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Dietary supplementation of brown seaweed (*Sargassum latifolium*) alleviates the environmental heat stress-induced toxicity in male Barki sheep (*Ovis aries*)

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ABSTRACT

Heat stress (HS) is the most potent environmental stressors for livestock in tropical and subtropical regions. HS induced splanchnic tissue hypoxia and intestinal oxidative damage, leading to endotoxemia and systemic inflammation. The present study evaluated and compared the modulatory effects of feeding Barki male sheep (*Ovis aries*) on a standard concentrated diet containing 2% or 4% of the brown seaweed (*Sargassum latifolium*) followed by roughage for 40 consecutive days on the toxicity-induced by exposure to severe environmental HS (temperature-humidity index = 28.55 ± 1.62). The present study showed that the diet containing *Sargassum latifolium* (especially 4%) modulated significantly ($P < 0.05$–0.001) almost all changes shown in the HS-exposed sheep including the increase in the thermo-respiratory responses (skin and rectal temperatures, and respiration rate) and the resulted dyslipidemia, anemia, and systemic inflammation (blood leukocytosis, the elevation in the erythrocyte sedimentation rate, and the increase in serum proinflammatory cytokines and heat shock protein-70 concentrations). In addition, *Sargassum latifolium* improved significantly ($P < 0.05$–0.001) the body-weight gain, kidney functions (especially at the high dose), and blood antioxidant defense system (total antioxidant capacity, and the activities of catalase and superoxide dismutase) in the HS-exposed sheep, as well as protected the animals from oxidative tissue damage and the risk of atherosclerosis. In conclusion, feeding sheep with the diet containing 4% of *Sargassum latifolium* was safe and suitable for animal nutrition, as well as efficiently alleviated the harmful effects of the environmental HS in Barki sheep through improving the animal antioxidant defense system, and regulating the thermo-respiratory and inflammatory responses.

1. Introduction

Heat stress (HS), the potential environmental stressor for livestock in the tropical and subtropical regions, is the most concerning issue nowadays in the ever changing climatic scenario. HS adversely affects the animal health, growth, lactation and reproductive performances, as well as increases the animals' mortality rate. Therefore, the environmental HS may cause a tremendous economic loss for the industries dependent on the livestock products (Al-Dawood, 2017). During HS, ruminants increase avenues of heat loss and reduce heat production in an attempt to maintain euthermia. The immediate responses of ruminants to environmental heat load are the increase in pulsation, respiration rate (RR), and water intake, as well as the decrease in feed intake (Sejian et al., 2017). Alterations in the hormonal profile of ruminants in response to environmental HS also aimed to decrease the endogenous heat production and protein anabolism, as well as to increase the heat dissipation and protein catabolism (Aleena et al., 2016). Continuous exposure of ruminants to high ambient temperature led to multi-organs

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CAT, catalase; CT, coat temperature; ESR, erythrocyte sedimentation rate; HDL, high-density lipoproteins; HS, heat stress; HSP(s), heat shock protein(s); IL, interleukin; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MDA, malondialdehyde; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; NOx, nitric oxide; PG, prostaglandins; RNS, reactive nitrogen species; ROS, reactive oxygen species; RR, respiration rate; RT, rectal temperature; SOD, superoxide dismutase; ST, skin temperature; TAC, total antioxidant capacity; THI, temperature humidity index; TNF, tumor necrosis factor.

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injury and circulatory dysfunction through increasing the blood flow to the skin and lungs, and hence reducing the blood flow to the critical splanchnic tissues such as the intestine and liver (Rathwa et al., 2017). The reduction of splanchnic blood flow caused cellular hypoxia, which enhanced the generation of oxygen- and nitrogen-derived free radicals over the cellular antioxidant capacity, leading to the possibility of intestinal oxidative damage and the translocation of bacteria "and their toxins" across the intestinal lumen into the blood circulation (de Punder and Pruimboom, 2015). Therefore, HS augmented the oxidative tissue damage and systemic inflammation.

Many reports suggested recently the beneficial effects of marine macroalgae, especially edible brown seaweeds, on animal health. Brown algae are rich in sulfated polysaccharides and polyphenols (e.g. flavonoids, phlorotannins, and bromophenols) that exhibit potential antioxidant properties. The most important powerful antioxidant pigments in brown algae are β-carotene, fucoxanthin, and tocopherol; since they are able to quench and scavenge the reactive oxygen species (ROS), and act as anticancer and antiobesity agents (Anis et al., 2017). In addition, brown seaweeds have been more exploited in animal feeding than other marine algal types, because of their large size and ease of harvesting. *Sargassum* spp. are brown algae that are widely distributed in subtropical and temperate oceans and seas. Therefore, they may be applied as feed additives to animals in such regions to relieve the negative effect of HS. They exhibited a large economic importance and ecological dominant among other brown algae (Makkar et al., 2016). There are many compounds that had been derived from *Sargassum* spp. with a broad range of biological activities. Sulfated polysaccharides are one of the isolated bioactive compounds from *Sargassum* spp. that possessed anticancer, antiapoptotic, antioxidant, and anti-inflammatory activities (Sanjeewa et al., 2017; Wu et al., 2016). Some studies including our previous study described the chemical constituents of the Egyptian *Sargassum* spp., which were collected from the Red Sea coasts, and reported that *Sargassum* spp. could be considered as promising natural sources of the antioxidants and anti-inflammatory compounds (Fawzy et al., 2017; Fouda et al., 2019). Among all collected *Sargassum* spp. from the Red Sea at Hurghada coast during spring season, *Sargassum latifolium* is the most abundant one that exists in large communities and forms large floating mats. In addition, *Sargassum latifolium* is rich in most of the nutritional and bioactive components (Fouda et al., 2019). Therefore, the present study aimed to evaluate and compare the alleviative effects of different doses of *Sargassum latifolium* "as dietary supplementation" on the toxicity-induced in Barki male sheep by exposure to the environmental HS; with special reference to the thermo-respiratory responses, oxidative stress, and inflammatory mediators. Furthermore, the present study investigated any deleterious effects caused by consuming *Sargassum latifolium*.

