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Application of microalgae biomass in poultry nutrition

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The aim of this review is to discuss the use of microalgae as a feed ingredient in poultry nutrition. Microalgae are unicellular, photosynthetic aquatic plants. They are introduced to poultry diets mainly as a rich source of n-3 long chain polyunsaturated fatty acids, including docohexaenoic and eicosapentaenoic acid, but they can also serve as a protein, microelement, vitamin and antioxidants source, as well as a pigmentation agent for skin and egg yolks. The majority of experiments have shown that microalgae, mainly *Spirulina* and *Chlorella* sourced as a defatted biomass from biofuel production, can be successfully used as a feed ingredient in poultry nutrition. They can have beneficial effects on meat and egg quality, *i.e.* via an increased concentration of n-3 polyunsaturated fatty acids and carotenoids, and in regards to performance indices and immune function. Positive results were obtained when fresh microalgae biomass was used to replace antibiotic growth promoters in poultry diets. In conclusion, because of their chemical composition, microalgae can be efficiently used in poultry nutrition to enhance the pigmentation and nutritional value of meat and eggs, as well as partial replacement of conventional dietary protein sources.

Keywords: microalgae; poultry; egg and meat quality; PUFA; carotenoids

Introduction

Microalgae, which are defined as microscopic algae, are unicellular, photosynthetic organisms which grow in salt or fresh water. As a rich source of nutrients and biologically active substances, including protein, amino acids, n-3 long chain polyunsaturated fatty acids (LCPUFA n-3), microelements, vitamins, antioxidants, and carotenoids, they have a long history of application as a food for humans (Belay *et al.*, 1996).

The increasing demand for human protein food sources has resulted in a need for new feed materials which provide a safe source of nutrients for poultry and livestock. Several feeding experiments have demonstrated that microalgae of different species can be

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successfully included into poultry diets, for example as a defatted biomass byproduct from biofuel production, and can have a beneficial influence on birds' health, performance, and the quality of meat and eggs. Especially important for the poultry industry are recent studies where microalgal biomass was efficiently used in the production of eggs containing health-promoting lipids, *i.e.* eggs enriched with health-promoting long-chain n-3 polyunsaturated fatty acids (LCPUFAs n-3). The traditional method of enriching eggs with LCPUFAs n-3 is to incorporate linseed or fish oil into the layer diet; however, this latter method is limited by the high demand for marine products and the risk of their contamination with heavy metals (Wu *et al.*, 2012). For this reason the use of some microalgae species, for instance *Nannochloropsis gaditana*, *Schizochytrium limacinum*, *Phaeodactylum tricornutum*, and *Isochrysis galbana*, in poultry nutrition could be of interest not only as a source of nutrients, but also as an alternative way of enriching of eggs with LCPUFAs n-3. The objective of this review is to discuss the results of current poultry studies where the effects of poultry feeding with microalgae have been examined.

Efficacy of microalgal biomass in poultry nutrition

SPIRULINA

The blue-green algae (*Spirulina*) is cultivated worldwide for use in the food and feed industries. Because of their prokaryotic cell type, this microalgae is sometimes called cyanobacteria and can be classified into two species: *Spirulina platensis* and *S. maxima*. Dried *Spirulina* biomass has a high nutritional value for human and animals as it contains about 60-70% protein, as well as being a good source of essential fatty acids, vitamins and minerals (Khan *et al.*, 2005). *Spirulina* is a rich source of carotenoids and contains around 6,000 mg total xanthophylls and 7,000 mg total carotenoids/kg in freeze-dried biomass (Anderson *et al.*, 1991). The study by Muhling *et al.* (2005) has shown a high concentration of gamma-linolenic acid in *Spirulina* biomass, which is an essential polyunsaturated fatty acid (12.9-29.4% total fatty acids).

