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Effects of Dietary Marine Algae (*Spirulina platensis*) on Egg Quality and Production Performance of Laying Hens

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ABSTRACT

In this experiment, a total of 128 Hy-line W_{36} hens at 63 weeks of age were used. The hens were put at random into 4 treatment groups (4 replicates and 32 hens per treatment) and were fed four different diets: three diets with different levels of Spirulina (1.5, 2.0 and 2.5%) and one control group based on wheat and soybean meal. All birds were housed in commercial cages, had ad libitum access to water, and were fed 100 g bird^{-1} per day. Egg production, feed intake, feed conversion ratio, egg weight, yolk index, Haugh unit, shell thickness, shell weight, specific gravity, egg yolk cholesterol, and yolk color were compared with the control group. Egg yolk color was measured by the BASF *Ovo-color* fan. Our results indicated that these traits did not show any significant changes with the Spirulina addition (P> 0.05), while a significant increase in egg yolk color was observed in the treatments that received the Spirulina (P< 0.0001). Yolk color scores of the control group and different levels of Spirulina (1.5, 2.0 and 2.5%) were 1.5, 10.5, 11.4 and 11.6 in BASF color fan, respectively. There were not significant differences between the treatments with 2.0 and 2.5% of Spirulina. In conclusion, this study can suggest use of 2.0~2.5% of Spirulina in diet to produce an aesthetically pleasing yolk color.

Keywords: Natural pigment, Marine algae (*Spirulina platensis*), Yolk cholesterol, Yolk color.

INTRODUCTION

The blue-green algae, *Spirulina platensis*, have been used for hundreds of years as a food source for humans and animals due to the excellent nutritional profile and high carotenoid content. Spirulina is relatively high in protein, with values ranging from 55-65%, and includes all of the essential amino acids (Anusuya Devi *et al*., 1981). The available energy has been determined to be 2.5-3.29 kcal g^{-1} and phosphorous availability is 41% (Lorenz, unpublished data, 2003, Available at: http://www.cyanotech.com/pdfs/spbul53.pdf). Chemical composition of *Spirulina platensis* is summarized in Table1 In poultry rations, algae up to a level of 5–10% can be used safely as partial replacement for conventional proteins (Spolaore *et al*., 2006). Prolonged feeding of algae at higher concentrations produces adverse effects (Spolaore *et al*., 2006). Spirulina is an excellent source of nutrition and provides a superior natural source of carotenoids that are extremely effective in coloring egg yolks (Lorenz, unpublished data, 2003). The yellow color of broiler skin and shanks as well as of egg yolk is the most important characteristic that can be influenced by feeding algae. In many markets, microalgae carotenoids are in competition with the synthetic form of the pigments (Spolaore *et al*., 2006). Although the synthetic forms are much less expensive than the natural ones, microalgae carotenoids have the advantage of supplying natural isomers in their natural ratio (Olaizola, 2003). In fact, 30% of the current world algal production is sold for

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Table 1. Chemical Composition of *Spirulina platensis* [a].

General Composition	%	Phytopigments	Mg 100 gr^{-1}
Protein	55-69	Total carotenoids	400-500
Carbohydrates	15-25	Carotens	160-260
Fats (Lipids)	5-6	Xanthophyll	170-240
Minerals (Ash)	6-9	Chlorophyll	1300-1700
Moisture	2.5-4.5	Phycocyanin	15000-19000

[a] http://www.parrynutraceuticals.com/organic-Spirulina.aspx or http://www.parrynutraceuticals.com/PDF/SPIRULINA_tablet_spec_sheet.pdf.

animal feed applications and over 50% of the current world production of Arthrospira is used as feed supplement (Spolaore *et al*., 2006). Different studies have been conducted on the applications of algae in poultry nutrition and other animals (Anderson *et al*., 1991; Gouveia *et al*., 1996; Grinstead *et al*., 2000; Toyomizu *et al*., 2001; Regunathan and Wesley, 2006; Venkatesh Kumar *et al*., 2009). However, there are few researches about the use of Spirulina as a feed supplement in laying hens. The aim of this work is to study the effects of dietary marine algae *(Spirulina platensis)* as a feed supplement on egg quality and laying hen's performance fed with diet based on wheat.

