infant development (Chadha, Naylor, and Tsappis 2015). In adults, high levels of dietary DHA and EPA cause lower rates of fatigue, poor memory, dry skin, suicidal behavior, schizophrenia, coronary heart disease, arrhythmias, atherosclerosis, inflammation, diabetes, and cancers such as breast, prostate and colon (Andrade et al. 2018). EPA is a precursor for prostaglandin-3, thromboxane-3 and leukotriene-5 group, which are part of the defense system against inflammation (Andrade et al. 2018).

The use of algae as a source of PUFA is increasingly recognized globally, and further research holds great potential for benefits in human pharmacology and nutrition (Robertson et al. 2013). Using microalgae in layers' diets enrich the ω -3 fatty acids content of eggs (Herber and Van Elswyk 1996; Chadha, Naylor, and Tsappis 2015; Ao et al. 2015). Changes in the fatty acid composition of DHA-enriched eggs do not affect the sensory and storage qualities of the eggs (Chadha, Naylor, and Tsappis 2015). The enriched eggs' appearance and taste are the same as those of conventional eggs, and their flavor is significantly preferred. The DHA-enriched eggs can be promoted as a dietary alternative source to fish (Chadha et al. 2015). Rajesha et al., (2009) reported that feeding layer hens with ω -3 PUFA sources along with a *Spirulina*-based diet (high in lutein and β - carotene) increased the total ω -3 PUFA content of eggs.

It is interesting to note that astaxanthin may have a positive effect on egg quality during storage time. Increasing the level of dietary astaxanthin decreased the amount of thio-barbituric acid-reactive substances (TBARS) in egg yolks (Yang et al. 2006). TBARS is a bio marker that shows the level of lipid oxidation; higher levels of TBARS exhibit more oxidative damages.

References

- Al-Harthi, M. A., and A. A. El-Deek. 2012. "Effect of different dietary concentrations of brown marine algae (*Sargassum dentifolium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens." *Italian Journal of Animal Science* 11 (4): e64.
- Anderson, D. W., C. S. Tang, E. and Ross. 1991. "The xanthophylls of *Spirulina* and their effect on egg yolk pigmentation." *Poultry Science* 70 (1): 115–19.
- Andrade, L. M., C. J. Andrade, M. Dias, C. A. O. Nascimento, and M. A. Mendes. 2018. "Chlorella and *Spirulina* Microalgae as Sources of Functional Foods." *Nutraceuticals, and Food Supplements*, an overview. 6(2): 1-14.
- Ao, T., L. M. Macalintal, M. A. Paul, A. J. Pescatore, A. H. Cantor, M. J. Ford, B. Timmons, and K. A. Dawson. 2015. "Effects of supplementing microalgae in laying hen diets on productive performance, fatty-acid profile, and oxidative stability of eggs." *Journal of Applied Poultry Research* 24 (3): 394–400.

- Ara, J., V. Sultana, R. Qasim, and V. U. Ahmad. 2002. "Hypolipidaemic activity of seaweed from Karachi coast." *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 16 (5): 479–83.
- Avila, E., and M. Cuca. 1974. "Efectividad de la alga *Spirulina geitleri* sobre la pigmentación de la yema de huevo." *Revista Mexicana de Ciencias Pecuarias* 1 (26): 47.
- Babica, P., L. Blaha, and B. Marsalek. 2006. "Exploring the natural role of microcystins—a review of effects on photoautotrophic organisms." *Journal of Phycology*, 42 (1): 9–20.
- Barford, D. 1996. "Molecular mechanisms of the protein serine/threonine phosphatases." *Trends in biochemical Sciences* 21 (11): 407–12.
- Blair, R. 2008. *Nutrition and Feeding of Organic Poultry*. Abingdon: CABI.
- Blum, J. C., and C. Calet. 1975. "Food value of spiruline algae for growth of the broiler-type chicken." *Annales de la Nutrition et de l'Alimentation* 29:651–74.
- Cardozo, K. H., T. Guaratini, M. P. Barros, V. R. Falcão, A. P. Tonon, N. P. Lopes, S. Campos, and E. Pinto. 2007. "Metabolites from algae with economical impact." *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 146 (1–2): 60–78.
- Chadha, C., A. Naylor, and A. Tsappis. 2015. "Microalgae in layer diets create functional, DHA enriched eggs." In *26th Annual Australian Poultry Science Symposium*, Sydney, page 32.
- Chen, T., Q. Wang, J. Cui, W. Yang, Q. Shi, Z. Hua, J. Ji, and P. Shen. 2005. "Induction of apoptosis in mouse liver by microcystin-LR: a combined transcriptomic, proteomic, and simulation strategy." *Molecular and Cellular Proteomics* 4 (7): 958–74.
- Chen, J., Y. Jiang, K. Y. Ma, F. Chen, and Z. Y. Chen. 2011. "Microalgae decreases plasma cholesterol by down-regulation of intestinal NPC1L1, hepatic LDL receptor, and HMG-CoA reductase." *Journal of Agricultural and Food Chemistry* 59 (12): 6790–97.
- Codd, G. A., L. F. Morrison, and J. S. Metcalf. 2005. "Cyanobacterial toxins: risk management for health protection." *Toxicology and Applied Pharmacology* 203(3): 264–72.
- Do Carmo Bittencourt-Oliveira, M., P. Kujbida, K. H. M. Cardozo, V. M. Carvalho, A. do Nascimento Moura, P. Colepicolo, and E. Pinto. 2005. "A novel rhythm of microcystin biosynthesis is described in the cyanobacterium *Microcystis panniformis* Komárek et al." *Biochemical and Biophysical Research Communications* 326 (3): 687–94.

- Dogan, S. C., M. Baylan, Z. Erdogan, G. C. Akpinar, A. Kucukgul, and V. Duzguner. 2016. "Performance, egg quality and serum parameters of Japanese quails fed diet supplemented with *Spirulina platensis*." *Fresenius Environmental Bulletin* 25 (12a): 5857–62.
- Franchini, A., and E. Padoa. 1996. "I pigmenti nell'alimentazione del pollo da carne." *Rivista di Avicoltura* 65:22–30.
- Gružauskas, R., R. Lekavičius, A. Racevičiūtė-Stupelienė, V. Šašytė, V. Tėvelis, and G. J. Švirmickas. 2004. "Viščiukų broilerių virškinimo procesų optimizavimas simbiotiniais preparatais." *Veterinarija ir zootechnika* 28 (50): 51–56.
- Gulledge, B. M., J. B. Aggen, H. Huang, A. C. Nairn, and A. R. Chamberlin. 2002. "The microcystins and nodularins: cyclic polypeptide inhibitors of PP1 and PP2A." *Current medicinal chemistry* 9 (22): 1991–2003.
- Hammershøj, M. 1995. "Effects of dietary fish oil with natural content of carotenoids on fatty acid composition, n-3 fatty acid content, yolk colour and egg quality of hen eggs." *Archiv für Geflügelkunde*. 59(3): 189–197.
- Hencken, H. 1992. "Chemical and physiological behavior of feed carotenoids and their effects on pigmentation." *Poultry Science* 71 (4): 711–17.
- Herber, S. M., and M. E. Van Elswyk. 1996. "Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs." *Poultry Science* 75 (12): 1501–07.
- Janczyk, P., B. Halle, and W. B. Souffrant. 2009. "Microbial community composition of the crop and ceca contents of laying hens fed diets supplemented with *Chlorella vulgaris*." *Poultry Science* 88 (11): 2324–32.
- Karunajeewa, H., R. J. Hughes, M. W. McDonald, and F. S. Shenstone. 1984. "A review of factors influencing pigmentation of egg yolks." *World's Poultry Science Journal* 40 (1): 52–65.
- Klaui, H., and C. J. Bauernfeind. 1981. In *Carotenoids as Colourants and Vitamin A Precursors*, edited by J. C. Bauernfeind. Academic Press. ISBN: 9780323139779.
- Kristensen, I. 1998. Organic egg, meat, and plant production—biotechnical results from farms, edited by T. Kristensen, *Report of the Danish institute of agriculture science*, (1): 95–169.
- Lahaye, M., and D. Jegou. 1993. "Chemical and physical-chemical characteristics of dietary fibres from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev." *Journal of Applied Phycology* 5 (2): 195.

- LiuFa, W., L. XuFang, and Z. Cheng. 1997. "Carotenoids from *Alocasia* leaf meal as xanthophyll sources for broiler pigmentation." *Tropical Science (United Kingdom)*. 37: 116-122.
- Lorenz, R. T. 1999. "A review of *Spirulina* and *Haematococcus* algae meal as a carotenoid and vitamin supplement for poultry." *Spirulina Pacifica Technical Bulletin* 53:13.
- Mariey, Y. A., H. R. Samak, and M. A. Ibrahim. 2012. "Effect of using *Spirulina platensis* algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens." *Egyptian Poultry Science* 32 (1): 201-15.
- Narahari, D., P. Michealraj, A. Kirubakaran, and T. Sujatha. 2005. "Antioxidant, cholesterol reducing, immunomodulating and other health promoting properties of herbal enriched designer eggs." Paper presented at the World's Poultry Science Association's 17th European Symposium on the Quality of Poultry Meat and XIth European Symposium on the Quality of Eggs and Egg Products, Doorwerth, the Netherlands, May 23-26 2005, 194-201.
- Nikodémusz, E., P. Páskai, L. Tóth, and J. Kozák. 2010. "Effect of dietary *Spirulina* supplementation on the reproductive performance of farmed pheasants." *Poultry Industry—Technical Articles*, 1-2.
- Olaizola, M. 2003. "Commercial development of microalgal biotechnology: from the test tube to the marketplace." *Biomolecular engineering* 20 (4-6): 459-66.
- Park, J. H., S. D. Upadhaya, and I. H. Kim. 2015. "Effect of dietary marine microalgae (*Schizochytrium*) powder on egg production, blood lipid profiles, egg quality, and fatty acid composition of egg yolk in layers." *Asian-Australasian Journal of animal Sciences* 28 (3): 391.
- Peipei, L., and Z. Sumin. 2018. "Effect of *Spirulina*-Chinese herbal medicine on quality of eggs and serum biochemical indexes of egg-laying hens." *Indian Journal of Animal Research* 52 (3): 373-77.
- Ponsano, E. H. G., M. F. Pinto, M. G. Neto, and P. M. Lacava. 2004. "*Rhodocyclus gelatinosus* biomass for egg yolk pigmentation." *Journal of Applied Poultry Research* 13 (3): 421-25.
- Qureshi, M. A., J. D. Garlich, and M. T. Kidd. 1996. "Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens." *Immunopharmacology and Immunotoxicology* 18 (3): 465-76.
- Rajasha, J., B. Madhusudhan, M. M. Swamy, R. J. Rao, G. A. Ravishankar, A. Ranga Rao, and M. Karunakumar. 2009. "Effects of flaxseed and spirulina biomass in layer diet on lipid profile and quality

- characteristics of egg yolk.” *Journal of Food Science and Technology (Mysore)* 46 (6): 509–14.
- Robertson, R., F. Guiheneuf, M. Schmid, D. B. Stengel, G. Fitzgerald, P. Ross, C. and Stanton. 2013. Nutrition and Diet Research Progress, Chapter 3.
- Ross, E. and W. Dominy. 1990. “The Nutritional Value of Dehydrated, Blue-Green Algae (*Spirulina platensis*) for Poultry.” *Poultry Science* 69 (5): 794–800. <https://doi.org/10.3382/ps.0690794>.
- Runnegar, M., N. Berndt, S. M. Kong, E. Y. Lee, and L. F. Zhang. 1995. “In vivo and in vitro binding of microcystin to protein phosphatase 1 and 2A.” *Biochemical and Biophysical Research Communications* 216 (1): 162–69.
- Saxena, P. N., M. R. Ahmad, R. Shyam, and D. V. Amla. 1983. “Cultivation of *Spirulina* in sewage for poultry feed.” *Experientia* 39 (10): 1077–83.
- Selim, S., E. Hussein, and R. Abou-Elkhair. 2018. “Effect of *Spirulina platensis* as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens.” *European Poultry Science* 82: 1-13.
- Sirri, F., N. Iaffaldano, G. Minelli, A. Meluzzi, M. P. Rosato. and A. Franchini. 2007. Comparative pigmentation efficiency of high dietary levels of apo-ester and marigold extract on quality traits of whole liquid egg of two strains of laying hens. *Journal of Applied Poultry Research*, 16(3): 429-437.
- Soares, R. M., V. F. Magalhães, and S. M. Azevedo. 2004. “Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (Cichlidae) under laboratory conditions.” *Aquatic Toxicology* 70 (1): 1–10.
- Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert. 2006. “Commercial applications of microalgae.” *Journal of Bioscience and Bioengineering* 101 (2): 87–96.
- Sulonen, J., R. Kärenlampi, U. Holma, and M. L. Hänninen. 2007. “Campylobacter in Finnish organic laying hens in autumn 2003 and spring 2004.” *Poultry Science* 86 (6): 1223–28.
- Takashi, S. 2003. “Effect of administration of *Spirulina* on egg quality and egg components.” *Animal Husbandry* 57:191–95.
- Tokusoglu, O., and M. K. Unal. 2003. “Biomass Nutrient Profiles of Three Microalgae: *Platensis*, *Chlorella Vulgaris* and *Isochrisisgalbana*.” *Journal of Food Science* 68 (4): 1144.

- Toyomizu, M., K. Sato, H. Taroda, T. Kato, and Y. Akiba. 2001. "Effects of dietary Spirulina on meat colour in muscle of broiler chickens." *British Poultry Science* 42 (2): 197–202.
- Venkataraman, L. V., T. Somasekaran, and E. W. Becker. 1994. "Replacement value of blue-green alga (*Spirulina platensis*) for fishmeal and a vitamin-mineral premix for broiler chicks." *British Poultry Science* 35 (3): 373–81.
- Werman, M. J., A. Sukenik, and S. Mokady. 2003. "Effects of the marine unicellular alga *Nannochloropsis* sp. to reduce the plasma and liver cholesterol levels in male rats fed on diets with cholesterol." *Bioscience, Biotechnology, and Biochemistry* 67 (10): 2266–68.
- Yang, Y. X., Y. J. Kim, Z. Jin, J. D. Lohakare, C. H. Kim, S. H. Ohh, S. H. Lee, J. Y. Choi, and B. J. Chae. 2006. "Effects of dietary supplementation of astaxanthin on production performance, egg quality in layers and meat quality in finishing pigs." *Asian Australasian Journal of Animal Sciences* 19 (7): 1019.
- Zahroojian, N., H. Moravej, and M. Shivazad. 2011. "Comparison of marine algae (*Spirulina platensis*) and synthetic pigment in enhancing egg yolk colour of laying hens." *British Poultry Science* 52 (5): 584–88.
- Zahroojian, N., H. Moravej, and M. Shivazad. 2013. "Effects of dietary marine algae (*Spirulina platensis*) on egg quality and production performance of laying hens." *Journal of Agricultural Science and Technology* 15:1353–60.

CHAPTER EIGHT

SPIRULINA IN BREEDER NUTRITION

“It's OK to have your eggs in one basket as long as you control what happens to that basket.”
—Elon Musk

8.1 Introduction

As mentioned previously, *Spirulina* is an edible marine ingredient that has high nutritional value with the potential of being used in organic feeding systems. It is well documented that feed quality of parent stock has a critical effect on the progeny health state and growth; this is because chick embryos rely on nutrients transferred from parents and stored in the egg to support normal growth and development (Surai 2000). *Spirulina* has significant antioxidant and free-radical scavenging properties because of its nutrient composition (Bhat and Madyastha 2000). It is well known that *Spirulina* contains high amounts of essential fatty acids (gamma linolenic acid, linoleic acid, stearidonic acid, EPA, docoshexaenoid acid and arachidonic acid), minerals—especially iron, and vitamins—especially vitamin B₁₂ and provitamin-A (i.e., β -carotene) (Alam et al. 2013). There are very limited reports about the effect of *Spirulina* on poultry breeders' performance; thus, this chapter will mainly discuss the effect of *Spirulina* nutrients on chick quality and parent stock reproductive traits.

8.2 Chick quality

It is interesting to note that several factors may affect the chick quality, including hen physiological status, hen nutrition, diet formulation, and farm and hatchery management (Chang, Halley, and Silva 2016). The bird egg is a semi-closed system in which chicken embryonic development occurs. In this system only exchange of gas and water happens. The success of embryonic development depends on egg composition and conditions of egg incubation. Therefore, a great body of evidence indicates that avian maternal nutrition is the major determinant of the health and

development of the progeny. All of the nutrients necessary for the development of the embryo are accumulated within the egg yolk and white (Speake, Murray, and Noble 1998; Surai, Sparks, and Noble 1999).

Among different nutrients in the maternal diet which may affect chick embryos' development and their viability in the early post-hatch life, natural antioxidants have been suggested to play a central role. It was documented that development of chick embryos has been associated with an accumulation of polyunsaturated fatty acids in tissue lipids (Speake, Murray, and Noble 1998), making embryos susceptible to lipid peroxidation (Surai 1999). At hatching time, oxidative stress may occur at a high rate due to long-chain PUFA accretion in tissues, exposure to atmospheric oxygen, onset of pulmonary respiration and sudden increase in the rate of oxidative metabolism (Speake, Murray, and Noble 1998). It was reported that the high amounts of endogenous antioxidants within the egg and embryonic tissues can help as a major adaptive mechanism for preventing oxidative stress at hatching time (Surai, Fisinin, and Karadas 2016). The chick's antioxidant system includes enzymes (e.g., super-oxide dismutase, glutathione peroxidase, glutathione reductase and catalase) and glutathione, vitamin A and E, and carotenoids (Surai, Sparks, and Noble 1999). Antioxidant distribution in the tissues of a newly hatched chick is shown in Table 8-1.

Table 8-1: Antioxidant distribution in the tissues of a newly hatched chick. (Surai, Fisinin, and Karadas 2016)

	Vitamin E, mg/g tissue	Vitamin A, mg/g tissue	Lutein + zeaxanthi n, mg/g tissue	Ascorbic acid, mg/g tissue	Glutat hione, nM/mg protein
Liver	678.16	10.34	30.76	150.95	43.31
Brain	6.62	0.11	nd	839.14	40.19
Kidney	19.48	1.26	2.33	130.60	54.71
Heart	24.25	0.35	3.32	59.01	32.94
Lung	21.03	<0.05	2.65	124.63	23.57
Thigh muscle	14.80	0.14	1.95	57.92	21.26
Yolk sac membrane	220.65	3.04	18.55	nd	4.19

nd = not detected

Spirulina platensis has antioxidant characteristics that relate to the presence of β -carotene, phycocyanin, vitamin C, vitamin E, selenium and manganese (Stivala et al. 1996; El-Demerdash 2001; Mazo, Gmoshinskiĭ, and Zilova 2004; Mueller and Boehm 2011). Previous studies showed that antioxidants from natural sources had higher bioavailability and higher protective efficacy than synthetic antioxidants (Gey 1998).

