



**EFFECT OF SUPPLEMENTING THE DIET WITH CAROTENOIDS-
ENRICHED SPIRULINA ALGAE**

**1. ON PRODUCTIVE PERFORMANCE AND PHSIOLOGICAL
RESPONSE OF DEVELOPED CHICKENS**

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ABSTRACT: The present experiment was conducted to study the effects of dietary supplementation with carotenoids-enriched *Spirulina platensis* algae (SA), flock age on carotenoids concentration in egg yolk, productive performance, egg quality and blood parameters of Bandarah chickens. One hundred and thirty five females with fifteen males from Bandarah chicken strain at 30weeks of age besides the same number from the same chicken strain at 56 weeks of age were housed in floor pens. The birds were weighed and randomly divided into five groups representing the dietary supplementations and commenced four months of laying production. The five experimental bird groups for each age were fed as follow: basal diet without supplementation of SA (control) , basal diet supplemented with 40 mg (group 2), 80 mg (group 3), 120 mg (group 4), 160 mg (group 5) SA/Kg diet. The results showed that there was a linear relationship between dietary SA concentration and carotenoids concentration in egg yolk. Supplementation the diet with 40 mg SA/Kg diet represented highest ($P \leq 0.05$) record of egg weight, egg mass and egg production compared to those for other treatment groups regardless of flock age. Moreover, the best significant improvement of feed conversion rate was recorded for group of chickens fed T2 and T5 treatments. The highest values for egg mass and egg production % were observed for interactions of T2 × young age and T5 × old age. Elder flock age represented significant increase for egg weight, egg shell index and yolk weight percentage compared to those for younger one. Also, supplementing the diets with 40 mg SA /Kg diet (T2) and 160 mg SA/Kg diet (T5) reduced($P \leq 0.05$) cholesterol and malonaldehyde. In conclusion, *Spirulina platensis* algae could be used safety in layer diets at levels of 40mg/kg diet for young flocks and 160 mg/kg diet for old ones for realizing the best productive performance and improving the yolk color and some blood parameters.

Key words: Spirulina algae, yolk color, carotenoids, antioxidant, chicken production

INTRODUCTION

Spirulina is a blue green microalga – and served as an important source of valuable bioactive compounds and good nutritional source with a high protein content (55-65 %) and a significant lipid content (10 - 140 g/kg) (Zahroojian et al., 2013). Spirulina is high in unsaturated and polyunsaturated fatty acids in particular (25% - 60% of the total fatty acids) (Habib et al., 2008). It has also been reported that the amino acid pattern of these microalgae could be comparable with or superior to that of other vegetable foods and feeds, and that they have a high nutrient digestibility (Alvarenga et al., 2011). In addition, Spirulina contains substances such as pigments (for example carotenoids such as β -carotene and zeaxanthin) (Maoka, 2011), phycobiliproteins (Eriksen, 2008), vitamins especially vitamin B₁₂ and pro-vitamin A (beta-carotene) (Becker, 1994), macro and micro mineral elements (Spolaore et al., 2006) and antioxidants (Christaki et al., 2013). The available energy content of Spirulina has been determined to be 2.50-3.29 kcal/gm and its phosphorous availability is 41 %. Algae compounds reveal potential biological properties such as anti-microbial, anti-oxidant, anti-cancer and anti-inflammatory or act as immune enhancers and colorants (Batista et al., 2013).

The higher carotenoid content of Spirulina helps in supplementation of vitamin A, provides antioxidant activity (Qureshi et al., 1996) . Also, Nikodémusz et al. (2010) reported that laying hen fed Spirulina-containing diets achieved superior productive performance compared to the control birds.

Spirulina platensis and its extract decreased the blood lipid values, phycocyanin and polyunsaturated fatty acids and has hypocholesterolemic effect (Henrikson, 2010). Also, Nagaoka et al. (2005) reported that cholesterol is lowered by inhibition of the cholesterol absorption from jejunum and bile acid resorption from ileum with

phycocyanin in *Spirulina platensis*. In addition, other researchers proposed that *Spirulina platensis* may have an effect on plasma total protein, albumin and globulin values (Mariey et al., 2012).

Spirulina platensis has been used in broiler and layer diets to enhance yolk color and flesh color (Ross and Dominy, 1990). Egg yolk color has also been found to intensify linearly with increased dietary *Spirulina* levels (Sujatha and Narahari , 2011). In white Leghorn layer hens, dietary *Spirulina* levels of 3-9% of the total ration was found to result in egg yolk colors best representative of consumer preferences (Saxena et al., 1982).

Carotenoids have also been demonstrated to affect broiler skin color, which is an important factor for consumer acceptance in many countries (Liu et al., 2008). Xanthophylls, which comprise a particular class of carotenoids, are the most prominent source of pigmentation in poultry feed (Goodwin, 1950); however, the typical corn and soybean-based commercial poultry diet does not supply the necessary amounts or types of Xanthophylls required to produce a deep yellow skin (Castaneda et al., 2005).

Several factors affect the performance of commercial poultry, such as breeder's age and egg weight at incubation (Correa et al., 2011 and Ayaşan, 2013). In broiler breeders, age has a direct influence on egg quality, composition and size, because egg production is reduced, egg (yolk and albumen) changes and egg weight increases as hens age (Rocha et al., 2008). The influence of female age is well described in laying hens, in which the egg size changes considerably, increasing egg size and weight and reducing the shell thickness and Haugh unit value (Roll et al., 2009), reflecting the overall quality of the eggshell.

Carotenoids could potentially be important age-specific mediators of life-history trade-offs (Pike et al., 2010). In breeding animals, carotenoid supplementation resulted in both enhanced antioxidant capacity and increased egg production (Bertrand et al., 2006).

Spirulina algae, yolk color, carotenoids, antioxidant, chicken production

This study was undertaken to assess the role of using small doses of Spirulina algae in layers diet on egg yolk color, egg pigmentation, productive performance and some blood constituents focusing on the effect of chicken age on these parameters.

