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EFFECTS OF *SPIRULINA PLATENSIS* ADMINISTRATION TO COPE THE NEGATIVE IMPACTS OF HEAT STRESS IN SUCKLING EGYPTIAN BUFFALOES CALVES

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ABSTRACT

Dietary manipulations play an important role in mitigating the negative impacts of heat stress (HS) on farm animals. Thus, this study was conducted to examine the modulatory role of *spirulina platensis* (SP) in improving the growth performance, biochemical parameters, antioxidant status, and immunological response of suckling Egyptian buffalo calves during summer season. A total of 24 newly born calves were randomly allotted to groups of six calves in each group. Group (1) served as the control. The other three groups were received suckling milk supplemented with SP powder at levels; 5 g/head/day (SP5), 10 g/head/day (SP10) and 15 g/head/day (SP15) for three months suckling period. Results showed that the values of average daily gain in the SP10 or SP15 groups were greater significantly ($P<0.05$) than those in the control group. The values of total protein, albumin, globulin, high density lipoproteins, red blood cells, hemoglobin, white blood cells, lymphocytes, and platelet count were significantly higher in SP- treated groups than those in the control group. Liver enzymes activities, urea, creatinine, low density lipoproteins, glucose and bilirubin did not affect significantly by the SP supplement. The concentrations of total cholesterol and triglycerides as well as lipid peroxidation were significantly lower ($p<0.05$) in the SP15 group than the control. Redox status and immunity parameters were improved in the SP10 and SP15 groups compared to the control. In conclusion, dietary SP 10 or 15 g SP/ Head /day can mitigate the harmful influences of heat stressed on suckling buffalo calves, via boosting the immunological parameters and antioxidant capacity.

INTRODUCTION

Heat stress is one of the most central issues with a harmful effect on livestock around the world, especially in the tropical climate. The exposure of farm animals to environmental heat stress directly affects homeostasis (Sheiha et al. 2020). Also, heat stress causes hormonal/metabolic imbalances, compromised immune function, induces the inflammatory reaction and alters animal behavior and reduction of antioxidants capacity by promoting free radical production which ultimately affects negatively the growth performance (Marai et al., 2002; El-Desoky et al., 2017; Hirakawa et al., 2020).

The associations between oxidative stress and heat stress (HS) have been established in farm animals (Kargar et al., 2015; Abdollah et al., 2016; Maha et al., 2018; El-Ratel et al., 2021). Oxidative stress occurs as a result of an imbalance between the efficiency of the antioxidant defense system and the generation of reactive oxygen species (ROS). At the same time that molecular oxygen is essential for normal cellular functions in mammals, increased ROS levels can cause many adverse effects on cells, organelles and tissues and disruption of physiology and normal metabolism (Long et al., 2017).

Oxidative stress in farm animals should be controlled either by reducing effects of substances that stimulate ROS or by adding natural antioxidant nutrients in animal diets (Ganaie et al., 2013). Nutritional intervention throughout natural antioxidant supplementation is considered a vital strategy to ameliorate the negative influence of HS in livestock (Al-Sagheer et al. 2017). microalga production as a good alternative dietary source of proteins has a great attention (Alvarenga et al., 2011). These microorganisms were classified into *Spirulina maxima*, *Spirulina platensis* and *Spirulina fusiformis* (Karkos et al., 2011). Spirulina alga (SA) has a complex composition and a simple structure. It has high feeding value with wide range of medicinal applications (Abu-Elala et al., 2016). The SA contains important compounds, in term of high protein content (approximately 60-70% based on dry matter) with all essential amino acids such as glutamic acid, aspartic acid, methionin, proline and phenylalanine (Farag et al., 2016; Ghattas, et al., 2019), poly-unsaturated fatty acids (γ-linolenic acid), minerals (K, Ca, Cr, Mn, Mg, Fe, Cu, P, Zn, Na and Se) and vitamins such as E, B1, B6, B12 and β-carotene (Hoseini et al., 2013; El-Ratel and Gabr 2020). It also contains many photosynthetic pigments (xanthophyll phytopigments and phycocyanobilin chlorophyll) as obtained by Gong et al. (2005) and Bermejo et al. (2008). Phytochemicals components in Spirulina has plentiful attributes of interest, including antioxidant (Kurd and Samavati, 2015), antitumor (Konickova et al., 2014), anti-inflammatory (Vide et al., 2015), antiviral, immune-modulatory (Sahan et al., 2015) and probiotics (Shanmugapriya et al., 2015) properties. Compared to other synthetic products, the animal performance was enhanced by spirulina treatment with good health status and low cost (Shanmugapriya et al., 2015). The possible role of SP as a natural antioxidant was studied on goat's milk and udder measurements (Khalifa et al., 2016), reproductive

performance (Ragab et al., 2019; El-Ratel and Gabr, 2020) and growth performance (EL-Sabagh et al., 2014). While the studies of the effects of SP on growing suckling calves especially under heat stress condition during summer season is limited. Therefore, the aim of this present study was to investigate the efficiency of SP on growth performance, some hemato-biochemical parameters, immune and hormonal response and redox status for buffaloes suckling calves under summer conditions.

MATERIAL AND METHODS

Meteorological parameters

Ambient air temperature (AT^0C) and relative humidity (RH, %) were assessed (at 1400 h) daily by an automatic thermo hygrometer (Dostmann GmbH and Co. KG, Wertheim, Germany) set in the farm throughout the entire experimental period. Temperature humidity index (THI) was calculated according to the equation proposed by amundson et al. (2006)

THI = (0.8×Ta °C) + [(RH %) × (Ta °C -14.4)/100)] + 46.4.

Where Ta is air temperature and RH is the relative humidity

Animals and housing

Twenty-four healthy newly born calves with average body weight 38 ±0.03 kg, were selected from Mehallet Moussa Experimental Station (Kafr El-Sheikh, North Delta), belongs to the Animal Production Research Institute, Ministry of agriculture, Egypt. Once the calves can stand after parturition, they begin to suckle their dams immediately within the 1 to 6 hours to get the colostrum. Calves separated to a special calf's pen after colostrum feeding which continues for about 4 to 5 days.

Diet

The calves were reared in individual pens and fed with whole pooled and warm milk (37oC) approximately at 10% of body weight at birth .Calves had the feed starter and berseem hay according APRI recommended requirements, while freshwater ad libitum. Milk was presented in two equal meals daily at 08:00am and 6:00pm. Calves were provided by *Spirulina platensis* (SP) powder after well mixing with whole milk to ensure that there is no sedimentation at the bottom of the bucket. The calves weaned at 90 kg body weight or three months of age.

