# *Spirulina Platensis* in Poultry Nutrition

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By

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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

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# 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** | *Acquired immune deficiency syndrome* |
| **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 *spirulan* |
| **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** | *European* production efficiency index |
| **EPR** | Egg production rate |
| **ESA** | *European* 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*** | *Gas* 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** | *Human immunodeficiency virus* |
| **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*** | *Malon dialdehyde* |

| | |
|---|---|
| **MDR-1** | Multidrug resistance-1 |
| **Mg** | Magnesium |
| **MIC** | *Minimum inhibitory* concentration |
| **Mn** | Manganese |
| **MOS** | Mannan oligosaccharides |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **NASA** | National aeronautics and space administration |
| **NDF** | Neutral detergent fiber |
| ***NDV*** | *Newcastle disease* virus |
| **NF-kB** | Nuclear factor kappa |
| **NO** | Nitric oxide |
| **ORAC** | Oxygen radical absorbance capacity |
| **P** | Phosphorus |
| **P.P.M** | Part per million |
| **Pb** | Lead |
| **Pc** | *Phycocyanin* |
| **PE** | Phycoerythrin |
| **PG** | *Prostaglandins* |
| **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** | *Schizochytrium sp*. 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** | *Total dissolved solids* |
| **TEAC** | Trolox equivalence antioxidant capacity |
| **TEC** | Total erythrocyte count |
| **TG** | Triacylglycerol |
| **THP** | T helper peptides |
| **TME** | True metabolizable energy |
| **TNF** | *Tumor necrosis factor* |
| **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, antihelmintical, 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 poly-carboxylic 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.). Poultries 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 $m^2$ 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 CaCl 2 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 $37.5\pm3.1 g.m^{-2}.d^{-1}$ 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.

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# 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.*

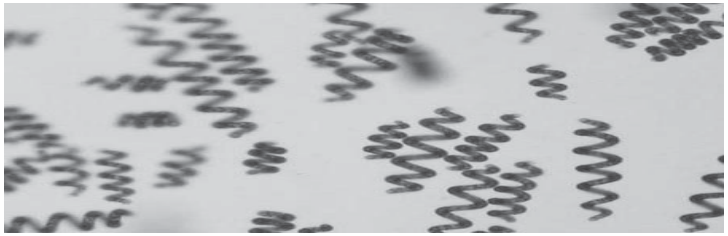

Figure 2-1: Microscopic view of the microalgae *Spirulina*. (Photograph by E. Koru 2014)

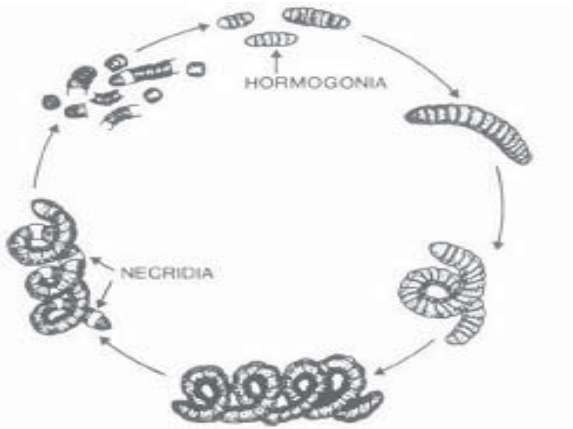


Figure 2-2: Life cycle of *Spirulina spp.* (Ciferri 1983)

*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.

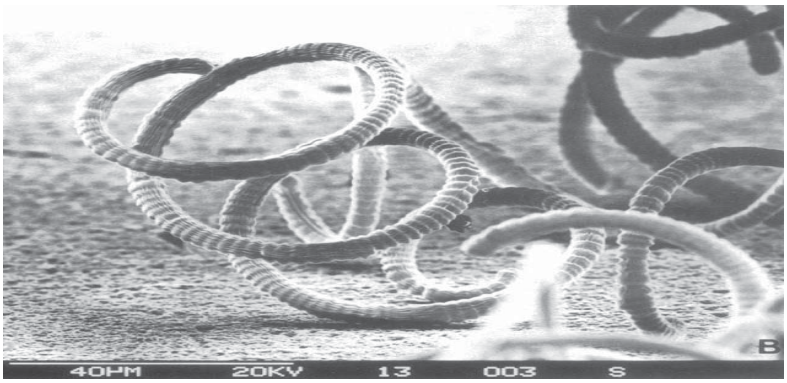


Figure 2-3: SEM image of a trichome of axenic *S. platensis* (Ciferri 1983, photograph by R. Locci).

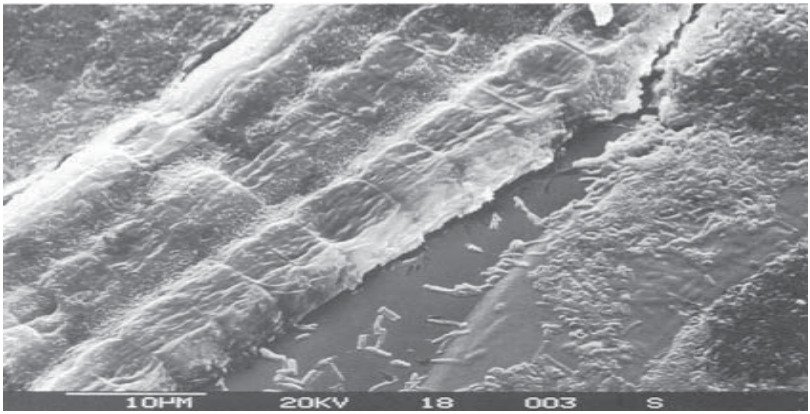


Figure 2-4: SEM image of non-axenic trichomes of *S. maxima* (Ciferri 1983, photograph by R. Locci).

*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).**

| *Spirulina* strain | *S. platensis* | *S. maxima* | *S. texcoco* | *S. crater* |
|---|---|---|---|---|
| Crude proteins | 55-60 | 49-54 | 53-58 | 56-58 |
| Total Carbohydrates | 15-18 | 13.5-15 | 10-12 | 11-13 |
| 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: growth rate = $\ln x_2 - \ln x_1/t_2 - t_1$, in which $x_1$ and $x_2$ were algae biomass concentrations and t1 and t2 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.

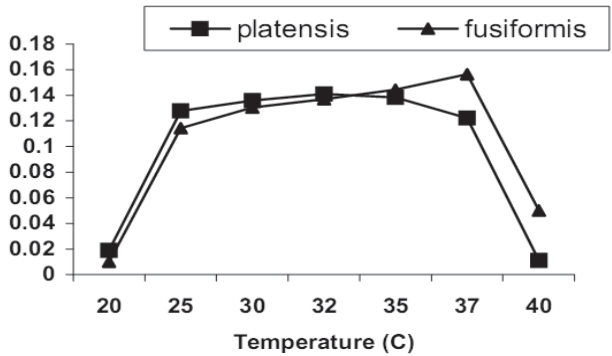


Figure 2-5: Growth rates of *S. platensis* and *S. fusiformis* at different temperatures. The values are means of three replicates. (Rafiqul Islam et al. 2003)

### 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 μmol photons $m^{-2}$ $s^{-1}$, 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 μmol photons $m^{-2}$ $s^{-1}$ 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: $l_c$: compensation light intensity, which shows that the photosynthesis and therefore algae growth increase with increasing light intensity; $l_s$: light saturation, which shows the algae growth reach plateau; and $l_h$: 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<br>630-675 | hydrophobic | Chlorophyll a<br>Chlorophyll b<br>Chlorophyll $c_1$, $c_2$, d |
| Phycobilins | blue, red | 500-650 | hydrophilic | phycocyanin<br>phycoerythrin<br>allophycocyanin |
| Carotenoids | yellow, orange | 400-550 | hydrophobic | β-carotene<br>a-carotene<br>lutein<br>violaxanthin<br>fucoxanthin |