MATERIALS AND METHODS

The present experiment was conducted at El-Sabahia Poultry Research Station (Alexandria), Animal Production Research Institute, Agriculture Research Center. One hundred and thirty five females with fifteen males from Bandarah chicken strain at 30-week of age besides the same numbers of females and males from the same chicken strain at 56-week of age were housed in floor pens. The birds were weighed and randomly divided into five groups representing the dietary supplementations within each experimental ages and kept in 15 floor pens with three replicates for each treatment. Sex ratio comprised 1 male for 9 females for each pen were used as replicate. The five experimental bird groups for each age were fed Spirulina powder as follows:

1. basal diet without supplementation of Spirulina Platensis algae (SA) (control, T1)
2. basal diet supplemented with 40 mg SA/ Kg diet (T2)
3. basal diet supplemented with 80 mg SA/ Kg diet (T3)
4. basal diet supplemented with 120 mg SA/ Kg diet (T4)
5. basal diet supplemented with 160 mg SA/ Kg diet (T5)

The ingredient profiles and nutrient composition of the experimental diets containing 16% crude protein and 2800 ME Kcal / Kg according to the recommendation of Animal Production Research Institute (Table 1). Crude protein, carbohydrate, fat and ash were determined by method of AOAC (2000) (Table 2). Extraction and analyses of carotenoids were performed using established methods of Surai et al. (2003) and Dvorska et al. (2002) (Table 3)

Birds were subjected to 16 hours light and 8 hours dark during the experimental period. Feed and water were provided *ad-libitum*.

Data collections

Birds were individually weighed (g) at the beginning of the experiment, and again after four months at the end of the experiment for both experimental ages. Body weight change was calculated by subtracting the initial average live weight from the average of final live weight. Feed consumption by gram was detected for each pen and for each bird every two weeks for each treatment and also during the whole experimental laying period for two ages. Feed conversion ratio was calculated as amount of consumed feed (g) required for producing a unit (g) of egg mass. No mortality was recorded during the experimental period. Egg production traits such as egg weight, egg production % and egg mass / hen/day were detected. At the end of the experimental periods (at 46 weeks of age for younger birds and 72 weeks of age for elder birds), three eggs from each replicate were randomly chosen from the same days of production and subjected to egg quality investigation. Egg shape index was calculated as the percentage of the greatest width to the greatest length. The shell was weighed with its membranes to the nearest 0.1 g. Shell with membranes thickness was measured by a micrometer to the nearest 0.01 mm. Yolk and albumen percentages were calculated relative to egg weight. The thick albumen height was measured by tripod micrometer to the nearest 0.01 mm. The yolk color score was determined by comparing with the roch yolk color (RYC) fan (F.Hoffmann – La – Roche Ltd., Basal, Switzerland). The RYC fan is standardized tool which shows the range of yolk colors from very light yellow to very dark yellow (Vulleumier, 1969). Pigments were separated using a modified method of Van-Heukelem and Thomas (2001). Thirty blood samples were randomly taken from branchial wing vein of 3 hens for each treatment group representing each age (46 and 72 wks). The blood

samples were collected immediately in heparinized tubes for measuring plasma total lipids (g/dl), plasma total protein (g/dl), globulin (g/dl), cholesterol (mg/dl), total antioxidant (mg/dl) and malondialdehyde (nmol/ml) using available commercial kits.

Statistical analyses

Data were analyzed using GLM procedure of SAS (2000) to study the effect of treatment, age and their interaction. The model was as follows:

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + e_{ijk}$$

where:

Y_{ijk} = an observation on the k^{th} individual,

μ = the overall mean,

T_i = the fixed effect of the i^{th} treatment.

A_j = the fixed effect of the j^{th} age.

TA_{ij} = the interaction between treatment and age

e_{ijk} = random error assumed to be independent normally distributed with mean = 0 and variance $\sigma^2 e$.

Significance among means was detected using Duncan's Multiple Range Test (Steel and Torrie, 1980).

Data of egg yolk colour grade was analyzed using Chi square test (Satorra and Bentler, 2001).

RESULTS AND DISCUSSION

Carotenoid profile of the egg yolk including β -carotene and zeaxanthin is demonstrated in Table 3. All concentrations of SA increased ($P \leq 0.05$) β -carotene and zeaxanthin compared to control with the maximum doses for T4 and T5 groups. Also, carotenoids concentrations were significantly increased in egg yolk with the increase of flock age. The lowest significant estimates of β -carotene and zeaxanthin were observed in interaction of T1 \times YA, while the highest ones were observed for interactions of T3, T4 and T5 with old age.

As can be seen from these data that there is a linear relationship between dietary SA concentration and carotenoids concentration in egg yolk. These results are keeping with those reported by Karadas et al.(2005). Furthermore, Kotrbacek et al. (2013)

reported significant increase in yolk carotenoids content as well as yolk color score in hens fed with chlorella algae supplementation. Moreover, Duarte et al.(2015) found that addition of canthaxanthin and 25-(oH)-D₃ improved the coloration of the yolk through increased deposition of pigment in the egg yolk.

Effects of dietary carotenoids- enriched Spirulina algae, flock age and their interactions on productive performance of Bandarah chickens are shown in Table 4. It is apparent from data of this table that supplementation the diets with SA as for T2 group represented the highest record ($P \leq 0.05$) of egg weight (g) and egg production % compared to those for other groups. Also, egg mass in the same group (T2) represented significantly highest record compared to others and sharing T5 group with the highest significant record. Also, dietary SA had no significant influence on feed consumption. Moreover, the best significant improvement of feed conversion (g feed/g egg mass) was recorded for group of chickens fed T2 treatment. Besides, birds of T5 group represented the same improvement of feed conversion without statistical change with the rest groups. Regarding the effect of chicken age on the previous mentioned parameters, weight was increased ($P \leq 0.05$) for eggs produced from elder age compared to younger one. Also, egg mass and feed consumption did not display any statistical change in relation to age. While egg production % and feed conversion ratio were significantly improved for younger chickens compared elder ones. Interaction between experimental treatment and flock age reveals that the best egg weight ($P \leq 0.05$) was recorded for interaction of T2 \times old age (54.09 g) and the worst one was observed for T4 \times young age interaction. The highest values for egg mass and egg production % were observed for T2 \times young age and T5 \times old age. While feed consumption was not statistically influenced among all

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interactions. Moreover, the best improvement of feed conversion was recorded for group of interaction between T2 × young age followed by those of T5 × old age, T4 × young age and T1 × young age without statistical change compared with the other interactions.

