Effect of *Spirulina platensis* as dietary Supplement on Some Biological Traits for Chickens under Heat Stress Condition

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ABSTRACT :
This work was performed to investigate the effect of *Spirulina platensis* levels and vitamin E under heat stress on the performance, some physiological and blood biochemical constituents, antioxidant and immune responses traits of growing chickens. A total of 150 male Gimmizah local strain chicks (4 weeks of age) were randomly distributed among five treatment groups in battery brooder placed in a temperature-controlled room until 14 weeks of age. The first treatment was fed the basal diet only without any supplementation and kept in the first sector under optimum temperature 22-24ºC and relative humidity (RH) 45-55 % (normal) used as negative control. Whereas, the other four treatments were kept in the last sector under chronic heat stress condition (38°C±1; 55-65 % RH) for three successive days a week from 11.00 am until 15.00 pm. The first heat stress group was fed the basal diet only without any supplementation and used as positive control (PC). Whereas in the other three treatments were fed the basal diet supplemented with *Spirulina platensis* at levels (0.5 and 1 g/kg diet) and vitamin E (75 mg/kg diet). The results indicated that different supplementations as *Spirulina* and VE decreased adverse effect of heat stress on growth performance and blood total protein, albumin, globulin, createinin, ALT, AST, total lipids, LDL, WBCs, RBCs, immunity of Chickens. In conclusion, addition of *Spirulina platensis* improved growth performance, immunity and can be decreased adverse effect stress on chickens under heat stress condition.
Keywords: *Spirulina*; antioxidant; immunity response; growth factor; heat stress.

INTRODUCTION:

Spirulina (Blue green algae) is a microscopic single cell alga which grows in fresh water and has a simple structure but a complex composition. It is a concentrated source of food containing nutraceutical, antioxidants, probiotics properties. Moreover, it is an important source of the blue photosynthetic pigmented protein C-phycocyanin, which has strong antioxidant and anti-inflammatory properties. Interestingly, *Spirulina* is known for its wide ranging biological activities, like prevention of anemia because of high iron and vitamin contents (Hemalatha et al., 2012), inhibition of herpes simplexinfection (Ferreira-Hermosillo et al., 2011). The phytochemical screening of the ethanolic extract of algae Spirulina platensis shows the presence of alkaloids, flavonoids, glycosides, tannins & phenolic compounds, steroids and saponins (Anbarasan et al., 2011). The ideal temperature for poultry is about 20-25 °C (North and Bell, 1990; Daghir, 2008; Tumová and Gous, 2012). Heat stress (HS) begins when the ambient temperature becomes higher than 27 °C and is readily apparent above 30 °C (Bollengier-lee et al., 1999; Attia et al., 2006). One of the major challenges facing the breeders/layers industry is the higher ambient temperature in the summer months that negatively affects growth performance and physiological traits (Mashaly et al., 2004; Daghir, 2008; Yoshida et al., 2011). Therefore, the present study was carried out to investigate the effect of *Spirulina platensis* levels and vitamin E under heat stress on the performance, some physiological and blood biochemical constituents, antioxidant and immune responses traits of the male Gimmizah exposed to chronic heat stress.

MARERIALS AND METHODS

The present study was conducted at El-Sabahia Poultry Research station, Animal production Research Institute, Agricultural Research Center, Egypt. Spirulina platensis powder prepared in The National Institute of Oceanography and Fisheries (NIOF); Egypt.

Chemical analysis of *Spirulina platensis*:
Samples of *Spirulina platensis* were taken for a complete chemical analysis according to methods of the AOAC (2000). Total Phenolic, Flavonoid and antioxidant activity content were determined according to the methods of (Antolovich et al., 2002), (Chang et al., 2002) and (Burits and Bucar, 2000), respectively.

