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Effects of Dietary Intervention Using Spirulina at Graded Levels on Productive Performance and Physiological Status of Quail Birds Reared under Elevated Temperatures

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Abstract: The current study aimed to explore the effect of *Spirulina platensis* (SP) inclusion at various levels in quail diets, in terms of their production performance, physiological traits, stress measurements, and immunological parameters under heat stress (HS) conditions. Four hundred Japanese quail (*Coturnix japonica*) chicks, one day old, were equally distributed into forty wire cages, and the cages were placed in two chambers with environmentally controlled systems (20 cages in each chamber). From 21 to 42 d of age, the quails were randomly subjected to a factorial design of two HS treatments × four SP treatments. To induce HS treatments in the quails, the first chamber was maintained at a thermoneutral temperature of 24 °C (TN group), while the temperature of the second chamber was elevated to 35 °C during the daytime (9:00–17:00 h), followed by a thermoneutral temperature for the remaining 24 h cycle (HS group). The birds in each chamber were further allocated into four SP treatments (5 replicate cages × 10 birds per cage in each treatment), where the quails were fed on a basal diet that included 0, 5, 10, or 15 g/kg SP (SP0, SP5, SP10, and SP15 groups, respectively). After exposure to the HS, a significant ($p < 0.05$) reduction of 5% in body weight and 9% in both weight gain and feed intake was recorded, and the slaughter performance of the quails was adversely ($p < 0.05$) affected. In addition, HS significantly ($p < 0.05$) impaired the physiological traits (total protein, albumin, globulin, alanine transferase, aspartate transferase, creatinine, uric acid, cholesterol, and triglycerides) and immunological parameters (total white blood cells, heterophil to lymphocyte ratio, and T- and B-lymphocyte stimulation indexes), but increased the stress measurements (corticosterone, malondialdehyde, interleukin-1β, and tumor necrosis factor-α). In contrast, most of these parameters were linearly ($p < 0.05$) improved by increasing the SP levels in the diets of the TN quail group. When the SP was included in the diets of the HS quail group, the deleterious effects of HS on the alanine and aspartate transferase activities, creatinine, uric acid, triglycerides, corticosterone, interleukin-1β, and tumor necrosis factor-α levels, heterophil to lymphocyte ratio, and T- and B-lymphocyte stimulation indexes were remarkably ($p < 0.05$) relieved. These results concluded that SP nutritional application can improve the production performance and the overall physiological homeostasis of the Japanese quail, especially when suffering from heat stress.

Keywords: spirulina; heat stress; productive performance; physiological status; stress indicators; immunological parameters; Japanese quail

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1. Introduction

The Japanese quail (*Coturnix japonica*) has become increasingly popular as an experimental animal model in scientific research due to its distinctive characteristics compared to

other poultry species [1]. These quail have a small body size, require low feed and rearing space, attain high egg rates, and produce several generations in a short time [2]. Japanese quails have been recently introduced in welfare studies measuring some physiological biochemical, behavioral, and productive parameters as indirect indices for the concept of bird's welfare [3]. In addition, meat and egg production from Japanese quail provides a considerable amount of featured animal protein and is preferred as an inexpensive food for consumers in some countries [4].

Similar to other poultry species, quails express a disturbance in their internal thermoregulation and physiological mechanisms when exposed to elevated temperatures, especially in some tropical countries [5,6]. Heat stress (HS) adversely affected the productive performance of poultry species, including broilers [7–10], laying hens [11–14], turkeys [15,16], and quails [17–21]. HS also impairs the carcass weight and composition in quail [22,23]. In addition, HS-treated birds express elevation in the pro-inflammatory cytokines [18], inhibition in the antioxidant defense system [17,22], and suppression in immune response [20] Furthermore, it was reported that HS increased lipid peroxidation and corticosterone hormone levels and decreased triiodothyronine hormone and metabolism in Japanese quail [20,21].

Blue-green microalgae, *Spirulina platensis* (SP), have recently gained much interest as a dietary supplement in poultry feeds due to their high nutritional value [24]. It was documented that SP is enriched with proteins, lipids, vitamins, and minerals, and contains considerable amounts of essential amino acids, polyunsaturated fatty acids, and phytopigments [25,26]. In Japanese quail, several studies reported beneficial effects of SP supplementation on their growth and egg laying performance [27–29]. It was also found that diets supplemented with 4% SP had a positive effect on the live body weight, feed intake, feed efficiency, carcass composition, and meat quality of Japanese quails [30]. In addition, SP has other biological properties that support work against inflammation [31], immunosuppression [32,33], and oxidative stress [34]. These characteristics encourage poultry nutritionists to use SP in poultry diets to relieve the deleterious effects of HS. For instance, it was reported that SP powder supplemented into the broiler diet at 0.5–2 g/kg enhanced the growth aspects, physiological status, immune function, and antioxidant activity under HS conditions [35,36]. Moreover, it was demonstrated that containing layer diets with 9% SP alleviated the cholesterol formation, oxidative stress, and inflammation induced by cyclic HS in laying hens [13,37].

In previous research, the harmful effects of HS on Japanese quail were successfully alleviated by using some antioxidants and natural compounds such as vitamins C and E [5,23], chromium [17,18], zinc [19], propolis [20,21], and lycopene [22]. In particular, Hajati et al. [37] found that dietary 0.5% SP supplementation can reduce the elevation of lipid peroxidation, the heterophil to lymphocyte ratio, and other stress indicators in laying quail suffering from HS. However, it is hard to find further studies investigating the protective impact of SP against HS in quail. Thus, the current study aimed to investigate the impact of SP supplementation at various levels into diets of quail chicks on their productive performance, physiological traits, stress measurements, and immunological parameters under HS conditions.