It is evident from the results of this table that, irrespective of flock age supplementation the diet with the minimal concentration of SA (40 mg/kg diet) significantly improved egg weight, egg production % and feed conversion rate compared to control group and other supplementation concentrations. Moreover, the increase of SA supplementation did not reveal any significant improvement of the mentioned traits. These results are in accordance with the previous researches reported by Mariey et al. (2012) and Kaoud (2013) who mentioned that birds fed dietary Spirulina had benefit effects on egg production, daily egg mass and feed conversion. Also, Mariey et al. (2012) reported that no differences were observed in feed intake of hens fed the experimental diets containing Spirulina, the significant improvement in feed conversion ratio may be due, at least in part, to an improvement in daily egg mass. On the other hand, Zahroojian et al. (2013) found that Spirulina addition did not have any influence on production performance. Also, others mentioned that pigment supplementation had not been associated with changes in production (Zahroojian et al., 2011).

The increase of egg weight with the increase of flock age in the current results is supported by Hasan and Aylin (2009). Contrary, Zemková et al. (2007) demonstrated that the egg weight was not influenced significantly by age. The results of egg production increase for younger flock age are keeping with those mentioned by Santos et al. (2015) who reported that the age of females influenced the egg production. The improvement of feed conversion for chickens of younger age in the present study could be due to the

increase of egg production % compared to the elder one.

It can be observed from interaction data of this table that supplementation the diet with 40 mg SA /kg diet could be recommended for improving egg weight, egg production and feed conversion ratio for young layer flock, while for old ones, there is need for increasing the concentration of Spirulina algae to 160 mg/kg diet to realize the best results of improvement.

Effects of Spirulina algae, flock age and their interactions on live body weights of Bandarah chickens are demonstrated in Table 5. No apparent significant influence of experimental treatment on either initial or final live body weights of Bandarah layers after 4 months of experiment. Also, the highest change of live body weight during the 4 months of experiment was observed for T3 group while the lowest change of body weight was detected for T2 group with no statistical change with the rest groups except between T2 and T3 groups. Regarding the effect of flock age, the elder flock represented significant ($P \leq 0.05$) change of final body weight compared with the younger one. The interactions between treatment × flock age with respect to body weight change during the 4 months of experiment represented the highest values for groups of T1 × old age and for T3 × old age and for the lowest ones were observed for T2 × young age and T3 × young age with variable significant change with the other interactions. The results of the body weight change during this experiment proved that no adverse effect of SA on final body weight and this result is in harmony with those reported by Ross et al. (1994). Whereas, other researchers reported that dietary Spirulina significantly improved weight gain of chickens compared with the control groups (Shanmugapriya and Saravanababu, 2014). Contradictory results are possibly due to the different Spirulina inclusion levels and quality in the present trials. In addition, secondary parameters, such as feed composition, housing conditions and production systems, might be reasons for the variation in the results of the present study.

The results of increase body weight change for elder flock age compared with those for younger one are in accordance with those reported by Koppenol et al. (2014) who found that body weight increased with age. Data of Table 6 display the effect of SA, flock age and their interactions on some parameters of egg quality. It can be observed that supplementation the diet with SA for groups of T2 and T5 represented the highest significant egg weights (52.50 and 51.34 g, respectively) compared to control T1 (48.85g) with no statistical change with the rest groups. While, the eggs produced from the same mentioned group birds (T2 and T5) represented the highest significant values of eggshell with membranes thickness and egg shape index compared to the other groups. The same mentioned groups realized the highest values of albumen height compared to the others without statistical change. Also, shell weight percentage was not statistically affected by algae treatment. Moreover, the highest significant values of albumen and yolk weights were observed for egg of T2 group compared to control with no statistical change with the others. With respect to flock age, elder flock age represented significant increase for egg weight, egg shape index and yolk weight percentage compared to those for younger one. The opposite trend was detected for eggshell with membranes thickness and albumen height as they increased for younger flock. While shell weight and albumen percentages did not represent any statistical differences. It can be observed that most of eggs produced from elder flock with all treatment groups except that of T2 × Young age recorded the highest interaction of egg weight. Supplementation the diet with the highest concentration of SA for elder flock age (T5 × old age) represented the highest significant value of eggshell with membranes thickness compared with all interactions except that for all interactions with younger flock. Furthermore, lowest egg shape index was

observed for interaction of T1 × YA while highest one was detected for T5 × OA interaction. Albumen height as good indicator for egg quality was generally superior for interactions between treatment and young age, while supplementation the diet with the highest concentration of algae for group T5 × OA represented marked improvement of albumen height. In addition, shell weight % did not represent any statistical change among the studied interactions. The lowest significant value of interaction for albumen weight % was detected for control group (T1 × YA), while most interactions of young flock with treatments represented significant increase compared to the other interactions. Regarding yolk weight %, the interactions revealed the increase of this parameter with increasing SA concentration with elder flock age, while treatment × YA represented the highest significant yolk weight % for interaction of T2 × YA compared to the rest groups of younger flock.

The increase of such egg quality parameters resulted of feeding SA with different concentrations compared to control are confirmed by different research – workers. Halle et al. (2009) observed positive effect of the microalgae on the egg quality. Also, Englmaierová et al. (2013) found that all carotenoids increased significantly the shell thickness. While, others indicated that diets had no significant effect on egg quality parameters (Mariey et al., 2012 and Zahroojian et al., 2013).

The increase of egg weight, egg shape index and yolk weight with advancing age is coincided with different researches. Koppenol et al. (2014) reported that the egg weight increase with the hen age is due to the increase of yolk weight. Moreover, the increases of eggshell with membranes thickness and albumen height for eggs produced from younger flock age are documented by different authors as Rizk et al. (2008) and Santos et al. (2015) reported that the age increase of females decreased

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the Haugh unit and eggshell with membranes thickness. While, Van den Brand et al. (2004) found no significant effect of the age of hen on eggshell characteristics and eggshell thickness.

