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Physiological and Behavioral Responses of Growing Barki Ram Lambs Exposed to Heat Stress and Fed Brown Seaweed as Additives under Semi-Arid Conditions

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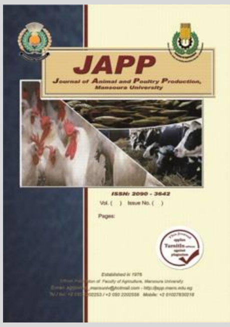

ABSTRACT

This study aimed to inspect the effects of consuming two different doses of the brown seaweeds "*Sargassum latifolium*" by heat stressed growing Barki ram lambs for 60 days on their growth performance, physiological and behavioral responses. Twenty Barki ram lambs 6–8 months old with average of 29.26 ± 2.09 kg live body weight were divided into four equal experimental groups. Lambs in the first group were kept in comfortable environmental conditions and were not offered seaweed and assigned as control (C). Animals in the second group were exposed to heat stress and were offered the diet without seaweed (T0). Animals in the third (T1) and fourth (T2) groups were exposed to heat stress and were supplemented with 2 and 4% (from CFM) brown seaweeds, respectively. Feed intake and body weight were recorded daily. Complete blood count and plasma biochemical parameters were assessed biweekly while behavioral responses were measured in the last week of the experiment. Animal had brown seaweeds in T1 and T2 groups increased (P<0.01) average daily gain and total body weight gain as compared to their counterparts exposed to heat stress (T0). Moreover, lambs of T2 achieved the best feed conversion ratio (8.37). Seaweed supplementations increased (P<0.05) leukocyte, hematocrit and hemoglobin concentration. Feeding lambs with a diet containing 4% brown seaweeds increased (P<0.01) serum total protein, albumin, potassium level, but decreased (P<0.01) serum total lipids, triglycerides, total cholesterol as compared with control ones. Supplementation with brown seaweeds affected significantly daily water intake and some behavioral parameters. It can be concluded that supplementation with brown seaweed (2% and 4% from CFM) to heat stressed Barki ram lamb in summer improved productive performance through improving growth rate, blood constituents, beside ingestive, standing and lying behavior.

***Keywords*:** Barki lambs, heat stress, Brown seaweeds, Thermoregulation, Blood, Behavioral responses.

INTRODUCTION

The animal and its environment make up an integrated system, where each act on the other (Ilori *et al.* 2011). So, the climatic changes will affect small ruminant performance through affecting their health, growth, reproduction, and disease... etc. In addition, heat stress has been generally related with detrimental effects on interior physiological equilibriums of small ruminants resulting in specific responses and reciprocal regulatory effects (Castanheira *et al.*, 2010). Accordingly, productive and reproductive performances of small ruminants are affected severely by heat stress (Shinde and Sejian, 2013; Al-Dawood, 2017). Consequently, the environmental heat stress in tropical and subtropical areas could cause a great economic loss for the livestock breeders (Marai *et al.*, 2007; Nardone *et al.*, 2010).

Seaweeds witnessed renewed interest as feed ingredients (Naylor, 1976; McHugh, 2002). Many researches indicated that seaweeds especially *Sargassum spp.* have been used as useful alternative feed to livestock because they contain sources of valuable foodstuffs such as essential and non-essential amino acids, some vitamins, antioxidant compounds, dyes and polyunsaturated fatty acids, that are beneficial to animal health (CEVA, 2005; Ananthi *et al.*, 2010; Evans and Critchley, 2014). In this respect, Abdoun *et al.* (2014) and Ekin *et al.* (2017) suggested that algae might positively impact metabolism and reduce lipid concentrations in growing lambs. These knowledge about seaweeds will open new avenues for investigations for exploiting potential of this neglected unconventional resource for the feed industry that could play a vital role in sustainable development of the livestock sector. However, there is a lack of published data in the scientific literature regarding the effects of algae on productive performance, blood constituents and behavioral patterns in lambs especially during hot season.

The present study was focused on examining the brown seaweeds (*Sargassum latifolium*) supplement on growth performance, blood constituents and behavioral patterns in Barki ram lambs under hot condition of the summer season.

MATERIALS AND METHODS

Study location:

This study was carried out at Ras Sudr Station (South Sinai), belongs to Desert Research Center, South Sinai Governorate. It is geographically is considered as a semi-arid region.

Animals and management:

Twenty growing healthy Barki ram lambs aging 6–8 months old (born in January) with average of 29.26 ± 2.09 kg live body weight were randomly divided into four similar

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experimental groups (5 lambs each). This trial conducted from 1st July to end of August 2017, which is a particular time period when the temperature-humidity index (THI) is proposed to be at maximum level. There was a 7-day adaptation period before start of the experiment.

All Animals in each group were provided with fresh tap water and fed on concentrate feed mixture(CFM) and wheat-hay as roughage. Lambs in the first group were kept in comfortable environmental conditions in a non-sunlit closed housing pen and offered the CFM without seaweed supplementation and assigned as control diet(C). Likewise, the animals in the second group were exposed to heat stress and were offered the CFM without seaweed supplementation (T0). Animals in the third (T1) and fourth (T2) groups were exposed to heat stress and received the control diet supplemented with 2 and 4% brown seaweeds (*Sargassum latifolium*) in the CFM, respectively.

Experimental animals were housed inside open pens except animals of control group (C) that were housed inside closed pens. All lambs were fed their nutrient requirements during growing stage according to Kearl (1982). Chemical composition of concentrate feed mixture and wheat-hay were determined according to A.O.A.C. (1990) and presented in Table (1). The chemical compositions, amino acids, phenolic compounds, total sugar, total flavonoids, carotenes, antioxidant activity an amount of vitamins of Sargassum latifolium is presented in Tables (2, 3, and 4). Drinking clean fresh water was made available twice a day over the experimental period.

