Effects of brown seaweed products on growth performance, plasma biochemistry, immune response, and antioxidant capacity of broiler chickens challenged with heat stress

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ABSTRACT

Brown seaweed (Ascophyllum nodosum) is an exceptional bioactive substance known for its excellent antioxidant ability. Given the potential benefits of brown seaweed, the current study was conducted to determine its efficacy on growth performance, blood biochemistry, immunoglobulins (IgG and IgM), and the antioxidant capacity of broiler chickens challenged with heat stress (HS). A total of 336 mixed-sex Ross 308 broiler chicks (one-day-old) were randomly assigned into two groups; The thermoneutral group (TN, broilers were raised at 24 ± 1°C); and the heat stress group (HS; broilers were exposed to 32°C to 34°C, 8 h/d from day 21 to 27; the temperature in the remaining time was same as TN group). All birds in each group were randomly allotted to 4 dietary treatments—Negative control (NC) (without seaweed), NC + 1 mL seaweed extract (SWE) in drinking water, NC + 2 mL SWE in drinking water, and NC + 2% seaweed meal (SWM) in feed. Each treatment was assigned to six replicates with 7 broilers/replicate. Average body weight gain (ABWG), average feed intake (AFI), average water intake (AWI), feed conversion ratio (FCR), and mortality were determined weekly. On day 28, two male birds/cage were euthanized to collect blood and immune organs for subsequent biochemical, antioxidant, and immune status analysis. Data were analyzed as a 4 × 2 factorial analysis of variance using the GLM procedure of Minitab software. Overall, 2% SWM inclusion significantly increased (P < 0.05) the AFI, ABWG, and AWI of broiler chickens irrespective of HS. HS significantly reduced (P < 0.05) AFI and increased (P < 0.05) the bird’s rectal temperature, plasma concentrations of sodium, chloride, glucose, amylase, and uric acid compared to TN birds. HS increased (P < 0.05) serum IgM and IgG and decreased plasma glutathione reductase and glutathione peroxidase compared to TN birds, while the activity of superoxide dismutase was not affected by HS and dietary treatments. 1 mL SWE in water and 2% SWM in feed significantly reduced (P < 0.05) the plasma activity of alanine aminotransferase and gamma-glutamyl transferase of heat-stressed broilers, respectively compared to other treatments. Conclusively, dietary supplementation of brown seaweed improved the growth performance of birds irrespective of HS and may help to reduce the negative effects of HS by improving the plasma enzyme activities of heat-stressed birds.

Key words: brown seaweed, heat stress, growth performance, immune status, broiler chickens

INTRODUCTION

Heat stress (HS) is a significant problem in modern poultry production (Ahmed-Farid et al., 2021; Calik et al., 2022; Huang et al., 2015; Liu et al., 2020a; Quinteiro-Filho et al., 2010; Zhang et al., 2017), causing considerable economic losses, which are conjected to surge in coming years with the rise of global temperature (Abdel-Moneim et al., 2021). Current poultry genotypes experience a fast growth rate, which reduces their tolerance to heat, leading to various metabolic disorders in chickens (Varasteh et al., 2015). Due to their high metabolic rate and physiological state, broiler chickens tend to be more vulnerable to temperature-related stress (Zhang et al., 2017). Under HS conditions, the physiological blood parameters of broilers are altered, which ultimately reduces feed intake, performance, and productivity (Liu et al., 2019; Khan et al., 2021; Oke et al., 2021). High ambient temperature can also weaken the immune system of broiler chickens (Siddiqui et al., 2022). The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Evidence showed lowered CD3+ and CD8+ T altered cytokine expression, and reduced immune organ weights and B-lymphocyte in response to HS (Honda et al., 2015; Quinteiro-Filho et al., 2010; Xu et al., 2018; Siddiqui et al., 2021). Moreover, inflammatory cytokines are secreted in response to stressors and can be used to determine animals’ immune status (Quinteiro-Filho et al., 2017). Proinflammatory cytokines mediate inflammatory damage while anti-inflammatory cytokines ameliorate inflammation and improve the healing process (Siddiqui et al., 2020). High expression of proinflammatory cytokines triggered by HS might be linked with reactive oxygen species (ROS) production (Jang et al., 2014). The ROS activates the production of primary regulators of inflammatory response known as cytokines via activation of the nuclear factor kappa-B pathway, which controls the expression of genes involved in inflammation and stress responses (Khan et al., 2019). High temperature can stimulate excessive ROS and cause an imbalance between oxidation and antioxidant enzymes resulting in oxidative damage in chickens (Lin et al., 2006). Previous studies have shown oxidative-induced damage in response to HS in broiler chickens (Yang et al., 2010; Huang et al., 2015). Exposure of broiler chicks to an acute temperature of 32°C for 6 h induced metabolic changes that triggered oxidative stress (Lin et al., 2006). A recent study by Liu et al. (2022a) further confirmed that chronic HS (32.5 ± 1.4°C for 8 h/d) for 4 wk induced oxidative stress in broilers evidenced by elevated MDA levels and reduced antioxidant enzymes activity in serum, muscles and small intestines. Enzymatic and non-enzymatic antioxidant defence systems can be internally synthesized or externally supplied in response to oxidative stress and increased due to defence mechanisms against heat-induced oxidative stress (Akbarian et al., 2016; Surai et al., 2019). The removal of free radicals in the body directly hinged on the depletion of the antioxidant factors (Zhang et al., 2020), which can alter the antioxidant capacity of broiler chickens (Hu et al., 2021).