It was documented that phycocyanin prevented peroxy radical-induced lipid peroxidation (Bhat and Madyastha 2001) and it may chelate heavy metals (Plazinski 2013). Previous research on *Spirulina* showed that the carotenoid fraction of the algae was 346 mg/100 g and consisted of 52% β -carotene, 21% zeaxanthin, 10% echinenone, 6% β -cryptoxanthin, 5% 3'-hydroxyechinenone and 7% unidentified carotenoids (Miki et al. 1986). It was documented that canthaxanthin supplementation of breeder diets led to lower embryo mortality in the first 48 hours of incubation and in the last week of incubation, and thus lowered total embryo mortality (Rosa et al. 2012). In a study on grey partridge (*Perdix perdix* L.), supplementation of diets with 2.7 mg/kg of β -carotene produced higher concentration levels of lysozyme, an enzyme with antibacterial activity, in the albumen of eggs laid by females with a higher amount of β -carotene. In addition, these eggs had higher hatching rates (Cucco et al. 2007).

Breeder diets and egg PUFA composition can regulate antioxidant activity in newly hatched chicks. There are two sources of ω -3 fats in poultry diets. ALA is derived from plant-based oils or oil seeds while long-chain PUFA (e.g., EPA, DPA, DHA) are derived from marine oil or algae (Cherian 2015). Supplementation of breeders' diets with n-3 FA, EPA and DHA incorporated in the yolk and transferred via the residual yolk to become available for the developing embryo may result in elevated EPA and DHA levels in the liver of the offspring at hatch (Koppenol et al. 2014a). This is important because ω -3 FAs have the potential of increasing glutathione reductase activity and decreasing lipid peroxide and ROS (Wu, Ying, and Gomez-Pinilla 2004).

On the other hand, the hatchability of fertile eggs in broiler breeders fed with diets that contained 5 and 10 ppm aflatoxin (AF) for four decreased from 95.0% (control group) to 68.9% and 48.5%, respectively (Howarth and Wyatt 1976). Also, mycotoxin residues in eggs have the potential to significantly affect progeny health. In a study, Manafi et al. (2012) evaluated *Spirulina* as a mycotoxin binder on broiler breeders induced with AF. They reported that *Spirulina* (0.1%) could decrease embryonic mortality. This may be due to the mycotoxin-binding effect of *Spirulina*

and subsequent prevention of hepatic damage. Inclusion of *Spirulina* at the level of 0.05% could partially compensate for the adverse effects of 300-ppm AF on growth rate and lymphoid organ weight of broiler chickens (Raju et al. 2005). It was also reported that *Spirulina* may be helpful for reducing the tissue burden of arsenic in ducks (Islam et al. 2009).

8.3 Breeder reproduction

Reproduction and hatchability are critical parts of the poultry industry, especially the hatching rate of settable eggs and male and female reproductive performance (Khatibjoo et al. 2018). The main factors affecting performance of commercial broiler breeders are shown in Figure 8-1.

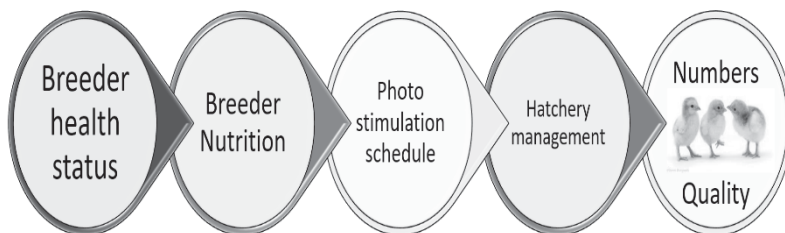


Figure 8-1: Factors affecting performance of commercial broiler breeders. (Designed by authors)

The oxidative process normally occurs in the body; whenever there is an imbalance between antioxidants and free radicals, normal physiological activity will be impeded (Rahal et al. 2014). Oxidative stress is one of the major causes of decreasing reproductive performance of both male and female breeders, and considering antioxidant levels in breeders' diets is critical to reaching the actual potential of the birds, especially when the flock face oxidative stressors such as obesity, high temperature and humidity during hot summer days (Surai 2000; Zaghari, Sedaghat, and Shivazad 2013). Birds sacrifice oxidative protection for reproduction (Wiersma et al. 2004). The positive effect of dietary *Chlorella* algae in laying hens was reported by Halle et al. (2009), who found that layers fed a diet supplemented with algae had higher egg hatchability, yolk color score and shell weight, but alga did not have any effect on egg performance and nitrogen balance. Zaghari, Sedaghat, and Shivazad (2013) reported that broiler breeder hens fed a diet fortified with four

times more than the recommended vitamin E requirement had a significantly higher egg production (Figure 8-2) and higher fertility.

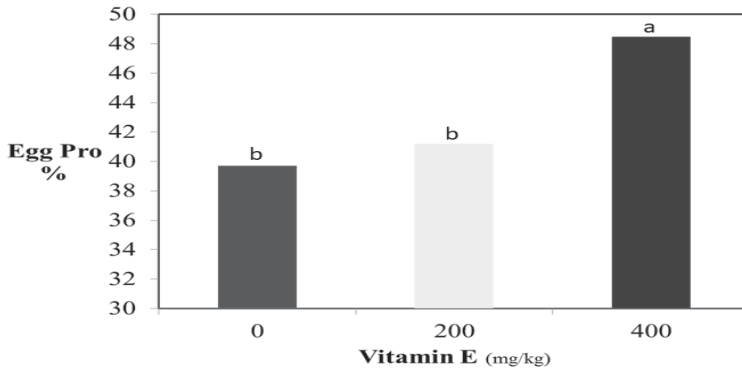


Figure 8-2: Effect of vitamin E (0, 200, and 400 mg α -tocopherol acetate/kg diet) on broiler breeder egg production. (Zaghari, Sedaghat, and Shivazad 2013)



Figure 8-3: A healthy commercial broiler breeder flock at 50 weeks of age. (Photograph by authors)

In addition to vitamin E and selenium (Se), it was documented that dietary n-3 and n-6 FAs have a great impact on avian metabolism, abdominal fat deposition and hatchability (Cherian and Sim 1997), fatty acid profile of egg yolk (Cherian 2008; Koppenol et al. 2014a; Koppenol et al. 2014b) and lipid metabolism in the chick (Ajuyah et al. 2003; Koppenol et al. 2015). It has been made clear that using *Spirulina* in broiler breeder flocks improved the fertility and hatchability rates (Inborr 1998). A healthy commercial broiler breeder flock at 50 weeks of age is shown in Figure 8-3.

8.4 Female breeder reproduction

It was demonstrated that there was a complex antioxidant system in the utero-vaginal portion of the fowl oviduct (Breque and Brillard 2002). In particular, GSH-Px activity in the utero-vaginal junction was 12-fold higher than in the liver. Hens fed *Spirulina*-containing diets had higher productive and reproductive performance (Ross and Dominy 1990; Nikodémusz et al. 2010). Using dietary *Spirulina* improved egg fertility from 87% to over 96% (Ross and Dominy 1990).

López, Gonzalez, and Sierra (2017) evaluated the effects of the dietary supplementation of a natural source of DHA (C22:6n-3 from *Schizochytrium* sp. algae, SA) on laying hen performance parameters and egg yolk fatty acid profile. They concluded that the dietary supplementation of 1.0% SA is able to increase egg yolk DHA content and decrease the n-6/n-3 ratio without affecting performance parameters.

Mobarez et al. (2018) studied the effect of *Spirulina platensis* algae (or turmeric powder) on golden montazah hens' reproductive performance. They found that supplementing hen diets with 2 or 3 g *Spirulina*/kg diet increased the hatchability percentage compared with the control group (82.8% and 84.2% vs. 80.6%). Effects of dietary *Spirulina platensis* or *Curcuma* powder supplementation on fertility and hatchability percentages in golden montazah hens are shown in Table 8-2.

Adding dietary *Spirulina* to the diets from 29 to 40 weeks of age may optimize the productive traits, improve immunity responses, increase interferon protein and support the birds against several enveloped viruses, including Newcastle disease virus (NDV) and influenza virus (Mobarez et al. 2018). Previous studies showed that birds fed a diet containing *Spirulina* had higher percentages of fertility and hatchability. This may be due to the high contents of tocopherols in *Spirulina*. El-Khimsawy (1985)

reported that tocopherols played a vital role in fertility and hatchability of poultry.

Table 8-2: Effects of *Spirulina platensis* or Turmeric powder supplementation on fertility and hatchability percentages of golden montazah hens at 40 weeks of age. (Mobarez et al. 2018)

	Control	<i>Spirulina</i>		Turmeric		SEM	Sig.
		2 g/kg	3 g/kg	4 g/kg	6 g/kg		
Fertility (%)	89.5	90.4	94.4	90.1	89.9	±0.294	**
Hatchability (%)	80.6	82.8	84.2	80.5	81.2	±0.331	**

Means having different letters at the same row differ significantly. * = (P<0.05), ** = (P<0.01) and NS = Not significant. SE: Standard error. Control (without supplementation).

In another study, Mariey, Samak, and Ibrahim (2012) used two local strains of laying hens (Sinai and Gimmizah). The hens were fed on experimental diets containing 4 levels of *Spirulina* powder (0, 0.10, 0.15 or 0.20%) from 28 to 52 weeks of age. They found that fertility and hatchability of eggs produced by birds fed diets containing *Spirulina* were significantly higher. The effects of different levels of dietary *Spirulina* and local hen strains on egg weight, egg weight loss, fertility, hatchability and chicks' weight of Sinai and Gimmizah laying hens are shown in Table 8-3.

It was revealed that dietary supplementation of vitamin E and organic Se supports the fertility of aging female breeder (Bréque, Surai, and Brillard 2003). *Spirulina* contains antioxidant substances that can inhibit elevation of testicular nitric oxide (NO) and tumor necrosis factor (TNF- α), thus lowering detrimental effects of oxidative stress (Bashandy et al. 2016). Supplementation of ingredients containing antioxidant nutrients such as vitamins E and C and carotenoids helps the enzymatic defense system to control the damage caused by free radicals in cells (Rosa et al. 2012). Surai et al. (2003) showed that including the carotenoid canthaxanthin in the maternal diet reduced the susceptibility of tissues of 1-day-old chicks to lipid peroxidation. Carotenoids are lipid-soluble pigments that may be synthesized by plants and photosynthetic microorganisms (Goodwin 1986; Olson 1996). These molecules can act as free-radical removal systems and physical attenuators, absorbing and dissipating the excess energy of highly reactive chemical species (Böhm et al. 1997).

Table 8-3: The effects of different levels of dietary *Spirulina* and local hen strains on egg weight, egg weight loss, fertility, hatchability and chicks' weight of Sinai and Gimmizah laying hens. (Mariey, Samak, and Ibrahim 2002)

Main effects	Egg weight (g)	Egg weight loss (%)	Egg fertility (%)	Egg hatchability (%)	Chick weight (g)
<i>Spirulina</i>					
level (%)					
Control	52.01	15.57	90.87 ^a	89.81 ^a	31.24
0.1	52.68	15.46	94.62 ^b	93.17 ^b	31.26
0.15	53.07	15.45	95.68 ^b	95.10 ^b	32.13
0.2	53.35	15.34	96.58 ^{bc}	95.75 ^b	32.22
SEM	0.423	0.106	0.396	0.976	0.407
Significance	NS	NS	*	*	NS
<i>Strain</i>					
Sinai	52.05	15.49	94.90	93.97	30.94 ^a
Gimmizah	53.05	15.42	93.97	93.00	32.73 ^b
SEM	0.299	0.075	0.396	0.684	0.284
Significance	NS	NS	NS	NS	*

a-c: Means with different superscripts within the same column for each variable are significantly different at $P \leq 0.05$. NS: not significant; *: significant at $P \leq 0.05$. 1: Standard error of the means.

Also, carotenoids can promote cell differentiation (Zhang, Cooney, and Bertram 1991), regulate cell proliferation (Krinsky 1991) and increase immunity responses (Bendich 1991). Koutsos et al. (2003) found that the carotenoid content in the maternal diet influenced the concentration of these compounds in progeny tissues until 28 days of age. In addition, carotenoids content of broiler breeder diets were the main factor affecting the carotenoid concentration in livers of chicks during the first week of life (Karadas et al. 2005). Regarding the important roles of carotenoids as antioxidants and immune-stimulating substances after hatching chicks, addition of carotenoids in the maternal diet may be a strategy to improve broiler breeders and their progeny performances. Souza et al. (2008) reported that supplementation of 6 mg canthaxanthin/kg of broiler breeders' diets decreased numbers of infertile eggs and embryonic mortality and improved the hatchability of the progeny.

8.5 Male breeder reproduction

Chicken spermatozoa are unique because of their structure and chemical composition. Avian spermatozoa are unique cells which have a membrane consisting of high concentrations of polyunsaturated fatty acids (Partyka, Łukaszewicz, and Nizański 2012). In fact, the high PUFA proportion is critical in order to maintain specific membrane properties (fluidity, flexibility, etc.). However, spermatozoa are very susceptible to lipid peroxidation, and the antioxidant defense system has a key role in maintaining semen quality (Surai, Sparks, and Speake 2006). Figure 8-4 shows the normal and abnormal avian sperm. Fully integrated and disintegrated membranes are clear in normal and abnormal cells, respectively.

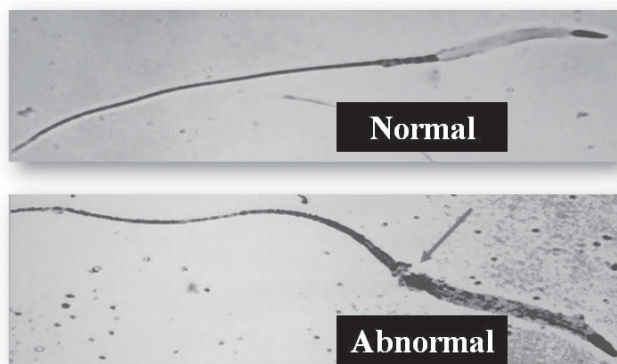


Figure 8-4: Normal and abnormal avian sperm membranes.

Oxidative stress is an important and probable cause of idiopathic male infertility (Agarwal et al. 2014). Sperm contains a large amount of unsaturated fatty acid; thus, it is susceptible to oxidation. Because of their high levels of polyunsaturated fatty acids, avian sperms are also very sensitive to reactive oxygen species, causing male infertility (Surai et al. 1997). Therefore, an increased antioxidant status in semen or spermatozoa is a prerequisite for the prevention of male infertility. Ajafar et al. (2018) observed significant reduction in the susceptibility of the semen to lipid peroxidation and increase in sperm penetration to the yolk perivitelline layer and plasma membrane functionality, sperm abnormality, semen, malondialdehyde (MDA) and blood testosterone concentrations ($Lsmean \pm SE$) of roosters fed different levels of α -tocopherol acetate in

heavy- and standard-weight roosters by adding α tocopherol acetate to the rooster's diet (Table 8-4).

Oxidative protection of birds' sperm and semen environments for improving male reproduction is crucial. Achieving a higher degree of protection requires appropriate tools. Abadjieva and Kistanova (2011) indicated that even a very small amount of biomass of micro-aquatic plants strengthens the immune system of animals, thereby stimulating their growth and improving their reproductive properties. Effects of dietary algae-rich and Economas E on semen quality of broiler breeder roosters were investigated by Macalintal et al. (2016). Results showed that the percentage of abnormal sperm cells was higher for the non-supplemented birds, compared with those fed the supplemented diet with the combination of algae-rich + Economas E. The number of normal cells, as a percentage of viable cells, was significantly higher for algae-rich + Economas-E-fed birds. These data suggest that a diet containing algae-rich + Economas E can be used to improve the semen quality of broiler breeder roosters. Logically, *Spirulina* is one of the important candidates for research in the future as an antioxidant additive in diet or semen for artificial insemination.

Table 0-4: Plasma membrane functionality, sperm abnormality, semen, MDA and blood testosterone concentrations (mean±SE) of roosters fed different levels of α -tocopherol acetate in both heavy- and standard-weight roosters. (Ajafar et al. 2018)

Weight category	α -TA doses	Traits				
		Semen concentration ($\times 10^9$ spermatozoa/mL)	Membrane functionality (%)	Sperm abnormality (%)	MDA (nM/mL)	Testosterone (ng/mL)
SW	0	3.19 ^a \pm 0.01	43.66 ^c \pm 0.14	9.21 ^a \pm 0.13	2.36 ^a \pm 0.12	1.58 ^{bc} \pm 0.04
	100	4.14 ^c \pm 0.01	48.66 ^b \pm 0.85	3.66 ^c \pm 0.14	1.55 ^a \pm 0.12	1.56 ^c \pm 0.02
	200	4.29 ^d \pm 0.02	48.36 ^b \pm 0.46	2.50 ^d \pm 0.10	1.29 ^b \pm 0.11	1.62 ^{bc} \pm 0.01
	300	4.60 ^b \pm 0.01	50.11 ^{ab} \pm 0.13	1.90 ^e \pm 0.10	0.91 ^{cd} \pm 0.08	1.66 ^b \pm 0.01
	400	4.67 ^a \pm 0.01	52.56 ^a \pm 0.24	1.78 ^f \pm 0.04	0.66 ^{cd} \pm 0.04	1.72 ^a \pm 0.01
HW	0	3.18 ^c \pm 0.01	48.01 ^d \pm 0.30	9.58 ^a \pm 0.20	2.72 ^a \pm 0.12	1.46 ^d \pm 0.02
	100	4.28 ^d \pm 0.03	49.98 ^{cd} \pm 0.31	4.83 ^b \pm 0.26	2.02 ^a \pm 0.08	1.57 ^c \pm 0.01
	200	4.41 ^c \pm 0.03	51.73 ^{bc} \pm 0.13	3.23 ^c \pm 0.23	1.44 ^b \pm 0.06	1.60 ^{bc} \pm 0.02
	300	4.62 ^b \pm 0.02	52.83 ^{ab} \pm 0.25	2.53 ^d \pm 0.16	1.01 ^c \pm 0.05	1.65 ^b \pm 0.01
	400	4.67 ^a \pm 0.01	55.23 ^a \pm 0.20	2.38 ^{ed} \pm 0.19	0.65 ^d \pm 0.05	1.77 ^a \pm 0.02
Pvalue	BW	<0.01	<0.01	<0.01	<0.01	0.07
	α -TA	<0.01	<0.01	<0.01	<0.01	<0.01
	BW \times α -TA	<0.01	0.02	<0.01	<0.01	<0.01

SW: standard weight, HW: heavy weight, BW \times α -TA: body weight and α -tocopherol acetate interaction. SE: standard error. Values with different superscripts (a-d) within each column are significantly ($P < 0.05$) different.

Spermatogenic cells with oxidatively damaged DNA undergo apoptotic elimination through p53-dependent and independent mechanisms, which can lead to infertility. In addition, researchers have reported that disorders such as poor fertilization, birth defects, poor embryonic development and even childhood cancer are correlated with high susceptibility of spermatozoa to oxidative disturbance (Fujii and Imai 2014; Wahab et al. 2015). Thus, antioxidants have the ability to improve various processes of male reproductive function such as spermatogenesis and steroidogenesis (Elumalai et al. 2009; Murugesan et al. 2007). Also, it was reported that *Spirulina platensis* increased levels of T₃ and T₄ hormones in oxidative stress situations (Bashandy et al. 2016), which have an effect on spermatogenesis (Ai et al. 2007). The n-3 and n-6 FAs and their ratio affect the avian semen characteristics, and nutritionists may notice dietary manipulation of sperm FA profiles in order to improve male breeders' fertility, which increases economic profits directly (Khatibjoo et al. 2018). The DHA can maintain fluidity and flexibility of spermatozoa membranes, and there is positive correlation between fertility and concentration of arachidonic acid (ARA) and DHA in male broiler breeders' sperm (Cerolini et al. 1997). Previous studies showed that n-3 FAs had no effect on avian semen volume, motile spermatozoa and spermatozoa concentration (Cerolini et al. 1997), but they had a negative effect on sperm concentration (Cerolini et al. 2001). On the other hand, n-6 FA-rich diets had positive effects on semen volume and total spermatozoa number (Surai 2000) but a negative effect on spermatozoa concentration (Cerolini et al. 2003), and the contradictory effects of n-3 and n-6 FA on male broiler breeder performance and sexual ability may be due to their competition (Khatibjoo et al. 2018).