All animals had the suitable veterinary care. They were vaccinated against sheep pox, clostridial diseases, foot-and-mouth disease and Rift Valley fever according to the program of the Ministry of Agriculture.

Table 1. Chemical composition (%) of experimental feed stuffs (as % on DM basis)

Ingredient	DM	OM	CP	EE	CF	NFE	Ash
Wheat-hay	93.0	75.0	3.6	1.2	30.9	39.3	25.0
Concentrate feed mixture	91.4	89.7	13.6	2.5	15.7	57.9	10.3

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, and nitrogen free extract.

Table 2. Chemical composition (%) of *Sargassum latifolium* collected from Red Sea at Hurghada coast

Brown algal species	Moisture %	DM %	Carbohydrates %	Protein %	Fat %	Ammonia (mg/g DM)	Ash
Sargassum latifolium	88.22	11.78	41.42	4.38	0.27	4.38	26.16

According to Fouda *et al.* (2019)

Table 3. The amino acid composition (mg/g DM) of *Sargassum latifolium*collected from Red Sea at Hurghada coast

Essential amino acids								Non-essential amino acids								
Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine	Alanine	Arginine	Aspartic acid	Cysteine	Glutamine	Glycine	Proline	Serine	Tyrosine
0.26	0.69	1.08	0.72	0.25	0.59	0.52	0.91	1.01	0.51	1.56	0.11	1.62	0.66	0.55	0.68	0.27
				5.02								6.97				

According to Fouda *et al.* (2019)

Table 4. Phenolic compounds, total sugar, total flavonoids, carotenes, antioxidant activity and amount of vitamins B2 & C (ppm) in *Sargassum latifolium* collected from Red Sea at Hurghada coast.

Phenolic compounds (ppm)				Vitamins (ppm)		Total sugars (%)	Total flavonoid(µg Catechin equivalent /g DM)	Carotenes (mg β-carotene equivalent/g DM)	Antioxidant activity (µg Ascorbic acid equivalent/g DM)
Kaempferol	Naphthalene	Phenanthrene	Resorcinol acid	Vitamin B_2	Vitamin C				
151.8	0.004	0.001	0.3	232.3	6.68	2.19	908.18	2.45	184.96

According to Fouda *et al.* (2019)

Climatic conditions:

Temperature-humidity index (THI) was calculated according to the equation of Nigm *et al.* (2015) under the Egyptian climatic conditions as follow:

$$\mathbf{THI = 32.783 + 1.478 \times AT + 0.056 \times RH}$$

Where: AT is the average of air temperature (°C) and RH is the average of relative humidity (%).

Table (5) and Figure (1) illustrated the environmental conditions around and inside the sheep yards of the control and heat stress groups at ٠7 a.m. and 02 p.m. throughout the experimental period. The control group was housed in the same yards in comfortable environmental conditions, while treatment groups (T0, T1 and T2) were exposed to heat stress and solar radiation in the summer season.

Table 5. Average of metrological data around and inside the sheep yards at ٠7.00 and 1٤.00 h during the experimental period

Climatic elements	At morning (07 a.m.)		Afternoon (02 p.m.)	
	C group	Treatment groups	C group	Treatment groups
Ambient temperature, °C	18.8	22.0	26.8	37.0
Humidity, %	65.8	66.8	30.5	27.8
Temperature-humidity index	64.2	69.0	74.0	89.0
Solar radiation,°C	19.6	25.1	28.3	44.9
Floor temperature,°C	19.3	22.6	27.9	49.1
Wall temperature,°C	18.6	23.1	30.4	48.3
Roof temperature,°C	19.6	Absent	33.6	Absent

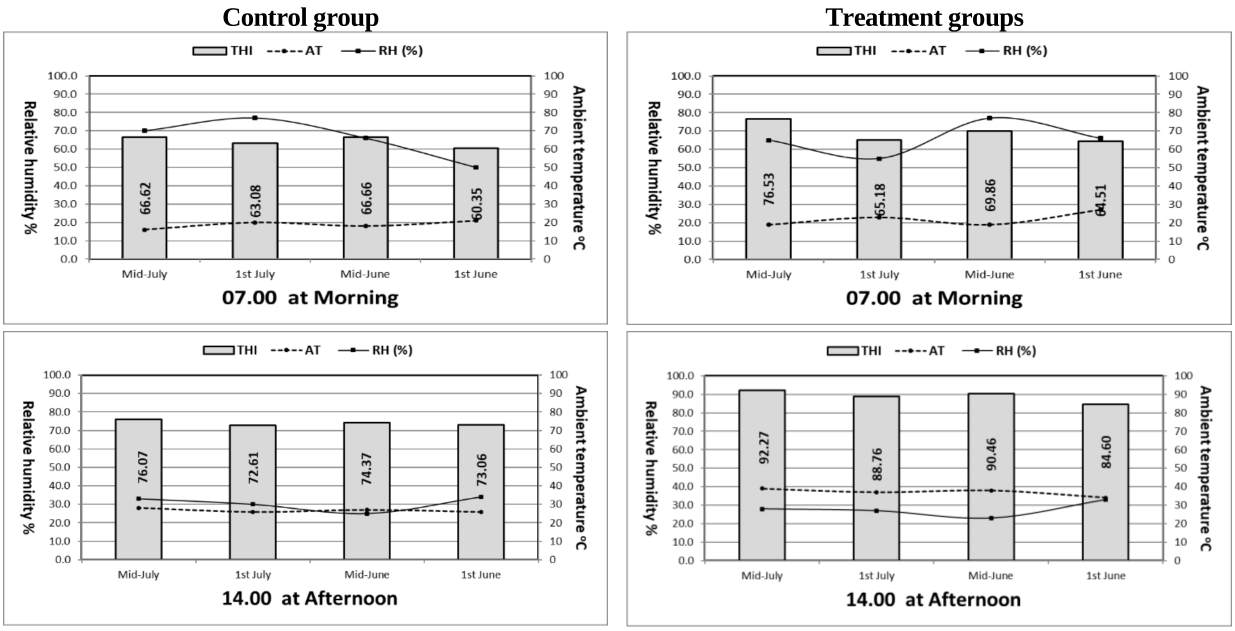

Figure 1. Graphical representation of the ambient temperature (expressed in °C), relative humidity (expressed in %) and temperature humidity index (THI) recorded during experimental period