Significant developments in management and nutritional strategies have been attained to mitigate HS and reduce the resulting animal production losses. However, the current increase in the incidence of heat waves due to climate change further heightens HS concerns (Chauhan et al., 2021). Recently, interests have involved the extensive use of bioactive substances to ameliorate HS in poultry (Saracila et al., 2021) due to their beneficial effects on health and production (Abdelli et al., 2021). These phytogenic feed additives can be incorporated into animal feed or water as extracts (Gadde et al., 2017). Brown seaweeds are exceptional bioactive substances, rich in phenolic compounds (chlorotetramin, flavonoids), pigments (chlorophyll, fucoxanthin) and contain high content of total polysaccharides such as fucoidan, laminarin, algic acid, and mannitol (Holdt and Kraan, 2011; Zheng et al., 2020). They have been reported to exert different biological activity, including antioxidant, anti-inflammatory, and anti-microbial roles (Lordan et al., 2013; Gullon et al., 2020; Meresse et al., 2020). For instance, fucoidan prevented liver injury in mice by deactivating the inflammatory signaling pathway (Li and Ye, 2015); laminarin enhanced growth performance, beneficial bacteria in chickens and increased the expression of claudins 1, toll-like receptor 2 and interleukins 17A in the small intestine (Venardou et al., 2021). Dietary seaweed (Enteromorpha prolifera) polysaccharide supplementation also improved broilers’ immunity and breast muscle growth (Zhao et al., 2021). The phenolic compounds in brown seaweed can protect the body against oxidative damage that occurs in response to environmental or nutrient changes (Li et al., 2017) and promote good health and productivity in animals (Michalak et al., 2022). Previous studies showed that the dietary supplementation of marine algae polysaccharides extracted from seaweed (E. prolifera) improved bursa weight, the intestinal and liver antioxidant enzymes (total superoxide dismutase, glutathione, catalase and glutathione peroxidase) and reduced malondialdehyde (MDA) contents of broilers (Liu et al., 2020b; Guo et al., 2021). In aged laying hens, marine algae polysaccharides improved the productive performance, egg quality and antioxidant capacity by increasing the activity of serum total superoxide dismutase, liver and jejunal catalase, and decreased jejunal MDA concentration (Guo et al., 2020). Studies have shown brown seaweed’s capacity in alleviating the adverse effects of HS in ruminant animals (Saker et al., 2003; Kannan et al., 2007) with no available data on broiler chickens. Therefore, our current study was conducted to test the hypothesis that the dietary supplementation of 2% brown seaweed meal (in feed), and 1 and 2 mL brown seaweed extract (in water) can mitigate HS negative effects in broiler chickens. The main objective was to evaluate the potential beneficial effect of brown seaweed meal and extract on the growth performance, blood biochemistry, immune response, and antioxidant status of broiler chickens challenged with heat stress.

**MATERIALS AND METHODS**

**Management and Housing**

All experimental procedures adopted in this study were endorsed by Dalhousie University Animal Use and Care Committee (Ethical code 2021-029), and all chickens were handled in agreement with the Canadian Council on Animal Care (CCAC-Canadian Council On Animal Care, 2009) guidelines. A total of 336 one-day-old broiler chicks (Ross 308) were gotten from a commercial source. Upon arrival, birds were weighed in groups of 6, then randomly assigned to each cage (50 cm × 60 cm) at a stocking density of 0.076 m²/bird. They were raised for 28 d in a controlled environment at the Atlantic Poultry Research Center, Dalhousie University, Faculty of Agriculture (Truro, NS, Canada). Room temperature was checked daily and steadily reduced from 33 to 24°C from d 0 to 28 in the
thermoneutral room. The lighting was programmed to produce 18 h of light and 6 h of darkness throughout the experimental period, and illumination was gradually reduced from 20 lux on day 0 to 5 lux (Adewole et al., 2020).

**Heat Stress Protocol**

Heat stress was induced on days 21 to 27 for 8 h/d. For the HS group, the temperature was gradually raised at 9 am, reached 28°C by 10 am, 32°C by noon, maintained at 34 ±1°C from noon to 4 pm, then slowly reduced to 28°C by 5 pm and dropped to thermoneutral (TN; 24°C±1) for the remainder of the day. TN birds were housed under the ambient temperature of 24°C±1 throughout the heat stress week. The TN and HS conditions were controlled by an automatic temperature control system and recorded twice daily. Relative humidity was observed and maintained at <40% for both rooms.

**Seaweed products, Diets, and Experimental Design**

The brown seaweed meal and extract used in this study were obtained from Sealife Seaplants (NS, Canada). Seaweed extract was derived from fresh Ascophyllum nodosum seaweed harvested from the North Atlantic coastal water of NS, Canada. The seaweed meal contained 90.4% dry matter, 3.7% crude protein, and 25.5% neutral detergent fiber on as fed basis. For both seaweed and experimental diets, dry matter was determined according to the Association of Official Analytical Chemists (AOAC, 1990), method (925.09) by oven drying a 5.0 g sample at 105°C overnight. The method of Goering and Van Soest (1970) was used to determine the content of neutral detergent fiber. The nitrogen content was determined using the combustion method (990.03; AOAC, 1990) with an N analyzer (Model Leco CN828 Carbon Nitrogen Determinator, St. Joseph, MO), and crude protein was calculated as N x 6.25. The ether extract in the diets was determined after hexane extraction (Method 920.39; AOAC, 1990) in an Ankom XT10 Fat Extractor system (Macedon, NY). The diets were further analyzed for minerals after they were ashed at 600°C for 12 h in a muffle furnace, using a Varian 725 ICP-OES inductively coupled plasma mass spectrometer (ICP-AES; Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005) (method 985.01). The seaweed extract is a water-soluble viscous brownish-black liquid containing minerals and carbohydrates (alginic acid, mannitol, and laminarin). Broiler chicks were randomly allotted to 8 treatment groups containing 6 replicate cages of 7 birds each. The experiment was designed as a 4 x 2 factorial arrangement consisting of 2 environmental controlled rooms; 1) Thermoneutral (TN; 24°C±1 on d 21–27), Heat stress (HS; 32-34°C for 8 h/d on d 21–27) and 4 dietary treatments: 1) Corn-wheat-soybean based diet negative control (NC)); 2) NC + 1 mL seaweed extract in drinking water (1 mL SWE), 3) NC + 2 mL seaweed extract in drinking water (2 mL SWE), and 4) NC + 2% seaweed meal in feed (2% SWM). All diets, including starter (d 0–14) and grower (d 15–28), were formulated to meet Ross 308 guidelines for nutrient requirements. Broiler chickens received feed (in mash form) and water ad libitum throughout the experimental period. Dietary treatments, ingredients, and nutritional composition are shown in Table 1.