In conclusion, phytochemical components of algae had the potential for improving the androgenic effect of testes and enhancing libido and gametogenesis, resulting in increased fertility and hatchability of commercial broiler breeder males (Figure 8-5).



Figure 8-5: Mating behavior of broiler breeder male enhanced by phytochemicals. (Durape 2007)

References

- Abadjieva, D., and E. Kistanova. 2011. Opportunities to stimulate reproductive function in female animals. *Niva Povoljaq. Zootech.* 4 (21): 71–75.
- Agarwal, A., G. Virk, C. Ong, and S. S. du Plessis. 2014. “Effect of oxidative stress on male reproduction.” *The World Journal of Men’s Health* 32 (1): 1–17.
- Ai, J., A. Zarifkar, M. A. Takhshid, J. Alavi, and M. Moradzadeh. 2007. “The effect of thyroid activity on adult rat spermatogenesis.” *Iranian Journal of Veterinary Research* 8 (2): 155–60.
- Ajafar, M., M. Zaghari, M. Zhandi, and L. Lotfi. 2018. “Effect of high dietary levels of α -tocopherol acetate on immune response of light and heavy weight male broiler breeders.” *Comparative Clinical Pathology*, 27 (5):1281–1288.
- Ajuyah, A. O., Y. Wang, H. Sunwoo, G. Cherian, and J. S. Sim. 2003. “Maternal Diet with Diverse Omega-6/Omega-3 Ratio Affects the Brain Docosahexaenoic Acid Content of Growing Chickens.” *Neonatology* 84 (1): 45–52.

- Alam, M. A., N. Haider, S. Ahmed, M. T. Alam, A. Azeez, and A. Perveen. 2013. "Tahlab (Spirulina) and few other medicinal plants having anti-oxidant and immunomodulatory properties described in Unani medicine—A review." *International Journal of Pharmaceutical Sciences and Research* 4 (11): 4158–4164.
- Bashandy, S. A., S. A. El Awdan, H. Ebaid, and I. M. Alhazza. 2016. "Antioxidant potential of *Spirulina platensis* mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats." *Oxidative Medicine and Cellular Longevity* 2016, Article ID 7174351.
- Bendich, A. 1991. "Beta-carotene and the immune response." *Proceedings of the Nutrition Society* 50:263–74.
- Bhat, V. B., and K. M. Madyastha. 2000. "C-phycocyanin: a potent peroxy radical scavenger in vivo and in vitro." *Biochemical and Biophysical Research Communications* 275 (1): 20–25.
- Bhat, V. B., and K. M. Madyastha. 2001. "Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA." *Biochemical and Biophysical Research Communications* 285 (2): 262–66.
- Böhm, F., R. Edge, E. J. Land, D. J. McGarvey, and T. G. Truscott. 1997. "Carotenoids enhance vitamin E antioxidant efficiency." *Journal of the American Chemical Society* 119 (3): 621–22.
- Breque, C., and J. P. Brillard. 2002. "Sperm storage in the avian oviduct: baselines for a complex antioxidant system in the sperm storage tubules." *Archiv für Geflügelkunde* 66:83.
- Bréque, C., P. Surai, and J. P. Brillard. 2003. "Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro." *Molecular Reproduction and Development: Incorporating Gamete Research* 66 (3): 314–23.
- Cerolini, S., K. A. Kelso, R. C. Noble, B. K. Speake, F. Pizzi, and L. G. Cavalchini. 1997. "Relationship between spermatozoan lipid composition and fertility during aging of chickens." *Biology of Reproduction* 57 (5): 976–80.
- Cerolini, S., A. Maldjian, F. Pizzi, and T. M. Gliozzi. 2001. "Changes in sperm quality and lipid composition during cryopreservation of boar semen." *Reproduction* 121 (3): 395–401.
- Cerolini, S., F. Pizzi, T. Gliozzi, A. Maldjian, L. Zaniboni, and L. Parodi. 2003. "Lipid manipulation of chicken semen by dietary means and its relation to fertility: a review." *World's Poultry Science Journal* 59 (1): 65–75.

- Cherian, G. 2008. "Egg quality and yolk polyunsaturated fatty acid status in relation to broiler breeder hen age and dietary n-3 oils." *Poultry Science* 87 (6): 1131–37.
- Cherian, G. 2015. "Nutrition and metabolism in poultry: role of lipids in early diet." *Journal of animal Science and Biotechnology* 6 (1): 28.
- Cherian, G. E. E. T. H. A., and J. S. Sim. 1997. "Egg yolk polyunsaturated fatty acids and vitamin E content alters the tocopherol status of hatched chicks." *Poultry Science* 76 (12): 1753–59.
- Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2007. "Effects of β -carotene on adult immune condition and antibacterial activity in the eggs of the Grey Partridge, *Perdix perdix*." *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 147 (4): 1038–46.
- Durape, N. M. 2007. "Phytochemicals improve semen quality and fertility." *Mortality* 3:2–4b.
- El-Demerdash, F. M. 2001. "Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver, and blood of rats." *Journal of Environmental Science and Health, Part B* 36 (4): 489–99.
- El-Khimsawy, K. A. 1985. "Feed additive in poultry feeds." [In Arabic.] *Dar. El-Hwda for publication*. Cairo. Egypt.
- Elumalai, P., G. Krishnamoorthy, K. Selvakumar, R. Arunkumar, P. Venkataraman, and J. Arunakaran. 2009. "Studies on the protective role of lycopene against polychlorinated biphenyls (Aroclor 1254)-induced changes in StAR protein and cytochrome P450 scc enzyme expression on Leydig cells of adult rats." *Reproductive Toxicology* 27 (1): 41–45.
- Fujii, J., and H. Imai. 2014. "Redox reactions in mammalian spermatogenesis and the potential targets of reactive oxygen species under oxidative stress." *Spermatogenesis* 4 (2): e979108.
- Gey, K. F. 1998. "Vitamins E plus C and interacting conutrients required for optimal health." *Biofactors* 7 (1, 2): 113–74.
- Goodwin, T. W. 1986. "Metabolism, nutrition, and function of carotenoids." *Annual review of nutrition* 6 (1): 273–97.
- Halle, I., P. Janczyk, G. Freyer, and W. B. Souffrant. 2009. "Effect of microalgae *Chlorella vulgaris* on laying hen performance." *Archiva Zootechnica* 12 (2): 5–13.
- Hashimoto, M., Y. Tanabe, Y. Fujii, T. Kikuta, H. Shibata, and O. Shido. 2005. "Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid β -infused rats." *The Journal of Nutrition* 135 (3): 549–55.

- Howarth, B., and R. D. Wyatt. 1976. "Effect of dietary aflatoxin on fertility, hatchability, and progeny performance of broiler breeder hens." *Applied and Environmental Microbiology* 31 (5): 680–84.
- Inbarr, J. 1998. "Haematococcus, the poultry pigmentor." *Feed Mix*. 6 (2): 31–34.
- Islam, M. S., M. A. Awal, M. Mostofa, F. Begum, A. Khair, and M. Myenuddin. 2009. "Effect of Spirulina on biochemical parameters and reduction of tissue arsenic concentration in arsenic induced toxicities in ducks." *International Journal of Poultry Science* 8 (1): 69–74.
- Karadas, F., A. C. Pappas, P. F. Surai, and B. K. Speake. 2005. "Embryonic development within carotenoid-enriched eggs influences the post-hatch carotenoid status of the chicken." *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 141 (2): 244–51.
- Khatibjoo, A., H. Kermanshahi, A. Golian, and M. Zaghari. 2018. "The effect of n-6/n-3 fatty acid ratios on broiler breeder performance, hatchability, fatty acid profile and reproduction." *Journal of Animal Physiology and Animal Nutrition*. 102(4): 986-998.
- Koppenol, A., J. Buyse, N. Everaert, E. Willems, Y. Wang, L. Franssens, and E. Delezie. 2014a. "Transition of maternal dietary n-3 fatty acids from the yolk to the liver of broiler breeder progeny via the residual yolk sac." *Poultry Science* 94 (1): 43–52.
- Koppenol, A., E. Delezie, J. Aerts, E. Willems, Y. Wang, L. Franssens, N. Everaert, and J. Buyse. 2014b. "Effect of the ratio of dietary n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid on broiler breeder performance, egg quality, and yolk fatty acid composition at different breeder ages." *Poultry Science* 93 (3): 564–73.
- Koppenol, A., E. Delezie, Y. Wang, L. Franssens, E. Willems, B. Ampe, J. Buyse, and N. Everaert. 2015. "Effects of maternal dietary EPA and DHA supplementation and breeder age on embryonic and post-hatch performance of broiler offspring: Age and n-3 pufa affect embryonic and post-hatch performance." *Journal of Animal Physiology and Animal Nutrition* 99:36–47.
- Koutsos, E. A., A. J. Clifford, C. C. Calvert, and K. C. Klasing. 2003. "Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (*Gallus gallus domesticus*)." *The Journal of Nutrition* 133 (4): 1132–38.
- Krinsky, N. I. 1991. "Effects of carotenoids in cellular and animal systems." *The American Journal of Clinical Nutrition* 53 (1): 238S–246S.

- López, L. B. B., G. D. Gonzalez, and A. C. Sierra. 2017. "Effects of the dietary supplementation with a Schizochytrium sp. algae on laying hen performance and egg yolk fatty acid composition." International Poultry Scientific Forum Georgia World Congress Center, Atlanta, GA, January 30–31, 2017.
- Macalintal, L., T. Ao, A. Pescatore, A. Cantor, P. Glenney, M. Ford, and K. Dawson. 2016. "Maternal dietary polyunsaturated fatty acids and antioxidant compound affect levels of trace minerals in eggs and docosahexaenoic acid content in progeny tissues." 2016 Southern Poultry Scientific Meeting. University of Kentucky Department of Animal and Food Sciences. <https://afs.ca.uky.edu/poultry/use-algae-products-poultry>
- Manafi, M., H. N. N. Murthy, K. Mohan, and H. D. Narayana Swamy. 2012. "Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced with Aflatoxin B: Effects on Fertility, Hatchability, Embryonic Mortality, Residues in Egg and Semen Quality." *Global Veterinaria* 8 (6): 642–48.
- Mariey, Y. A., H. R. Samak, and M. A. Ibrahim. 2012. "Effect of using Spirulina platensis algae as a feed additive for poultry diets: 1–productive and reproductive performances of local laying hens." *Egyptian Poultry Science* 32 (1): 201–15.
- Mazo, V. K., I. V. Gmshinskiĭ, and I. S. Zilova. 2004. "Microalgae Spirulina in human nutrition." *Voprosy pitaniia* 73 (1): 45–53.
- Miki M., K. Yamaguchi, and S. Konosu. 1986. "Carotenoid composition of Spirulina maxima." *Nippon Suisan Gakkaishi* 52:1225–27.
- Mobarez S. M., A. M. Rizk, A. M. Abdelatif, and Osama A. El-Sayed. 2018. "Effect of supplementing diet with *Spirulina platensis* algae or turmeric on productive and reproductive performance of golden montazah layers." *Egyptian Poultry Science Journal* 38 (I): 109–25.
- Mueller, L., and V. Boehm. 2011. "Antioxidant activity of β -carotene compounds in different in vitro assays." *Molecules* 16 (2): 1055–69.
- Murugesan, P., T. Muthusamy, K. Balasubramanian, and J. Arunakaran. 2007. "Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells." *Toxicology* 232 (3): 170–82.
- Nikodémusz, E., P. Páskai, L. Tóth, and J. Kozák. 2010. "Effect of dietary Spirulina supplementation on the reproductive performance of farmed pheasants." *Poultry Industry—Technical Articles* 1–2.
- Olson, J. A. 1996. "Biochemistry of vitamin A and carotenoids." In *Vitamin A Deficiency: Health, Survival, and Vision*, edited by A.

- Sommer and K. P. West, 221–50. New York, NY: Oxford University Press.
- Partyka, A., E. Łukaszewicz, and W. Nizański. 2012. “Lipid peroxidation and antioxidant enzymes activity in avian semen.” *Animal reproduction Science* 134 (3–4): 184–90.
- Plazinski, W. 2013. “Binding of heavy metals by algal biosorbents. Theoretical models of kinetics, equilibria and thermodynamics.” *Advances in Colloid and Interface Science* 197:58–67.
- Rahal, A., A. Kumar, V. Singh, B. Yadav, R. Tiwari, S. Chakraborty, and K. Dhama. 2014. “Oxidative stress, prooxidants, and antioxidants: the interplay.” *BioMed Research International* 2014, Article ID 761264.
- Raju, M. V. L. N., S. V. Rao, K. Radhika, and M. M. Chawak. 2005. “Dietary supplementation of Spirulina and its effects on broiler chicken exposed to aflatoxicosis.” *Indian Journal of Poultry Science* 40 (1): 36–40.
- Rosa, A. P., A. Scher, J. O. B. Sorbara, L. S. Boemo, J. Forgiarini, and A. Londero. 2012. “Effects of canthaxanthin on the productive and reproductive performance of broiler breeders.” *Poultry Science* 91 (3): 660–66.
- Ross, E. and W. Dominy. 1990. “The Nutritional Value of Dehydrated, Blue-Green Algae (*Spirulina platensis*) for Poultry.” *Poultry Science* 69 (5): 794–800. <https://doi.org/10.3382/ps.0690794>.
- Souza, R. A., P. A. Souza, R. C. Souza, and A. C. R. S. Neves. 2008. “Efeito da utilização de Carophyll Red nos índices reprodutivos de matrizes de frangos de corte.” *Revista Brasileira de Ciência Avícola* 10:32.
- Speake, B. K., A. M. Murray, and R. C. Noble. 1998. “Transport and transformations of yolk lipids during development of the avian embryo.” *Progress in Lipid Research* 37 (1): 1–32.
- Stivala, L. A., M. Savio, O. Cazzalini, R. Pizzala, L. Rehak, L. Bianchi, V. Vannini, and E. Prosperi. 1996. “Effect of β -carotene on cell cycle progression of human fibroblasts.” *Carcinogenesis* 17 (11): 2395–401.
- Surai, A. P., P. F. Surai, W. Steinberg, W. G. Wakeman, B. K. Speake, and N. H. C. Sparks. 2003. “Effect of canthaxanthin content of the maternal diet on the antioxidant system of the developing chick.” *British Poultry Science* 44 (4): 612–19.
- Surai, P. F. 1999. “Vitamin E in avian reproduction.” *Poultry and Avian Biology Reviews* 10 (1): 1.
- Surai, P. F. 2000. “Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick.” *British Poultry Science* 41 (2): 235–43.

- Surai, P. F., N. H. C. Sparks, and R. C. Noble. 1999. "Antioxidant systems of the avian embryo: tissue-specific accumulation and distribution of vitamin E in the turkey embryo during development." *British Poultry Science* 40 (4): 458–66.
- Surai, P. F., V. I. Fisinin, and F. Karadas. 2016. "Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium." *Animal Nutrition* 2 (1): 1–11.
- Surai, P. F., N. H. C. Sparks, and B. K. Speake. 2006. "The role of antioxidants in reproduction and fertility of poultry." XII European Poultry Conference, September 10–14, Verona, Italy, 2006.
- Surai, P. F., E. Kutz, G. J. Wishart, R. C. Noble, and B. K. Speake. 1997. "The relationship between the dietary provision of alpha-tocopherol and the concentration of this vitamin in the semen of chicken: effects on lipid composition and susceptibility to peroxidation." *Reproduction* 110 (1): 47–51.
- Tang, G., and P. M. Suter. 2011. "Vitamin A, nutrition, and health values of algae: Spirulina, Chlorella, and Dunaliella." *Journal of Pharmacy and Nutrition Sciences* 1 (2): 111–118.
- Wahab, A., A. Yazmie, M. Isa, and M. Lokman. 2015. "The future of Azospermic Patients: In vitro spermatogenesis." *Andrology (Los Angel)* 4 (2): 1–5.
- Wiersma, P., C. Selman, J. R. Speakman, and S. Verhulst. 2004. "Birds sacrifice oxidative protection for reproduction." *Proceedings of the Royal Society of London B: Biological Sciences* 271 (Suppl 5): S360–S363.
- Wu, A., Z. Ying, and F. Gomez-Pinilla. 2004. "Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats." *Journal of Neurotrauma* 21 (10): 1457–67.
- Zaghari, M., V. Sedaghat, and M. Shivazad. 2013. "Effect of vitamin E on reproductive performance of heavy broiler breeder hens." *Journal of Applied Poultry Research* 22 (4): 808–13.
- Zhang, L. X., R. V. Cooney, and J. S. Bertram. 1991. "Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: relationship to their cancer chemopreventive action." *Carcinogenesis* 12 (11): 2109–14.

CHAPTER NINE

SPIRULINA IN QUAIL NUTRITION

“Study nature, love nature, stay close to nature. It will never fail you.”
—Frank Lloyd Wright

9.1 Introduction

Quails are small game birds with high growth and maturity rates, and nowadays they are considered in commercial meat and egg production (Onyewuchi, Ofor, and Okoli 2013; Abouelezz 2017). In comparison with broilers and layers, quails have more resistance to diseases. They can be reared in a limited space with low investment (Cheong et al. 2015). The amount of nutrients in quails’ eggs may be about 3–4 times higher than the nutrient value of chicken eggs (Abduljaleel, Shuhaimi-Othman, and Babji 2011; Tunsaringkarn, Tungjaroenchai, and Siriwong 2013). *Coturnix coturnix japonica* is the most common quail that is reared for human consumption. The bird distribution is over large areas of Asia, Europe and Africa, but they were first domesticated in Japan (Mizutani 2003). The average of body weight in Japanese adult quails is about 250–300 g, sexual maturity occurs at 42–48 days, egg production is up to 290/year with average egg weight about 9–10 g, and the carcass yield is around 75–78% (Mizutani 2003). It was documented that Japanese quail eggs have therapeutic effects due to the presence of bioactive compounds such as lysozymes, ovomucoid and cystatin in the eggs (Kovacs-Nolan, Phillips, and Mine 2005). *Spirulina platensis* is a blue-green alga that has high nutritive value due to its valuable nutrient content (Hajati and Zaghari 2018). It was documented that *Spirulina* could replace about 5–10% of conventional proteins in poultry diets (Spolaore et al. 2006). Also, there is some evidence that shows that *Spirulina* improves poultry health, immune function and livability (Qureshi et al. 1996a; Kanagaraju and Omprakash 2016). The aim of this chapter is to provide an overview of *Spirulina*’s effects on quails’ health and performance.

