Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A COMPREHENSIVE REVIEW ON ALGAE AND PROPOLIS-CHARACTERISATION AND THE IMPLICATIONS OF THEIR USE IN THE LAYING HEN DIET

Mihaela SARACILA, Tatiana Dumitra PANAITE, Arabela Elena UNTEA

National Research-Development Institute for Animal Biology and Nutrition (IBNA),
1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania

Corresponding author email: mihaela.saracila@yahoo.com

Abstract

Microalgae represent a new field of interest for laying hens' nutrition as they constitute a novel and valuable nutrient source, due to their nutritional composition and richness in polyphenols, polysaccharides and fatty and amino acids. Many studies have studied the effect of using microalgae in laying hen nutrition and their ability to improve health, production and egg quality. Propolis, like microalgae, is a natural source of nutrients with a long tradition in natural medicine. The literature has shown many benefits of using propolis in the diets of laying hens, such as improved productive performance and egg production, health, egg quality. This review makes it clear that including microalgae and propolis in laying hen diet can be an undeniable future nutritional strategy, enhancing standard feed formulations to the benefit of health and egg quality.

Key words: *diet, egg quality, laying hen, microalgae, nutrients, propolis.*

INTRODUCTION

Nowadays there is an increasing demand for functional foods for human consumption that provide various benefits in addition to the nutrients. The farming industry has thus become interested in using natural forms of vitamins and minerals instead of synthetically produced ones. Considering these dynamics, the possibility of using microalgae as a new source of nutrients and health additives in animal feed formulations has been evaluated. Eggs can be enriched with certain nutrients through dietary manipulation to create products that could possibly provide health benefits for humans (Saracila et al., 2017; Panaite et al., 2021).

'Algae' is a generic term that groups brown, green, and red types of both macro- and microalgae (Coudert et al., 2020). Global demand for macroalgae and microalgae is growing. There is substantial evidence for health benefits, but it is challenging to study the effects of including them in poultry feed as natural sources of vitamins and minerals. In general, the growing trend in global nutritional demand for algae comes from a greater focus on health. In addition to their nutritional value, algae are increasingly being marketed as functional foods or nutraceuticals. In recent years, much interest has been focused on the potential of microalgae biotechnology, mainly due to the identification of several substances synthesized by these microorganisms (Andrade et al., 2018). Chlorella and Spirulina are two of the best-known genera of microalgae (Andrade et al., 2018). These marine plants may play a key role in the future for poultry production, as they constitute a new and valuable nutrient source, thanks to their nutritional composition and richness in as polyphenols, polysaccharides and fatty and amino acids. Algae are a valuable source of B vitamins (especially B1, B12), as well as vitamin A (derived from the β-carotene carotenoid) and vitamin E. Algae provide one of the few plant alternatives to cobalamin (vitamin B12) in the diet (Andrade et al., 2018). Microalgae produced through fermentation contains high levels of DHA (Zeller et al., 2001) and could be potentially used in animal diets. Ao et al. (2015) showed that supplements of 2 or 3% microalgae significantly improved the color and nutritional quality of egg yolk.

The global interest and the growing awareness of consumers, especially in terms of the nutritional and medicinal value of what they eat or drink, arouse the concept of a return to natural products, especially bee products. Propolis has attracted a lot of attention from food supplements and food processing industries due to its high value for health.

Propolis (bee glue) is a natural product collected by bees from various plants, particularly from flowers and leaf buds (Abdel-Kareem & El-Sheikh, 2015). Propolis contains resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%) as well as both pollen and other substances (5%) as organic debris (Burdock, 1998). Literature showed many benefits of using propolis in laying hen diets such as improved productive performance and egg production (Abdel-Kareem & El-Sheikh, 2015), health promoting effect (El-Neney et al., 2014), egg quality (Casagrande et al., 2021).

This review describes the main nutritional characteristics of microalgae and propolis and the current knowledge on their effects in laying hen production, impacts on health, performance and egg quality.