**Growth Performance and Sample Collection**

Feed intake (FI) and body weight (BW) were recorded weekly, and average feed intake (AFI), and average body weight (ABW) were calculated. The feed

| Ingredients | Starter (1–14 d) | Grower (15–28 d) |
|-------------|-----------------|------------------|
|             | Negative control (NC) | 2% SWM | Negative control (NC) | 2% SWM |
| Corn (ground) | 42.42 | 39.12 | 45.75 | 42.45 |
| Soybean meal (46.5) | 40.12 | 40.58 | 36.19 | 36.65 |
| Wheat | 10.00 | 10.00 | 10.00 | 10.00 |
| Vegetable Oil Young | 2.80 | 3.92 | 3.82 | 4.94 |
| Brown seaweed meal | 0.00 | 2.00 | 0.00 | 2.00 |
| Dicalcium phosphate | 1.57 | 1.57 | 1.39 | 1.39 |
| Limestone | 1.32 | 1.34 | 1.32 | 1.21 |
| DL Methionine Premix¹ | 0.61 | 0.61 | 0.53 | 0.53 |
| Vitamin-Mineral Premix²,³ | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt | 0.37 | 0.21 | 0.38 | 0.22 |
| Lysine HCl | 0.16 | 0.15 | 0.12 | 0.11 |
| Total | 100 | 100 | 100 | 100 |

1Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.
2Start vitamin-mineral premix contained the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg DL Ca-pantothenate; 0.012 mg vitamin B12; 20.7 mg niacin; 1.0 mg folie acid, 801 mg choline; 0.5 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc; 25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543 mg wheat middlings; 500 mg ground limestone.
3Grower andFinisher vitamin-mineral premix contained the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg DL Ca-pantothenate; 0.012 mg vitamin B12; 20.7 mg niacin; 1.0 mg folie acid; 801 mg choline; 0.5 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc; 25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543 mg wheat middling’s; 500 mg ground limestone.

Table 1. Ingredients, calculated, and analyzed compositions of experimental diets (as-fed basis, % unless otherwise stated).
conversion ratio (FCR) was obtained by dividing the amount of feed consumed by body weight gain. Mortality was recorded daily and used to correct the AFI and FCR. Dead birds were sent to Animal Health Laboratory (Truro, NS) for necropsy examination. On day 28, 2 male birds per cage (12 replicates per treatment group) were randomly selected and euthanized by electrical stunning and exsanguination. Following euthanizing, blood samples were collected from the jugular vein of each bird into 10 mL serum and 10 mL heparinized tubes, and centrifuged at 2,000 × g for 15 min. The supernatant was further divided into several aliquots tubes to avoid repeated thawing. The serum and plasma samples were stored immediately at −80 °C for serum enzyme-linked immunosorbent assay (ELISA) and at −20 °C for plasma biochemistry and other analysis.

**Plasma Biochemistry**

Plasma samples for biochemical analysis were shipped on ice to Atlantic Veterinary College, University of Prince Edward Island Pathology Laboratory, for analysis using cobas6000 analyzer series (Roche Diagnostics, Indianapolis, IN).

**Immune Parameters**

The bursa and spleen weights collected on day 28 were recorded as a percentage of the live BW of the slaughtered chicken (g/Kg BW). Serum samples were used to analyze the concentrations of immunoglobulins G (IgG) and immunoglobulins M (IgM) using ELISA kits specified for chickens (Bethyl Laboratories, Montgomery, TX; Catalog No. E33-104-200210 and E33-102-21109, respectively) following the company protocol. Plasma samples were used to determine the concentrations of chicken interleukin 6 (IL6) and interleukin 10 (IL10) using ELISA kits (MyBioSource.com Catalog No. MBS037319 and MBS007312, respectively). The optical density (OD) values for IgG, IgM, IL6 and IL10 were determined on a microplate reader (Bio-Tek Instrument Inc., Winooski, VT) using a software program (KC4, version #3.3, Bio Tek Instruments). The 4-parameter logistic model was used to calculate immunoglobulins concentration, while curve fitting software was used to generate standard linear curves and calculate IL6 and IL10 analyte levels.

**Antioxidant Activities Analysis**

Antioxidant activities of Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Glutathione reductase (GR) were assessed using the Superoxide dismutase Assay Kits (Item No. 706002), Glutathione Peroxidase Assay Kit (Item No. 703102), Glutathione Reductase Assay Kits (Item No. 703202), respectively, following the manufacturers’ instructions (Cayman Chemical Company, Ann Arbor, MI). Absorbance for serum SOD was measured at 450 nm while absorbance for plasma GPx and GR were read once every minute at 340 nm using a plate reader (Bio-Tek Instrument Inc., Winooski, VT) and a software program (KC4, version #3.3, Bio Tek Instruments) to obtain at least 5-time points. The concentration of GPx and GR were expressed as nmol/min/mL while SOD was expressed as U/mL equivalent.

**Stress Indicators**

The rectal temperature of broilers (2 birds/replicate) on d 21 and 27 were measured using a digital thermometer (Accuflex5; A.M.G. Medical Inc.) to confirm induced HS on d 27. The entire probe was inserted into the bird’s cloaca, and the data were automatically displayed on the device. Serum corticosterone was measured using a commercial ELISA kit (Corticosterone Enzyme Immunoassay Kit; Catalog Number K014-H1/H5) following the instructions and recommendations from the manufacturer (Arbor Assays, Ann Arbor, MI).

**Statistical Analysis**

Data were analyzed as a 4 × 2 factorial analysis of variance (ANOVA) using the General Linear Model of Minitab LLC (2019) software for data collected on day 28. Statistically significant differences among dietary treatments (NC, 1 mL SWE, 2 mL SWE and 2% SWM) were identified using one-way ANOVA before the onset of HS. Following ANOVA, significant means were separated using Tukey’s honest significant difference test. Analyzed data were presented as means, standard error of the mean (SEM), and probability values. P-values less than or equal to 0.05 were considered significant.

