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Effects of alginates on the growth, haematological, immunity, antioxidant and pro-inflammatory responses of rabbits under high temperature

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ABSTRACT

Heat stress (HS) is one of the most severe hurdles impacting rabbit growth, immunity, homeostasis, and productivity. Alginate oligosaccharides (AOS) have considerable beneficial effects due to their plausible antioxidant and immune-stimulatory properties. This work was planned to explore the preventive function of AOS as a new bio-feed additive against the harmful effects caused by environmental HS on growing rabbits. Rabbits were allotted in four experimental groups (25 animals in each group) and fed on a basal diet supplemented with 0.0 (AOS0), 50 (AOS50), 100 (AOS100), and 150 (AOS150) mg AOS/kg diet reared under summer conditions. Dietary AOS supplementation improved significantly ($P \leq 0.001$) feed conversion rate, while both AOS100 and AOS150 significantly ($P \leq 0.001$) enhanced the final body weight and body weight gain. All AOS addition significantly increased nitric oxide and lysosome activity and significantly reduced interferon-gamma (IFNγ) compared with those in the control group. Tumor necrosis factor α (TNFα), interleukin1β (IL-1β), myeloperoxidase and protein carbonyl levels were significantly reduced in rabbits fed diets containing AOS (100 and 150 mg/kg) compared with those in the control group under heat stress conditions. In addition, glutathione (GSH) and catalase (CAT) were significantly ($P \leq 0.001$) improved with increasing AOS dietary levels compared with the control group. Still, total antioxidant capacity (TAC), malondialdehyde (MDA), hematocrit, mean corpuscular volume (MCV), eosinophils, and lymphocytes did not change. Erythrocyte's indices improved significantly ($P \leq 0.001$), while neutrophils and white blood cell counts were decreased by dietary AOS inclusion. Immunological (IgM and IgG) were markedly reduced in AOS-treated groups compared with the control group. The current investigation exemplified that AOS as a novel bio-feed additive that could be an effective strategy to extenuate prejudicial effects in heat-stressed rabbits via enhancing immunity, and antioxidant defence system, further regulating the inflammation cytokines.

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1. Introduction

With the increasing warming of the global climate, integrated with the rising environmental heat stress (HS) on animals, has become more serious and is one of the utmost significant stress-triggering factors in the world. HS is a serious factor facing the rabbit industry in tropical and semi-tropical regions. Rabbits exposed to cyclic or chronic HS were connected with a decrease in growth performance (Sheiha et al., 2020), altered the haematological variables, and suppressed humoral and cell-mediated immune responses (Madkour et al., 2020; El-Desoky et al., 2021). It is well documented that HS harms immunity and causes disturbance of homeostasis profile in rabbits (Abdelnour et al., 2019 and 2020). Due to the declining number of sweat glands in rabbits, they are more vulnerable to HS (Marai et al., 2002). HS induced a substantial release in the levels of pro-inflammatory cytokines like interleukins (ILs) and interferon-gamma (IFN-γ) in rabbits (Abdelnour et al., 2020; Sheiha et al., 2020). Exposing rabbits to HS-induced liver dysfunction (Abdelnour et al., 2020), reduced absorption of nutrients (Ayyat et al., 2021), increased oxidative stress (Abdelnour et al., 2020), lowered antioxidant capacity (El-Desoky et al., 2021; El-Ratel et al., 2021), and reduced the immunity (Madkour et al., 2020). A negative influence of HS on rabbit feed efficiency, growth indices, fecundity, and viability were also detected (El-Desoky et al., 2021; Sirotkin et al., 2021). To support animals in enhancing their productivity under hot climates and mitigating the negative effects of HS, it is essential to use nutritional approaches for this intention.