Experimental Parameters:
A total of 150 male Gimmizah local strain chicks (4 weeks of age) {a cross between White Plymouth Rock × Dokki-4} (Mahmoud et al., 1982) were transferred to an environmentally controlled house (close system) in order to run the experiment. Male chicks were randomly allocated in a completely randomized design considering

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five treatment groups with three replicates (each contained 10 chicks). All chicks were raised in battery brooder placed in a temperature-controlled room until 14 weeks of age. The first treatment was fed the basal diet (corn-soybean meal diet, Table 1) only without any supplementation and kept in the first sector under optimum temperature 22-24ºC and relative humidity (RH) 45-55 % (normal) used as negative control (NC). Whereas, the other four treatments were kept in the last sector under chronic heat stress condition **(CHS**) (38°C±1; 55-65 % RH) for three successive days a week from 11.00 am until 15.00 pm. The first heat stress group was fed the basal diet only without any supplementation and used as positive control (PC). Whereas in the other three treatments, one group was fed the basal diet supplemented with *Spirulina platensis* (0.5 g/ kg diet), or basal diet supplemented with *Spirulina platensis* (1 g/kg diet) and vitamin E (75 mg/kg diet). Feed and water were provided *ad-libitum* throughout the experimental period. Vaccination and medical program were done according to common veterinarian care practice. The photoperiod was 23 hours of light per day throughout the experimental period. The feed intake (FI), body weight and mortality were examined. At the end of the experimental period (14 weeks of age), four birds from each treatment were randomly selected, weighed and then slaughtered to complete bleeding. Liver and breast meat sampel were carefully excised from bird and immediately immersed in a saline solution (0.9% NaCl). Liver and breast meat sampel (10%) were prepared in 0.01M Tris-HCl buffers (pH 7.5). At the end of the experiment, four birds from each treatment were randomly chosen for collection of blood samples. The tubes were kept at room temperature for approximately half-hour to allow clotting of blood. The blood samples were centrifuged at 3000 r. p. m. for 20 minutes to separate the serum. The serum was collected and preserved in a deep freezer at – 18° C until analysis of total protein, albumin, Createinin, ALT, AST, Triglyceride, cholesterol, Total Lipids, HDL and LDL by using commercial kits (from Biomerieux, Poains, France). Half of the blood sample was to determine red blood cells (RBC'S), 5 The 4th Inter. white blood cells (WBC'S) counts; hemoglobin (Hb) content and packed cell volume (PCV) according to Wintrobe (1967). Differential leucocytic count, Blood film was prepared according to the method described by (Lucky, 1977). The percentage and absolute value for each type of cells were calculated according to (Schalm, 1986). The homogenate were centrifuged at 4000 r.p.m for 15 min, and the resultant supernatants were frozen at -20°C for hepatic parametersassay. Lipid peroxidation in breast and meat were estimated calorimetrically by measuring Malondialdehyde (MDA) by the thiobarbituric acid assay procedure using kit purchased from Bio- Diagnostic, Egypt (CAT NO. MD 2529). Glutathione peroxidase (GPx) wasdetermined using kit purchased from Bio- Diagnostic, Egypt (CAT NO. GP 2524). Serum immunoglobulins (IgG, IgM and IgA) were determined using commercial ELISA kits (Kamiya Biomedical Company, USA). Total antibody production specific for NDV vaccine was determined in serum by means of an ELISA using commercial ELISA test kits (Jeffery et al., 1996). HI test was applied for determination of antibody response according to King and Seal (1998). Computerizes one -way analysis of variance, and Duncan's multiple range test procedures using (SAS software, 2001) were run to comparison of treatment groups.

RESULTS AND DISCUSSION

Chemical analysis of *Spirulina Platensis*

Tables 2 and 3 showed that *Spirulina Platensis* content of moisture, crude protein, ether extract, crude fiber, ash and NFE were 14.23, 57.66, 1.75, 3.6, 9.83and 12.93 percentages, respectively. Total phenolic and total flavonoid contents and total antioxidant activity in *Spirulina Platensis* presented in table 3 were 47.11, 44.4 and 64.38, respectively. Generally, the chemical analysis and Phytochemical screening values of *Spirulina platensis* are agreement with those reported by (Gershwin and Belay 2008; Henrikson, 2009; Bhakuni and Rawat, 2005; Anbarasan et al., 2011).