MATERIALS AND METHODS

In this review, we used 45 specialized articles, using databases such as Google academic, Science direct, etc. Recent research papers (last 5-10 years), well-designed experimental design, large number of animals taken in the experiment were the search criteria.

RESULTS AND DISCUSSIONS

1. Microalgae - chemical characterisation and implication in laying hen diet

Microalgae represent a new field of interest for animal nutrition and health, both biologically and economically. The economic importance is related to the wide range of applicability of microalgae worldwide (Andrade et al., 2018) and the number of publications devoted to or related to this subject in recent years has increased regularly (Coudert et al., 2020). Microalgae, microscopic single-celled organisms, can be used to produce a wide range of metabolites, such as proteins, fats, carbohydrates, vitamins, and organic minerals. The cultivation of microalgae was carried out with the aim of producing biomass both for food and also for obtaining value-added natural compounds. These natural compounds include polyunsaturated fatty acids, carotenoids, polysaccharides, vitamins, sterols, and many natural bioactive compounds, such as antioxidants, that can be used primarily for functional food production. Chlorella and Spirulina are two of the best-known genera of microalgae (Andrade et al., 2018).

1.1. Microalgae - chemical characterisation

Chlorella and Spirulina microalgae live in freshwater and are rich in bioactive compounds such as proteins, vitamins, pigments, long chain polyunsaturated fatty acids, sterols and other compounds that make these microalgae very interesting in terms of health benefits (Andrade et al., 2018).

Vitamins are essential organic micronutrients that an organism cannot synthesize directly in sufficient quantities and therefore must be obtained from the diet. Algae are a source of B vitamins (especially B1, B12), as well as vitamin A (derived from the β-carotene carotenoid) and vitamin E (tocopherol) (Andrade et al., 2018). Algae provide one of the few plant alternatives to cobalamin (vitamin B12) in the diet. Chlorella and Spirulina microalgae produce vitamin A (beta-carotene), vitamin C, vitamin E, thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9) and cobalamin (B12). These vitamins are used to nourish the body, detoxify and normalize bowel function, as well as stimulate the immune system and regenerate cells (Andrade et al., 2018). Chlorella biosynthesizes vitamin A with a concentration of 30.77 mg/100 grams dry mass, while spirulina contains 0.34 mg/100 g dry mass. In fact, vitamin A is the most abundant vitamin produced by microalgae. Niacin (vitamin B3) is also abundantly biosynthesized by microalgae. Chlorella contains 23.8 mg Vitamin B3/100 g dry mass, and spirulina contains 12.08 mg vitamin B3/100 g dry mass (Andrade et al., 2018). Vitamin B12 (cyanocobalamin) is present in microalgae at a low level (0.1 μg) Vitamin B12 in microalgae from Chlorella sp. has better bioavailability than Spirulina microalgae (Solomons, 2012). Chlorella and Spirulina microalgae have a high concentration of folic acid (94 μg/100 g dry mass), which is necessary for cell formation and maintaining metabolism, preserving the skin and mucous membranes and for the normal development of bones and teeth (Andrade et al., 2018). Compared to Chlorella, Spirulina are richer sources of vitamin E, vitamin B1 and vitamin B7. Chlorella contains substantial amounts of vitamins D2 and B12, both of which are well known to be absent in plants. Commercially available Chlorella (*C. vulgaris*) products contain higher amounts of folate (approximately 2.5 mg/100 g dry weight) than spinach (Woortman et al., 2020). Vitamin D, a major regulator of calcium absorption, reduces the risk of osteomalacia in adults and rickets in children (Taofiq et al., 2017). The two main food forms of vitamin D are vitamin D2 and D3, which are found in mushrooms and foods of animal origin, such as fish and fish products, respectively. Ingestion of small amounts of microalgae (biomass) can help meet all vitamin requirements in both animal feed and human food. Vitamins from microalgae can increase the nutritional value of algae - applied as a supplement.