Experiment design:

Calves were haphazardly allotted to 4 four groups of six calves in each group. Group (1) served as control fed the basal diet only. Groups 2, 3, and 4 supplemented with SP powder, that was added in the milk of each calve at a rate of 5, 10, and 15 gm/ calve/ day, respectively for 75 days.

Spirulina platensis analysis

The SP powder was obtained from AB CHEM Company, Mansoura, Dakahlia, Governate , Egypt. It was analyzed to its nutritional constituents according to AOAC. (2000) as seen in Table 1.

Table 1. The chemical compositions and antioxidant compounds of *Spirulina platensis* (SP)

Composition quantities	Estimates
Chemical compositions (Dry matter basis)	
Moisture	5.9
Crude protein	58.715
Fat	6.06
Crude fiber	4.12
Ash	10.505
Total carbohydrates	14.7
Amino acid profile	
Glutamic acid (mg/m)	12.8
Aspartic acid (mg/g)	25.2
Arginine (mg/g)	8.4
Proline (mg/g)	28.2
Methionin (mg/g)	68.98
Alanine (mg/g)	16.6
Glycine (mg/g)	9.3
Phenylalanine (mg/g)	15.29
Mineral	
Calcium(mg/100g)	164
Iron (mg/100g)	106
Manganese (mg/100g)	5.6
Selenium(μg/100g)	6.5
Phosphorus (mg/100g)	98
Sodium (mg/100g)	840
Zinc (mg/100g)	2.7
Vitamins	
Vitamin E(mg/100g)	12.10
Thiamin (mg/100g)	2.23
Riboflavin (mg/100g)	3.83
Vit. B12(mg/100g)	0.15

Body weight and rectal temperature determination

All calves in the four groups were weighted before treatment and bi-weekly thereafter till the end of the experiment. Also, rectal temperature was monitored and recorded every two weeks for each calf.

Blood sampling

At the end of the experment, five calves in each experimental group were selected for blood collection. Two blood samples were collected via jugular vein puncture. The first sample collected on anticoagulant EDETA for hematological parameters by an automated hematology analyser (Hospitex Hema Screen 18, Sesto Fiorentino, Italy). The second sample was collected in clean, sterilized tubes and centrifuged at 3000 rpm for 20 minutes by a centrifuge (T32c; Janetzki, Wallhausen, Germany). Serum samples were separated and kept at -20 0C until the biochemical analysis.

Blood constituents

Blood serum metabolites including total protein (TP), albumin, globulin, urea, creatinine, triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), high density

lipoprotein (HDL), glucose, bilirubin, aspartate transaminase (AST), and alanine transaminase (ALT) were determined spectrophotometrically using commercial kits from Biodiagnostic Company (Giza, Egypt). Immunoglobulins (IgG and IgA) were measured based on the method termed by Akiba et al. (1982). Serum thyroxin (T4) and triiodothyronine (T3) concentrations were determined by ELISA (Enzyme-Linked Immune-Sorbent Assay) technique using EIA kits (Prechek Bio, Inc., Atlaslink technology, California-USA).

Triiodothyronine/thyroxin ratio was calculated. With respect to oxidative stress and antioxidant indices assessments, the activities of the enzymes of reduced glutathione (GSH), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), malondialdehyde (MDA) levels and the total antioxidant capacity (TAC) in serum were determined using commercial kits (Bio-diagnostic Research Egypt) by spectrophotometric methods (Shimadzu, Kyoto, Japan) according to manufacturer's instructions.

Statistical model and analysis procedure

Data were edited in MS Excel (Microsoft Corporation, Redmond, WA, USA). The Levene and Shapiro–Wilk tests were conducted to check for normality and homogeneity of variance (Razali and Wah 2011).The mixed model of statistical analysis system (SAS., 2012 version 8, Cary, NC, USA) was used for assessing body weight, rectal temperature, haemato-biochemical attributes, redox status indicators, and immunity status. SP levels (as a fixed factor), while calve individual (as a random factor), was introduced in the statistical model. The linear, quadratic or cubic trends of each depended variable to different SP levels were determined by orthogonal contrast statements. Sample size was detected according to Thompson equation at CI=95%, Z= 1.96, α = 0.05, D=0.05 and P=0.50 (Thompson, 2012). Multiple comparisons among means were carried out by the Duncan's Multiple Range Test (DMRT, Steel and Torrie, 1980). The statistical significance was accepted at probability less than 0.05.

RESULTS AND DISCUSSION

Meteorological parameters

Results in Table 2 show the mean values of AT, RH and THI during the whole experimental period were 34.12 ± 0.30°C, 75.29 ± 3.04%, and 88.95 ± 2.69, respectively. According to Amundson et al. (2006) the THI in the present study indicated that the growing suckling calves suffer from severe heat stress. Gaafar et al., (2011) referred that expose Friesian cows in Egypt to heat stress during the period from June to September. Management strategies are needed to minimize heat stress and attain optimal animal performance.

Table 2. Mean ambient temperature, relative humidity, and temperature–humidity index during the experimental period.

Parameters[1]	Mid-June	July	August	Over all	SEM	*p*-value
AT (^{0}C)	33.62^b	35.22^a	34.15^{a,b}	34.12	0.30	0.0281
RH (%)	75.36	73.41	77.12	75.29	3.04	0.2317
THI	87.78	89.85	88.95	88.86	2.69	0.3152

[1]AT: ambient temperature; RH: relative humidity; THI: temperature–humidity index. ^{a,b} Mean values followed by different superscript

letters in the same row are significantly different ($p < 0.05$).

