EFFECT OF SPIRULINA PLATENSIS ALGAE SUPPLEMENTATION ON GROWTH PERFORMANCE, PHYSIOLOGICAL STATUS OF BROILERS DURING SUMMER SEASON

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SUMMARY

The current study was designed to investigate the effects of dietary Spirulina inclusion at different levels on growth performance, antioxidant status, carcass traits and blood hematology of broilers subjected to cyclic heat stress. A total of 150 one-day-old Ross broiler chicks were randomly divided into three experimental groups with 5 replicates with 10 birds for each. The first group was provided with a basal diet and served as a control. Meanwhile, others were fed a basal diet supplemented with Spirulina at a concentration of 1 or 2 g/kg diet, respectively. The experiment was conducted during hot climate of July with ambient temperature of 30 ± 1 ◦C, Spirulina supplementation to heat-stressed broilers was able to alleviate the negative impacts of heat stress on the final average daily gain, body weight and feed conversion ratio, with the best impacts on the chickens fed Spirulina at level of 2 gm/kg diet. Further, Spirulina supplementation significantly affected blood lipid profile by reducing serum total cholesterol, low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL) levels. While the thyroid hormones T3 and T4 were increased by Spirulina supplementation. In addition, spirulina supplementation at 1 or 2 g improved carcass dressing percentages. It concluded that dietary Spirulina supplementation at 1 or 2 g/kg diet to broilers reared under heat stress conditions can effectively improve broiler production performance, blood constituents and physiological status.

Keywords: *Spirulina, broilers, performance, blood constituents, thyroid hormones*

INTRODUCTION

Early interest in a spirulina focused mainly on its rich content of proteins, vitamins especially vitamin B12 and provitamin-A (β-carotene), provides antioxidant activity and enhance immunity, helps in hormonal regulation and plays additional roles in growth, reproduction and sexual maturation (Alam et al., 2013), Spirulina contains essential amino acids, minerals especially iron and essential fatty acids, as well as a pigmentation agent for skin and egg yolks (Swiatkiewicz *et al.,* 2015).

Several studies have demonstrated the growth –promoting effects of spirulina platensis on broiler chickens. Abaza *et al.* (2021) showed that feeding spirulina platens at level 2, 4 g\kg diets increased the body weight, weight gain and feed conversion ratio of broiler chickens. The yellowness of muscles, skin, fat, and liver increased with an increasing dietary level of microalgae, being more attractive for consumers in certain markets (Swiatkiewicz *et al.,* 2015).

Feeding broilers with spirulina at 1% and 2% significantly reduced the serum levels of cholesterol, triglycerides and total lipids as compared with control; (Jamil *et al.,* 2015).

Some of the important biochemical and physiological consequences of heat stress are reduced immunity function, increased production of free radicals and lipid peroxidation of cell membranes (EL-Ratel and Gabr, 2019).

The heat shock proteins (HSPs) act as molecular chaperons that help in protein folding and assembly, assist in restoring the native state of protein, regulate degradation of protein and in translocation across membranes. HSPs synthesis up-regulation under different stress condition is an adaptive phenomenon resulting in improved tolerance (Murugesan *et al.,* 2017). The synergetic effect of chemical constituents (total phenolic and flavonoid contents and total antioxidants) present in spirulina had antioxidant action, alkaloids, flavonoids, glycosides, tannins, and phenolic compounds (Zeweil *et al.,* 2019).

The present study was therefore designed to evaluate the effect of incorporation of various spirulina levels in diet on growth performance, carcass traits and some blood constituents in broilers.

MATERIALS AND METHODS

Experimental design, broiler chickens, and diets:

A total number of 150 one-day-old Ross 308 broiler chicks (average weight of 45 g,) were obtained from a commercial hatchery. The birds were fed a basal diet to match the feeding requirements of the Ross 308 recommendations during the starter (1–21 days) and grower (22–35 days) phases. Broilers were raised under hot climate environment during season of July month. The chicks were randomly allocated to three experimental groups fed with basal diet with different amounts of the algae added for an experimental period of 5 weeks. Each experimental group contained 50 chicks and replicated Five times with 10 each for a total of 150 chicks for the entire study. The 1st group received a standard basal diet without any additives (control group) formulated according to Nutrient Requirements of Poultry following the National Research Center (NRC) guidelines. The 2nd and 3rd groups were fed the basal diet with Spirulina added at 1 or 2 g/kg over basal diet, respectively, (Table 1). Chicks were allowed ad libitum access to feed and water throughout the experimental period. Feed consumption and body weights were recorded at weekly intervals and used to calculate performance parameters. At the end of the experiment (day 35), blood was collected from the wing veins into EDTA-containing tubes for further analyses.