Algae contain significant amounts of iodine and iron (Wells et al., 2017). In particular, Chlorella contains substantial amounts of iron (104 mg/100 g dry matter) and potassium (986 mg/100 g dry matter), of which adequate intake prevents anaemia and hypertension, respect-tively. Spirulina contains minerals such as iron, magnesium, calcium and phosphorus (Soni et al., 2017). The iron, calcium and phosphorus content of Spirulina are 1.7, 15 and 10 mg/ sample, respectively (Deasy Liestianty et al., 2019). Spirulina is a splendid source of iron that contains 20 times more iron than 1 gram of wheat (Soni et al., 2017). Microalgae can accumulate Se in high concentrations (100 μg Se/g dry weight) (Doucha et al., 2009). It is essential for many algae and works to protect them from oxidative damage. Selenium is an essential oligomineral that serves as a fundamental nutrient for human health. It is a component of selenoproteins such as thioredoxin reductase and glutathione peroxidases and protects against intercellular oxidative damage.

1.2. Microalgae-implication in laying hen diet

Microalgae have been recognized as natural, sustainable and economically feasible resources of unconventional ingredients capable of promoting the benefits of animal husbandry and improving meat quality (de Medeiros et al., 2021). About 30% of the world's algae are currently used in animal feed (Li et al., 2002).

Once microalgae biomass is included in animal feed, these compounds meet the energy-protein needs for breeding animals with satisfactory performance. In parallel with the positive impact on the nutritional, technological and sensory quality of meat, microalgae biomass improves feed digestibility and improves the animal's immune response (Kibria and Kim, 2019). Microalgae have been introduced into animal feed mainly by incorporating the entire biomass of microalgae in the form of dry powder, high moisture extruded biomass or microalgae extracts (Van Vo et al., 2020).

Table 1 presents a summary of microalgae applications in hen diet. Literature research revealed that microalgae supplementation in laying hen diet improve productive parameters. Zheng et al. (2011) showed that dietary supplementation in 80-week-old Hy-Line Brown layers with 2% of *Chlorella vulgaris* increased egg productivity from 55.4% in the control to 59% in the supplemented group. An et al. (2014) revealed that 1% conventional or lutein-fortified Chlorella improved egg production.

Some microalgae are able to positively influence egg physical and nutritional quality (Table 1). Providing dietary 1.25% *C. vulgaris*, Englmaierová et al. (2013) showed an increase in egg weight (62.3 g vs. 61.1 g for control hens), shell weight (6.1 g vs. 5.9 g for control hens), and yolk colour, which was more intense (increased redness and yellowness) for the supplemented group.

Ao et al. (2015) showed that supplements of 2 or 3% All-G-RichTM significantly increased redness (a*) and decreased lightness (L) of egg yolk. Dietary 1% or 1.25% of *Chlorella vulgaris* in 56- to 63-week-old and 25- to 39-week-old ISA brown laying hens, significantly increased lutein, zeaxanthin and beta-carotene in egg yolk (Kotrbáček et al., 2015). Several studies have reported that microalgae supplementation significantly change the fatty acid composition of eggs. Adding All-G-RichTM in layer diets can produce DHA-enriched eggs with no negative impact on egg quality (Ao et al., 2015). Similar results were found by using Spirulina microalgae instead of Chlorella (Luo et al., 2015). Fraeye et al. (2012) showed that hens fed microalgae had a n-3 LC FA content 3.8- to 7.0-fold higher in eggs. Although enrichment in LC-PUFA accelerates the oxidative processes of lipids, some authors have shown that Yolk fatty-acid oxidation, as measured by thiobarbituric acid reactive substances (TBARS), was not affected by All-G-RichTM in eggs stored up to 30 days at 4°C (Ao et al., 2015).