The results of the experiments on *Spirulina* inclusion use in broiler diets are summarised in *Table 1*. In recent work, Evans *et al.* (2015) showed that dried full-fat *Spirulina* algae had an energy value equal to 90% the energy of corn (2839 kcal TMEn/kg), as well as containing a high level of crude protein (76%) and essential amino acids. They also reported that up to 16% of dried algae can be incorporated into a broiler starter diet without any negative effects on the performance of chicks. Similar results were obtained in work by Ross and Dominy (1990) who found no significant differences in performance of broilers fed a diet containing 1.5, 3, 6 or 12% dehydrated *Spirulina* in feed. They concluded that *Spirulina* at up to 12% of the diet may be substituted for other protein sources in broiler diets with good growth and FCR. Toyomizu *et al.* (2001) reported no difference in growth performance of broilers fed with or without 4 or 8% of *Spirulina* biomass in the diet. However, the yellowness of muscles, skin, fat and liver increased with an increasing dietary level of microalgae, being more attractive for consumers in certain markets. Hence, dietary *Spirulina* is useful for the manipulation of chicken meat colour, especially as the range where the fillets produced by feeding *Spirulina* do not fall under the extremes of either dark or light meat (Toyomizu *et al.*, 2001). Similar results were reported by Venkataraman *et al.* (1994) who demonstrated no effect of dried *Spirulina* (included at 14 or 17% in the diet) as a replacement for dietary fish meal or groundnut cake protein on performance, dressing percentage and histopathology in the various organs of broiler. However they found a more intensive meat colour in the case of birds fed algal-supplemented diets. In contrast to the above

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authors, Shanmugapriya *et al.* (2015) recently observed improved body weight gain (BWG), FCR and villus length in broilers fed a diet containing *Spirulina* biomass. Mariey *et al.* (2014) reported that a low dietary level of *Spirulina* biomass (0.02 or 0.03%) not only improved performance in broilers, but also increased dressing percentage, meat colour score, weight of lymphoid organs, improved blood morphology and decreased relative abdominal fat weight, blood cholesterol, triglycerides and total lipids.

Table 1 Results of selected studies on the effects of *Spirulina* inclusion into poultry diets.

Dietary concentration of algae	Animals, duration of the study and studied characteristics	Results	References
1.5, 3, 6, or 12%	Leghorn cockerel chicks, 1-21 d. Performance indices	No significant effect of *Spirulina* on performance.	Ross and Dominy (1990)
0.001, 0.01, 0.1, 1.0%	White Leghorns and broiler chicks, 1-49 1-21 d. Growth performance, Immune characteristics	No effect of *Spirulina* on performance. Leghorn chicks in *Spirulina*-dietary groups had increased total anti-SRBC titters; birds of both strains had increased phagocytic potential of macrophages and NK-cell activity	Qureshi *et al.* (1996)
4 or 8%	Broiler chickens, 21-37 d. Performance and pigmentation of the muscles	No effect of *Spirulina* on performance and relative weights of internal organs. Pigmentation (yellowness) of muscles, skin, fat, and liver increased with an increasing dietary level of *Spirulina*	Toyomizu *et al.* (2001)
0.01, 0.02, or 0.03%	Broiler chickens, 1-42 d. Performance, carcass and meat quality, blood haematology and biochemistry, weight of lymphoid organs	0.02 or 0.03% of *Spirulina* increased BWG, feed efficiency, meat colour score, weight of bursa, thymus and spleen, blood total protein, globulin and albumin, and red and white blood cells count, as well as lowered relative abdominal fat weight, blood plasma cholesterol, triglycerides, and total lipids	Mariey *et al.* (2014)
6, 11, 16, or 21%	Broiler chickens, 1-21 d. Performance, content of digestible amino acids in the diet	Dietary levels up to 16% algae resulted in a similar performance as in control group. The positive effect of algae inclusion on the digestible methionine content in the diet	Evans *et al.* (2015)
0.5, 1.0, or 1.5%	Broiler chickens, 1-21 d. Performance indices, histological measurements	A positive effect of 1% *Spirulina* on BWG, FCR, and villus length	Shanmugapriya *et al.* (2015)
1.5, 2.0, or 2.5%	Laying hens, 63-67 wk. Laying performance, yolk colour	*Spirulina* increased yolk colour without an effect on egg performance	Zahroojian *et al.* (2011)
1.5, 2.0, or 2.5%	Laying hens, 63-67 wk. Performance, egg quality, yolk cholesterol content	No significant effect of *Spirulina* on studied indices, except yolk colour, which was increased by dietary algae addition	Zahroojian *et al.* (2013)

