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The protective role of algae (Spirulina platensis) and garlic (Allium Sativum), and their combination on alleviation of heat stress in broiler chicken

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Abstract. the study aimed to determine the effects of dietary Spirulina platensis (SP) and/or garlic powder (GP) addition on broiler growth performance, carcass characteristics and some blood parameters. Six-hundred, Ross-308 one-day old broiler chicks were enlisted. Heat stresses were created starting from day 22 to 35 of age, and birds were randomly divided into five treatment groups with 6 replicates × 20 birds per each, where the first one was provided with the basal diet and reared under normal thermal conditions (26 ± 1˚C) to serve as a negative control (NC). Meanwhile, the other four groups were exposed to cyclic heat stress (35 ± 1˚C for 9 h per day) and were fed a basal diet with no supplementation to serve as a positive control (PC), PC diet supplemented with 1 gm SP, 200 mg GP and 1g SP + 200 mg GP respectively. The findings demonstrated that supplementing broilers' diets under heat stress with SP and/or garlic powder significantly ($P \leq 0.05$) reduced the effects of heat stress on growth performance. Supplementing with 1g of SP and / or 200 mg of garlic powder increased the proportion of carcass dressing. It can be concluded that feeding broiler chickens raised under heat stress diets supplemented with 1 g of SP or 200 mg of garlic powder significantly ($P \leq 0.05$) improved their performance and carcass traits. The best results were seen in the chicks fed SP with GP.
Keywords: heat stress, broiler chicks, spirulina, garlic, growth performance, carcass.

1. Intoduction

In In tropical climates, heat stress is a significant factor in performance decline and even poultry fatalities. The most typical symptoms of high ambient temperatures include reduction in feed intake, live weight gain, and feed efficiency (Siegel, 1995). In poultry, environmental stress results in bodily oxidative stress, which in turn causes an imbalance in the body's antioxidant level (Sahin et al., 2001). Additionally, stress from the environment causes a decline in the nutritional value and usefulness of muscle proteins (Wang et al., 2009). According to Feng et al. (2006), daily high ambient temperatures cause the pectoral muscle to experience oxidative stress, denatured muscle protein, decreased pH, increased drip loss, lightness, and shear force.

Lipid oxidation is a major factor in the decline of muscle quality and has a direct impact on the safety, nutritious value, flavour, colour, and texture of meat (Buckley et al., 1995). The pH gradually decreases from 7.4 in living muscle to about 5.6 - 5.7 in 6 - 8 hours post-mortem, and then reaches its final pH at 24 hours of about 5.3 - 5.7. (Briskey and Wismer-Pedersen, 1961). Numerous studies have shown how heat stress affects chicken skeletal muscle oxidative damage and increased generation of reactive oxygen species (ROS) (Mujahid et al., 2009). In a study, broiler chickens exposed to prolonged heat stress (34ºC) had higher levels of malondialdehyde (MDA) in their skeletal muscles when they were four weeks old (Azad et al., 2010). Furthermore, ongoing exposure to heat stress inhibits the innate immune response and results in immunological diseases by changing the immune functioning of the organs and the amounts of circulating antibodies (Abo Ghanima et al., 2020). Apoptosis increased intestinal barrier permeability, and the subsequent translocation of hazardous chemicals into the body are all results of oxidative stress that is also brought on by heat stress (Hirakawa et al., 2020). As a result, it's critical to put mitigation techniques in place for environmental stressors and climate change that can lessen their negative impacts on broiler chicken growth performance, immunological functions, and antioxidant capacity, particularly in situations of heat stress.

Spirulina platensis (SP), a filamentous cyanobacterium, is a microalgae with a number of therapeutic uses, health advantages, and other biological functions. Because to SP has great nutritional value, notably its high quantity of proteins, vital amino acids, and fatty acids, it is thought to have medical and pharmacological effects (Hassan et al., 2021). In addition to spirulina's nutritional importance, various medical benefits and therapeutic potential have also been observed (Mendiola et al., 2007). A variety of bioactive substances, such as phycocyanin, polysaccharides, carotenoids, chlorophyll, phenolic compounds, and desirable vitamins and minerals, are present in spirulina (Farag et al., 2016). Spirulina has been shown to have immunostimulatory, hepatoprotective, antiinflammatory, antimicrobial, antiviral, and antioxidative properties that increase disease resistance, stimulate the production of antibodies and cytokines, effectively scavenge free radicals, and inhibit lipid peroxidation, leading to increased poultry production and high profitability (Aladaileh et al., 2020).

Since it can be grown all year round, garlic (*Allium sativum*), the most significant species of the onion genus Allium, belongs to the family Alliaceae. It is the second mosteaten spice in the world, and its popularity has been boosted by the growing understanding of its health advantages (FAO, 1992). Numerous advantageous bioactive compounds can be found in garlic (*Allium sativum*). According to a study by Ziarlarimi et al. (2011), garlic powder (GP) contains a variety of organosulfur compounds, including allicin, alliin, ajoene, diallylsulfide, dithiin, and S-allylcysteine, making it a successful natural feed additive for broilers. Additionally, garlic has long been used as a growth promoter or feed additive (Teshika et al., 2019), and it has also been discovered to be a medicinal plant for the prevention and treatment of numerous heart, intestinal, and metabolic diseases, including atherosclerosis, thrombosis, dementia, cancer, and diabetes (Kim et al., 2009). Garlic has been shown in several studies to have a variety of positive health effects, including being antibacterial, antioxidant, and antithrombotic in broilers (Ur Rahman et al., 2017). Even while SP or GP have been shown to have positive benefits on host physiological systems, there is very little information available on feeding broiler chickens SP, GP, or their combination, especially when they are under heat stress. This study's amied to investigate the positive effects of nutritional supplementation with SP, GP, and their combinations on heat-stressed broiler chickens' growth performance, meat quality and blood biochemistry.