Growth performance

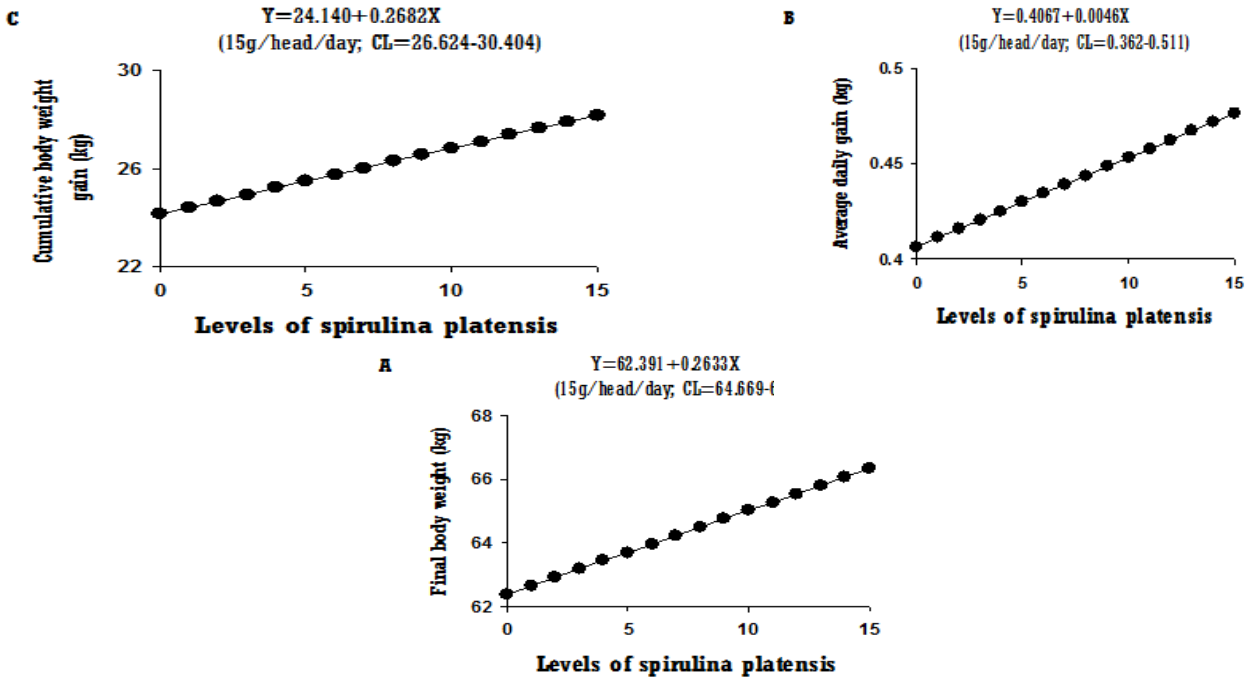
Results in Table 3 and Figure 1 show the effect of SP on the growth performance of suckling calves exposed to high ambient temperature. The present results indicated that the treatment with SP affected linearly final body weight ($p = 0.0124$), average daily gain ($p = 0.0195$), and cumulative body weight gain ($p = 0.0264$). Non-significant differences ($p>0.05$) were observed between the groups treated with SP at the highest levels (10 and 15 g /head/ day) whereas the significant differences with the control were observed ($p<0.05$). Significant improvements in the growth performance of suckling calves supplemented by SP in the present study may be due to the pronounced stimulating effect of SP on the metabolism during the period of intensive growth of animal organs (Glebova et al., 2018). Additionally, our results could be supported by the findings of Farag et al. (2016) who reported that the Spirulina have a wide variety of natural carotene and xanthophyll phytopigments in addition to good quality proteins, minerals and vitamins which make SP impressive and unique nutrient composition could be used as a good dietary supplement not only for therapeutic aspects but also to enhance nutritional qualities. In this context, Spirulina can be digested simply as its cell wall had not cellulose structure and thus it stimulates the performance of animal growth (Moreira et al., 2011; Seyidoglu and Galip, 2014; Seyidoglu et al., 2017). Additionally, Kamel (2012) showed significant improvements of rabbit buck fed diets contained SP due to the several antioxidant properties of SP in similar pattern with other dietary antioxidants sources such as folic acid, selenium and their combinations. Herein, the greatest values of live body weight and average daily gain study were observed at the level of 15g SP / head/ day. In parallel, El-Ratel and Gabr (2020) reported significant effects of adding SP in drinking water on the growth performance only at the highest level (300 mg/L). The present results corresponded with the previous studies of Simkus et al. (2013) and Nedeva et al. (2014), they reported that adding SP to pig's diets resulted in a significant increase in live body weight compared to control. Similarly, Zhang et al. (2019) postulated that adding 0.5 % SP to weaned piglet's diet increased both of body weight gain and average daily gain significantly, this effect could be attributed to the ability of animals to fight stress due to their perfect immunity. In lambs, EL-Sabagh et al. (2014) reported a significant ($P<0.05$) increase in daily weight gain and the final body weights of animals treated by SP powder (1 g/10 kg BW/ day) compared to the control group. On the contrary, Ghattas et al. (2019) observed non-significant increase in the body weight of in Holstein suckling calves supplemented with SP (6g/ head/ day) compared to the control.

Eweedah et al. (2022) showed that both niacin and nutrition C, as well as their combination, were efficient in increasing feed intake, digestibility, rumen fermentation, blood parameters, growth rate, feed conversion, and financial efficiency in suckling Friesian calves under heat stress during summer season.

Table 3.Concentration-dependent effects of *Spirulina platensis* supplements on body weight, average daily gain, growing suckling calves (n=6/ treatment) exposed to high ambient temperature.

Variables[1]	*Spirulina platensis* (SP, g / h/ day)				SEM	*p*-value			
	Control	5	10	15		T	Linear	Quadratic	Cubic
IBW (kg)	38.103	38.241	38.117	38.201	0.172	0.9802	-	-	-
FBW (kg)	62.332^{b}	63.667^{a,b}	65.283^{a}	66.182^{a}	0.882	0.0391	0.0124	0.2897	0.2707
ADG (kg/calve)	0.406^{b}	0.427^{a,b}	0.461^{a}	0.472^{a}	0.016	0.0467	0.0195	0.3336	0.3143
WG (Kg/calve)	24.113^{b}	25.414^{a,b}	27.039^{a}	28.042^{a}	0.651	0.0481	0.0264	0.2114	0.3914

[1] IBW: initial body weight; FBW: Final body weight; ADG; Average daily gain; SEM: Pooled standard error of least square means. ^{a,b} Means in the same row with different superscript letter following them are significantly different ($p < 0.05$).



Figure, 1: Dose–response curve of final body weight (A), average daily gain (B), cumulative body weight gain (C) for different levels of dietary supplemental, x and y are the dependent (*Spirulina platensis* levels) and the independent variables of the regression equation, respectively. The figure only shows significant relationships ($p < 0.05$).