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The results of several trials have shown that *Spirulina* can be used to enhance the immune function of birds. Quereshi *et al.* (1996) reported that broiler chicks fed diets containing 1% *Spirulina* had increased phytohaemagglutinin-mediated lymphocyte proliferation and phagocytic activity of macrophages compared to control treatment. Raju *et al.* (2005) found that dietary *Spirulina* (0.05% in feed) can partially alleviate the negative effects of aflatoxin on weight of immune organs and BWG in broilers.

Experiments with laying hens have been mainly focussed on evaluating the efficiency of *Spirulina* biomass as a source of carotenoids for pigmentation of egg yolks. In experiments with laying hens, Zahroojian *et al.* (2011; 2013) demonstrated that algal carotenoids were well absorbed and accumulated in the egg yolk, and 2.0-2.5% dietary *Spirulina* could be used to produce eggs with increased yolk colour with similar efficiency to a synthetic pigment. An earlier study with quail (Anderson *et al.*, 1991) showed that optimal yolk colour was achieved when 1% of *Spirulina* biomass was added to the diet. Mariey *et al.* (2012) reported improved egg production, hatchability and yolk colour when laying hens were fed a diet with a low level of *Spirulina* inclusion (0.1-0.2%).

A study with Japanese quail by Ross and Dominy (1990) evaluated the effect of *Spirulina* included at 1.5, 3.0, 6.0, or 12.0% in the diet on growth performance, egg production and quality. The authors observed no significant differences due to the dietary microalgae level, except for increased yolk colour and fertility in birds fed with *Spirulina*, and concluded that up to 12% of *Spirulina* biomass could be included into diets. The results of the study with growing quail (aged 15-35 days) showed no negative effects in growth performance and meat quality when included in levels up to 4% of *Spirulina* in feed (Cheong *et al.*, 2015).

CHLORELLA

Chlorella, a unicellular, freshwater green microalgae used mainly for human food and biofuel production, has been studied in several animal experiments as a potential source of high quality protein (approximately 60%), essential amino acids, vitamins, minerals, and antioxidants. *Chlorell*a biomass is a very good source of carotenoids, as it contains 1.2-1.3% of total pigments in dry mass (Batista *et al.*, 2013). As indicated by Kotrbacek *et al.* (2015), this microalgae is too expensive to be used as protein material for animals, however, due to the content of many bioactive substances, even a low, economically acceptable dietary level of *Chlorella* biomass may beneficially affect animal performance.

A very early study with chickens (Combs, 1952) demonstrated that dried *Chlorella*, included into the diet at 10% could serve as a rich source of certain nutrients, *i.e.* carotene, riboflavin and vitamin B12, and increased performance in birds when the diet was deficient in these nutrients. Grau and Klein (1957) reported that *Chlorella* biomass grown in sewage was a rich source of protein and xanthophyll pigments, and levels up to 20% in the diet was well tolerated by chicks. Similarly, Lipstein and Hurwitz (1983) found that *Chlorella* was a suitable protein supplement in broiler diets and, used at 5 or 10% dietary level, had no adverse effect on growth performance.