Experimental procedures:

All experimental growing lambs were weighed weekly early morning before feeding and drinking to the nearest 10 gm. From each lamb, two blood samples before serving food and drink were taken biweekly from the external jugular vein; the first into vial containing ethylene diamine tetra acetic acid powder (EDTA) as an anticoagulant to be used for counting blood cells (red (RBCs) and white (WBCs) blood cells; hematocrit value (Ht); hemoglobin concentration (Hb); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration, MCHC) using blood counter apparatus model Rayto Product RT-7600 Auto Hematology Analyzer. The second sample was drawn in heparinized tube and plasma was carefully separated after centrifugation at 3000 rpm for 30 min. Plasma was stored at -18 ^{0}C in glass vials with plastic stoppers until chemical assays.

Concentration of total proteins (TP) was measured by a kit supplied by Egyptian- American Company for Laboratory Services by Biuret method after Gornal *et al.* (1949), while albumin (AL) was measured conferring to Doumas *et al.* (1971). Values of globulin (GL) was obtained by subtracting and AL/GL ratio was calculated. Plasma total lipids (Schmit, 1964), total cholesterol (Roeschlau *et al.*, 1974), e glucose (Tietz, 1986) were deterined. Activity of both alanine (ALT) and aspartate (AST) amino transferases (Reitman and Frankel, 1957), alkaline phosphatase (ALP) enzyme (Belfield and Goldberg, 1971) was also determined in blood plasma. Plasma creatinine and urea concentrations as pointers for kidney function were measured using bio-diagnostic kits according to Fawcett and Soctt (1960) and Schirmeister *et al.*, (1964), respectively. Radioimmunoassay assay (RIA) was performed for plasma triiodothyronine (T3) and thyroxin (T4) hormones using ready antibody coated tubes kits constructed by Immunotech, Beckman Counter Company, France.

At the last week of the experimental period, behavioral patterns including drinking, feeding, ruminating, idling, standing and lying were personally recorded for each animal during 12 hours/day. For behavioral observations, the control group animals were kept in a non-sunlit closed housing pen (observation pen) with dimensions of ٦ × 6 m and a floor made from cement lined with rice straw. While the animals of the other three groups are kept in an open housing and exposed to the sun with the same dimensions and specifications of the control group housing. Each animal was color marked on its two sides to help personal observation and recording behavior.

Statistical analysis:

Statistical analysis of other parameters was performed one-way repeated analysis of variance (ANOVA). All parameters were analyzed by generalized linear model using statistical software Minitab 12.1 (SAS, 2004).

RESULTS AND DISCUSSION

Hematological Parameters:

Table (6) shows the effect of brown seaweed supplementation on erythrocyte cell counts (RBCs), white blood cells (WBCs), blood hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of growing Barki male lambs during hot season months.

Results show that only hematocrit value decreased significantly ($P<0.05$) in heat-exposure lambs (T0) than non-exposure ones (C) (Table 6). These results agreed with those of Cwynar *et al.* (2014), who reported that environmental heat stress had adverse effects on hematocrit value and caused anemia, which might be attributed to: (a) heat stress-induced oxidative stress, where the reactive oxygen species can cause deterioration of cell membrane and formation of lipid peroxidation resulting in a demolition of erythrocytes, (b) inadequate nutrient availability for hemoglobin synthesis through decreasing feed intake under the heat stress conditions.

The diets containing *Sargassum latifolium*, especially at a level of 4% Seaweed significantly (P<0.05) improved hematological parameters of heat-stressed group (T0) by increasing WBCs, Ht and Hb. However, diet containing 2% seaweed significantly(P<0.05) increased Ht and Hb of heat stressed lambs (T0, Table 6). In accordance with the present results, Ina and Kamei (2006) and García-Casal *et al.* (2009) reported that marine brown seaweeds especially *Sargassum spp.* are good sources of anti-anemic agents such as iron and vitamins B. Brown seaweeds have a considerable antioxidant capacity, as determined by its contents of alkaloids, flavonoids, kaempherol, carotenes and ascorbic acid, which could protect animals from the intestinal damage/hemorrhage caused by the heat stress (Park *et al.*, 2005; Budhiyanti *et al.*, 2012). Also, Saker *et al.* (2004) found that the brown seaweed *Ascophyllum nodosum* partially improved the antioxidant status in heat-stressed lambs.

It is of interest to note that erythrocyte indices (MCV, MCH, and MCHC) were not affected by heat stress or *Sargassum latifolium* addition to the diet of lambs.