Biochemical variables

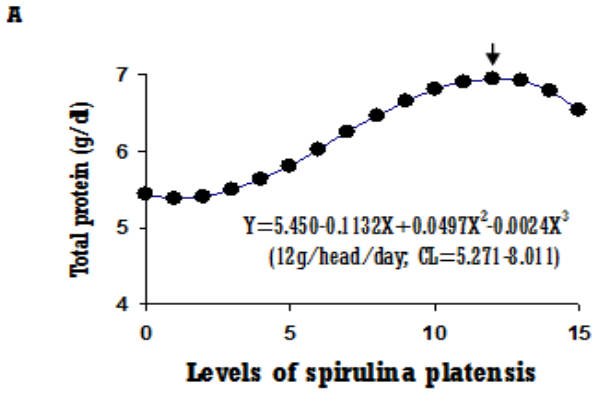
Table 4 and Figure 2 provides the effects of various levels of SP on blood serum biochemical variables of growing suckling calves exposed to high ambient temperature during summer season. Increasing the levels of SP cubically affected concentrations of TP ($p < 0.0001$), albumin ($p < 0.0001$), and globulin ($p = 0.0012$), non-significant differences were showed between the groups treated with SP at levels of 10 and 15g /head/ day ($p > 0.05$). The present results agreed with previous literature showed significant increase in blood protein profile in SP treated group compared to the control in rabbits (El-Ratel, 2017) and cattle (Glebova et al., 2018). Higher concentrations of blood serum protein and its main components (Albumin and Globulin) in treated groups compared to the control may be attributed to the high contents of protein, essential amino acids minerals, vitamins, phospholipids and antioxidants in SP (Gershwin and Belay, 2008; Farag et al., 2016). The higher blood albumin in the present study is coupled with the higher total leukocytic count which reflected a stronger innate response in heat stressed suckling calves (Matanović et al., 2007). Additionally, albumin concentration is considered an indicator for long-term dietary protein intake (Sargison and Scott, 2010). On the contrary, Ragab et al. (2019) mentioned that dietary SP supplementation (0.3 and 0.6 g/kg diet) in rabbit does diets during gestation and suckling periods did not exhibit significant effect on blood parameters (TP, albumin, and globulin). Feeding does of SP significantly affected lipid profile in blood serum in terms of TG ($p=0.0007$), high density lipoprotein ($p=0.0002$), and total cholesterol ($p < 0.0001$) compared to the control. Regression analysis showed that there was a cubic relationship between dietary SP and triglycerides. In this context, there was a linear increase ($p < 0.0001$) in high density lipoprotein and a linear decrease ($p < 0.0001$) in the total cholesterol. However low density lipoprotein concentration was not affected significantly by the treatment ($p=0.0917$). Improved lipid profile in the present study in terms of increasing high density lipoprotein and decreasing concentration of triglycerides and total cholesterol in blood of suckling calves agreed with the results of El-Ratel (2017) and Ragab et al (2019) in rabbits. On the contrary, EL-Sabagh et al. (2014) showed significant increase in TG in blood serum of lambs fed diet contained SP may be due to the insufficient dose of SP to decrease blood TG or the experimental period was not long enough for SP to exert its properties in lipid modulating. They added that the SP lipid extracts from had antimicrobial and antioxidant activity hence, the lipid extract from SP presented a promising potential as a safe alternative to synthetic antimicrobials and antioxidants. Additionally, SP has a hypolipidemic effect due to the presence of C-phycocyanin protein which frustrates the activity of pancreatic lipase in a dose-dependent manne (Torres-Duran et al., 2007). The hypocholesterolemic actions of SP reduce liver and plasma cholesterol as a result of increasing the hepatic triglyceride lipase activity and lipoprotein lipase (Karkos et al., 2008) in addition to modifying the metabolism of lipoproteins by increasing high density lipoprotein and decreasing low density lipoprotein (Torres-duran et al., 2007). Furthermore, SP treatment did not affect

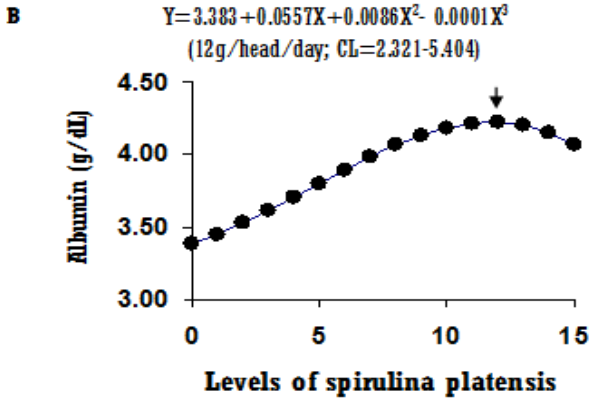
neither kidney function with respect to urea (p=0.1254) and creatinine (p=0.8093) nor liver function in terms of bilirubin (p=0.8374) and both of AST (p=0.2122) and ALT (p=0.4130) enzymes and all estimates were in normal range compared to the untreated groups.

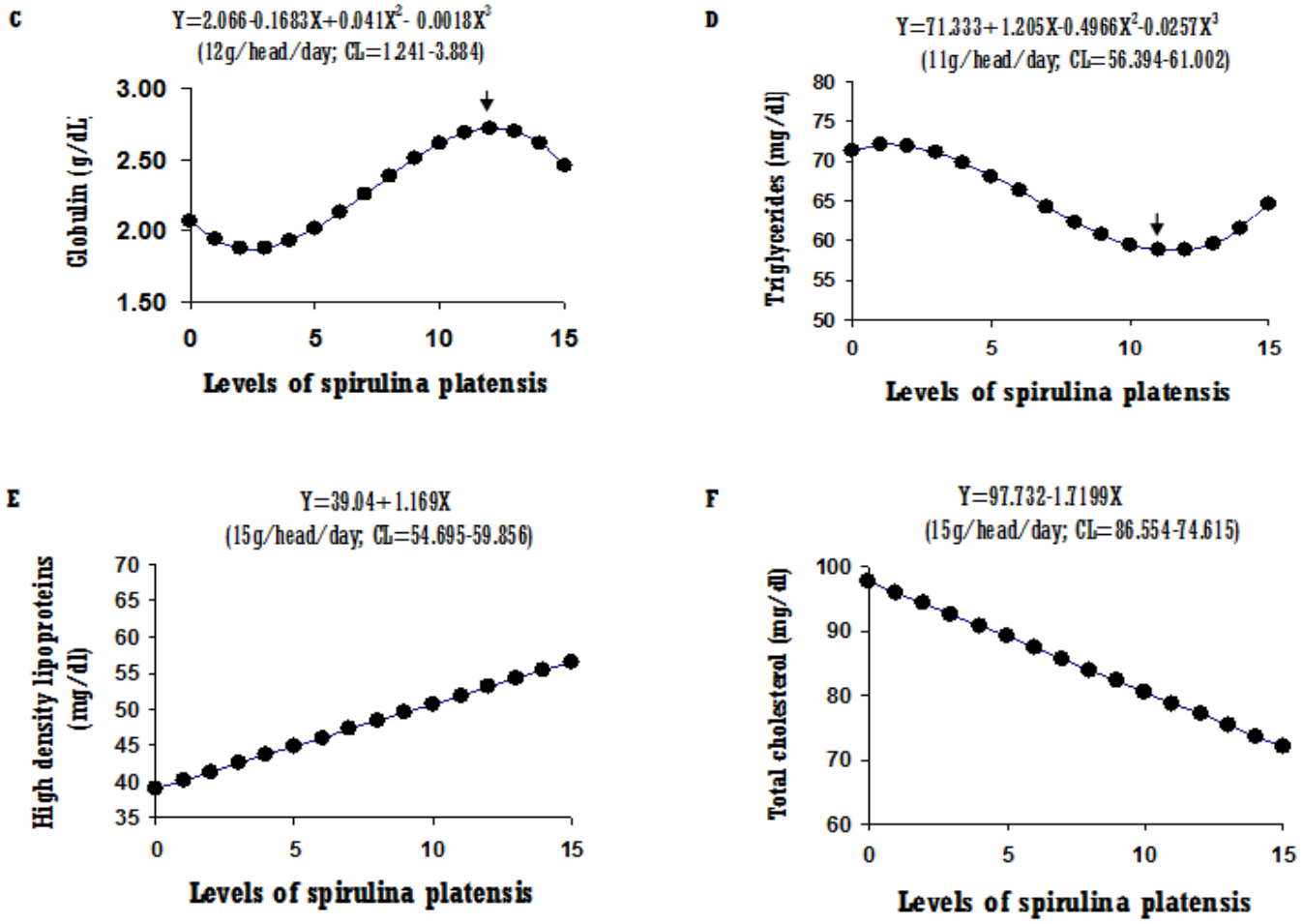
Table 4. Concentration-dependent effects of *Spirulina platensis* supplements on biochemical variables of growing suckling calves (n=5/ treatment) exposed to high ambient temperature.