Kang *et al.* (2013) studied the effects of the replacement of antibiotic growth promoter with different forms of *Chlorella* on performance, immune indices and the intestinal microfloral population. They found that *Chlorella* in its fresh liquid form included at a 1% dietary level beneficially affected BWG, some immune characteristics (*e.g.* number of white blood cells and lymphocytes, plasma IgA, IgM, and IgG concentrations) and the intestinal production of *Lactobacillus* bacteria (*Table 2*). Such an effect of dietary *Chlorella* appears to be based on multiple components, and the fibre fraction, among others including a polysaccharide named immurella, glycoprotein, and peptides contained in *Chlorella,* stimulate the immune response of birds (Kang *et al.*, 2013). Likewise,

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Kotrbacek *et al.* (1994) found that broilers fed a diet with 0.5% *Chlorella* significantly increased the phagocytic activity of leucocytes and lymphatic tissue development. Rezvani *et al.* (2012) observed a numeric increase in response to phytohemagglutinin-P, which was accompanied by improved FCR in broilers fed supplementary *Chlorella*.

Table 2 Results of selected studies on the effects of *Chlorella* inclusion to poultry diets.

Dietary concentration of algae	Animals, duration of the study and studied characteristics	Results	References
Selenium-enriched *Chlorella* added in the amount supplying 0.3 mg Se/kg of the diet	Broiler chickens, 1-42 d. Performance, Se concentration and activity of glutathione peroxidase in meat, oxidative stability of meat lipids	Positive effect of algae on BWG, Se content and glutathione peroxidase activity in breast meat. Decreased oxidation of stored breast meat of birds fed a diet with Se-enriched *Chlorella*	Dlouha *et al.* (2008)
0.07, 0.14, or 0.21%	Broiler chickens, 1-42 d. Performance, immune response indices	Improved FCR and a numerical increase in response to phytohemagglutinin-P in broilers fed with dietary *Chlorella* biomass	Rezvani *et al.* (2012)
1%, to replace antibiotic growth promoter (dried powder, or fresh liquid *Chlorella*)	Broiler chickens, 1-28 d. Performance, immune indices, intestinal bacteria population	Fresh liquid *Chlorella* positively affected BWG, the immune characteristics and *Lactobacillus* bacteria count in the intestine	Kang *et al.* (2013)
0.25, 0.50, 0.75% (in the form of spray dried or bullet milled and spray dried biomass)	Laying hens, 22-54 wk. Laying performance, egg quality and hatchability, nitrogen balance	*Chlorella* improved yolk colour, shell weight and egg hatchability, without affecting performance and nitrogen balance	Halle *et al.* (2009)
1.25%	Laying hens, 25-39 wk. Performance, egg quality, oxidative stability of yolk lipids	Positive effect of *Chlorella* on egg weight, FCR, shell quality, yolk colour, yolk lutein and zeaxanthin, as well as oxidative stability of yolk lipids of fresh and stored eggs.	Englmaierova *et al.* (2013)
1 or 2%	Laying hens, 56-63 wk. Egg quality, yolk carotenoids content, blood triacylglycerol and cholesterol level	*Chlorella* increased yolk carotenoids, lutein, β-carotene and zeaxanthin content and yolk colour score. It decreased FI and yolk weight in hens fed a diet with 2% of *Chlorella*	Kotrbacek *et al.* (2013)
1% (conventional or lutein-fortified. *Chlorella*) (Exp 1), 0.1 or 0.2% lutein-fortified *Chlorella* in the diet (Exp. 2)	Laying hens, 70-72 wk (Exp. 1), 60-62 wk of age (Exp. 2). Performance, egg quality, lutein content in the body of hens and eggs.	1% conventional or lutein-fortified *Chlorella* improved egg production, yolk colour and lutein content in the serum, liver and growing oocytes. 0.2% of lutein- fortified *Chlorella* increased egg weight, yolk colour and lutein content in eggs	An *et al.* (2014)

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Table 2 Continued

Dietary concentration of algae	Animals, duration of the study and studied characteristics	Results	References
0.1 or 0.2% (fermented *Chlorella* biomass)	Laying hens, 80-86 wk. Performance, egg quality, intestinal microflora profile	*Chlorella* improved egg production, yolk colour, Haugh units and lactic acid bacteria cecal population	Zheng *et al.* (2012)
0.1 or 0.2% (fermented *Chlorella* biomass)	Pekin ducks, 1-42 d. Growth performance, meat quality, cecal microflora, tibia bones quality	Positive effect of *Chlorella* on BWG, FI, meat quality and tibia breaking strength, without differences in cecal microflora	Oh *et al.* (2015)