The interaction results imply that egg weight, egg shape index and yolk weight could be improved with supplementation with 40 mg/Kg diet SA for young layer flock. While, supplementation the diet of old layers with 160 mg/Kg diet SA could improve eggshell with membranes thickness and albumen height.

Statistical analyses of data of color pigmentation in egg yolk with chi square reveal that there was a linear increase of yellow color grade (score) with the increase of SA concentration in the diet (Table 7). Moreover, yolk color from eggs produced from young flock represented the highly majority of medium yellow and yellow colors, while old age represented the majority of yellow and dark yellow colors compared with other colors of studied yolk fan, irrespective of flock age. Furthermore, data of interaction demonstrated that increasing of SA concentration in the diet recorded the highest percentage for yellow color, while the highest percentages for dark yellow and very dark yellow were observed for interactions of T4 and T5 with OA.

Regardless of flock age, there is a linear relationship between SA concentration and egg yolk pigmentation. These results parallel earlier studies by Mariey et al. (2012) and Zahroojian et al. (2013) who reported that feeding SA gave significant increases in yolk color score compared with those of the control group. In respect of dietary treatment, The present results indicated that eggs of old flock age had significant increase of yolk color score compared with those for young one. In this regard, Koppenol et al. (2014) observed that yolk color increased with age ($P < 0.001$).

Effects of dietary supplementation with carotenoids-enriched *Spirulina* algae, flock age and their interactions on blood parameters of Bandarah chickens are shown in Table 8. Supplementation the diets with 40 mg SA/Kg diet (T2) and 160 mg SA/Kg diet (T5) recorded significant ($P \leq 0.05$) reduction of cholesterol and

malonaldehyde besides significant increase of total protein and globulin compared with those for other experimental treatments. Moreover, the same groups of T2 and T5 represented numerical reduction of total lipid besides increase of total antioxidant compared to others treatments. Moreover, blood of younger birds represented significant reduction of total lipid, cholesterol and malonaldehyde, besides significant increase of total protein, globulin and total antioxidant compared with those for elder chickens, regardless of experimental treatments. Significant interactions between dietary supplementation with different concentrations of SA and flock age were found. Regarding young flock age, the interaction of T2 \times YA represented highest diminish of total lipid, cholesterol and malonaldehyde compared with the other interactions, while, supplementation the diet of elder flock with T5 reduced the same previous mentioned parameters compared with the interactions of elder flock. Moreover, the same mentioned interactions (T2 \times YA) and (T5 \times OA) represented the highest increase of total antioxidant, total protein and globulin.

The results of decreasing plasma cholesterol and total lipids for chickens fed dietary SA are keeping with that reported by Mariey et al. (2014) and Kanagaraju and Omprakash (2016) who mentioned that the reduction in plasma cholesterol by SA feeding could be attributed to reducing the absorption and/ or synthesis of cholesterol in the gastrointestinal tract and increase *Lactobacillus* population. *Lactobacillus* has found to have a high bile salt hydrolytic activity and to reduce the cholesterol in the blood by deconjugating bile salts in the intestine (Surono, 2003). Also, Radwan et al. (2008) reported that the decrease plasma total cholesterol and total lipids may be attributed to lowering effect of the herbal antioxidant on HMG-COA that is needed for cholesterol synthesis in liver. Moreover, the present results of the significant increase of total antioxidant due to SA supplementation are compatible with the results of Karadas et al. (2016). The current results of total antioxidant increase and Malonaldehyde (MDA) decrease may be due to SA has high antioxidant and carotenoids contents as detected by Christaki et al. (2013). The decrease of MDA concentration protect the

body from the harmful effects of free radicals and reflected in the improvements in the general health and performance of chicken (Attia et al., 2015). The observed increase in the levels of plasma total protein as SA levels (40,160 mg/kg diet) may be related to the high protein contents in Spirulina (with values ranging from 55-65% and includes all of the essential amino acids). Also, SA has antimicrobial and immune enhancing (Christaki et al., 2013) and could be the main reason for increasing the plasma globulin concentration. These results are in harmony with the findings of Eggum, (1989) who stated that total serum protein and globulin were directly responsive to both protein quantity and quality.

Respective of flock age, younger chickens represented significant reduction in cholesterol and total lipid, Peebles et al. (1997) have drawn the same conclusion. Supporting to our results regarding the significant decrease in total lipid and cholesterol, Latour et al.(1998) mentioned that serum lipids and cholesterol tended to be higher in elder hens than the younger ones . While, other researchers did not show any significant association between age and cholesterol concentration in broiler (Sarikhah et al. 2009). Also Eshratkhah et al.(2011) detected that the concentrations of total protein and globulin in blood serum of layer breeder are affected by age and egg laying and the considerable increase of total protein occur just prior and the peak of egg laying.

The reduction of plasma total antioxidant for elder flock age may be due to that as animals age, they experience a decline in immune competence and antioxidant capacity (Devevey et al., 2010 and Beamonte-Barrientos and Verhulst, 2013).

It is evident from the foregoing results that the concentration of SA algae supplementation with the age of layer flock have to be taken into account in order to maximize the chicken performance. Therefore, it is concluded that supplementation the diet with 40 mg/ Kg diet could be recommended for young layers to improve egg weight, eggshell index, yolk weight, egg production and feed conversion ratio, while for old layers, there is need for increasing the concentration of SA to 160 mg/ Kg diet to realize the best results of improvement besides eggshell with membranes thickness and albumen height. Moreover, the most interesting aspect of the present work for enhancing the yolk color pigmentation and antioxidants had been realized and results proved that there is linear relationship between SA concentration and yolk pigmentation including carotenoids. Due to the yolk pigmentation and its contents with carotenoids, further researches are needed to assess the role of SA on the embryogenesis.

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Table (1): Composition * and the nutritive value of the basal diets .