Variables[1]	*Spirulina platensis* (SP, g / h/ day)				SEM	*p*-value			
	Control	5	10	15		T	Linear	Quadratic	Cubic
TP (g/dl)	5.450^{c}	5.816^{b}	6.800^{a}	6.533^{a}	0.097	<0.0001	<0.0001	0.0040	0.0004
Albumin (g/dL)	3.383^{c}	3.800^{b}	4.183^{a}	4.066^{a}	0.085	<0.0001	<0.0001	0.0053	0.2346
Globulin (g/dL)	2.066^{b}	2.016^{b}	2.616^{a}	2.466^{a}	0.106	0.0012	0.0003	0.6441	0.0082
Urea(mg/dL)	40.000	33.500	38.333	39.666	4.169	0.1254	0.3654	0.0654	0.4351
Creatinine (mg/dL)	1.186	1.155	1.180	1.146	0.022	0.8093	0.9226	0.6425	0.4015
TG (mg/dL)	71.333^{a}	68.166^{a}	60.500^{b}	64.666^{a,b}	2.215	0.0079	0.0090	0.0747	0.0452
TC (mg/dL)	98.166^{a}	88.666^{b}	80.166^{b,c}	72.333^{c}	2.933	<0.0001	<0.0001	0.7792	0.9800
LDL(mg/dL)	30.416	26.950	29.816	25.516	3.178	0.0917	0.0796	0.1453	0.2365
HDL(mg/dL)	38.733^{c}	44.950^{b,c}	51.600^{a,b}	56.016^{a}	2.653	0.0010	<0.0001	0.7381	0.8245
Glucose (mg/dL)	88.000	82.666	83.000	83.666	1.876	0.1900	0.0609	0.2993	0.6955
Bilirubin (mg/dL)	0.600	0.500	0.531	0.566	0.080	0.8374	0.8558	0.4200	0.7166
AST (U/mL)	49.500	49.000	46.500	43.700	5.126	0.2122	0.1017	0.7280	0.3820
ALT (U/mL)	38.166	37.166	35.833	34.166	2.052	0.4130	0.2413	0.8726	0.4760

[1] TP: total protein; TG: triglycerides; LDL: low density lipoproteins; HDL: high density lipoproteins; AST: aspartate transaminase; ALT: alanine transaminase. SEM: Pooled standard error of least square means. ^{a,b,c} Means in the same row with different superscript letter following them are significantly different ($p < 0.05$).







Figure, 2: Dose–response curve of total protein (A) albumin (B), globulin (C), triglycerides (D), high density lipoproteins (E), total cholesterol (F) for different levels of dietary supplemental, x and y are the dependent (*Spirulina platensis* levels) and the independent variables of the regression equation, respectively. The figure only shows significant relationships ($p < 0.05$).

Redox status

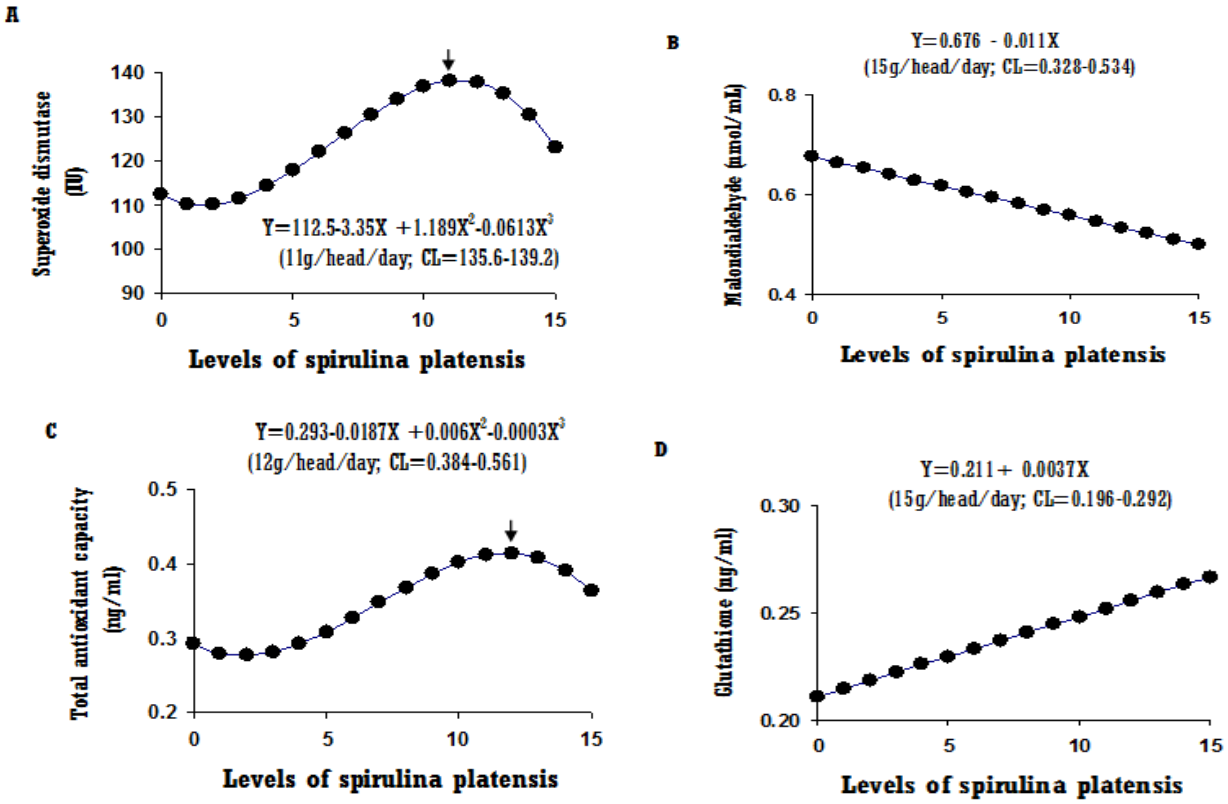
A cubic relationship was observed between dietary SP and blood serum superoxide dismutase (SOD; *p*-value =0.0034), total antioxidant capacity (TAC; *p*-value =0.0315), glutathione peroxidase (GPX; *p*-value =0.0466) and catalase activity (CAT; p-value =0.0174). Non-significant differences were observed between the groups treated with SP at levels of 10 and 15g /head/ day ($p>0.05$) for TAC, GPX and CAT. A linear relationship was observed between dietary SP and glutathione concentration (*p*-value=0.0112) (Table 5 and Figure 3). With respect to lipid peroxidation, the dietary treatment also affected MDA linearly (*p*-value=0.0020), being in a reverse trend with the ascending levels of SP. The activity of antioxidants enzymes (SOD and GPX) decreased under heat stress, while the lipid peroxidation increased occurring oxidative stress (Georgieva et al., 2006). Bioactive components in SP such as α-tocopherol, ß-carotene and phycocyanin had great activates of scavenging acting on free radicals either in synergy or individually (Kurd and Samavatl, 2015).