MATERIAL AND METHODS:

The experiment was conducted on 600 unsexed day-old healthy Ross-308 broiler chicks under standard management conditions in an experimental broiler house. The birds were individually weighed and randomly assigned to five groups. Each group consisted of six replicates (20 birds / replicate). The total duration of the experimental trial was 35 days. The temperature of 35 ± 1°C and relative humidity (RH) of 65% was maintained by day 1 of the experiment. The temperature decreased gradually by 3°C per week to reach 26 °C at day21 of the investigation. From day 22 onwards, the temperature of 26 °C and RH of 65 ± 5 % were maintained for the negative control group. While for the remaining heat-stressed groups, the temperature and RH were kept at 35 ± 1°C and 75 ± 5%, respectively, from 9:00 to 18:00 (9 hours daily) followed by 26 °C and 65 ± 5% RH for 15 hours during the remaining experimental period. The birds were fed a corn-soybean-based diet. The basal diet was formulated using NRC (1994) guidelines, and its composition is presented in

Table 1. Two phases of feed i.e., starter-grower (1 - 21 days), and finisher (22 - 35 days) were adopted. Mash feed and water were provided *ad libitum*.

The experimental groups were arranged as follows: (1) a negative control (chickens were reared under normal thermal conditions (26 ± 1◦C) from 1 to 35days) and fed the basal diet with no supplementation. Meanwhile, the other four groups were exposed to cyclic heat stress (35 ± 1◦C for 9 h per day) and were fed a basal diet with no supplementation to serve as a positive control, (2) a positive control diet with no supplementation; (3) a positive control diet supplemented with 1g SP; (4) a positive control diet supplemented with 200 mg GP; and (5) a positive control diet supplemented with 1 g SP plus 200 mg GP.

Throughout the first week, the chicks were exposed to 24 h of light per day and then reduced to 22 h per day. The chicks, at day 7, were injected subcutaneously in the back of the neck with inactivated Newcastle disease (NDV) plus inactivated avian influenza (H9N1) vaccines while they were vaccinated against infectious bursal disease (IBD) at day 14.

***Spirulina platensis* and garlic powder sources:**

The dried powder of *Spirulina platensis* was supplied by the project entitled "Utilization of marine algae for salt fodders, milk, meat, and fish production under saline conditions," Desert Research Center of Egypt.

Garlic powder

Garlic is sold under the trade name Garllicin, and each kilogram of the compound contains 750 grams of a carrier (Silicon Dioxide) and 250 grams of Garlic Extract (Allicin C_6 $H_{10}S_3$). Gloryvet Company manufactured this product. 50 to 200 g/ton is the dosage range suggested by the company.

Table (1): Composition and calculated analysis of Starter and Finisher diets.

Ingredients	Starter-grower (1-21d)	Finisher (22-35d)
Yellow corn	54.40	62.00
Soybean meal, 44%	27.00	24.05
Corn Gluten meal, 60%	10.00	6.19
Soy bean oil	4.55	4.00
Limestone	1.10	1.00
Di-calcium phosphate	2.20	2.05

Vit& min. premix*	0.30	0.30
DL-Methionine	0.05	0.01
L-lysine (HCl)	0.15	0.15
Na Cl	0.25	0.25
Total	100	100
Calculated analysis: **		
CP, %	23.03	20.02
ME (Kcal/kg)	3204	3201
Calcium, %	1.05	0.97
Available phosphorus, %	0.45	0.42
Lysine, %	1.14	1.03
Methionine, %	0.52	0.41
TSAA, %	0.90	0.73

*Each 3kg contain: VitA 12000000IU, Vit D3 2000 000 IU, Vit E 10g, Vit K3 2g, Vit B1 1g, Vit B2 5g, Vit B6 1.5g, Vit B12 10mg, Nicotinic acid 30g, Pantothenic acid 10g, Folic acid 1g, Biotin 50mg, Choline chloride 250g, Iron 30g, Copper 10g, Zinc 50g, Manganese 60g, Iodine 1g, Selenium 0.1g, Cobalt 0.1gand carrier (CaCo3) to 3 kg. **According to tables of NRC (1994).

Measurements:

The response of the chicks was assessed in terms of weekly body weights, feed intake, and feed conversion ratio (FCR).

The rectal temperature (RT) of three birds randomly selected out of each replicate was measured with adigital thermometer (0.1^{o}Caccuracy) inserted into the rectum (colon) of the birds for one minuteas previously described by Yahav and McMurtry (2001). Respiratoryrate (RR) of the birds was takenas the number of breaths per minute. Data on RT and RR were collected two consecutive days in every week.

At the end of the trial, 6 chicks from each group were sacrificed, scalded, and de-feathered, and carcasses were eviscerated. Data on dressing yields and weights of visceral organs, and abdominal fat pads were collected. The heart, gizzard and liver were excised and weighed.

Blood sampling and measurements:

Blood samples (6 samples per treatment) were taken at the time of slaughter using sterile tubes that weren't heparinized. The samples were then centrifuged at 3400 g for 9 min. The serum was then extracted from the samples and placed in Eppendorf tubes, which were then stored at -20°C until a subsequent biochemical examination.

Using a commercial kit, serum total proteins, albumin, and glucose were measured using a colorimetric approach (Egyptian company for biotechnology, Egypt). By deducting serum albumin from serum total protein, serum globulin was calculated. Using total T3 commercial ELISA kits, serum triiodothyronine (T3) and thyroxin (T4) were measured using a solid phase enzyme immunoassay (Catalog Number; BC-1005; BioCheck, Inc .323 Vintage Park Drive. Foster City, CA 94404, USA).

Meat Quality Measurements

During slaughter, two parts of the upper right thigh were cut off per bird (6 bird per treatment). The pH and nutritional makeup of the first component were calculated. Using a digital pH metre, the pH of the thigh meat was determined (TitroLine Easy, Schott Instruments, Mainz, Germany). According to the AOAC (2000) procedures, the contents of dry matter (DM), crude protein (CP), ether extract (EE), and ash were measured. The other portion of thigh meat was utilised to examine meat colour indices and Thiobarbituric acid reactive (TBARS). The surface colour of thigh meat was measured through the packaging film, following calibration against a white tile covered with the same film. Colour measurements were describe in terms of lightness (L*), redness (a*) and yellowness (b*). The L*, a*, and b* of sample meat were measured with a Minolta Chroma Meter CR-400 (Minolta Inc., Tokyo, Japan).