Because *Chlorella* is grown in the presence of high levels of selenite, it accumulates cellular selenium and there is a growing interest in the use of this algae as a rich source of Se for animals (Kotrbacek *et al.*, 2015). In a study with broilers, Dlouha *et al.* (2008) found that dietary addition of Se-enriched *Chlorella* biomass not only positively affected BWG but also increased Se content and glutathione peroxidase activity in breast meat, as well as decreasing the oxidation of breast meat stored under refrigeration.

A positive effect of *Chlorella* as a feed material for laying hens was found by Halle *et al.* (2009), who reported that layers fed a diet supplemented with dietary algae had increased egg hatchability, yolk colour and shell weight without affecting egg performance and nitrogen balance. In a subsequent study, the same authors showed a higher diversity of the microbiota community in the intestinal tract of hens fed a diet containing *Chlorella* and suggested that it could be responsible for the effects on egg quality (Janczyk *et al.*, 2009). A beneficial influence of feeding *Chlorella* on laying performance, egg quality, and caecal lactic bacteria population was observed by Zheng *et al.* (2012). Skrivan *et al.* (2008) reported that Se-enriched *Chlorella* was a more efficient source of Se than sodium selenite as, despite equal doses of Se supplementation, a higher Se content was found in eggs from hens fed diet supplemented with *Chlorella*. An *et al.* (2014) found that diet supplementation with conventional or lutein-enriched *Chlorella* could positively affect egg performance, yolk colour and lutein concentration in eggs. Hence, the use of *Chlorella* is a valuable tool for the production of chicken eggs enriched with natural lutein, and increasing consumption of this compound can prevent macular degeneration in the human ageing population. Englmaierova *et al.* (2013) showed that supplementing layers with *Chlorella* not only increased the concentration of lutein and zeaxanthin, but also improved FCR, shell quality, and the oxidative stability of yolk lipids of fresh and stored eggs. In agreement, Kotrbacek *et al.* (2013) reported significantly increased yolk carotenoids' content as well as yolk colour score in hens fed with *Chlorella* supplementation, however, dietary microalgae decreased feed intake and yolk weight.

OTHER MICROALGAE SPECIES

The results of an early study by Lipstein and Hurwitz (1981) showed that the microalgae *Micractinium* could be a useful protein source for broilers, and supplementing up to a 6% in the diet had no negative effect on growth performance. However, chickens fed a higher inclusion level of this algae had decreased feed intake and BWG. The study by Austic *et al.* (2013) evaluated the effects of *Staurosira*

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incorporation into the broilers' diet, and the results indicated that *Staurosira* may be used to substitute 7.5% of soybean meal without any negative influence on performance or plasma and liver biomarkers, when an appropriate amino acids dietary level was maintained.

The aim of the study by Waldenstedt *et al.* (2003) was to evaluate the efficacy of an increasing dietary level of *Haematococcus pluvalis* meal, used as an astaxanthin source, in broiler chickens infected with *Campylobacter jejuni*. The authors showed no influence of algal meal on performance, but tissue astaxanthin concentrations were significantly higher with increasing levels of dietary algae. Caecal *Campylobacter jejuni* populations was not affected by *Haematococcus pluvalis* inclusion, however a diet with 0.18% algal meal reduced caecal *Clostridium perfringens* counts. Yan and Kim (2013) showed that adding 0.1 or 0.2% *Schizochytrium* to the diet improved the fatty acid composition of breast meat lipids, without affecting BWG in broilers.

