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Effects of Adding *Spirulina platensis* to Laying Hen Rations on Performance, Egg Quality, and Some Blood Parameters

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ABSTRACT: This study was conducted to determine the effect of adding different levels of *Spirulina platensis* (*SP*) to laying hen diets on performance, egg quality, egg yolk color and some blood parameters. Sixty 60-week-old Lohmann LSL Classic laying hens were used in the study. The experiment was conducted in 15 subgroups with 4 hens in each cage, with 5 replications in 3 treatment groups. Three different diets were offered to laying hens; one control (based to wheat) and two supplemented with different levels (1 and 2%) of *SP*. As it was observed, the addition of *SP* to laying hen diets had no significant effect on live-weight change (LWC), egg yield (EY), egg mass, egg weight (EW), feed intake (FI), and feed conversion ratio (FCR) (p>0.05). Concerning the egg quality characteristics that were examined, the effects of *SP* on shell strength (SS), shell ratio (SR), shell thickness (ST), shape index (SI), albumen index (AI) and serum parameters were not statistically significant (p>0.05); egg yolk color characteristics (Roche Color Scale and L*, a*, b*) were affected by dietary *SP* supplementation (p<0.01). Addition of different levels of *SP* (1% and 2%) to laying hen diets caused a significant increase in egg yellow color values compared to the control group. According to the results of the study, the addition of 1% and 2% SP to the laying hen rations had a significant positive effect on egg yolk color, but did not cause a significant change in other parameters.

Keywords: Egg quality; Laying hen; Performance; *Spirulina platensis*; Serum parameters

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INTRODUCTION

In recent years, the latest trend in the feed sector has been towards the use of natural ingredients as alternatives to antibiotics, synthetic color agent, and other chemicals. *Spirulina platensis* (*SP*), also known as blue-green algae, one of these natural ingredients, is one of the high-quality natural feed additives that can be used in the diets of ruminants and poultry. There are two species of Spirulina that are widely used worldwide: *Spirulina platensis* and *Spirulina maxima* (Oliveira et al., 1999). *Spirulina platensis*, with its nutrient profile and high protein and carotenoid content, has been used as an excellent food source for humans and animals for hundreds of years (Mostolizadeh et al., 2020; Sharmin et al., 2020) and is commonly found in Africa, Asia, and South America (Vonshak, 2002). It is rich in unsaturated and polyunsaturated fatty acids, especially that oleic acid, linoleic acid, gamma-linolenic acid, and docosahexaenoic acid (Hue et al., 2002; Yukino et al., 2005; Habib et al., 2008; Sharmin et al., 2020).

Zeweil et al. (2016) reported that *SP* consist of 85.77% dry matter, 57.66% crude protein, 1.75% crude oil, 3.6% crude cellulose, and 9.83% crude ash. At the same time, *SP* has a protein content ranging from 55% and 70% and contains all the essential amino acids (Seyidoğlu and Galip, 2013, Seyidoğlu et al., 2017). In addition, the energy content of *SP* was determined to be 2500-3290 kcal ME/kg and its phosphorus availability was 41% (Zahroojian et al., 2013). *Spirulina platensis* is also rich in thiamine, riboflavin, pyridoxine, vitamin B_{12}, vitamin C, and carotenoids. Also, *SP* is widely used as a feed additive for the improvement of the egg yolk color in laying hens and the meat quality in broilers (Ross and Dominy, 1990). It was reported in a study that the addition of 3-9% *SP* to the diet of laying hens resulted in egg yolk colors that best represented consumer preferences (Saxena et al., 1983). This effect of *SP* on egg yolk is due to its rich content of zeaxanthin, xanthophyll, and other carotenoid pigments, especially β-carotene (Takashi, 2003; Maoka, 2011). *SP* has also been reported to fortify human organism against disorders such as diabetes and rheumatism (Parikh et al., 2001; Rasool et al., 2006). It was also stated in literature that *SP* has immune system-stimulating effects and antiviral activity (Khan et al., 2005).