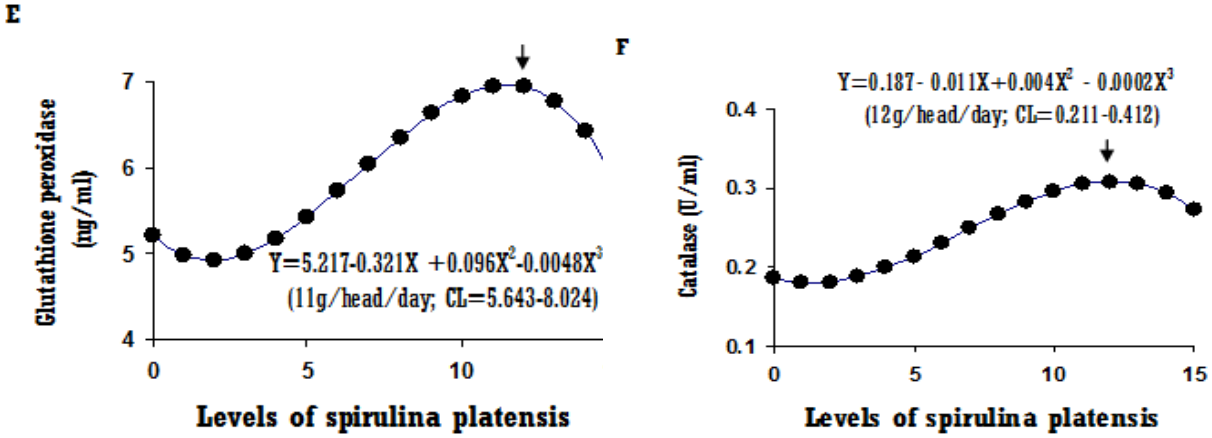
The antioxidant activity of phycocyanin is about 20 times higher efficient than vitamin C (Gershwin and Belay, 2008). Some antioxidant compounds can be produced from SP (Abd El-Baky, 2003) may be due to its high contents of bioactive components such as C-phycocyanin, carotenoids and a potent antioxidant agent as one of its major constituents (Mittler et al., 2004 and Abd El-Baky et al., 2007). Estrada et al., (2001) attributed the antioxidant agent to the presence of phycocyanin and polyunsaturated fatty acids in SP. Additionally, superoxide dismutase in SP acts indirectly by slowing down the rate of oxygen radical generating reactions (Belay, 2002). Further, SP had the ability to maintain cellular antioxidant enzymes and increase the levels of glutathione in these cells (El-Ratel and Gabr, 2019). The present results were in conformity with Jeyaprakash and Chinnaswamy (2007) they displayed that SP improved the antioxidant capacity in heat stressed rats. In heat stressed rabbit does, the supplementation of 300 mg SP/kg diet presented greater levels of GPX, TAC, GSH and CAT and lower contents of MDA in blood serum (El-Ratel and Gabr, 2019).

Table 5.Concentration-dependent effects of *Spirulina platensis* supplements on redox status of growing suckling calves (n=6/ treatment) exposed to high ambient temperature.

Variables[1]	*Spirulina platensis* (SP, g / h/ day)				SEM	*p*-value			
	Control	5	10	15		T	Linear	Quadratic	Cubic
SOD (IU)	112.50^{c}	117.83c^{b}	136.66^{b}	123.00^{a}	3.051	0.0001	0.0036	0.0098	0.0034
MDA (nmol/ml)	0.683^{a}	0.620^{a}	0.533^{b}	0.515^{b}	0.048	0.0124	0.0020	0.8506	0.2675
TAC(ng/ml)	0.293^{c}	0.308^{c,b}	0.401^{a}	0.363^{a,b}	0.011	0.0037	0.0028	0.2073	0.0315
GSH (ng/ml)	0.201^{b}	0.236^{a,b}	0.266^{a}	0.253^{a}	0.014	0.0314	0.0112	0.1181	0.5689
GPX (ng/ml)	5.217^{b}	5.420^{b}	6.836^{a}	5.865^{a,b}	0.109	0.0313	0.0615	0.1375	0.0466
CAT (U/ml)	0.187^{a}	0.214^{a}	0.296^{b}	0.274^{b}	0.002	0.0001	0.0644	0.0312	0.0174

[1] SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; GSH: glutathione; GPX: glutathione peroxidase; CAT: catalase; LYZ: lysosome activity; SEM: Pooled standard error of least square means. [a,b,c] Means in the same row with different superscript letter following them are significantly different ($p < 0.05$).