Table 6. Effect of diet containing *Sargassum latifolium* on the hematological parameters of growing Barki sheep exposed to heat stress

Item	Control group (C)	Treatment groups T0	T1	T2	±SEM
Erythrocytes cell counts (RBCs), x10^6/µL	9.08	8.57	9.26	9.53	0.31^{NS}
White blood cells (WBCs), x10^3/µL	18.85^{ab}	16.05^{b}	19.74^{ab}	22.05^{a}	1.43 *
Hematocrit (Ht), %	32.84^{b}	28.34^{c}	32.59^{b}	35.85^{a}	0.42 **
Hemoglobin (Hb), g/dl	9.87^{ab}	8.60^{b}	10.34^{a}	11.06^{a}	0.53 *
Mean corpuscular volume (MCV), fl	36.34	33.22	35.60	37.94	1.29 ^{NS}
Mean corpuscular hemoglobin (MCH), pg	10.93	10.03	11.13	11.74	0.58 ^{NS}
Mean corpuscular hemoglobin concentration (MCHC), %	30.10	30.27	31.90	31.02	1.38 ^{NS}

***, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c= values in the same row with different super scripts are significantly differed (P< 0.05).**

Metabolic parameters:

There was no difference in plasma total proteins concentration of control and heat-stressed lambs in T0 (Table 7). This result agreed with those reported by Alhidary *et al.* (2015) on sheep. There were no significant differences among the 4 groups in Triiodothyronine, thyroxin, sodium, and potassium (Table 7).

Plasma albumin, AL/GL ratio, glucose, total lipids, cholesterol and triglycerides significantly (P<0.05) increased by 11.96, 62.1, 25.11, 11.33, 8.99 and 40.43%, while globulin significantly (P<0.05) decreased of heat stressed lambs compared to the control lambs. Many researchers reported that serum glucose and cholesterol levels increased significantly during heat stress in sheep and in goats (Ellamie, 2013; Rashid *et al.*, 2013; Sejian *et al.*, 2013; Okoruwa, 2014).

Adding *Sargassum latifolium* only at a level of 4% significantly increased (P<0.05) albumin, globulin, and total proteins, and significantly (P<0.05) improved AL/GL ratio as compared to T0. However, Adding *Sargassum latifolium* only at a level of 4% significantly (P<0.05) decreased glucose level and lipid profile of heat stressed lambs. Yet, this treatment significantly (P<0.05) increased K content in blood plasma of heat stressed lambs (Table 7).

Table 7. Effect of diet containing *Sargassum latifolium* on the metabolic parameters of growing Barki sheep exposed to heat stress

Parameter	Control group (C)	Treatment groups T0	T1	T2	±SEM
Total proteins, g/dl	6.20^{b}	5.65^{b}	6.08^{b}	7.54^{a}	0.12 **
Albumin, g/dl	3.01^{c}	3.37^{b}	3.47^{b}	3.99^{a}	0.12 **
Globulin, g/dl	3.19^{ab}	2.27^{c}	2.61^{bc}	3.54^{a}	0.13 **
AL/GL ratio	0.95^{b}	1.54^{a}	1.46^{a}	1.21^{ab}	0.13 *
Glucose, mg/dl	54.63^{c}	68.35^{a}	61.35^{b}	56.04^{bc}	2.20 **
Total lipids, g/l	437.51^{b}	487.07^{a}	402.51^{c}	350.01^{d}	9.81 **
Cholesterol, g/dl	77.30^{b}	84.25^{a}	63.38^{c}	56.86^{d}	1.26 **
Triglycerides, g/dl	63.07^{b}	88.57^{a}	52.98^{c}	48.57^{c}	3.04 **
Triiodothyronine, pg/ml	3.22	2.09	2.77	3.19	0.31^{NS}
Thyroxine, ng/ml	10.99	8.64	9.94	10.11	0.89^{NS}
Na (mmol/l)	121.12	135.65	132.1	128.9	4.42 ^{NS}
K (mmol/l)	5.91^{b}	6.10^{b}	7.33 ^{a}	7.54 ^{a}	0.45 **

***, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c, d= values in the same row with different super scripts are significantly differed (P< 0.05).**

Sargassum spp. have a substantial content of minerals, e.g. potassium, calcium, iron, magnesium, phosphorus and zinc (Valentina *et al.*, 2015). Zinc and magnesium potentially enhance protein metabolism due to their several roles in the metabolic processes. Furthermore, zinc is an essential component of a large number of enzymes contributing in the synthesis and destruction of carbohydrates, lipids, proteins and nucleic acids (Papet *et al.*, 2008; Roohani *et al.*, 2013). Magnesium has a key role in many important biological processes such as nucleic acids and protein synthesis (Noronha and Matuschak, 2002; Swaminathan, 2003). Also, potassium may overpower the progress of atherosclerosis and deterioration the atherosclerotic burden. It reduced the formation of the free radical, proliferation of vascular smooth muscle cells, arterial thrombosis, platelet aggregation and deposition of cholesterol ester in walls of the blood vessels (Sica *et al.*, 2002; Lai *et al.*, 2015). Therefore, the obtained results may suggest that *Sargassum spp.* can protect animals from the metabolic disorders and the risk of the atherosclerosis.

Simultaneously, adding *Sargassum latifolium* resulted in decreasing glucose, total lipids, cholesterol and triglycerides. In agreement with our findings, Kannan *et al.* (2007a, b) and Ekin *et al.* (2017) recorded that blood cholesterol tended to decrease in goats, dairy cows and lambs fed diets containing algae during stress. *Sargassum latifolium* is rich in sulfated polysaccharides such as fucoidans and alginate (Mohsen *et al.*, 2007; Gamal-Eldeen *et al.*, 2009). These polysaccharides have a capacity to inhibit lipid absorption in the gastrointestinal tract, resulting in lowering serum cholesterol levels (Jimenez-Escrig and Sanchez-Muniz, 2000). In addition, fucoidans from a brown seaweed "*Laminaria japonica*" modulated dyslipidemia in hyperlipidemic rats (Huang *et al.*, 2010).