**RESULTS**

**Growth Performance**

The effect of experimental treatments on the growth performance of broiler chickens is shown in Table 2. Two percent SWM inclusion significantly increased \((P = 0.023)\) the body weight gain and significantly improved \((P = 0.024)\) FCR at the early phase of growth (D 0−7) and significantly increased \((P = 0.001)\) average feed intake at D 7−14 compared to NC. There was no significant interaction between dietary treatment and the temperature model for all the growth performance parameters (D 21−28). HS significantly reduced feed intake \((P < 0.001)\) and FCR \((P = 0.012)\) compared to TN birds. Among the heat-stressed group, broiler chickens fed with 2% SWM and those that received 1 mL and 2 mL SWE in drinking water had numerically improved FCR (19.4%, 26.7%, 22.1%, respectively) compared to those fed the control diet. Overall, the dietary treatment significantly affected \((P < 0.05)\) all the growth performance parameters except for FCR. Birds fed 2% SWM had significantly higher AFI \((P = 0.019)\), ABWG
(P = 0.020), and AWI (P = 0.026) than those that were fed with the control diet alone. Birds that received 1 mL and 2 mL SWE in drinking water had marginally improved (P < 0.05) growth performance parameters, compared to the control.

### Plasma Biochemistry

The effect of the experimental treatments on the blood biochemical indices is presented in Table 3. There was a significant interaction between dietary treatments and HS for plasma lipase (P = 0.002) and alanine aminotransferase (ALT; P < 0.001). In the heat-stressed group, birds that received 1 mL and 2 mL SWE in drinking water had higher (P = 0.042) plasma magnesium concentrations compared to those in the NC treatment. Also, birds fed with 2% SWM had lower plasma lipase (P < 0.001), uric acid (P = 0.017) and gamma-glutamyl transferase (GGT; P = 0.033) concentrations compared to other treatments. The plasma activity of ALT significantly reduced (P < 0.001) in the heat-stressed broilers that received 1 mL SWE in drinking water compared to those that received other treatments. In-water supplementation of 2 mL SWE significantly increased plasma albumin in heat-stressed birds, while 1 mL SWE and 2% SWM marginally increased albumin levels compared to the control treatment (P = 0.039). Concerning the temperature model, significantly higher plasma concentrations of sodium (P = 0.038), chloride (P = 0.024), glucose (P < 0.001), amylase (P = 0.046), and uric acid (P = 0.012) and reduced concentrations of urea (P = 0.002), lipase (P < 0.001), and creatine kinase (P = 0.045) concentrations were recorded among the

### Table 2. Effect of brown seaweed meal and extract on the growth performance parameters of broiler chickens before and during HS conditions.

| Parameters | NC | 1 mL SWE | 2 mL SWE | 2% SWM | Diet | Temp. Model | P-values |
|------------|----|----------|----------|--------|------|-------------|---------|
| AFI (g/bird) | 158| 152 | 161 | 160 | - | - | 7.00 | 0.068 | - |
| BWG(g/bird) | 105 | 109 | 111 | 117 | - | - | 1.44 | 0.026 | - |
| BWG (g/bird) | 65.5 | 68.9 | 70.8 | 77.5 | - | - | 1.45 | 0.023 | - |
| FCR | 2.3 | 2.2 | 2.1 | 1.9 | - | - | 0.11 | 0.024 | - |
| AWI (L) | 0.4 | 0.3 | 0.4 | 0.4 | - | - | 0.01 | 0.427 | - |
| AFI(g/bird) | 190 | 218 | 209 | 235 | - | - | 7.64 | 0.001 | - |
| BWG(g/bird) | 306 | 322 | 323 | 341 | - | - | 5.06 | 0.069 | - |
| BWG (g/bird) | 201 | 212 | 211 | 227 | - | - | 4.03 | 0.159 | - |
| FCR | 1.0 | 1.0 | 1.0 | 1.1 | - | - | 0.04 | 0.508 | - |
| AWI (L) | 0.6 | 0.6 | 0.6 | 0.6 | - | - | 0.02 | 0.793 | - |
| AFI(g/bird) | 537 | 572 | 578 | 611 | - | - | 12.4 | 0.039 | - |
| BWG(g/bird) | 647 | 687 | 688 | 743 | - | - | 13.4 | 0.097 | - |
| BWG (g/bird) | 341 | 366 | 364 | 399 | - | - | 8.97 | 0.166 | - |
| FCR | 1.6 | 1.6 | 1.6 | 1.5 | - | - | 0.03 | 0.739 | - |
| AWI (L) | 0.9 | 1.0 | 1.0 | 1.1 | - | - | 0.02 | 0.016 | - |
| AFI(g/bird) | 627 | 598 | 628 | 687 | 802 | 802 | 19.1 | 0.106 | <0.001 | 0.641 |
| TN | 740 | 808 | 768 | 804 | - | - | 804 | 0.019 | 0.773 | 0.610 |
| BWG(g/bird) | 1037 | 1100 | 1181 | 1272 | 1165 | 1150 | 27.6 | 0.019 | 0.773 | 0.610 |
| TN | 1007 | 1242 | 1128 | 1288 | - | - | 1288 | 0.026 | 0.237 | 0.565 |
| BW (g/bird) | 373 | 429 | 476 | 504 | 485 | 453 | 17.8 | 0.026 | 0.237 | 0.565 |
| TN | 405 | 558 | 480 | 581 | - | - | 581 | 0.025 | 0.354 | 0.333 |
| FCR | 1.66 | 1.39 | 1.31 | 1.36 | 1.6 | 1.4 | 0.07 | 0.161 | 0.012 | 0.582 |
| AWI (L) | 1.53 | 1.80 | 1.68 | 1.85 | 1.7 | 1.7 | 0.03 | 0.025 | 0.354 | 0.333 |
| AFI(g/bird) | 1590 | 1648 | 1662 | 1796 | 1796 | 802 | 19.1 | 0.106 | <0.001 | 0.641 |
| TN | 2088 | 2275 | 2262 | 2474 | - | - | 2474 | 0.019 | - | - |
| BWG(g/bird) | 1130 | 1110 | 1110 | 1241 | - | - | 27.7 | 0.020 | - | - |
| TN | 1.6 | 1.5 | 1.5 | 1.4 | - | - | 0.03 | 0.156 | - | - |
| AFI (g/bird) | 3.4 | 3.7 | 3.5 | 3.9 | - | - | 0.05 | 0.026 | - | - |

a,b, means assigned different lowercase superscript letters are significantly different, P < 0.05 (Tukey’s procedure).