Figure, 3: Dose–response curve of superoxide dismutase (A) malondialdehyde (B), total antioxidant capacity (C), glutathione (D), glutathione peroxidase (E), catalase (F) for different levels of dietary supplemental, x and y are the dependent (*Spirulina platensis* levels) and the independent variables of the regression equation, respectively. The figure only shows significant relationships ($p < 0.05$).

Immunity status and pro-inflammatory cytokinesis

Increasing the levels of SP cubically affected immunity status represented by immunoglobulin A (IgA; p-value =0.0003) and G (IgG; p-value =0.0002) in addition to lysosome activity (LYZ; p-value = 0.0199); regression analysis showed that the greatest value of IgA and IgG was at level of 12g SP /head/ day (Figure 6A and B), whereas it was at level of 11g SP /head/ day for LYZ (Figure 6C). With respect to pro-inflammatory cytokines, ascending levels of SP increased linearly both of interleukin (p-value <0.0001; Figure 6D) and interferon γ (p-value=0.0022 Figure 6E). Significant differences were observed in the three treatment groups (5, 10 and 15g SP /head/ day) compared with the control group. However, the differences between the levels of 11 and 12g SP /head/ day were not significant.

The broadly informed health benefits of SP (antioxidant, anti-inflammatory, immune-modulatory, and chemo-protective properties), could be elucidated by the activities of biological constituents in SP (Sitohy et al. 2015; El-Shall et al. 2019). Improved immune status under heat stress in the present study because SP may be able to increase the activity of macrophage phagocytic, stimulate the secretion of both of cytokines and antibodies, in addition to mobilization and activation of T and B cells as mentioned by Gad et al. (2011). Under high AT, the upper contents of pro-inflammatory cytokines such as INF-α and IL-4 in blood plasma were induced Also, levels of LYZ and antioxidants indices such as GPX, GSH, SOD, and TAC decreased upon heat stress (Abd El-Hack et al. 2020). Spirulina enhances the immune status, particularly the primary response

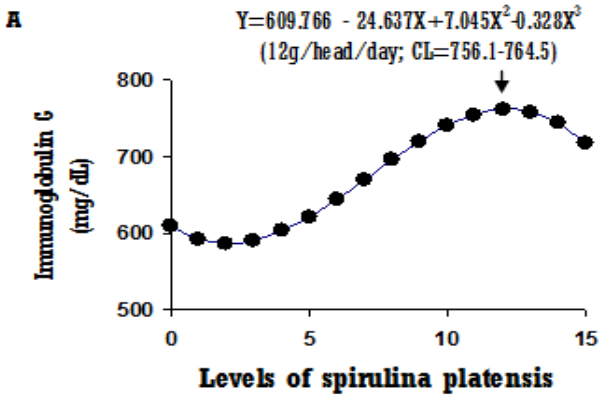
through reduces the synthesis of IL-4 and INF- γ or the augmented the GSH and TAC leading to growth stimulation (Yadav and Kumar, 2018; Abdelnour et al., 2020). Phycocyanin can inhibits the generation of pro-inflammatory cytokines and improve lipid peroxidation (Hwang et al. 2011a).

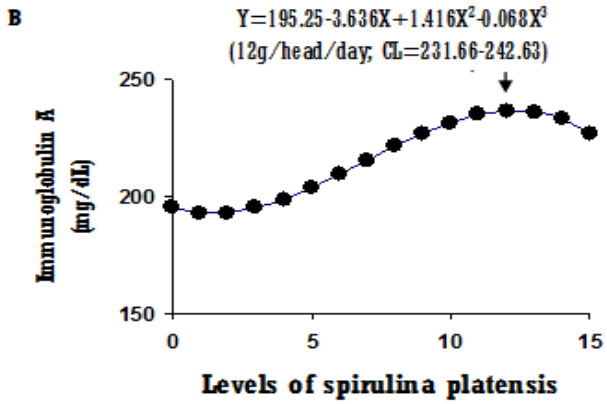
The inclusion of phycocyanin as a bioactive component isolated from SP at levels of 50 or 100 mg/kg diet in heat stressed growing rabbits declined the levels of IL-β and INF-α in the blood plasma significantly, but increased both of nitric acid and lysosome activity significantly in those groups (Abdelnour et al., 2020).

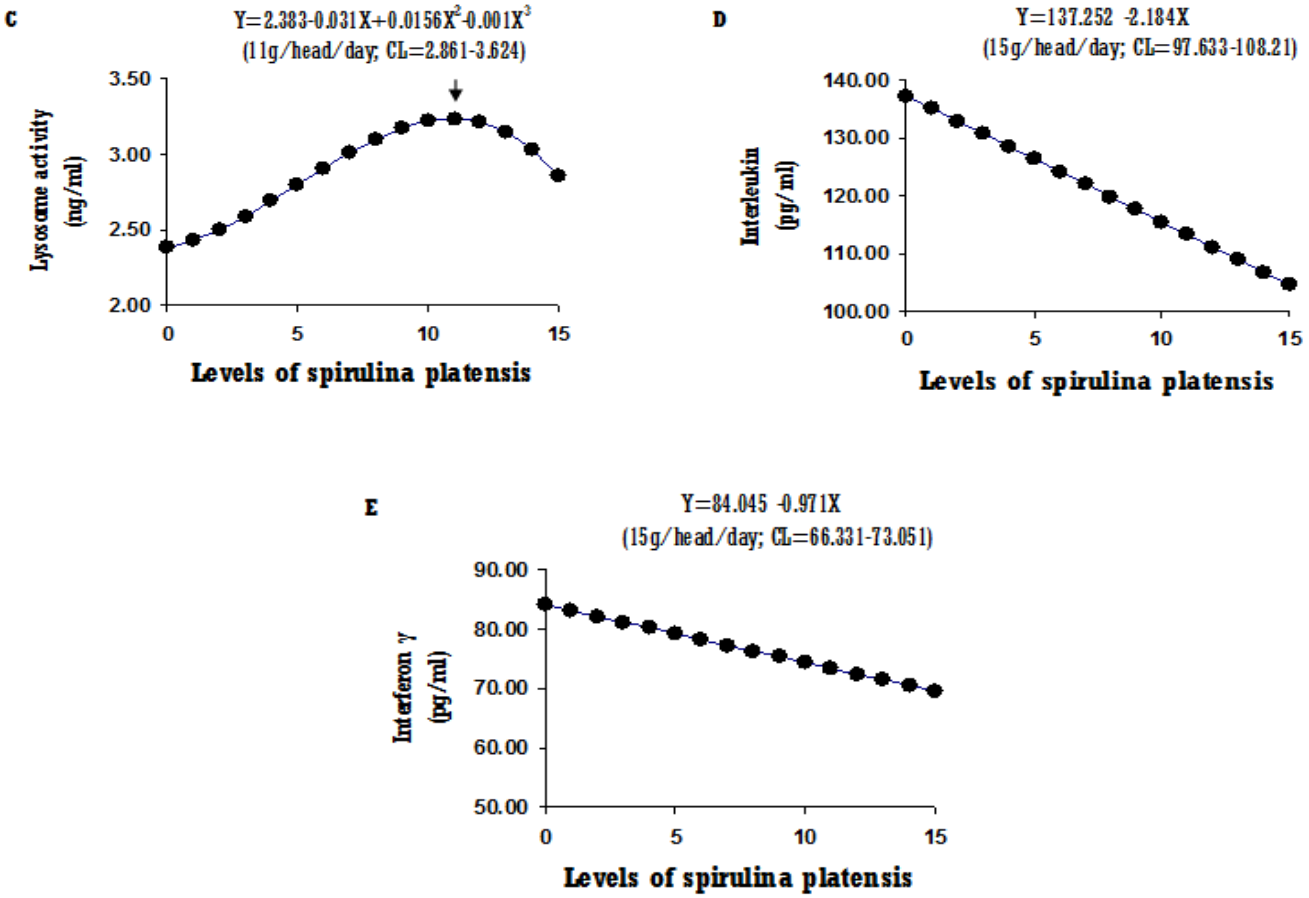
Table 6.Concentration-dependent effects of *Spirulina platensis* supplements on immunity status of growing suckling calves (n=6/ treatment) exposed to high ambient temperature.