2. Materials and Methods

2.1. Spirulina Analysis

The SP algae were attained as a powder compound from a commercial vendor (Rejuve Biotech Co., Ordos, Inner Mongolia, China) and used freshly by mixing with the experimental diets on the day of feeding. Three samples of the SP were analyzed for the basic nutritional composition using the procedures of the 'Association of Official Analysis Chemists' AOAC [38]. The SP total polyphenolic and flavonoid contents were analyzed following the methods described in a previous study [39]. The radical scavenging activity of the SP was also detected as described in previous protocols [40]. The data derived from SP analysis are shown in Table 1.

Table 1. The chemical analysis, polyphenols, flavonoids, and total antioxidant activity of the *Spirulina platensis* (SP).

Item	Values (% of DM) [1]
Dry matter (DM)	94.4 ± 1.7
Protein	56.4 ± 0.5
Lipids	7.2 ± 0.3
Carbohydrate	14.2 ± 0.7
Fiber	0.02 ± 0.004
Total ash	7.5 ± 0.4
Energy (kcal)	436.2 ± 2.6
Polyphenolic content (GAE) [2]	221.3 ± 7.4
Flavonoid contents (QE) [3]	66.8 ± 3.1
Total antioxidant activity (IC50, µg/mL) [4]	29.2 ± 1.1

[1] Data are mean values analyzed on dry matter basis ± SD of three determinations. [2] Polyphenolic contents were calculated as gallic acid equivalent (GAE). [3] Flavonoid contents were calculated as quercetin equivalent (QE). [4] Total antioxidant activity was presented in terms of IC50 (the sample concentration that achieves 50% inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals).

2.2. Birds and Experimental Design

Four hundred Japanese quail (*Coturnix japonica*) chicks, one day old, were obtained from a commercial farm in Saudi Arabia. The chicks were equally distributed into 40 wire cages measuring 60 cm length × 50 cm width × 50 cm height. The cages were placed in two chambers with environmentally controlled systems (20 cages in each room). Chicks in both chambers were maintained during the first 20 days of age under the same hygienic, environmental and managerial conditions. During this period, the chicks received a starter basal diet, and from d 21 onwards, the chicks received a grower basal diet. The chicks had free access to diets and fresh water. The experiment started on the 21st day of age and continued until the 42nd day. During this period, quails were allotted into a wholly randomized 2 × 4 factorial design with HS and SP treatments, respectively. To induce HS treatments in the quails, the first chamber was maintained at a thermoneutral temperature of 24 °C (TN group), while the temperature of the second chamber was elevated to 35 °C during the daytime (9:00–17:00 h), followed by a thermoneutral temperature for the remaining 24 h cycle (HS group). The birds in each chamber were further allocated into four SP treatments (5 replicate cages × 10 birds per cage in each treatment), where the quails were fed on grower basal diets including 0, 5, 10, or 15 g/kg SP (SP0, SP5, SP10, and SP15 groups, respectively). The diets were formulated according to the nutritional requirements of Japanese quail recommended by NRC [41], and the chemical analysis of the diets was determined by the international methods of the AOAC [38]. The ingredients and nutritional analysis of the diets were presented in Table 2.

Birds were under constant veterinary supervision and examined daily during the application of HS treatment to identify any stress symptoms. If any abnormal indications appeared in the breath, appetite, or general health of the birds, they were submitted to euthanasia to prevent suffering induced by the stress. The current protocol was revised and approved by the research ethical committee of Saudi Arabia's King Faisal University (Ref. No. KFU-REC-2023-FEB-ETHICS606).

2.3. Productive Performance

The average body weight and feed intake for birds in each cage were recorded every week. The body weight gain (BWG, g/bird) was calculated from the difference between the initial body weight (IBW) and the final body weight (FBW), which were recorded during the experimental period on the 21st d and 42nd d of age, respectively. The total feed intake (TFI, g/bird) was also determined during the same period by subtracting the left-over feed from the quantity originally supplied to the birds in each cage. The feed conversion ratio was then calculated by dividing the TFI by the BWG.

Table 2. Ingredients and nutritional analysis of the diets.

Ingredients (g/kg as Fed)	Starter Diet (1–20 d)	Grower Diets (21–42 d)			
		SP0	SP5	SP10	SP15
Spirulina [1]	0	0	5	10	15
Yellow Corn (8.5% CP)	558	595	595	595	595
Soybean meal (44% CP)	324	282	282	282	282
Gluten meal (62% CP)	81	73	73	73	73
Premix [2]	3	3	3	3	3
Di-calcium phosphate	15.8	18.5	18.5	18.5	18.5
Limestone	13.1	23.1	23.1	23.1	23.1
Sodium chloride	3.4	3.5	3.5	3.5	3.5
Methionine	0.5	0.5	0.5	0.5	0.5
Lysin	1.2	1.4	1.4	1.4	1.4
Calculated nutrients (g/kg)					
Crude protein, CP	241.7	221.2	223.0	226.8	229.7
Metabolizable energy, kcal	2915	2926	2948	2969	2991
Calcium	8.0	10.2	10.6	11.0	11.5
Available phosphorus	4.5	5.2	5.3	5.4	5.6
Determined nutrients					
Crude protein	240.2	220.8	223.6	226.5	230.5
Crude fiber	33.8	32.4	32.4	32.4	32.4
Ether extract	27.6	29.4	29.4	29.4	29.4
Lysin	13.0	13.5	15.9	17.3	20.2
Therionine	3.1	2.8	5.5	7.3	9.7
Tryptophan	2.1	1.7	2.2	2.8	3.2
Methionine	5.0	6.2	7.7	8.6	9.8

[1] Spirulina was supplemented to the quail's grower diet at 0, 5, 10, and 15 g/kg (SP0, SP5, SP10, and SP15, respectively). [2] Each 3 Kg of premix contained: Vit. A 10,000,000 I.U., Vit. D_3 2250,000 I.U., Vit. E 10 g, Vit. K_2 1 g, Vit. B_1 1 g, Vit. B_6 1.5 g, Vit. B_{12} 10 mg, Pantothenic acid 10 g, Niacin 20 g, Folic acid 1 g, Biotin 50 mg, Choline chloride 500 g, Iron 30 g, Manganese 40 g, Zinc 45 g, Cupper 3 g, Cobalt 100 mg, Iodin 300 mg, and Selenium 100 mg.