2. Materials and methods

2.1. Collection and biochemical analyses of Sargassum latifolium

Sargassum latifolium (order: Fucales, family: Sargassaceae) were collected from the Red Sea at Hurghada coast (Egypt) during spring season, from April to June. Epiphytic and extraneous matters were removed by washing several times with tap water and then distilled water to separate different impurities. The algal samples were dried in the air at room temperature to constant weight, powdered, and kept in plastic bags. Almost all biochemical analyses of *Sargassum latifolium* were previously performed by us using the official methods of analysis of the Association of Official Analytical Chemists, inductively coupled argon plasma (ICAP 6500, Duo Thermos scientific, England), automatic amino acid analyzer (S 433, Sykam GmbH, Eresing, Germany), and high performance liquid chromatograph (HP 3000 series, Agilent Technologies, Newtown, PA, USA) equipped with an auto-sampler and a diode-array detector (Fouda et al., 2019). The biochemical analyses revealed that *Sargassum latifolium* had a high content of carbohydrates (41.4%), fibers (7.0%), and high quality proteins (4.4%, essential amino acids index = 41.9), but very low amount of fat (0.3%). The amounts of vitamin B12 and vitamin C were 232.3 ppm and 6.9 ppm, respectively, in *Sargassum latifolium*. In addition, *Sargassum latifolium* provided the essential minerals required for animals health such as potassium (35.0 ppm), calcium (15.2 ppm), magnesium (9.264 ppm), iron (0.7 ppm), phosphor (0.6 ppm), and zinc (0.3 ppm); while the concentrations of heavy metals in *Sargassum latifolium* were below the toxic limits (Fouda et al., 2019). *Sargassum latifolium* was also rich in the most active antioxidant components such as alkaloids (760.0 μg/g dry weight), phenolic compounds (987.6 μg gallic acid equivalent/g dry weight) especially kaempherol (151.8 ppm), and flavonoids (908.2 μg catechin equivalent/g dry weight) as estimated by a Folin-Ciocalteu reagent (Singleton et al., 1999), in addition to carotenes (2.5 mg β-carotene equivalent/g dry weight) as determined by a spectrophotometric method (Costache et al., 2012). The *in vitro* free radical scavenging activity of *Sargassum latifolium* was 184.96 μg ascorbic acid equivalent/g dry weight, as measured by using 1,1-diphenyl-2-picryl-hydrazyl (Grzegorczyk et al., 2007).

2.2. Animals

Healthy growing Barki male sheep (*Ovis aries*) during pre-puberty stage, 8–10 months old and weighing 29.7 ± 0.7 kg, were obtained from the Ras Sedr station of the Desert Research Center. The animals were housed in suitable yards under veterinary care according to the program of the Ministry of Agriculture; and the study design was carried out in accordance with the EC Directive 86/609/EEC guidelines for the animal experiments, and approved by the Research Ethics Committee at Faculty of Science, Ain Shams University (6/2014) prior to the commencement of the study. Animals were provided with fresh tap water and fed daily on a preservative ruminant diet: standard concentrated pellets (2.5% of animal's body weight), with/without *Sargassum latifolium*, and wheat-hay (1.5% of animal's body weight) as roughage. The concentrated pellets contain 50% under-corticated cotton seed, 18% wheat-bran, 15% yellow maize, 11% rice polish, 3% molasses, 2% lime stone, and 1% common salt. The concentrated pellets have 45% carbohydrates, 14% proteins, 2% fats, and 15% fibers. Table 1 showed the overall composition of the standard concentrated diet containing 0, 2%, 4% *Sargassum latifolium*.

2.3. Measurements of environmental conditions

The environmental conditions around the animals were measured daily at 2:00 p.m. Some environmental conditions as day length, humidity, ultra violet index (UVI), wind speed, and dew point were obtained from the Egyptian Meteorological Authority, Cairo. Solar radiation was recorded inside each yard by using black globe thermometer to determine the actual heat load on the animals (Purswell and Davis, 2008). Floor and wall temperatures were also measured in the animals' yards by using a digital telethermometer. The

Table 1
Overall composition of the standard concentrated diet containing 0, 2%, or 4% *Sargassum latifolium*.

	Standard concentrated diet with/without brown seaweeds		
	0% *Sargassum latifolium*	2% *Sargassum latifolium*	4% *Sargassum latifolium*
Carbohydrate (%)	45.00	44.93	44.86
Protein (%)	14.00	13.81	13.62
Fat (%)	2.00	1.96	1.93
Fiber (%)	15.00	14.84	14.68
Ash (%)	9.00	9.34	9.68
Moister (%)	12.00	13.52	15.05

severity of environmental HS was estimated by temperature-humidity index (THI) according to the following equation:

THI = Ambient temperature (°C) – [(0.31 – 0.31 relative humidity) × (Ambient temperature – 14.4)]

Where, THI < 22.2 means absence of HS, THI = 22.2 and < 23.3 means moderate HS, and THI ≥ 23.3 means severe HS (Marai et al., 2001).

2.4. Experimental design and treatment schedule

The present study was performed in Ras Sedr station of the Desert Research Center. After 10 days of animal acclimatization to the yard conditions, thirty animals were randomly and equally divided into 6 groups (3 control groups and 3 HS groups) as follows: In the control groups, animals lived in comfortable environmental conditions in the spring season (THI was < 22.2) without exposure to solar radiation and were fed on a standard concentrated diet containing 0, 2%, or 4% *Sargassum latifolium* followed by roughage for consecutive 40 days. In HS groups, animals were exposed daily to solar radiation from 8:00 am to 5:00 pm in the summer season (THI was ≥ 23.3) and were fed on a standard concentrated diet containing 0, 2%, or 4% *Sargassum latifolium* followed by roughage for consecutive 40 days. The daily environmental conditions, at 2:00 pm, during the experimental period around the control and HS groups were shown in the Table 2. The HS groups were exposed to significantly higher ($P < 0.05$–0.001) ambient temperature, THI, and solar radiation compared with the control groups indicating severe HS (THI was 28.55 ± 1.62 in the HS groups *versus* 18.77 ± 1.17 in the control groups, Table 2).

