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Supplementation of spirulina (*Spirulina platensis*) on yolk colour, egg quality and production performance of laying hens

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Abstract

A total of 96 Shaver 579 laying hens of 78 weeks of age were studied for the effects on egg production of dietary supplementation with spirulina (*Spirulina platensis*). The hens were divided into four dietary treatments with six replications of four birds in each group. Birds of three groups were supplemented with 0.1%, 0.2% and 0.3% spirulina and one group was kept as control. Yolk colour significantly improved ($P<0.01$) as compared to control, without affecting any other production performances. It is suggested that dietary supplementation of spirulina at 0.2 to 0.3% levels in aged laying hens enhanced egg yolk colour. (*Bangl. vet*. 2017. Vol. 34, No. 2, 71–78)

Introduction

Organic feeding in poultry is pricier but organic products are safer than conventional poultry products (Blair, 2008). Algae are used in organic poultry production, having high nutritional value (Gerrard *et al*., 2015). Two types of algae are widely used in poultry diet. Microscopic algae are blue-green algae, which have the ability to grow under different environmental conditions and are a source of carbohydrates, proteins, amino acids, lipids, vitamins A, B (especially B_{12}) and C, and colourants (Al-Harthi and El-Deek, 2012). They also increase carotene, lutein and zeaxanthin content (Athukarala *et al.,* 2006). *Spirulina platensis* is ablue-green algae with antimicrobial, antiviral, anti-inflammatory and hypocholesterole micactivities (Mayada Farag *et al*., 2016). *Spirulina platensis* also enhances immune function by increasing antigen processing and T-cell activity (Qureshi *et al.,* 1994). In addition, spirulina is a source of carotenoids, which colour yolks (Lorenz, 2003).

Egg quality, which is important for producers and consumers, is determined by the age of hens, egg storage temperature, humidity, laying season and feeding. Yolk colour, texture, firmness and smell are the main determinants of yolk quality. Yolk colour can be increased by incorporating carotenes and xanthophylls (Nys, 2000). Xanthophyll is a plant pigment, which does not affect taste but meets market demand. Natural or synthetic xanthophylls can be incorporated in layer diet. Excess pigment in diet causes orange-red yolks (Coutts and Wilson, 1990) while low doses cause olive or salmon coloured yolks (Beyer, 2005). Most people prefer golden yellow to orange

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yolks. Consumers believe that eggs with pale yolks are neither tasty nor nutritious. Layer birds need to be fed pigment. The study was undertaken to determine the optimal levels of dietary *S. platensis* to improve egg yolk colour, egg quality and production of laying hens aged 78 - 84 weeks.

Materials and Methods

Experimental birds and management

A total of 96 Shaver 579 laying hens at 78 weeks of age were separated and kept in an open-sided house with routine management at Bangladesh Agricultural University (BAU) Poultry Farm, Mymensingh. The hens were divided into four groups and each group was divided into six sub-groups, each containing four birds. First group was control without supplementation and the other three groups were supplemented with 0.1%, 0.2% and 0.3% spirulina (*Spirulina platensis*), respectively. The trial lasted seven weeks. The bird shed was cleaned and disinfected. All feeders and drinkers were attached in the three tier California cage system. The experimental cage was divided into 24 equal sections, each containing four hens. Strict hygiene and bio-security were followed. A digital thermo-hygrometer was used to maintain the poultry shed temperature and relative humidity.

Preparation of experimental diets

A corn-soybean meal-based diet was formulated with local ingredients to meet the nutrient requirements of the hens (Table 1). Processed spirulina powder was collected from June Pharmaceuticals Ltd. Myanmar (Table 2) and was mixed thoroughly into the basal diet according to experimental layout.