Spirulina platensis has been reported to improve the health status and survival ability in poultry and enhance immune function (Qureshi et al., 1996; Kanagaraju and Omprakash, 2016). In this context, some researchers reported that the poultry fed with diets containing *SP* gained superior reproductive performance compared to the control group (Ross and Dominy, 1990; Nikodémusz et al., 2010; Mariey et al., 2012; Samia et al., 2018). It has been shown that *SP* is an effective way of improving the quality of product obtained from poultry to meet consumer preferences. For example, the total cholesterol content of eggs can be reduced by the addition of *SP* to hens diets (Sujatha and Narahari, 2011). This can mainly be attributed to the high antioxidant and omega-3 polyunsaturated fatty acid content of *SP*, which enhance the nutritional value of eggs (Rajesha et al., 2011; Sujatha and Narahari, 2011).

In another study, it was reported that the addition of *SP* to the diet increased egg yield from 87% to 96% (Ross and Dominy, 1990). Raju et al. (2005) found that the addition of *SP* by 0.05% to the diet could partially offset the negative effect of 300-ppm aflatoxin on the growth rate and lymphoid organ weight of broilers. Islam et al. (2009) reported that *SP* could help in reducing the arsenic accumulation in the tissues of ducks. Selim et al. (2018) stated that *SP* dietary supplementation at the level of 0.3% could be suggested for laying hens, and the egg yield increased in the 0.3% *SP* added group compared to the other groups. Considering the age of 60-weeks-old laying hen, the hypothesis that *SP* has no effect on laying hen of this age is the original aspect of this study. The purpose of this study was to determine the effect of adding *Spirulina platensis,* which was obtained from a commercial company, to the laying hen diets on performance, egg internal and external quality characteristics, and some blood parameters.

MATERIALS AND METHODS

In this study, sixty 60-week-old Lohmann LSL Classic laying hens were used as animal material. The average live weight of the hens was 1652.8 g. A trial was conducted with three treatment groups (5 replications of 20 hens per treatment). In study, three different diets were offered one of which was the control (without SP addition) and 2 that were supplemented with different levels (1% and 2%) of *Spirulina platensis*.

The experimental diets were prepared based on wheat-soybean meal and the nutrient needs of laying hens were formulated according to recommendations of NRC (1994). The study lasted 28 days, the feed and water were provided to the animals *ad libitum* throughout the study, and 16 hours of lighting was applied. The composition of the experimental diets used in the study are shown in the Table 1.

Table 1. The composition of experimental diets

Ingredients	Dietary Spirulina levels, %		
	0	1	2
Wheat	60.00	63.70	62.50
Soybean meal (43.8% CP)	17.00	15.00	15.00
Sunflower seed meal (32% CP)	8.50	8.00	6.70
Spirulina (65% CP)	0.00	1.00	2.00
Vegetable oil (8800 Kcal ME/kg)	3.20	2.00	2.5
Limestone	9.10	9.10	9.10
Dicalcium phosphate, DCP	1.50	1.50	1.50
Salt	0.25	0.25	0.25
Premix	0.25	0.25	0.25
Methionine	0.20	0.20	0.20
Calculated nutrient composition			
Metabolizable energy, ME (Kcal/kg)	2770	2753	2767
Crude Protein, CP (%)	16.64	16.66	16.70
Calcium (%)	3.89	3.89	3.88
Available phosphorus (%)	0.40	0.40	0.41
L- Lysine (%)	0.76	0.75	0.76
DL-Methionine (%)	0.44	0.44	0.45

Premix (in 1 kg of the ration): vitamin A, 8.800 IU; vitamin D3, 2.200 IU; vitamin E, 11 mg; nicotinic acid, 44 mg; Cal-DPantothenate, 8.8 mg; riboflavin 4.4 mg; tiamine 2.5 mg; vitamin B12, 6.6 mg; folic acid, 1 mg; D-Biotin, 0.11 mg; colin, 220 mg; manganese, 80 mg; copper, 5 mg; iron, 60 mg; zinc, 60 mg; cobalt, 0.20 mg; iodine, 1 mg; selenium, 0.15 mg

The live weights of the animals were determined at the beginning and end of the trial. The feed intake (FI) and egg weights (EW) were measured in biweekly and the egg production was recorded daily. Using the data of egg yield (EY) and egg weight (EW), the egg mass was calculated by the following formula: Egg mass (EM) = (egg yield x egg weight)/period (day). The feed conversion ratio (FCR; feed g/egg g) was calculated by the following formula: FCR = FI (g feed/hen/period) / EM (g egg/hen/period).