2.5. Blood sampling

Blood samples were collected from the jugular vein of each animal every 10 days during the experimental period at 7:00 am before offering the ration and water. The blood samples were collected into clean centrifuge tubes with or without EDTA. A portion of blood with EDTA was used for performing the complete blood picture analysis by HA-VET coulter (Clinding, Belgium), determining the erythrocyte sedimentation rate (ESR) by Westergren method, the erythrocyte superoxide dismutase (SOD) activity, and separating plasma. Another blood portion without EDTA was left to coagulate at room temperature and the clotting time was recorded using a stopwatch. After separating the clot by centrifugation in a cooling centrifuge (IEC centra-4R; International Equipment Co., Needham Heights, MA, USA) for 30 min at 3000 rpm and 4 °C, the serum was collected by a long Pasteur pipette, divided into samples and preserved at −80 °C.

Table 2
The environmental conditions (at 2:00 pm) around and inside the sheep yards during the experimental period (40 days) of the control and HS groups.

Environmental conditions	Control groups	HS groups
Ambient temperature (°C)	23.48 ± 0.75	37.00 ± 0.82**
Dew point (°C)	9.03 ± 1.91	13.63 ± 2.35
Sheep yards floor temperature (°C)	22.75 ± 0.69	59.10 ± 2.72***
Sheep yards wall temperature (°C)	25.18 ± 0.63	47.04 ± 1.13***
Solar radiation (°C)	34.50 ± 0.65	51.25 ± 1.32***
Wind speed (km/h)	15.00 ± 0.71	13.98 ± 2.76
Humidity (%)	33.75 ± 2.66	39.50 ± 4.44
THI	21.59 ± 0.53	32.75 ± 0.58*
UVI	6.25 ± 0.25	8.00 ± 0.41

Data are presented as mean ± standard errors. HS: heat stress, THI: temperature humidity index, UVI: ultraviolet index. Day length did not significantly change ($P > 0.05$) between the time of performance the experiments in spring (12.28 ± 0.19 h) and summer (13.23 ± 0.16 h) seasons. Mean values were significantly different compared with the control group: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Repeated Measures ANOVA with Bonferroni's Multiple Comparison Test).

2.6. Measurements

The animal thermal responses such as coat, skin and rectal temperatures (CT, ST, and RT), as well as RR, were measured for all animals at 2:00 pm every 10 days during the experimental period. The animals were weighted before morning feeding and drinking at the beginning of the experiment (day 0) and every 10 days during the experimental period to calculate the body-weight gain or loss.

Serum glucose, total protein, albumin, total lipids, triacylglycerol, total cholesterol, high-density lipoproteins (HDL)-cholesterol, creatinine, urea, and malondialdehyde (MDA) concentrations, serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and lactate dehydrogenase (LDH) activities, plasma catalase (CAT) and erythrocyte SOD activities, and plasma total antioxidant capacity (TAC) were determined by using commercial kits from Spectrum Diagnostics (Cairo, Egypt), Reactivos GPL (Barcelona, Spain), and Bio-diagnostic (Giza, Egypt). Serum globulins, low-density lipoprotein (LDL)-cholesterol, and blood urea nitrogen concentrations, in addition to the atherogenic indices, were calculated by the following equations:

Serum globulins concentration (g/dL) = Serum total protein (g/dL) – Serum albumin (g/dL)

Serum LDL-cholesterol concentration (mg/dL) = Serum total cholesterol (mg/dL) – [Serum triacylglycerol (mg/dL)/5] – Serum HDL-cholesterol (mg/dL)

Blood urea nitrogen concentration (mg/dL) = Serum urea (mg/dL) / 2.14

Serum atherogenic index$^{(1)}$ = Total cholesterol (mg/dL) / HDL-cholesterol (mg/dL)

Serum atherogenic index$^{(2)}$ = LDL-cholesterol (mg/dL) / HDL-cholesterol (mg/dL)

Serum immunoglobulins (IgM and IgG), interleukin (IL)-6, and tumor necrosis factor (TNF)-α concentrations were quantitatively determined by competitive inhibition ELISA, while the concentration of serum heat shock protein (HSP)-70 was quantitatively determined by quantitative sandwich ELISA, using Cusabio Biotech ELISA kits (College Park, MD. USA).

2.7. Statistical analysis

Statistical analysis was performed with Repeated Measure and One-way Analysis of Variance (ANOVA); and the differences among groups were determined by Bonferroni's multiple comparison test (Turner and Thayer, 2001), using GraphPad Prism version 4.03 for windows (GraphPad software; San Diego, CA, USA). *P* values of < 0.05, < 0.01 and < 0.001 were considered statistically significant, highly significant, and very highly significant, respectively.

3. Results

3.1. Sargassum latifolium regulated the thermo-respiratory responses and improved the body-weight gain of HS sheep

The present study indicated that the diet containing 4% *Sargassum latifolium* significantly decreased ($P < 0.01$) RR of the non-stressed sheep, which may be comfortable for animals (Fig. 1). The environmental HS increased significantly ($P < 0.001$) the thermo-respiratory responses (CT, ST, RT, and RR) of the animals compared with the control group (Fig. 1). On the other hand, the diet containing 2% or 4% *Sargassum latifolium* modulated significantly ($P < 0.05$–0.001) the increase shown in thermo-respiratory responses of the HS sheep, except CT (Fig. 1). Although the environmental HS caused a significant loss ($P < 0.01$) of sheep body weight by 52.5 ± 6.1% on day 40, the diet containing *Sargassum latifolium* returned the body weight of HS sheep to the control value (Fig. 2).