Table 1: Ingredients and composition of basal diet

Feed ingredients	Basal diet (amounts in kg)	Chemical composition (%)	
Maize	58	ME (Kcal/kg)	2809.2
Protein concentrate	4	CP	17.57
Soya meal	23	Ca	3.77
Limestone	9	Total P	0.77
DCP	2	Av. P	0.47
Soybean oil	3	Lysine	0.91
Vit-Min Premix	0.25	Methionine	0.45
Lysine	0.04	Tryptophan	0.45
Methionine	0.15		
Enzyme	0.04		
Salt	0.52		

DCP = Di–calcium phosphate; Vit-Min Premix = Vitamin-Mineral premix; CP = Crude protein; Ca = Calcium; P = Phosphorus; ME = Metabolizable energy

Table 2: Chemical composition of *Spirulina platensis*

General composition	%	Phytopigments	Mg 100 g^{-1}
Protein	55-59	Total carotenoids	400-500
Carbohydrates	15-25	Carotenes	160-260
Fats (Lipids)	5-6	Xanthophyll	170-240
Minerals (Ash)	6-9	Chlorophyll	1300-1700
Moisture	2.5-4.5	Phycocyanin	15000-19000

Data collection

Hen day egg production (HDEP), egg weight and egg mass outputs were recorded. Two eggs from each sub-group were collected. The egg shape index, shell thickness and shell percentage were measured. The albumen index, Haugh unit, yolk index and yolk colour score were measured. The length and width were measured with the help of slide callipers for determining the shape index**.** Shell thickness was measured by means of an eggshell thickness meter (Ogawa Seiki Company, Tokyo, Japan). Yolk Index was calculated by the ratio of yolk height and yolk width. The Haugh unit was calculated by the formula suggested by Haugh (1937). Yolk colour score was determined by comparing with the Roche Yolk colour fan (RYC, F. Hoffman-La Roche and Ltd., Switzerland) depending on visual comparison according to Vuilleumier (1969). Best yolk colour was observed by Chromameter that represents L* (lightness), a* (redness) and b* (yellowness) values (Fig. 1). In a uniform colour scale, the differences between points plotted in the colour space correspond to visual differences between the colours plotted.

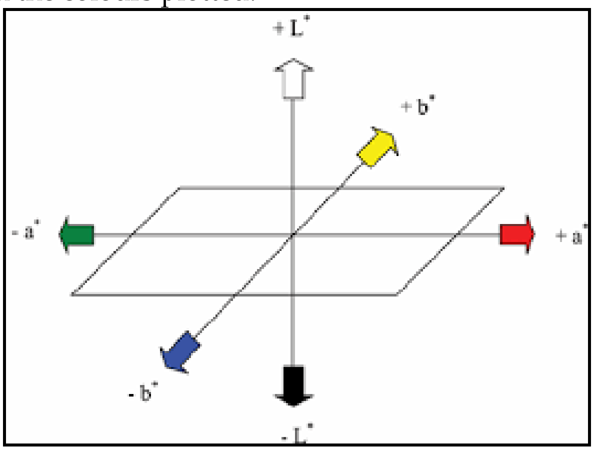

Fig. 1: L*, a*, b* values on egg yolk colour space.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) employing SAS (2002) statistical computer package program. The least significant differences (LSD) were calculated to compare treatment means.

Results and Discussion

Egg production performance

No differences in the hen day egg production (HDEP) were caused by the addition of spirulina (Table 3). This result is consistent with the result of other researchers (Garcia *et al.,* 2002; Zahroojian *et al.,* 2011) where they reported that dietary marine algae supplementation had no effect on egg production. Halle *et al.* (2009) observed the same at different levels of addition of algae. On the other hand, Nikodémusz *et al.* (2010); Mariey *et al*. (2012**)** found significantly better HDEP after supplementation of spirulina-containing diet in laying hens which are not in agreement with present finding.

Table 3: Egg production of hens fed spirulina-supplemented diet at 78-84 weeks of age

Parameters	Treatments	Age (week)						
		1st	2nd	3rd	4th	5th	6th	7th
Hen -day egg production (%)	Control	71.4 ± 6.1	86.9 ± 7.0	89.3 ± 4.4	84.5 ± 4.3	80.9 ± 4.8	85.7 ± 2.6	82.1 ± 3.1
	0.1% Spirulina	72.6 ± 4.3	89.3 ± 3.1	85.7 ± 3.2	85.7 ± 1.8	83.3 ± 5.1	79.8 ± 3.9	79.8 ± 4.3
	0.2% Spirulina	77.4 ± 5.9	86.9 ± 7.7	89.3 ± 5.5	82.1 ± 5.5	80.9 ± 6.6	85.7 ± 4.5	80.9 ± 6.8
	0.3% Spirulina	78.6 ± 8.7	85.7 ± 7.6	83.3 ± 8.4	86.9 ± 4.7	80.9 ± 3.5	79.8 ± 6.5	82.1 ± 7.9
	P-Value	NS	NS	NS	NS	NS	NS	NS

NS = No significant, Mean ± SE

Egg quality parameters

Egg weight and egg mass output data are shown in Table 4. There was similar egg weight found among the dietary treatments. In other studies, spirulina-supplemented diet increased egg weight (Sakaida Takashi, 2003; Nikodémusz *et al*., 2010). Ross *et al.* (1994) found that egg weight was significantly higher that containing increased levels of spirulina, which are in agreement with our findings at the end of 3rd week but at the end of 7th week egg weight was almost similar among different spirulina levels.