MATERIALS AND METHODS

Animals and Diets

A total of 128 Hy-line W_{36} hens at 63 weeks of age were used (the average weight of birds at the beginning of the experiment was 1500±40 g). The hens were put at random into 4 treatment groups (4 replicates and 32 hens per treatment) and were fed four diets: three diets with different levels of Spirulina (1.5, 2.0 and 2.5%) and one control group based on wheat and soybean meal without Spirulina in the ration. The composition of the control diet is shown in Table 2. Diets were formulated using UFFDA feed formulation package according to the nutrient requirements of white laying hens (NRC, 1994). Samples of the wheat, soybean meal, and wheat bran were ground in a laboratory mill fitted with 1-mm mesh

Table 2. The ingredients and composition of basal diet.

Ingredients	%
Wheat	60.00
Soybean meal (48% Protein)	19.21
Wheat bran	3.03
Dical. Phos.	1.74
Fatty acid	4.10
Common salt	0.33
Limestone	10.88
Vitamin-Mineral premix [a]	0.50
DL-Methionine	0.15
L-Lysine HCL	0.06
Analysis results	
ME (Kcal Kg^{-1})	2800
CP (%)	15.31
Calcium (%)	4.6125
Available phosphorus (%)	0.41
Lysine (%)	0.78
TSAA (%)	0.6653

[a] Provided per kilogram of diet: Retinol (vitamin A), 7700 IU; Cholecalciferol (vitamin D_3), 3300 IU; DL-alpha-tocopherol acetate (vitamin E), 6.6 IU; Menadione (vitamin K_3), 0.55 mg; Thiamine, 1.5 mg; Nboflavin, 4.4 mg; Pantothenic acid, 22 mg; Niacin, 5.5 mg; Pyridoxine, 3 mg; Choline chloride, 275 mg; Folic acid 1.1 mg ; Biotin 0.055 mg; Vitamin B_{12} (cyanocobalamin), 0.088 mg; Antioxidant,1 mg; Manganese, 66 mg; Zinc, 66 mg; Iron, 33 mg; Copper, 8.8 mg; Iodine, 0.9 mg; Selenium, 0.3 mg.

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screen. The dry matter (DM), crude protein (CP), ether extract (EE), ash and crude fiber (CF) contents of the ingredients which were used as feed were determined by the methods of AOAC (2000). The crude protein content of the samples was calculated from its nitrogen composition (N×6.25). The Ca and P contents were measured by flame atomic absorption spectrophotometry (atomic absorption flame emission AA670, Made in Japan) as an analytical technique (AOAC, 2000). Also, MEn were determined by prediction formulas based on the recommendation of the NRC (1994). The dry matter (DM), crude protein (CP), ether extract (EE), ash and crude fiber (CF), MEn, Ca, and P contents of the feed ingredient are presented in Table 3.

Sampling

Number and weight of eggs and yolk color were recorded daily. Yolk color was determined by comparison with the BASF *Ovo-color* fan. Feed intake was recorded each 10-day interval. Feed conversion ratio (FCR) was calculated by dividing the feed consumption by the egg mass produced during the time that feed consumption was measured. The height of the albumen and yolk was determined using a standard tripod micrometer and the diameter of yolk determined by caliper. Shells were washed under running water, dried, and weighed. Shell thickness was measured by micrometer. Specific gravity of eggs was determined by using the saline flotation method (Butcher and Miles, 1991). Salt solutions were made in incremental concentrations of 0.005 in the range from 1.065 to 1.09 specific gravity. Haugh units were calculated using the HU formula, based on the height of albumen determined by a micrometer and egg weight. The Haugh unit was calculated using the following formula:

$$\text{Haugh unit} = 100\log HA + 7.57 - 1.7WE^{0.37}$$

Where, *HA* is albumen height and *WE* is egg weight (Doyon *et al*., 1986). Yolk index was calculated by dividing the yolk height by the yolk diameter. The cholesterol values were obtained by the enzyme method solubilized samples (Pasin *et al*., 1998).