Table 1. Summary of microalgae applications in hen nutrition

Type of microalgae	Animals	Dose of inclusion	Effect	Reference
All-G-RichTM		2 or 3%	- increased redness (a*) and decreased lightness (L) of egg yolk - enriched eggs yolk in DHA	Ao et al. (2015)
Spirulina platensis	Hy-line W36 hens, 63-67 wk	1.5, 2.0, 2.5%	Increased yolk colour	Zahroojian and Morajev (2013)
Chlorella vulgaris	ISA brown hens, 25-39 wks	1.25%	Increased egg weight, shell quality, yolk colour, lutein and zeaxanthin concentrations	Englmaierová et al. (2013)
Chlorella vulgaris	Laying hens, 56-63 wks	1.0%	Increased yolk carotenoids (lutein, beta-carotene and zeaxanthin)	Kotrbáček et al. (2015)
Spirulina platensis - Supercritical Extract	ISA Brown, 36 weeks of age	0.2%	increase the concentration of DPA, EDA reduce the content of saturated fatty acids such as pentadecanoic acid	Michalak et al., (2020)
Lutein-fortified Chlorella	Laying hens, 70-72 wk (Exp. 1), 60-62 wk (Exp. 2)	0.1 or 0.2%	1% conventional or lutein-fortified Chlorella improved egg production, yolk colour and lutein content in the serum, liver and growing oocytes. 0.2% lutein-fortified quality, lutein of lutein- fortified Chlorella increased egg weight, yolk colour and lutein content in egg	An et al. (2014)
Fermented Chlorella biomass	Laying hens, 80-86 wk	0.1 or 0.2%	-improved egg production, yolk colour, Haugh units and lactic intestinal acid bacteria cecal population	Zheng et al. (2012)

Some microalgae may influence egg lipid composition. Several studies have reported that microalgae supplementation can significantly reduce egg cholesterol (Table 1). This was the case for supplementation with *Spirulina platensis* (1.5, 2 and 3% in feed) in 63- to 67-week-old Hy-line W36 hens (Zahroojian & Morajev, 2013), with *Chlorella vulgaris* in 80-week-old Hy-line Brown layers (Zheng et al., 2011). Few studies revealed that microalgae supplementation in laying hen diet could improve gastrointestinal health (Table 1). Zheng et al. (2011) showed that 0.1 or 0.2% of fermented Chlorella biomass supplementation in laying hen diet increased the number of lactic acid bacteria in the cecum.

2. Propolis - chemical characterisation and implication in laying hen diet

The application of propolis in the formulation of feed is ongoing (Kostić et al., 2020). In recent decades, the use of natural products has been promoted to improve the performance and meat quality (Saracila et al., 2021a; 2021b; Untea et al., 2021). Therefore, propolis is one of the natural candidates for this purpose.

Propolis is considered a valuable ingredient for animal nutrition due to its active components that have significant health properties (Abdel-Kareem & El-Sheikh, 2017). It is generally marketed as a functional and affordable food with promising future industrial potential.

2.1. Propolis - chemical characterisation

Propolis is collected from buds, leaves and similar parts of trees and plants like pine, oak, eucalyptus, poplar, chestnut, etc. by bees and mixed with wax (Valle, 2000). Chemically propolis is composed of more than 180 different types of chemicals (Kuropatnicki et al., 2013). Propolis contains resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%) as well as both pollen and other substances (5%) as organic debris (Burdock, 1998). Generally, the known major components of propolis are aromatic acids, flavonoids, diterpenoid acids, phenolic compounds and triterpenoids (Elnakady et al., 2017). Cecere et al. (2021) showed a content in total phenolic compounds of 916.28±23.22 mg equivalent in gallic acid/mL; 158.15±4.47 µg/mL antioxidant activity (IC50); and concentrations of gallic acid of 0.57 mg/mL, p-coumaric acid of 3.10 mg/mL, and chlorogenic acid of 1.41 mg/mL.

Devequi-Nunes et al. (2018) analysed three varieties of propolis (red, green and brown) and showed a concentration of protein that ranges from 2.12-2.49%, of lipids 8.19-15.61% and fiber 68.72-70.82%. The same authors analysed the main chemical classes present in propolis. They are flavonoids, phenolics, and aromatic compounds. The content of phenolic compounds varied from 113.41±0.01 (Brown SCO2) to 481.59±0.02 mg EAG/g (Red EtOH), whereas the content of flavonoids varied from 29.67±0.01 (Brown EtOH) to 186.96±0.01 mg EQ/g (Red EtOH) among other samples, and the antioxidant capacity varied from 371.12±0.01 (Brown SCO2) to 89.90±0.02 (Red EtOH) (IC50).

