

*Spirulina Platensis*  
in Poultry Nutrition



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By

Hosna Hajati and Mojtaba Zaghari

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

—M. Zaghari

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

—H. Hajati



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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

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

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

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

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

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

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

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



# CHAPTER ONE

## ORGANIC FEED

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

### **1.1 Introduction**

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

### **1.2 Herbal additives in poultry diet**

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

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

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

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

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

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

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

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

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

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

### 1.3 Algae

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

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

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

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

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

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

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

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

Algae consumption may prevent heart defects due to their considerable  $\omega$ -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,