A 50 mL test tube containing 10 g of meat samples and 1 mL of 0.1% BHT was used to measure TBARS. Following the addition of 35 mL of 5% trichloroacetic acid (TCA), the meat sample was homogenised using an Ultra-turrax T-25 by Janke and Kunkel IKA-Labortechnik in Staufen, Germany, at a speed of 13500 rpm for 30 seconds. 5 mL of the filtrate and 5 mL of thiobarbituric acid solution (0.02 mM) were added to the test tube after filtering. The absorbance was measured at 532 nm against a blank containing a 5 mL TCA and 5 mL TBA solution after the tubes had been heated in a boiling water bath for 1 h at 100°C then cooled. As mg per kg of sample, TBARS values were indicated (Ulu, 2004).

At the end of the experimental period, ten panellists performed a sensory evaluation of cooked samples of minced broiler chicken breast meat from five different birds for each treatment. Color, juiciness, flavour, tenderness, and general acceptability are among the criteria the panellists consider. Each piece of meat was coded and served to the panellists one at a time. After evaluating each meat sample, each participant cleaned their mouth with warm water to prevent carryover effects. Using a nine (9) point hedonic scale, the panellists determined scores by: (i) Dislike extremely, (ii) Dislike very much, (iii) Dislike moderately, (iv) Dislike slightly, (v) Intermediate, (vi) Like slightly, (vii) Like moderately, (viii) Like very much, (ix) Like extremely

Nutrient digestibility and Digestive enzymes

Six chicks from each group were selected at the conclusion of the experiment at the age of 35 days (representing the average weight of each experimental group), and each bird was weighed before being placed separately in the digestion cage and starved for 12 hours to empty the digestive tract before the digestion experiment began. The excreta were collected twice daily (every 12 hours), weighed, and dried for 24 hours at 70 degrees Celsius to estimate the required analyses. Estimates were made for ether extract (EE), dry matter (DM), and crude protein (CP) (AOAC. 2000).In order to measure the activity of digestive enzymes, Najaf et al. (2006) recommended taking 3 cm of the ileum digesta from six broiler chicks per group, draining the contents into sterile tubes, and then doing an immediate analysis (trypsin and amylase) .

Statistical analysis:

Statistical analysis was performed using the Statistical Analysis Software (SAS, 2004). Means were compared using Duncan's multiple-range test, and significance was determined at $p < 0.05$ (Duncan, 1955). The following model was used to study the effect of treatments on the parameters investigated as follows: $Y_{ij} = \mu + T_i + e_{ij}$. Where: Y_{ij} = an observation, μ = overall mean, T_i = effect of treatment (i=1,2,3,…..5) and e_{ij} = experimental random error.

RESULTS

Physiological parameters:

Results in Figures 1 and 2 show that rectal temperature was insignificantly increased, however, respiration rate was significantly ($p \leq 0.05$) increased for broiler exposure to heat stress as compared with those reared under thermoneutral condition. Addition of algae and/ or garlic alleviated the adverse effects of heat stress on rectal temperature and respiratory rate compared with the negative control group.

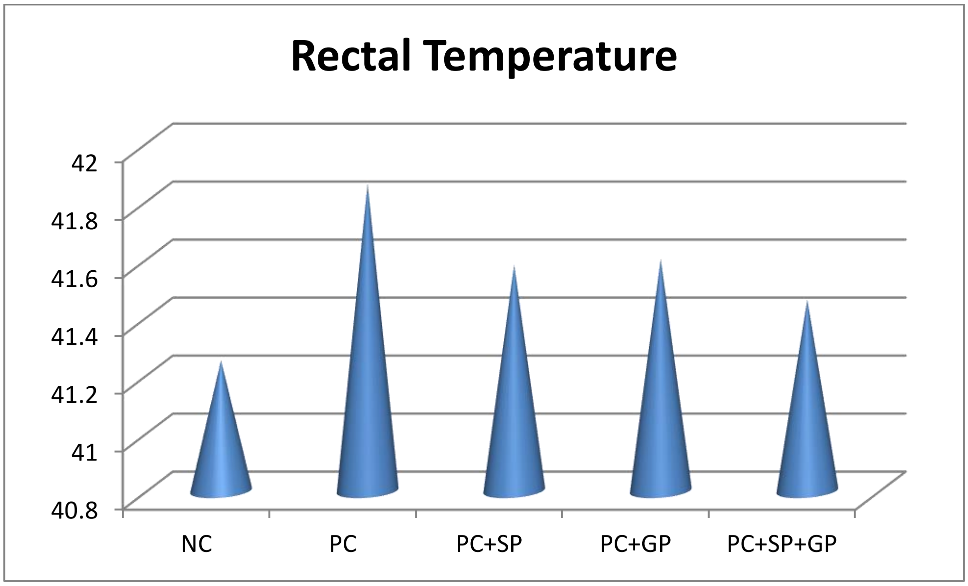

Figure (1): Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on rectal temperature of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