Statistical Analysis

The data were analyzed as completely randomized design, using analysis of variance procedures by the GLM procedure of statistical analysis software, SAS (2002). Differences between treatment means were tested using Duncan multiple comparison test and statistical significance was declared at a probability of $P < 0.05$ (Duncan, 1955)

RESULTS AND DISCUSSION

Laying Hen Performance

The results showed that egg production, feed intake, feed conversion ratio (FCR), and egg weight were not significantly affected by the dietary treatments ($P > 0.05$), (Table 4). In other words, Spirulina addition did not have any influence on production performance. These results are consistent with the results of other researchers that reported; Pigment

Table 3. Nutrition values of feed ingredient used in the experiment (As Fed).

Feed ingredient	DM[a] (%)	Ash (%)	CP[b] (%)	EE[c] (%)	CF[d] (%)	Ca[e] (%)	P[f] (%)	MEn
Wheat	95.21	1.72	9.75	2.47	2.97	0.05	0.45	3181.323
Soybean meal	92.99	-	46.84	2.43	4.66	0.23	0.77	2419.857
Wheat Bran	91.84	4.19	15.25	4.47	6.33	0.043	1.16	2167.415
DCP	97.69	85.83	-	-	-	23.08	14.86	-

[a] Dry Matter; [b] Crude Protein; [c] Ether Extract; [d] Crude Fiber; [e] Calcium, [f] Phosphorus.

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Table 4. Effect of Spirulina on laying hen performance.

Spirulina levels (%)	Egg production (%)	Feed intake (g hen^{-1} d^{-1})	FCR (g^{-1} Feed g^{-1} egg)	Egg weight (g)
0	74.16	100.27	1.56	63.92
1.5	78.33	97.80	1.55	63.19
2.0	82.08	96.49	1.52	63.59
2.5	73.64	97.39	1.53	63.47
P-value	0.1215	0.3642	0.7971	0.9499
SEM	2.566	1.499	0.032	0.884
CV	6.66	3.06	4.17	2.79

supplementation had not been associated with changes in production (Ross and Dominy, 1990; Garcia *et al*., 2002; Zahroojian *et al*., 2011). It seems possible that these results are due to the use of low levels of algae in those studies. However, Halle *et al.* (2009) observed decrease in feed intake and no effect on the overall egg production with higher levels of algae. In contrast, in a field study, an increase of the laying performance of hens was observed, however, the hens used in that study were of low productivity (Janczyk, 2005). Also, several authors reported that laying hens fed Spirulina-containing diets attained the best means of egg production and feed conversion compared with those of the control group (Ross *et al*., 1994; Nikodémusz *et al*., 2010; Mariey *et al*., 2012). In these experiments, the increase in egg weight for hens fed with the Spirulina-diets may be attributed to heavier egg yolks. Different results in these studies may be due to differences in strains of laying hens.

Egg Quality Parameters

Egg quality measurements, after 4 weeks of feeding, are summarized in Table 5.

Yolk index, Haugh unit, egg yolk cholesterol, yolk color, shell thickness, shell weight and specific gravity were compared with the control group. Our results indicated that diets with Spirulina addition had no effect on egg quality parameters, except for yolk color score. These results are in line with those obtained by Inborr (1998) and Mariey *et al.* (2012), who reported that there were no significant differences in egg shell percentage, yolk index, albumen percentage, and Haugh unit as a result of feeding experimental diets containing Spirulina. In contrast, Mariey *et al.* (2012) reported that there were significant ($P< 0.05$) reductions in yolk cholesterol and total lipids as the level of dietary Spirulina was increased. This reduction in yolk total lipids and cholesterol contents may be related to their lower levels in blood plasma of hens fed with the Spirulina-containing diets (Mariey *et al.,* 2012).