2.2. Propolis - implication in laying hen diet

In general, the use of propolis is pronouncedly increasing in medical science, but very limited data is available regarding its use in the field of poultry production. This subsection aimed to present the effect of supplementing the diets of laying hens.

Table 2 presents a summary of propolis applications in hen diet. Some researchers showed a beneficial influence on daily gain, feed intake and conversion in different animal species, including poultry (Guclu-Kocaoglu, 2010; Mathivanan et al. 2013). Galal et al. (2008) found that the feed consumption for laying hens fed diets that contained 100 and 150 mg propolis/kg diet increased significantly compared with control group.

Table 2. Summary of propolis applications in hen nutrition

Type	Animals	Dose of inclusion	Effect	Reference
Propolis	28-weeks-old Lohmann LSL hybrid layers	250, 500 and 1000 mg/kg diet	- improved egg production, blood constituent and haematological parameters of the commercial laying hens.	Abdel-Kareem and El-Sheikh (2017)
Green propolis	brown Hy-line laying hens	10 20, 30 g/kg of feed	- reduced bacterial contamination in the eggshells reduced the lipid peroxidation of fresh and stored eggs	Casagrande et al. (2021)
Propolis	55-wk-old Isa Brown® layers	0, 1, 2 and 3%	- did not improve performance and worsened the eggs' quality improves the integrity of the gastric tract	Belloni et al. (2015)
Propolis	Hy-Line White strain 46-54wk	50, 100 and 150 mg	- 100 or 150 mg is beneficial for improving the performance and immunity and for exploiting the full genetic potential of the commercial laying hens.	Galal, et al. (2008)
Propolis	Hyline White Leghorn, 42-week-old (chronic heat stress)	3 g/kg	- exhibited the same efficiency than antibiotic for restoring performances, nutrient digestibility and egg qualities in laying hens chronically exposed to heat stress.	Seven et al. (2011)
Propolis	White Leghorn layer hens	0.5, 1, 3, and 6 g/kg of diet	- 3 g/kg of diet may have a positive effect on humoral immunity of laying hens.	Çetin et al. (2010)

Contradictory results were observed by Abdel-Kareem and El-Sheikh (2017), respectively the final body weight and egg weight of hens that received different levels of propolis were not significantly different in comparison with control group. Belloni et al. (2015) also showed that the propolis supplementation did not improve performance and worsened the eggs' quality.

Regarding the egg production, the result showed that dietary propolis supplementation improved egg numbers and egg production rate for hens treated with propolis at 100 and 150 mg/kg diet significantly than those of the control group (Galal et al., 2008). The same authors showed that the averages of increased while the eggshell thickness for eggs produced from treated laying hens was significantly higher as compared to the control group. Dietary supplementations of laying hens with flavomycin or propolis have significantly reduced the negative effects of heat stress on performances, nutrient digestibility and eggshell characteristics (Seven et al., 2011).

Dietary propolis supplementation has an effective role in improve laying hen's health. In this line, El-Neney et al. (2014) showed that plasma cholesterol was significantly reduced in Dokki 4 laying hens fed propolis compared to control. Plasma total protein, albumin and globulin were significantly lower for control than those fed propolis. Using different dietary propolis levels of treated groups led to a significant increase in RBC and WBC, Hb, lymphocytes, eosinophils and monocytes percentages, while the basophils percentage was insignificantly affected. Due to the antibacterial and antioxidant activity, some studies showed that propolis can reduce the bacterial contamination and delay the lipid oxidation process in eggs (Casagrande et al., 2021).