The egg shell breaking strength, egg shell thickness, egg shell weight, egg shape index, yolk and albumen index, and egg yolk color criteria (Roche scale and CIELAB as L*, a*, b*) were measured in the eggs collected in the last two days of the trial. The yolk and albumen heights were determined using a digital height gauge, and yolk diameter and albumen length and diameter were determined using a digital caliper.

The following formulas were used to calculate the yolk index and albumen index: Yolk index (%) = (yolk height/yolk diameter) x 100 and Albumen index (%) = (albumen height / (albumen length + albumen width) x 100. The egg quality analyses were completed within 24 hours after the eggs were collected. The shell membrane weight (%) was calculated using the following formula: Eggshell weight (g)/egg weight x 100. The eggshell strength was measured by applying the assisted system pressure to the blunt part of the egg (Egg Force Reader, Orka Food Technology, Israel). The shell membrane thickness was calculated by averaging the values measured from three points on the eggs (2 from the equatorial region, 1 from the blunt and pointed part) using a micrometer (Mitutoyo, 0.01 mm, Japan). A colorimeter was used for the assessment of yolk color (Minolta Chroma Meter CR 400 (Minolta Co., Osaka, Japan) (Romero et al., 2002). Egg yolk color was measured with Roche color scale.

At the end of the trial, 10 hens randomly selected from each group (2 hens from each replicate) a were slaughtered and approximately 5 ml of blood was collected into the vacuum tubes with clotting activators and delivered to the laboratory by the cold chain. The serum tubes brought to the laboratory were centrifuged at 2700 x g for 10 minutes and then their serum were collected and put into the Eppendorf tubes. Serum cholesterol, HDL, albumin, total protein, phosphorus, calcium, uric acid, and globulin analyses were carried out using the commercial kits in the autoanalyzer device (DDS ® Spectrophotometric Kits, Diasis Diagnostic Systems Co., Istanbul, Turkey).

Data were subjected to one-way ANOVA using Minitab (2000). Duncan's multiple range tests were applied to detect diffirences among means (Duncan 1955). Statements of statistical significance are based on a probability of $p<0.05$.

RESULTS

The effects of the addition of 1% and 2% *SP* to the laying hen rations on LWC, EY, FC, EW, EM, and FCR are presented in the Table 2. It was observed that the addition of *SP* to laying hen rations had no significant effect on LWC, EY, EM, EW, FC, and FCR ($p>0.05$).

Table 2. Effects of the rations containing different levels (1% and 2%) of *Spirulina platensis* on performance parameters of laying hens.

Parameters	Treatments			SE	*P*-Value
	Control	SPR1	SPR2		
ILW, g	1629.5	1654.0	1674.9	30.8	0.59
FLW, g	1594.9	1652.9	1670.2	38.5	0.38
LWC, g	-34.7	-1.1	-4.7	51.4	0.41
EY, %	89.1	93.8	93.1	3.4	0.60
FI, g/hen/day	120.3	116.9	118.6	1.8	0.46
EW, g	63.8	65.2	66.5	1.4	0.45
EM, g/hen/day	56.9	61.2	61.7	2.4	0.34
FCR, FC/EM	2.1	1.9	1.9	0.1	0.31

ILW: Initial live weight, FLW: Final live weight, LWC: Live weight change, EY: Egg yield, EW: Egg weight, EM: Egg mass, FI: Feed intake, FCR: Feed conversion ratio, SE: Standard error. SPR1: 1% *Spirulina platensis,* SPR2: 2% *Spirulina platensis*.

The effects of the addition of *SP* (1% and 2%) to the laying hen rations on the shell strength, shell ratio, shell thickness, shape index, albumen index, yolk index, and yolk color values (L, a, b) are shown in the Table 3. When we compared different doses of *SP* with the control group, it was found that its effect on yolk color values (L, a, b) was statistically significant ($p<0.01$). The effect of *SP* addition on yolk index was also significant ($p<0.05$).