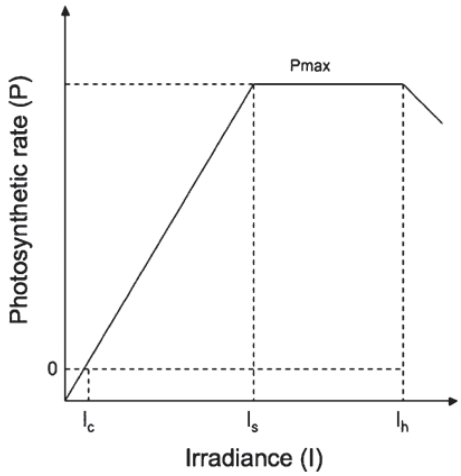


Figure 2-6: The relation between light irradiance and photosynthetic rate. (Carvalho et al. 2011)

### 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 $CO_2$ 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 (Venkatraman 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 $KNO_3$, 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 $KNO_3$/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/ $m^2$ per day for *Chlorella*, and 4.3 g/$m^2$ 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)

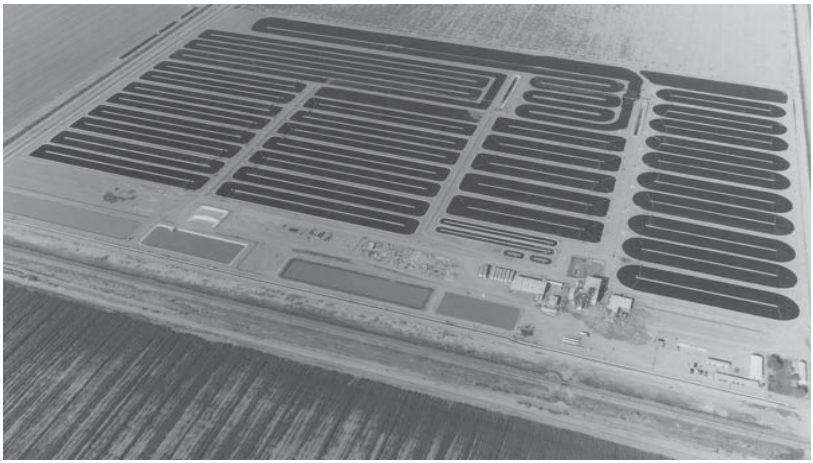

Figure 2-9: *Spirulina* production in California. (Photograph by Borowitzka)

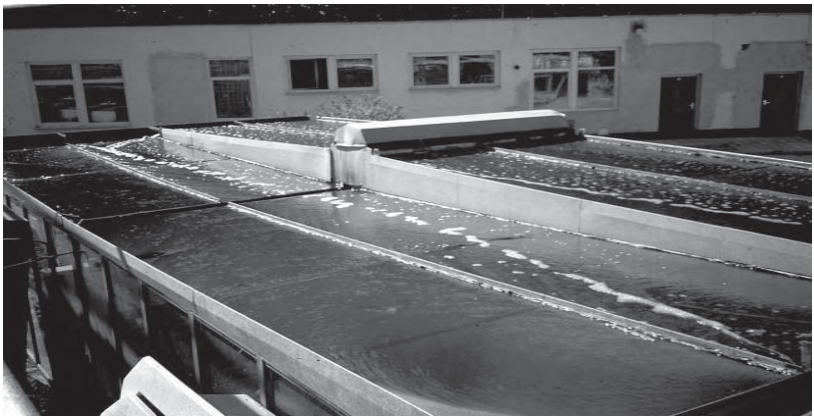

Figure 2-10: *Spirulina* production in the Czech Republic. (Photograph by Borowitzka)

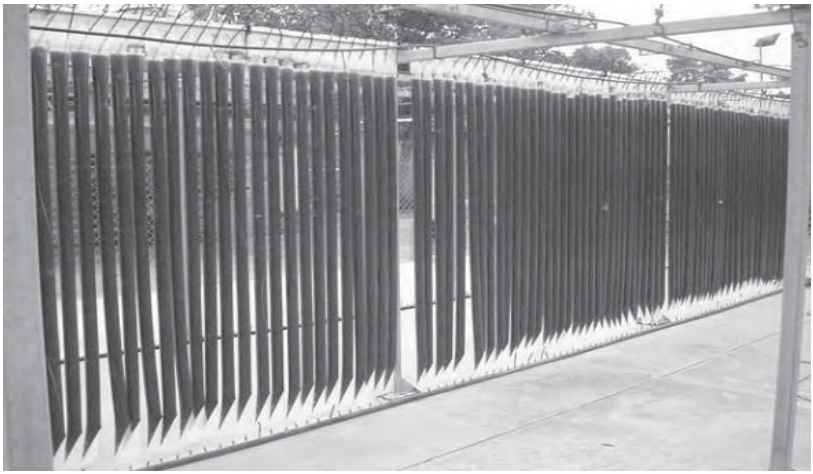

Figure 2-11: *Spirulina* bag culture in Israel. (Photograph by Borowitzka)

## 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);

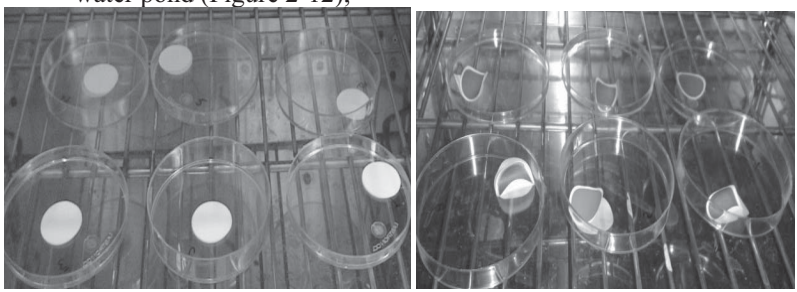

Figure 2-12: Using filter 0.45 µ for evaluating *Spirulina* biomass. (Photograph by authors)

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:

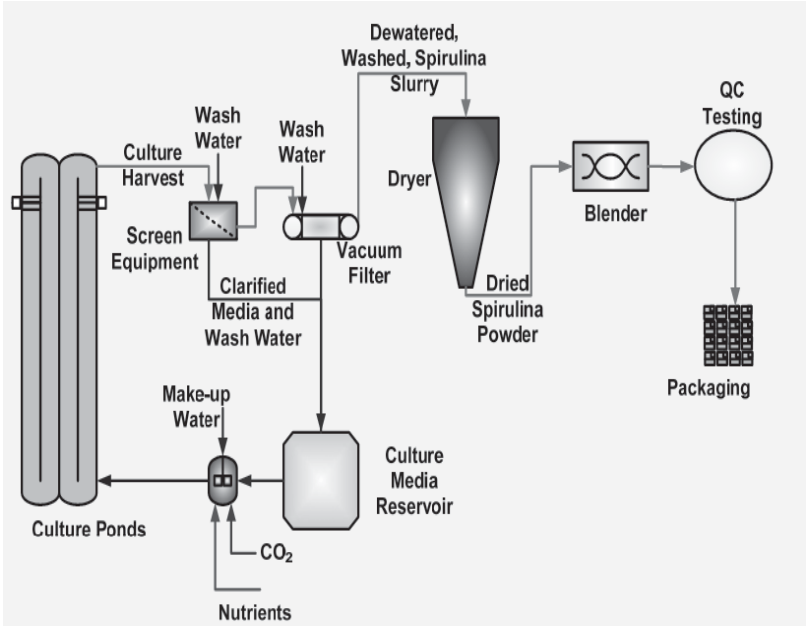


Figure 2-13: *Spirulina* production flow chart. (Cysewski 2010)