2.4. Slaughter Performance

After experiment completion at 42 d of age, two quails from each cage were weighed alive then slaughtered. After complete bleeding, the birds were heated in 54 °C water for 2 min, followed by plucking of the feathers and removal of the head, viscera, and shanks. The clean carcass was then weighed again to determine the carcass yield as a percentage of live weight (CY%). The weights of the liver, gizzard, heart, intestines, and abdominal fat were recorded and expressed as a percentage of the live body weight.

2.5. Physiological Traits

Two birds from each cage were randomly selected at the end of the experiment (42 d of age) to obtain blood samples into heparinized tubes. The samples were centrifuged at 2000× *g* for 10 min at 4 °C to separate the plasma. The plasma samples were then frozen at −20 °C until further assay. The total protein (TP), albumin (ALB), globulin (GLB), creatinine (CRT), uric acid (UA), cholesterol (CHO), and triglycerides (TG) concentrations, as well as the alanine transferase (ALT) and aspartate transferase (AST) enzyme activities were analyzed in the plasma using a scanning spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK) and commercial colorimetric kits (Spectrum-Diagnostic, Giza, Egypt). More details regarding the protocols of each analysis can be downloaded at: https://dx.doi.org/10.17504/protocols.io.s7yehpw, accessed on 26 March 2023.

2.6. Stress Measurements

Plasma samples were separated from 2 birds randomly selected from each cage at the end of the experimental period (42 d of age) and frozen for further analysis, as mentioned above. The corticosterone (CORT), malondialdehyde (MDA), interleukin-1β (IL-1

β), and tumor necrosis factor-α (TNF-α) levels were determined in the plasma as stress indicators [14]. The chicken-specific ELISA kits protocols (MyBioSource Incorporation, San Diego, CA, USA) were followed to assay the CORT, IL-1β, and TNF-α (Catalog no. MBS701668, MBS761055, and MBS2509660, respectively). The intra and inter CV% assays were <8 and <10% for CORT, <10 and <12% for IL-1 β, and <5.57 and <5.89% for TNF-α, respectively. The sensitivities and detection ranges were <0.5 and 0.5–20 ng/mL, 20 and 30–2000 pg/mL, and 18.75 and 31.25–2000 pg/mL for CORT, IL-1β, and TNF-α, respectively. The plasma MDA level was measured using a colorimetric assay kit (MBS9718963, MyBioSource). Briefly, a mixture of the sample with thiobarbituric acid (TBA) reagent was incubated to raise a color, where the absorbance could be detected by a microplate reader (ELx808TM BioTek Instruments, Winooski, VT, USA).

2.7. Immunological Parameters

At the end of the experimental period (42 d of age), blood samples were collected from 2 birds selected randomly from each cage and placed into heparinized tubes. These samples were used to measure the total white blood cells (TWBC), heterophil to lymphocyte cells ratio (H/L ratio), and the lymphocyte proliferation, as described in a previous study [14]. In brief, 10 µL of the blood sample was vortexed with 490 µL of brilliant cresyl-blue stain. A drop of this solution was pipetted in a hemocytometer slide (Bright-Line™, American Optical, Buffalo, NY, USA) and examined under a microscope at 200× magnification to detect the TWBC [24]. A slide smear of another 10 µL of the blood sample was processed with HEMA-3 stains (Fisher Scientific, Pittsburg, PA, USA). The slide was examined under a microscope at 1000× magnification with an oil immersion lens to differentiate the leukocyte cells into heterophils, eosinophils, basophils, monocytes, and lymphocytes. The H/L ratio was then determined in a total of 200 differentiated leukocytes per sample. The remaining part of the blood sample was overlayed on a similar volume of separation medium (Histopaque-1077, Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at 1030× *g* for 20 min at 4 °C. The peripheral blood mononuclear cells (PBMC), which were isolated as a layer on the histopaque interface, were carefully aspirated, washed twice, and then resuspended in 1 mL of RPMI-1640 (Invitrogen Corp., Grand Island, NY, USA). After that, the Trypan Blue Dye was used to detect and re-adjust the viable lymphocyte concentration in the samples to 1×10^7 cells/mL. To stimulate T- or B-lymphocyte proliferation, the viable lymphocytes were placed in triplicates in 96-well plates, and then 50 µL of either 5% Concanavalin-A mitogen or 1% Lipopolysaccharide was supplemented to the wells, respectively. In contrast, control wells were supplemented only with 50 µL RPMI-1640. All wells were incubated with 15 µL of MTT solution (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide). One hundred µL of sodium dodecyl sulfates (10% in HCl 0.04 M) was added to the wells to raise a color, which can be scanned using an automated ELISA (Bio-Rad Laboratories Inc., Hercules, CA, USA). Finally, stimulating indexes for T- and B-lymphocytes (TSI and BSI, respectively) were identified by reading the optical density at 570 nm (OD570) and calculating the OD570 ratio for stimulated to unstimulated cells in each sample.