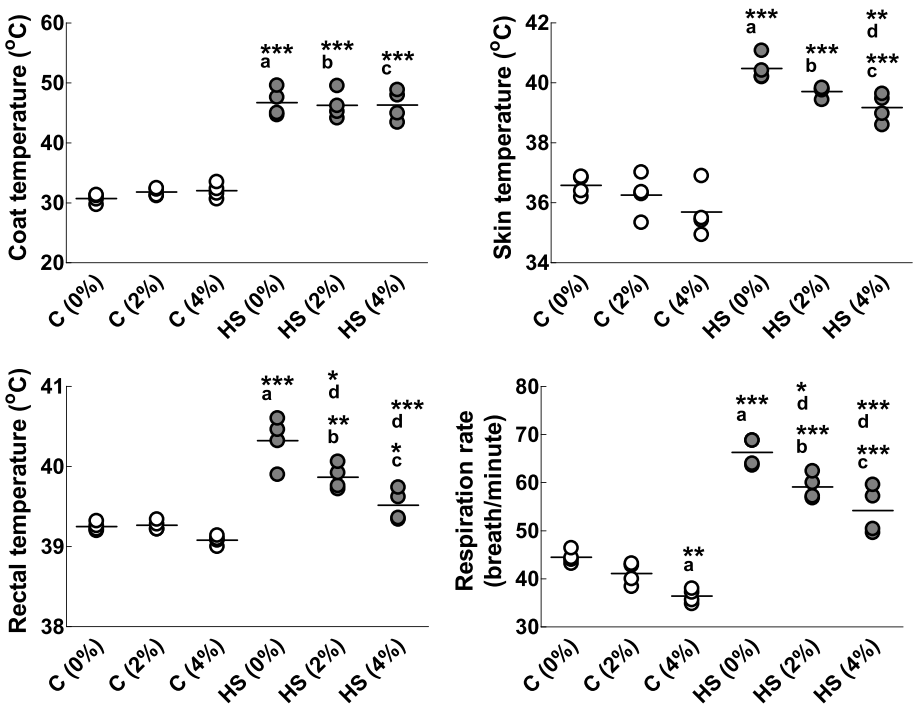

Fig. 1. Effects of diet containing *Sargassum latifolium* on the thermo-respiratory responses of the control sheep (C) and sheep exposed to heat stress (HS) during the experimental period (40 days) 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. a: compared with C (0%), b: compared with C (2%), c: compared with C (4%), d: compared with HS (0%). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Repeated Measures ANOVA with Bonferroni's Multiple Comparison Test).

3.2. *Sargassum latifolium alleviated blood hyperglycemia and dyslipidemia in the HS sheep*

The present study showed that the environmental HS induced serum hyperglycemia ($P < 0.05$), hyperlipidemia ($P < 0.05$), hypertriacylglycerolemia ($P < 0.001$) and hypercholesterolemia ($P < 0.05$), as well as increased significantly ($P < 0.01$) the risk of atherosclerosis, in the male Barki sheep (Table 3). On the other hand, the diet containing 2% or 4% *Sargassum latifolium* reverted the serum glucose level, lipid profile, and the atherogenic indices of the HS sheep to near the control values of non-stressed animals (Table 3). In addition, they reduced significantly ($P < 0.05$–0.001) the risk of dyslipidemia and atherosclerosis in the non-stressed animals (Table 3). Moreover, the modulatory effect of *Sargassum latifolium* on serum dyslipidemia of the HS group was dose-dependent.

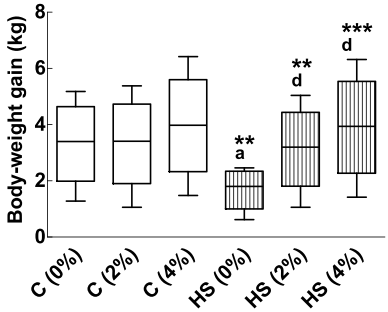

Fig. 2. Effects of diet containing *Sargassum latifolium* on the body-weight gain of the control sheep (C) and sheep exposed to heat stress (HS) during the experimental period (40 days) 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. a: compared with C (0%), d: compared with HS (0%). $^{**}P < 0.01$, $^{***}P < 0.001$ (Repeated Measures ANOVA with Bonferroni's Multiple Comparison Test).

3.3. *Sargassum latifolium alleviated the tissue injury, and improved the antioxidant capacity and kidney functions in the HS sheep*

The present study indicated that the diet containing 4% *Sargassum latifolium* reduced significantly ($P < 0.05$) the concentration of serum MDA and improved significantly ($P < 0.001$) the blood antioxidant defense system of the non-stressed sheep (Table 4). However, the environmental HS caused a significant increase ($P < 0.05$–0.001) in the serum markers for tissue injury (MDA concentration, as well as ALAT and LDH activities) and a significant alteration ($P < 0.01$–0.001) in the kidney functions of male Barki sheep (Table 4). In addition, HS caused a significant decrease ($P < 0.01$–0.001) in plasma TAC, as well as plasma CAT and erythrocyte SOD activities, of animals. On the other hand, the diet containing 2% or 4% *Sargassum latifolium* reduced significantly ($P < 0.05$–0.001) the elevation in serum markers for tissue injury and improved significantly ($P < 0.01$–0.001) the blood antioxidant defense system of the HS sheep (Table 4). However, only the diet containing 4% *Sargassum latifolium* completely modulated ($P < 0.05$–0.001) the elevation shown in the blood creatinine, urea, and BUN concentrations of the HS sheep (Table 4).