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# 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 $B_{12}$ (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)** | | | |
| Alanine | 4590 | **Phytonutrients** | |
| Arginine | 4310 | Phycocyanin | 17.2% |
| Aspartic acid | 5990 | Chlorophyll | 1.2% |
| Cystine | 590 | Super oxide dismutase (SOD) | 531.000 IU |
| Glutamic acid | 9130 | Gamma linolenic acid (GLnA) | 1080 mg |
| Glycine | 3130 | Total carotenoids | 504 mg |
| Proline | 2380 | β-carotene | 211 mg |
| Serine | 2760 | Zeaxanthin | 101 mg |
| 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 (Kyntäjä 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±0.05 | 4.63±0.07 | 8.37±0.13 | 3.42±0.1 | 4.1±0.08 | 7.04±0.14 | 5.37±0.11 | 1.98±0.05 | 0.6±0.03 |
| AMINODAT® 5.0 (EVONIK COMPANY) | 1.14 | 2.69 | 4.32 | - | 2.49 | 6.14 | 4.68 | 0.91 | 0.51 |
| Kyntäjä 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±0.06 | 4.94±0.07 | 2.81±0.06 | 3.35±0.06 | 4.02±0.06 | 4.02±0.06 | 3.85±0.1 | 10.81±0.14 | 6.66±0.1 |
| AMINODAT® 5.0 (EVONIK COMPANY) | 2.21 | 3.24 | 0.88 | 2.39 | 2.07 | 3.04 | 2.38 | 3.91 | 2.74 |
| Kyntäjä 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 *Spirulina* 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 | *Spirulina* | 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 | | | | | |
|---|---|---|---|---|---|---|
| | SID Lys | SID Met | SID Cys | SID Thr | SID Val | Reference feed ingredient |
| Algae *Spirulina* UK 2012 | 27.5 | 14.8 | 6.2 | 28.8 | 37.3 | Fish meal, CP>680 g/kg |

## 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, xantophylls 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 (Sotiroudis and Sotiroudis 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.

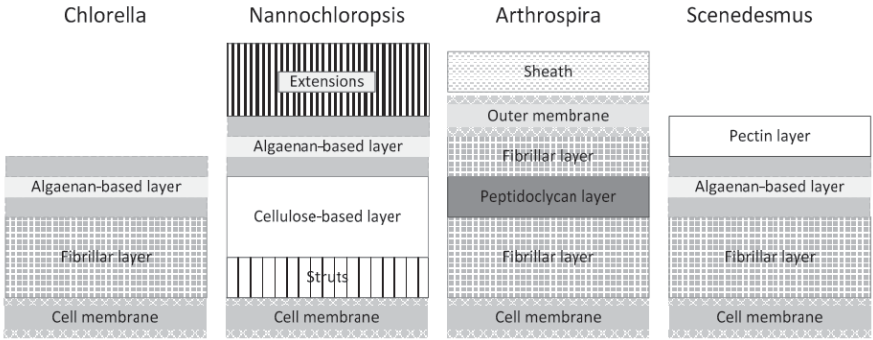


Figure 3-1: Comparing the cell wall structures of four microalgae (*Chlorella vulgaris, Nannochloropsis oculata, Arthorspira platensis* and *Scenedesmus rubescens*). (Kröger, Klemm, and Nelles 2018)

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 Ötleş and Pire (2001).

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

| Fatty acid | Content (%) |
|---|---|
| ($C_{14}$) Myristic acid | 0.23 |
| ($C_{16}$) Palmitic acid | 46.07 |
| ($C_{16:1}$) $\Delta^{9}$ Palmitoleic acid | 1.26 |
| ($C_{18:1}$) $\Delta^{9}$ Oleic acid | 5.26 |
| ($C_{18:2}$) $\Delta^{9,12}$ Linoleic acid | 17.43 |
| ($C_{18:3}$) $\Delta^{9,12,15}$ y-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 $B_6$, biotin, pantothenic acid, folate, and vitamin $B_{12}$. 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.

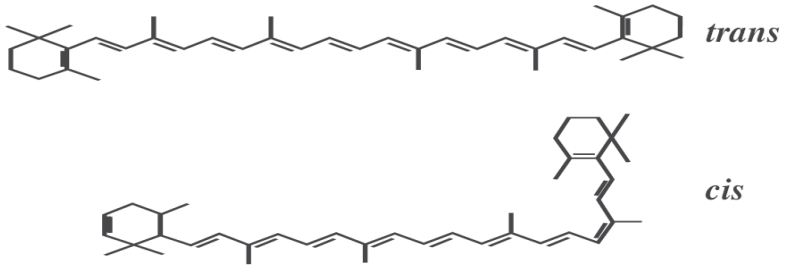


Figure 3-2: Molecular structure of two forms of β-carotene. (Moorhead et al. 2011)

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 $B_{12}$ is a bioavailable source for mammals. However, previous studies showed that pseudo vitamin $B_{12}$ (an inactive corrinoid), which predominated in the *Spirulina* tablets, is not suitable for use as a vitamin $B_{12}$ source (Watanabe et al. 2006). *Spirulina* has an exceptionally high content of vitamin $B_{12}$; 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 $B_{12}$. This vitamin is essential for normal growth and neurological function. Vitamin $B_{12}$ deficiency causes fatigue and moodiness, and eventually neurological damage. In addition, *Spirulina* is a good natural source of vitamins $K_1$ and $K_2$. Vitamin $K_1$, 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 $K_1$ has benefits for bone health, and it has beneficial effects on Alzheimer's disease patients. Vitamin $K_2$ 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 $K_2$ 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<br><br>Minerals | Belay (1997, Earthrise farms) | Kyntäjä et al. (2014) |
|---|---|---|
| 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 |
|---|---|---|
| *Spirulina* | 10 g | 10 |
| *Chlorella* | 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 μmol photon/$m^2$/second and 144 μmol photon/$m^2$/second) in batch culture. A third species of *Spirulina*, cultivated in a "Complex" medium and exposed to 66 μmol photon/$m^2$/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 μmol photon/$m^2$/second. It was reported that the chemical composition of *Spirulina sp.* cells did have a strong influence on their adsorption capacities (qmax) 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 gmax 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 Gershwin 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 Gershwin 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 Gershwin 2007)**

| | *As* | *Pb* | *Hg* | *Cd* | *reference* |
|---|---|---|---|---|---|
| Maximum allowance | *2.0* | *5.0* | *0.1* | *1.0* | IUPAC 1974 |
| *Spirulina* (India) | *0.97* | *3.95* | *0.07* | *0.62* | Becker and Venkataraman 1982 |
| *Spirulina* (Mexico) | *2.9* | *5.1* | *0.5* | *0.5* | Boudene et al. 1975 |
| *Spirulina* (Chad) | *1.8* | *3.7* | *0.5* | - | Boudene et al. 1975 |
| *Spirulina* (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 Gershwin 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.