Table (1): Composition and calculated chemical analysis of the experimental diets.

Ingredient (%)	Starter Phase (0-3 wks)	Crower Phase (3-5 wks)
Yellow corn	55.80	59.71
Soybean meal (44%)	34.32	30.00
Corn gluten	3.33	2.80
Vegetable oil	2.79	4.00
Dicalcium phosphate	1.94	1.67
Limestone	1.14	1.14
Common salt	0.25	0.25
Vit & min. premix*	0.25	0.25
DL. methionine	0.18	0.18
Total	100	100
Calculated Composition**		
Crude protein, %	22.00	20.00
Metabolizable energy, kcal/kg	3000	3194
Calcium, %	1.00	0.91
Available Phosphorus, %	0.50	0.45
Methionine + Cysteine, %	0.93	0.78
Lysine, %	1.10	1.10

** Composition of vitamin and minerals premix. Each 3 kg of vitamin and minerals mixture contain: 12000000 IU vitamin A; 2000000 IU vit. D3; 10 g vit. E; 1 vit. K; 1g vit. B_1; 5g vit. B_2; 1500 mg vit. B_6; 10 mg vit. B_{12}; 10g pantothenic acid; 20g Nicotinic acid 1g Folic acid; 50mg Biotin, 500g choline chloride; 4g copper; 300mg iodine; 30g iron; 60g manganese; 50g zinc; and 100mg selenium (Ahmed El-Batal, Co.).*
*** Calculated composition according to NRC (1994).*

Blood biochemical parameters:

Blood samples were collected at 35 days of age from the slaughtered chicks immediately into tube with anticoagulant, to obtain plasma by centrifugation of blood sample at 4000 rpm for 10 minutes, then plasma was transferred into sterilized tube, tightly closed and stored at-20° C until to be used. The methods of biochemical measurements were photometrical method using spectrophotometer (Apel 310 Spectro-photometer, Japan). The biochemical characteristics of blood constituents were calorimetrically determined using specific commercial Kits. The radioimmunoassay (RIA) method was used for the determination of triiodothyronine (T3), thyroxine (T4) and insulin like growth factor (IGF). Plasma T3 and T4 were determined by RIA technique using commercial RIA kits (Immunotech, Beckman Coult. Company) as reported by Britton *et al.* (1975).

Statistical analysis:

Pen means were the experimental unit for all obtained data. Data were subjected to one way ANOVA analysis of variance General Linear Model (GLM) procedure of SAS software SAS (1998) user's guide according to the following model:

$$Yij = \mu + Ti + eij$$

Where; μ = overall mean, T i = dietary treatment, e ij = experimental error. Individual effects of dietary treatments were compared using Duncan (1955) multiple range tests at α level equal to 0.05 or 0.01.

RESULTS AND DISCUSSIONS

Productive performance and carcass traits:

Table 2. Illustrates the effect of Spirulina (SP) administration on growth performance of broiler at 35 days of age. The results indicated that live body weight was significantly (P≤0.01) higher in chicks given diet supplemented with 1 or 2 g Spirulina as compared to the control. Similar results had been observed by Opoola *et al.* (2019).

On the other hand, Abaza *et al.* (2021) found that the final body weight was not significant differed among different experimental groups at the end of experiment. Algae implies the presence of more than one group of growth–prompting substances such as (auxins, cytokinins and gibberellins) which may be influenced the body growth (Michalak *et al.,* 2016). The use of microalgae as a supplement has been recommended to benefit poultry involving growth, survival, feed utilization and carcass quality (Cheong *et al.,* 2015).