The exploration for practical, safe and effective naturally occurring feed supplements is a promising key that can be useful to combat the harmful impacts of HS in livestock species. In the last decades, some studies have revealed that marine algae contain a rich natural bioactive compound with multi-beneficial health effects (Gamal-Eldeen et al., 2013; Saadaoui et al., 2021; Ming et al., 2021). Algae-derived bioactive compounds have numerous commercial applications like dietary supplementation, feed additives and pharmaceutical applications (Gomez-Zavaglia et al., 2019; Most and Yates, 2021). In this regard, *Sargassum dentifolium* is brown seaweed and belongs to the Sargassaceae family. This seaweed is widely distributed in Egyptian coastal like the red sea and the Mediterranean Sea and is considered the most effective source of alginates polysaccharides (El-deen, 2011; Gamal-Eldeen et al., 2013). The methanolic extracts of *Sargassum dentifolium* exhibited several biological activities, including anti-genotoxic and promising anti-mutagenic action (Gamal-Eldeen et al., 2013). Among many active compounds isolated from seaweed is alginate, which consisting of β-d-mannuronic acid and α-l-guluronic (Ming et al., 2021). Alginate oligosaccharides extracted from marine algae have considerable beneficial effects on the productive traits in livestock and poultry (Wan et al., 2017; Ming et al., 2021). Several studies reported that AOS improved the growth performance in the broiler (Yan et al., 2011; Yang et al., 2016) and pigs (Wan et al., 2017) by improving the antioxidant capacity and reducing the pathogens bacteria (Wang et al., 2006), as well as improving the intestinal barrier function. Generally, AOS derived from brown algae could be used as a favourable natural antioxidant, antimicrobial and anti-inflammatory constituent.

Nevertheless, utmost of the previous publications did not reflect the utilization of AOS in stressed rabbit diets to relieve the deleterious influences of HS by regulating the immunity, redox status, and pro-inflammatory responses. It is inevitable to detect innovative bio-natural feed supplement strategies to prevent the antagonistic effects of HS in animals. Previous reports are few considering the potentiality of using AOS in livestock feed additives (Ming et al., 2021), especially its safety and applicability as an anti-heat stress agent in rabbits. Based on the literature (Yan et al., 2011; Yang et al., 2016; Wan et al., 2017), we postulated that the dietary inclusion of AOS could boost the health, immunity, redox status, and pro-inflammatory in rabbits exposed to HS due to its antioxidant, anti-inflammatory, and immune-booster effects.

2. Materials and methods

2.1. Isolation and characterization of Alginate oligosaccharides (AOS)

Sargassum dentifolium (SD) is brown algae and belongs to the class Phaeophyceae. SD was collected from marine ecosystems in Alexandria. As previously performed, AOS was extracted from the SD dried via the adjusted protocol (Larsen et al., 2003). A spectrophotometric system detected the levels of the AOS. Algae-derived AOS was dried by lyophilization protocol and included in the diets according to the study procedures. All in-vivo animal treatments complied with the regulations established by the Institutional Animal Care and Use Committee, Zagazig University (Approval no. ZU-IACUC/2//F/367/2022). All efforts were assumed to safeguard minimal distress to rabbits during the trial.

2.2. Animal management and experimental design

One hundred and twenty growing rabbit males at weaning (7 weeks of age) with an initial body weight (IBW) of 801.5+ 13.7 g were included in the current experiment. The existing work was carried out at the Rabbit Research Unit, Faculty of Agriculture, Zagazig University, Egypt. Rabbits were individually reared in standard galvanized wire cages (40 cm × 50 cm × 35 cm) equipped with a manual feeder and automatic drinkers. Animals were randomly assigned into four identical experimental groups, each of which (25 rabbits), for ten consecutive weeks during the summer conditions. The experimental groups were as follows: the control group (AOS0) received a basal diet without supplementation; the other three treated groups received a basal diet +50 (AOS50), 100 (AOS100), and 150 mg (AOS150) of alginate oligosaccharides/kg diet. Ration constituents and their chemical composition are depicted in Table 1, conferring the optimal requirements for growing rabbits (NRC, 1977). To confirm the severity of environmental HS exposure on growing rabbits, the valuation of relative humidity (RH), ambient temperature (AM), and temperature-humidity index (THI) were verified indoors on the farm according to a natural environment of HS in Sharkia Province, Egypt conferring to the method of (Marai et al., 2002). The THI was considered as the subsequent equation: THI = db °C-((0.31–0.31RH) (db °C-14.4)), where t °C = dry bulb temperature in degrees Celsius. Depending on the thermos-neutral zone of an animal environment, the THI values were categorized as follows: <27.8 = absence of heat stress; 27.8–28.9 = moderate heat stress; 29.0–30.0 =

Table 1
Ingredients and nutrient contents of the basal diet of growing rabbits (as fed).

Items	Basal diet
Ingredient	%
Maize	20
Soybean meal	20
Wheat bran	16
Berseem hay	30
Barley grain	10
Molasses	2
Limestone	1
NaCl	0.5
Premix*	0.5
Approximate analysis, %**	
ME, MJ/kg	7.95
Crude protein	17.50
Calcium	0.88
Available phosphorus	0.20

* Each 1 kg of premix (minerals and vitamin mixture) contains vit. A, 20,000 IU; vit. D3, 15,000 IU; vit. E, 8.33 g; vit. K, 0.33 g; vit. B1, 0.33 g; vit. B2, 1.0 g; vit. B6, 0.33 g; vit. B5, 8.33 g; vit. B12, 1.7 mg; pantothenic acid, 3.33 g; biotin, 33 mg; folic acid, 0.83 g; choline chloride, 200 g.