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# 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), antinephratoxic (Khan et al. 2006) and anti-hepatoxic 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 | *Anabaena cylindrical Chlorococcum humicola, Spirulina platensis, Nostoc* | *E. coli, S. Typhimurium, K. pneuminiae, v. Cholera, S. Aureus, B. Subtilis, Streptococcus sp., Pseudomonas sp., Bacillus sp., Staphylococcus sp., E. coli, Enterobacteria aerogens* | Goud, Seshikala, and Charya (2007), Bhagavathy, Sumathi, and Madhushree (2011), Muthulakshmi et al. (2012), Fan et al. (2013) |
| Fatty acids and lipids | *Dunaliella salina, Haematococcus pluvialis, Phaeodactylum tricornutum, Chaetoceros muelleri, Spirulina platensis* | *Escherichia coli, Staphylococcus aureus, MRSA, Listonella anguillarum, Lactococcus garvieae, Vibrio spp.* | Xue et al. (2002), Herrero et al. (2006), Santoyo et al. (2009) |
| Carbohydrates | *Anabaena sphaerica, Chroococcus turgidus, Oscillatoria limnetica, S. Platensis, Porphyridium cruentum* | *E. coli, S. Typhimurium, S. Faecalis* | O'Doherty et al. (2010) |
| Polyphenols | *Anabaena sphaerica, Chroococcus turgidus, Oscillatoria limnetica Spirulina platensis* | *Salmonella typhi, Streptococcus, E. coli, Staphylococcus aureus* | Gao and Zhang (2010), Klejdus 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. pnuemoniae*. 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-dimethyethyl) | 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 | Hepatcosane | 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-dimethyethyl) | 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 | Pentafluropropionic 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 | Hepataflurobutanoic acid, heptadecyl ester | 0.94 |
| 21 | 33.65 | Octacosane | 28.66 |
| 22 | 35.86 | 1-Decanol, 2-hexyl | 3.25 |
| 23 | 36.49 | Tetrapantacontane, 1,54 -dibromo | 1.86 |
| 24 | 37.16 | Tetrapantacontane, 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-methyhexyl 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. marcescencs*) 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* |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 10 | 1.25 | 1.25 | 5 | 2.50 | 5 |
| *Streptococcus pyogenes* | 10 | 1.25 | 2.50 | 10 | 5 | 10 |
| *Streptococcus epidermidis* | 20 | 1.25 | 2.50 | 10 | 5 | 10 |
| *Proteus mirabilis* | 5 | 1.25 | 1.25 | 5 | 2.50 | 10 |
| *Bacillus cereus* | 10 | 1.25 | 1.25 | 5 | 2.50 | 10 |
| *Escherichia coli* | 10 | 2.50 | 2.50 | 10 | 5 | 5 |
| *Pseudomonas aeruginosa* | 20 | 2.50 | 5 | 10 | 10 | 5 |
| *Vibrio cholerae* | 80 | 5 | 10 | 40 | 20 | 20 |
| *Salmonella typhi* | 40 | 5 | 5 | 20 | 10 | 20 |
| *Klebsiella pneumoniae* | 10 | 1.25 | 2.50 | 5 | 2.50 | 10 |
| *Shigella flexneri* | 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, xanthones, 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* |
|---|---|---|---|---|---|---|
| *Aspergillus flavus* | 32 | 8 | 16 | 32 | 16 | 8 |
| *Aspergillus niger* | 16 | 4 | 8 | 16 | 8 | 8 |
| *Aspergillus fumigatus* | 32 | 8 | 8 | 16 | 16 | 8 |
| *Candida tropicalis* | 64 | 16 | 32 | 32 | 64 | 32 |
| *Candida albicans* | 16 | 2 | 4 | 8 | 8 | 4 |
| *Candida glabrata* | 32 | 4 | 8 | 16 | 8 | 4 |

*Flucanozole (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 Baturo 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.

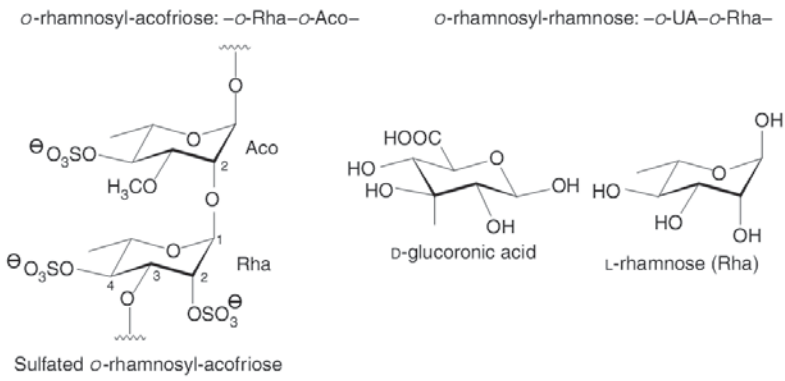


Figure 4-1: Two kinds of disaccharide repeating units of calcium-*Spirulan*. (Belay and Gershwin 2007)

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-phycocianin (Belay and Gershwin 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 algaes 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.

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# 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

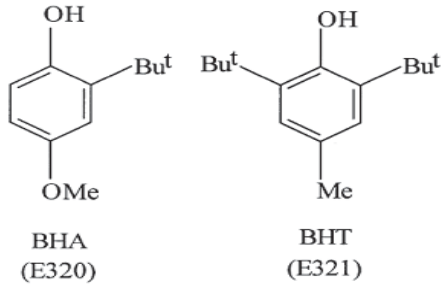


Figure 5-1: The structures of BHA and BHT.

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 | *Spirulina* (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 ß-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.

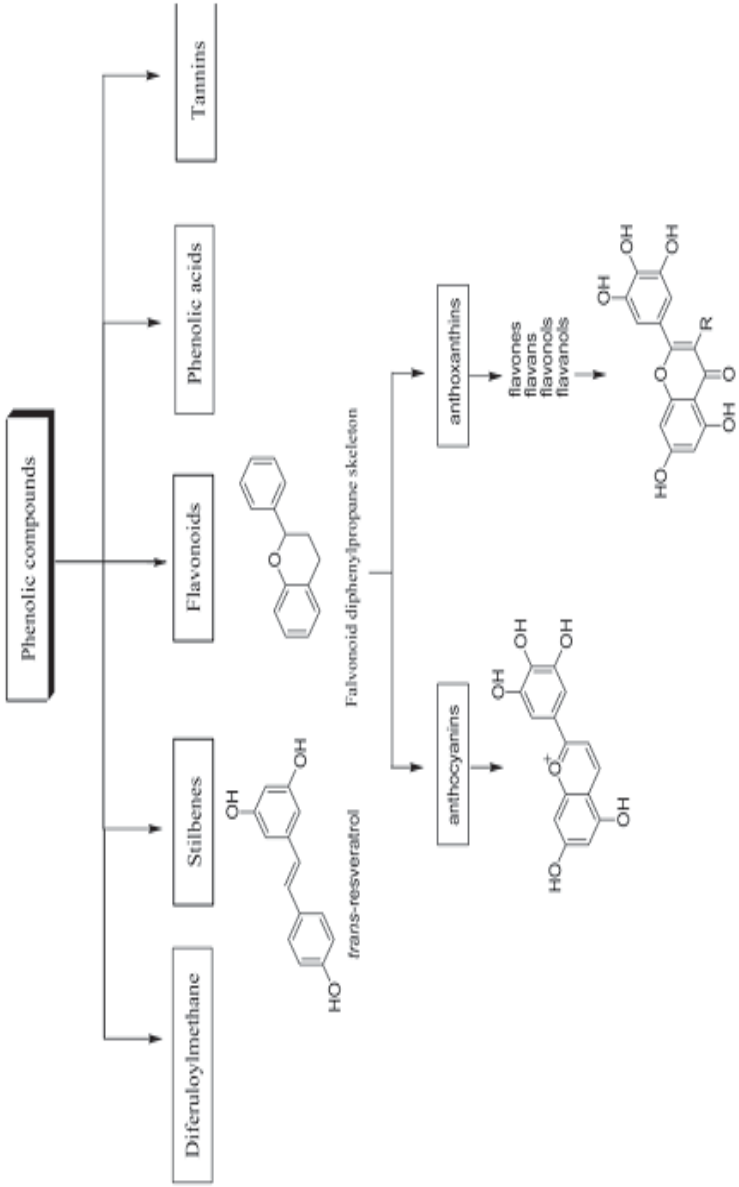


Figure 5-2: Classification of polyphenols. (Han, Shen, and Lou 2007)