CONCLUSIONS

It is undeniable that there is an increasing demand for functional foods for human consumption that offer various benefits in addition to nutrients. Dietary microalgae and propolis could be effective in improve the nutritional quality of eggs without affecting laying hen's performance. This observation result from the valuable chemical composition: microalgae contain important percent of vitamins (especially B1, B12, A), carotenoids, minerals (Fe, K, Ca, Se) and propolis is characterised by aromatic acids, flavonoids, diterpenoid acids, phenolic compounds and triterpenoids with high antioxidant capacity. Many studies confirmed the advantages of using microalgae and propolis in laying hen nutrition and their ability to improve health, productive parameters, egg nutritional quality (fatty acids, carotenoids, reduced cholesterol) and to inhibit lipid oxidative processes in eggs.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Research and Digitalization, grant number PN 19 09 0102.

REFERENCES

Abdel-Kareem, A.A.A., & El-Sheikh, T.M. (2015). Impact of supplementing diets with propolis on productive performance, egg quality traits and some haematological variables of laying hens. *Journal of Animal Physiology and Animal Nutrition*, *101*(3), 441–448. doi:10.1111/jpn.12407.

An, B.K., Kim, K.E., & Jeon, J.Y. (2016). Effect of dried Chlorella vulgaris and Chlorella growth factor on growth performance, meat qualities and humoral immune responses in broiler chickens. *SpringerPlus* 5, 718. https://doi.org/10.1186/s40064-016-2373-4.

Andrade, L.M., Andrade, C.J., & Dias, M. (2018). Chlorella and spirulina microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. *MOJ Food Process Technol.* *6*(1):45–58. DOI: 10.15406/mojfpt.2018.06.00144.

Ao, T., Macalintal, L.M., Paul, M.A., Pescatore, A.J., Cantor, A.H., Ford, M.J., & Dawson, A.K. (2015). Effects of supplementing microalgae in laying hen diets on productive performance, fatty-acid profile, and oxidative stability of eggs. *Journal of Applied Poultry Research*, *24*(3), 394-400.

Belloni, M., Almeida Paz, I.C.L., Nääs, I.A., Alves, M.C.F., Garcia, R.G., Caldara, F.R., & Seno, L.O. (2015). Productive, qualitative, and physiological aspects of layer hens fed with propolis. *Brazilian Journal of Poultry Science*, *1*(4), 467-472 http://dx.doi.org/10.1590/1516-635x1704467-472.

Burdock, G.A. (1998). Review of the biological properties and toxicity of bee propolis (propolis). *Food and Chemical toxicology, 36*(4), 347-363.

Casagrande, A.C., Machado, G.C., Brunetto, A.L., Galli, G.M., Rosa, G.D., Araujo, D.N., & Silva, A.S. (2021). The addition of green propolis to laying hens had positive effects on egg quality: lower bacteria counts in the shell and lipid peroxidation in the yolk. *Anais da Academia Brasileira de Ciências*, 93.

Cécere, B.G., da Silva, A.S., Molosse, V.L., Alba, D.F., Leal, K.W., da Rosa, G., & Vedovatto, M. (2021).

Addition of propolis to milk improves lactating lamb's growth: Effect on antimicrobial, antioxidant and immune responses in animals. *Small Ruminant Research*, 194, 106265.

Çetin, E., Silici, S., Çetin, N., & Güçlü, B.K. (2010). Effects of diets containing different concentrations of propolis on hematological and immunological variables in laying hens. *Poultry Science*, *89*(8), 1703-1708, https://doi.org/10.3382/ps.2009-00546.

Coudert, E., Elisabeth, E. & Berri, C. (2020). Use of algae in poultry production: a review. *World's Poultry Science Journal*, DOI: 10.1080/00439339.2020.1830012

de Medeiros, V P.B., Pimentel, T.C., Sant'Ana, A.S., & Magnani, M. (2021). Microalgae in the meat processing chain: feed for animal production or source of techno-functional ingredients. *Current Opinion in Food Science*, *37*, 125-134.

Deasy Liestianty et al. (2019). *IOP Conf. Ser.: Mater. Sci. Eng*., 509 012031.