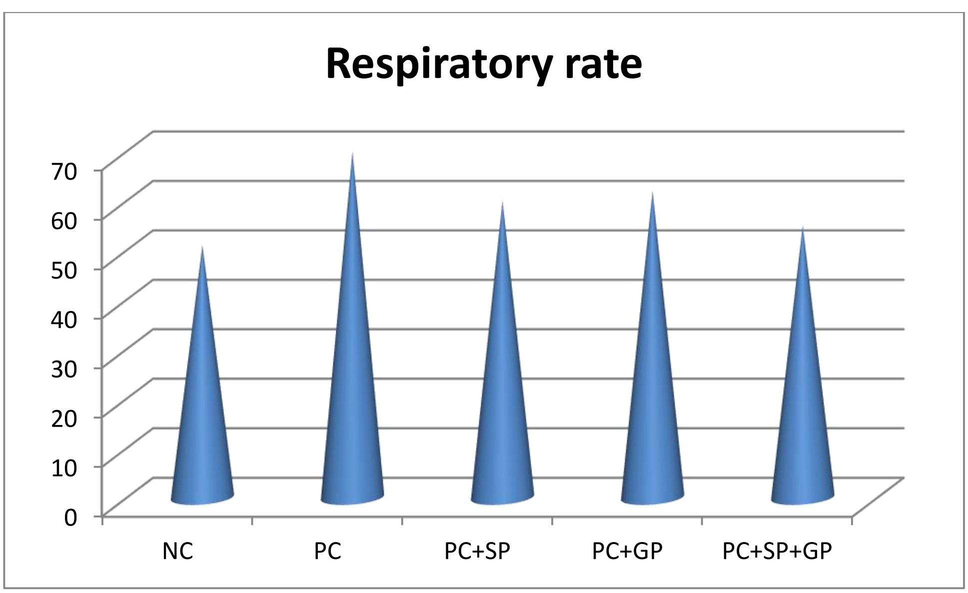

Figure (2): Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on respiratory rate of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Growth performance:

Table 2 shows the effects of varying dietary SP and/or GP supplementation levels on the growth performance of broiler chicks exposed to cyclic heat stress. Heat stress exposure had a negative effect on growth performance. The positive control group's final body weight, body weight gain, and feed intake were all significantly ($p < 0.05$) lower than those of the negative control group. SP and/or GP supplementation significantly boosted final body weight, weight gain, and feed intake under heat stress conditions compared to the positive control group. Additionally, supplementing with SP and/or GP enhanced the feed conversion ratio compared to the positive control group.

Serum metabolites

Broiler blood serum biochemical parameters of the different experimental groups are shown in Table 3. The results demonstrated the presence of significant changes in blood metabolites related to heat stress exposure. Results showed significant decrease in serum total protein, albumin, globulin, hemoglobin, T3 and T4 levels and a increase in glucose level in positive control group compared to the negative control group. Results demonstrated that Spirulina and / or garlic powder supplementation generally revoked the negative impact of heat stress on serum biochemical parameters. Spirulina and /or garlic powder supplementation was able to significantly decrease serum glucose level while increase serum total protein, albumin, globulin, hemoglobin, T3 and T4 levels compared to the positive control group.

Table 2. Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on growth performance of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
IBW, g	40.5	40.2	40.0	40.1	40.2	1.025	0.895
FBW, g	2135[a]	1850[c]	2035[b]	2020[b]	2100[a]	25.22	0.005
DWG, g	59.84[a]	51.70[c]	57.00[b]	56.56[b]	58.85[ab]	1.658	0.045
DFI, g/d	108.0	103.4	104.3	104.1	105.7	10.22	0.058
FCR	1.80[b]	2.00[a]	1.83[b]	1.84[b]	1.80[b]	0.257	0.028

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulina platensis; GP, Garlic powder; IBW, initial body weight; FBW, final body weight; DWG, daily weight gain; DFI, daily feed intake; FCR, feed conversion ratio.

Table 3. Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on serum biochemicals and thyroid hormones of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
Total protein, g/dl	6.20^{a}	4.06^{c}	5.46^{b}	5.44^{b}	5.50^{ab}	0.15	0.002
Albumin (A), g/dl	3.25^{a}	2.17^{b}	3.20^{a}	3.22^{a}	3.22^{a}	0.06	0.026
Globulin (G), g/dl	2.95^{a}	1.89^{b}	2.26^{ab}	2.22^{ab}	2.28^{ab}	0.152	0.001
Glucose, mg/dl	136.3^{c}	198.5^{a}	150.0^{b}	130.8^{c}	139.0^{c}	0.528	0.025
hemoglobin (g/dl)	11.05^{a}	9.15^{c}	10.58^{ab}	10.28^{b}	10.77^{ab}	0.445	0.012
T3, ng/ml	1.93^{a}	1.52^{c}	1.73^{b}	1.75^{b}	1.87^{ab}	0.485	0.002
T4 (µg/dl)	3.56^{a}	2.16^{c}	3.05^{b}	3.12^{b}	3.55^{a}	0.14	0.001

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulina platensis; GP, Garlic powder; T3, triiodothyronine.

Carcass characteristics:

Carcass characteristics were calculated and presented in (Table 4). Dressing percentage, decreased significantly with heat stress exposure. No effect was found on the heart and gizzard proportions among the different experimental groups. Meanwhile, carcass fat percentage and liver were significantly lower in the positive control group compared to the negative control group. Under heat stress, dietary Spirulina and / or garlic powder supplementations improved carcass dressing percentage. Furthermore, the intestine percentage was higher in Spirulina and /or garlic powder supplemented groups compared to the positive control group. These findings indicate that Spirulina and / or garlic powder supplementation has a positive effect on carcass characteristics in broilers reared under heat stress, with the best outcome observed among chickens fed Spirulina plus garlic powder supplemented levels.

Table 4. Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on carcass traits of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
Dressing %	70.52[a]	68.58[b]	69.89[a]	70.05[a]	70.22[a]	11.05	0.035
Liver, %	2.88[a]	2.55[b]	2.82[a]	2.80[a]	2.82[a]	0.558	0.039
Heart, %	0.653	0.485	0.532	0.545	0.555	0.158	0.085
Gizzard, %	1.58	1.38	1.45	1.44	1.50	0.115	0.065
Intestine, %	6.35[a]	6.05[b]	6.58[a]	6.42[a]	6.60[a]	1.358	0.002
Abdominafat, %	1.66[b]	1.985[a]	1.358[c]	1.320[c]	1.258[c]	0.052	0.001

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulina platensis; GP, Garlic powder.

Meat Quality Measurements

Meat is the end product of the broiler industry. Thus its quality is crucial for the breeders' final profitability. In the present study, meat quality parameters showed no changes across the positive control group compared to the negative control group (Table 5). Nevertheless, Spirulina and / or garlic powder supplementation, increased the meat yellowness (b*) compared to the positive control group.