No differences were observed on egg mass, yolk weight, percent shell and Haugh unit after supplementation of spirulina. It has been reported that the egg weight and yolk weight were reduced significantly by dietary inclusion of higher level (4.8%) of marine algae (Al-Harthiand El-Deek, 2012). There was no effect of spirulina in layer diet on the shape index, shell thickness and shell percentage of egg. Inborr (1998) and Mariey *et al*. (2012) reported that spirulina had no effect on eggshell percentage, similar to our results. Spirulina had no influence on albumen index and Haugh Unit up to the end of 7th week.

Table 4: Egg weight and egg mass output of laying hens fed Spirulina-supplemented diet at 78-84 weeks of age

Para-meters	Treat-ments	Age (week)						
		1st	2nd	3rd	4th	5th	6th	7th
Egg weight (g)	Control	65.7 ± 3.1	63.6 ± 1.7	61.6^{b} ± 1.7	65.4 ± 1.7	63.8 ± 1.8	64.4 ± 2.4	69.0 ± 2.8
	0.1% Spirulina	66.2 ± 2.9	65.1 ± 1.5	63.4^{ab} ± 1.8	64.2 ± 1.3	62.6 ± 2.7	65.9 ± 2.8	67.1 ± 2.3
	0.2% Spirulina	67.8 ± 2.3	67.4 ± 1.0	67.3^{a} ± 2.3	66.1 ± 2.0	65.7 ± 2.8	68.0 ± 3.0	68.7 ± 3. 7
	0.3% Spirulina	67.8 ± 5.9	66.7 ± 2.7	68.5^{a} ± 1.3	65.7 ± 1.9	64.5 ± 2.2	67.5 ± 3.3	69.7 ± 3.1
	P-Value	NS	NS	*	NS	NS	NS	NS
Egg mass output (g) (egg/hen/day)	Control	46.3 ± 3.2	54.1 ± 3.4	55.7 ± 2.8	55.8 ± 1.5	49.8 ± 2.3	52. 5 ± 0.9	56.3 ± 0.7
	0.1% Spirulina	49.1 ± 2.4	58.1 ± 1.3	52.8 ± 3.1	53.1 ± 0.7	49.4 ± 1.9	50.9 ± 2.6	53.9 ± 1.4
	0.2% Spirulina	52.1 ± 3.6	53.5 ± 4.9	58.1 ± 5.4	52.9 ± 1.6	51.3 ± 2.6	57.5 ± 2.9	55.8 ± 3.2
	0.3% Spirulina	52.7 ± 4.3	55.8 ± 4.8	54.7 ± 6.2	56.4 ± 1.4	51.7 ± 2.7	52.5 ± 2.9	56.2 ± 4. 5
	P-Value	NS	NS	NS	NS	NS	NS	NS

NS = Not significant; Mean ± SE; * = P<0.05; a, b, c = means bearing dissimilar superscript in a column differ significantly

Yolk index and yolk colour

Yolk index and yolk colour score data are presented in Table 5. Yolk index was not affected by addition of spirulina in layer diet. This result is consisted with Zahroojian *et al*. (2013) who found no significant variation of yolk index after dietary supplementation of spirulina. All groups showed similar results in yolk colour score up to 2nd week of feeding trial. At 4th week of production, the maximum yolk colour score (7.50) was found in 0.3% spirulina group and it was significant (P<0.01) when compared with control group. There was a strong positive correlation between the inclusion of spirulina supplementation and egg yolk colour at the end of 4th week. Spirulina-containing diet enhanced the yolk colour scores of egg yolks of hens by RYCF and Chromameter. In general, the yolk colour score depends on the levels of natural pigments, mainly xanthophyll and canthaxanthin in the diet and the type and ratio of these compounds. Enhancement of yolk colour score is supported by several workers (Ross *et al*., 1994; Mariey *et al*., 2012**)** who observed that spirulina-supplemented diet significantly increased yolk colour score.