Devequi-Nunes, D., Machado, B.A.S., Barreto, G.d.A., Rebouc, as Silva, J., da Silva, D.F., & da Rocha, J.L.C. (2018). Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. *PLoS ONE*, *13*(12), e0207676. https://doi.org/ 10.1371/ journal. pone.0207676,

Doucha, J., Lívanský, K., Kotrbáček, V., & Zachleder, V. (2009). Production of Chlorella biomass enriched by selenium and its use in animal nutrition: A review. *Appl. Microbiol. Biotechnol*. *83*, 1001–1008.

Elnakady, Y., Rushdi, A., & Franke, R. (2017). Characteristics, chemical compositions and biological activities of propolis from Al-Bahah, Saudi Arabia. *Sci Rep, 7*, 41453, https://doi.org/10.1038/srep41453.

El-Neney, B.A.M., Awadien, N.B., & Eid, Y.Z. (2014). The use of propolis as a source of natural additives to improve the productive performance and immune system of chicken's local. 1 – Effect on laying hen performance. 7 *International Poultry Conference – Proceeding*, 58–79.

Englmaierova, M., Skřivan M., & Bubancova I. (2013). A comparison of lutein, spray-dried chlorella, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens. *Czech Journal of Animal Science*, *58*, 412–419. doi:10.17221/6941-CJAS.

Fraeye, I., Bruneel C., Lemahieu C., Buyse J., Muylaert K., & Foubert I. (2012). Dietary enrichment of eggs with omega-3 fatty acids: A Review. *Food Research International*, 48, 961–969. doi:10.1016/ j.foodres.2012.03.014.

Galal, A., Abd El – Motaal, A.M., Ahmed, A.M.H., & Zaki, T.G. (2008). Productive performance and immune response of laying hens as affected by dietary propolis supplementation. *International Journal of Poultry Science*, *7*, 272-278.

Guclu-Kocaoglu, B. (2010). Effect of dietary addition of propolis and caffeic acid on the growth performance, production and hatching performance, egg quality and some biochemical parameters of quail (*Coturnix Coturnix Japonica*). *Ere University Journal of Health Science*, *19*, 94–104.

Kibria, S., & Kim, I.H. (2019). Impacts of dietary microalgae (Schizochytrium JB5) on growth performance, blood profiles, apparent total tract digestibility, and ileal nutrient digestibility in weaning pigs. *Journal of the Science of Food and Agriculture*, *99*(13), 6084-6088.

Kostić, A.Ž., Milinčić, D.D., Barać, M.B., Ali Shariati, M., Tešić, Ž.L., & Pešić, M.B. (2020). The application of pollen as a functional food and feed ingredient—the present and perspectives. *Biomolecules*, *10*(1), 84.

Kotrbáček, V., Doubek, J., & Doucha, J. (2015). The chlorococcalean alga Chlorella in animal nutrition: a review. *Journal of Applied Phycology*, *27*(6), 2173-2180.

Kuropatnicki, A.K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-Based Complementary and Alternative Medicine*, 2013.

Li, X., Fan, X., Han, L., & Lou, Q. (2002). Fatty acids of some algae from the Bohai Sea. *Phytochemistry*, *59*(2), 157-161.

Luo, X., Su, P., & Zhang, W. (2015). Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Mar Drugs*, *13*(7), 4231-54. doi: 10.3390/md13074231.

Mathivanan, V., Shah, Gh., Manzoor, M., Mir, G.M., Selvisabhanayakam. (2013). A review on propolis – as a novel folk medicine. *Indian Journal of Science*, *2*, 23–30.

Panaite, T.D., Nour, V., Saracila, M., Turcu, R.P., Untea, A.E., & Vlaicu, P.A. (2021). Effects of linseed meal and carotenoids from different sources on egg characteristics, yolk fatty acid and carotenoid profile and lipid peroxidation. *Foods*, *10*(6), 1246.

Sărăcilă, M., Panaite, T.D., Untea, A., Ropota, M., Vlaicu, P.A., Șoica, C., & Mitoi, M. (2017). Evaluation of oxidation stability and antioxidant activity in eggs enriched in ω-3 polyunsaturated fatty acids. *Archiva Zootechnica*, *20*(1), 51-64.