9.2 Growth performance

Recently, poultry nutritionists considered microalgae such as *Chlorella vulgaris* and *Spirulina platensis* as interesting feedstuffs due to their high nutritional and functional properties (Jamil et al. 2015; Sugiharto and Lauridsen 2016). Regarding Chapter Three, *Spirulina* has high nutritive value because it contains essential amino acids, provitamin A, vitamin B₁₂, linolenic and γ -linolenic acid (Sánchez et al. 2003), ω_3 and ω_6 polyunsaturated fatty acids (Wu et al. 2005; Beheshtipour et al. 2013; Holman and Malau-Aduli 2013), carotenoids, chlorophyll, and phycobiliproteins (Hsiao et al. 2005). A selenium-containing phycocyanin has been isolated from *Spirulina platensis* (Huang et al. 2007). Also, *Spirulina platensis* is rich in polysaccharides which may function as a prebiotic (Beheshtipour et al. 2013; de Jesus Raposo, de Moraes, and de Moraes 2016). In a study, the effect of a dietary prebiotic (derived from *Saccharomyces cerevisiae*, 25% MOS, 30% β -glucan) on Japanese quails growth performance was studied (Hajati and Hassanabadi 2016). Quails fed with this prebiotic during 7 to 42 days of age showed better growth performance. It has been claimed that the benefits of MOS are based on its specific properties such as modification of the intestinal flora, changes in intestinal mucosal structure and digestive enzyme activity, and modulation of the immune system including those related to gut-associated lymphoid tissues (GALT) (Schley and Field 2002; Hajati and Rezaei 2010; Pan and Yu 2014). Prebiotic supplementation improved FCR of broiler breeders numerically; however, the differences were not significant statistically (Hajati, Hassanabadi, and Yansari 2014).

Cheong et al. (2015) evaluated the effects of different levels of *Spirulina* (*Arthrospira platensis*) inclusion in feed on live performance, carcass composition and meat quality of Japanese quails. Dietary treatments included *Spirulina* at the levels of 0, 1, 2, 4 or 8% of diet and were fed to quails from 15 days to 35 days of age. The researchers found that *Spirulina* inclusion of up to 4% of diet caused the best live performance, carcass composition and meat quality. However, they found that using over 4% of the algae had adverse effects on growth performance of the birds. Kanagaraju and Omprakash (2016) demonstrated that the Japanese quails fed a diet that included 1% *Spirulina* had a higher BW than that of the control group. Danny et al. (2016) reported that dietary inclusion of *Spirulina* at the levels of 1, 2 or 4% improved growth performance of meat quails, but higher levels had adverse effects. Abouelezz (2017) found that using *Spirulina* powder in quails' diets at the level of 1% in the feed or at the level of 0.25% in the drinking water caused higher body weight gain

and lower FCR than those of the control group. Dogan et al. (2016) reported that using *Spirulina platensis* in quails' diets decreased blood levels of LDL cholesterol while blood levels of HDL cholesterol increased.

Recently, we assessed the effects of dietary inclusion of *Spirulina platensis* on Japanese quails' performance (Hajati and Zaghari 2018). The experimental diets included a control diet (with no additive) and four levels of algae (2.5, 5, 10 or 20 g/kg diet) that were fed to birds from 1 to 35 days of age. The birds' feed intake, body weight gain and FCR were measured weekly. The percentages of carcass yield, breast, drumstick and femur of quails were studied at 35 days. Results showed that feeding quails with 5 g *Spirulina*/kg diet resulted in a higher feed intake, body weight gain, European production efficiency index and breast percentage compared to the control group. Quails that received 20 g *Spirulina*/kg diet consumed more feed; however, they had a lower carcass yield compared to the control group. There was no significant difference among the percentages of drumstick and thigh of quails at 35 days.

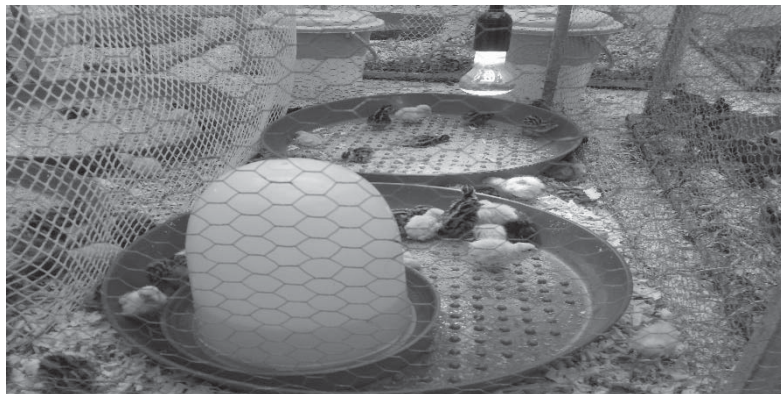


Figure 9-1: Experimental pens of Japanese quail chicks at 2 days post hatch fed with a diet containing *Spirulina platensis*. (Photograph by authors)

9.3 Meat quality

“Meat quality” is a term that relates to overall meat characteristics including its physical, chemical, morphological, biochemical, microbial, sensory, technological, hygienic, nutritional and culinary properties (Ingr 1989). The most important meat characteristics that may influence the

quality judgment by meat consumers are appearance, texture, juiciness, wateriness, firmness, tenderness, odor and flavor (Cross, Durland, and Seideman 1986). Also, quantifiable parameters of meat such as water holding capacity, intramuscular fat, acto-myosin complex, quality of collagen (Coro, Youssef, and Shimokomaki 2002), shear force, drip loss, cook loss, pH, shelf life, collagen content, protein solubility, cohesiveness and fat binding capacity are very important for processors in the manufacture of processing meat products (Allen et al. 1998). Color is one of the main factors affecting consumer acceptance (Saláková et al. 2009).

Microalgae have a superior fatty-acid profile with high abundance of EPA and/or DHA compared to traditional animal feed protein supplements (Fredriksson et al. 2006; Guschina and Harwood 2006; Kalogeropoulos et al. 2010). It was found that using microalgae in poultry diets improved the overall n-3 fatty-acid status in egg yolks (Fredriksson et al. 2006) and breast muscle (Mooney et al. 1998). Previous studies confirmed that consuming diets with high amounts of long-chain polyunsaturated fatty acids (PUFAs), in particular n-3 FAs, may decrease risk of cardiovascular disease, diabetes, arthritis and cancer (Daviglus et al. 1997; Albert et al. 1998; Ruggiero et al. 2009; Sala-Vila and Calder 2011; Delgado-Lista et al. 2012). Also, *Spirulina* is rich in β -carotene and zeaxanthin and it could improve redness of breast muscle in quails (Cheong et al. 2015). Cheong et al. (2015) studied the inclusion of 4 levels of *Spirulina* (1, 2, 4 and 8%) in quail diets. They found that *Spirulina* improved the redness of breast muscle in quails. They also reported that using dietary *Spirulina* reduced the toughness of quail meat. *Spirulina* improved meat tenderness and the meat quality was found to be higher mainly due to the lower drip loss and cooking loss (Cheong et al. 2015; Park, Lee, and Kim 2018).

It was reported that using *Spirulina platensis* in drinking water decreased the relative weight of abdominal fat in Japanese quails (Ibrahim et al. 2018). This is interesting for consumers who have limitations in consuming lipid. The hypo-lipidemic characteristic of *Spirulina* may be due to its high GLA content (Gershwin and Belay 2007). GLA is a precursor of prostaglandins (PG). It is well known that the prostaglandin PGE1 is essential for regulating blood pressure, cholesterol synthesis, inflammation and cell proliferation (Henrikson 1989; Cronin 2000). Also, consumption of high levels of arginine decreases serum cholesterol levels; however, eating low levels of methionine suppresses the incidence of coronary heart disease (Anderson et al. 1999; Anderson, Johnstone, and Cook-Newell 1995; Teixeira et al. 2000). Previous studies indicated that the hypo-cholesterolemic effect of *Spirulina* may be due to the effect of its

amino acids, fiber, phytonutrients and antioxidants content (Anderson and Hanna 1999; Anderson et al. 1999; Henrikson 1989; Cronin 2000; Hermansen et al. 2001).

9.4 Immunity

Previous studies reported that feeding chicks *Spirulina platensis* enhanced their humoral and cellular immune responses and lymphoid organ development (Qureshi and Ali 1996; Raju et al. 2004). It is well known that macrophages and T and B lymphocytes have a key role in all immune responses. Indeed, other cells in the tissues participate in immune responses by signaling to the lymphocytes and responding to the cytokines such as interleukins (ILs) and interferon γ (IFN γ). Cell-mediated immunity is mediated by phagocytes and lymphocytes; however, humoral immunity is mediated by antibodies in the circulating blood and lymph (Gershwin and Belay 2007). Figure 9-2 illustrates the humoral- and cell-mediated immunity pathways. Many factors such as cytokines (IL-12, IL-6, TNF and IL-18), co-stimulators (CD80, CD86) and nitrogen oxide are up-regulated through toll and cytoplasmic signaling, causing stimulation of the cellular immunity (Tsuiji et al. 2000; Seya 2003; Seya et al. 2006).

Spirulina platensis has immune-modulating effects in animals and humans (Khan, Bhadouria, and Bisen 2005). Effects of *Spirulina* on body immune responses are shown in Figure 9-3. *Spirulina* products may improve innate immune functions and promote both the humoral and the cellular responses (Gershwin and Belay 2007). The sulfated-polysaccharides isolated from a water extract of *Spirulina*, called calcium-spirulan (Ca-SP), showed immunomodulatory and antiviral activities (Pugh et al. 2001; Hernández-Corona et al. 2002; Mao, Water, and Gershwin 2005; Balachandran et al. 2006). Also, immolina, a high-molecular-weight polysaccharide fraction of *Spirulina*, promotes chemokine expression in human monocytic (T helper peptides) THP-1 cells (Grzanna et al. 2006). Polysaccharides and phycocyanin content of *Spirulina* increase antibody titers and white blood cell numbers (Hayashi, Katoh, and Okuwaki 1994; Qureshi and Ali 1996; Qureshi, Garlich, and Kidd 1996b; Al-Batshan et al. 2001), support bone marrow reproduction, thymus growth and spleen cell proliferation, and increase immunity responses in animal models such as mice (Gershwin and Belay 2007). Furthermore, it was shown that *Spirulina* up-regulated the immune system against stress caused by environmental toxins, bacteria and viruses (Pugh et al. 2001; Hernández-Corona et al. 2002; Mao, Water, and Gershwin 2005; Balachandran et al.

2006; Grzanna et al. 2006; Qureshi and Ali 1996; Qureshi, Garlich, and Kidd 1996b; Al-Batshan et al. 2001).

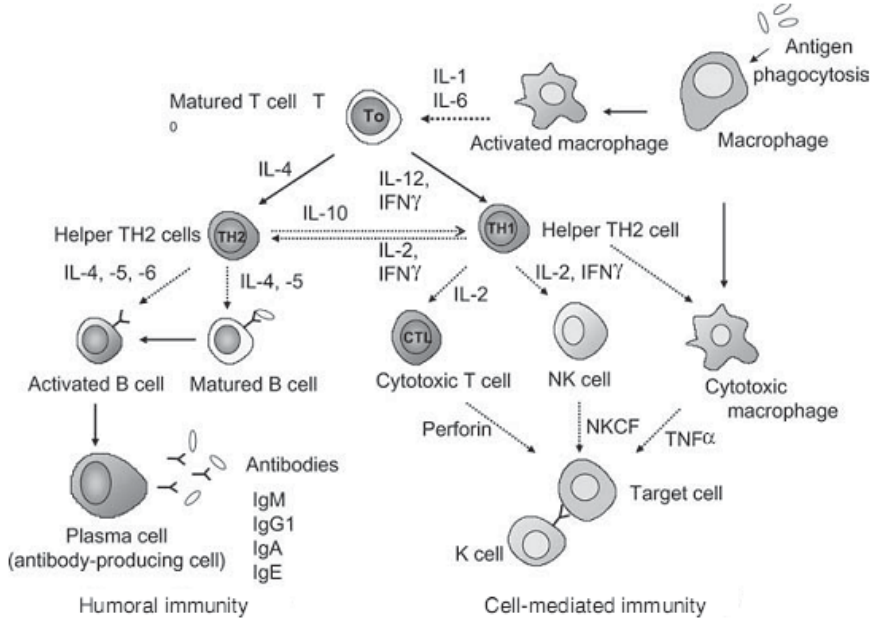


Figure 9-2: Humoral and cell immunity systems. (Gershwin and Belay 2007)

Jamil et al. (2015) reported that *Spirulina* has the potential to modulate growth and immunity of broiler chicks. It was documented that using *Spirulina* in drinking water increased the serum antibody titers against Newcastle virus on days 3, 7 and 9 post vaccinations of Japanese quails (Ibrahim et al. 2018). Using *Spirulina* in mice diets (at the levels of 50 and 150 mg/kg body weight) increased the antibody production after vaccination, which shows the supporting effect of *Spirulina* on the immunity system (Chu, Van Quynh, and Radhakrishnan 2013).

Using *Spirulina* in poultry diets may improve the immune system and total health condition of the birds (Qureshi et al. 1994). Recently, Lokapirnasari et al. (2016) showed that dietary *Spirulina platensis* increased the number of leukocytes and decreased the mortality rate of broiler chicks. The antimicrobial, anti-inflammatory and immunomodulatory capacities as

well as antioxidant potential of *Spirulina platensis* seemed to be responsible for the health promoting effect of poultry (Farag et al. 2016).

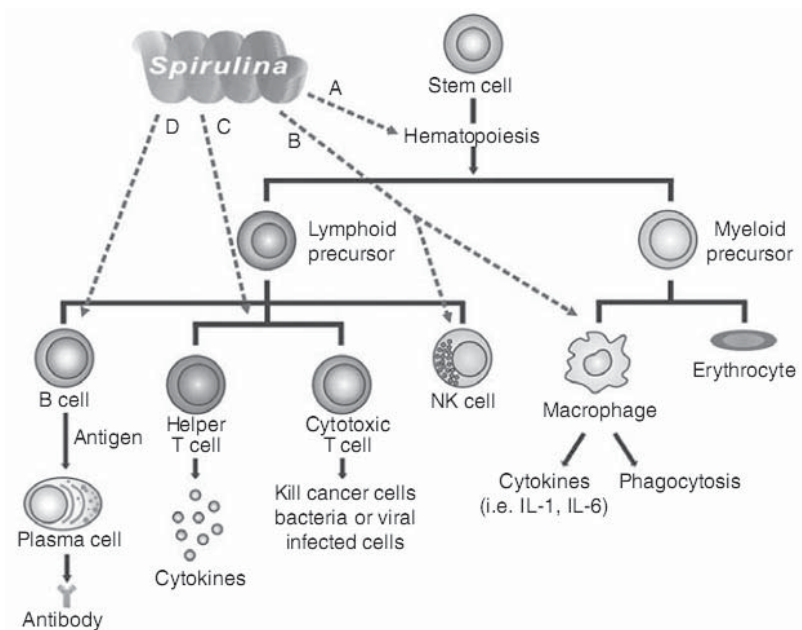


Figure 9-3: Effects of *Spirulina* on body immune responses. (Gershwin and Belay 2007). A = *Spirulina* promotes hematopoiesis to produce more erythrocytes and lymphocytes; B = *Spirulina* has a direct effect on innate immunity by activating macrophages and natural killer (NK) cells; C = *Spirulina* activates T-helper cells and T-cytotoxic cells; D = *Spirulina* promotes the maturity of B-cells for the production of antibodies.

9.5 Egg production

Anderson, Tang, and Ross (1991) studied the effect of feeding freeze dried *Spirulina* to Japanese quails. They analyzed *Spirulina* carotenoid content with HPLC and found that the algae had 5,787 mg xanthophyll per kg of *Spirulina*. The experimental diets included graded levels of *Spirulina* between 0.25 and 4% that were fed to quails for 21 days. They reported that using *Spirulina* at the level of 1% of diet caused optimum yolk color pigmentation. In a study about the effects of *Spirulina platensis* in laying

quails, Dogan et al. (2016) fed Japanese quails with different levels of *Spirulina* (0, 0.5, 1.0 and 2.0%) for 8 weeks. They found that *Spirulina* had no significant effect on FI, FCR, egg production, egg weight, shape index, eggshell thickness and haugh unit ($P>0.05$). Abouelezz (2017) studied the effects of *Spirulina platensis* supplementation in the feed (1%) and drinking water (0.25%) of the Japanese quails during the laying period. They found that *Spirulina platensis* had no significant effect on egg production. They recommended using higher levels of dietary *Spirulina* to see its effect on egg production traits. The effects of *Spirulina platensis* on egg production of Japanese quails is shown in Table 9-1.

Table 9-1: The effects of *Spirulina platensis* on egg production of Japanese quails. (Abouelezz 2017)

	Control	Inclusion of <i>Spirulina</i> <i>platensis</i> (1%)	<i>Spirulina platensis</i> in the drinking water (0.25%)
Final body weight	226.7±4.7 ^b	237.6±11.7 ^a	239.6±4.0 ^a
Egg laying rate (%)	61.50±5.10	62.70±4.51	61.95±4.26
Egg weight (g)	11.19±0.26	11.20±0.40	11.25±0.04
Daily egg mass (g/day)	6.88±0.72	7.02±0.64	6.97±0.53
FI (g/day)	24.44±0.67	24.60±0.72	25.06±1.50
FCR (g feed: g egg)	3.55±0.24	3.50±0.19	3.60±0.24

Means ± SE within the same row with different superscripts are significantly different ($P<0.05$).

Recently, we studied the effects of *Spirulina platensis* supplementation on egg production performance of Japanese laying quails (*Coturnix coturnix japonica*). Experimental diets included a control diet (with no additive) and diets containing three levels of *Spirulina platensis* (1, 3 or 5 g/kg diet). Figure 9-4 shows the Japanese laying quails fed with diets that contained *Spirulina platensis*.



Figure 9-4: Experimental house of Japanese laying quails fed with diet contained *Spirulina platensis*. (Photograph by authors)

Our result showed that *Spirulina platensis* did not have any significant effect on laying percentage, average egg weight, egg length, egg width and egg surface area ($P>0.05$). The results are shown in Table 9-2.

Table 9-2: Effects of *Spirulina platensis* on egg quantity and quality traits of laying quails. (Hajati and Zaghari, unpublished data)

Treatment	Laying (Hen day %)	Average egg weight (g)	Egg length (mm)	Egg width (mm)	Egg surface area
Control	87.41	12.21	34.08	26.50	26.02
SP1	87.64	12.29	33.69	26.40	26.15
SP3	87.86	12.32	34.18	26.48	26.20
SP5	86.83	12.28	34.03	26.38	26.13
SEM	0.766	0.033	0.260	0.281	0.151
Pvalue	0.9961	0.7896	0.3660	0.3373	0.2920

SP1: 1 g *Spirulina platensis*/kg diet; SP3: 3 g *Spirulina platensis*/kg diet; SP5: 5 g *Spirulina platensis*/kg diet.

The means within the same column with at least one common letter do not have significant difference ($P>0.05$).

SEM: standard error of the means.

9.6 Egg quality

Spirulina is a microalgae that has a high amount of carotenoids including zeaxanthin (Ciferri 1983; Annapurna, Deosthale, and Bamji 1991; Miranda et al. 1998; Gireesh, Nair, and Sudhakaran 2004; Yu et al. 2012). Singh, Pathak, and Akhilesh (2012) stated that pigment enrichment of egg yolk has the following advantages: preventing macular degeneration, attractive color, antioxidant and anti-carcinogenic effects, and safeguard to the retina. It is documented that zeaxanthin and lutein are important for the development and maintenance of normal distribution of the retinal pigment epithelium in animal models (Malinow et al. 1980; Leung et al. 2004). Indeed, retinal zeaxanthin may protect the photoreceptors from light-induced damage in quail eyes (Thomson et al. 2002).