Ingredients	%
Yellow corn	66.33
Soybean meal (44%)	24.2
Dicalcium phosphate	1.32
Limestone	7.5
Salt (Nacl)	0.25
DL – methionine	0.15
Vit& Min mix.*	0.25
Total	100.00
Calculated analysis:	
Metabolizable energy (Kcal/kg)	2777
Crude protein %	16.97
Calcium %	3.1
Available phosphate %	0.37
Methionine % + cyctine %	0.67
Lysine %	0.8

** Composition of premix in 3 kg is : Vit. A, 10.000 IU ; Vit . D₃, 100.000 IU ; Vit E , 10.000 mg ; Vit. E,10.000 mg; Vit. K₃,1.000 mg; Vit . B₁ , 1 mg ; vit . B₂ , 4 mg ; Vit B₆ ,1.5 mg ; Vit . B₁₂ , 10 mcg ; Niacin , 20.000 mg ; Pantothenic acid 10.000 mg ; Folec acid , 1 mg ; Biotin , 50 mg ; Choline chloride , 500 mg ; Copper , 4 mg ; Iron , 30 mg ; Manganese , 40.000 mg ; Zinc , 45.000 mg ; Cu , 3.000 mg ; Iodine , 300 mg ; Selenium , 0.1 mg ; Cobalt , 0.1 mg .

Table (2): Chemical Composition of *Spirulina platensis*.

General Composition	%
Protein	62.3
Carbohydrates	21.8
Fats (Lipids)	5.43
Ash	7.5
Moisture	3.5
	Mg 100 gr-1
Total carotenoids	455
Carotens	210
Xanthophyll	205
Chlorophyll	1550
Phycocyanin	16500

Table (3): Carotenoid profile of the egg yolk representing two flock ages supplemented with dietary *Spirulina* algae.

Traits	Total carotenoids	
	β .carotene (%)	Zeaxanthin (%)
Main effect Treatment (TRT)		
T1 (Control)	4.35 ^B ±0.14	9.40 ^C ±1.12
T2 (40mg/Kg diet)	5.29 ^A ±0.16	10.55 ^B ±1.18
T3 (80mg/Kg diet)	5.30 ^A ±0.18	10.90 ^{AB} ±1.17
T4 (120mg/Kg diet)	5.33 ^A ±0.15	11.25 ^A ±1.35
T5 (160 mg/Kg diet)	5.72 ^A ±0.13	11.89 ^A ±1.53
Age (wk)		
Young (YA) (30 – wk)	4.93 ^B ±0.13	9.93 ^B ±1.56
Old (OA) (56 – wk)	5.38 ^A ±0.25	11.01 ^A ±1.61
Interaction (TRT*Age)		
T1*YA	4.10 ^D ±0.16	9.00 ^D ±1.31
T2*YA	5.13 ^B ±0.13	10.21 ^B ±1.20
T3*YA	5.20 ^B ±0.15	10.42 ^B ±1.12
T4*YA	5.35 ^B ±0.20	10.61 ^B ±1.32
T5*YA	5.61 ^A ±0.19	10.90 ^{AB} ±1.41
T1*OA	4.60 ^C ±0.21	10.00 ^C ±1.42
T2*OA	5.44 ^B ±0.18	10.91 ^{AB} ±1.53
T3*OA	5.50 ^{AB} ±0.19	11.13 ^A ±1.43
T4*OA	5.60 ^A ±0.23	11.30 ^A ±1.35
T5*OA	5.65 ^A ±0.20	11.66 ^A ±1.45

A , B , C and D Means within the same column in the same trait with different superscripts are significantly different ($P \leq 0.05$) .

Table (4): Effects of dietary supplementation with carotenoids- enriched Spirulina algae, flock age and their interactions on productive performance of Bandarah chickens

Traits	Egg weight (g)	Egg mass (g/hen/day)	Egg production (%)	Feed consumption (g/hen/day)	Feed conversion (g feed / g egg mass)
Main effect Treatment(TRT)					
T1 (Control)	51.76 ^B ±0.09	23.67 ^{BC} ± 1.67	46.35 ^C ±3.48	124.85 ± 5.25	5.27 ^A ± 0.44
T2 (40mg/Kg diet)	52.75 ^A ± 0.09	33.19 ^A ± 2.52	64.05 ^A ± 4.99	121.74 ±6.83	3.66 ^B ±0.40
T3 (80mg/Kg diet)	51.67 ^B ±0.11	20.89 ^C ± 1.63	40.99 ^C ±3.33	113.57 ±3.67	5.43 ^A ± 0.45
T4 (120mg/Kg diet)	51.78 ^B ± 0.09	23.3 ^{BC} ± 1.67	45.96 ^C ± 3.24	121.04 ±4.36	5.19 ^A ± 0.41
T5 (160 mg/Kg diet)	51.34 ^B ± 0.08	27.77 ^{AB} ± 2.60	55.06 ^B ± 4.84	125.85 ± 5.87	4.53 ^{AB} ± 0.45
Age (wk)					
Young (YA) (30–wk)	50.69 ^B ± 0.05	27.37± 1.63	54.04 ^A ± 4.04	119.07 ±3.55	4.35 ^B ± 0.30
Old (OA) (56 –wk)	53.05 ^A ± 0.06	24.22± 1.66	46.92 ^B ±3.22	123.74 ±2.86	5.10 ^A ± 0.32
Interaction(TRT*Age)					
T1*YA	51.03 ^E ± 0.12	26.02 ^B ± 2.74	51.19 ^C ±11.35	124.91 ± 8.20	4.80 ^{ABC} ± 0.57
T2*YA	51.42 ^D ± 0.10	38.56 ^A ± 1.16	75.00 ^A ±0.01	119.15 ±12.86	3.08 ^C ± 0.49
T3*YA	50.47 ^F ±0.12	22.20 ^C ± 3.11	44.04 ^D ± 8.58	111.96 ± 6.52	5.04 ^{AB} ± 0.67
T4*YA	50.57 ^F ± 0.12	26.80 ^B ± 2.45	53.57 ^C ± 6.18	118.10 ±5.49	4.40 ^{ABC} ± 0.49
T5*YA	49.98 ^G ± 0.10	22.99 ^C ± 3.12	46.42 ^{CD} ±5.45	121.21 ± 9.81	5.33 ^{AB} ± 0.68
T1*OA	52.50 ^{CD} ± 0.13	21.33 ^C ± 2.15	41.51 ^D ± 4.96	124.79 ± 8.34	5.85 ^{AB} ±0.73
T2*OA	54.09 ^A ±0.15	27.83 ^B ± 4.18	53.10 ^C ± 7.89	124.32 ±7.68	4.46 ^{ABC} ± 0.46
T3*OA	52.87 ^{BC} ±0.19	19.58 ^C ± 1.78	37.95 ^D ± 2.83	115.18 ± 3.81	5.88 ^A ± 0.73
T4*OA	52.99 ^B ± 0.15	19.80 ^C ± 1.77	38.35 ^D ± 2.77	123.97 ± 7.33	6.26 ^A ± 0.39
T5*OA	52.70 ^{BCD} ± 0.11	32.55 ^{AB} ± 1.74	63.70 ^B ±3.14	130.43 ± 6.70	4.00 ^{BC} ± 0.40