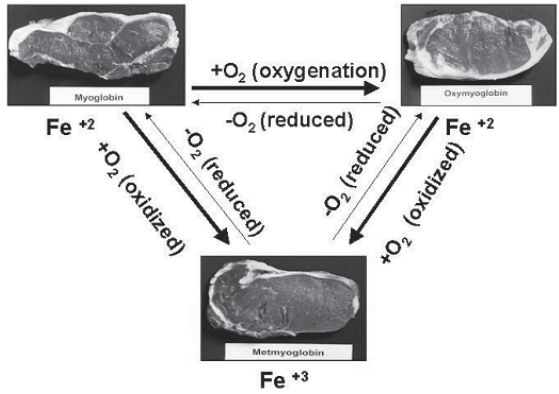


Figure 5-3: Oxidation of meat pigments. (Boles and Pegg 2010)

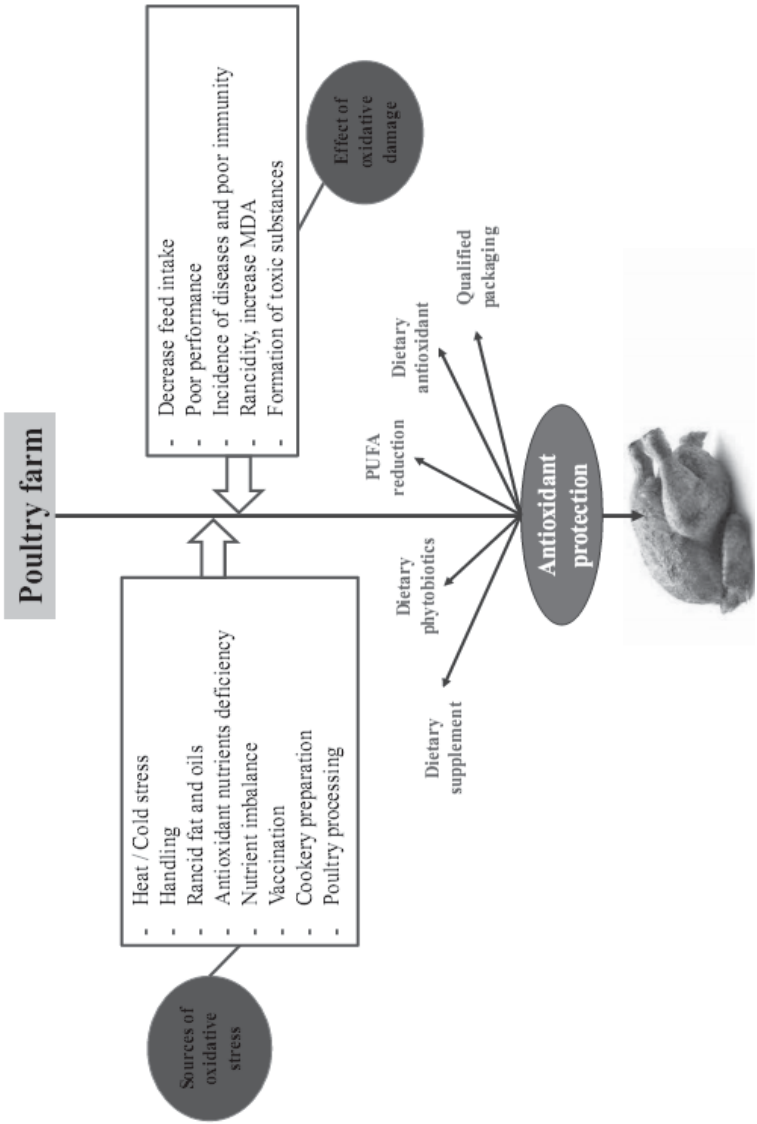


Figure 5-4: Oxidative stressors, effect of oxidation and antioxidants. (Adapted from Estévez 2015)

## 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.

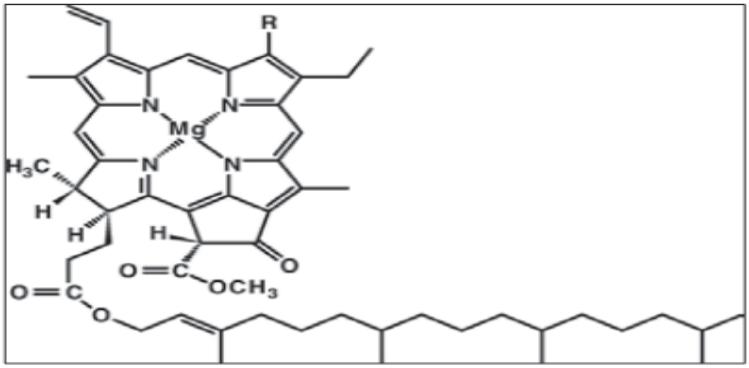


Figure 5-5: Chemical structure of chlorophyll (Asghari et al. 2016).

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 fusiform*is 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.

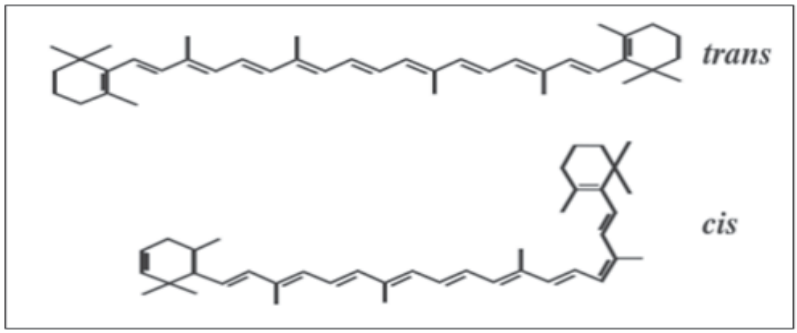


Figure 5-6: Chemical structures of two forms of β-carotene. (Asghari et al. 2016)

*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 peroxyl 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.

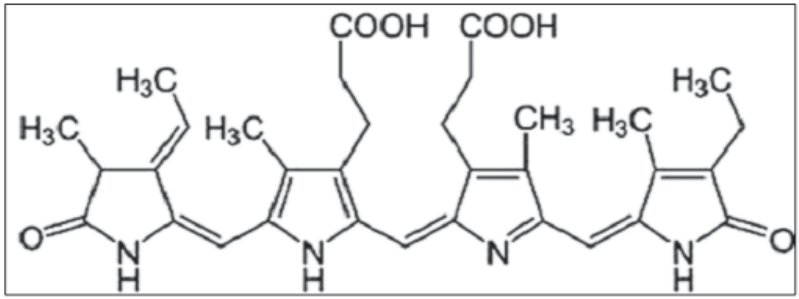


Figure 5-7: Chemical structure of C-phycocyanin. (Asghari et al. 2016)

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 $H_2O_2$ 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; Yücetepe and Ozcelik 2016). *Spirulina platensis* may control pro-inflammatory cytokine expression and secretion through repression of nuclear factor kappa (NF-kB). It was reported that activation of the NF-kB 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 cisplastin 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 ($CCl_4$) 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 peroxyl 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 ≈ 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 ± 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 |
|---|---|---|---|---|---|
| *Spirulina platensis* | $43.2\pm1.0^{a}$ | $17.0\pm0.5^{b}$ | $23.9\pm0.1^{c}$ | $18.4\pm0.1^{d}$ | $24.4\pm0.2^{c}$ |

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.

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# 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 Morais, and de Morais 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 | *Spirulina* | 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 | |
|---|---|---|---|---|
| | *Spirulina* | SBM | *Spirulina* | 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).**

*Spirulina platensis* (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)**

| | *Spirulina platensis* (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 Morais, and de Morais 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 Ibrahem 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)**

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

| | *Spirulina platensis* (g/kg diet) | | | | | |
|---|---|---|---|---|---|---|
| | Control | 1 | 1.5 | 2 | SEM | Pr>F |
| **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

Immolina, 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, 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 ß-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).