In our recent study about the effects of using dietary *Spirulina* on the eggs of Japanese quails, the haugh unit and yolk color were decreased and increased, respectively ($P < 0.05$).

Table 9-3: Effects of *Spirulina platensis* on egg quantity and quality traits of layer quails. (Hajati and Zaghari, unpublished data)

Treatment	control	SP1	SP3	SP5	SEM	p-value
Haugh unit	91.94 ^a	90.57 ^{bc}	90.73 ^b	90.09 ^c	0.159	0.0004
Yolk color	5.53 ^d	7.80 ^c	9.45 ^b	10.69 ^a	0.140	0.0001

SP1: 1 g *Spirulina platensis*/kg diet; SP3: 3 g *Spirulina platensis*/kg diet; SP5: 5 g *Spirulina platensis*/kg diet.

The means within the same column with at least one common letter do not have significant difference ($P > 0.05$).

SEM: standard error of the means.

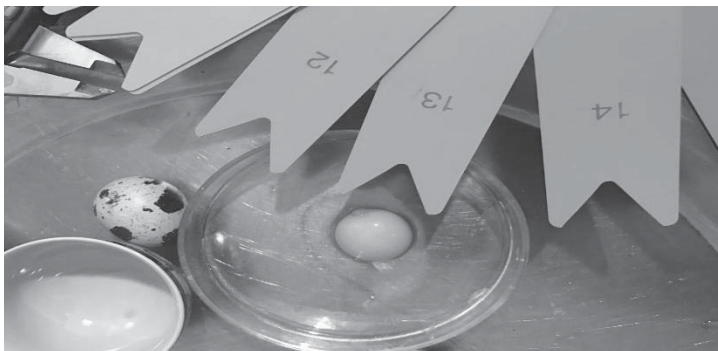


Figure 9-5: Evaluating egg yolk color of Japanese laying quails fed with diets containing *Spirulina platensis*. (Photograph by authors)

Dogan et al. (2016) found that dietary inclusion of *Spirulina platensis* at the levels of 1 or 2% of diet decreased egg yolk cholesterol levels by 19.65 and 18.93%, respectively. This may be important for some consumers such as those suffering from arteriosclerosis or obesity diseases.

9.7 Fertility, hatchability and chick quality

Algae is a natural source of astaxanthin, a pigment in xanthophylls family (Higuera-Ciapara, Felix-Valenzuela, and Goycoolea 2006). It was shown that the antioxidant characteristics of astaxanthin are more than those of β -carotene or even α -tocopherol (Miki 1991). It was documented that using astaxanthin in hens could improve their health state and fertility. Also, their progeny mortality decreased, possibly due to decreased *Salmonella* infections (Lignell, Nicolin, and Larsson 1998).

Ibrahim, Wakwak, and Al-Gamal (2018) studied the effects of using *Spirulina* extract (0.5, 1 and 2 g/liter) in drinking water on the productive performance and immune response of growing Japanese quails. They reported that using *Spirulina* in the drinking water of quails could increase the relative weight of sex organs (testes and ovaries). They concluded that *Spirulina* contained essential fatty acids (e.g., α -linolenic acid) that influenced the accumulation of fatty acid in sex organs of quails. Also, the antioxidant and anti-proliferative properties of the selenium content of *Spirulina* may increase the relative weight of sex organs (Chen and Wong 2008).

Abouelezz (2017) reported positive effects of *Spirulina* on quails' fertility. The researcher stated that this result was due to the high content of several biologically active nutrients in *Spirulina*. It was reported that the high content of n-3 long-chain polyunsaturated fatty acids (DHA and EPA), protein, antioxidants and vitamins in *Spirulina platensis* (Świątkiewicz et al. 2015; Belay et al. 1996) were required for producing high-quality spermatozoa with an intact and normal structure. As well as the importance for pigmentation (Hill, Inouye, and Montgomerie 2002), carotenoids have several physiological and immunological functions in both the laying females and developing embryos, and they are the nutrients that the mothers use for the requirements of themselves and their progeny (Moller et al. 2000; Cucco et al. 2007). With regards to the transmission of these fat-soluble nutrients from hen to egg, it has been documented that adding dietary carotenoid can increase the yolk carotenoid contents (Surai and Speake 1998; Blount et al. 2002; Bortolotti et al. 2003; Surai et al. 2003; Karadas et al. 2005a, 2005b). Domestic hen *Gallus domesticus* embryos with high concentrations of maternally derived carotenoids have higher levels of antioxidant protection (Surai et al. 2001; Surai, Speake, and Sparks 2001) and improved lymphocyte synthesis in the newly hatched chicks (Haq, Bailey, and Chinnah 1996). It was shown that dietary carotenoids supported the immunity responses in the progeny of the barn swallow (Saino et al. 2002a), moorhen (Fenoglio, Cucco, and Malacarne 2002) and grey partridge (Cucco et al. 2006). Supplementation of β -carotene in female grey partridges increased the concentration of lysozyme, an enzyme with antibacterial activity, in the albumen of eggs, and the hatching rate was higher (Cucco et al. 2007). Lysozyme is an antibacterial immune enzyme that digests bacterial cell walls (Sato and Watanabe 1976), and it has positive effects on the chick livability (Saino et al. 2002b). This substance affects egg hatchability and anti-parasite defense and viability of the offspring (Cucco et al. 2007). Since lysozyme is secreted by leukocytes, Amar et al. (2004) suggested that the increased levels of lysozyme by β -carotene could be due to its stimulation of phagocytic cells.

References

- Abduljaleel, S. A., M. Shuhaimi-Othman, and A. Babji. 2011. "Variation in trace elements levels among chicken, quail, guinea fowl and pigeon eggshell and egg content." *Research Journal of Environmental Toxicology* 5 (5): 301–8.

- Abouelezz, F. M. K. 2017. "Evaluation of *Spirulina* algae (*Spirulina platensis*) as a feed Supplement for Japanese quail: Nutritional effects on growth performance, egg production, egg quality, blood metabolites, sperm-egg penetration and fertility." *Egyptian Poultry Science Journal* 37 (3): 709–21.
- Al-Batshan, H. A., S. I. Al-Mufarrej, A. A. Al-Homaidan, and M. A. Qureshi. 2001. "Enhancement of chicken macrophage phagocytic function and nitrite production by dietary *Spirulina platensis*." *Immunopharmacology and Immunotoxicology* 23 (2): 281–89.
- Albert, C. M., C. H. Hennekens, C. J. O'donnell, U. A. Ajani, V. J. Carey, W. C. Willett, J. N. Ruskin, and J. E. Manson. 1998. "Fish consumption and risk of sudden cardiac death." *Jama* 279 (1): 23–28.
- Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. "The relationship of broiler breast colour to meat quality and shelf-life." *Poultry Science* 77 (2): 361–66.
- Amar, E. C., V. Kiron, S. Satoh, and T. Watanabe. 2004. "Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products." *Fish and Shellfish Immunology* 16 (4): 527–37.
- Anderson, D. W., C. S. Tang, E. and Ross. 1991. "The xanthophylls of *Spirulina* and their effect on egg yolk pigmentation." *Poultry Science* 70 (1): 115–19.
- Anderson, J. W., and T. J. Hanna. 1999. "Impact of nondigestible carbohydrates on serum lipoproteins and risk for cardiovascular disease." *The Journal of Nutrition* 129 (7): 1457S-1466S.
- Anderson, J. W., L. D. Allgood, J. Turner, P. R. Oeltgen, and B. P. Daggy. 1999. "Effects of psyllium on glucose and serum lipid responses in men with type 2 diabetes and hypercholesterolemia." *The American Journal of Clinical Nutrition* 70 (4): 466–73.
- Anderson, J. W., B. M. Johnstone, and M. E. Cook-Newell. 1995. "Meta-analysis of the effects of soy protein intake on serum lipids." *New England Journal of Medicine* 333 (5): 276–82.
- Annapurna, V. V., Y. G. Deosthale, and M. S. Bamji. 1991. "Spirulina as a source of vitamin A." *Plant Foods for Human Nutrition* 41 (2): 125–34.
- Balachandran, P., N. D. Pugh, G. Ma, and D. S. Pasco. 2006. "Toll-like receptor 2-dependent activation of monocytes by *Spirulina* polysaccharide and its immune enhancing action in mice." *International Immunopharmacology* 6 (12): 1808–14.
- Beheshtipour, H., A. M. Mortazavian, R. Mohammadi, S. Sohrabvandi, and K. Khosravi-Darani. 2013. "Supplementation of *Spirulina platensis*

- and *Chlorella vulgaris* algae into probiotic fermented milks.” *Comprehensive Reviews in Food Science and Food Safety* 12 (2): 144–54.
- Belay, A., T. Kato, and Y. Ota. 1996. “Spirulina (Arthrospira): potential application as an animal feed supplement.” *Journal of Applied Phycology* 8:303–11.
- Blount, J. D., P. F. Surai, D. C. Houston, and A. P. Møller. 2002. “Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization.” *Functional Ecology* 16 (4): 445–53.
- Bortolotti, G. R., J. J. Negro, P. F. Surai, and P. Prieto. 2003. “Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output.” *Physiological and Biochemical Zoology* 76 (3): 367–74.
- Chen, T., and Y. S. Wong. 2008. “In vitro antioxidant and antiproliferative activities of selenium-containing phycocyanin from selenium-enriched *Spirulina platensis*.” *Journal of Agricultural and Food Chemistry* 56 (12): 4352–58.
- Cheong, D. S. W., A. Kasim, A. Q. Sazili, O. M. A. R. Hishamuddin, and J. Y. Teoh. 2015. “Effect of supplementing Spirulina on live performance, carcass composition and meat quality of Japanese quail.” *Walailak Journal of Science and Technology (WJST)* 13 (2): 77–84.
- Chu, W. L., L. Van Quynh, and A. K. Radhakrishnan. 2013. “Effect of Spirulina (Arthrospira) supplementation on the immune response to tetanus toxoid vaccination in a mouse model.” *Journal of Dietary Supplements* 10 (3): 229–40.
- Ciferri, O. 1983. “Spirulina, the edible microorganism.” *Microbiological Reviews* 47 (4): 551.
- Coro, F. A., E. Y. Youssef, and M. Shimokomaki. 2002. “Age related changes in poultry breast meat collagen pyridinoline and texture.” *Journal of Food Biochemistry* 26 (6): 533–41.
- Cronin, J. R. 2000. “Gamma linolenic acid: a building block for good health.” *Alternative and Complementary Therapies* 6 (4): 218–21.
- Cross, H. R., P. R. Durland, and S. C. Seideman. 1986. “Sensory qualities of meat.” *Muscle as Food*, Academic Press, Orlando, pp 279–320.
- Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2006. “Effects of β -carotene supplementation on chick growth, immune status and behaviour in the grey partridge, *Perdix perdix*.” *Behavioural Processes* 73 (3): 325–32.
- Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2007. “Effects of β -carotene on adult immune condition and antibacterial activity in the

- eggs of the Grey Partridge, *Perdix perdix*." *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 147 (4): 1038–46.
- Danny, S. W. C., K. Azhar, Q. S. Awis, O. Hishamuddin, and Y. T. Jia. 2016. "Effect of supplementing Spirulina on live performance, carcass composition and meat quality of Japanese Quail. Walailak." *Journal of Science and Technology* 13:77–84.
- Daviglus, M. L., J. Stamler, A. J. Orenchia, A. R. Dyer, K. Liu, P. Greenland, M. K. Walsh, D. Morris, and R. B. Shekelle. 1997. "Fish consumption and the 30-year risk of fatal myocardial infarction." *New England Journal of Medicine* 336 (15): 1046–53.
- De Jesus Raposo, M. F., A. M. M. B. de Moraes, and R. M. S. C. de Moraes. 2016. "Emergent sources of prebiotics: seaweeds and microalgae." *Marine Drugs* 14 (2): 27.
- Delgado-Lista, J., P. Perez-Martinez, J. Lopez-Miranda, and F. Perez-Jimenez. 2012. "Long chain omega-3 fatty acids and cardiovascular disease: a systematic review." *British Journal of Nutrition* 107 (S2): S201–S213.
- Dogan, S. C., M. Baylan, Z. Erdogan, G. C. Akpinar, A. Kucukgul, and V. Duzguner. 2016. "Performance, egg quality and serum parameters of Japanese quails fed diet supplemented with Spirulina platensis." *Fresenius Environmental Bulletin* 25 (12a): 5857–62.
- Farag, M. R., M. Alagawany, M. A. El-Hack, K. and Dhama. 2016. "Nutritional and healthical aspects of Spirulina (Arthrospira) for poultry, animals and human." *International Journal of Pharmacology* 12 (12): 36–51.
- Fenoglio, S., M. Cucco, and G. Malacarne. 2002. "The effect of a carotenoid-rich diet on immunocompetence and behavioural performances in Moorhen chicks." *Ethology Ecology and Evolution* 14 (2): 149–56.
- Fredriksson, S., K. Elwinger, and J. Pickova. 2006. "Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens." *Food Chemistry* 99 (3): 530–37.
- Gershwin, M. E., and A. Belay, eds. 2007. *Spirulina in Human Nutrition and Health*. CRC Press. Taylor and Francis group. ISBN 9781420052565
- Gireesh, T., P. P. Nair, and P. R. Sudhakaran. 2004. "Studies on the bioavailability of the provitamin A carotenoid, β -carotene, using human exfoliated colonic epithelial cells." *British Journal of Nutrition* 92 (2): 241–45.

- Grzanna, R., A. Polotsky, P. V. Phan, N. Pugh, D. Pasco, and C. G. Frondoza. 2006. "Immulina, a High-Molecular-Weight Polysaccharide Fraction of Spirulina, Enhances Chemokine Expression in Human Monocytic THP-1 Cells." *Journal of Alternative and Complementary Medicine* 12 (5): 429–35.
- Guschina, I. A., and J. L. Harwood. 2006. "Lipids and lipid metabolism in eukaryotic algae." *Progress in Lipid Research* 45 (2): 160–86.
- Hajati, H., and A. Hassanabadi. 2016. "The Effect of a Dietary Prebiotic on Japanese Quails Growth Performance." In *5th International Veterinary Poultry Congress*, January 31–February 1, 2016, Tehran, Iran.
- Hajati, H., and M. Rezaei. 2010. "The application of prebiotics in poultry production." *International Journal of Poultry Science* 9 (3): 298–304.
- Hajati, H., and M. Zaghari. 2018. "Scrutinizing of *Spirulina Platensis* on growth performance and carcass characteristics of Japanese quail." *Proceedings of the British Society of Animal Science in association with the Agricultural Research Forum*, BSAS Annual Conference, 2018, Ireland, page 195.
- Hajati, H., A. Hassanabadi, and A. T. Yansari. 2014. "The effect of dietary supplementation of prebiotic and probiotic on performance, humoral immunity responses and egg hatchability in broiler breeders." *Poultry Science Journal* 2 (1): 1–13.
- Haq, A. U., C. A. Bailey, and A. Chinnah. 1996. "Effect of β -carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets." *Poultry Science* 75 (9): 1092–97.
- Hayashi, O., T. Katoh, and Y. Okuwaki. 1994. "Enhancement of antibody production in mice by dietary *Spirulina platensis*." *Journal of Nutritional Science and Vitaminology* 40 (5): 431–41.
- Henrikson, R. 1989. *Earth Food Spirulina*. Laguna Beach, CA: Ronore Enterprises, Inc.
- Hermansen, K., M. Søndergaard, L. Høie, M. Carstensen, and B. Brock. 2001. "Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects." *Diabetes Care* 24 (2): 228–33.
- Hernández-Corona, A., I. Nieves, M. Meckes, G. Chamorro, and B. L. Barron. 2002. "Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2." *Antiviral Research* 56 (3): 279–85.
- Higuera-Ciapara, I., L. Felix-Valenzuela, and F. M. Goycoolea. 2006. "Astaxanthin: a review of its chemistry and applications." *Critical Reviews in Food Science and Nutrition* 46 (2): 185–96.

- Hill, G. E., C. Y. Inouye, and R. Montgomerie. 2002. "Dietary carotenoids predict plumage colouration in wild house finches." *Proceedings of the Royal Society of London B: Biological Sciences* 269 (1496): 1119–24.
- Holman, B. W. B., and A. E. O. Malau-Aduli. 2013. "Spirulina as a livestock supplement and animal feed." *Journal of Animal Physiology and Animal Nutrition* 97 (4): 615–23.
- Hsiao, G., P. H. Chou, M. Y. Shen, D. S. Chou, C. H. Lin, and J. R. Sheu. 2005. "C-phycocyanin, a very potent and novel platelet aggregation inhibitor from *Spirulina platensis*." *Journal of Agricultural and Food Chemistry* 53 (20): 7734–40.
- Huang, Z., B. J. Guo, R. N. S. Wong, and Y. Jiang. 2007. "Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*." *Food Chemistry* 100 (3): 1137–43.
- Ibrahim, N. S., M. M. Wakwak, and M. A. Al-Gamal. 2018. "Productive performance and immune response in growing Japanese quail supplemented with *Spirulina* algae extract (*Arthrospira platensis*) in drinking water." *Egyptian Poultry Science Journal* 38 (2): 409–26.
- Iji, P. A., and D. R. Tivey. 1998. "Natural and synthetic oligosaccharides in broiler chicken diets." *World's Poultry Science Journal* 54 (2): 129–43.
- Ingr, I. 1989. "Meat quality. Defining the term by modern standards." *Fleischwirtschaft (Germany, FR)*. ISSN: 0015-363X.
- Jamil, A. R., M. R. Akanda, M. M. Rahman, M. A. Hossain, and M. S. Islam. 2015. "Prebiotic competence of spirulina on the production performance of broiler chickens." *Journal of Advanced Veterinary and Animal Research* 2 (3): 304–9.
- Kalogeropoulos, N., A. Chiou, M. Ioannou, V. T. Karathanos, M. Hassapidou, and N. K. Andrikopoulos. 2010. "Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries." *Food Chemistry* 121 (3): 682–90.
- Kanagaraju, P., and A. V. Omprakash. 2016. "Effect of *Spirulina platensis* algae Powder Supplementation as a Feed Additive on the Growth Performance of Japanese quails." *Indian Veterinary Journal* 93:31–33.
- Karadas, F., A. C. Pappas, P. F. Surai, and B. K. Speake. 2005a. "Embryonic development within carotenoid-enriched eggs influences the post-hatch carotenoid status of the chicken." *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 141 (2): 244–51.