Saracila, M., Panaite, T.D., & Dragotoiu, D. (2021). Implication of dietary phytogenic mixture in modulating the intestinal microflora of broilers raised in thermoneutral and heat stress conditions. *Scientific Papers. Series D. Animal Science*, Volume LXIV, *1*, 205-209.

Saracila, M., Panaite, T.D., Mironeasa, S., & Untea, A.E. (2021b). Dietary supplementation of some antioxidants as attenuators of heat stress on chicken meat characteristics. *Agriculture*, *11*, 638.

Saracila, M., Panaite, T.D., Papuc, C.P., & Criste, R.D. (2021a). Heat stress in broiler chickens and the effect of dietary polyphenols, with special reference to willow (Salix spp.) bark supplements—a review. *Antioxidants*, *10*, 686. https://doi.org/10.3390/ antiox10050686

Seven, I., Tatli, S.P., & Silici, S. (2011). Effects of dietary Turkish propolis as alternative to antibiotic on growth and laying performances, nutrient digestibility and egg quality in laying hens under heat stress. *Rev Med Vet*, 162, 186-19.

Solomons, N.W. (2012). Vitamin A. *In Present Knowledge in Nutrition* (eds J.W. Erdman, I.A.

Macdonald and S.H. Zeisel). https://doi.org/10.1002/9781119946045.ch11
Soni, R.A., Sudhakar, K., & Rana, R.S. (2017). Spirulina–From growth to nutritional product: A review. *Trends in food science & technology*, *69*, 157-171.
Taofiq, O., Fernandes, A., Barreiro, M.F., & Ferreira, I.C.F.R. (2017). UV-irradiated mushrooms as a source of vitamin D2: A review. *Trends Food Sci. Technol*., *70*, 82–94. doi: 10.1016/j.tifs.2017.10.008.
Untea, A.E., Varzaru, I., Turcu, R.P., Panaite, T.D., & Saracila, M. (2021). The use of dietary chromium associated with vitamins and minerals (synthetic and natural source) to improve some quality aspects of broiler thigh meat reared under heat stress condition, *Italian Journal of Animal Science*.
Valle, M.L. (2000). Quantitative determination of antibacterian capacities of propolis. *Apiacta*. *35*, 152-161.
Van Vo, B., Siddik, M.A., Fotedar, R., Chaklader, M.R., Hanif, M.A., Foysal, M.J., & Nguyen, H.Q. (2020). Progressive replacement of fishmeal by raw and enzyme-treated alga, Spirulina platensis influences growth, intestinal micromorphology and stress response in juvenile barramundi, Lates calcarifer. *Aquaculture*, *529*, 735741.
Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E., Smith, A.G., Camire, M.E., & Brawley, S.H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of applied phycology*, *29*(2), 949–982. https://doi.org/10.1007/s10811-016-0974-5.
Woortman, D.V., Fuchs, T., Striegel, L., Fuchs, M., Weber, N., Brück, T.B., & Rychlik, M. (2020). Microalae a superior source of folates: Quantification of folates in halophile microalgae by stable isotope dilution assay. *Front. Bioeng. Biotechnol*, *7*, 481. doi: 10.3389/fbioe.2019.00481.
Zahroojian, N., & Morajev, H.M.S. (2013). Effects of dietary marine algae (Spirulina platensis) on egg quality and production performance of laying hens. *Journal of Agricultural Science and Technology, 15*, 1353–1360.
Zeller, S., Barclay, W., & Abril, R. (2001). Production of docosahexaenoic acid from microalgae. 108–124 in *Omega-3 fatty acids: chemistry nutrition and health effects.* Shahidi, F., and J. Finley, ed., American Chemical Society, Washington, DC.
Zheng, L., Oh, S.T. Jeon, J.Y. Moon, B.H. Kwon, H.S. Lim, S.U. An, B.K., & Kang, C.W. (2011). The dietary effects of fermented Chlorella vulgaris (CBT) on production performance, liver lipids and intestinal microflora in laying hens. *Asian-Australasian Journal of Animal Sciences*, *25*, 261–266. doi:10.5713/ajas.2011.11273.