Growth performance

The effect of CHS and different supplements on growth performance during 4-14 wk of age are shown in Table (4). The initial BW (4-wk old) was not significantly different among the experimental groups. The results of body weight and the body weight gain indicated that insignificant differences between all treatments. This results are agreement with those reported by (Manafi et al., 2012 and P. V. Pandav & P. R. Puranik., 2015). The data of feed intake (FI) indicated significant differences among treatments. Heat exposure significantly decreased FI for chicks fed positive control (PC) while addition of 0.5 g and 1 g *Spirulina* / kg diet significantly increased feed intake without significant than the negative control (NC). While, the data of feed conversion indicated insignificant differences between all treatments. Different supplementations as *Spirulina* and VE decreased adverse effect of CHS on growth performance. These improvements may be due to the synergetic effect of the chemical constituents (Total phenolic and flavonoid contents and total antioxidant) presented in *Spirulina.* These chemical constituents had antioxidant action (Miranda et al., 1998), alkaloids, flavonoids, glycosides, tannins & phenolic compounds, steroids and saponins (Anbarasan et al., 2011), anti-inflammatory action (Remirez et al., 2002).

Blood parameters

Results presented in Table (4, 5 and 6) showed that Different supplementations as *Spirulina* and VE decreased adverse effect of CHS on total protein, albumin, globulin, createinin, ALT, AST, total lipids, LDL, WBCs, RBCs, PCV, Hb, total antioxidant, IgA, IgM, HIN, HIA, HI7 , HI 14 in blood and malondialdehyde in liver values. Thus, addition of different levels of *Spirulina* and Vit. E restored it to the control (NC) group. But the highest significant total cholesterol value recorded in the group received control (NC) diet followed by those fed (PC), T3, T5 and T4, respectively. The highest significant HDL, IgG in blood and glutathione peroxidase in liver and meat and the lowest significant malondialdehyde in meat values were recorded with the chicks fed 1 g *Spirulina* / kg diet under heat stress compared with the control (NC) group, whereas the lowest significant HDL in blood and glutathione peroxidase in liver values were recorded with the chicks fed VE. The results show that heat stress had significant adverse effect

on fractions of WBCs (monocyte, eosinophil and heterophil) except for basophil, and basophil percentage.

Table (1): Composition and calculated analysis of the basal experimental diet.

Ingredients %	Diet
Yellow corn	63.01
Soybean meal (44% CP)	29.7
Corn gluten 60%	3.40
Dicalcium phosphate	1.70
Limestone	1.47
DL-Methionine	0.03
NaCl	0.39
Vit.and mineral (premix)[1]	0.30
Total	100
Calculated analysis[2]	
Crude protein (%)	20.04
ME (Kcal/kg diet)	2899.7
C/P ratio	144.7
Crude fat (%)	2.91
Crude fiber (%)	3.69
Calcium (%)	1.05
Phosphorus available (%)	0.455
Methionine (%)	0.405
Methionine + Cysteine (%)	0.74

[1]Three kg of vitamin- mineral premix per ton of feed supplied each kg of diet with Vit. A 12000 IU; Vit. D_3 2000 IU; Vit. E. 10mg; Vit. k_3 2mg; Vit.B_1 1mg; Vit. $B_2$4mg; Vit. B_6 1.5 mg; Pantothenic acid 10mg; Vit.B_{12} 0.01mg; Folic acid 1mg; Niacin 20mg; Biotin 0.05mg; Choline chloride (50% choline) 500 mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; ethoxyquin 3000 mg.

[2]Calculated analysis was according to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

Table (2): Proximate analysis of Spirulina platensis used in the experimental diets.

Parameter	Moisture %	C.P. %	C.fat %	C.F. %	Ash. %	N.F.E. %
Spirulina platensis	14.23	57.66	1.75	3.6	9.83	12.93

The data determined on air dried basis

Table (3): Total phenolic contents and total flavonoid contents and total antioxidant activity in *Spirulina Platensis* used in the experimental diets.

Concentration of phenolic compounds (mg gallic acid equivalent / ml)	Concentration of Flavonoid compounds (mg Rutin equivalent / ml)	antioxidant activity %
47.11 ±0.05	44.4 ± 0.1	64.38 ± 0.08

Table (4): Effect of *Spirulina platensis* supplementation under heat stress on growth performance, blood biochemical traits responses of chicks