Table (2): Effect of Spirulina administration on growth performance of broiler at 35 days of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
Initial LBW on 1day old (g)	40.69±0.043	40.53±0.084	40.54±0.091	NS
Final LBW at 35 days of age (kg)	1.21^{b}±0.02	1.73^{a}±0.026	1.77^{a}±0.030	**
Body weight gain,1-35 days (kg)	1.196^{b}±0.052	1.696^{a}±0.0361	1.729^{a}±0.0531	**
Feed consumption, 1-35 days (kg)	2.057^{b}±0.033	2.764^{a}±0.071	2.852^{a}±0.054	**
Feed conversion ratio, 1-35days	1.76^{a}±0.010	1.63^{b}±0.0151	1.65^{b}±0.005	**

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. ** P≤ 0.01, NS= non-significant.*
a and b, Means within Treatments (S) with no common superscript differ significantly.

In the present study, both levels of SP algae supplementation significantly affected the relative weight of carcass, liver, heart, body fat. On other hand gizzard was significantly decreased by increasing levels of SP, (Table 3). These results indicate an improvement in chicks' growth performance when consumed SP enriched diet up to 2%. These results are in agreement with Abou-Zeid *et al.* (2015) who found the obtained results cleared those birds fed 2 g spirulina\kg diet achieved superior means of body weight, significant difference in carcass percentage and abdominal fat percentage and no significant difference among groups in liver, heart, gizzard.

Table (3): Effect of Spirulina administration on carcass characteristics of broiler 35 day of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
Carcass weight %	61.76^{b}±0.371	67.89^{a}±0.564	68.41^{a}±0.629	**
Abdominal fat %	0.583^{a}±0.010	0.485^{b}±0.012	0.506^{b}±0.018	**
Heart weight %	0.446^{c}±0.009	0.520^{a}±0.008	0.483^{b}±0.007	**
Liver weight %	2.25^{b}±0.073	2.49^{a}±0.043	2.26^{b}±0.045	*
Gizzard weight %	1.160±0.0172	1.193±0.021	1.21±0.029	NS

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. * P≤ 0.05, ** P≤ 0.01, NS= non-significant.*
a,b,c Means within Treatments (S) with no common superscript differ significantly.

That spirulina improved absorption of minerals, protect from diarrhea and optimized nutrient digestion process. The positive impact of spirulina supplementation on broiler performance can partially be justified by the high apparent metabolizable energy and amino acid digestibility of spirulina supplemented diet (Levine *et al.,* 2018) especially with the negative impact of heat stress on intestinal morphology and feed consumption.

Blood parameters:

Thyroid hormones:

The result in Table (4) showed a marked increase in plasma Thyroxin (T4) level in the treated groups with Spirulina compared to control. This agreement with Ibrahim *et al.* (2018) serum thyroxin (T4) concentration was significantly (P≤0.05) increased in all supplemented groups. The observed positive effect of Spirulina supplementation in diet on treated chicks appeared the role of spirulina as growth and immunity promoter agent as described by Jamil *et al.* (2015) who reported that spirulina can act as good growth and immune modulatory agent for broiler chicks. Thyroid hormones are considered as the key controllers of metabolic heat production which is necessary for the maintenance of high and constant body temperature in homoeothermic birds Abdel-Hack *et al.* (2019).

Table (4): Effect of dietary treatments on plasma thyroid hormones of broiler at 35 days of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
T3	2.27^{b}±0.14	6.41^{a}±0.08	5.27^{a}±0.07	**
T4	16.64^{b}±0.16	20.68^{a}±0.24	16.10^{b}±0.023	**

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. ** P≤ 0.01, NS= non-significant.*
T3: Triiodothyronine, T4: Thyroxine
a and b, Means within Treatments (S) with no common superscript differ significantly.

Total proteins:

The effect of dietary treatments with spirulina on plasma parameters in Table (5) presents in sharp increased total protein and serum glucose levels slightly above. in agreement with the present study (Pestana *et al.,* 2020) when they found increased total protein (P≤ 0.001) compared to birds of the remaining groups. The better digestibility of protein observed in spirulina supplemented diets may be result of better absorption, which enhanced growth in broiler chickens (Park *et al.,* 2018).