2.8. Statistical Analysis

The experimental unit was the cage for the data of productive performance (n = 5 replicate cages per treatment group), while the bird represents the experimental unit for the remaining parameters (n = 10 replicate birds per treatment group). The data were set in a 2 × 4 factorial design and subjected to two-way analysis of variance (ANOVA) to clear the main effects of HS, SP, and HS × SP interaction. A polynomial contrast test was added to the analysis to explore linear and quadratic trends for the increasing SP levels. Data were also analyzed as one-way ANOVA to obtain the differences among all 8 groups (2 HS treatments with 4 SP treatments). The significant differences among the treatment means were separated by Duncan's post hoc test at a p-value less than 0.05. The statistical analysis

was computed using the multivariate procedure of the IBM-SPSS software program (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Productive Performance

The results of quails' productive performance as affected by HS, SP, and HS × SP are presented in Table 3. There was a significant ($p < 0.05$) decrease in the FBW, BWG, and TFI by 5%, 9%, and 9%, respectively, due to the HS treatment. The dietary SP treatment significantly ($p < 0.05$) improved the FBW, BWG, TFI, and FCR of the quails in a linear response to the increased SP level in the diet. No interaction effect was obtained for the HS × SP on the production performance of the quails ($p > 0.05$).

Table 3. Heat stress, spirulina, and their interaction effects on the production performance of Japanese quail chicks.

Treatment Groups [1]	*n*	IBW, g	FBW, g	BWG, g	TFI, g	FCR
Heat stress						
TN	20	118.9	263.6 [a]	144.6 [a]	498.1 [a]	3.49
HS	20	119.2	250.7 [b]	131.5 [b]	452.3 [b]	3.48
SEM		0.44	0.94	1.12	1.32	0.032
p-value		0.667	<0.001	<0.001	<0.001	0.839
Spirulina						
SP0	10	119.1	229.6 [d]	110.6 [d]	420.6 [d]	3.81 [a]
SP5	10	119.5	247.7 [c]	128.2 [c]	460.4 [c]	3.60 [b]
SP10	10	119.2	266.3 [b]	147.1 [b]	490.7 [b]	3.34 [c]
SP15	10	118.5	284.8 [a]	166.3 [a]	529.1 [a]	3.18 [d]
SEM		0.62	1.33	1.59	1.86	0.046
p-value		0.731	<0.001	<0.001	<0.001	<0.001
SP—Linear contrast		0.482	<0.001	<0.001	<0.001	<0.001
SP—Quadratic contrast		0.392	0.873	0.638	0.712	0.585
Interaction						
TN × SP0	5	119.8	235.6	115.8	439.8	3.80
TN × SP5	5	119.8	253.6	133.8	485.6	3.65
TN × SP10	5	118.9	274.8	155.9	512.7	3.29
TN × SP15	5	117.2	290.2	173.0	554.3	3.21
HS × SP0	5	118.4	223.6	105.3	401.3	3.81
HS × SP5	5	119.2	241.9	122.7	435.1	3.55
HS × SP10	5	119.4	257.8	138.4	468.7	3.39
HS × SP15	5	119.8	279.5	159.6	503.9	3.16
SEM		0.88	1.88	2.25	2.63	0.065
p-value		0.137	0.344	0.406	0.086	0.497

Means within the main effect in the column with different superscripts (a–d) significantly differ at $p < 0.05$. [1] Treatment groups: heat stress—the quail chicks were maintained in a thermo-neutral chamber at 24 °C (TN) or heat stress chamber at 35 °C (HS); Spirulina—the quail chicks were fed a soybean–corn diet supplemented with 0, 5, 10, and 15 g *Spirulina platensis* per kg diet (SP0, SP5, SP10, and SP15, respectively). *n*: number of replicates per treatment group. SEM: pooled standard error of the mean. IBW: initial body weight at 21 d of age. FBW: final body weight at 42 d of age. BWG body weight gain through 21–42 d of age. TFI: total feed intake per bird through 21–42 d of age. FCR: feed conversion ratio (TFI/BWG).

3.2. Slaughter Performance

The slaughter performances of Japanese quail affected by HS, SP, and HS × SP are presented in Table 4. The HS treatment significantly ($p < 0.05$) decreased the LBW, CY, and negatively affected the other slaughter parameters. In contrast, the SP treatment linearly ($p < 0.05$) increased the LBW, CY, liver, gizzard, heart, intestines, and ABF as the dietary SP level increased in quail diets. Moreover, it was observed that SP supplementation at increasing levels into quail diets significantly ($p < 0.05$) ameliorated the reduction induced by HS in the heart and ABF percent.

Table 4. Heat stress, spirulina, and their interaction effects on the slaughter performance of Japanese quail chicks.