3.4. *Sargassum latifolium alleviated the anemia and systemic inflammation in the HS sheep*

The environmental HS induced a normochromic normocytic anemia in the male Barki sheep on the 30th day of the experimental period (Supplement 3), which developed into a hypochromic normocytic anemia at the end of the experiment, day 40 (Table 5). Also, the HS sheep had blood leukocytosis (due to lymphocytosis and granulocytosis) and a significant increase ($P < 0.001$) in the ESR and the concentrations of serum IgM, HSP-70, IL-6, and TNF-α compared with the control sheep, indicating a systemic inflammation (Table 5 and Fig. 3). On the other hand, the diet containing 2% or 4% *Sargassum latifolium* reverted all alterations in the hematological parameters of the HS sheep to near the control values (Table 5). In addition, they modulated significantly ($P < 0.01$–0.001), in a dose dependent manner, the elevation in the serum IL-

Table 3
Effects of diet containing *Sargassum latifolium* on serum glucose and lipid profile (on day 40) of the control sheep (C) and sheep exposed to heat stress (HS).

	Control (±) *Sargassum latifolium* groups			HS (±) *Sargassum latifolium* groups		
	C (0%)	C (2%)	C (4%)	HS (0%)	HS (2%)	HS (4%)
Glucose (mg/dL)	51.94 ± 1.43	54.57 ± 5.43	57.46 ± 6.35	71.85 ± 3.09 **a***	60.40 ± 4.69	57.88 ± 4.11
Total lipids (mg/dL)	427.4 ± 14.8	382.1 ± 6.4	323.3 ± 15.6 **a*****	491.0 ± 10.0 **a***	412.6 ± 9.8 **d****	333.8 ± 17.7 **d*****
Triacylglycerol (mg/dL)	62.67 ± 2.69	40.27 ± 3.14 **a*****	37.33 ± 1.45 **a*****	91.60 ± 1.53 **a*****	52.10 ± 2.35 **b*, d*****	46.93 ± 2.12 **d*****
Total cholesterol (mg/dL)	76.79 ± 2.53	62.86 ± 1.81 **a***	57.33 ± 2.26 **a*****	89.89 ± 3.88 **a***	67.40 ± 3.83 **d*****	58.11 ± 2.80 **d*****
Atherogenic index(1)	3.64 ± 0.28	2.22 ± 0.21 **a***	1.87 ± 0.10 **a****	5.71 ± 0.56 **a****	3.50 ± 0.39 **d*****	2.27 ± 0.22 **d*****
Atherogenic index(2)	2.05 ± 0.24	0.94 ± 0.20	0.63 ± 0.10 **a***	3.56 ± 0.49 **a****	1.96 ± 0.34 **d****	0.90 ± 0.18 **d*****

Data are presented as mean ± standard errors. 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. a: compared with C (0%), b: compared with C (2%), d: compared with HS (0%). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (One-way ANOVA with Bonferroni's Multiple Comparison Test).
Atherogenic index(1) = Serum total cholesterol (mg/dL)/Serum HDL-cholesterol (mg/dL).
Atherogenic index(2) = Serum LDL-cholesterol (mg/dL)/Serum HDL-cholesterol (mg/dL).

Table 4
Effects of diet containing *Sargassum latifolium* on serum markers for tissue injury, blood antioxidant capacity, and kidney functions (on day 40) of the control sheep (C) and sheep exposed to heat stress (HS).

	Control (±) *Sargassum latifolium* groups			HS (±) *Sargassum latifolium* groups		
	C (0%)	C (2%)	C (4%)	HS (0%)	HS (2%)	HS (4%)
Serum markers for tissue injury						
MDA (nmol/mL)	7.84 ± 0.19	6.64 ± 0.35	6.08 ± 0.23 **a***	12.27 ± 0.63 **a*****	8.81 ± 0.44 **b**, d*****	7.77 ± 0.23 **c*, d*****
ALAT activity (U/L)	17.80 ± 1.43	16.15 ± 0.93	15.91 ± 0.83	22.80 ± 0.92 **a***	17.82 ± 1.44 **d***	16.20 ± 1.16 **d****
LDH activity (U/L)	340.0 ± 16.2	291.4 ± 15.1	259.0 ± 20.6	574.7 ± 23.6 **a*****	404.8 ± 28.6 **b**, d*****	337.7 ± 21.2 **d*****
Blood antioxidant capacity						
TAC (mmol/L)	4.00 ± 0.27	4.93 ± 0.29	5.95 ± 0.10 **a*****	2.45 ± 0.22 **a****	4.10 ± 0.36 **d****	5.20 ± 0.37 **d*****
CAT activity (U/L)	386.4 ± 19.0	500.0 ± 16.1 **a*****	550.0 ± 19.6 **a*****	232.9 ± 13.6 **a*****	390.9 ± 13.3 **b**, d*****	504.5 ± 19.6 **d*****
SOD activity (U/L)	69.75 ± 2.54	78.75 ± 2.65	87.75 ± 3.48 **a*****	48.75 ± 2.65 **a*****	71.25 ± 2.65 **d*****	80.25 ± 1.91 **d*****
Kidney functions						
Creatinine (mg/dL)	1.16 ± 0.07	1.05 ± 0.07	1.00 ± 0.09	1.70 ± 0.11 **a****	1.38 ± 0.09	1.25 ± 0.13 **d***
Urea (mg/dL)	44.35 ± 2.93	38.13 ± 1.53	36.50 ± 1.26	70.03 ± 6.75 **a*****	60.08 ± 2.48 **b****	45.19 ± 3.35 **d*****
BUN (mg/dL)	20.73 ± 1.37	17.82 ± 0.72	17.06 ± 0.59	32.72 ± 3.16 **a*****	28.08 ± 1.16 **b****	21.12 ± 1.57 **d*****

Data are presented as mean ± standard errors. 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. ALAT: alanine aminotransferase, BUN: blood urea nitrogen, CAT: catalase, LDH: lactate dehydrogenase, MDA: malondialdehyde, SOD: superoxide dismutase, TAC: total antioxidant capacity. The activity of serum aspartate aminotransferase (ASAT) did not significantly affect ($P > 0.05$) among all groups. a: compared with C (0%), b: compared with C (2%), c: compared with C (4%), d: compared with HS (0%). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (One-way ANOVA with Bonferroni's Multiple Comparison Test).