Sensory evaluation of the different experimental groups are shown in Table 5. The results demonstrated the presence of not significant changes in sensory evaluation related to heat stress exposure. Results showed not significant effect in sensory evaluation in positive control group compared to the negative control group. Results demonstrated that Spirulina and / or garlic powder supplementation generally improved sensory evaluation which caused by heat stress.

Table 5. Effect of dietary *Spirulinaplatensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on meat quality of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
Meat color and pH							
Lightness (L*)	48.2	49.0	48.0	48.4	48.0	1.185	0.065
Yellowness (b*)	4.45[c]	4.80[c]	10.25[ab]	8.44[b]	10.45[a]	0.528	0.001
Redness (a*)	4.62[b]	4.20[b]	5.52[a]	4.85[ab]	5.95[a]	1.058	0.001
pH	5.74	5.60	5.78	5.72	5.80	0.155	0.085
Sensory evaluation							
Color	8.05	7.10	7.75	7.68	7.90	0.24	0.225
Flavor	7.50	6.35	6.80	6.75	7.0	0.19	0.352
Juiciness	7.00	6.55	6.80	6.67	6.85	0.28	0.065
Tenderness	7.5	6.60	6.77	6.80	7.00	0.22	0.058
Acceptability	9.00	7.00	8.00	8.09	9.00	0.24	0.465

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulinaplatensis; GP, Garlic powder.

Nutrient composition of thigh meat in broilers

The effects of dietary treatments on ash, fat, protein, moisture, pH and TBARS of thigh meat of broilers under heat stress at 35 days of age are indicated in Table 6. There were no significant differences between the treatments in the levels of the ash, and EE while there was significantly ($p < 0.05$) decresed in CP of the thigh meat for chicks in negative control group. Dietary supplementation of SP and GP separately or in combination increased thigh moisture compared with the positive control group ($p < 0.05$). Supplementation of SP and / or GP numerically increased the thigh CP. Furthermore, all the dietary supplements resulted in a lower ($p < 0.05$) thigh TBARS concentration.

Table 6. Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on carcass quality parameters,of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
Crude protein, %	19.05^{a}	18.00^{c}	18.60^{b}	18.70^{b}	18.85^{ab}	0.03	0.140
Moisture, %	77.8^{a}	74.65^{b}	76.05^{a}	76.00^{a}	76.80^{a}	0.24	0.035
Ether extract, %	2.00	1.69	1.80	1.78	1.90	0.03	0.215
Ash, %	1.90	2.15	2.01	2.11	2.00	0.02	0.133
TBARS, mg/kg	1.05^{c}	2.25^{a}	1.50^{b}	1.38^{b}	1.15^{bc}	0.052	0.001

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulina platensis; GP, Garlic powder.TBARS, Thiobarbituric acid reactive.

Table 7. Effect of dietary *Spirulina platensis* (1gm/kg diet) and /or garlic powder (200 mg /kg diet) supplementation on digestibility of broiler chickens subjected to cyclic heat stress starting from 22 to 35 days of age.

Traits	NC	Heat stress treatments				SEM	p-value
		PC	PC+ SP	PC+ GP	PC+ SP+GP		
Dry matter , %	79.80^{a}	76.6^{b}	78.8^{ab}	78.0^{ab}	79.0^{a}	1.08	0.012
Protein , %	74.0^{a}	70.0^{c}	73.0^{b}	72.9^{b}	73.8^{ab}	0.88	0.015
Fat, %	73.0	72.6	71.6	72.0	73.0	2.44	0.125
Digestive enzyme activity							
Amylase, u/ml	8.2^{a}	4.8^{d}	6.85^{b}	7.00^{b}	7.32^{ab}	0.84	0.004
Trypsin, u/ml	30.20^{a}	22.80^{c}	27.0^{b}	27.8^{b}	29.0^{a}	1.125	0.035

Means within a row with different superscripts significantly differ ($p < 0.05$). SEM, Standard error of the mean, NC, fed the basal diet and reared under thermoneutral condition; PC, fed the basal diet and reared under heat stress condition; SP, Spirulina platensis; GP, Garlic powder.

Nutrient digestibility and digestive enzymes

Nutrient digestibility and digestive enzymes of the different experimental groups are shown in Table 7. The results demonstrated the presence of significant changes in Nutrient digestibility and digestive enzymes related to heat stress exposure.

Results showed significant decrease in dry matter, crude protein digestibility, activity of amylase and trypsin in positive control group compared to the negative control group. Results demonstrated that Spirulina and / or garlic powder supplementation generally revoked the negative impact of heat stress on nutrient digestibility and digestive enzymes. Spirulina and /or garlic powder supplementation was able to significantly increase nutrient digestibility and digestive enzymes compared to the positive control group.

DISCUSSION

Oxidative stress deteriorates physiological parameters, and by reducing feed intake by 5% for every 1°C increase in the temperature range of 32-38°C, environmental heat stress severely affects broiler chicks' growth performances (Attia and Hassan, 2017 and Attia et al., 2017). In this study, heat stress significantly reduced the final body weight, weight gain, feed intake,feed conversion ratio, and gut health indices.

Physiological parameters:

Results in Figures1 and 2 show that rectal temperature was insignificantly increased, however, respiration rate was significantly ($p \leq 0.05$) increased for broiler exposure to heat stress as compared with those reared under thermoneutral condition. The results are inagreement with those reported by Borges et al. (2004) who found that exposure to heat stress caused an increase in respiratory rate (panting) and resulted in a reduction in blood pCO_2. A thigh temperatures, birds increase their respiration rate to regulate heat loss through watere vaporation from their lungs (Okela et al., 2003). This panting behavior, increases CO_2 loss from the lungs and partial pressure of CO_2 in blood. The decreased of CO_2 causes a decrease of HCO_3 concentrations due to the increase in HCO_3 excretion with a reduction of H+ excretion by the kidneys to maintain the acid-base balance in the bird. Lowered H+ concentration raises the level of blood plasma pH, a leading to respiratory alkalosis (Borges et al., 2007). This acid-base imbalance alters Na: Cl ratio, thus reduced feed consumption (Naseem et al., 2005). Moreover, broiler diets supplemented with SP and / or GP lead to a significant ($p \leq 0.05$) decrease in respiration rate compared to those exposed to only early age thermal condition or with heat stress.