1Dietary treatment includes NC (negative control [corn-soybean meal-wheat−based diet]), 1 mL SWE (chicks supplied brown seaweed extract in water at 1 mL/L), 2 mL SWE (chicks supplied brown seaweed extract in water at 1 mL/L), 2% SWM (chicks fed NC + 2% brown seaweed in feed).

2HS (Heat stress).

3TN (Thermoneutral).

4SEM, Standard error of means. Abbreviations: AFI, average feed intake; AWI, average water intake; BW, body weight; BWG, body weight gain; FCR, feed conversion ratio; Temp, Temperature.
Table 3. Effect of brown seaweed meal and extract on blood biochemistry parameters in broiler chickens under TN and HS conditions.

| Parameters                  | Thermoneutral (TN) Dietary treatments | Heat stress (HS) Dietary treatments | Temp. Model | P-value          |
|-----------------------------|---------------------------------------|-------------------------------------|-------------|-----------------|
|                             | NC 1 mL SWE 2 mL SWE 2% SWM           | NC 1 mL SWE 2 mL SWE 2% SWM         | TN HS       | PooledSEM<sup>2</sup> |
|                             |                                       |                                     | Diet Temp.  | Diet X Temp.    |
| Electrolytes and minerals, mmol<sup>-1</sup> |                                       |                                     |             |                 |
| Sodium                      | 144 144 146 146                       | 147 147 148 146                     | 145.5<sup>b</sup> 147.2<sup>b</sup> | 0.45          |
| Potassium                   | 7.56 6.84 7.10 6.36                    | 6.5 7.5 6.6 6.5                     | 0.67 6.66    | 0.16            |
| Chloride                    | 107 106 107 108                       | 109 109 109 108                     | 107<sup>c</sup> 109<sup>c</sup> | 0.4            |
| Calcium                     | 1.91 1.70 1.77 1.94                    | 1.88 1.89 2.11 1.93                 | 1.8 1.9      | 0.04            |
| Phosphorus                  | 2.12 1.72 1.99 1.85                    | 1.84 1.95 1.82 1.87                 | 1.8 1.8      | 0.05            |
| Magnesium                   | 0.75 0.68 0.73 0.69                    | 0.66<sup>c</sup> 0.72<sup>c</sup> 0.73<sup>b</sup> 0.69<sup>b</sup> | 0.7 0.7      | 0.01            |
| Urea                        | 0.38 0.40 0.42 0.42                    | 0.37 0.33 0.36 0.34                 | 0.4<sup>a</sup> 0.3<sup>b</sup> | 0.01            |
| Glucose                     | 13.0 12.8 13.0 13.1                    | 13.2 14.0 13.7 13.4                 | 12.9<sup>b</sup> 13.6<sup>b</sup> | 0.08            |
| Cholesterol                 | 2.52 2.68 2.71 2.59                    | 2.69 2.69 2.78 2.57                 | 2.6 2.7      | 0.03            |
| Uric acid                   | 320 336 338 349                       | 340<sup>b</sup> 386<sup>b</sup> 485<sup>a</sup> 336<sup>b</sup> | 324<sup>b</sup> 373<sup>b</sup> | 13.4           |
| Metabolites, mmol<sup>-1</sup> |                                       |                                     |             |                 |
| Alanine                     | 472 509 545 527                       | 594 560 608 529                     | 479<sup>b</sup> 594<sup>a</sup> | 28.6           |
| Creatine kinase             | 4382 6266 4087 5872                    | 5294 4043 5264 3387                 | 4364 4420    | 28.6           |
| Cholesterol                 | 14420 7550 11630 12502                 | 4337 4338 4478 5002                 | 6001<sup>a</sup> 4527<sup>b</sup> | 1045           |
| Lipase                      | 10.1 10.7 10.3 11.3                    | 11.9<sup>b</sup> 13.2<sup>a</sup> 11.8<sup>b</sup> 9.5<sup>b</sup> | 10.3 11.4   | 0.34           |
| ALT<sup>6</sup>             | 21.1 21.0 27.4 22.3                    | 18.0<sup>a</sup> 21.0<sup>a</sup> 18.4<sup>a</sup> 10.3<sup>b</sup> | 22.5<sup>a</sup> 17.1<sup>b</sup> | 0.8            |
| GGT<sup>5</sup>             | 10.5 11.5<sup>c</sup> 10<sup>b</sup>   | 7<sup>c</sup> 7.9<sup>b</sup> 6.2<sup>b</sup> 9.5<sup>b</sup> | 7.8 8.9    | 0.42           |
| Proteins, gL<sup>-1</sup>   |                                       |                                     |             |                 |
| Total protein               | 25.67 25.42 25.50 25.83                 | 25.5 25.1 28.7 26.0                  | 25.2 26.2   | 0.34           |
| Albumin                     | 10.4 10.4 10.4 10.3                    | 10.2 10.4 11.3 10.8                 | 10.4 10.7   | 0.1            |
| Globulin                    | 15.3 15.0 15.1 15.5                    | 15.1 14.9 16.3 15.2                 | 14.8 15.4   | 0.28           |

<sup>a, b, c</sup> means assigned different lowercase superscript letters are significantly different, <i>P</i> < 0.05 (Tukey’s procedure).