A , B , C, D, E, F and G Means within the same column in the same trait with different superscripts are significantly different ($P \leq 0.05$) .

Table (5): Effects of dietary supplementation with carotenoids- enriched Spirulina algae, flock age and their interactions on live body weights of Bandarah chickens

Traits	Live body weight (g)		
	Initial weight	Final weight after 4 months	Body weight change during 4 months
Main effect Treatment(TRT)			
T1 (Control)	1575.46±27.24	1803.6±37.55	228.14 ^{AB} ± 19.55
T2 (40mg/Kg diet)	1566.55±24.92	1741.73± 32.29	175.18 ^B ± 20.97
T3 (80mg/Kg diet)	1573.69± 30.40	1812.17± 35.13	238.42 ^A ± 20.90
T4 (120mg/Kg diet)	1564.72± 31.35	1798.77 ± 32.92	225.83 ^{AB} ± 17.99
T5 (160 mg/Kg diet)	1572.94± 25.94	1769.01 ± 42.04	205.00 ^{AB} ± 22.31
Age (wk)			
Young (YA)(30–wk)	1561.00± 15.37	1757.52 ^B ±19.45	196.51 ^B ± 12.29
Old (OA)(56 –wk)	1580.06 ±18.74	1812.57 ^A ± 24.49	232.42 ^A ± 13.45
Interaction(TRT*Age)			
T1*YA	1560.00± 33.88	1772.04 ^{AB} ±50.44	212.03 ^{AB} ± 27.37
T2*YA	1552.41± 31.45	1703.15 ^B ± 36.97	150.74 ^B ± 30.02
T3*YA	1566.67± 31.58	1750.37 ^{AB} ± 37.44	183.70 ^B ± 23.35
T4*YA	1557.78± 45.20	1774.07 ^{AB} ±53.54	216.29 ^{AB} ± 30.47
T5*YA	1568.15± 30.13	1787.96 ^{AB} ± 37.46	219.81 ^{AB} ± 25.31
T1*OA	1590.93± 40.77	1835.16 ^A ±53.72	244.23 ^{AB} ±28.10
T2*OA	1580.70±36.28	1780.32 ^{AB} ±49.06	199.62 ^B ± 29.09
T3*OA	1580.70 ± 51.07	1873.84 ^A ± 53.39	293.14 ^A ±31.70
T4*OA	1570.26 ± 42.25	1809.59 ^A ±53.03	231.85 ^{AB} ± 26.01
T5*OA	1577.74 ± 40.97	1763.96 ^{AB} ± 65.07	193.70 ^B ± 33.03

A , B Means within the same column in the same trait with different superscripts are significantly different (P≤0.05).

Table (6): Effects of dietary supplementation with carotenoids- enriched Spirulina algae, flock age and their interactions on some parameters of egg quality of Bandarah chickens.