Generally, the results of the present study proved that the heat stress induced hyperglycemia, dyslipidemia, and increased the risk of atherosclerosis in Barki lambs. Fortunately, *Sargassum latifolium* completely modulated the resulted hyperglycemia as well as dyslipidemia, and hence protected the animals from the risk of atherosclerosis in heat stress groups

Liver and kidney functions:

Data in Table (8) showed insignificant effect of heat stress or adding *Sargassum latifolium* on activity of AST, ALT and ALP in blood plasma of lambs,

Contrary, many researches indicated that the activities of serum ALT, AST and ALP increased in sheep and goats during the heat stress (Cwynar *et al.*, 2014; Hooda and Upadhyay, 2014; Aleena *et al.*, 2016). It was found that serum aminotransferases enzymes are sensitive markers of tissue injury and the elevation of their levels in serum is indicative of cellular leakages and loss of functional integrity of the cell membrane (Brancaccio *et al.*, 2010; Khan *et al.*, 2013). In the present study however, heat stressed lambs did not attain the risk of liver failure. Feeding lambs diet containing *Sargassum latifolium* did not significantly alter the liver function compared with the control lambs.

In addition, the present results indicated significant alteration in the kidney functions, whereas plasma creatinine and urea levels increased significantly (P<0.05) in heat stressed lambs (T0) compared with the control ones (Table 8). The normal level of creatinine in sheep ranging between 0.8 and 1.4 mg/100 ml and increased with failing glomerular filtration. Levels of urea and creatinine in animal blood are a very important tool in the diagnosis of diseases, some metabolic disorders and kidney glomerular filtration (Kour *et al.*, 2014). Several authors (Srikandakumar *et al.*, 2003; Kour *et al.*, 2014; Rathwa *et al.*, 2017) illustrated that the observed increase in urea and creatinine concentration during the environmental heat stress was attributed to the increase in blood flow in the peripheral capillaries and the decrease in the renal blood flow and glomerular filtration rate during the heat stress.

Dietary adding *Sargassum latifolium* significantly (P<0.05) modulate the alterations shown in kidney functions in heat stressed lambs in group T0, by decreasing creatinine and urea levels to be near to the normal values of the control lambs. In this respect, Josephine *et al.*, (2006) reported that sulfated polysaccharides isolated from *Sargassum wightii* had a significant nephron-protective role against oxidative renal injury induced by cyclosporine A in rats. It was also proved that the pretreatment with ethanolic extracts of *Sargassum ilicifolium* and *Sargassum swartzii* for 14 days protected significantly the liver and kidney from injury in carbon tetrachloride and acetaminophen intoxication rats (Hira *et al.*, 2017).

All previous findings supported the use of brown seaweeds, especially *Sargassum spp.*, to protect animals from the tissue damage and dysfunction of the important visceral organs such as liver and kidney caused by the environmental heat stress.

Table 8. Effect of diet containing *Sargassum latifolium* on activity of AST, ALT and ALP in blood plasma of growing Barki sheep exposed to heat stress

Parameter	Control group (C)	Treatment groups T0	T1	T2	±SEM
Aspartate amino transferase (AST), IU	27.57	35.22	29.28	28.69	3.15 NS
Alanine amino transferase (ALT), IU	27.63	30.27	28.53	28.98	1.71 NS
Alkaline phosphatase (ALP), IU	145.46	158.48	144.23	150.21	3.78 NS
Urea, mg/dl	48.03 c	72.70 a	62.88 b	48.71 c	2.48 **
Creatinine, mg/dl	1.18 c	1.77 a	1.47 b	1.30 bc	0.06 **

***, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c= values in the same row with different super scripts are significantly differed (P< 0.05).**

Live body weight changes:

Results in Table (9) revealed insignificant effect of heat stress or adding *Sargassum latifolium* on

final body weight of growing lambs, but average daily gain (ADG), total gain (TG), LBW change and feed intake were significantly (P<0.05) decreased in heat stressed than lambs in control. These results approved with those obtained by Júnior *et al.* (2014), who reported that heat stress in tropical and subtropical regions adversely affected the animal productivity such as the animal growth rate, which may be partially attributed to the decrease in feed intake. Also, Marai *et al.* (1997) and Padua *et al.* (1997) found that lambs' ADG was lower in hot season than in cold season as well as in a psychometric chamber (30–40 ºC) compared to a shelter (20–30 ºC). Similarly, body weight, growth rate, total body solids and body solids daily gain were impaired following the exposure to elevated temperatures (Marai *et al.*, 1997). Moreover, Abdel-Fattah (2014) found a loss in LBW of Baladi and Damascus goats due to 4 days exposure to direct solar radiation under desert conditions. He attributed the LBW loss under heat stress to losses in body fluids. Jaber *et al.* (2004) add other possible explanations for this reduction in LBW to be the consequent mobilization of fat (and possibly muscle) used for energy metabolism to compensate the less feed intake under heat stress.

Dietary addition of *Sargassum latifolium* (T1 and T2) significantly (P<0.05) increased lamb body weights compared to lambs of the group exposed to thermal stress (T0). Diet containing 2% *Sargassum latifolium* reverted the decrease in growth parameters to near the normal values of control lambs. Furthermore, diet containing 4% *Sargassum latifolium* significantly (P<0.01) increased daily gain, total gain and dry matter intake than all other groups, achieving insignificantly the best feed conversion ratio.

Table 9. Effect of diet containing *Sargassum latifolium* on growth performance parameters of growing Barki sheep exposed to heat stress

Item	Control group (C)	Treatment groups			±SEM
		T0	T1	T2	
Initial body weight, Kg	29.18	29.12	29.05	29.7	2.09 NS
Final body weight, Kg	37.10	33.80	36.94	39.05	2.31 NS
Average daily gain, gm/day	132.28^{b}	77.60^{c}	131.28^{b}	166.25^{a}	10.27 **
Total gain, Kg	7.92^{b}	4.67^{c}	7.88^{b}	9.97^{a}	0.62 **
Body weight Changes, %	27.83^{b}	16.10^{c}	27.62^{b}	36.66^{a}	2.80**
Average daily dry matter intake, kg	1.22 ^{a}	0.98 ^{b}	1.33 ^{a}	1.39 ^{a}	0.07 **
Feed conversion ratio	9.24^{c}	12.59^{a}	10.13^{b}	8.37^{d}	0.98**

***, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c, d= values in the same row with different super scripts are significantly differed (P< 0.05).**

Behavioral responses:

This type of study provides the vision to understand the necessity of different types of behavior for indorsing productivity and welfare (Altinçekiç and Koyuncu 2012). The patterns of basic behavioral changes like dinking, feeding, ruminating, idling, lying and standing in growing lambs were personally measured.