Table 5
Effects of diet containing *Sargassum latifolium* on the hematological parameters (on day 40) of the control sheep (C) and sheep exposed to heat stress (HS).

	Control (±) *Sargassum latifolium* groups			HS (±) *Sargassum latifolium* groups		
	C (0%)	C (2%)	C (4%)	HS (0%)	HS (2%)	HS (4%)
Hemoglobin (g/dL)	10.27 ± 0.35	10.91 ± 0.13	11.26 ± 0.29	8.62 ± 0.18 **a****	10.24 ± 0.28 **d****	11.31 ± 0.22 **d*****
Hematocrit (%)	33.36 ± 0.71	33.46 ± 0.47	35.23 ± 0.74	28.88 ± 0.72 **a***	33.08 ± 1.14 **d***	35.68 ± 1.39 **d*****
MCH (pg)	11.38 ± 0.45	11.95 ± 0.19	11.91 ± 0.46	9.61 ± 0.24 **a***	10.68 ± 0.39	11.68 ± 0.27 **d****
Total leucocytes ($10^3/mm^3$)	6.77 ± 0.54	6.26 ± 0.62	5.16 ± 0.32	10.47 ± 0.66 **a****	8.48 ± 0.85	7.72 ± 0.69 **d***
Lymphocytes ($10^3/mm^3$)	3.85 ± 0.33	4.00 ± 0.24	3.56 ± 0.19	6.58 ± 0.41 **a*****	5.56 ± 0.57	5.22 ± 0.46
Granulocytes ($10^3/mm^3$)	2.51 ± 0.20	1.85 ± 0.43	1.43 ± 0.14	3.74 ± 0.24 **a***	2.41 ± 0.33 **d***	2.16 ± 0.21 **d****
ESR 1st hour (mm)	2.0 ± 0.4	1.4 ± 0.2	1.2 ± 0.2	6.0 ± 0.4 **a*****	2.4 ± 0.4 **d*****	1.8 ± 0.4 **d*****
ESR 2nd hour (mm)	3.8 ± 0.7	3.0 ± 0.3	2.8 ± 0.4	10.0 ± 0.7 **a*****	5.0 ± 0.8 **d*****	3.2 ± 0.5 **d*****

Data are presented as mean ± standard errors. 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. ESR: erythrocyte sedimentation rate, MCH: mean corpuscular hemoglobin. Erythrocytes and monocytes counts, mean corpuscular volume, MCH concentration, and clotting time did not significantly affect ($P > 0.05$) among all groups. a: compared with C (0%), d: compared with HS (0%). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (One-way ANOVA with Bonferroni's Multiple Comparison Test).

6 (only by diet containing 4% *Sargassum latifolium*), HSP-70, and TNF-α concentrations of the HS sheep (Fig. 3). Moreover, they increased significantly ($P < 0.05$–0.001) the serum IgM concentration of non-stressed and HS sheep, indicating an enhancement of animal humoral immune response (Fig. 3).

4. Discussion

To the best of our knowledge, the present study is the 1st one that explored the anti-HS activity of *Sargassum latifolium* in Barki sheep. During the HS, ruminants (like other homoeothermic animals) increased avenues of heat loss and reduced internal heat production to maintain euthermia. The thermo-respiratory responses (e.g. ST, RT, and RR) reflected both the environmental HS and the internal heat load on the animals (Al-Dawood, 2017). The results of the present study clarified that the diet containing *Sargassum latifolium* modulated significantly almost all changes in the thermo-respiratory responses of the HS groups except CT, which indicated the surrounding HS conditions. Lowering ST, RT, and RR in the HS sheep received *Sargassum latifolium* indicated that they were less stressed than those did not receive the brown seaweeds.