Traits	Egg weight (g)	Egg shell with membranes thickness (mm)	Egg shape Index	Albumen height (mm)	Shell weight (%)	Albumen weight (%)	Yolk weight (%)
Main effect Treatment(TRT)							
T1 (Control)	48.85 ^B ±0.11	0.35 ^B ±0.033	73.81 ^B ±0.9	7.50 ^{AB}	12.10±0.2	54.61 ^B ±0.38	30.89 ^B ±0.35
T2 (40mg/Kg diet)	52.50 ^A ±0.10	0.39 ^A ±0.040	78.12 ^A ±0.8	9.00 ^A ± 0.19	11.59±0.2	57.77 ^A ±0.49	33.28 ^A ±0.41
T3 (80mg/Kg diet)	51.26 ^{AB} ±0.11	0.36 ^B ±0.013	74.65 ^B ±0.8	7.91 ^{AB} ±0.21	11.79±0.2	56.60 ^{AB} ±0.4	31.59 ^{AB} ±0.5
T4 (120mg/Kg diet)	51.11 ^{AB} ±0.09	0.37 ^B ±0.010	74.52 ^B ±0.7	7.62 ^{AB} ±0.20	12.35±0.2	55.60 ^{AB} ±0.2	32.04 ^{AB} ±0.4
T5 (160 mg/Kg diet)	52.10 ^A ±0.10	0.40 ^A ±0.010	77.21 ^A ±0.4	8.85 ^A ±0.17	11.92±0.2	55.66 ^{AB} ±0.1	31.90 ^{AB} ±0.3
Age (wk)							
Young (YA) (30–wk)	49.2 ^B ±0.05	0.39 ^A ±0.003	74.23 ^B ±0.4	9.01 ^A ±0.19	12.18±0.2	56.80±0.50	30.81 ^B ±0.38
Old age(OA) (56 –wk)	53.1 ^A ±0.07	0.35 ^B ±0.003	77.10 ^A ±0.5	7.35 ^B ±0.22	11.72±0.2	55.19±0.51	33.07 ^A ±0.44
Interaction TRT*Age							
T1*YA	46.6 ^{CD} ±0.08	0.38 ^{AB} ±0.013	70.79 ^C ±0.4	9.00 ^A ± 0.18	12.49±0.2	53.80 ^C ±0.36	28.91 ^{BC} ±0.50
T2*YA	53.03 ^A ±0.10	0.41 ^A ±0.033	77.96 ^A ±0.6	10.00 ^A ±0.19	11.75±0.2	58.58 ^A ±0.38	34.43 ^A ±0.39
T3*YA	49.01 ^C ±0.15	0.40 ^A ±0.043	72.96 ^{BC} ±0.	9.43 ^A ±0.18	12.07±0.2	57.62 ^A ±0.51	30.29 ^B ±0.41
T4*YA	48.10 ^C ±0.11	0.39 ^{AB} ±0.013	74.00 ^B ±0.7	8.45 ^{AB} ±0.19	12.36±0.2	57.02 ^{AB} ±0.4	30.61 ^B ±0.45
T5*YA	49.20 ^C ±0.12	0.40 ^A ±0.010	75.43 ^B ±0.6	8.16 ^{AB} ±0.17	12.22±0.2	56.97 ^{AB} ±0.3	29.79 ^{BC} ±0.39
T1*OA	51.10 ^B ±0.09	0.33 ^{BC} ±0.011	76.84 ^{AB} ±0.	6.01 ^B ±0.20	11.71±0.2	55.42 ^B ±0.50	32.86 ^{AB} ±0.3
T2*OA	52.02 ^{AB} ±0.10	0.36 ^B ±0.019	78.27 ^A ±0.7	8.00 ^{AB} ±	11.43±0.2	56.44 ^{AB} ±0.5	32.12 ^{AB} ±0.3
T3*OA	53.40 ^A ±0.09	0.33 ^{BC} ±0.009	76.34 ^{AB} ±0.	6.4 ^B ± 0.13	11.51±0.2	55.58 ^B ±0.51	32.89 ^{AB} ±0.5
T4*OA	54.20 ^A ±0.13	0.35 ^B ±0.013	75.04 ^B ±0.4	6.8 ^B ± 0.15	12.33±0.2	54.18 ^B ±0.59	33.47 ^A ±0.48
T5*OA	55.10 ^A ±0.11	0.40 ^A ±0.015	79.00 ^A ±0.4	9.55 ^A ± 0.16	11.63±0.2	54.35 ^B ±0.50	34.00 ^A ±0.40

A , B , C and D Means within the same column in the same trait with different superscripts are significantly different ($P \leq 0.05$) .

Table (7): Effects of dietary supplementation with carotenoids- enriched Spirulina algae, flock age and their interactions on yolk color for eggs produced from Bandarah chickens

Traits	Very light yellow	Light yellow	Medium yellow	Yellow	Dark yellow	Very dark yellow
Main effect						
Treatment(TRT)						
T1 (Control)	4.5	24.25	59	6.5	5.75	0
T2 (40mg/Kg diet)	1	3	32.5	45.75	17.75	0
T3 (80mg/Kg diet)	0.75	2.5	29.25	61.00	5	1.5
T4 (120mg/Kg diet)	0	1	32.25	27.25	32.75	6.75
T5 (160 mg/Kg diet)	0	0.5	15.75	20	28.75	35
Chi square value	45.10					
Significance	P < 0.0001					
Age (wk)						
Young (YA) (30–wk)	2.50	10.00	27.00	22.50	5.00	2.50
Old (OA) (56–wk)	0.00	2.50	12.50	45.00	27.50	12.5
Chi square value	26.2689					
Significance	P < 0.0001					
Interaction TRT*Age						
T1*YA	9	40	28	13	10	0
T2*YA	2	4	15	79	0	0
T3*YA	1.5	3	23.5	72	0	0
T4*YA	0	2	34.5	54.5	5	4
T5*YA	0	1	11.5	40	27.5	20
T1*OA	0	8.5	90	0	1.5	0
T2*OA	0	2	50	12.5	35.5	0
T3*OA	0	2	35	50	10	3
T4*OA	0	0	30	0	60.5	9.5
T5*OA	0	0	20	0	30	50
Chi square value	30.10					
Significance	P < 0.0001					

Table (8): Effects of dietary supplementation with carotenoids- enriched Spirulina algae, flock age and their interactions on blood parameters of Bandarah chickens