Table (5): Effect of Spirulina administration on some Biochemical plasma parameters of broiler at 35 days of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
Total Proteins (g/dl)	5.26^{b}±0.02	6.66^{a}±0.35	5.10^{b}±0.18	**
Albumin (g/dl)	2.43^{a}±0.05	2.41^{a}±0.08	2.15^{a}±0.04	*
Globulin (g/dl)	2.21^{c}±0.16	4.45^{a}±0.44	3.29^{b}±0.05	**
Glucose (g/dl)	86.14^{b}±2.30	95.27^{a}±0.58	88.21^{a}±2.25	**

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. * P≤ 0.05, ** P≤ 0.01.*
a,b and c Means within Treatments (S) with no common superscript differ significantly.

Glucose is an important source of energy, while total proteins, including albumin, reflect the synthesis of proteins in liver, which may be associated with broilers growth and physiological status (Pestana *et al.,* 2020). Chicks fed diets supplemented with spirulina at levels of 1, 2 g\kg diet had higher significant values of globulin compared with the control diet. This results in agreement with (Fathi *et al.,* 2018), Oopoola *et al.* (2019) when they found chickens fed diets supplemented with spirulina at levels of 6,12 and 18 g\kg diet had higher values of globulin, glucose, total protein compared with control diet. These researchers suggested that the high value of serum protein, globulin and albumin may be due to protein quality and quantity of spirulina platensis, that being is rich in polyunsaturated fatty acids phycocyanin (Hassanein *et al.,* 2014).

Plasma lipids profile:

Plasma cholesterol and total lipids had lower levels in all supplemented groups than control as shown in Table (6). similar results were reported by (Abdel-Hady and EI-Ghalid, 2018) when they found the supplementation of Spirulina 3%, 6% to broiler diet, serum levels of total lipid, triglyceride, cholesterol, and low-density lipoprotein were significant decreased in both groups compared to the control group. The decrease in serum lipid profile of broiler chickens fed dietary spirulina may reflect the hypogastria–intestinal tract by Spirulina supplementations which increase lactobacillus population (Mariey *et al.*, 2012). Serum high density lipoprotein (HDL) concentration of broilers of 1, 2 gm spirulina significantly ($P \leq 0.05$) increased. This cholesterol serum reduction has been stated as the effect of spirulina on lipoproteins metabolism and the increase of lipoprotein enzyme activity levels, the hypocholesterolemic actions of spirulina involve reducing plasma and liver cholesterol levels due to the increase in lipoprotein lipase and hepatic triglyceride lipase activity (Hassanein *et al.*, 2014).

Table (6): Effect of Spirulina administration on plasma Lipid profile of broiler 35 days of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
Total cholesterol (mg\dl)	$203.27^{a}\pm1.35$	$178.60^{b}\pm1.415$	$189.24^{c}\pm1.365$	**
Triglycerides (mg\dl)	$223.41^{a}\pm2.603$	$156.82^{b}\pm0.440$	$148.90^{b}1.478$	**
HDL (mg\dl)	$33.62^{a}\pm4.276$	$43.33^{b}\pm1.353$	$37.04^{b}\pm1.906$	*
VLDL (mg\dl)	$58.96^{a}\pm4.90$	$43.84^{b}\pm0.602$	$42.52^{b}\pm4.189$	*
LDL (mg\dl)	$138.38^{b}\pm2.157$	$85.39^{b}\pm0.854$	$73.78^{a}\pm1.586$	**

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. * P≤ 0.05, ** P≤ 0.01, NS= non-significant.*
HDL: (high density lipoprotein cholesterol) VLDL: (very low-density lipoprotein) LDL: (low density lipoprotein cholesterol).
a,b,c Means within Treatments (S) with no common superscript differ significantly.