- Karadas, F., P. F. Surai, N. H. Sparks, and E. Grammenidis. 2005b. "Effects of maternal dietary supplementation with three sources of carotenoids on the retinyl esters of egg yolk and developing quail liver." *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 140 (4): 430–35.
- Khan, Z., P. Bhadouria, and P. S. Bisen. 2005. "Nutritional and therapeutic potential of Spirulina." *Current Pharmaceutical Biotechnology* 6 (5): 373–79.
- Kovacs-Nolan, J., M. Phillips, and Y. Mine. 2005. "Advances in the value of eggs and egg components for human health." *Journal of Agricultural and Food Chemistry* 53 (22): 8421–31.
- Leung, I. Y. F., M. M. Sandstrom, C. L. Zucker, M. Neuringer, and D. M. Snodderly. 2004. "Nutritional manipulation of primate retinas, II: Effects of age, n-3 fatty acids, lutein, and zeaxanthin on retinal pigment epithelium." *Investigative Ophthalmology and Visual Science* 45 (9): 3244–56.
- Lignell, A., C. Nicolin, L.-H. Larsson. 1998. Method for increasing the production of/in breeding and production animals in the poultry industry. US Patent 5,744,502. issued April 28, 1998.
- Lokapinrasari, W. P., A. B. Yulianto, and D. Legowo. 2016. "The effect of Spirulina as feed additive to myocardial necrosis and leukocyte of chicken with avian influenza (H5N1) virus infection." *Procedia Chemistry* 18:213–17.
- Malinow, M. R., L. Feeney-Burns, L. H. Peterson, M. L. Klein, and M. Neuringer. 1980. "Diet-related macular anomalies in monkeys." *Investigative Ophthalmology and Visual Science* 19 (8): 857–63.
- Mao, T. K., J. V. D. Water, and M. E. Gershwin. 2005. "Effects of a Spirulina-based dietary supplement on cytokine production from allergic rhinitis patients." *Journal of Medicinal Food* 8 (1): 27–30.
- Miki, W. 1991. "Biological functions and activities of animal carotenoids." *Pure and Applied Chemistry* 63 (1): 141–46.
- Miranda, M. S., R. G. Cintra, S. B. M. Barros, and J. Mancini-Filho. 1998. "Antioxidant activity of the microalga *Spirulina maxima*." *Brazilian Journal of Medical and Biological Research* 31 (8): 1075–79.
- Mizutani, M. 2003. The Japanese quail. *Laboratory Animal Research Station, Nippon Institute for Biological Science, Kobuchizawa, Yamanashi, Japan*, 143–163.
- Moller, A. P., C. Biard, J. D. Blount, D. C. Houston, P. Ninni, N. Saino, and P. F. Surai. 2000. "Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability?" *Poultry and Avian Biology Reviews* 11 (3): 137–60.

- Mooney, J. W., E. M. Hirschler, A. K. Kennedy, A. R. Sams, and M. E. Van Elswyk. 1998. "Lipid and flavour quality of stored breast meat from broilers fed marine algae." *Journal of the Science of Food and Agriculture* 78 (1): 134–40.
- Onyewuchi, U. U., I. R. Offor, and C. F. Okoli. 2013. "Profitability of quail bird and egg production in IMO state." *Nigerian Journal of Agriculture, Food and Environment* 9 (1): 40–44.
- Pan, D., and Z. Yu. 2014. "Intestinal microbiome of poultry and its interaction with host and diet." *Gut Microbes* 5 (1): 108–19.
- Park, J. H., S. I. Lee, and I. H. Kim. 2018. "Effect of dietary *Spirulina* (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens." *Poultry Science* 97 (7): 2451–59.
- Pugh, N., S. A. Ross, H. N. ElSohly, M. A. ElSohly, and D. S. Pasco. 2001. "Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*." *Planta Medica* 67 (08): 737–42.
- Qureshi, M. A., and R. A. Ali. 1996. "Spirulina platensis exposure enhances macrophage phagocytic function in cats." *Immunopharmacology and Immunotoxicology* 18 (3): 457–63.
- Qureshi, M. A., J. D. Garlich, and M. T. Kidd. 1996. "Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens." *Immunopharmacology and Immunotoxicology* 18 (3): 465–76.
- Qureshi, M. A., D. Garlich, M. T. Kidd, and R. A. Ali. 1994. "Immune enhancement potential of *Spirulina platensis* in chickens." *Poultry Science* 73:46.
- Raju, M. V. L. N., S. V. Rama Rao, K. Radhika, and M. M. Chawak. 2004. "Effects of *Spirulina platensis* or furazolidone on the performance and immune response of broiler chickens fed with aflatoxin contaminated diet." *Indian Journal of Animal Nutrition* 21 (1): 40–44.
- Ruggiero, C., F. Lattanzio, F. Lauretani, B. Gasperini, C. Andres-Lacueva, and A. Cherubini. 2009. "Ω-3 polyunsaturated fatty acids and immune-mediated diseases: inflammatory bowel disease and rheumatoid arthritis." *Current Pharmaceutical Design* 15 (36): 4135–48.
- Saino, N., V. Bertacche, R. P. Ferrari, R. Martinelli, A. P. Møller, and R. Stradi. 2002a. "Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal

- ornamentation.” *Proceedings of the Royal Society of London B: Biological Sciences* 269 (1501): 1729–33.
- Saino, N., P. Dall’Ara, R. Martinelli, and A. P. Møller. 2002b. “Early maternal effects and antibacterial immune factors in the eggs, nestlings and adults of the barn swallow.” *Journal of Evolutionary Biology* 15 (5): 735–43.
- Saláková, A., E. Straková, V. Válková, H. Buchtová, and I. Steinhauserová. 2009. “Quality indicators of chicken broiler raw and cooked meat depending on their sex.” *Acta Veterinaria Brno* 78 (3): 497–504.
- Sala-Vila, A., and P. C. Calder. 2011. “Update on the relationship of fish intake with prostate, breast, and colorectal cancers.” *Critical Reviews in Food Science and Nutrition* 51 (9): 855–71.
- Sánchez, M., J. Bernal-Castillo, C. Roza, and I. Rodríguez. 2003. “Spirulina (Arthrospira): an edible microorganism: a review.” *Universitas Scientiarum* 8 (1): 7–24.
- Sato, Y., and K. Watanabe. 1976. “Lysozyme in hen blood serum.” *Poultry Science* 55 (5): 1749–56.
- Schley, P. D., and C. J. Field. 2002. “The immune-enhancing effects of dietary fibres and prebiotics.” *British Journal of Nutrition* 87 (S2): S221–S230.
- Seya, T., 2003. Innate immune therapy for cancer: application of BCG-CWS and spirulina to patients with lung cancer. *Anticancer Res*, 23, pp.4369-76.
- Seya, T., T. Akazawa, T. Tsujita, and M. Matsumoto. 2006. “Application of Toll-like receptor agonists to vaccine adjuvant therapy.” *ECAM* 3:31–38.
- Singh, V., V. Pathak, and K. V. Akhilesh. 2012. “Modified or enriched eggs: A smart approach in egg industry: A.” *American Journal of Food Technology* 7 (5): 266–77.
- Spolaore, P., C. Joannis-Cassan, E. Duran, and A. Isambert. 2006. “Commercial applications of microalgae.” *Journal of Bioscience and Bioengineering* 101 (2): 87–96.
- Sugiharto, S., and C. Lauridsen. 2016. “Dietary Chlorella supplementation effect on immune responses and growth performances of broiler chickens exposed to post hatch holding time.” *Livestock Research of Rural Development* 28 (7): 1-6.
- Surai, P. F., and B. K. Speake. 1998. “Distribution of carotenoids from the yolk to the tissues of the chick embryo1.” *The Journal of Nutritional Biochemistry* 9 (11): 645–51.

- Surai, P. F., B. K. Speake, and N. H. C. Sparks. 2001. "Carotenoids in avian nutrition and embryonic development. 1. Absorption, availability and levels in plasma and egg yolk." *The Journal of Poultry Science* 38 (1): 1–27.
- Surai, P. F., Speake, B. K., Wood, N. A., Blount, J. D., Bortolotti, G. R., and Sparks, N. H. 2001. Carotenoid discrimination by the avian embryo: a lesson from wild birds. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 128(4): 743–750.
- Surai, A. P., P. F. Surai, W. Steinberg, W. G. Wakeman, B. K. Speake, and N. H. C. Sparks. 2003. "Effect of canthaxanthin content of the maternal diet on the antioxidant system of the developing chick." *British Poultry Science* 44 (4): 612–19.
- Świątkiewicz, S., A. Arczewska-Włosek, and D. Józefiak. 2015. Application of microalgae biomass in poultry nutrition. *World's Poultry Science Journal*, 71(4): 663–672.
- Teixeira, S. R., S. M. Potter, R. Weigel, S. Hannum, J. W. Erdman Jr., and C. M. Hasler. 2000. "Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men." *The American Journal of Clinical Nutrition* 71 (5): 1077–84.
- Thomson, L. R., Y. Toyoda, A. Langner, F. C. Delori, K. M. Garnett, N. Craft, C. R. Nichols, K. M. Cheng, and C. K. Dorey. 2002. "Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail." *Investigative Ophthalmology and Visual Science* 43 (11): 3538–49.
- Tsuji, S., M. Matsumoto, O. Takeuchi, S. Akira, I. Azuma, A. Hayashi, K. Toyoshima, and T. Seya. 2000. "Maturation of human dendritic cells by cell wall skeleton of *Mycobacterium bovis* bacillus Calmette-Guerin: involvement of toll-like receptors." *Infection and Immunity* 68 (12): 6883–90.
- Tunsaringkarn, T., W. Tungjaroenchai, and W. Siri Wong. 2013. "Nutrient benefits of quail (*Coturnix coturnix japonica*) eggs." *International Journal of Scientific and Research Publications* 3 (5): 1–8.
- Wu, L. C., J. A. A. Ho, M. C. Shieh, I. W. and Lu. 2005. "Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts." *Journal of Agricultural and Food Chemistry* 53 (10): 4207–12.
- Yu, B., J. Wang, P. M. Suter, R. M. Russell, M. A. Grusak, Y. Wang, Z. Wang, S. Yin, and G. Tang. 2012. "Spirulina is an effective dietary source of zeaxanthin to humans." *British Journal of Nutrition* 108 (4): 611–19.

CHAPTER TEN

SPIRULINA IN WATERFOWL AND PET BIRD NUTRITION

“In every walk with nature one receives far more than he seeks.”

—John Muir

10.1 Introduction

Waterfowls mainly include ducks, coots, geese, swans and flamingos which live near lakes in nature. They need water to be able to breed and grow well (Van Der Meulen and Den Dikken 2004). Today, ducks and geese are reared for eggs and meat. Other products which can also be sold include down, feathers and fattened livers called foie gras (Van Der Meulen and Den Dikken 2004). Waterfowl may consume the microalgae of the lakes in nature, and also their excreta can help the growth of microalgae (Van Der Meulen and Den Dikken 2004; Henrikson 2010). It was well documented that the use of microalgae as a feed supplement improved the yellow coloration of the bird skin and eggs' yolks in poultries (Sánchez et al. 2003). Using microalgae may improve fatty acids profiles of the birds' eggs and meat (Mooney et al. 1998; Abril 2000; Fredriksson et al. 2006; Cheng et al. 2006; Sujatha and Narahari 2011; Andrade et al. 2018). Also, *Spirulina* contains pigments that have positive effects on the color of birds' plumage (Wanjari and Dabhade 2015). Although there is such limited information about the effects of dietary *Spirulina* on waterfowls and pet birds, the aim of this chapter is to provide an outline about the effects of *Spirulina* in waterfowls' and pet birds' health and performance.

10.2 *Spirulina* in waterfowl habitat

It was demonstrated that lakes Bodou and Rombou in Chad have stable monocultures of *Spirulina* (Henrikson 2010). It is a major species in Kenya's lakes Nakuru and Elementeita and Ethiopia's lakes Aranguadi

and Kilotes, and flocks of flamingo feed entirely on algae when it is abundant (Henrikson 2010). Figure 10-1 shows the pink flamingos in an African lake.



Figure 10-1: Pink flamingos in an African lake fed on *Spirulina*. (Henrikson 2010)

It was found that using blue-green algae improved feather color and shine and promotes beneficial bacteria in the digestive tract (Chakdar et al. 2012). It was well documented that *Spirulina platensis* contains chlorophyll and two major groups of pigments called as phycobiliproteins and carotenoids (Walter et al. 2011). *Spirulina* has small amounts of phycoerythrin (PE) and allophycocyanin (AP); however, the amount of phycocyanin (PC) is the highest among phycobiliproteins of the algae (Walter et al. 2011). PC may be used as a natural colorant. It is environmentally friendly, non-toxic and non-carcinogenic, able to scavenge alkoxyl, hydroxyl and peroxy radicals in vitro, and inhibit microsomal lipid peroxidation (Chakdar et al. 2012). Carotenoids are natural colorants that support the antioxidant and immune systems (Amotz 1987). Chlorophyll-a extracts from *Spirulina* have iron oxide and alcohols such as cetyl and stearyl, which can absorb odors. Also, the alcohol moiety of chlorophyll is a precursor for synthesis of vitamin A, E and K₂ (Chakdar et al. 2012).

On the other hand, it has been reported that the mortality rate of lesser flamingos (*Phoenicopeterus minor*) was considerable in the alkaline-saline Rift Valley lakes in East Africa (Thomas, Hunter, and Atkinson 2008). Further research showed that neurological signs of poisoning were seen in the birds, and their intestinal contents and fecal pellets had high concentrations of microcystins and anatoxins. This evidence suggests that

cyanotoxins may have contributed to birds' mortality. More investigation revealed that *Arthrospira fusiformis* was one of the toxin sources isolated from Lake Bogoria. It was found that the algae had the potential of producing both microcystin-YR and anatoxin-a (Ballot et al. 2004).

10.3 Algae in domestic waterfowl

Previous studies indicated that the dietary FA can be deposited into egg yolks and into other tissues of avian species (Cruickshank 1934; Donaldson 1967; Ding and Lilburn 1997). Also, it was documented that the unsaturated FA composition of egg yolks is modified by dietary FA (Cruickshank 1934). It was shown that DHA are important nutrients in reducing plasma triacylglycerol (TG) and cholesterol levels, preventing cardiovascular diseases, hypertension, platelet aggregation and arthritis in humans (Innis 1992; Simopoulos 2000). Thus, several researchers increasing n-3 PUFA levels in animal diets using fish oil, fish meal, algae, linseed, flaxseed or red crab meal that may have valuable effects on human health (Navarro et al. 1972; Hargis, Van Elswyk, and Hargis 1991; Nitsan, Mokady, and Sukenik 1999; Schumann, Squires, and Leeson 2000; López-Ferrer et al. 2001; Howe et al. 2002; Carrillo-Dominguez et al. 2005).

Schiavone et al. (2004) reported that using dietary fish oil increased the amount of long-chain PUFAs in duck meat. However, the European Union banned the use of animal proteins in animal nutrition (decision00/766/EU). Also, considering the consumers' preferences increased the interest in formulating diets for birds using vegetable feedstuffs (Schiavone et al. 2007). Cheng et al. (2006) arranged a trial to evaluate the effects of dietary DHA on fatty acid profiles in egg yolks and various tissues of laying *Tsaiya* ducks. Experimental diets contained 0% (control diet), 0.5% or 2% algal DHA oil. They found that using algal DHA oil increased the DHA content in egg yolks, plasma, liver and skeletal muscle of the ducks. Dietary DHA treatments had no significant effect on the levels of triacylglycerol (TG) and cholesterol in plasma of laying *Tsaiya* ducks. Also, the mRNA abundance of sterol regulatory element-binding protein (SREBP1 and SREBP2) in the livers of laying *Tsaiya* ducks was not affected by dietary DHA. It was well documented that SREBP1 and SREBP2 regulate two important enzymes involved in FA synthesis in the liver: fatty acid synthase (FAS) and 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (Semenkovich 1997; Horton et al. 1998; Gondret et al. 2001; Cheng et al. 2006).

El-Deek and Brikaa (2009a) conducted two trials to evaluate the effect of seaweed as a feedstuff in starter and finisher diets of ducks. In trial 1, experimental diets included 0, 4, 8 and 12% seaweed that ducks had access to from 1 day to 5 weeks of age. In trial 2, experimental diets contained 0, 5, 10 and 15% seaweed, and ducks had access to it during 35–63 days of age. They concluded that seaweed can be used in ducks' starter and finisher diets up to 12% and 15%, respectively, without any adverse effect on growth performance and carcass quality of the birds.

In another study, El-Deek and Brikaa (2009b) studied the nutritional value of red seaweed (*Polysiphonia spp.*) as a feedstuff for poultry and assessed the use of algae as a pellet binder in duck diets. Chemical analysis revealed that the algae had considerable amounts of protein (32.4%), EE (17.7%), crude fiber (14.9%), ash (6.0%) and nitrogen-free extract (23.4%). The estimation of essential amino acid index (EAAI) was 63.34%, the average of total protein efficiency (TPE) was 1.26 and the metabolizable energy value of marine seaweed was 3518 kcal/kg. El-Deek and Brikaa (2009b) found that using red seaweed at the level of 3% could improve pellet hardness quality of duck feed, besides improving the overall nutrient profile of the diet.

Islam et al. (2009) stated that *Spirulina* had the potential of reducing heavy metals and nephrotoxic substances from the bodies of ducks. The mentioned researchers evaluated the effects of *Spirulina* in arsenic-induced toxicities in ducks. Ducklings were fed with 100 mg arsenic trioxide/L drinking water and three levels of algae (30, 60 and 120 mg *Spirulina*/L drinking water) for 90 days starting from day 15. The results showed that using *Spirulina* in drinking water could improve the reduction of BWG, total erythrocyte count (TEC), hemoglobin (Hb) and packed cell volume (PCV) of ducks suffering from arsenic toxicity. Islam et al. (2009) concluded that *Spirulina* may be helpful for reducing the tissue burden of arsenic in ducks.

Oh et al. (2015) evaluated the effects of fermented *Chlorella* biomass (at the levels of 0.1 or 0.2%) on growth performance, meat quality, cecal microflora and tibia quality of *Pekin* ducks. They found that the microalgae had a positive effect on FI, BWG, meat quality and tibia breaking strength; however, there was no difference in the population of cecal microflora.

Schiavone et al. (2007) conducted a trial to study the effects of dietary supplementation of microalgae (*Cryptocodinium cohnii*) in *Muscovy*

ducks (*Cairina moschata domestica* L.). They demonstrated that microalgae at the level of 5g/kg diet could increase DHA concentration in ducks' meat without affecting growth performances or slaughter traits. The chemical composition, color, pH, oxidative stability and sensory characteristics of the breast meat were not affected by dietary treatments. Figure 10-2 shows a closed system for rearing meat-type ducks.



Figure 10-2: Rearing *Pekin* ducks for meat production in a closed system. (Photograph by authors)

Also, duck rearing may be managed with fish production. The manure of the ducks fertilizes the pond and increases the growth of water plants, algae and planktons which are duck and fish feed (Van der Meulen and Den Dikken 2004). In a recent study, our research team examined the effect of dietary supplementation *Spirulina platensis* (0, 0.1, 0.15 and 0.2% of diet) on rainbow trout performance for eight weeks. Figure 10-3 shows the trial pools. The initial body weight of trout was about 10.06 g. Results showed that adding *Spirulina platensis* at the level of 0.2% improved BWG, FCR and livability of rainbow trout (Gholizadeh et al. 2017).

Spirulina may be used by aquaculture companies to promote the growth rates, augment disease resistance, support survival rates, decrease medication requirements, and increase quality and coloration of various fish and shellfish (Moorhead, Capelli, and Cysewski 2011).