<sup>1</sup>Dietary treatment includes NC (negative control [corn-soybean meal-wheat-based diet]), 1 mL SWE (chicks supplied brown seaweed extract in water at 1 mL/L), 2 mL SWE (chicks supplied brown seaweed extract in water at 1 mL/L), 2% SWM (chicks fed NC + 2% brown seaweed in feed).

<sup>2</sup>SEM, Standard error of means.

<sup>3</sup>ALP, Alkaline phosphatase.

<sup>4</sup>AST, Aspartate Aminotransferase.

<sup>5</sup>GGT, Gamma-Glutamyl Transferase.

<sup>6</sup>ALT, Alanine Transferase.

AKINYEMI AND ADEWOLE
heat-stressed chicks, compared to TN birds. The remaining tested plasma biochemistry parameters were not significantly affected by heat stress and brown seaweed products supplementation.

**Stress Indicators, Immune Response, and Antioxidants Status**

The effect of HS on broilers’ rectal temperature and corticosterone concentration is shown in Figure 1. On d 21, before the exposure to HS, the rectal temperatures of birds in the 2 rooms were not different (average of 41.1°C in each room). However, on d 27, high ambient temperature significantly elevated ($P < 0.001$) the rectal temperature of birds in the HS room (average of 42.2°C) compared to those in the TN rooms (average of 41.6°C). The dietary treatments had no significant effect on the bird’s rectal temperature ($P = 0.752$). Corticosterone level was not affected by HS ($P = 0.459$) and dietary treatments ($P = 0.621$), though the corticosterone level of heat-stressed birds was numerically higher than the TN birds (5.2%).

The effect of SWM and SWE on the relative weight of immune organs, serum immunoglobulins, interleukins, and antioxidant status in broiler chickens challenged with or without HS is shown in Table 4. There was no significant interaction between dietary treatments and the temperature model. The immune organs’ weights (bursa and spleen), serum IgG, IgM, plasma IL6, IL10, serum SOD, plasma GPx, and GR were not significantly affected by SWM and SWE supplementation in-feed and in-water. HS increased plasma IL6 ($P < 0.001$), serum IgM ($P < 0.001$) and IgG ($P = 0.002$) concentrations, compared to TN birds. Broiler chickens challenged with HS exhibited lower ($P < 0.001$) plasma GR and GPx compared to TN birds, while the activity of serum SOD, plasma IL10, and immune organ weights were not affected by HS.

**DISCUSSION**

Seaweed contains unique bioactive compounds and has been applied in poultry feed because of its richness in carbohydrates, minerals, protein, vitamins, and
dietary fibers, with relatively stable amino acid profiles and growth-stimulating properties (Kulshreshtha et al., 2020). Several studies have reported the potential benefits of different species of seaweed in layers (Choi et al., 2014; Kulshreshtha et al., 2014; Borzouie et al., 2020), ducks (El-Deek and Brikaa, 2009), and broiler chickens (Abudabos et al., 2013; Mohammadighisar et al., 2020) in terms of performance, blood parameters and gut health. However, there are limited studies on the potential effect of brown seaweed (Ascophyllum nodosum) meal and extract on broiler chickens challenged with HS. Here, we investigated for the first time the effect of brown seaweed meal and extract on the growth performance, plasma biochemistry indices, immune response, and antioxidant capacity of broiler chickens challenged or unchallenged with HS.

Our study showed that 2% SWM inclusion improved the BWG, FCR, AFI, and AWI overall regardless of HS. This was in accordance with Choi et al. (2014) and Kulshreshtha et al. (2014), who found a significant improvement in the growth performance of layers fed with 0.5% brown and red seaweed, respectively. In a study by Kumar (2018), dietary supplementation of Sargassum wightii (genus of brown seaweed) at 1%, 2%, 3%, and 4% enhanced BW, FI, FCR, and meat quality of broilers. Conversely, 0.5%, 1%, and 2% brown seaweed in broiler chickens’ diet did not influence the ABG, FI, or FCR of broiler chickens in the study by Bonos et al. (2016). The authors attributed this to several factors like housing conditions, basal diet, and production system (Bonos et al., 2016). The growth-promoting effects of brown seaweed leading to the increment of AFI and BWG in our study could be ascribed to its richness in fiber content (Table 1) and laminarin. Evidence revealed that dietary fiber could improve digestibility, feed intake, and overall growth performance in poultry production (Rezaei et al., 2014; Varastegani and Dahan, 2014; Nassar et al., 2019). Laminarin interacts with a surface layer of the small intestine to improve nutrient digestibility, positively affecting broiler chickens’ growth performance parameters (Sweeney et al., 2017). In addition, HS reduced the AFI of broiler chickens by 20.9% (about 167 g/bird) compared to TN birds which was consistent with past findings (Awad et al., 2020; Kikusato et al., 2021; Iyasere et al., 2021) while the body weight was unaffected by HS in our study. The decrease in feed intake among HS birds is a recovery mechanism to reduce body-heat increment (Song et al., 2013). There was no significant interaction between the dietary treatments and HS for growth performance parameters; however, among heat-stressed birds, we observed that the inclusion of brown seaweed meal and 2 mL brown seaweed extract numerically improved FCR by 18 and 21%, respectively, compared to NC. Studies on ruminants indicated that dietary supplementation with 2 and 4% brown seaweed meal improved productive performance through improving growth rate and blood constituents in heat-stressed Barki ram lamb (Ibrahim et al., 2020). Similarly, 4% Sargassum latifolium significantly improved body weight gain in heat-stressed exposed sheep (Ellamie et al., 2020). Meanwhile, dietary supplementation of seaweed (Ulva lactuca) did not enhance production performance in growing lambs reared under HS conditions (Abdoun et al., 2014). Taken together, our results suggest that dietary supplementation of 2% SWM improved palatability while increasing AFI, leading to improved BWG of broiler chickens compared to those fed the control diets.