Drinking behavior:

Table (10) shows the effect of brown seaweed supplementation on drinking behavior (daily water intake, drinking time, drinking frequency, drinking period and drinking rate) of growing Barki male lambs on an observation period (12 hours) basis during hot season months.

The obtained results of the study designate that daily water intake and total drinking time significantly (P< 0.05) increased in heat stress group compared with the control group. The increase in drinking time in the thermal stress groups was due to the increased intake of water as a result of exposure of growing lambs to heat, to compensate for the loss of body water by evaporation throughout the respiratory system and the skin surface. Likewise, previous studies of Indu *et al.*, (2015) and Sejian *et al.* (2010) also recorded higher water intake in sheep under environmental stress. Under warm conditions, the rumen is consumed as a water reservoir during the heat of the day when it is most needed and as a fermentation tub when dryness rates are lower (Kadzere *et al.* 2002), which might be the possible cause for decrease of rumination during the warmer part of the day with the rise of water intake. Thus, the decrease of rumination plays a necessary role in the adversative effect of heat stress (Moallem *et al.* 2010).

It was found that the diet containing *Sargassum latifolium* significantly (P< 0.05) increased daily water intake and time spent for drinking during observation period. However, the differences in drinking frequency, drinking period and drinking rate were not significant (P ≥ 0.05) between ram lambs in all groups (Table 10).

Table 10. Effect of diet containing *Sargassum latifolium* on drinking behavior parameters of growing Barki sheep exposed to heat stress.

Drinking behavior	Control group (C)	Treatment groups			±SEM
		T0	T1	T2	
Daily water intake, liter / day	3.96^{c}	5.27^{a}	4.78^{b}	4.30^{b}	0.51 *
Daily water intake, ml/kg BW	118.87	170.02	149.68	133.77	21.72 NS
Total drinking time, min	2.50^{b}	4.00^{a}	3.25^{ab}	3.50^{ab}	0.31 *
Drinking frequency, time/ day	5.00	6.50	5.50	6.00	0.61 NS
Drinking period (min/ once), min	0.51	0.62	0.65	0.60	0.09 NS
Drinking rate, l/ min	1.61	1.35	1.48	1.24	0.20 NS

Total observation time, 720 min; *, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c= values in the same row with different super scripts are significantly differed (P< 0.05).

Feeding behavior:

Table (11) shows the effect of brown seaweed additives on feeding behavior (time, frequency, period, and rate) of growing Barki male lambs on an observation period (12 hours) basis during hot season months.

The obtained results of the study indicate that, time, frequency and rate of feeding significantly (P<0.05) decreased with the increase of temperature in heat stress group. These results are in agreement with Paranhos da Costa *et al.* (1992) and Kalyan *et al.*, (2017). It might be a result of voluntary adaptive recession of metabolic rate correlated with reduced appetite in heat-stressed lambs (Silanikove, 2000b). The result of the study indicates that the exposure of growing lambs to high ambient temperature severely modulates the behavior of growing lambs which is directed to circumvent the effect of the stressor.

Effect of brown seaweed supplementation (especially 4%) significantly (P<0.05) increased time spent, frequency and rate of feeding compared with lambs of heat stress group (T0). However, feeding period did not differ significantly between ram lambs in all groups (Table 11). The higher feed intake of T1 and T2 groups as compared to the control and T0 groups might be a compensatory feeding to meet their dry matter requirement, and it might be an adaptive mechanism of heat-exposed sheep to reduce their heat production in the warmer hour of the day and produce heat in the cooler part of the day. Diet containing 2 or 4% of *Sargassum latifolium* (T1&T2) reverted an increase in all parameters of feeding behavior in the heat stress group (T0) to near the normal values compared with the control group (C).

Table 11. Effect of diet containing *Sargassum latifolium* on feeding behavior parameters of growing Barki sheep exposed to heat stress.

Feeding behavior	Control group (C)	Treatment groups T0	T1	T2	±SEM
Feeding time, min	233.00^{a}	172.80^{b}	224.00^{ab}	248.70^{a}	21.82 *
Total feeding time as % of total observation time*	32.36^{a}	24.00^{b}	30.21^{ab}	34.54^{a}	3.00 *
Feeding frequency, time/ day	29.00^{a}	20.00^{c}	24.50^{b}	28.25^{a}	1.13**
Feeding period(min/ once), min	8.05	8.75	9.15	9.02	1.04 ^{NS}
Rate of feeding (bites/minute)	7.25^{a}	4.75^{b}	5.50^{b}	7.50^{a}	0.44 **

Total observation time, 720 min; *, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b= values in the same row with different super scripts are significantly differed (P< 0.05).

Ruminating behavior:

Table (12) shows the effect of brown seaweed supplementation on ruminating behavior (ruminating time, ruminating frequency, ruminating period, number of chews per minute) in growing Barki male lambs on an observation period (12 hours) basis during hot season months.

All parameters of ruminating behavior except ruminating period decreased each day with the increase of temperature in heat stress group (T0) compared with control group. Under hot conditions, the rumen is used as a water reservoir during the heat days when it is most needed and as a fermentation vat when dehydration rates are lower (Kadzere *et al.* 2002), which might be the possible reason for reduction of rumination during the hotter part of the day with the increase of water intake. Thus, the reduction of rumination plays a crucial role in the adverse effect of heat stress (Moallem *et al.* 2010).