Poultry products enriched with n-3 long chain polyunsaturated fatty acids are good examples of a functional food, *i.e.* food that, in addition to possessing traditionally understood nutritional value, can beneficially affect the metabolic and health status of consumers, thus reducing the risk of various chronic lifestyle diseases (Pietras and Orczewska-Dudek, 2013; Yanovych *et al.*, 2013; Zdunczyk and Jankowski, 2013). The results of several experiments have shown that microalgae, as a rich source of LCPUFAs n-3, can be introduced into the diet of laying hens to produce functional foods, *i.e.* designer eggs with naturally increased LCPUFAs n-3 concentration. For instance, Bruneel *et al.* (2013) reported an increased content of DHA in egg yolks of hens fed a diet containing *Nannochloropsis gaditana* and suggested that this algae may be used as an alternative to current sources of LCPUFA n-3 for the production of DHA-enriched eggs. A similar effect was seen on enhanced DHA yolk concentration through diet supplementation with the marine microalgae *Schizochytrium limacinum* (Rizzi *et al.*, 2009). What is important here is that the sensory characteristics of eggs enriched with LCPUFA n-3 by a addition of *Schizochytrium* were not altered (Parpinello *et al.*, 2006). The results of recent work by Park *et al.* (2015) have shown that the addition of *Schizochytrium* to layers' diet not only significantly improved the fatty acids profile of the yolks but also positively affected laying performance and egg quality.

Lemahieu *et al.* (2013) compared the efficacy of four different algae species (*Phaeodactylum tricornutum*, *Nannochloropsis oculata*, *Isochrysis galbana* and *Chlorella fusca*) on the enrichment of egg yolks in LCPUFA n-3. They reported that the highest enrichment with PUFA n-3 as well as increased yolk colour was achieved with supplementation using *Phaeodactylum* or *Isochrysis*, and these two microalgae could be used as an alternative to current sources for the enrichment of eggs. Subsequent studies proved the suitability of *Isochrysis* as an LCPUFA n-3 source and showed that 2.4% dietary supplementation with *Isochrysis* lead to the highest LCPUFA n-3 enrichment in the yolk, and that this supplementation level should be considered as the optimal dose (Lemahieu *et al.*, 2014; 2015).

Because of a high content of lipids, certain microalgal species can be used as a suitable material for the production of biofuels. Once defatted, algae can provide a rich source of crude protein in poultry diets. Leng *et al.* (2014) showed no adverse effect of feeding layers with 7.5% defatted Staurosira spp. when used for partial replacement of soybean meal. However, higher dietary levels (15%) worsened egg performance, feed intake and FCR. These authors indicated that such a decrease in performance was likely to be due to the high ash and sodium chloride concentrations of the algae. The results of a recent study by Ekmay *et al.* (2015) demonstrated that defatted *Desmodesmus* and *Staurosira* spp. could be used in laying hen diets at relatively high levels (up to 25% in the diet), as a source of well-digested dietary protein, without any negative effect on egg production.

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A study with Muscovy ducks investigated the effects of diet supplementation with 0.5% microalgae *Crypthecodinium cohnii* (Schiavone *et al.*, 2007). They demonstrated the positive effect of this microalgae on the fatty acid profile in breast meat lipids, without affecting growth performances or slaughter traits, as well as chemical composition, colour, pH, oxidative stability and sensory characteristics of the breast meat. An experiment with Japanese quail showed that diet supplementation with *Schizochytrium* sp. could be an effective way of bio-fortifying egg LCPUFA n-3 levels, as the yolks of birds fed a diet with 0.5% of this microalgae significantly increased DHA concentration, as well decreasing n-6/n-3 PUFA ratio and cholesterol content in yolk lipids (Gladkowski *et al.*, 2014; Trziszka *et al.*, 2014).

Conclusions

Summarising the literature available, it can be concluded that, although chemical composition of different microalgal biomasses is diverse, many can safely be added to poultry diets. Several *Spirulina*, *Chlorella* and other microalgae species may be used to increase the pigmentation and nutritional value of meat and eggs for human consumption, *e.g.* to enhance these products with LCPUFA n-3 and carotenoids, as well as to partially replace conventional protein sources, mainly soybean meal.

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