Table 3. The effects of the rations containing different levels (1% and 2%) of *Spirulina platensis* on egg quality parameters of laying hens

Parameters	Treatments			SE	*P*-Value
	Control	SPR1	SPR2		
SS, kg	3.88	3.79	3.86	0.20	0.95
SR, %	9.32	9.59	9.28	0.14	0.31
ST, mm	0.36	0.38	0.37	0.00	0.22
SI, %	72.17	73.64	73.64	0.89	0.44
AI, %	8.34	8.76	8.12	0.25	0.23
YI, %	44.74^{a}	42.21^{b}	44.51^{a}	0.70	0.05
Color Characteristics					
Roche	2.35^{C}	9.20^{B}	11.20^{A}	0.23	<0.001
CIELAB					
L^*	54.24^{A}	51.44^{B}	49.87^{B}	0.66	<0.001
a^*	-5.84^{C}	1.34^{B}	4.92^{A}	0.27	0.002
b^*	20.24^{B}	34.50^{A}	35.68^{A}	1.18	<0.001

SS: Shell strength, SR: Shell ratio (% of egg weight), ST: Shell thickness, SI: Shape Index, AI: Albumen index, YI: Yolk index, SE: Standard error. SPR1: 1% *Spirulina platensis,* SPR2: 2% *Spirulina platensis*.

^{A,B,C} Differences shown with different letters in the same row are statistically significant ($p<0.01$); ^{a,b}:$p<0.05$.

The effects of the addition of 1% and 2% of *SP* to the laying hen rations on the serum cholesterol, HDL, albumin, globulin, total protein, uric acid, calcium, and phosphorus parameters are given in the Table 4. The effect of *SP* addition on serum parameters was not found statistically significant (p>0.05).

Table 4. Effects of the rations containing different levels of *Spirulina platensis* on serum parameters of laying hens

Parameters	Treatments			SE	*P*-Value
	Control	SPR1	SPR2		
Cholesterol,mg/dl	132.40	146.40	128.00	9.56	0.39
HDL, mg/dl	19.00	18.60	20.20	1.21	0.63
Albumin, g/dl	1.76	1.92	1.80	0.05	0.11
Globulin, g/dl	4.68	5.78	4.66	0.34	0.06
Total Protein, g/dl	6.44	7.70	6.46	0.37	0.06
Uric Acid, mg/dl	5.90	5.70	6.10	0.56	0.88
Calcium, mg/dl	30.60	30.04	30.54	1.00	0.91
Phosphorus, mg/dl	5.62	5.14	5.00	0.50	0.66

SE: Standard error. SPR1: 1% *Spirulina platensis,* SPR2: 2% *Spirulina platensis*

DISCUSSION

Performance parameters

According to the Table 2, the effect of 1% and 2% *SP* addition to the laying hen rations on the performance parameters was not significant (p>0.05).

In a study, the high dose of *SP* (15%) reduced the albumin content in the egg, causing a decrease in EW (Blum et al., 1975). Takashi, (2003) and Nikodemusz et al. (2010) reported that the production performances of the laying hens fed on the rations containing *SP* were positively affected. Another study reported that the addition of 10% or above *SP* to ration could suppress the growth of poultry (Ross and Dominy, 1990). In contrast, some researchers reported that *SP* positively improved the LWC and FCR of hens compared to the control group (Kharde et al., 2012; Shanmugapriya and Saravana Babu, 2014).

Mariey et al. (2012) found that 0.2% addition of *SP* to the laying hen rations had a statistically positive effect on EY, EW, EM, and FCR (p<0.05). Furthermore, Bellof and Alarcon (2013) observed that the addition of *SP* under the conditions of organic farming significantly improved the growth and carcass performances of broilers. Bonos et al. (2016) found that the addition of *SP* to the broiler rations did not cause a statistically significant difference in terms of LWC, FCR, and mortality compared to the control group. Similarly, Zeweil et al. (2016) stated that *SP* addition did not affect the LWC of broilers under temperature stress. In contrast to our findings in the trial, Michalak et al. (2020) emphasized that the effect of *SP* addition on FCR was significant.