Variables[1]	*Spirulina platensis* (SP, g / h/ day)				SEM‡	*p*-value			
	Control	5	10	15		T	Linear	Quadratic	Cubic
IgG (mg/dL)	609.766^{b}	621.650^{b}	739.433^{a}	716.750^{a}	12.090	<0.0001	<0.0001	0.1683	0.0002
IgA (mg/dL)	190.250^{c}	203.866^{b}	231.666^{a}	227.033^{a}	2.391	<0.0001	<0.0001	0.0011	0.0003
LYZ (ng/ml)	2.383^{c}	2.800^{b}	3.216^{a}	2.850^{b}	0.052	<0.0001	0.0014	0.0007	0.0199
IL4 (pg/ml)	138.303^{a}	125.833^{b}	113.166^{c}	106.151^{c}	2.898	<0.0001	<0.0001	0.3540	0.6575
INF-γ (pg/ml)	86.137^{a}	76.211^{b}	74.000^{b}	70.676^{b}	3.149	0.0133	0.0022	0.3024	0.5452

[1] IgG: immunoglobulin G; IgA: immunoglobulin A; LYZ: lysosome activity; IL4: interleukin; INF- γ: interferon γ; SEM: Pooled standard error of least square means. a,b,c Means in the same row with different superscript letter following them are significantly different ($p < 0.05$).







Figure, 4: Dose–response curve of immunoglobulin G (A) immunoglobulin A (B), lysosome activity (C), interleukin (D), interferon γ (E) for different levels of dietary supplemental, x and y are the dependent (*Spirulina platensis* levels) and the independent variables of the regression equation, respectively. The figure only shows significant relationships ($p < 0.05$).

CONCLUSION

Supplementing suckling buffalo calves diets with SP at a dose of 10 or 15 g/head/ day can be an effective intervention that positively affects growth performance, biochemical parameters, and immune-oxidative status.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest

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Conceptualization, A.A.E. M.M.H.. S.A.A., and M.M.E.; methodology, A.A.E., M.M.E., M.M.H., and S.A.S; software, A.A.E.; validation, A.A.E., M.M.E. and M.M.H.; formal analysis, A.A.E.; resources, A.A.E., M.M.E., M.M.H. and S.A.S; data curation, A.A.E, M.M.H., and S.A.S writing—original draft preparation, A.A.E. and S.A.S; writing—review and editing, A.A.E.; visualization, A.A.E. and M.M.H.; supervision, A.A.E.; project administration, M.M.E. All authors have read and agreed to the published version of the manuscript

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تأثير إضافة طحلب الاسبيرولينا في مواجهة التأثيرات السلبية للإجهاد الحراري على عجول الجاموس المصري الرضيعة

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تلعب السياسات التغذاوية دورًا هاماً في التخفيف من التأثيرات السلبية للإجهاد الحراري على الحيوانات المزرعية. لذلك فقد أحريت هذه الدراسة للوقوف على مدى مقدرة طحلب الإسبيرولينا على تحسين أداء النمو وخصائص الدم وحالة الأكسدة والإستجابة المناعية للعجول الجاموس الرضيعة. تم تقسيم ٢٤ عجل جاموس رضيع عشوائياً على أربعة مجموعات تجريبية (٦ حيوانات بكل مجموعة). المجموعة الأولى غير خاضعة لأى معاملات (كنترول) بينما تم إضافة ٥ و ١٠ و ١٥ جرام لكل حيوان يومياً فى المجموعات الثانية والثالثة والرابعة على التوالى. أظهرت النتائج تحسن معنوى في أوازن الجسم فى المجموعات المعاملة ب ١٠ و ١٥ جرام يومياً مقارنة بالمجموعة الكنترول.كما اظهرت النتائج ايضا تحسن معنوي فى المجموعات المعاملة بطحلب الإسبيرولينا فيما يتعلق ببروتين الدم ومشتقاته (الألبيومين والجلوبيولين) وكذلك المواد البروتينية عالية الكثافة وكرات الدم البيضاء والحمراء والخلايا الليمفاوية والصفائح الدموية. بينما لم يكن هناك فروق معنوية بين المجموعات المعاملة والمجموعة الكنترول فيما يتعلق بمستويات انزيمات الكبد واليوريا والكرياتينين والمواد البروتينية منخفضة الكثافة والجلوكوز والبروليولين. كانت مستويات الكوليسترول والجليسريدات الثلاثية أقل فى المجموعة المعاملة ب ١٥ جرام اسبيرولينا مقارنة بالمجموعة الكنترول. كذلك أظهرت المجموعات المعاملة ب ١٠ و ١٥ جرام يومياً تحسن معنوي فى حالة الأكسدة والاستجابة المناعية مقارنة بالمجموعة الكنترول. فى الخلاصة، يمكن لطحلب الإسبيرولينا التخفيف فى التأثيرات السلبية للإجهاد الحراري على العجول الجاموس الرضيعة من خلال تعزيز الحالة المناعية ومضادات الأكسدة.

الكلمات المفتاحية: طحلب الإسبيرولينا ، الجاموس المصري،الإجهاد الحراري ، مضادات الأكسدة ، الإستجابة المناعية.