Growth performance:

It has been noted that birds subjected to cyclic heat stress performed poorly in terms of growth; the decreased performance of heat-stressed birds appears to be caused by decreased appetite and reduced feed intake, a strategy that enables birds to minimize metabolic heat production (Abo Ghanima et al. 2020; Attia and Hassan, 2017).

This work demonstrates the synergistic effect of SP and GP on the performance of heat-stressed broiler chickens, even though other studies have examined the significance of dietary supplementation of SP or GP individually in broiler chickens. Due to its excellent nutritional content and valuable qualities, SP has traditionally been employed as a dietary element and functional food component (Alwaleed, et al., 2021). The effects of a feed supplementation with various SP doses up to 200 g/kg on growth performance have been studied over the past few years, with wildly divergent results (Tavernari et al., 2018).

Spirulina supplementation boosted FCR, which increased the final body weight of the heat-stressed chickens even though it did not affect feed intake in the current study. Japanese quails treated with 5 g/kg Spirulina showed a considerable increase in final body weight and the European production efficiency factor, according to Hajati and Zaghari (2019). Additionally, adding SP at doses of 0.25, 0.5, 0.75, or 1.0% enhanced body weight gain, feed conversion ratio, and the European production index linearly (Park et al., 2018). The SP-supplemented diet's high apparent metabolizable energy and high amino acid digestibility can explain the beneficial effects of SP on broiler performance (Tavernari et al., 2018), especially in the presence of the detrimental effects of heat stress on intestinal morphology and feed intake.

In addition, SP supplementation has been shown to improve gut morphology, with longer villi and more goblet cells, as well as enhanced healthy intestinal microbial community, with an increase in Lactobacillus sp. and a decrease in E. coli (Alwaleed et al., 2021). The considerable rise in relative intestine weight seen in the current study with the addition of SP may be related to the adaptation to low feed intake, which then helps to make up for the decrease in feed efficiency of birds under heat stress (De Verdal et al., 2010). In addition to the fact that the amino acids in spirulina protein are readily digestible, phenolic compounds, carotenoids, vitamins, minerals, and other bioactive components of SP may have physiological effects on broiler chickens under heat stress. (Agustini et al., 2015)

The findings of El-katcha et al. (2016), who observed the same trend in body weight gain, concur with the findings of the current study regarding growth performance. They concluded that adding garlic (in the form of allicin extract) had little impact on weight gain during the first three weeks of the experimental trial. Still, this effect was significantly improved by the end of the study. Garlic's active ingredients, such as allicin, oregano sulfur compounds, and antibacterial compound dialkylpolysulphide, support intestinal flora performance by inhibiting pathogenic bacteria and fungi leading to better nutrient utilization and their translation to meat. This may be responsible for improved weight gain in groups supplemented with garlic. Additionally, the active components in garlic increase the activity of pancreatic enzymes and create an environment that is conducive to nutrient absorption from the digestive system.

Blood constituents:

By measuring clinical serum biochemicals, the health state of the birds was evaluated in the current study. The concentration of circulating total protein and globulins reduced under heat stress, indicating that an increase in temperature changes how proteins are metabolised (Attia et al., 2011). In cases of heat stress, blood protein levels were noticeably lower. According to Maajid et al. (2015), oxidative stress increases the formation of ROS, which causes biomolecules including nucleic acids, proteins, and enzymes to become denaturized. The decrease in serum protein may be related to the elevated oxidative stress brought on by heat stress, which may in part be brought on by the lower protein intake and lack of necessary amino acids (Laudadio et al., 2012). Low blood protein levels could potentially result from reduced protein digestion under heat stress (Bonnet et al., 1997). Our findings are in line with those published previously by Faisal et al. (2008), who found that heat stress lowers blood protein levels in Japanese quails.

According to prior research (Quinteiro-Filho et al., 2012), heat stress can raise glucocorticoids, which can lead to an increase in glucose concentration. This could be the cause of the rise in glucose concentration in the current study. Proteins from muscle tissue are encouraged to produce gluconeogenesis by glucocorticoids. Plasma glucose and cholesterol levels rose while total protein levels fell as a result of heat stress (Rashidi et al., 2010).

The low levels of haemoglobin reported in heat-stressed chicken can be attributed to the negative effects of heat exposure on ion absorption, which reduces haemoglobin production. As a result of its high mineral content and capacity to alter intestinal integrity and permeability, supplementing with SP also caused a direct alteration in blood haemoglobin levels (Yu et al., 2020). It was also demonstrated that using SP in broiler diets at a rate of 5 or 10 g/kg feed raised haemoglobin levels (Alwaleed et al., 2021). These findings demonstrate that supplementation with SP helps to restore the normal blood metabolite and haematological profiles of heat-stressed broilers.

The control of thermoregulation and nutrition metabolism, which are regulated by both internal and external variables, such as exposure to various stressors, is greatly aided by thyroid hormones (T3 and T4) (Mancini et al., 2016; Garasto et al., 2017). For homoeothermic animals like chickens to maintain body temperature through energy metabolism, regulation of thyroid activities, such as T3 and T4 levels, is crucial. Significantly lower blood T3 levels have been observed in broilers subjected to thermal stress under a variety of conditions, including 36°C for 4 to 6 hours per day from days 22 to 42 (Zaglool et al., 2019), 38°C for 3 hours per day from days 35 to 40 (Tollba and Hassan, 2003), and 38°C for 1 hour at day 40 (Zaglool et al., 2019). In stressed broilers, a lower T3 state may prevent harmful catabolic consequences. On the other hand, broilers exposed to acute heat stress at 50°C for one hour at five days old showed considerably higher blood T3 and T4 concentrations, according to Bowen and Washburn (1985). While May et al. (1986) discovered that broilers subjected to heat at 41°C for 4 to 6 hours on the fourth day did not have their plasma T3 and T4 levels impacted. These findings suggest that a number of variables, including stress intensity, duration, and bird age, have an impact on the thyroid hormones in broilers. When compared to the broilers in the negative control group, the heat-stressed broilers in the current study had lower levels of T3 and T4 hormone. High ambient temperatures in broiler chicken cause the thyroid gland's size and activity to decrease, and vice versa (Gonzalez-Rivas et al., 2020). Drop in T3 and T4 plasma levels is typically caused by high ambient temperature. By reducing metabolic heat generation, lowering maintenance energy needs, and promoting fat deposition by inhibiting lipolysis, this system acts as an adaptive tool to cope with additional heat load (Collin et al., 2005).When compared to the group of heat-stressed broilers fed SP + GP, the blood T3 and T4 concentrations showed no diffeences compard with negative control group.