These results of the study may indicate that, the effect of brown seaweed supplementation had a significant (P < 0.05) increase of ruminating time, ruminating frequency and number of chews per minute during observation period compared with lambs of heat stress group (T0). However, ruminating period did not differ significantly between ram lambs in all groups (Table 12).

Diet containing 2 or 4% of *Sargassum latifolium* (T1&T2) reverted the increase shown in all parameters of ruminating behavior in the heat stress group (T0) to near the normal values compared with the control group (C).

Table 12. Effect of diet containing *Sargassum latifolium* on ruminating behavior parameters of growing Barki sheep exposed to heat stress.

Ruminating behavior	Control group (C)	Treatment groups T0	T1	T2	±SEM
Total ruminating time, min/day	40.00^{a}	28.75^{b}	43.75^{a}	44.00^{a}	3.56 *
Ruminating time as % of total observation time	5.56^{a}	3.99^{b}	6.07^{a}	6.11^{a}	0.49 *
Ruminating frequency, time/ day	4.75^{a}	3.25^{b}	4.50^{a}	5.25^{a}	0.39 *
Ruminating period (min/ once), min	8.89	9.44	9.73	8.41	1.19 ^{NS}
Number chews per minute, chew	55.00^{a}	47.75^{b}	52.25^{ab}	54.00^{a}	1.89 *

Total observation time, 720 min; *, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c= values in the same row with different super scripts are significantly differed (P< 0.05).

Idling behavior:

Table (13) shows the effect of brown seaweed additives on idling behavior (idling time, idling frequency, idling period) in growing Barki male lambs on an observation period (12 hours) basis during hot season months.

The time spent in idling and idling period was significantly (P< 0.05) higher in heat stress group animals (T0) as compared to the control animals. Whereas, the frequency of idling decreased significantly (P<0.05) in the lambs of the heat stress group compared to the lambs of control group. Where, the time obtainable for idling depends on the time not used for other behaviors (drinking, feeding and ruminating).

Table 13. Effect of diet containing *Sargassum latifolium* on idling behavior parameters of growing Barki sheep exposed to heat stress.

Idling behavior	Control group (C)	Treatment groups T0	T1	T2	±SEM
Total idling time, min	444.50^{b}	514.35^{a}	448.80^{b}	424.00^{b}	22.90 *
Total idling time as %of total observation time	61.68^{ab}	71.44^{a}	62.33^{ab}	58.86^{b}	3.18 ^{NS}
Idling frequency, time/ day	38.75^{a}	29.75^{b}	34.50^{ab}	39.50^{a}	1.64 **
Idling period (min/ once), min	11.49^{b}	17.50^{a}	13.00^{b}	10.74^{b}	0.73 **

Total observation time, 720 min; *, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b= values in the same row with different super scripts are significantly differed (P< 0.05).

It is correspondent to the time between periods of dinking, eating and ruminating, whether the animals are standing or lying. Thus, the time spent idling varied inversely with the time feeding and ruminating, so that the periodicity of idling is the opposite of that for the active behavior (drinking, feeding and ruminating).

Results also revealed that adding brown seaweed supplementation significantly ($P < 0.05$) decreased idling time and idling period during observation period compared with lambs of heat stress group (T0). While, an opposite trend occurred in repetition of idling behavior (Table 13). These results indicated that diet containing 2 or 4% of *Sargassum latifolium* (T1&T2) reverted the change shown in all parameters of idling behavior in the heat stress group (T0) to near the normal values compared with the control group (C).

Standing and laying behavior:

Standing and lying time (ST & LT), standing and lying frequency (SF and LF) and standing and lying period (SP & LP) during observation period (12 hours) are presented in Table (14).

Heat stress did not affect significantly any component of standing and lying behavior, except ST, LT, and LP. During hot summer, experimental lambs in T0 increased ST (700.65 min) on the account of LT (19.35 min). Increasing standing time in a group of animals exposed to heat stress (T0) helps the animals to get rid of the extra heat by using convection. The same trend was found in ST% and LT%. Decreasing LT in heat stress group was achieved by decreasing LP.

In the present study, the longer proportion of standing time and shorter lying time of the T0 group might be assigned to their effort to improve heat reduction by fascinating advantage of increased rational water loss, radiating surface expanse, and air crusade or convection by increasing its body surface area exposed to heat stress (Allen *et al.*2015). In agreement with our findings, Dikmen *et al.* (2011) also recorded lower lying and more standing behavior in shorn Awassi lambs to dissipate heat during the warmer part of the day. Animals occupy more time in standing without feeding and less time in lying as heat load increased (Legrand *et al.* 2011; Kanjanapruthipong *et al.*2015). The less lying and higher standing time in the current study is a mark of a lack of welfare in which the lambs tend to adjust their other behavior to compensate the bad effects of heat stress (Dikmen *et al.* 2011; Silanikove 2000a).

Effect of brown seaweed supplementation (especially 4%) was significant ($P<0.01$) on decreasing standing time during observation period compared with lambs of heat stress group (T0). While, an opposite trend occurred in repetition of lying behavior (Table 14). These results revealed that diet containing or 4% of *Sargassum latifolium* (T1&T2) reverted the change shown in all parameters of standing ang lying behavior in the heat stress group (T0) to near the normal values compared with the control group.