Table 6 represents the colour of the yolk. Yellowness was indicated by b* values. The 0.3% spirulina group was significantly more yellow (P<0.05) than the control group. Negative a* values indicated greenish colour, and L* indicates lightness (+ white, - black) of yolk, which were not significantly different between groups. Similar results were reported by Herber-McNeill and Van-Elswyk, 1998. Zeller *et al.* (2001) pointed out that *Schizochytrium sp*. algae contained carotenoids, specifically canthaxanthin and beta-carotene, which are major sources for yolk pigmentation.

Table 5: Yolk index and yolk colour of the eggs of laying hens fed spirulina-supplemented diet at 78-84 weeks of age

Parameters	Treatments	Age (week)						
		1st	2nd	3rd	4th	5th	6th	7th
Yolk Index	Control	0.39 ± 0.0	0.40 ± 0.0	0.41 ± 0.0	0.40 ± 0.0	0.42 ± 0.0	0.40 ± 0.0	0.40 ± 0.0
	0.1% Spirulina	0.39 ± 0.0	0.40 ± 0.0	0.40 ± 0.0	0.41 ± 0.0	0.40 ± 0.0	0.41 ± 0.0	0.40 ± 0.0
	0.2% Spirulina	0.39 ± 0.0	0.40 ± 0.0	0.41 ± 0.0	0.40 ± 0.0	0.40 ± 0.0	0.40 ± 0.0	0.41 ± 0.0
	0.3% Spiru lina	0.40 ± 0.0	0.40 ± 0.0	0.40 ± -0.0	0.40 ± 0.0	0.42 ± 0.0	0.42 ± 0.0	0.40 ± 0.0
	P-Value	NS	NS	NS	NS	NS	NS	NS
Yolk Colour Score	Control	6.33 ± 0.2	6.50 ± 0.4	6.50^{b} ± 0.2	6.67^{b} ± 0.3	6.83^{ab} ± 0.2	6.50^{b} ± 0.2	6.50 ± 1.1
	0.1% Spirulina	6.83 ± 0.2	6.50 ± 0.2	7.50^{a} ± 0.2	7.00^{ab} ± 0.1	7.00^{ab} ± 0.1	6.83^{ab} ± 0.1	6.83 ± 0.3
	0.2% Spirulina	6.17 ± 0.3	6.67 ± 0.2	7.33^{ab} ± 0.2	7.33^{ab} ± 0.1	7.33^{a} ± 0.1	6.67^{ab} ± 0.1	7.00 ± 0.2
	0.3% Spirulina	6.67 ± 0.2	7.17 ± 0.3	7.17^{ab} ± 0.2	7.50^{a} ± 0.1	7.33^{a} ± 0.1	7.00^{a} ± 0.0	7.50 ± 0.5
	P-Value	NS	NS	*	**	*	*	NS

NS = No significant, Mean ± SE; * = P<0.05, ** = P<0.01, a, b, c = means bearing dissimilar superscript in a column differ significantly

Table 6: Yolk colour score by chromameter of laying hens fed spirulina-supplemented diet at 78-84 weeks of age

Parameter	Dietary treatment				P-value
	Control	0.1% Spirulina	0.2% Spirulina	0.3% Spirulina	
L*	35.67 ± 1.5	35.68 ± 2.2	36.07 ± 1.5	38.93 ± 1.2	NS
a*	-1.28 ± 0.1	-1.42 ± 0.2	-1.45 ± 0.1	-1.53 ± 0.1	NS
b*	16.64^{bc} ± 0.6	16.78^{bc} ± 0.4	17.38^{b} ± 0.6	19.20^{a} ± 0.9	*

L* (lightness), a* (redness), b* (yellowness) of yolk colour, NS = No significant, Mean ± SE; * = P<0.05 a, b, c = means bearing dissimilar superscript in a row differ significantly

Conclusions

Spirulina supplementation in diet of laying hens had no detrimental effect on egg production performance and egg quality characteristics. Spirulina algae could be safely used in laying hen diets, at level of 0.3% with good effects on egg yolk colouration in aged layers.

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