However, Halle et al. (2009) observed a decrease in the feed intake of hens; however, the ration with higher amounts of algae did not affect the egg production. In another study, it was emphasized that the addition of *SP* did not affect the FI of quails (Hayati et al., 2020). Moreover, Omri et al. (2019) found that the *SP* addition did not affect the FI of quails, which is consistent with the finding of the present study.

Furthermore, some researchers found that the hens fed with *SP* supplemented ration achieved the highest egg production rates and FCR compared to the control group (Ross et al., 1994; Nikodémusz et al., 2010; Mariey et al., 2012). The increase in the egg weight of hens fed on *SP* ration in these studies may be associated with the heavier yolks. Accordingly, the results of the present study were similar to those found by Mariey et al. (2012), Zahroojian et al. (2013), and Selim et al. (2018). The different results in the studies may be due to the differences in the breeds and age of the laying hens.

Zahroojian et al. (2013) reported that *SP* had no statistically significant effect on EY, FI, FCR, and EW (p>0.05). Moreover, Hayati et al. (2020) stated that *SP* did not affect FCR, EW, and EY. It can be suggested that the differences in these results may stem from the inability to accurately determine the *SP* doses added to the rations, the problems regarding the housing conditions of animals, different species of animals used in the studies, the feed composition, and the production systems, etc.

Egg quality characteristics

As can be observed in the Table 3, there was no statistical difference between adding 1% and 2% of *SP* to the laying hen rations in terms of shell strength, shell weight, shell thickness, shape index, albumen index ($p>0.05$). In contrast, the effect of *SP* supplementation was significant on the yolk index ($p<0.05$) and yolk colour "L*, a*, b*" values ($p<0.01$).

Selim et al. (2018) found that the addition of different levels of *SP* to the diet did not affect SI, eggshell percentage, albumen (%), albumen index (%), yolk weight (%), and Haugh unit ($p>0.05$); whereas the yolk color score was higher in the group in which 0.3% *SP* was added to the diet.

The yolk color scores, one of the egg quality parameters, were examined and it was found that the addition of different doses of *SP* to the laying hen rations to increase the yolk color scores compared to the control group. Based on this, the highest Roche score was found at the dose of 2%. These results were similar to those found in the previous studies on yolk color scores (Mariey et al., 2012, Zahroojian et al., 2013). Algae increase the total carotenoid and antioxidant content in the egg and, as a result, reduce egg yolk cholesterol; therefore, they have some positive effects on human health (Zahroojian et al., 2013; Al-Harthi, 2014; Park et al., 2015). According to Selim et al. (2018), the increase in yolk color due to the increase in the dose of *SP* added to the laying hen rations compared to the control group most probably reflected an increase in the yolk pigmentation, enabling the accumulation of carotenoids in the egg yolk. Omri et al. (2019) stated that the *SP* addition significantly affected the L*, a*, and b* values, the color characteristics of egg yolk.

The effect of adding different doses of *SP* to the laying hen rations on the lightness (L), one of the color characteristics of egg yolk, was found to be statistically significant ($p<0.01$). The addition of different doses of *SP* to the ration reduced the lightness (L) of the egg yolk compared to the control group (54.24). No statistical difference was observed between the administered doses ($p>0.05$). The red/green value of the egg yolk (a) ranged between -5.84 and 4.92 in the study ($p<0.01$).

Egg selection by consumers is based not only on the cholesterol content of egg yolks or fatty acid profiles but also on the color of egg yolks (Englmairova et al., 2013). The degree of pigmentation required for the eggs varies depending on the geographical regions of countries, and the golden yellow colors are generally considered more attractive (Baiao et al., 1999). The yolk color intensity varies depending on the amount of carotenoid content found in the ration. Laying hens are unable to synthesize carotenoids; so, they have to absorb them from the rations (Park et al., 2015).