Treatment Groups [1]	*n*	LBW, g	CY, %	Liver, %	Gizzard, %	Heart, %	Intestines, %	ABF, %
Heat stress								
TN	40	247.8 ^{a}	71.49 ^{a}	3.86 ^{a}	2.29 ^{a}	0.93 ^{a}	11.40 ^{a}	1.04 ^{a}
HS	40	224.3 ^{b}	66.99 ^{b}	3.62 ^{b}	2.14 ^{b}	0.79 ^{b}	9.63 ^{b}	0.68 ^{b}
SEM		2.66	0.410	0.022	0.030	0.010	0.170	0.012
p-value		<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Spirulina								
SP0	20	199.0 ^{d}	66.85 ^{c}	2.67 ^{d}	1.95 ^{d}	0.76 ^{c}	9.78 ^{b}	0.76 ^{c}
SP5	20	226.3 ^{c}	68.65 ^{b}	3.43 ^{c}	2.11 ^{c}	0.82 ^{b}	10.58 ^{a}	0.81 ^{b}
SP10	20	242.0 ^{b}	69.85 ^{b}	4.19 ^{b}	2.32 ^{b}	0.85 ^{b}	10.70 ^{a}	0.86 ^{b}
SP15	20	277.0 ^{a}	71.60 ^{a}	4.66 ^{a}	2.46 ^{a}	0.99 ^{a}	11.02 ^{a}	1.00 ^{a}
SEM		3.76	0.580	0.031	0.042	0.014	0.240	0.018
p-value		<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
SP—Linear contrast		<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
SP—Quadratic contrast		0.307	0.966	<0.001	0.803	0.003	0.317	0.013
Interaction								
TN × SP0	10	214.7	69.65	2.79	2.04	0.81 ^{d}	10.37	0.970 ^{b}
TN × SP5	10	234.0	70.25	3.52	2.15	0.88 ^{b}	11.62	1.021 ^{b}
TN × SP10	10	253.6	71.85	4.31	2.38	0.89 ^{b}	11.61	1.037 ^{b}
TN × SP15	10	289.0	74.20	4.82	2.58	1.12 ^{a}	12.02	1.110 ^{a}
HS × SP0	10	183.3	64.05	2.56	1.86	0.72 ^{e}	9.18	0.546 ^{e}
HS × SP5	10	218.6	67.05	3.35	2.07	0.75 ^{e}	9.54	0.608 ^{de}
HS × SP10	10	230.3	67.85	4.07	2.27	0.82 ^{cd}	9.78	0.676 ^{d}
HS × SP15	10	265.0	69.00	4.49	2.35	0.87 ^{bc}	10.02	0.896 ^{c}
SEM		5.32	0.820	0.044	0.059	0.020	0.339	0.025
p-value		0.523	0.445	0.260	0.611	<0.001	0.550	<0.001

Means within the main effect in the column with different superscripts (a–e) significantly differ at $p < 0.05$. [1] Treatment groups: heat stress—the quail chicks were maintained in a thermo-neutral chamber at 24 °C (TN) or heat stress chamber at 35 °C (HS); Spirulina—the quail chicks were fed soybean–corn diet supplemented with 0, 5, 10, and 15 g *Spirulina platensis* per kg diet (SP0, SP5, SP10, and SP15, respectively). *n*: number of replicates per treatment group. SEM: pooled standard error of the mean. LBW: live body weight; CY: carcass yield; ABF: abdominal fats. All percentages were calculated based on live weight.

3.3. Physiological Traits

The effects of HS, SP, and HS × SP on the physiological traits of quail are summarized in Table 5. The results showed that HS treatment induced a significant ($p < 0.05$) decrease in the plasma TP, ALB, and GLB, and a significant increase in the ALT, AST, CRT, UA, CHO, and TG. On the contrary, dietary SP supplementation at increasing levels into quail diets induced a linear ($p < 0.05$) increase in the TP, ALB, and GLB levels, and a linear ($p < 0.05$) decrease in the ALT, AST, CRT, UA, CHO, and TG levels in the plasma. Under the HS condition, it was found that SP treatment significantly ($p < 0.05$) reduced the elevation in the ALT, AST, CRT, UA, and TG. In contrast, no interaction effect was found for the HS × SP on the TP, ALB, GLB, and CHO levels.

3.4. Stress Measurements

The effects of HS, SP, and HS × SP on the stress measurements of quail are presented in Table 6. HS significantly ($p < 0.05$) increased all stress indicators in the quails, including the plasma CORT, MDA, IL-β1, and TNF-α concentrations. On the contrary, these parameters linearly ($p < 0.05$) decreased as the dietary SP supplementation into the quail diet increased. Further, there was a significant ($p < 0.05$) interaction effect for HS × SP treatments on the CORT, MDA, and IL-β1, while no interaction effect on the TNF-α concentration was observed.

Table 5. Heat stress, spirulina, and their interaction effects on the physiological traits of Japanese quail chicks.

Treatment Groups [1]	*n*	TP, g/dL	ALB, g/dL	GLB, g/dL	ALT, U/mL	AST, U/mL	CRT, mg/dL	UA, mg/dL	CH, mg/dL	TG, mg/dL
Heat stress										
TN	40	5.41 ^{a}	2.71 ^{a}	2.71 ^{a}	20.41 ^{b}	26.92 ^{b}	0.53 ^{b}	2.40 ^{b}	150.35 ^{b}	197.04 ^{b}
HS	40	4.54 ^{b}	2.29 ^{b}	2.25 ^{b}	25.40 ^{a}	34.35 ^{a}	0.68 ^{a}	3.04 ^{a}	193.20 ^{a}	260.63 ^{a}
SEM		0.029	0.054	0.060	0.154	0.418	0.005	0.021	1.240	1.001
p-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Spirulina										
SP0	20	4.15 ^{d}	2.39 ^{bc}	1.78 ^{c}	28.70 ^{a}	35.11 ^{a}	0.81 ^{a}	3.65 ^{a}	194.08 ^{a}	254.61 ^{a}
SP5	20	4.86 ^{c}	2.24 ^{c}	2.62 ^{b}	23.80 ^{b}	34.82 ^{a}	0.67 ^{b}	3.03 ^{b}	180.24 ^{b}	239.02 ^{b}
SP10	20	5.06 ^{b}	2.51 ^{b}	2.55 ^{b}	21.57 ^{c}	26.86 ^{b}	0.54 ^{c}	2.44 ^{c}	165.14 ^{c}	221.91 ^{c}
SP15	20	5.83 ^{a}	2.86 ^{a}	2.97 ^{a}	17.55 ^{d}	25.76 ^{b}	0.39 ^{d}	1.77 ^{d}	147.64 ^{d}	199.80 ^{d}
SEM		0.041	0.077	0.085	0.218	0.591	0.007	0.029	1.754	1.416
p-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear contrast		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic contrast		0.406	0.002	0.016	0.050	0.499	0.447	0.447	0.300	0.024
Interaction										
TN × SP0	10	4.54	2.70	1.88	25.50 ^{c}	30.12 ^{d}	0.72 ^{c}	3.24 ^{c}	173.92	230.89 ^{e}
TN × SP5	10	5.30	2.34	2.96	20.60 ^{e}	32.65 ^{c}	0.57 ^{d}	2.59 ^{d}	160.48	210.83 ^{f}
TN × SP10	10	5.47	2.61	2.86	19.81 ^{ef}	22.65 ^{e}	0.49 ^{e}	2.20 ^{e}	144.43	186.66 ^{g}
TN × SP15	10	6.33	3.18	3.15	15.71 ^{g}	22.25 ^{e}	0.35 ^{g}	1.58 ^{g}	122.57	159.78 ^{h}
HS × SP0	10	3.76	2.09	1.67	31.89 ^{a}	40.10 ^{a}	0.90 ^{a}	4.05 ^{a}	214.24	278.33 ^{a}
HS × SP5	10	4.42	2.13	2.29	27.01 ^{b}	36.98 ^{b}	0.77 ^{b}	3.47 ^{b}	199.99	267.20 ^{b}
HS × SP10	10	4.64	2.40	2.24	23.33 ^{d}	31.07 ^{cd}	0.59 ^{d}	2.67 ^{d}	185.86	257.17 ^{c}
HS × SP15	10	5.33	2.53	2.79	19.38 ^{f}	29.27 ^{d}	0.44 ^{f}	1.97 ^{f}	172.72	239.82 ^{d}
SEM		0.058	0.109	0.121	0.309	0.836	0.009	0.042	2.480	2.002
p-value		0.259	0.070	0.184	<0.001	0.009	<0.001	<0.001	0.126	<0.001