** Calculated according to NRC (1977).

severe heat stress; 30.0 = very severe heat stress.

2.3. Growth performance

The body weight (BW) of rabbits was documented for the assessment of final body weight (FBW) and daily body weight gain (BWG). Feed intake (FI) also was recorded daily in each group to analyze average daily feed intake (ADFI). The feed conversion ratio (FCR) was assessed at the termination of the experiment.

2.4. Haematological variables and physiological responses

Blood samples were gathered from the marginal ear vein of each rabbit (6 animals per group) using heparinized tubes (Moore et al., 2015). Each blood sample was allocated into two subsamples: the first subsample was hired to evaluate haematological indices. In contrast, the second subsample was centrifuged at 2000 ×*g* for 20 min at 4 °C to attain plasma. The plasma samples were kept for biochemical analysis (at −20 °C). The whole blood was used to determine erythrocytes [red blood cells (RBCs, 106); mean corpuscular haemoglobin (MCH, %); mean corpuscular volume (MCV, %); mean corpuscular haemoglobin concentration, (MCHC,%)] using an automated haematology analyser (Hospitex Hema Screen 18, Sesto Fiorentino, Italy), while leucocytes variables [white blood cells (WBCs), lymphocytes, eosinophils monocytes and basophils] were assessed according to the method of (Weiss and Wardrop, 2011). The physiological responses, including respiration rate (RR; breaths/min) and rectal temperature (°C) were monitored and documented weekly for each animal.

2.5. Redox status and immunity

The assessment of protein carbonyl (PC; ab126287) was detected using a quantitative calorimetric ELISA kit (sensitivity up to 0.015 μM) purchased from Abcam Company (Cambridge, CB2 0AX, UK). Diagnostic ELISA kit was used for assessing the levels of malondialdehyde (MDA) (MyBioSource, San Diego, CA, USA; cat No: MBS8806802) based on the colorimetric procedures reported by (Ohkawa et al., 1979) with values 31.25–2000 ng/mL for detection range and the value of sensitivity was up to 9.15 ng/mL. The antioxidant enzymes, including catalase (CAT), glutathione (GSH) total and antioxidant capacity (TAC), were determined by applying the commercial diagnostic kits (Bio diagnostic, Egypt). The total plasma immunoglobulin M (IgM) and G (IgG) levels were measured using ELISA kits according to the methodology described in (Humam et al., 2019).

2.6. Pro-inflammatory cytokines

The ELISA kits provided by CUSABIO Company (Houston, TX, USA) were used for assessing the tumor necrosis factor-α (TNF-α) in rabbits. This ELISA (CSB-E06998Rb) kit has values of detection range between 78 and 5000 pg/mL and sensitivity up to 19.5 pg/mL. The plasma levels of myeloperoxidase (Catalog No. MBS266621), interleukin1β (Catalog No. MBS266621) and interferon-gamma (Catalog No. MBS220076) were determined using ELISA kits provided by MyBioSource Company (San Diego, CA, USA). The turbidimetric technique assessed lysozyme activity (Abdelnour et al., 2020; Ohkawa et al., 1979). The nitric oxide (NO) level was analyzed in the rabbit plasma with Griess reagent (Rajaraman et al., 1998).

2.7. Statistical analysis

All results were examined using SPSS 25.0 program with a one-way ANOVA (apply diet as a fixed factor) using the post hoc Tukey's test. All results were expressed as (mean ± SEM). The statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1. THI values, growth performance and physiological responses

During the study period, the THI values were estimated and presented in Table 2. Overall, the THI was 31.83 in all experimental periods. The predictable THI values fluctuated from 31.00 to 33.57, representing a very serve degree of environmental HS on growing rabbits. As shown in Table 3, exposure to heat stress persuaded significant decay in the final body weight (FBW). In AOS100 and AOS150 groups, a significant increase was detected in the FBW and body weight gain (BWG), while FCR presented a significant decline in all supplemented groups compared with those documented in the heat stress group. No significant effect was recorded among all experimental groups and the HS group. Overall, the inclusion of AOS in the diets of heat-stressed growing rabbits significantly improved the growth performance by enhancing the FBW, BWG and feed efficiency. Additionally, rabbits fed AOS had lower respiration rates (RR) and rectal temperatures (RT) when compared with the heat-stressed group (AOS) (Table 3). Dietary inclusion of AOS in rabbit diets effectively reduced the increment of RR and RT induced by HS.