Parameters	Normal	Under heat stress			
	T1 Basal diet (B)	T2 Basal diet (B)	T3 B + 0.5 g Spirulina / Kg	T4 B + 1 mg Spirulina / Kg	T5 B + 75 mg V E/ Kg
Growth Performance					
Initial body weight, g	376.65 ±17.41	379.55 ±6.71	387.25 ±19.29	381.25 ±17.92	376.05 ±28.73
Final body weight, g	1280.70±51.53	1248.65± 45.01	1342.40±11.77	1346.25±51.87	1343.05±13.02
Body weight gain, g/day	10.88± 0.42	10.59±0.51	11.37±0.19	11.61±0.59	11.63 ±0.32
Feed consumption ,g/day	47.14±1.12 [a*]	42.58 ±0.56 [b]	47.60 ±0.92 [a]	47.47±1.75 [a]	45.17 ±0.83 [ab]
Feed conversion ratio	4.33 ±0.13	4.02±0.24	4.22±0.07	4.10 ±0.31	3.92 ±0.07
Blood biochemical traits					
Total protein(g/dl)	7.53 ±0.66	6.70 ±0.40	6.99 ±0.35	7.43 ±0.71	6.42 ±0.52
Albumin(g/dl)	3.23 ±0.13	3.16 ±0.04	3.27 ±0.14	2.98 ±0.10	3.31 ±0.06
Globulin(g/dl)	4.29 ±0.79	3.54 ±0.44	3.72 ±0.24	4.45 ±0.81	3.12 ±0.46
Createinin (mg/dl)	0.57 ±0.01	0.74 ±0.03	0.61 ±0.02	0.67 ±36.19	0.75 ±0.12
ALT (U/L)	32.80 ±1.40	35.67 ±1.76	33.67 ±0.88	30.50 ±10.20	35.67 ±0.88
AST (U/L)	59.0 ±0.57	60.33 ±0.33	57.67 ±1.47	45.0 ±7.50	57.33 ±1.45
Triglyceride (mg/dl)	117.28 ±2.41	117.94 ±1.56	115.61±1.30	116.57 ±77.50	116.42 ±0.85
Total Cholesterol (mg/dl)	156.73 ±6.65 [a]	153.12 ±6.87 [a]	148.89 ±7.17 [ab]	90.67±38.0 [b]	143.15 ±3.20 [ab]
Total Lipids (mg/dl)	422.94 ±18.63	372.29 ±13.32	344.68 ±72.44	262.69 ±0.01	423.59 ±47.22
HDL (mg/dl)	37.33 ±2.60 [a]	35.10 ±2.80 [a]	32.0 ±2.30[ab]	39.33±2.90 [a]	26.0 ±2.08 [b]
LDL (mg/dl)	95.94 ±9.22	94.44 ±9.51	93.76 ±8.38	73.85 ±10.45	101.66 ±9.56

The results are presented as means ± SE of 30 chicks. Letters (a-b) mean within a raw not sharing similar superscripts in each classification are * significantly different (P ≤0.05)

Table (5): Effect of *Spirulina platensis* supplementation under heat stress on blood hematology and differential leucocytic count traits responses of chicks

Parameters	Normal	Under heat stress			
	T1 Basal diet (B)	T2 Basal diet (B)	T3 B + 0.5 g Spirulina / Kg	T4 B + 1 mg Spirulina / Kg	T5 B + 75 mg V E/ Kg
Blood hematology traits					
WBCs	25.33 ±0.66	23.66 ±0.88	24.0 ±1.73	25.33 ±0.66	24.67 ±0.66
RBCs /10	15.0 ±0.57	11.33 ±0.33	12.33±0.88	13.0 ±1.52	13.0 ±1.52
PCV	35.0 ±2.08	32.0 ±1.15	32.0 ±0.57	30.67 ±1.45	31.0 ±0.57
Hb	12.33 ±0.88	11.0 ±0.57	10.66 ±0.33	10.67 ±0.88	10.66±0.33
Differential leucocytic count					
Lemph.	36.0±1.15 ^{ab*}	38.33 ±0.33 ^{a}	38.33 ±1.45 ^{a}	37.33 ±0.88 ^{ab}	34.33 ±0.88 ^{b}
Mono.	15.0 ±0.57 ^{a}	11.66 ±0.33 ^{c}	11.33 ±0.33 ^{c}	11.67 ±0.33 ^{c}	13.33 ±0.33 ^{b}
Baso.	1.0 ±0.01 ^{ab}	0.33 ±0.33 ^{b}	1.33±0.33 ^{a}	1.0 ±0.01 ^{ab}	1.0 ±0.01 ^{ab}
Easin.	16.33 ±0.33 ^{a}	12.0 ±0.57 ^{b}	12.0 ±1.52 ^{b}	11.33 ±0.33 ^{b}	12.33 ±0.88 ^{b}
Hetero.	24.66 ±0.66 ^{a}	22.33 ±0.88 ^{b}	21.33 ±0.33 ^{b}	21.66 ±0.33 ^{b}	23.0 ±0.57 ^{ab}
P.A	19.0 ±1.15 ^{b}	18.67 ±1.45 ^{b}	18.33 ±1.45 ^{b}	23.33 ±0.66 ^{a}	19.67 ± 0.88 ^{ab}
P.I /10	16.0 ±0.57	16.0 ±0.57	13.33 ±1.45	16.33 ±0.88	15.0 ± 0.57