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# 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.

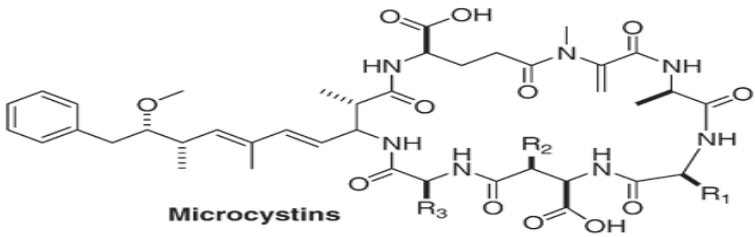


Figure 7-1: Chemical structure of microcystins. (Cardozo et al. 2007)

Some researchers reported that *Spirulina*-containing diets had a beneficial effect on FCR of laying hens (Nikodémusz et al. 2010; Mariey, Samak, and Ibrahem 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 Ibrahem 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) | $0.76^{a}\pm0.028$ | $100.275^{a}\pm1.356$ | $1.562^{a}\pm 0.029$ | $63.923^{a}$ $\pm0.801$ | $1.55^{d}\pm 0.111$ |
| 1.5% *Spirulina* | $0.81^{a}\pm0.028$ | $97.803^{a}\pm1.356$ | $1.550^{a}\pm 0.029$ | $63.198^{a}$ $\pm0.801$ | $10.55^{c}\pm0.111$ |
| 2.0% *Spirulina* | $0.85^{a}\pm0.028$ | $96.495^{a}\pm1.356$ | $1.520^{a}\pm 0.029$ | $63.598^{a}$ $\pm0.801$ | $11.43^{b}\pm0.111$ |
| 2.5% *Spirulina* | $0.76^{a}\pm0.028$ | $97.393^{a}\pm1.356$ | $1.532^{a}\pm 0.029$ | $63.473^{a}$ $\pm0.801$ | $11.66^{ab}\pm0.111$ |
| Synthetic pigment | $0.81^{a}\pm0.028$ | $98.148^{a}\pm1.356$ | $1.567^{a}\pm 0.029$ | $62.663^{a}$ $\pm0.801$ | $11.93^{a}\pm 0.111$ |
| 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 Ibrahem 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.

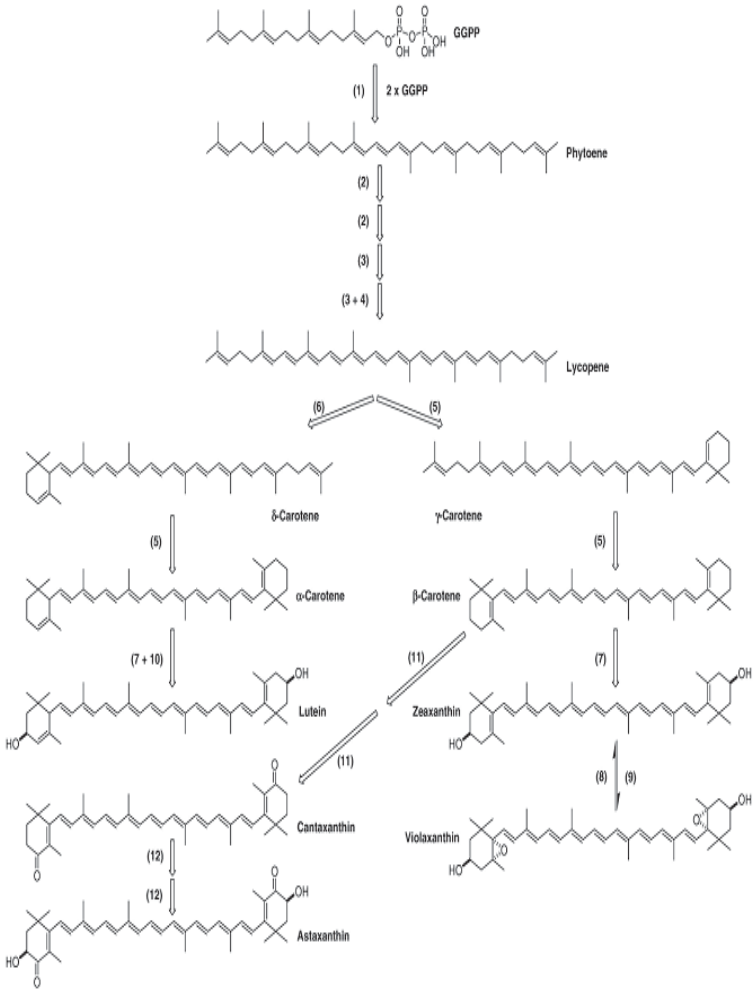


Figure 7-2: Biosynthetic pathway of some carotenoids. GGPP: geranylgeranyl diphosphate. Enzymes: (1) phytoenesynthase, (2) phytoenedesaturase, (3) ζ-carotene desaturase, (4) carotene isomerase, (5, 6) lycopene α-or β-cyclase, (7) β-ring hydroxylase, (8) zeaxanthine poxidase, (9) violaxanthinde-epoxidase, (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.

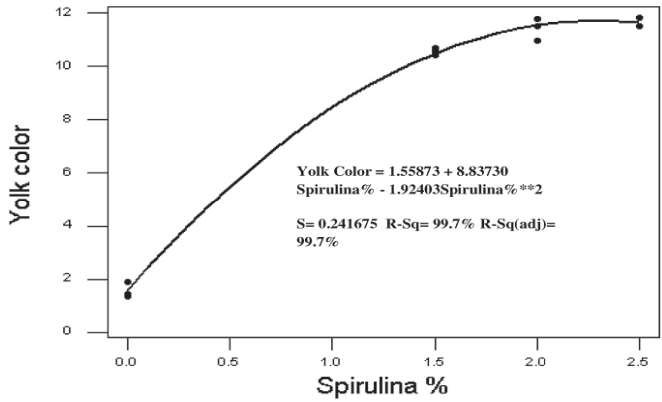


Figure 7-3: Regression between BASF yolk color fan values and level of *Spirulina* in the diet. (Zahroojian, Moravej, and Shivazad 2013)

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.

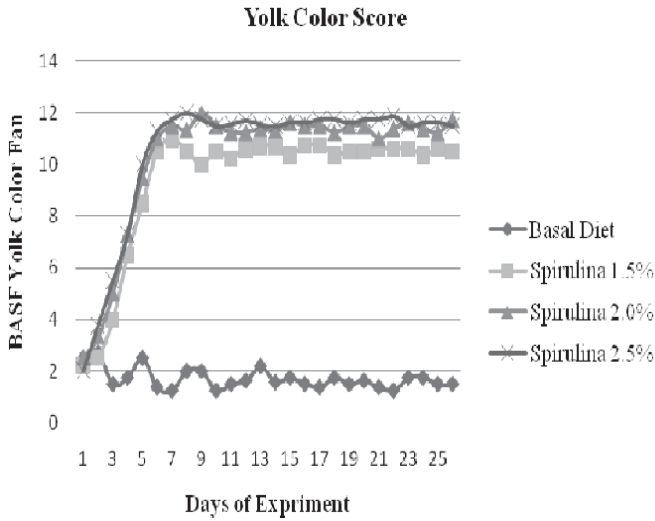


Figure 7-4: Effect of treatments on egg yolk color score. (Zahroojian, Moravej, and Shivazad 2013)

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 Ibrahem 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 Ibrahem (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 Ibrahem 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% *Spirulina* |
|---|---|
| Serum TG (mg/dl) | $715^{ab}\pm3.9$ |
| Serum TC (mg/dl) | $138^{b}\pm1.3$ |
| Serum LDL (mg/dl) | $23.8^{b}\pm0.16$ |
| Serum VLDL (mg/dl) | $79.5^{b}\pm1.1$ |
| Serum HDL (mg/dl) | $34.7^{b}\pm1.03$ |
| Yolk cholesterol (mg/g) | $9.90^{cd}\pm0.22$ |

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

Microalgae are a hopeful source of $W_3$ and $W_6$ (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.