Algae, plants, fungi, and some bacteria synthesize carotenoids. In this study, the yolk color intensity was achieved by adding *SP* to the laying hen rations. A researcher reported that the addition of *SP* to the ration caused an increase in the egg yolk redness and a decrease in the yellowness intensity (Omri et al., 2019). Herber and Van Elswyk, (1996) reported that the addition of microalgae product to laying hen rations increased the amount of docosahexaenoic acid and also darkened the egg yolk color, the taste of the egg did not change, and the red/green value (a) was found to be high in the color analysis, and this was due to the increase in the color quality of the egg. Mariey et al. (2012) reported that the addition of different levels of *SP* (0.1%, 0.15%, and 0.2%) to the ration increased the yolk color score compared to the control group.

Zahroojian et al. (2013) found that the highest dose (2.5%) of *SP* yielded the highest value for the yolk color score among the groups in which 1.5%, 2%, and 2.5% *SP* were added to the ration, compared to the control group. Selim et al. (2018) found that the addition of *SP* to the ration increased the yolk color score due to the increase in dose and there was a strong positive correlation between the yolk color and the ration's containing *SP* from the 42nd week to the end of the 46th week. Anderson et al. (1991) reported that the addition of *SP* to quail rations gradually increased the egg yolk color scores from the beginning to the end of the trial. Park et al. (2015) found that the addition of 0.5% and 1.0% *Schizochytrium*, a microalgae species, to the laying hen rations increased the yolk color score compared to the control group at the end of the 6-week trial.

Serum parameters

The effects of the addition of different levels of *SP* to the laying hen rations on the serum cholesterol, HDL, albumin, globulin, total protein, uric acid, calcium, and phosphorus were not found significant ($p>0.05$).

Mariey et al. (2012) found that the addition of different levels of *SP* to the diet caused a significant decrease in cholesterol of blood concentrations.

Another study concluded that the decline of algae was associated with the docosahexaenoic acid and the ration fibers in its content (Lahaye and Jegou, 1993; Park et al., 2015). However, some researchers attributed this decline to the content of polyunsaturated fatty acids in *SP* and the inclusion of newly formed cholesterol esters in HDL (high-density lipoprotein) by omega 3 fatty acids' stimulating the LCAT (lecithin cholesterol acyltransferase) activity, an enzyme responsible for esterification of serum cholesterol (Rajaram and Barter, 1986; Vaysse-Boue et al., 2007; Ferchaud-Roucher et al., 2014). Furthermore, Chen et al. (2011) reported that docosahexaenoic acid (DHA) found in microalgae could inhibit the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by reducing serum cholesterol concentration.

A study found that the effect of *SP* addition on total protein, albumin, and globulin was statistically insignificant, whereas its effect on cholesterol, HDL, and LDL was found to be significant (Zeweil et al., 2016). Omri et al. (2019) stated that the effect of *SP* addition on cholesterol levels in egg yolks was statistically insignificant.

Many studies assert that algae have rich mineral content, which means that these plants can improve the health of poultry and the quality of products obtained from it. Studies have shown that the *SP* applied to laying hen rations does not have any effect on the calcium and phosphorus content in the blood of the animals studied (Konkol et al., 2018). In contrast, Michalak et al. (2011) reported that macroalgae-fed chickens had higher calcium content in blood. Finally, in a study examining the effect of *SP* addition on the serum parameters of quails, Abouelezz, (2017) reported that the *SP* addition did not statistically affect the total protein, albumin, and globulin values, but the low dose decreased the free fatty acids and cholesterol more than the high dose.

CONCLUSION

In conclusion, it was observed that the addition of *SP* to the laying hen rations had no significant effect on the performance and serum parameters ($p>0.05$), and it was effective only on the color characteristics among the egg quality characteristics examined in this study ($p<0.01$). Various researchers explained that the reasons for this were related to the fact that *SP* contained high amounts of carotenoids. In the study, the addition of *SP* to the ration increased the Roche score. It can be stated that the algae used commercially in poultry rations cause an increase in the egg yolk pigmentation by accumulating carotenoids in egg yolk. Based on the findings on its nutritional profile, it can be asserted that *SP* undoubtedly appears to be a strong feed supplement for poultry. For a better understanding of its nutritional value, it should also be examined in the studies to be carried out on various poultry species. For this reason, there is a need for further studies on the use of *SP* in poultry farming and its effects on the performance and egg quality characteristics.

CONFLICT OF INTEREST

None declared.

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