The results are presented as means ± SE of 30 chicks. Letters (a-c) mean within a raw not sharing similar superscripts in each classification are * significantly different (P ≤0.05)

Table (6): Effect of *Spirulina platensis* supplementation under heat stress on antioxidant and immune responses traits responses of chicks

Parameters	Normal	Under heat stress			
	T1 Basal diet (B)	T2 Basal diet (B)	T3 B + 0.5 g Spirulina / Kg	T4 B + 1 mg Spirulina / Kg	T5 B + 75 mg V E/ Kg
Antioxidant					
Total antioxidant capacity in blood (µmol/L)	0.33 ±0.03	0.33 ±0.01	0.36 ±0.02	0.37 ±0.01	0.34 ±0.01
Malondialdehyde In liver (µmol/L)	8.18 ±0.26	8.67 ±0.88	9.27 ±1.27	7.69 ±0.04	8.94 ±0.76
Malondialdehyde In meat (µmol/L)	9.93 ±0.46 ^{ab}	10.30 ±0.01 ^{a}	9.37 ±8.87 ^{ab}	8.55 ±0.45 ^{b}	9.08 ±0.38 ^{ab}
Glutathione peroxidase In liver (GSH-Px) (µmol/ml)	193.07 ± 25.37 ^{b}	198.87 ±1.15 ^{ab}	216.91 ±0.73 ^{ab}	250.10 ±20.88 ^{a}	203.26 ±3.99 ^{ab}
Glutathione peroxidase In meat (GSH-Px) (µmol/ml)	169.40± 9.58 ^{ab}	143.57 ±18.57 ^{b}	150.51 ±8.34 ^{b}	199.32 ±12.04 ^{a}	198.89 ±4.82 ^{a}
Immune responses					
IgA	77.0 ±0.57	78.67 ±0.88	76.0 ±0.57	79.0 ±1.52	78.33 ±1.45
IgM	220.33 ±2.33	225.33 ±2.33	223.66 ±1.45	221.67 ±1.85	222.0 ±2.08
IgG	967.67 ±1.76 ^{b}	973.67 ±2.18 ^{ab}	973.33 ±1.45 ^{ab}	977.0 ±1.73 ^{a}	970.66 ±2.02 ^{b}
HIN	1.67 ±0.33	1.67 ±0.33	1.0 ±0.01	1.66 ±0.33	1.33 ±0.33
HI IB	2.0 ±0.01 ^{a}	1.0 ±0.01 ^{b}	1.33 ±0.33 ^{ab}	1.67 ±0.33 ^{ab}	1.33 ±0.33 ^{ab}
HI A	1.0 ±0.01	1.33 ±0.01	0.67 ±0.33	0.67 ±0.33	1.0 ±0.01
HI 7	2.33 ±0.33	3.33 ±0.33	3.0 ±0.57	3.0 ±0.57	3.33 ±0.06
HI 14	3.33 ±0.66	4.0 ±0.01	3.66 ±0.33	3.66 ±0.33	4.0 ±0.01

The results are presented as means ± SE of 30 chicks. Letters (a-b) mean within a raw not sharing similar superscripts in each classification are * significantly different (P ≤0.05)

Different supplementations resulted in diminishing the negative effect of heat stress on WBCs, lymphocyte and basophil, showing complete recovery with similar response among different agents. This results are agreement with those reported by (Seyidoglu and Galip, 2014; Heidarpour et al., 2011; Kim et al., 2013; Mariey et al., 2014; Ashgan et al., 2015 ; Abdel-Daim et al., 2013; Manafi et al., 2012).

In conclusion, addition of *Spirulina platensis* improved growth performance, immunity and can be decreased adverse effect stress on chickens under heat stress condition.

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