Figure 10-3: Pools of reared rainbow trout fed with diets containing *Spirulina platensis*. (Photograph by authors)

10.4 Pet birds

In most birds, red, orange and yellow plumage coloration is produced by carotenoid pigments (McGraw 2006). No birds can synthesize carotenoids *de novo*, but some birds can biochemically modify the carotenoids that they ingest (Brush 1981). Adding *Spirulina* for pet birds seems to improve the birds' appearance, healthiness and livability (Henrikson 2010). The guts of the birds absorb carotenoids efficiently (Ziswiler and Farner 1972; Fisher 1972). Ingested carotenoids are transported to different organs by the plasma. This may occur as free molecules, but carotenoids are usually associated with plasma lipoproteins (Cheesman, Lee, and Zagalsky 1967; Trams 1969). Also, the presence of a carotenoid-binding protein in the blood of laying hens was reported by Lush (1963). Carotenoids may be stored in the liver, fat bodies or integument and often go through chemical modification (Brush and Power 1976). It was shown that house finches without a dietary source of suitable carotenoids regenerated feathers with abnormal colors that were usually yellow (Brush and Power 1976).

Canaries have the ability to deposit xanthophylls in their feathers for producing the yellow feather coloration (Stradi 1999). With regards to the report of Koch, McGraw, and Hill (2016), red-factor canaries have the ability to convert β -carotene, lutein, zeaxanthin, and β -cryptoxanthin into the red ketocarotenoids, a-doradexanthin, canthaxanthin, and echinenone (Figure 10-4).

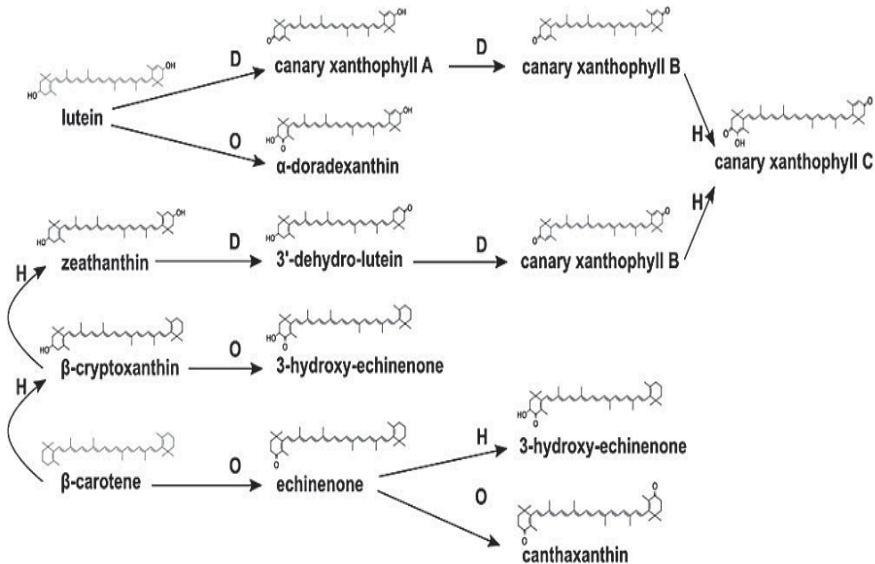


Figure 10-4: Chemical structures and conversion pathways of four dietary carotenoids (lutein, zeaxanthin, β -cryptoxanthin and β -carotene) into the metabolically altered carotenoids in canary feathers (canary xanthophylls A, B, and C, echinenone, canthaxanthin and α -doradexanthin). The capital letters above the arrows show the nature of the reaction: D = dehydrogenation, H = hydroxylation, and O = oxidation (McGraw 2006).

Producers of canaries, finches, parrots, lovebirds and other pet birds can use *Spirulina* to increase coloration and accelerate growth rate and sexual maturity, and improve fertility rates (Henrikson 2010). Figure 10-5 shows a healthy bird of the *Psittaciformes* order (Malango parrot) fed with a diet that contained *Spirulina* as an organic additive.

Also, ostrich and turkey breeders may use *Spirulina* to increase fertility and reproduction rates. It enhances desirable yellow skin coloration in chickens and increases the deep yellow color of egg yolks (Henrikson 2010).



Figure 10-5: A healthy Malango parrot fed on *Spirulina* as a feed additive. (Photograph by authors)

References

- Abril, J. R. 2000. "Safe use of microalgae (DHA GOLDTM) in laying hen feed for the production of DHA-enriched eggs." *Egg Nutrition and Biotechnology* 197–202.
- Amotz, B. 1987. "Presentation to polysaccharides from micro algae." Workshop, Duke University, USA.
- Andrade, L. M., C. J. Andrade, M. Dias, C. A. O. Nascimento, and M. A. Mendes. 2018. "Chlorella and Spirulina Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; An Overview." *MOJ Food Processing & Technology* 6 (1): 45–58.
- Ballot, A., L. Krienitz, K. Kotut, C. Wiegand, J. S. Metcalf, G. A. Codd, and S. Pflugmacher. 2004. "Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita." *Journal of Plankton Research* 26 (8): 925–35.
- Brush, A. H. 1981. "Carotenoids in wild and captive birds." In *Carotenoids as Colourants and Vitamin A Precursors*, 539–62. Academic Press, New York-London.
- Brush, A. H., and D. M. Power. 1976. "House finch pigmentation: carotenoid metabolism and the effect of diet." *The Auk* 93 (4): 725–39.
- Carrillo-Dominguez, S., M. E. Carranco-Jauregui, R. M. Castillo-Dominguez, M. I. Castro-Gonzalez, E. Avila-Gonzalez, and F. Perez-Gil. 2005. "Cholesterol and n-3 and n-6 fatty acid content in eggs from laying hens fed with red crab meal (*Pleuroncodes planipes*)." *Poultry Science* 84 (1): 167–72.

- Chakdar, H., S. D. Jadhav, D. W. Dhar, and S. Pabbi. 2012. "Potential applications of blue green algae." *Journal of Scientific and Industrial Research* 71:13–20.
- Cheesman, D. F., W. L. Lee, and P. F. Zagalsky. 1967. "Carotenoproteins in invertebrates." *Biological Reviews* 42 (1): 131–60.
- Cheng, C. H., B. R. Ou, T. F. Shen, and S. T. Ding. 2006. "Effects of dietary algal docosaheaxaenoic acid oil supplementation on fatty acid deposition and gene expression in laying Tsaiya ducks." *Asian Australian Journal Of Animal Sciences* 19 (7): 1047–53.
- Cruickshank, E. M. 1934. "Studies in fat metabolism in the fowl: The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amounts of different fats." *Biochemical Journal* 28 (3): 965.
- Ding, S. T., and M. S. Lilburn. 1997. "Inclusion of coconut oil in diets for turkey breeders and its effects on embryonic yolk and liver fatty acids." *Poultry Science* 76 (12): 1714–21.
- Donaldson, W. E. 1967. "Lipid composition of chick embryo and yolk as affected by stage of incubation and maternal diet." *Poultry Science* 46 (3): 693–97.
- El-Deek, A. A., and A. M. Brikaa. 2009a. "Effect of different levels of seaweed in starter and finisher diets in pellet and mash form on performance and carcass quality of ducks." *International Journal of Poultry Science* 8 (8): 1014–21.
- El-Deek, A. A., and M. A. Brikaa. 2009b. "Nutritional and biological evaluation of marine seaweed as a feedstuff and as a pellet binder in poultry diet." *International Journal of Poultry Science* 8 (9): 875–81.
- Fisher, H. 1972. "The nutrition of birds." *Avian Biology* 2:431–69.
- Fredriksson, S., K. Elwinger, and J. Pickova. 2006. "Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens." *Food Chemistry* 99 (3): 530–37.
- Gholizadeh, F., M. S. Ansari, H. Hajati, N. Soltani, A. Farhadi, S. Karimzadeh, S. M. Alavi. 2017. "The effect of different levels of *Spirulina platensis* algae in performance of rainbow trout fish." *Algae and Water Plants National Conference*, October 30 and 31, 2017, Tehran, Iran.
- Gondret, F., P. Ferré, and I. Dugail. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. *Journal of Lipid Research*, 42(1), 106–113.
- Hargis, P. S., M. E. Van Elswyk, and B. M. Hargis. 1991. "Dietary modification of yolk lipid with menhaden oil." *Poultry Science* 70 (4): 874–83.

- Henrikson, R. 2010. *Spirulina World Food: How this micro algae can transform your health and our planet*. Ronore Enterprises, Incorporated, Hana, Maui, Hawaii
- Horton, J. D., Y. Bashmakov, I. Shimomura, and H. Shimano. 1998. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proceedings of the National Academy of Sciences*, 95(11), 5987-5992.
- Howe, P. R., J. A. Downing, B. F. Grenyer, E. M. Grigonis-Deane, and W. L. Bryden. 2002. "Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs." *Lipids* 37 (11): 1067-76.
- Innis, S. M. 1992. "n-3 Fatty acid requirements of the newborn." *Lipids* 27 (11): 879-85.
- Islam, M. S., M. A. Awal, M. Mostofa, F. Begum, A. Khair, and M. Myenuddin. 2009. "Effect of Spirulina on biochemical parameters and reduction of tissue arsenic concentration in arsenic induced toxicities in ducks." *International Journal of Poultry Science* 8 (1): 69-74.
- Koch, R. E., K. J. McGraw, and G. E. Hill. 2016. "Effects of diet on plumage colouration and carotenoid deposition in red and yellow domestic canaries (*Serinus canaria*)." *The Wilson Journal of Ornithology* 128 (2): 328-33.
- López-Ferrer, S., M. D. Baucells, A. C. Barroeta, J. Galobart, and M. A. Grashorn. 2001. "n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: linseed oil." *Poultry Science* 80 (6): 753-61.
- Lush, I. E. 1963. "The relationship of egg-laying to changes in the plasma proteins of the domestic fowl." *British Poultry Science* 4 (3): 255-60.
- McGraw, K. J., and G. E. Hill. 2006. "Mechanics of carotenoid-based colouration." *Bird Colouration* 1:177-242.
- Mooney, J. W., E. M. Hirschler, A. K. Kennedy, A. R. Sams, and M. E. Van Elswyk. 1998. "Lipid and flavour quality of stored breast meat from broilers fed marine algae." *Journal of the Science of Food and Agriculture* 78 (1): 134-40.
- Moorhead, K., B. Capelli, and G. R. Cysewski. 2011. *Spirulina: Nature's Superfood*. Kailua Kona, HI: Cyanotech Corporation.
- Navarro, J. G., J. C. Saavedra, F. B. Borie, and M. M. Caiozzi. 1972. "Influence of dietary fish meal on egg fatty acid composition." *Journal of the Science of Food and Agriculture* 23 (11): 1287-92.
- Nitsan, Z., S. Mokady, and A. Sukenik. 1999. "Enrichment of poultry products with ω 3 fatty acids by dietary supplementation with the alga *Nannochloropsis* and mantur oil." *Journal of Agricultural and Food Chemistry* 47 (12): 5127-32.

- Oh, S. T., L. Zheng, H. J. Kwon, Y. K. Choo, K. W. Lee, C. W. Kang, and B. K. An. 2015. "Effects of dietary fermented *Chlorella vulgaris* (CBT®) on growth performance, relative organ weights, cecal microflora, tibia bone characteristics, and meat qualities in Pekin ducks." *Asian-Australasian Journal of Animal Sciences* 28 (1): 95.
- Sánchez, M., J. Bernal-Castillo, C. Rozo, and I. Rodríguez. 2003. "Spirulina (Arthrospira): an edible microorganism: a review." *Universitas Scientiarum* 8 (1): 7–24.
- Schiavone, A., R. Chiarini, M. Marzoni, A. Castillo, S. Tassone, and I. Romboli. 2007. "Breast meat traits of Muscovy ducks fed on a microalga (*Cryptocodinium cohnii*) meal supplemented diet." *British Poultry Science* 48 (5): 573–79.
- Schiavone, A., I. Romboli, R. Chiarini, and M. Marzoni. 2004. "Influence of dietary lipid source and genotype on fatty acid composition of Muscovy duck meat." *Journal of Animal Physiology and Animal Nutrition*, 88:88–93.
- Schumann, B. E., E. J. Squires, and S. Leeson. 2000. "Effect of dietary flaxseed, flax oil and n-3 fatty acid supplement on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens." *British Poultry Science* 41 (4): 465–72.
- Semenkovich, C. F. (1997). Regulation of fatty acid synthase (FAS). *Progress in lipid research*, 36(1), 43-53.
- Simopoulos, A. P. 2000. "Human requirement for N-3 polyunsaturated fatty acids." *Poultry Science* 79 (7): 961–70.
- Stradi, R. 1999. Pigmenti e sistematica degli uccelli. In: Brambilla L, Canali G, Mannucci E, Massa R, Saino N, Stradi R, Zerbi G, editors. Colori in volo: il piumaggio degli uccelli. Università degli Studi di Milano; Milan, Italy. pp. 117–46.
- Sujatha, T., and D. Narahari. 2011. "Effect of designer diets on egg yolk composition of 'White Leghorn' hens." *Journal of Food Science and Technology* 48 (4): 494–97.
- Thomas, N. J., D. B. Hunter, and C. T. Atkinson, eds. 2008. *Infectious Diseases of Wild Birds*. Blackwell publishing. 484 pages.
- Trams, E. G. 1969. "Carotenoid transport in the plasma of the scarlet ibis (*Eudocimus ruber*)." *Comparative Biochemistry and Physiology* 28 (3): 1177–84.
- Van der Meulen, S. J., and G. Den Dikken. 2004. "Duck keeping in the tropics." 2nd ed. Edited by Arno Overgaag. Digigrafi, Wageningen, the Netherlands. ISBN: 90-77073-85-X. 80 pages.
- Walter, A., J. C. D. Carvalho, V. T. Soccol, A. B. B. D. Faria, V. Ghiggi, and C. R. Soccol. 2011. "Study of phycocyanin production from

- Spirulina platensis under different light spectra.” *Brazilian Archives of Biology and Technology* 54 (4): 675–82.
- Wanjari, H. V., and D. S. Dabhade. 2015. “Lonar Crater Lake of India: An Abundant Source of Highaly Economic Important Spirulina.” *ICSTS (2015) International Journal Of Researches in Biosciences, Agricultuer and Technology. Vishvashanti Multipurpose Society, Publication IJBAT* 2 (3): 249–41.
- Ziswiler, V., and D. S. Farner. 1972. “Digestion and the digestive system.” *Avian Biology* 2:343–430.

GLOSSARY

Albumen:	White of eggs
Algae:	A large, diverse group of photosynthetic organisms that are not necessarily closely related. They are capable of using sunlight to convert carbon dioxide into sugars and oxygen during the process of photosynthesis
Amino acids:	The building blocks of protein
Antibiotic:	Substances produced by moulds and bacteria capable of destroying or preventing the growth of bacteria
Antibody:	Substances formed in the blood tending to inhibit or destroy harmful bacteria, etc.
Antioxidant:	Substances that prevent oxidation
Astaxanthin:	A red-orange carotenoid pigment that is a powerful biological antioxidant
Avian:	Relating to birds
Bacteriocins:	Substances produced by some bacteria which are encoded in the plasmids with the purpose of killing or inhibiting closely related species or even different strains of the same species
Battery cage:	Series of boxes in which hens are kept for laying eggs or for fattening
Biologic value (BV):	A measure of the nutrient retained within the body in relation to the nutrient absorbed

Broiler Breeder:	Female and male birds who are the parents of broiler chickens. These hens and roosters mate to produce fertilized eggs, which are transferred to broiler hatcheries for incubation.
Broiler chicken:	Fast-growing chickens that are raised for meat
Carcass:	Dead body of an animal that was slaughtered or died naturally
Carbohydrate:	Organic compounds containing carbon, hydrogen and oxygen, such as starch and sugars. They are considered as energy sources in poultry diets.
Chick:	Young bird just before or after hatching
<i>Chlorella</i>:	Genus of green microsalgae (family <i>Chlorellaceae</i>) found either singly or clustered in fresh or salt water and in soil
Chlorophyll:	Green coloring matter in the leaves of plants
Coccidiosis:	A parasitic disease of birds and animals that mainly affects the intestines
Crop:	Bag-like part of a birds' esophagus where feed is retained for soaking before passing into the stomach
Digestibility coefficient (DC):	The proportion of nutrients contained in feeds that are actually absorbed
Digestive tract:	The alimentary canal
Droppings:	Waste matter dropped from the body of the bird; bird manure
Embryo:	Offspring of an animal in the early stage of its development before birth (or before coming out of an egg)

Endogenous:	Growing or originating from within an organism
Essential amino acids:	Amino acids that the body cannot synthesize in enough quantities to meet the body requirement and must be supplied by the diet
Feather:	One of the light coverings that grow from a bird's skin
Feed intake:	The amount of feed consumed by a bird
Feed conversion ratio:	The ratio of feed consumed per unit of increase in birds' weight
Feedstuffs:	Food ingredients for animals
Fertility:	The ability of producing normal gametes with the potential of producing a zygote from which an embryo can develop
Fowl:	Any bird, but commonly refers to domestic birds
Finch:	A kind of small bird
Flamingo:	Large, long-legged, long-necked wading bird with pink feathers
Foie gras:	The French term means "fatty liver"; foie gras is a luxury food product made of the liver of a duck or goose that has been specially fattened
Hatch:	Break out of an egg by natural or artificial incubation
Hatchability:	The ability of an embryo to hatch from the egg
Haugh unit:	An index of egg protein quality based on the height of the egg white
Hen:	A female fowl that is more than one year old

Lake:	Large area of water enclosed by land
Layers:	Hens producing eggs for food
Macrophage:	The specialized cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms
Minerals:	The ash portions of feed which are essential for birds to reach their productive performance
Net protein utilization (NPU):	The percentage of protein ingested that remains within the organism
Nutrition:	The process of supplying and receiving nourishment
Organic acids:	Organic compounds that possess acidic properties. Organic acids have been used for thousands of years as food preservatives and have recently been used as a feed additive for maintaining poultry gut health.
Ovary:	Either of the two reproductive organs in which ova are produced in female animals.
Oxidative stress:	Disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses
Palatability:	The attractiveness of the feed for birds
Parrot:	A sort of bird with a hooked bill and brightly colored feathers, some kinds of which can be trained to imitate human speech
Partridge:	A sort of bird of the same family as the pheasant
Pathogens:	An agent causing infectious disease
Pheasant:	Long-tailed game bird

Phosphorus:	A mineral that is present in bones, egg shells, nerves and other tissues
Plumage:	Poultry feathers
Poultry:	Large domestic fowl kept for eating or egg laying
Prebiotics:	A non-digestible feed ingredient that promotes the growth of beneficial microorganisms in the intestines, like FOS, MOS, GOS, XOS and inulin
Probiotics:	Live microbial supplements or feed ingredients that have a beneficial effect on the consumer intestinal health. <i>Bifidobacterium</i> and <i>Lactobacillus</i> genera are the main probiotic microorganisms.
Progeny:	Offspring
Proteins:	Complex combination of amino acids that present in large amounts in body tissues
Protein efficiency ratio (PER):	Weight gain of a test bird divided by its protein intake during the specific experimental period
Rancid:	With the smell or taste of stale, decaying fat or butter
Salmon:	Large fish, valued for food and the sport of catching it with rod and line
Semen:	Fertilizing sperm-bearing fluid of male animals
Spirulina:	A blue-green alga with high nutrient content
Symbiotic:	Combining probiotics and prebiotics in feed
Toxin:	A poisonous substance, produced within living cells or microorganisms that are capable of causing particular diseases
Viability:	Ability to exist