Traits	Total Lipid (g/dl)	Cholesterol (mg/dl)	Total Antioxidant(mg/dl)	Malondialdhide (nmol/ml)	Total Protein(g/dl)	Globulin(g/dl)
Main effect Treatment(TRT)						
T1 (Control)	5.46 ^A ±0.26	170.6 ^A ±15.7	400.0 ^C ±25.7	2.2 ^A ±0.37	5.68 ^B ±0.23	2.39 ^B ±0.18
T2 (40mg/Kg diet)	4.38 ^{BC} ±0.3	141.0 ^C ±13.7	475.0 ^A ±34.5	1.40 ^B ±0.57	6.33 ^A ±0.11	2.90 ^A ±0.05
T3 (80mg/Kg diet)	4.60 ^B ±0.23	155.1 ^B ±9.32	430.0 ^{BC} ±20.5	2.20 ^A ±0.19	5.94 ^B ±0.16	2.45 ^B ±0.17
T4 (120mg/Kg diet)	4.68 ^B ±0.19	159.5 ^B ±10.8	445.0 ^{AB} ±24.8	1.65 ^B ±0.25	5.82 ^B ±0.17	2.49 ^B ±0.16
T5 (160 mg/Kg diet)	4.10 ^C ±0.13	132.7 ^C ±3.17	485.0 ^A ±19.7	1.03 ^C ±0.07	6.41 ^A ±0.21	2.91 ^A ±0.05
Age (wk)						
Young (YA) (30 –wk)	4.24 ^B ±0.11	137.9 ^B ±4.9	484.00 ^A ±11.5	1.12 ^B ±0.06	6.34 ^A ±0.13	2.88 ^A ±0.11
Old age(OA) (56 –wk)	5.05 ^A ±0.18	165.4 ^A ±8.6	410.0 ^B ±16.5	2.29 ^A ±0.23	5.73 ^B ±0.06	2.37 ^B ±0.03
Interaction TRT*Age						
T1*YA	4.90 ^B ±0.05	160.6 ^{CDE} ±3.84	450.0 ^{BCD} ±26.	1.40 ^C ±0.20	6.20 ^{BC} ±0.08	2.76 ^B ±0.03
T2*YA	3.70 ^D ±0.26	110.0 ^H ±3.16	550.0 ^A ±5.7	0.76 ^E ±0.03	6.80 ^A ±0.2	2.99 ^{AB} ±0.08
T3*YA	4.10 ^{CD} ±0.0	140.2 ^{FG} ±2.65	470.0 ^B ±10.40	1.20 ^{CD} ±0.05	6.20 ^{BC} ±0.24	2.90 ^{AB} ±2.14
T4*YA	4.30 ^C ±0.15	149.0 ^{EF} ±6.17	490.0 ^{AB} ±15.2	1.10 ^{CDE} ±0.05	6.18 ^{BC} ±0.1	2.85 ^{AB} ±0.08
T5*YA	4.20 ^{CD} ±0.0	130.0 ^G ±5.80	460.0 ^{BC} ±20.8	1.16 ^{CDE} ±0.06	6.33 ^B ±0.0	2.93 ^{AB} ±0.05
T1*OA	6.03 ^A ±0.17	180.6 ^A ±1.76	350.0 ^E ±10.40	3.03 ^A ±0.03	5.16 ^E ±0.0	2.03 ^C ±0.0
T2*OA	5.06 ^B ±0.06	172.0 ^{BC} ±5.7	400.0 ^{CDE} ±17.	2.03 ^B ±0.14	5.86 ^{CD} ±0.08	2.80 ^B ±0.0
T3*OA	5.10 ^B ±0.11	170.0 ^{BC} ±5.77	390.0 ^{DE} ±20.0	3.30 ^A ±0.25	5.69 ^D ±0.0	2.00 ^C ±0.1
T4*OA	5.06 ^B ±0.16	170.0 ^{BC} ±11.54	400.0 ^{CDE} ±28.	2.20 ^B ±0.15	5.46 ^{DE} ±0.0	2.13 ^C ±0.0
T5*OA	4.00 ^{CD} ±0.2	135.3 ^{FG} ±3.12	510.0 ^{AB} ±30.0	0.90 ^{DE} ±0.05	6.50 ^{AB} ±0.10	2.90 ^{AB} ±0.0

A , B , C, D, E, F, G and H Means within the same column in the same trait with different superscripts are significantly different (P≤0.05) .

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الملخص العربي

تأثير إضافة طحلب الإسبيرولينا الغنى بالكاروتينات للعليقة

1. على الأداء الإنتاجي والإستجابة الفسيولوجية للدجاج المستنبت

إبتسام السيد إبراهيم عراقى – منى محمود أحمد – وسام أديب فارس – على عبد الهادى سعد البرلسى –

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أجريت هذه التجربة لدراسة تأثير إضافة طحلب الإسبيرولينا الغنى بالكاروتينات وكذلك عمر القطيع على تركيز الكاروتينات في صفار البيض، و جودة البيض و الأداء الإنتاجي، وصفات الدم لدجاج البندرة . و اجريت التجربة على مائة وخمسة وثلاثون أنثى مع خمسة عشر ذكر من سلالة دجاج البندرة عند عمر 30 أسبوع إلى جانب عدد مماثل من نفس السلالة عند عمر 56 أسبوع فى بيوت تربية ارضية و استغرقت التجربة أربعة شهور إنتاجية. وتم وزن الطيور وقسمت عشوائيا إلى خمس مجموعات تمثل الإضافات الغذائية لكل عمر كما يلي: العليقة المقارنة بدون اى اضافات (المجموعة الأولى، كنترول)، العليقة المقارنة مضاف اليها 40 ملجم (المجموعة الثانية)، العليقة المقارنة مضاف اليها 80 ملجم (المجموعة الثالثة)، العليقة المقارنة مضاف اليها 120 ملجم (المجموعة الرابعة)، العليقة المقارنة مضاف اليها 160 ملجم (المجموعة الخامسة) طحلب الإسبيرولينا/كجم علف. وأظهرت النتائج أن هناك علاقة خطية بين تركيز الطحلب وتركيز الكاروتينات في صفار البيض و سجلت مجموعة الدجاج المغذاة على 40 ملجم طحلب الإسبيرولينا/كجم علف أعلى وزن و كتلة البيض و إنتاجية البيض مقارنة بالمجاميع الأخرى بغض النظر عن عمر القطيع. بالإضافة لذلك فقد حدث تحسن معنوى فى معدل التحويل الغذائى لدجاج المجموعتين الثانية و الخامسة (40 و 160 ملجم طحلب الإسبيرولينا/كجم علف) . و أظهرت قيم التداخل كذلك أن أعلى قيمة لكتلة البيض و نسبة إنتاج البيض قد تم تسجيلها نتيجة تغذية القطيع الأصغر عمرا على 40 ملجم طحلب الإسبيرولينا/كجم علف (المجموعة الخامسة) و أظهرت كذلك النتائج زيادة معنوية فى كل من وزن البيض و دليل شكل البيض و وزن الصفار للقطيع الأكبر مقارنة بالأصغر عمرا . و أظهرت النتائج كذلك أن إضافة 40 ملجم طحلب الإسبيرولينا/كجم علف (المجموعة الثانية) ، 160 ملجم طحلب الإسبيرولينا/كجم علف (المجموعة الخامسة) أدى إلى حدوث إنخفاض معنوى ($P \leq 0.05$) فى كل من الكوليسترول و المألونالدهيد. ونستخلص من نتائج هذه الدراسة الى انه يمكن استخدام طحلب الإسبيرولينا بأمان فى علائق الدجاج البياض عند مستويات 40 مجم/كجم علف للعمر الصغير و 160 ملجم/كجم علف للعمر الكبير لتحقيق أفضل أداء إنتاجي وتحسين لون صفار البيض وبعض مقاييس الدم.