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# 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, docoschexaenoid acid and arechidonic acid), minerals—especially iron, and vitamins—especially vitamin $B_{12}$ 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 + zeaxanthin, mg/g tissue | Ascorbic acid, mg/g tissue | Glutathione, 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 peroxyl 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 (Per-dix 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.

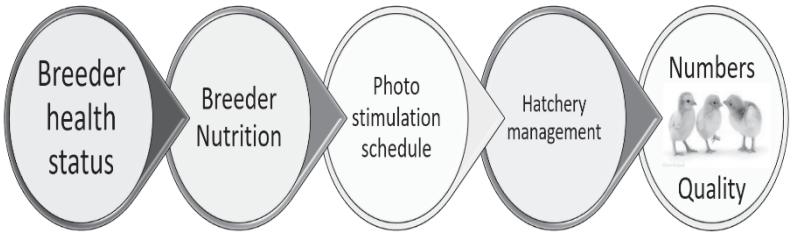


Figure 8-1: Factors affecting performance of commercial broiler breeders. (Designed by authors)

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.

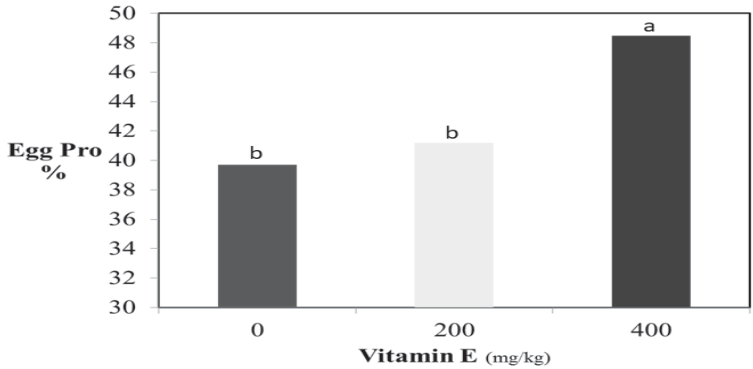


Figure 8-2: Effect of vitamin E (0, 200, and 400 mg α -tocopherol acetate/kg diet) on broiler breeder egg production. (Zaghari, Sedaghat, and Shivazad 2013)

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 | *Spirulina* | | Turmeric | | | |
|---|---|---|---|---|---|---|---|
| | | 2 g/kg | 3 g/kg | 4 g/kg | 6 g/kg | SEM | Sig. |
| 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 Ibrahem (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 Ibrahem 2002)**

| Main effects | Egg weight (g) | Egg weight loss (%) | Egg fertility (%) | Egg hatchability (%) | Chick weight (g) |
|---|---|---|---|---|---|
| *Spirulina* 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 |
| Strain | | | | | |
| 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≤0.05. NS: not significant; *: significant at P≤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 Niżański 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.

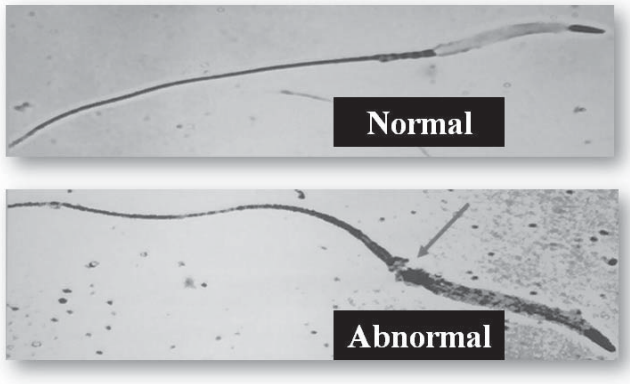


Figure 8-4: Normal and abnormal avian sperm membranes.

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±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 | $3.19^{e}\pm0.01$ | $43.66^{c}\pm0.14$ | $9.21^{a}\pm0.13$ | $2.36^{a}\pm0.12$ | $1.58^{bc}\pm0.04$ |
| | 100 | $4.14^{c}\pm0.01$ | $48.66^{b}\pm0.85$ | $3.66^{c}\pm0.14$ | $1.55^{a}\pm0.12$ | $1.56^{c}\pm0.02$ |
| | 200 | $4.29^{d}\pm0.02$ | $48.36^{b}\pm0.46$ | $2.50^{d}\pm0.10$ | $1.29^{b}\pm0.11$ | $1.62^{bc}\pm0.01$ |
| | 300 | $4.60^{b}\pm0.01$ | $50.11^{ab}\pm0.13$ | $1.90^{ef}\pm0.10$ | $0.91^{cd}\pm0.08$ | $1.66^{b}\pm0.01$ |
| | 400 | $4.67^{a}\pm0.01$ | $52.56^{a}\pm0.24$ | $1.78^{f}\pm0.04$ | $0.66^{cd}\pm0.04$ | $1.72^{a}\pm0.01$ |
| HW | 0 | $3.18^{e}\pm0.01$ | $48.01^{d}\pm0.30$ | $9.58^{a}\pm0.20$ | $2.72^{a}\pm0.12$ | $1.46^{d}\pm0.02$ |
| | 100 | $4.28^{d}\pm0.03$ | $49.98^{cd}\pm0.31$ | $4.83^{b}\pm0.26$ | $2.02^{a}\pm0.08$ | $1.57^{c}\pm0.01$ |
| | 200 | $4.41^{c}\pm0.03$ | $51.73^{bc}\pm0.13$ | $3.23^{c}\pm0.23$ | $1.44^{b}\pm0.06$ | $1.60^{bc}\pm0.02$ |
| | 300 | $4.62^{b}\pm0.02$ | $52.83^{ab}\pm0.25$ | $2.53^{d}\pm0.16$ | $1.01^{c}\pm0.05$ | $1.65^{b}\pm0.01$ |
| | 400 | $4.67^{a}\pm0.01$ | $55.23^{a}\pm0.20$ | $2.38^{ed}\pm0.19$ | $0.65^{d}\pm0.05$ | $1.77^{a}\pm0.02$ |
| *P*value | BW | <0.01 | <0.01 | <0.01 | <0.01 | 0.07 |
| | α-TA | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| | BW× α-TA | <0.01 | 0.02 | <0.01 | <0.01 | <0.01 |

SW: standard weight, HW: heavy weight, BW× 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 $T_3$ and $T_4$ 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)

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# 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, Offor, 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 to 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 $B_{12}$, linolenic and γ-linolenic acid (Sánchez et al. 2003), $\omega_3$ and $\omega_6$ 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 Morais, and de Morais 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.

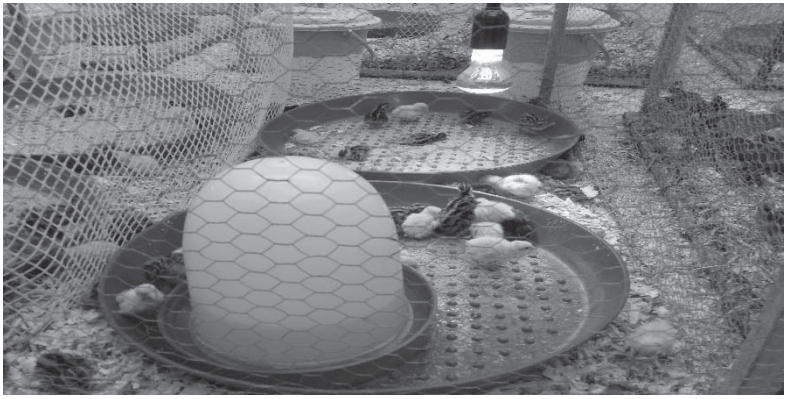

Figure 9-1: Experimental pens of Japanese quail chicks at 2 days post hatch fed with a diet containing *Spirulina platensis*. (Photograph by authors)

## 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 (IFNγ). 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 (Tsuji 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 piptides) THP-1cells (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).