Means within the main effect in the column with different superscripts (a–h) significantly differ at $p < 0.05$. [1] Treatment groups: heat stress—the quail chicks were maintained in a thermo-neutral chamber at 24 °C (TN) or heat stress chamber at 35 °C (HS); Spirulina—the quail chicks were fed soybean–corn diet supplemented with 0, 5, 10, and 15 g *Spirulina platensis* per kg diet (SP0, SP5, SP10, and SP15, respectively). *n*: number of replicates per treatment group. SEM: pooled standard error of the mean. TP: total protein; ALB: albumin; GLB: globulin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRT: creatinine; UA: uric acid; CH: cholesterol; TG: triglycerides.

Table 6. Heat stress, spirulina, and their interaction effects on the stress measurements of Japanese quail chicks.

Treatment Groups [1]	*n*	CORT, ng/mL	MDA, nmol/mL	IL-β1, pg/mL	TNF-α, pg/mL
Heat stress					
TN	40	2.63 ^{b}	1.79 ^{b}	251.27 ^{b}	89.53 ^{b}
HS	40	5.99 ^{a}	4.28 ^{a}	776.98 ^{a}	140.80 ^{a}
SEM		0.063	0.058	14.162	2.198
p-value		<0.001	<0.001	<0.001	<0.001
Spirulina					
SP0	20	4.88 ^{a}	3.24 ^{a}	613.12 ^{a}	123.90 ^{a}
SP5	20	4.41 ^{b}	3.14 ^{a}	544.00 ^{b}	119.60 ^{a}
SP10	20	4.12 ^{c}	3.02 ^{a}	488.87 ^{b}	109.55 ^{b}
SP15	20	3.85 ^{d}	2.74 ^{b}	410.53 ^{c}	107.60 ^{b}
SEM		0.089	0.082	20.028	3.108
p-value		<0.001	<0.001	<0.001	0.001
SP—Linear contrast		<0.001	<0.001	<0.001	<0.001
SP—Quadratic contrast		0.255	0.297	0.819	0.707
Interaction					
TN × SP0	10	2.74 ^{e}	1.91 ^{c}	261.85 ^{e}	95.40
TN × SP5	10	2.60 ^{e}	1.85 ^{c}	261.21 ^{e}	92.20

Table 6. *Cont.*

Treatment Groups [1]	*n*	CORT, ng/mL	MDA, nmol/mL	IL-β1, pg/mL	TNF-α, pg/mL
TN × SP10	10	2.62 ^{e}	1.70 ^{c}	243.77 ^{e}	84.80
TN × SP15	10	2.57 ^{e}	1.71 ^{c}	238.27 ^{e}	85.70
HS × SP0	10	7.01 ^{a}	4.58 ^{a}	964.38 ^{a}	152.40
HS × SP5	10	6.21 ^{b}	4.44 ^{a}	826.78 ^{b}	147.00
HS × SP10	10	5.61 ^{c}	4.34 ^{a}	733.98 ^{c}	134.30
HS × SP15	10	5.13 ^{d}	3.77 ^{b}	582.79 ^{d}	129.50
SEM		0.126	0.116	28.324	4.396
p-value		<0.001	0.031	<0.001	0.446

Means within the main effect in the column with different superscripts (a–e) significantly differ at $p < 0.05$. [1] Treatment groups: heat stress—the quail chicks were maintained in a thermo-neutral chamber at 24 °C (TN) or heat stress chamber at 35 °C (HS); Spirulina—the quail chicks were fed soybean–corn diet supplemented with 0, 5, 10, and 15 g *Spirulina platensis* per kg diet (SP0, SP5, SP10, and SP15, respectively). *n*: number of replicates per treatment group. SEM: pooled standard error of the mean. CORT: corticosterone; MDA: malondialdehyde; IL-β1: interleukin-beta 1; TNF-α: tumor necrosis factor-alpha.