INDEX

- A. jenneri* 24
Abdominal fat 114, 150, 167
ABTS 98
Aflatoxin 147
Alanine 42, 44, 110, 115, 137
Albumen percentage 136
Algae 1, 4, 5, 6, 7, 8, 9, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 41, 43, 44, 45, 46, 47, 48, 50, 51, 53, 54, 55, 64, 66, 67, 73, 77, 91, 95, 97, 98, 99, 108, 109, 112, 115, 116, 119, 120, 131, 132, 133, 135, 136, 137, 138, 139, 147, 148, 150, 154, 156, 165, 166, 170, 173, 185, 186, 187, 188, 189
Alkaloids 2, 64, 65
Alkoxy 97, 186
Alpha-linolenic acid 138
Amides 63
Anatoxin-a 187
Anthocyanins 74
Anthraquinones 74
Antibacterial 2, 3, 63, 65, 66, 67, 68, 69, 73, 74, 90, 117, 147, 175
Antibiotics 1, 2, 63, 108, 130
Antibody 77, 130, 168, 169
Antifungal 63, 74, 109
Anti-inflammatory 2, 109, 119, 137, 169
Antimicrobial 2, 3, 63, 64, 65, 66, 68, 69, 116, 117, 119, 169,
Antioxidant 3, 4, 7, 49, 64, 88, 89, 90, 91, 94, 95, 96, 97, 98, 99, 118, 119, 120, 137, 145, 146, 147, 148, 150, 151, 153, 154, 156, 68, 170, 173, 174, 175
Antiviral 2, 48, 63, 75, 76, 77, 168
AOAC 40,
Aphanizomenon sp. 8
Appearance 40, 139, 167, 190
Aqueous extract 68, 98, 120
Arginine 42, 44, 110, 167
Arsenic 55, 98, 148, 188
Artemisia 4
Arteriosclerosis 174
Arthrospira Maxima 7, 22, 95
Arthrospira fusiformis 23, 24, 95, 187
Aspartic acid 42, 44
Avian 77, 145, 150, 153, 156, 187
B. cereus 73
B. subtilis 67, 73
Barn Swallow 175
Battery cages 130
BHA 89, 90
BHT 89, 90
Bioavailability 55, 99, 147
Biofuel 5, 8, 30
Biologic value 45
Biomass 5, 7, 8, 24, 25, 26, 33, 34, 54, 112, 157, 188
Biotin 49
Black cumin 4
Bone marrow 118, 168
Breast 7, 114, 138, 166, 167, 189
Broiler 3, 4, 7, 40, 45, 99, 108, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 130, 164, 169
Broiler breeder 147, 148, 149, 150, 152, 154, 156, 157, 165
Caffeic 64, 95, 120
Cairina moschata domestica L. 189,
Calcium 29, 40, 41, 42, 48, 49, 51, 52, 53, 64, 75, 76, 96, 109, 111, 118, 119, 136, 168
Calcium-Spirulan 64, 75, 76, 168

- Camellia* 4
Campylobacter 130
 Canary 191
 Cancer 3, 6, 47, 49, 50, 51, 88, 89,
 90, 91, 95, 96, 109, 118, 131,
 138, 156, 167
Candida albicans 64, 68, 75
 Canthaxanthin 147, 151, 152, 190,
 191
 Carbohydrates 24, 40, 47, 49, 67,
 89, 109,
 Carcass 4, 114, 164, 165, 166, 169,
 170, 193
 Carotenoids 20, 24, 27, 42, 46, 50,
 51, 56, 66, 77, 90, 95, 96, 98,
 131, 133, 134, 135, 146, 147,
 151, 152, 165, 173, 175, 186,
 190, 191,
 Catalase 90, 98, 120, 121, 146
 Catechins 74
 CD80 168
 CD86 168
 Cecal 7, 188
 Cellular 4, 6, 20, 50, 53, 54, 63, 64,
 66, 89, 90, 99, 117, 119, 168
 Chalcones 74
 Chemokine 168
Chlorella 7, 29, 30, 47, 48, 53, 65,
 69, 98, 108, 117, 120, 133, 148,
 165, 188
 Chlorogenic 64, 95, 120
 Chlorophyll 20, 24, 27, 28, 42, 46,
 65, 95, 98, 165, 186
 Cholesterol 3, 5, 6, 42, 63, 109, 118,
 119, 137, 138, 166, 167, 174,
 187
 Citrinin 55
Cobb 500 40
 Cohesiveness 167
 Collagen 167
 Colorant 46, 133, 186
 Commercial 5, 7, 29, 112, 114, 148,
 149, 150, 156, 164
 Cook loss 167
 Copper 51, 52, 53
 Corn 108, 110
Coturnix coturnix japonica 164, 171
 COX-2 99
 Creatine kinase 96
 Crop 1, 5, 6, 133
 Crypt depth 115, 116
Cryptocodium cohnii 188
 Cyanobacteria 4, 6, 28, 48, 53, 63,
 69, 131
 Cyanobacterium 8, 54, 108
 Cyanocobalamin 40
Cyanophyceae 23
 Cyanotoxins 120, 131, 187
 Cystatin 164
 Cystine 42, 43, 44, 110, 111
 Cytokines 168
 Digestibility 7, 43, 45, 111, 115
 DPPH 90, 98
 Drip loss 7, 167
 Droppings 130
 Drumstick 114, 166
 Ducks 185, 187, 188, 189
Dunaliella 8, 29, 67
 Duodenum 115, 116
E. coli 3, 4, 65, 67, 68, 69, 116, 117
Echineneone 190, 191
 Egg 3, 5, 43, 93, 99, 130, 131, 132,
 133, 135, 136, 137, 139, 145,
 146, 147, 148, 149, 150, 151,
 152, 164, 167, 170, 171, 172,
 173, 174, 175, 185, 187, 191
 Egg length 172
 Egg shell percentage 136
 Egg surface area 172
 Egg weight 131, 132, 151, 152, 164,
 171, 172
 Egg width 172
 Egg yolk 131, 133, 135, 136, 137,
 139, 146, 150, 167, 173, 174,
 187, 191
 Enterobacteriaceae 68
Enterococcus faecalis 68, 117
 Enzymes 40, 65, 66, 90, 98, 115,
 134, 146, 187
 Erythrocytes 99, 120, 170
 European production efficiency 3,
 111, 112, 113, 166

- FAO 4, 6, 55
 Farm 2, 20, 29, 30, 52, 53, 112, 114, 117, 130, 145
 Fat binding capacity 167
 Fatty acids 40, 48, 63, 66, 67, 76, 98, 118, 138, 139, 145, 146, 153, 165, 167, 174, 175, 185
 FDA 7
 Feather 133, 185, 186, 190, 191
 Fecal pellets 186
 Feed 1, 2, 3, 4, 5, 7, 9, 45, 46, 50, 55, 63, 77, 108, 111, 112, 117, 119, 120, 130, 132, 133, 137, 138, 139, 145, 165, 166, 167, 168, 170, 171, 185, 186, 187, 188, 189, 192
 Finch 190, 191
 Finisher 112, 188
 Fish 5, 8, 43, 45, 46, 138, 139, 187, 189, 190
 Flamingo 185, 186
 Flavonoids 65, 66, 74
 Flavor 139, 167
 Foie gras 185
 Folate 49
 Food 4, 5, 6, 7, 8, 20, 22, 30, 46, 49, 55, 88, 89, 97, 99, 130, 133
 FRAP 90
 Free radicals 50, 89, 91, 97, 98, 120, 148, 151
 Fructose 64
 Fucoidans 91
 Functional feed 1, 108
Fungi 5, 64, 75, 116
Fusarium graminearum 65
 GAE 99
 Galactose 64
 Galacturonic acid 64
 Gas emission 116, 117
 Genetically modified 1, 130
 Glucosamines 47
 Glucose 3, 48, 64, 118, 119, 121
 Glucuronic acid 64
 Glutamic acid 42, 44, 115
 Glycine 42, 44, 110, 115
 GPx 90, 120
 Grape seed extract 3
 Grapefruit 4
 GRAS 7, 98
 Gross Energy 109, 110, 111
 Grower 112
 Gut-associated lymphoid tissues 165
 Hemoglobin 95, 118, 120, 188
 Halotolerant 8
 Haugh unit 136, 171, 173
 HDL 138, 166
 Health 1, 3, 4, 5, 6, 7, 22, 30, 43, 51, 52, 53, 55, 63, 88, 89, 90, 108, 119, 145, 147, 164, 169, 170, 174, 185, 187
 Hen 5, 45, 99, 130, 131, 132, 133, 135, 136, 137, 138, 139, 145, 148, 150, 151, 152, 172, 174, 175, 190
 Hepatotoxin 120
 Heptadecane 64, 66, 68, 69, 71, 72
Hermetia illucens 109, 125
 Herpes simplex virus 64, 75
 Histidine 42, 44, 110
 Homeostatic 89
 Hormones 1, 49, 130, 156
 HSP70 3
 Humoral 119, 168, 169
 Hydrogen peroxide 88
 IL-12 168
 IL-18 168
 IL-6 168
 Ileum 4, 115, 116, 117
 Imbalance 89, 148
 Immolina 119, 168
In ovo feeding 4
 Infection 3, 64, 77, 89, 108, 174
 Inflammation 136, 167
 Interferon 150, 168
 Interleukins 168
 Iron 40, 42, 49, 51, 52, 53, 145, 186
 Isoleucine 42, 44, 110
 Jejunum 115, 116
 Juiciness 167
K. pneumonia 73
Klebsiella pneumoniae 68, 74, 116,
Lactobacillus acidophilus 117

- Lactobacillus* 4, 7, 117
Lactobacillus casei 117
 Lauric 63, 117
 LDL 90, 138, 166
 Leucine 42, 44, 110, 115
 Leukocytes 119, 169, 175
 Linoleic acid 48, 49, 145
 Lipid 5, 8, 24, 40, 48, 49, 54, 64, 66, 67, 89, 90, 97, 99, 118, 137, 138, 139, 146, 147, 150, 151, 153, 167, 186
 Litter 117
Livability 164, 175, 179, 189, 190
 Liver 51, 53, 97, 98, 112, 120, 185, 187, 190, 133, 146, 147, 150, 152
 Lutein 27, 139, 146, 173, 190, 191
 Lycopene α -or β -cyclase 134
 Lymphoid organ 119, 148, 168
 Lysine 42, 43, 44, 110, 115
 Lysozyme 21, 66, 147, 164, 175
M. luteus 73
 Macroalgae 4, 6
 Magnesium 29, 42, 51, 52, 53
 Malango 191, 192
 Manganese 42, 51, 52, 53, 98, 147
 Maturity 76, 133, 164, 170, 191
 MDR-1 99
 Measles virus 64
 Meat quality 108, 120, 165, 166, 167, 188
 Metabolic 47, 49, 88, 112
 Methionine 42, 43, 44, 110, 111, 115, 167
 Metmyoglobin 91
 Mice 168, 169
 Microalgae 4, 5, 6, 7, 8, 20, 21, 22, 26, 27, 40, 47, 48, 54, 67, 77, 91, 97, 108, 109, 112, 116, 119, 133, 136, 138, 139, 165, 167, 173, 185, 188, 189
 Microcystins (MCs) 131
 Microflora 116, 188
 Minerals 40, 42, 49, 51, 52, 53, 54, 55, 90, 108, 109, 112, 131, 132, 135, 136, 145
 Moorhen 175
 Muramic acid 47
Muscovy 188
 Mycotoxin 65, 89, 147
 Myristic acid 49
 Myxoxanthophyll 50, 95
Nannochloropsis oculata 47
 Natural 2, 3, 4, 8, 49, 50, 51, 52, 53, 63, 64, 75, 88, 89, 90, 91, 95, 109, 130, 133, 135, 146, 147, 150, 170, 174, 186
 Net protein utilization 43, 45
 Newcastle 77, 150, 169
 Niacin 42, 49, 51
 Nitric oxide 89, 97, 151
 Nucleic acids 40, 46, 111
 Obesity 30, 148, 174
 Ochratoxin A 55
 Odor 26, 167, 186
 Oleic acid 48, 49
 Organic 1, 2, 3, 5, 7, 9, 25, 28, 47, 49, 54, 55, 63, 65, 88, 95, 111, 114, 130, 145, 151, 191
Oscillatoriaceae 23
 Ostrich 191
 Ovomucoid 164
 Oxidative 88, 89, 90, 91, 94, 97, 98, 99, 139, 146, 148, 151, 153, 154, 156, 189
 Oxymyoglobin 91
P. aeruginosa 69
Palmaria palmata (dulce) 6
 Palmitic acid 48, 49
 Palmitoleic acid 49, 63, 117
 Pancreatin 115
 Pantothenic acid 49
 Parrot 191, 192
 Partridge 147, 175
 Pathogens 1, 67, 69, 130
 Patulin 55
 Penicillinacid 55
 Pepsin 115
 Performance 2, 3, 4, 5, 7, 88, 111, 112, 113, 119, 120, 145, 148, 150, 152, 156, 164, 165, 166, 171, 174, 185, 188, 189

- Phenolic acids 2, 74
 Phenylalanine 42, 44, 110
 Phoenicopter minor 186
 Phosphorus 41, 42, 47, 51, 52, 54, 109, 110, 111, 118, 119
 Phycobiliproteins 68, 97, 165, 186
 Phycocyanin 20, 27, 42, 46, 52, 63, 65, 68, 91, 95, 96, 97, 98, 99, 117, 118, 120, 121, 147, 165, 168, 186
 Phycoerythrin 20, 27, 91, 95, 186
 Phytobiotics 2
 Phytonutrients 42, 168
 Plumage 185, 190
 Polypeptides 2, 97
 Polyphenols 2, 3, 66, 67, 74, 90, 92
Porphyra spp.(laver) 6
Porphyridium 8, 67
 Poultry 28, 45, 46, 50, 90, 91, 101, 111, 121, 135, 148, 150, 151, 153, 168, 171, 173, 193,
 Poultry nutritionists 1, 108, 109, 165
 Prebiotic 1, 2, 108, 117, 165
 Precursor 49, 138, 167, 186
 Probiotic 1, 2
 Proline 42, 44, 110, 115
 Pronase 115
 Pro-oxidant 50, 96
 Prostaglandin-3 138,
 Protein efficiency 43, 45, 188
 Protein solubility 167
 Proteins 2, 6, 24, 40, 43, 49, 51, 55, 74, 89, 90, 97, 109, 164, 187
Proteus vulgaris 116
Pseudomonas aeruginosa 64, 68, 116
Psittaciformes 191
 Pulmonary 146
Pyropia spp.(nori) 6
 Quail 4, 164, 165, 166, 167, 169, 170, 171, 172, 173, 174, 175
 Rainbow trout 189, 190
 RBC 118, 119, 121
 Redness 167
 Redox status 99
 Reproduction 21, 108, 148, 150, 153, 154, 168, 191
 Reproductive 5, 88, 145, 148, 150, 154, 156
 Retina 173
 Rhamnose 48, 64
 Riboflavin 42, 49
 Ribose 64
 Rooster 153, 154, 155
 ROS 99, 147
 Ross 308 3, 7
S. aureus 67, 68, 69, 73, 116, 117
S. crater 24
S. labyrinthiformis 24
S. laxa 24
S. laxissima 24
S. lonar 24
S. marcescens 73
S. nodosa 24
S. platensis NIES-39 23
S. princeps 24
S. subsalsa 23, 53
S. texcoco 24
S. typhi 3, 67, 116, 117
Saccharomyces Cerevisiae 64, 165
 Salicylic 64, 95, 120
 Salmon 138
Salmonella 67, 68, 69, 74, 174
Saragassum latifolium 69
Saragassum wightii 69
Scenedesmus rubescens 47
Scenedesmus sp. 8
 Serine 42, 44, 110
Schizochytrium 108, 136
 Selenium 42, 51, 52, 53, 98, 147, 150, 165, 174
 Serum 3, 96, 118, 120, 137, 138, 167, 169
 Sexual maturity 133, 164, 191
 Shelf life 56, 89, 91, 167
 Shell thickness 136, 137, 171
 Shellfish 189
Shigella shigae 116
Shigella flexneri 74
Shigella sp. 69

- Skin 50, 96, 97, 133, 135, 138, 185, 191
- Sodium 25, 29, 42, 51, 52, 53, 54, 98
- Spermine 64
- Spirulina fusiformis* 25, 68, 95, 96
- Spirulina platensis* 7, 23, 24, 25, 26, 28, 40, 41, 43, 47, 48, 49, 54, 63, 64, 65, 66, 67, 68, 69, 73, 74, 75, 77, 88, 90, 91, 95, 96, 97, 98, 99, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 132, 136, 137, 147, 150, 151, 156, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 186, 189, 190
- Spleen 168
- Starter 3, 112, 188
- Sterigmatocystin 55
- Stillbenes 74
- Staphylococcus aureus* 65, 67, 68, 74, 116
- Streptococcus Aureus* 64
- Streptococcus epidermidis* 74
- Streptococcus pyogenes* 68, 74
- Streptococcus thermophiles* 117
- T helper piptides 168
- Tannins 2, 74, 76
- Taste 139
- Temperature 8, 20, 22, 25, 26, 53, 64, 88, 99
- Tenderness 167
- Tetradecane 64, 70, 71, 72
- Tetramine 64
- Tetraselmis* sp. 8
- Thiamin 42, 49
- Thigh 114, 120, 146, 166
- Threonine 42, 44, 110
- Trichothecene 55
- Thromboxane-3 138
- Thymus 168
- Tissue 1, 2, 50, 95, 98, 99, 118, 146, 148, 151, 152, 165, 168, 187, 188
- Toxic 2, 5, 54, 55, 132
- Tryptophan 42, 44
- Turkey 30, 53, 191
- Tyrosine 42, 44, 110
- Ulva* spp. 6
- Uronic acids 48
- Valine 42, 44, 110
- Viability 146, 175
- Villi 111, 115
- Virus 48, 64, 75, 76, 77, 150, 168, 169
- Vitamin A 42, 49, 51, 89, 96, 146, 186
- Vitamin B₁₂ 40, 42, 49, 50, 51, 145, 165
- Vitamin B₆ 42, 49
- Vitamin C 50, 51, 90, 98, 99, 120, 147
- Vitamin E 90, 96, 98, 99, 119, 146, 147, 149, 150, 151
- Vitamin K 42, 51
- Vitamins 3, 40, 42, 49, 51, 55, 108, 109, 112, 117, 132, 135, 145, 151, 175
- VLDL 138
- W₃ 138
- W₆ 138
- Water holding capacity 167
- Waterfowl 185, 187
- Wateriness 167
- WBC 118, 119
- Welfare 1, 4
- WHO 4, 55
- Xanthophyll 46, 95, 133, 170, 174, 190, 191
- γ -Linolenic acid 49, 63, 117, 145, 165
- Zearalenone 55
- Zeaxanthin 42, 50, 51, 95, 96, 98, 134, 147, 167, 173, 190, 191
- Zinc 42, 51, 52, 53, 98
- α -doradexanthin 191
- α -tocopherol 120, 153, 155, 174,
- β -cryptoxanthin 50, 95, 147, 190, 191
- β -carotene 8, 27, 40, 42, 46, 49, 50, 51, 91, 95, 96, 98, 134, 139, 145, 147, 167, 174, 175, 190, 91