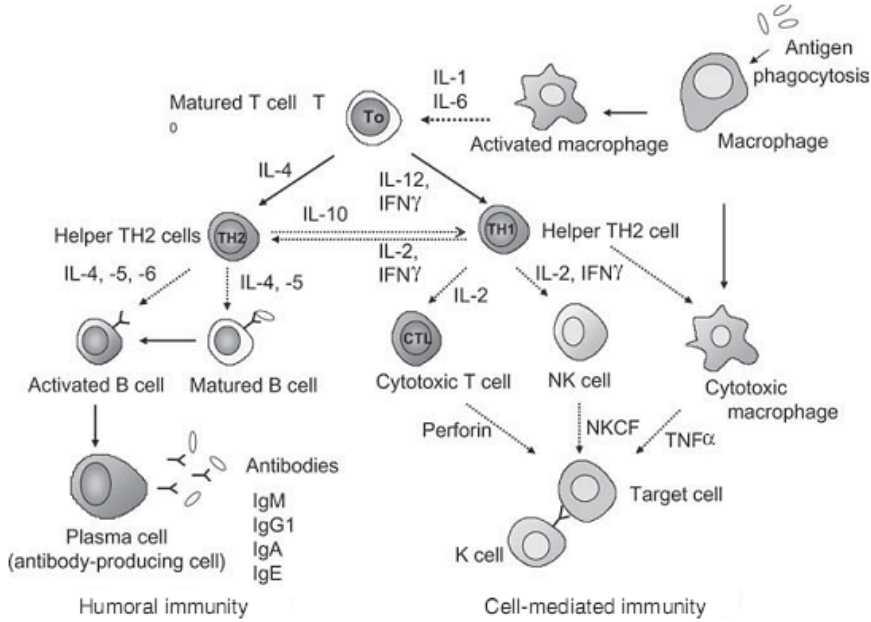


Figure 9-2: Humoral and cell immunity systems. (Gershwin and Belay 2007)

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).

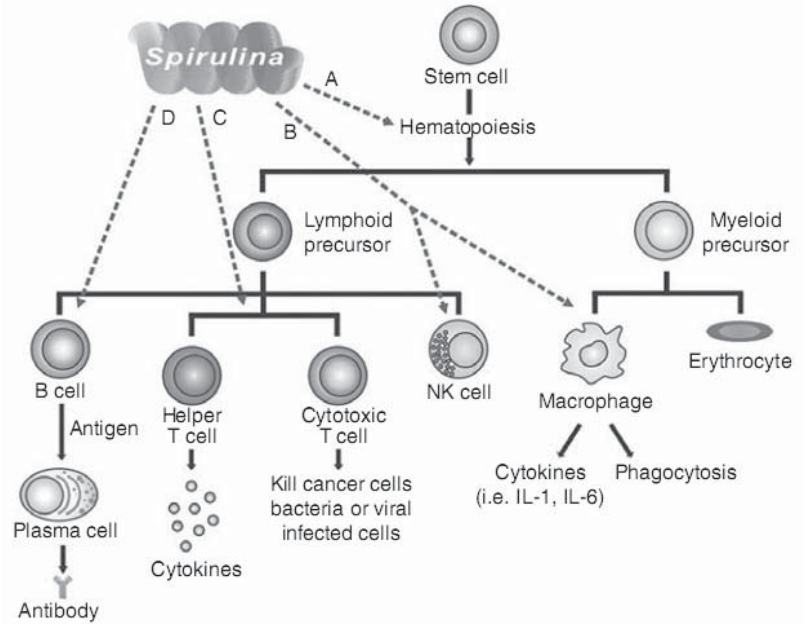


Figure 9-3: Effects of *Spirulina* on body immune responses. (Gershwin and Belay 2007). A = *Spirulina* promotes hematopoiesis to produce more erythrocytes and lymphocytes; B = *Spirulina* has a direct effect on innate immunity by activating macrophages and natural killer (NK) cells; C = *Spirulina* 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 *Spirulina platensis* (1%) | *Spirulina platensis* 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.

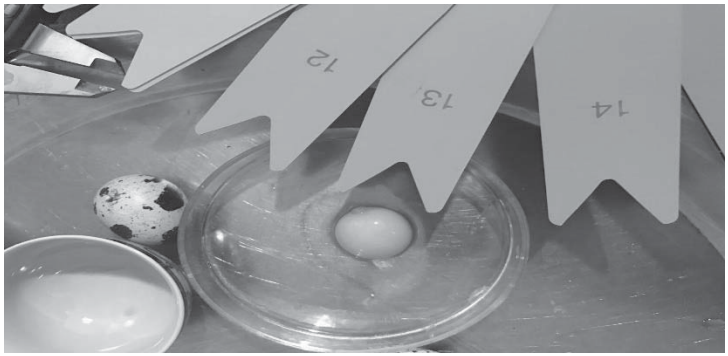


Figure 9-5: Evaluating egg yolk color of Japanese laying quails fed with diets containing *Spirulina platensis*. (Photograph by authors)

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., a-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.

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# 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 peroxyl 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 $K_2$ (Chakdar et al. 2012).

On the other hand, it has been reported that the mortality rate of lesser flamingos (Phoenicopterus 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 (*Crypthecodinium 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).

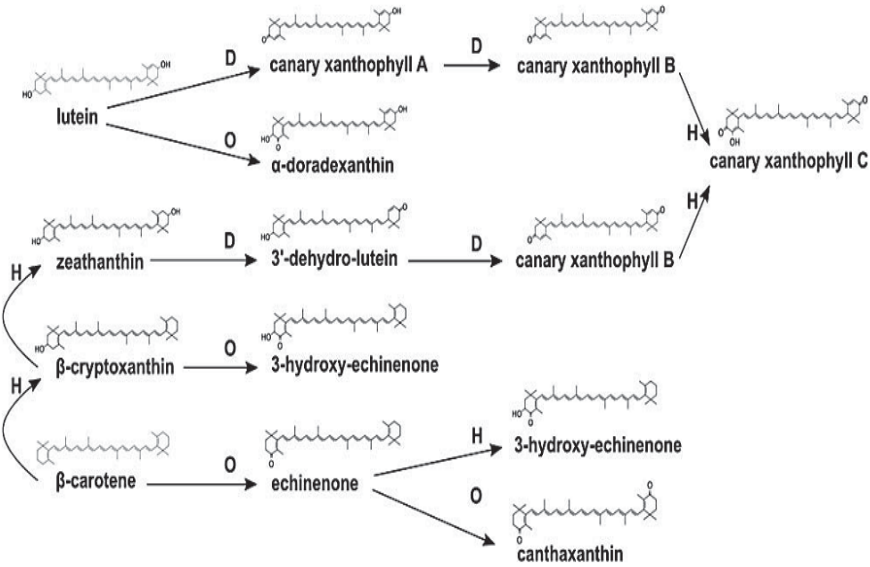


Figure 10-4: Chemical structures and conversion pathways of four dietary carotenoids (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) into the metabolically altered carotenoids in canary feathers (canary xanthophylls A, B, and C, echinenone, canthaxanthin and **α**-doradexanthin). The capital letters 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)

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# 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

***Chlorella*:** Genus of green microsalgae (family *Chlorellaceae*) 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. *Bifidobacterium* and *Lactobacillus* 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