The cloaca temperature and corticosterone level can reflect the degree of HS in broiler chickens. The cloaca temperature is a sign of balance between heat loss and gain, reflecting the central body temperature (Farag and Alagawany, 2018). The balance will be disrupted when the thermoregulatory mechanisms of birds are compromised (Selvam et al., 2018). The present results revealed that the rectal temperature of broiler chickens under HS treatment significantly increased, following HS exposure at 8hr/day for 7 d, indicating that the birds were stressed. Similarly, Altan et al. (2000) reported that broiler chickens challenged with HS (38 ± 1°C) for 2 h had significantly increased rectal temperature compared to the TN group. He et al. (2019) revealed that heat-stressed birds had significantly increased rectal temperature from the third time point to the end of the cyclic HS while a polyphenolic compound suppressed the increase. In our study, dietary treatments did not influence the rectal temperature. Aside from rectal temperature, corticosterone is secreted in response to physiological stress (Zeng et al., 2022). Studies have shown that cyclic HS markedly increased serum corticosterone concentration in broilers compared to the control group (Cheng et al., 2018; He et al., 2018). On the contrary, the corticosterone level was not affected by HS in our study; this agrees with Sun et al. (2015), who reported that exposing broiler chickens to 10 h HS (32°C) for 7 d did not influence their plasma corticosterone. The authors further explained that the effect of HS on plasma corticosterone is associated with the length of heat exposure (Sun et al., 2015). Acute heat exposure (32°C) for 3 and 6 h did not change the corticosterone level of birds as well (Lin et al., 2006). HS triggers the activity of the neuroendocrine system, resulting in activation of the hypothalamic-pituitary-adrenal axis, which subsequently elevates corticosterone levels (He et al., 2018).

HS can further cause metabolic disorders in broiler chickens. Our blood biochemistry analysis showed altered plasma electrolytes and metabolites in response to HS. The increase in plasma sodium, chloride, glucose, and uric acid among heat-stressed birds has been confirmed by past studies (Harsini et al., 2012; Hajati et al., 2015; Xie et al., 2015; Huang et al., 2018). Huang et al. (2018) observed increased sodium and decreased chloride concentration among birds exposed to 2 h HS (38 ± 1°C). Alterations in sodium and chloride concentrations can indicate kidney damage. As sodium and chloride excretion levels increase, their loss could result in acid-base disorder, which is detrimental to the kidney (Farag and Alagawany, 2018). Glucose and uric
acid levels are good indicators of stress. Uric acid is excreted as an end product of nitrogen metabolism and can scavenge free radicals in birds (Hajati et al., 2015; Kikusato et al., 2021). Under HS conditions, catecholamines deplete liver glycogen and increased glucose level in the blood (Wasti et al., 2020). In the current study, dietary treatments did not influence sodium, chloride, and glucose level but 2% SWM supplementation suppressed plasma uric acid concentration in heat-stressed treated chickens. Hence, 2% SWM might mitigate oxidative damage by eliminating the need to produce uric acid (Kikusato et al., 2021). Phytogenic feed additives can lower blood uric acid through their antioxidant ability to depress the adeno-corticotrophic hormone responsible for uric acid production (Kanani et al., 2016).

The increase in blood enzymes levels denote consequent enzyme leakage and could result from hepatocyte deterioration (Tessari et al., 2010). Specifically, high levels of AST, ALT, and GGT are sensitive indicators of liver damage (Will Castro et al., 2016). HS can cause liver damage that can enhance the serum levels of AST and ALT in chickens because of enzyme leakages from hepatocytes (Chang et al., 2020). In the current study, 2% SWM and 1 mL SWE decreased the plasma ALT and GGT of birds under HS conditions; respectively, which implies an amelioration of hepatic damage (Erinle et al., 2022) among treated birds. Dietary inclusion of galactofucan sulfate in brown seaweed reduced the activity of ALT, GGT, and AST in rats (Will Castro et al., 2016). We speculated that the hepatoprotective activity of seaweed on heat-stressed birds is associated with its richness in fucoidans that have been found to prevent induced liver damage via regulating inflammatory mediators (Lim et al., 2015). Fucoidan, a sulfated polysaccharide extract from brown seaweeds, consistently reduced plasma activity of ALT and AST and ameliorated hepatic accumulation in mice (Zheng et al., 2018). Brown seaweed meal and extract also increased plasma albumin concentration among heat-stressed birds in our study, indicating better liver functioning (Nhlane et al., 2020). Choi et al. (2018) reported the greatest blood albumin level among birds fed with brown seaweed products compared to other treatments. A linear increase in albumin concentration due to seaweed supplementation was previously observed by Balasubramanian et al. (2021).

Moreover, HS can impair intestinal absorption by reducing plasma digestive enzymes (Al-Zghoul et al., 2019). In the current study, HS decreased lipase activity compared to TN birds, and this finding agrees with Song et al. (2018), who reported reduced jejunal lipase activity after HS treatments. The lipase activity was enhanced by 1 mL SWE supplementation compared to 2% SWM in heat-stressed birds in our study, indicating that 1 mL SWE exerts a positive effect on the digestive capacity of broilers under HS and could be related to the anti-inflammatory and antibacterial activity of the seaweed extract (Wu, 2018). Several studies have revealed that polysaccharides from bioactive substances can improve the activities of lipase in broiler chickens (Hashemipour et al., 2013; Long et al., 2020). The significant difference in the effects of brown seaweed extract and meal on digestive enzymes might be due to the ability of the polyphenol contents of seaweed to bind with feed and gut contents forming insoluble complexes (Abdel-Moneim et al., 2020). In-water treatments are generally absorbed better than in-feed treatments. We also observed an increase in amylase activity in response to HS, contrary to previous studies (Huang et al., 2018; Jaiswal et al., 2018). The reason amylase activity increased with HS was not entirely understood; a possible explanation may be due to modulatory actions of brown seaweed in HS groups. An alternative hypothesis could be that, during heat stress, the body wants to limit the overall heat produced by reducing feed intake. It is possible that a higher amylase activity may help this process by improving starch digestion and that ultimately may help to reduce the feed intake. Among the heat-stressed birds, birds that received SWM and SWE had higher amylase levels than the control diet; however, this was not statistically significant. Overall, our results indicated that brown seaweed products might serve as a mitigative nutritional strategy to sustain liver functioning and improve chickens’ digestive enzymes under HS.