The color scores of egg yolks of hens that were fed a diet containing Spirulina were higher than those that were fed a control diet. This result agreed with a previous study (Anderson *et al*., 1991). Also, it was reported that feeding Spirulina-diets achieved a significant increase in yolk color score (Sakaida, 2003; Mariey *et al.,* 2012) and egg yolk color score from quails fed dried Spirulina (Ross *et al.,* 1994). The degree of yolk color preferred by consumers varies widely throughout the world, though deeper hues bring significant premiums in most markets. Baking operations and the food processing industry prefer darker colored yolks over adding artificial coloring agents. Although Spirulina powder appears as a bluish-green color, in fact, it contains one of the highest levels of carotenoids of any natural food source when properly manufactured and packaged (Miki *et al*., 1986).Yolk color scores of the control group

Table 5. Effect of Spirulina on egg quality parameters.

Spirulina levels (%)	Yolk index	Haugh unit	Yolk cholesterol(mg g^{-1} yolk)	Yolk color	Shell thickness (mm)	Shell weight (g)	Specific gravity
0	0.41^{a}	80.5^{a}	11.44^{a}	1.55^{c}	0.357^{a}	5.53^{a}	1.079^{a}
1.5	0.405^{a}	77.09^{a}	10.00^{a}	10.55^{b}	0.362^{a}	6.08^{a}	1.077^{a}
2.0	0.407^{a}	76.4^{a}	10.59^{a}	11.43^{a}	0.367^{a}	5.36^{a}	1.079^{a}
2.5	0.417^{a}	79.81^{a}	11.81^{a}	11.66^{a}	0.360^{a}	5.53^{a}	1.078^{a}
P-value	0.5793	0.4190	0.6549	$<.0001$	0.5621	0.8054	0.6862
SEM	0.006	1.990	1.100	0.119	0.005	0.180	0.001
CV	3.18	5.07	20.07	2.72	2.79	6.61	0.19

^{abc}Means in a column with different superscripts differ significantly ($P< 0.05$).

and different levels of Spirulina (1.5, 2.0 and 2.5%) were 1.5, 10.5, 11.4 and 11.6 in BASF color fan, respectively. The effect of Spirulina concentration on yolk color was better explained by polynomial effect response curves, as shown in Figure 1.

The optimal level of yolk color (between 11.4 and 11.6 on the BASF yolk color fan) was achieved with 2.0-2.5% Spirulina in diet after only 7 days, the color levels of the egg yolks remained stable as long as the supplementation continued (Figure 2).

There were no significant differences between the treatments with 2.0 and 2.5% of Spirulina.

In conclusion, this study can suggest use of 2.0~2.5% of Spirulina in the egg industry to produce an aesthetically pleasing yolk color without any negative effects on production performance.

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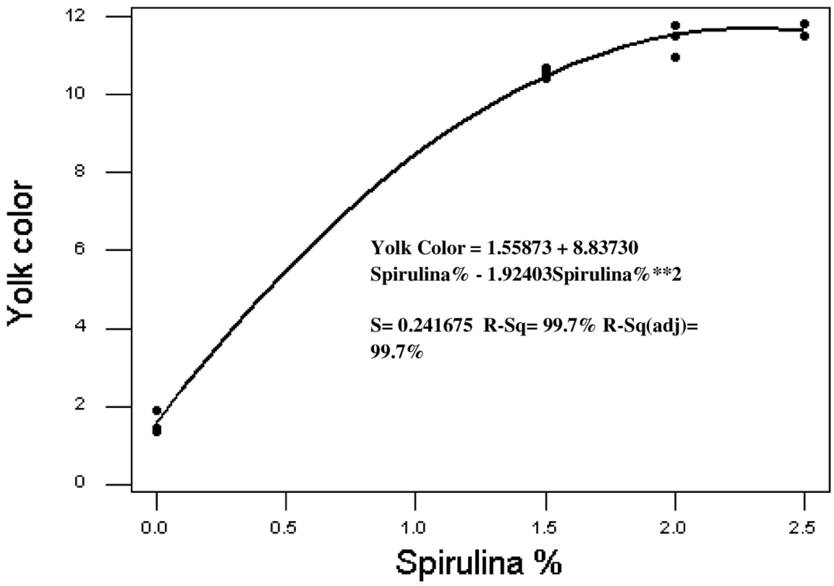

Figure1. Regression between BASF yolk color fan values and level of Spirulina in the diet.