3.5. Immunological Parameters

The effects of HS, SP, and HS × SP on the immunological parameters of quail are shown in Table 7. The results showed that HS treatment induced a significant ($p < 0.05$) decrease in the TWBC, TSI, and BSI. In contrast, HS treatment significantly increased the H/L ratio. As the dietary SP supplementation level increased, the TWBC, TSI, and BSI linearly and quadratically increased ($p < 0.05$), while the H/L ratio significantly decreased ($p < 0.05$). In HS-birds, the H/L ratio elevation and the TSI and BSI reduction were significantly ($p < 0.05$) ameliorated by the SP treatment. In contrast, no interaction effect was observed in the TWBC.

Table 7. Heat stress, spirulina, and their interaction effects on the immunological parameters of Japanese quail chicks.

Treatment Groups [1]	*n*	TWBC, 10^3/mL	H/L Ratio	TSI	BSI
Heat stress					
TN	40	106.15 ^{a}	0.37 ^{b}	5.14 ^{a}	3.27 ^{a}
HS	40	85.38 ^{b}	0.73 ^{a}	2.21 ^{b}	1.37 ^{b}
SEM		0.962	0.004	0.037	0.036
p-value		<0.001	<0.001	<0.001	<0.001
Spirulina					
SP0	20	65.60 ^{d}	0.66 ^{a}	2.69 ^{d}	1.43 ^{d}
SP5	20	76.40 ^{c}	0.59 ^{b}	2.98 ^{c}	1.67 ^{c}
SP10	20	111.90 ^{b}	0.54 ^{c}	4.14 ^{b}	2.86 ^{b}
SP15	20	129.15 ^{a}	0.42 ^{d}	4.91 ^{a}	3.32 ^{a}
SEM		1.360	0.005	0.053	0.051
p-value		<0.001	<0.001	<0.001	<0.001
SP—Linear contrast		<0.001	<0.001	<0.001	<0.001
SP—Quadratic contrast		0.020	<0.001	<0.001	0.031
Interaction					
TN × SP0	10	75.80	0.46 ^{e}	3.94 ^{d}	2.18 ^{d}
TN × SP5	10	87.00	0.39 ^{f}	4.50 ^{c}	2.58 ^{c}
TN × SP10	10	122.00	0.38 ^{f}	5.57 ^{b}	3.82 ^{b}
TN × SP15	10	139.80	0.27 ^{g}	6.56 ^{a}	4.48 ^{a}
HS × SP0	10	55.40	0.87 ^{a}	1.43 ^{g}	0.67 ^{f}
HS × SP5	10	65.80	0.79 ^{b}	1.46 ^{g}	0.75 ^{f}
HS × SP10	10	101.80	0.69 ^{c}	2.70 ^{f}	1.89 ^{e}
HS × SP15	10	118.50	0.56 ^{d}	3.26 ^{e}	2.16 ^{d}

Table 7. *Cont.*

Treatment Groups [1]	*n*	TWBC, 10^3/mL	H/L Ratio	TSI	BSI
SEM		1.923	0.007	0.074	0.072
p-value		0.989	<0.001	<0.001	<0.001

Means within the main effect in the column with different superscripts (a–g) significantly differ at $p < 0.05$. [1] Treatment groups: heat stress—the quail chicks were maintained in a thermo-neutral chamber at 24 °C (TN) or heat stress chamber at 35 °C (HS); Spirulina—the quail chicks were fed soybean–corn diet supplemented with 0, 5, 10, and 15 g *Spirulina platensis* per kg diet (SP0, SP5, SP10, and SP15, respectively). *N*: number of replicates per treatment group. SEM: pooled standard error of the mean. TWBC: total white blood cells; H/L ratio: heterophil/lymphocyte ratio; TSI: T-lymphocyte stimulation index; BSI: B-lymphocytes stimulation index.

4. Discussion

Heat stress induces a substantial drop in poultry products, which comprise an essential source of animal protein for millions of people [42]. It was documented that exposure to HS severely deteriorates the well-being and the productive performance of poultry species [43]. Our results displayed that the FI and growth aspects (FBW and BWG) of the quails exposed to HS were significantly reduced. Such HS deleterious impacts on the production aspects of quail birds were also evidenced in other previous studies [20,21,23,37,44,45]. The disruption of feed consumption by HS mainly impairs growth and body composition due to the subsequent reduction in intestinal function, energy production, digestibility, and metabolism [46]. In addition, a low slaughter performance was recorded for quails exposed to HS, as also reported in a previous study [22]. The same drop has also been induced by heat stress to broiler production [8,47,48]. Therefore, HS is one of the significant factors that cause a considerable economic loss for the meat production sector in the poultry industry [49].

Recently, SP has been potentially included in poultry diets to enhance their performance under HS conditions [13,35,50,51]. The results showed a linear increase in all production and slaughter performance traits of quail due to the SP inclusion at increasing levels from 5 to 15 g/kg of the diet. It was reported that adding 5 g/kg Spirulina to quail diets could be applied as a probiotic to maximize the production of quail at thermoneutral [29] or HS [37] conditions. The effective production of quail in the SP treatment group seems to be associated with the high nutritional value of SP-supplemented diets [52]. As presented in Table 2, the diets supplemented with 15 g/kg SP caused an elevation in the protein and metabolizable energy by 4% and 2%, respectively, in addition to an increase of the amino acids by 3.5-fold threonine, 1.9-fold tryptophan, 1.6-fold methionine, and 1.5-fold lysine, in comparison with diets lacking SP supplementation. Moreover, SP may improve the quails' growth and feed efficiency by enhancing the intestinal acidosis and population of beneficial bacteria [53,54], as well as increasing the intestinal weight, villi length, and goblet cells [36,55].