Carcass

Stress had a considerable impact on the current experiment's carcass features and internal organ weights, which are crucial health indicators. Focus is on the decreased carcass weight. These findings concur with those of Akşit et al (2006). Poor nutrient use and reduced feed intake may be the causes of the poor carcass features. One of the body's most important organs, the liver serves as the life support system for birds. This essential immunological organ in the body exhibited shrinkage as a result of HS and also plays a significant part in the detoxification, digestion, metabolism, and utilisation of feed nutrients. It also serves as a hub for several digestive, metabolic, and productive functions. This result was previously noted by Kucuk et al. (2003) and Ahmed and ElGhamdi (2008). This can be explained by cardiovascular adjustment in response to heat stress, which includes vasodilatation in the cutaneous vascular bed and vasoconstriction in the hepato-splanchnic vascular area (Richardson et al., 1991). This result was manifested by a reduction in the size of the liver (Sritharet et al., 2002). Additionally, heat stress reduced the weights of the gizzard and heart. Kucuk et al. (2003) and Keambou et al. (2003) had already noted these results.

The results of the current study showed that food supplementations improved carcass output and dressing percentage. These results are consistent with those reported by Moustafa et al. (2021), who discovered that improving carcass qualities in broilers reared in hot and thermoneutral settings by adding SP powder to the meal at concentrations of 0.25, 0.5, and 1% improved carcass characteristics. The potential for SP powder to provide sufficient amounts of nutrients and metabolizable energy, promoting muscle growth and nutrient conversion to lean meat, can explain the improvement in carcass characteristics (Tavernari et al. 2018). Garlic supplementation enhances carcass weight and carcass yield because it includes allicin, an antibiotic-like substance that lowers the amount of harmful bacteria and fungi that create aflatoxin in bird intestines, enhancing nutrition absorption and boosting carcass weight (Kharde and Soujanya, 2014). The lowest abdominal fat pad seen in birds fed a combination of SP and garlic, demonstrates the ability of the test ingredients to synergistically reduce bird abdomen fat. Our findings then suggested that customers are also benefiting from consuming lean, functional, and nutraceutical meats as farmers are obtaining more live weight of birds. Additionally, eating meat from these birds will promote customers' health and wellbeing rather than put them at danger for disease.

Meat quality parameters

Broilers exposed to prolonged heat stress reportedly had poorer meat quality (Awad et al., 2020). Heat stress has been linked to lower final pH, higher lightness (L*), cooking loss, and shear force in broiler meat (Gonzalez-Rivas et al., 2020). In their study, Lu et al. (2017) examined how chronic heat stress affected the quality of broiler breast muscle and found that it resulted in decreased pH, increased lightness, drip loss, and intramuscular fat deposition. They went on to explain these alterations in terms of the detrimental effects of heat stress on mitochondrial activity, which results in an increase in glycolysis and fat deposition and a decrease in aerobic metabolism of fat and glucose.Because of the high carotenoid concentration of spirulina, supplementation led to an increase in the yellowness (b*) of meat (El-Desoky et al., 2013). According to Pestana et al. (2020), the meat of chicken breast and thigh that had been fed 15% spirulina had greater yellowness values and total carotenoids. Spirulina's beneficial effects on meat quality and carcass composition can be explained by the increased energy partitioning in favour of muscle growth. Spirulina also benefits from its high nutrient content, which improves feed efficiency and the conversion of nutrients into lean meat. Studies in the past have demonstrated that broiler leg and breast muscles treated with black garlic extract can greatly enhance meat colour (Barido et al., 2021). The a* value of broilers tended to rise when garlic powder was added ($p < 0.05$). The shape and composition of myoglobin mostly determine the colour of meat (Barido et al., 2021). Because of the antioxidant properties of garlic powder's antioxidants, Choi et al. (2010) thought that giving 5% of it to broilers could increase a* value of broilers.

According to Songsang et al. (2008), effect of garlic powder on breast meat had no noticeable effect on the colour, flavour, or overall appeal of the meat. In a different study, Koreleski and Swiatkiewicz (2007) discovered that there were no alterations in the sensory qualities of raw breast flesh when coneflower, thyme, and sage extracts were supplemented (flavour, taste, tenderness, juiciness). Marcincak et al. (2011) shown that feeding clove in the feed along with agrimony in water or clove in the feed along with lemon balm extracts in the water tended to have a little beneficial influence on the sensory evaluation of breast and thigh. Kim et al. (2009) found no difference in meat juiciness when dietary garlic bulb and hush powder were used, however sensory panellists gave the samples with garlic supplementation higher values for hardness and flavour. Because sensory panellists discovered that the thigh meat from birds given a garlic-supplemented diet had better texture and flavour, these findings imply that adding garlic to broiler chicken diets can improve eating quality. Additionally, Pawer et al. (2007) came to the conclusion that flavor-producing events that take place during cooking may have contributed to the improvement in flavour of ginger-treated samples. The acceptance profile (tenderness, juiciness, taste, overall acceptance), L*, a*, pH24, and intramuscular fat of breast meat of female medium-growing broilers at the age of 9 weeks were not affected by two concentrations of oregano essential oil (100 or 250 mg/kg) in the feed, according to Symeon et al. (2009).