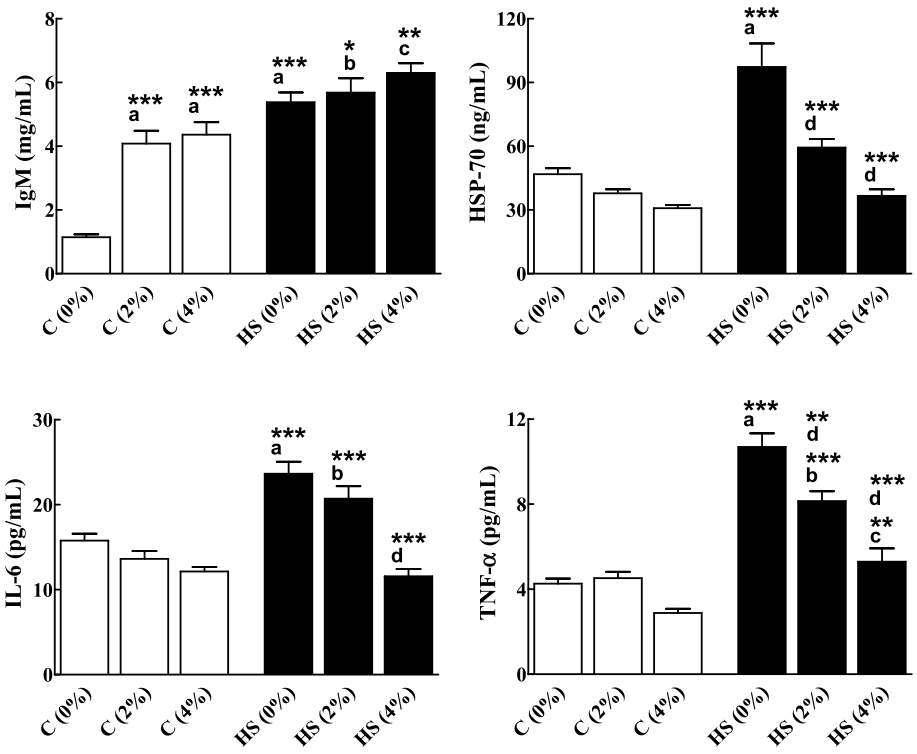

Fig. 3. Effects of diet containing *Sargassum latifolium* on serum immunoglobulins (Ig), heat shock protein (HSP)-70, and inflammatory cytokines (on day 40) of the control sheep (C) and sheep exposed to heat stress (HS). Data are presented as mean ± standard errors. 0%, 2%, or 4%: diet containing 0%, 2%, or 4% *Sargassum latifolium*, respectively. IL: interleukin, TNF: tumor necrosis factor. Serum IgG level did not significantly affect ($P > 0.05$) among all groups. a: compared with C (0%), b: compared with C (2%), c: compared with C (4%), d: compared with HS (0%). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (One-way ANOVA with Bonferroni's Multiple Comparison Test).

Other studies reported that feeding lambs and beef cattle exposed to the elevated ambient temperature during walking and transportation or during daytime in animals' yards on *Ascophyllum nodosum* (brown seaweed) or its extract (Tasco) lowered significantly their ear canal temperatures and RT, respectively (Archer et al., 2007; Williams et al., 2009).

Actually, the environmental HS increased the consumption of water and decreased the feed intake, as well as promoted the production of free radicals and affected the endocrine functions, which may disturb the animal metabolism especially the glucose and lipid metabolism (Belhadj Slimen et al., 2016). Indeed, the data of the present study proved that the environmental HS induced hyperglycemia, dyslipidemia, and increased the risk of atherosclerosis in male Barki sheep. The environmental HS did not significantly affect the levels of serum total protein and albumin in the animals (data not shown). On the other hand, the diet containing *Sargassum latifolium* modulated significantly the metabolic disorders shown in the HS group, and hence protected the animals from the risk of atherosclerosis. Moreover, *Sargassum latifolium* reduced the risk of atherosclerosis in non-stressed animals. A previous study assured that *Sargassum ringgoldianum* extract decreased the postprandial blood glucose level in streptozotocin-induced diabetic mice *via* diminishing the amount of α-glucosidase in the brush border of the small intestine, which affected the digestion of starch and disaccharides into glucose in the small intestine, and thus decreased the glucose absorption (Lee and Han, 2012). Also, the sulfated polysaccharides of *Sargassum polycystum*, such as fucoidans, enhanced insulin sensitivity and glucose uptake in target tissues, as well as had a capacity to inhibit lipid absorption in the gastrointestinal tract, lower serum cholesterol levels, and modulate dyslipidemia in a type 2 diabetic rat model (Motshakeri et al., 2013). In addition, *Sargassum* spp. have a considerable content of minerals, e.g. potassium, and magnesium (Fouda et al., 2019). Magnesium reduced dyslipidemia by raising the serum HDL-cholesterol level and lowering the serum triacylglycerol level (Schwalfenberg and Genuis, 2017). Also, potassium suppressed the development of atherosclerosis and reduced the atherosclerotic burden through its ability to decrease the formation of free radicals, proliferation of vascular smooth muscle cells, platelet aggregation, arterial thrombosis, and deposition of cholesterol ester in walls of the blood vessels (Lai et al., 2015). Therefore, *Sargassum* spp. had the ability to protect the animals from the metabolic disorders and the risk of the atherosclerosis.

The animals increased the peripheral capillaries blood flow and decreased the visceral blood flow in order to facilitate heat dissipation and prevent hyperthermia caused by the environmental HS. The reduction of splanchnic blood flow leads to multi-organs injury and dysfunction, e.g. liver, kidney, and intestine (Kour et al., 2014). In addition, the environmental HS contributed in inducing an oxidative stress in livestock animals either through enhancing ROS and reactive nitrogen species (RNS) production or decreasing the endogenous antioxidant defenses of the animal (Rathwa et al., 2017). In the present study, the environmental HS increased significantly the serum markers for tissue damage (MDA, ALAT, and LDH), altered the kidney functions, and decreased significantly the antioxidant defenses in Barki sheep. The elevated serum creatinine concentration shown in the HS sheep may also be attributed to the decrease in glomerular filtration rate (Kour et al., 2014). The reduction in the activities of blood enzymic antioxidants (SOD and CAT) in the HS sheep may be explained by the utilization of these enzymes to detoxify the free radicals produced by the HS, and to preserve the redox steady state. However, SOD and CAT are important antioxidant enzymes in preventing lipid peroxidation, and maintaining the structure and function of cell membranes (Belhadj Slimen et al., 2016). On the other hand, the diet containing *Sargassum latifolium* (especially 4%) modulated significantly the resulted tissue damage, improved the kidney functions, and protected the animals from the oxidative stress in the HS group through improving the TAC. Previous reports demonstrated that the ethanolic extracts of *Sargassum* spp. (*Sargassum fluitans*, *Sargassum ilicifolium*, *Sargassum lanceolatum*, and *Sargassum swartzii*) protected significantly the liver and kidney from injury in carbon tetrachloride and acetaminophen intoxicated murine models (Hira et al., 2017; Quintal-Novelo et al., 2018). Also, *Sargassum polycystum* prevented the excessive depletion of hepatic SOD and CAT in galactosamine-induced hepatitis in rats (Meena et al., 2008). The potential anti-lipid peroxidation activity of *Sargassum* spp. may be attributed to their richness in the antioxidant compounds such as alkaloids, polyphenols, flavonoids, and carotenes. Phlorotannins (a class of tannins

found mainly in brown seaweeds) of *Sargassum hystrix* were effective in inhibiting UV-induced lipid peroxidation by acting as superoxide anion scavengers (Harnita et al., 2013). Also, fucoxanthin of *Sargassum wightii* exhibited a strong *in vitro* free radicals scavenging activity (Sujatha et al., 2017).