Table 14. Effect of diet containing *Sargassum latifolium* on standing and lying parameters of growing Barki sheep exposed to heat stress,

Standing and lying behavior	Control group (C)	Treatment groups T0	T1	T2	±SEM
Total standing time, min	642.50 ^{c}	700.65 ^{a}	680.31 ^{ab}	667.10 ^{bc}	9.65**
Total standing time as % of total observation time	89.23 ^{c}	97.31 ^{a}	94.49 ^{ab}	92.65 ^{bc}	1.34**
Standing frequency, time/ day	3.50	3.50	4.25	4.25	0.40^{NS}
Standing period, (min/ once), min	187.60	204.30	166.44	163.09	17.60^{NS}
Total lying time, min	77.50 ^{a}	19.35 ^{c}	39.69 ^{bc}	52.90 ^{ab}	9.65**
Total lying time as % of total observation time	10.77 ^{a}	2.69 ^{c}	5.51 ^{bc}	7.35 ^{ab}	1.34**
Lying frequency, Time/ day	2.50	2.50	3.25	3.25	0.39^{NS}
Lying period, (min/ once), min	31.87 ^{a}	8.17 ^{b}	14.77 ^{ab}	19.35 ^{ab}	5.91*

Total observation time, 720 min; *, P<0.05; **, P<0.01; NS, non-significant; C, lambs were kept in comfortable environmental conditions and were not offered seaweed; T0, lambs were exposed to heat stress and were not offered seaweeds; T1, lambs were exposed to heat stress and were supplemented with 2% brown seaweeds; T2, lambs were exposed to heat stress and were supplemented with 4% brown seaweeds; a, b, c= values in the same row with different super scripts are significantly differed (P< 0.05).

CONCLUSION

Growing lambs are considered a highly sensitive animal to heat load and thus productivity is affected as far as its ability to adapt in adverse climatic conditions without compromising production. Through the current study, heat stress had a severe effect on body weight gain and blood constituents as well as the normal behavior of developing lambs and their level of well-being.

We have concluded in this study that the addition of brown seaweed "*Sargassum latifolium*" (2 and 4% from concentrate feed mixture) to lamb diets in summer showed beneficial effects on feed intake, growth rate, feed conversion ratio, blood hematology and biochemicals, and drinking, feeding, ruminating, idling, standing, and lying behavior Dietary addition of *Sargassum latifolium* at a level of 4% to CFM not only alleviate adverse effects of heat stress, but also achieved higher growth performance during heat stress.

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Competing interests

The authors emphasize that, about the manuscript, there is no conflict of interest between them about research, authoring and publishing. The authors also confirm that they have no competing interests.

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الإستجابات الفسيولوجية والسلوكية لذكور الحملان البرقي النامية المعرضة للإجهاد الحراري والمغذاه علي الطحالب البنية كإضافات علفية تحت الظروف شبه الجافة

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هدفت هذه الدراسة إلى فحص آثار إستخدام جرعتين مختلفتين من الأعشاب البحرية البنية "*Sargassum latifolium*" باستخدام ذكور الحملان البرقي النامية المعرضة للإجهاد الحراري لمدة ٦٠ يومًا وذلك على أداء نموها واستجاباتها الفسيولوجية والسلوكية. تم استخدام عشرين ذكر حمل برقي نامي عمرها ٦-٨ أشهر بمتوسط وزن الجسم الحي ٢٩,٢٦ ± ٢,٠٩ كجم وتم تقسيمها بشكل عشوائي إلى أربع مجموعات تجريبية متساوية. بالنسبة للمجموعة الأولي تم الاحتفاظ بالحملان في في ظروف بيئية مريحة (غير معرضة للإجهاد الحراري) ولم يتم تقديم الأعشاب البحرية لها وعينت كمجموعة ضابطة، كما تعرضت الحيوانات في المجموعة الثانية للضغط الحراري وقدمت لهم الغذاء بدون الأعشاب البحرية، في حين تعرضت حيوانات في المجموعة الثالثة والرابعة للإجهاد الحراري وتم تزويدها بنسبة ٢ و ٤٪ (من خليط التغذية المركزة) من الأعشاب البحرية البنية على التوالي. تم تسجيل العلف المأكول ووزن الجسم يوميا. تم تقدير صورة دم كاملة وكذلك الأدلة البيوكيميائية للبلازما كل أسبوعين بينما تم قياس الاستجابات السلوكية للحملان البرقي النامية في الأسبوع الأخير من التجربة. زادت حيوانات المجموعتين اللتان تم استكمال غذائهما بالطحالب البنيه *Sargassum latifolium* في متوسط الزيادة اليومية وإجمالي وزن الجسم مقارنة بحيوانات المجموعة المعرضة للإجهاد الحراري فقط، علاوة على ذلك حققت الحملان المغذاه على ٤% من الطحلب أفضل نسبة تحويل تغذية (٨,٣٧%). زادت مكملات الأعشاب البحرية ($P < 0.05$) تركيز كرات الدم البيضاء والهيماتوكريت وتركيز الهيموجلوبين. كما أن تغذية الحملان بنظام غذائي يحتوي على ٤ ٪ من طحلب *Sargassum latifolium* عمل على زيادة ($P < 0.01$) البروتين الكلي في السيرم والألبومين ومستوى البوتاسيوم لكنه خفض ($P < 0.01$) من إجمالي الدهون في السيرم وثلاثي الجليسيرول وإجمالي الكوليسترول مقارنة مع حيوانات المجموعة الضابطة.أثرت مكملات الأعشاب البحرية البنية بشكل كبير على معدل استهلاك المياه اليومي وبعض الإستجابات السلوكية.يمكن استنتاج أن مكملات الأعشاب البحرية البنية *Sargassum latifolium*بنسبة ٢ ٪ و ٤ ٪ من مخلوط التغذية المركزة لذكور الحملان البرقي المعرضة للإجهاد الحراري في الصيف أدى إلى تحسين الأداء الإنتاجي من خلال تحسين معدل النمو ومكونات الدم إلى جانب سلوك الإغتذاء وسلوك الوقوف والرقاد.

الكلمات الدالة:الحملان البرقي - الإجهاد الحراري- الأعشاب البحرية البنية- التنظيم الحراري- الدم- الاستجابات السلوكية.