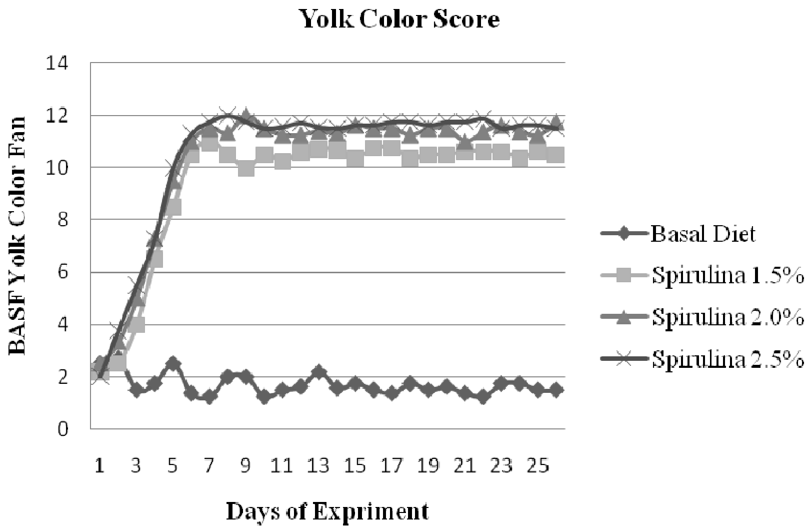

Figure2. Effect of treatments on egg yolk color score.

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بررسی اثر جلبک دریایی *اسپیرولینا پلاتنسیس* بر صفات کیفی تخم مرغ و عملکرد تولیدی مرغ‌های تخم‌گذار

چکیده

در این تحقیق ۱۲۸ قطعه مرغ تخم‌گذار نژاد های-لاین W_{36} در سن ۶۳ هفتگی استفاده شد. مرغ‌ها به طور تصادفی در ۴ تیمار (۴ تکرار و ۳۲ مرغ در هر تیمار) قرار داده شدند. مرغ‌ها با ۴ جیره‌ی غذایی تغذیه شدند: یک جیره شاهد بر پایه گندم و کنجاله سویا و ۳ جیره شامل جیره پایه بعلاوه سطوح مختلف اسپیرولینا(۱/۵، ۲ و ۲/۵ درصد اسپیرولینا) بود. مرغ‌ها به طور آزاد به آب دسترسی داشتند و به هر مرغ روزانه ۱۰۰ گرم غذا داده شد. تولید تخم‌مرغ، مصرف خوراک، ضریب تبدیل خوراک، وزن تخم‌مرغ روزانه، شاخص زرده، واحد هاو، ضخامت پوسته، وزن پوسته، وزن مخصوص تخم مرغ، کلسترول زرده و رنگ زرده در این آزمایش تعیین شد و با تیمار پایه مورد مقایسه قرار گرفت. رنگ زرده بر اساس شاخص شرکت *BASF* اندازه‌گیری شد. نتایج نشان داد که صفات عملکردی مرغ‌های تخم‌گذار و همین‌طور صفات کیفی تخم‌مرغ تحت تاثیر جیره‌های آزمایشی قرار نگرفت ($P>۰/۰۵$) ، درحالیکه رنگ زرده در همه‌ی تیمارهای حاوی اسپیرولینا به طور معنی داری افزایش نشان داد ($P<۰/۰۰۰۱$). شاخص رنگ زرده برای تیمار شاهد، درصدهای مختلف اسپیرولینا (۱/۵، ۲/۰ و ۲/۵٪) به ترتیب ۱/۵، ۱۰/۵، ۱۱/۴ و ۱۱/۶ بود. تفاوت معنی داری بین سطوح ۲ و ۲/۵ درصد اسپیرولینا از نظر رنگ زرده وجود ندارد و در نهایت استفاده از هر یک از سطوح ۲~۲/۵ درصد اسپیرولینا در صنعت تولید تخم‌مرغ باعث تولید رنگ زرده خوشایند و مورد قبول می‌شود.

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