HS caused splanchnic tissue hypoxia, leading to an oxidative damage of the internal organs. Lower integrity of the intestinal epithelium of livestock may cause endotoxemia by facilitating the translocation of bacteria and their endotoxins into the portal and systemic blood circulation, and thus enhanced the inflammatory responses and their related disorders (de Punder and Pruimboom, 2015; Inbaraj et al., 2016). The present study indicated that the environmental HS augmented the systemic inflammation in male Barki sheep by: (a) enhancing the release of serum proinflammatory cytokines and HSP-70; (b) inducing blood granulocytosis and lymphocytosis; (c) increasing the ESR and humoral immune response. In addition, HS caused hypochromic normocytic anemia in male Barki sheep, which was firstly started as a normochromic normocytic anemia (a typical type of anemia of inflammation). The resulted anemia in HS animals may be due to the intestinal injury/-bleeding and the appetite loss caused by enhancing the release of free radicals and proinflammatory cytokines, respectively (Mittal et al., 2014). In addition, the proinflammatory cytokines enhanced liver synthesis of acute phase proteins such as C-reactive protein and fibrinogen, a pro-sedimentation factor that increased ESR (Papageorgiou et al., 2010). It was also reported that ruminants under heat stress showed an increase in the blood leucocytes count, which could be associated with an active infection (Alam et al., 2011). On the other hand, the diet containing *Sargassum latifolium* (especially 4%) modulated significantly the resulted anemia and systemic inflammation in the HS sheep. Some studies, including our previous study, indicated that marine algae especially *Sargassum* spp. are good sources of antianemic, antioxidant, and anti-inflammatory agents such as iron, vitamins B and C, sulfated polysaccharides, etc. (Budhiyanti et al., 2012; Fouda et al., 2019; Wu et al., 2016). In addition, phlorotannins of brown seaweed (*Ascophyllum nodosum*) had the ability to inhibit the release of TNF-α and IL-6 *in vitro* (Dutot et al., 2012). Also, *Sargassum fulvellum* showed anti-inflammatory activity and was efficient in protecting the BALB/c mice from UV-induced skin damage (Lee et al., 2013).

Induction of oxidative stress in livestock during HS caused oxidative damage to proteins and DNA, which was an essential factor to enhance the intracellular HSPs (especially HSP-70) production to protect cellular proteins from damage, facilitate protein refolding, and maintain the structural function of proteins (Archana et al., 2017). The most remarkable intracellular effect of HSP-70 is the inhibition of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) activation, which has intense implications in inflammation and cell apoptosis (Wang et al., 2017). In addition, HSP-70 interacted with prostaglandins-(PG)-E synthase forming PGE synthase-HSP-70 complex, which negatively inhibited the production of PGE_2 in bovine primary dermal fibroblast cells exposed to heat stress. This mechanism was considered as one of the essential regulatory processes for protecting cells from damage (Richter et al., 2015). On the contrary, HSP-70 released to the extracellular environment during continuous oxidative-induced cell injury. Extracellular HSP-70 enhanced systemic inflammation by stimulating the release of proinflammatory mediators such as nitric oxide (NOx), TNF-α, and IL-6 through its binding with different surface receptors on immune cells such as monocytes and tissue macrophages (Calderwood et al., 2016). The anti-inflammatory activity of the *Sargassum latifolium* (especially at the high dose) shown in the present study may be in part due to its flavonoids (especially kaempherol), which had the ability to inhibit NOx production, the inflammatory mediators-induced synthesis of PGE_2, and TNF-α-enhanced NF-κB expression in the intestinal epithelial cell line (Lopez-Posadas et al., 2010). Also, the sulfated polysaccharides isolated from *Sargassum horneri* inhibited the secretion of pro-inflammatory cytokines in lipopolysaccharide-stimulated murine macrophage cell line (Sanjeewa et al., 2017, 2018). Therefore, *Sargassum* spp. had obvious anti-inflammatory activity. The anti-HS activity of *Sargassum latifolium* was noticed in the present study after 20–30 days of treatment (Supplements 1–3). In addition, the doses used of *Sargassum latifolium* were safe, did not affect the taste of the concentrated diet or decrease the feed intake of animals, and did not induce any harmful effect for non-stressed or HS sheep.

5. Conclusion

The present study proved that the diet containing 4% of *Sargassum latifolium* was safe and efficient in protecting male Barki sheep from the harmful effects of the environmental HS through improving the antioxidant defense system, and regulating the thermo-respiratory and inflammatory responses of the animals.

Author contributions

G. R. and A. M. E. planned the study, designed all experiments, and summarized, discussed and interpreted the results. W. A. F. and W. M. I. collected, identified, and characterized the brown seaweeds. W. A. F. carried out the experiments, performed the statistical analysis, and drafted the manuscript with assistance from G. R and A. M. E.

Declaration of competing interest

Patent registration number: 1716/2019 (Patent Office, Academy of Scientific Research and Technology, Ministry of Scientific Research, Cairo, Egypt); inventors (authors): Wafaa A. Fouda, Gamal Ramadan, Ashgan M. Ellamie, and Wael M. Ibrahim. Authors hereby disclose that there is no other actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

CRediT authorship contribution statement

Ashgan M. Ellamie: Conceptualization, Methodology, Data curation, Formal analysis, Resources. **Wafaa A. Fouda:** Data curation, Formal analysis, Visualization, Investigation, Resources, Writing - original draft. **Wael M. Ibrahim:** Data curation, Formal analysis, Investigation. **Gamal Ramadan:** Conceptualization, Methodology, Data curation, Formal analysis, Validation, Software, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2020.102561.

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