Indeed, other physiological events were noted in the HS quail in the present study, and these physiological mechanisms may contribute to the low performance under HS conditions [49]. The plasma TP, ALB, and GLB were remarkedly reduced under HS conditions, while they increased linearly as a result of dietary SP supplementation (Table 5). It was previously reported that SP increased the plasma proteins in chickens [24], which was attributed to the abundant amount of protein with essential amino acids in the SP [56]. However, no interaction effect for HS × SP on the plasma proteins was observed in the present study. The elevated levels of ALT, AST, CRT, and UA in the blood usually denote a deterioration incidence in the liver, kidney, and muscle tissues which may be caused by excessive stress [57]. The linear decrease in these parameters as a response to the increased SP level supplementation could assume a protective effect for SP on the hepatic and muscle cells [58], as well as the renal function [59], especially in the HS quail birds. The hepatoprotective impacts of SP may allow the release of albumin from the liver to blood circulation [41], as previously evidenced in our results. Furthermore, HS increased the CHO and TG levels in the quail. It was reported that HS provokes the biosynthetic

pathway of cholesterol formation [60] and raises the levels of CHO and TG in the liver and bloodstream [61,62]. In contrast, SP supplementation to quail diets linearly decreased the CHO and TG levels. Moreover, adding SP at the same time as HS lowered the TG levels in the quails. These results agree with the hypocholesterolemic effect of SP demonstrated in broiler [36,63] and layer [13] chickens exposed to HS. Deng and Chow [64] attributed the SP hypolipidemic activity to the existence of γ-linoleic acid, which participates in CH catabolism and TG breakdown [65].

Under heat stress conditions, some physiological events are closely correlated with stress, such as oxidative stress and inflammation, which mainly occur in poultry species [6,66]. In the current study, the plasma CORT, MDA, IL-β1, and TNF-α levels were measured as stress indicators in the quail. The elevation in the CORT concentration is a frontal result of the hypothalamic–pituitary–adrenal (HPA) axis activation by HS in birds [10]. MDA is a measurement product of lipid peroxidation in the tissues, and its levels in circulation increase due to the destructive effect of reactive oxygen species (ROS) produced under HS conditions [67]. Consistent with the results of previous research [17,20,21], exposure of Japanese quail to HS induced a serious elevation in the CORT and MDA levels in the present study. CORT may also activate the inflammation pathways in birds [68]; this may be why the IL-β1 and TNF-α elevation occurred in the HS quail. In contrast, the increasing levels of SP supplementation in the current study displayed a linear reduction in the stress measurements in the quail, and presented an interaction effect on the CORT, MDA, and IL-β1 levels in the HS quails. In agreement with our results, Hajati et al. [37] reported that SP supplementation at the level of 5 g/kg reduced the MDA levels in HS-laying quails. The positive impact of SP and its capacity to counteract the oxidative stress in the HS quail could be explained by the antioxidant activity of polyphenolic and flavonoid compounds, which occurred in high quantities within the analyzed SP (Table 1). It was reported that SP contains some vitamins such as α-tocopherol and ascorbic acid, minerals such as selenium, and phytopigments such as β-carotene, which antagonize the metabolic reaction of ROS and free radicals [69,70]. Furthermore, the ameliorative action of SP for raising IL-β1 and TNF-α in the HS quail, evidenced in the current study, was previously indicated in another study on HS-laying hens [13]. Baxter et al. [71] reported that SP algae include considerable amounts of zinc, manganese, iron, magnesium, potassium, and calcium, which are pivotal minerals when it comes to reducing the stress and the release of pro-inflammatory cytokines in the blood. In a recent study, it was reported that environmental welfare enrichment of Japanese quail displayed a significant reduction in certain physiological stress indicators such as low CORT, AST, and ALT levels, as well as low H/L ratio [3]. Such indices could explain the positive effects of SP on the HS quails in the present study.

In line with previous studies [20,21], our results indicate that HS deteriorated some immunological parameters of Japanese quail. As shown in Table 7, HS reduced 20% in the TWBC and >50% in the lymphocyte stimulation indexes and, in contrast, doubled the H/L ratio. This could occur as a response to the increased CORT level in the HS-birds [72]. The reduction in TWBC resulting from heat stress may be due to the decrease in lymphocytes and monocytes contents in the blood [73]. It may also be explained by the complete destruction of leukocyte cells in heat-stressed birds and the absorption of such broken cells by the bone marrow [74]. The reduction in T- and B-lymphocyte proliferation in HS quails could be attributed to the insufficient macro-energy sources and/or micro-nutrients consumed by the lymphoid tissues under the HS condition. Another reason for the deterioration of such immunological parameters was explained previously by Kamel et al. [8], who reported a critical depression of the leukocyte protein synthesis pathway in birds exposed to HS. On the contrary, SP is well characterized by immune function activation [75–77]. When the quails were fed on diets complemented with increased levels of SP, the immunological parameters were linearly improved in both TN and HS birds. In a particular study by Hajati et al. [37], it was found that 5 g/kg SP supplementation in the diets of laying quails significantly decreased the levels of heterophils, increased the levels of lymphocytes, and consequently reduced the H/L ratio under HS conditions. This

could be ascribed to the high quantity and quality of protein in the SP [78]. As indicated in Table 2, increasing SP levels in quail diets enriched the diet's contents of essential amino acids, maintaining the integrity and function of immune cells [79,80].

5. Conclusions

Exposure of Japanese quail to cyclic HS impaired the live body weight, feed efficiency, slaughter performance, physiological status, immunological parameters, and stress indicators. In contrast, SP supplementation at increasing levels within 5–15 g/kg in quail diets resulted in a linear improvement in their productive performance, slaughter yield, physiological status, and immunological parameters. Furthermore, the positive impact of SP was explored in the HS quails, and the deleterious effects of HS were remarkably alleviated when SP was included in their diets. These results concluded that SP could be used as a potential supplement in quail diets to improve their production aspects and overall physiological homeostasis, especially in birds suffering from heat stress.

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