Liver function tests:

The liver is the main metabolic organ of the body; it indicates the hepatoprotective activities of spirulina due to antioxidative and anti-inflammatory quality (Rawshon *et al.*, 2015). AST and ALT concentration significant decrease for the Spirulina groups (Table 7.) this results in agreement with Abaza *et al.* (2021) and Jamil *et al.* (2015) who reported that ALT and AST activity significantly decreased in all treatment groups supplemented with spirulina , therefore Zeweil *et al.* (2019) results indicated that different supplementations as Spirulina 0.5 and 1 g\kg diet decreased adverse effect of heat stress on ALT and AST of chickens, and there was a slight decrease in plasma ALP and liver enzymes activities the obtained values are fell within the normal range groups in the present study and may reflect normal liver function. The decrease in ALP, ALT and AST actives were observed among supplemented groups. Indicated that Spirulina has hepatoprotective effect that can normalize the elevation of liver enzymes activates led to enhancing the liver health (Abdel-Daim et al., 2015). Since the increasing serum ALP, ALT and AST activities have all been associated with physiological stressful condition. This isn't agreement with Sugiharto *et al.* (2018) who found supplements with 1% of spirulina platensis for first seven days, 21 days and 35 days, treatments had no significant effect on AST, ALT. considering the contradictory results, the difference in nutritional and functional properties of spirulina platensis used in the studies may to some extent be responsible.

Table (7): Effect of Spirulina administration on plasma liver and kidney functions of broiler at 35 days of age.

Trait	Spirulina (S)			Significance
	S0	S1	S2	
ALT, U\L	$8.72^{c}\pm0.116$	$11.74^{a}\pm0.150$	$10.66\pm^{b}0.230$	**
AST, U\L	$9.01^{c}\pm0.179$	$14.68^{a}\pm0.248$	$11.99^{b}\pm0.186$	**
LDH, U\L	$8.57^{b}\pm2.775$	$12.52^{a}\pm3.099$	$7.82^{b}\pm0.970$	*
ALP, U\L	$208.57^{c}\pm3.750$	$286.55^{a}\pm3.444$	$235.50^{a}\pm3.550$	NS

*S0: Control received a basal diet without supplementation, S1: received a basal diet supplemented with 1g\kg, S2: received a basal diet supplemented with 2g\kg. * P≤ 0.05, ** P≤ 0.01, NS= non-significant.*
ALT: (alanine aminotransferase), AST: (aspartate aminotransferase), LDH: (lactate dehydrogenase), ALP: (alkaline phosphatase). a,b and c Means within Treatments (S) with no common superscript differ significantly.

CONCLUSION

It could be concluded that, dietary supplementation with spirulina dry leaves powder with 1 or 2 g/kg diet as alternatives growth promoters may enhancing productive performance of broiler reared under heat stress conditions, without any adverse effect on their physiological responses.

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تأثير إضافة طحلب الأسبيرولينا على الأداء الإنتاجي والفسيولوجي لدجاج التسمين خلال فصل الصيف

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تم إجراء هذه الدراسة خلال الفترة من يوليو الى اغسطس 2020 بهدف دراسة تأثير أضافة طحلب الأسبيرولينا في علائق كتاكيت التسمين علي الأداء الإنتاجي وبعض الصفات الفسيولوجية .استخدمت في هذه الدراسة 150 كتكوت غير مجنس من سلالة الروس وزعت عشوائيا الى ثلاث معاملات كل معامله 50 طائر مقسمة الى خمسة مكررات بكل مكرر 10 طيور وموزعة كالتي: المعاملة الأولى تحتوي على العليقة الأساسية (مجموعة الكنترول)، والمعاملات االأخرى تحتوي في علائقها علي طحلب الأسبيرولينا بنسب 1 و 2 جم / كجمعلف. وقد أظهرت نتائج التجربة أن الكتاكيت المغذاه علي علائق تحتوي علي طحلب الأسبيرولينا تحسنت معنويا في وزن الجسم، ومعدل الزيادة في وزن الجسم، ومعدل التحويل الغذائي، ومعدل النمو، والأداء الإنتاجي، وبعض صفات مقاييس الدم ويستخلص من نتائج الدراسة أن: اضافة االسبيرولينا بنسبة 1 أو 2 جرام لكل كيلو جرام عليقة أدت الى تحسين الأداء الإنتاجي والفسيولوجي للكتاكيت.

الكلمات المفتاحية: *طحلب الإسبيرولينا – كتاكتيت التسمين – الأداء – مكونات الدم - هرمونات الغدة الدرقية*