Regarding immunoglobulins, there was no interaction between the dietary treatment and HS in the current study. Meanwhile, HS increased serum concentrations of IgG and IgM compared to TN birds in our present study, similar to Honda et al. (2015) findings. Our findings also agree with Nanto-HARA et al. (2021), who reported that HS (33°C for 14 d) activated immune responses by increasing plasma levels of immunoglobulins (IgY and IgM). Contradictorily, several articles have shown lower levels of IgA, IgM, and IgG in broiler chickens exposed to HS (Awad et al., 2020; Hu et al., 2022; Abdel-Moneim et al., 2022). Variation in results might be attributed to the duration of HS exposure, level of HS, time of sample collection, and presence of other stressors. For example, Honda et al. (2015) reported increased levels of IgG and IgM in heat-stressed chickens on d 7, and other results revealed lower concentrations of IgM on d 19 in heat-stressed vaccinated birds. Stressors can increase the levels of immunoglobulins at least 24 h after the end of stress stimuli application (Honda et al., 2015). Acute HS has also been discussed to improve immune function, while chronic heat stress has been shown to suppress immunity (Han et al., 2010).

Furthermore, the present study had no treatment effect on the relative weight of the bursa and spleen. Matshogo et al. (2020) reported no changes on the intestinal organs except the decrease in the spleen in response to SWM intake in broilers. On the other hand, the dietary inclusion of Laminaria japonica, a type of seaweed, increased the weights of spleen, bursa, and thymus in 21 and 41-day-old broilers (Bai et al., 2019). Additionally, 0.4% red seaweed (Chondrus crispus) increased the relative weights of thymus and bursa (Martínez et al., 2019). Immune organ weights can reflect the immune status of chickens. The present findings signify that our HS protocol and dietary brown seaweed used in this
study had no detrimental effects on the immune organs of chickens.

Cytokines are also important markers of immunity that can enhance host defence for controlling infection (Kaiser et al., 2009). Interleukins are major cytokines that play vital roles in stimulating inflammation and immune responses under HS conditions (Goel et al., 2021), in which excessive expression may lead to tissue damage. The present study showed an increase in the secretion of IL6 in response to HS while the IL10 remain unaltered. IL6 could act as a proinflammatory and anti-inflammatory cytokine. IL10 is produced simultaneously with proinflammatory cytokines and plays a central role in the anti-inflammatory response by preventing inflammatory pathology (Hietbrink et al., 2006). Brown seaweed extract can inhibit inflammatory responses by reducing the secretion of IL6 (Saeed et al., 2021). Similarly, dietary supplementation of seaweed-derived polysaccharides reduced the levels of pro-inflammatory cytokines and splenic inflammatory response of heat-stressed birds by suppressing the nuclear factor-kappa B signaling (Liu et al., 2022b). Nonetheless, dietary supplementation of brown seaweed meal and extract had no significant effects on the expression of IL6, and IL10 in chickens challenged or unchallenged with HS in the current study.

Brown seaweed can also improve the animal antioxidant defence systems and regulate their inflammatory responses (Ramadan et al., 2020; Michalak et al., 2022). Our results showed that dietary supplementation of brown seaweed meal and extract did not influence antioxidant enzymes (GPx, GR, and SOD). This agrees with Paul et al. (2020) that red seaweed (Kappaphycus alvarezii) did not affect most of the antioxidant enzymes in broiler chickens. In contrast, Kannan et al. (2007) observed that the dietary supplementation of brown seaweed powder increased the antioxidant status of goats exposed to preslaughter stress. Liu et al. (2021) found that dietary algae-derived polysaccharides supplementation improved the antioxidant capacity in the duodenum of broilers through the regulation of the nuclear factor erythroid 2-related factor 2 signaling pathway observed in the study (Liu et al., 2021). Ellamie et al. (2020) reported that 4% Sargassum latifolium improved the total antioxidant capacity and the activities of catalase and SOD in the sheep exposed to HS (Ellamie et al., 2020). Similarly, Saker et al. (2004) observed increased GPx and SOD and decreased MDA in heat-stressed lambs fed with brown seaweed (Tasco). SOD, GPx, and GR are essential antioxidant enzymes in the body, and their activities indirectly show the body’s capability to combat oxidative stress (Hu et al., 2020). SOD is the first line of defence against ROS, while GPx catalyzes the reduction of hydrogen peroxides by reduced glutathione to preserve cells from oxidative damage (Nagami et al., 2005). HS further reduced GPx and GR while SOD was unaffected. The decreased antioxidant enzymes observed in this study confirmed the claim from multiple studies (Ahmed-Farid et al., 2021; Song et al., 2018; Hu et al., 2021; Wang et al., 2021) that HS can induce oxidative stress by disrupting the redox status (Wang et al., 2021). The antioxidant capacity and radical scavenging activity of seaweed are positively correlated with flavonoid concentration and the presence of flavonoids can be influenced by seaweed geographical area, harvest time, period of collection and extraction methods (Cotas et al., 2020). The effect of brown seaweed on the antioxidant status of chicken in our present study might be affected by several factors such as concentration and composition of polyphenols, the bioavailability of molecules and the type of chickens (Gessner et al., 2017). Liu et al. (2022b) speculated that the effect of seaweed treatments on the antioxidants-related gene expression could be dose-dependent. Lowered polyphenol contents in seaweed species are associated with oxidation and polymerization of phlorotannins, reflecting the antiproliferative effects and the quantity of secondary metabolites in seaweed (Yuan and Walsh, 2006).

In conclusion, our results indicated that HS reduced feed intake and affected blood biochemical parameters and immunoglobulins, while dietary supplementation of brown seaweed products improved the growth performance of birds irrespective of HS and may help to mitigate the negative effects of HS by improving the plasma and digestive enzyme activities of heat-stressed birds.

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DISCLOSURES

The authors declare no conflict of interest.

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12 AKINYEMI AND ADEWOLE

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