Nutrient composition of thigh meat in broilers

Regarding the lower levels of protein in the flesh of chicks exposed to heat stress without the use of additives, earlier research showed that chronic HS had a significant negative impact on protein metabolism and lower levels of protein synthesis in muscles. Dietary protein cannot make up for this dropping level of protein because doing so aggravates HS by increasing metabolic heat (Attia et al., 2018). According to reports, heat stress lowers the pH of meat (Cornforth, 1994). Meat with a high pH has a greater ability to bind water, according to Cornforth (2002). The oxidation of cellular lipid, DNA, and carbohydrates by oxidative free radicals can impair the normal function of cells (Bianchi and Antunes, 1999). In addition to enhancing the organoleptic qualities (taste, sight, smell, and touch) and quality of meat, the combined use of antioxidants may lessen the effects of stress due to their oxi-reduction activities, which may play a significant role in the quenching and neutralisation of free radicals (Jem et al., 2008).

In the current investigation, the TBARS in the thigh meat was decreased by SP and GP powders. This may be the result of these materials' antioxidant components. Saleh et al. (2018), who indicated broiler thigh meat may be enriched successfully with long chain polyunstaturated fatty acids n-3 and its antioxidant potential and fuctional quality characteristics may be improved by supplementing the diet. Polyphenols are natural antioxidant that showed antioxidant and animicrobial activities (Lorenzo et al., 2014) and it can prevent lipid oxidation by preventing chain inhibition by scavening initiating radicals, breaking chain reaction, decomposing peroxides, decreasing localized oxygen concentration and binding chain intiating catalyst such as metal ions (Juntachote et al., 2006).

Digestability

The intestinal epithelial cells of broilers are vulnerable to osmotic stress during heat stress because a high ambient temperature may cause a water imbalance and modify cell permeability due to dehydration (Ratriyanto and Mosenthin, 2018). Additionally, alterations in intestinal structure and digestive function may result from fluid transfer in the digestive system under heat stress (Song et al., 2013). In fact, chicks exposed to heat stress in the present investigation had worse digestability ratings. this result agree with Attia et al. (2009) showed that heat stress affected the nitrogen digestibility and digestive enzyme activity of broilers. The results of the current investigation demonstrated that adding SP and/or GP to the diet improved the digestibility scores in heat-stressed broilers.

The primary mechanisms by which phytoadditives exert their beneficial effect on nutrient digestibility include increased digestive secretion (Puvaca et al. 2013), enhancement of intestinal trypsin, lipase, and amylase activities (Lee et al. 2004), and improved gut morphological characteristics (Jamroz et al. 2006). Garlic's active components and phenolic compounds, which are able to lower the amount of intestinal pathogens and, in turn, minimise nutrient loss and improve performance, may be the cause of the improved digestibility shown in diets supplemented with garlic. In turn, the bodily tissues begin to accumulate proteins. In addition to conventional growth promoters, garlic has been shown to have excellent effects on growth and digestibility in chicken (Bampidis et al., 2005).

Conclusion

Broiler hens given a corn-soybean-based basic diet and raised under heat stress had better growth performance and carcass yield with the addition of SP, GP, or their combination. The findings also demonstrated that when food supplements were given in combination, birds benefited more from them due to their synergistic interactions. These findings point to the potential for using 1 g of SP or 200 mg of GP in broiler chicken diets to reduce the adverse effects of heat stress and boost performance.

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الدور الوقائي لطحلب سبيرولينا (Spirulina platensis) والثوم (Allium Sativum) ، وخلطهما معا في التخفيف من الإجهاد الحراري في دجاج التسمين

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مستخلص. هدفت الدراسة إلى تحديد آثار إضافة سبيرولينا بلاتنسيس و/ أو مسحوق الثوم على أداء نمو دجاج التسمين وخصائص الذبيحة وبعض مقاييس الدم. تم أستخدام ستمائة من كتاكيت اللاحم روس –٣٠٨ عمرها يوم واحد. تم إنشاء ضغوط حرارية بدءًا من اليوم ٢٢ إلى ٣٥ من العمر، وتم تقسيم الطيور عشوائيًا إلى خمس مجموعات معالجة مع ٦ مكررات × ٢٠ طائرًا لكل مجموعة، حيث تم تزويد المجموعة الأولى بالنظام الغذائي الأساسي وتربيتها في ظل ظروف حرارية عادية (٢٦ ± ١ درجة مئوية) لتكون بمثابة عنصر تحكم سلبي. في هذه الأثناء، تعرضت المجموعات الأربع الأخرى إلى إجهاد حراري دوري (٣٥ ± ١ درجة مئوية لمدة ٩ ساعات في اليوم) وتم إطعامهم نظامًا غذائيًا أساسيًا بدون مكملات ليكون بمثابة تحكم إيجابي، ونظام غذائي مكمل بـ ١ جم سبيرولينا بلاتنسيس ، ٢٠٠ مجم مسحوق ثوم و١ جم سبيرولينا بلاتنسيس+ ٢٠٠ مجم مسحوق الثوم على التوالي. أظهرت النتائج أن استكمال وجبات دجاج التسمين تحت الإجهاد الحراري باستخدام سبيرولينا بلاتنسيس و/ أو مسحوق الثوم يقلل بشكل فعال من آثار الإجهاد الحراري على أداء النمو. أدت إضافة ١ جم من سبيرولينا بلاتنسيس و / أو ٢٠٠ مجم من مسحوق الثوم إلى زيادة نسبة تصافى الذبيحة. يمكن الاستنتاج أن تغذية الدجاج اللاحم المربى تحت علائق الإجهاد الحراري المضاف إليها ١جم من سبيرولينا بلاتنسيس أو ٢٠٠ مجم من مسحوق الثوم تحسن بشكل كبير من أدائها وخصائص الذبيحة. شوهدت أفضل النتائج في الكتاكيت التي تغذت على سبيرولينا بلاتنسيس مع مسحوق الثوم.

الكلمات المفتاحية: الإجهاد الحراري، كتاكيت اللاحم، طحلب سبيرولينا، الثوم، أداء النمو، الذبيحة.