Global warming is one of the principal climatological issues that may affect the livestock industry in the upcoming era. Heat stress (HS) exhibited many harmful effects on animals' whole cellular biological activity, altering internal body environments and reducing their productive traits. It is necessary to search for new policies to promote the body's defence system in environmental HS conditions and neutralize the deleterious consequences. With the present research, rabbits were hired to explore the ameliorative function of dietary marine oligosaccharides (especially AOS) supplementation as a new bio-feed supplement against HS adverse effects on the growth, haematological, humoral immune indices, redox status and pro-inflammatory responses. Data obtained exhibited that the dietary presence of AOS effectively boosted growth performance (FBW, BWG and FCR), haematological, immune status, redox status, and inflammatory cytokines in growing rabbits reared under high environmental conditions. For the first time, our research examined the use of AOS as a new bio-feed supplement produced by brown algae (*Sargassum dentifolium*; SAG), in the animal or rabbit diets to mitigate the destructive influences of HS.

Our data exhibited that the AOS inclusion significantly enhanced the FBW, BWG and feed efficiency in stressed rabbits. Ellamie et al. (2020) clarified that the dietary SAG (2–4%) could restore the body loss induced by HS in male Barki sheep. Moreover, thermo-respiratory responses of the HS sheep were significantly ($P < 0.05$–0.001) modulated by SAG dietary supplementation (Ellamie et al., 2020). Studies have reported that microalgae or their secondary metabolites considerably impact reducing the negative influences of HS via restoring the BWG, FBW and feed efficiency in heat-stressed rabbits (Abdelnour et al., 2020). Moreover, Kang et al. (2013) described that dietary inclusion with fresh liquid algae (1%) improves the BWG, immune features and significantly increases the Lactobacillus bacteria community in the intestinal of broiler chickens. Gumus et al. (2018) indicated an enhancement in the FBW by 19.3% and no effect was observed in FI as affected by fucoxanthin pigment (200 mg/kg) supplemented with the broiler

Table 2
Calculated temperature-humidity index (THI) throughout the experimental period in Sharkia Province, Egypt.

Item*	July	August	September	*P* value
Temperature (°C)	32.78 ± 0.40[a]	31.42 ± 0.25[b]	31.20 ± 0.11[b]	0.006
Relative humidity (%)	68.78 ± 1.26	70.42 ± 1.65	71.97 ± 1.79	0.407
THI	31.10 ± 0.31[a]	29.93 ± 0.20[b]	29.67 ± 0.22[b]	0.004

* THI; temperature humidity index.

Table 3
Effect of supplemental dietary AOS on growth performance and physiological responses of growing rabbits under heat stress.

Items	AOS level[1]				Pooled SME	*P*-value
	AOS0	AOS50	AOS100	AOS150		
Growth performance						
Initial body weight (IBW; g)	801.25	812.5	797.5	796.25	13.797	0.8337
Final body weight (FBW; g)	1960.0^{c}	2037.5^{b}	2126.2^{a}	2113.7^{a}	21.096	0.0004
Feed conversion ratio (FCR; g feed/g gain)	3.54^{a}	3.26^{b}	3.25^{b}	3.21^{b}	0.058	0.0067
Feed intake	136.83	133.00	143.92	141.08	6.78	0.130
Body Weight Gain (BWG;g)	1158.75	1225.0^{b}	1328.7^{a}	1317.5^{a}	24.5	0.001
Rectal Temperature (°C)						
Week1	39.46^{a}	39.08^{ab}	38.63^{bc}	38.83^{c}	0.15	0.0021
Week2	39.63^{a}	39.30^{ab}	39.07^{b}	38.36^{c}	0.21	<0.0001
Week3	38.80^{a}	38.57ab	38.58^{ab}	38.23^{b}	0.14	0.0483
Week4	38.61	37.98	38.67	38.57	0.11	0.2104
Week5	39.05^{a}	38.52^{b}	38.53^{b}	38.22^{b}	0.1	0.0014
Week6	39.61^{a}	39.12^{a}	39.28^{a}	38.58^{b}	0.14	0.0014
Week7	39.08^{ab}	39.30^{a}	38.82^{b}	38.75^{b}	0.1	0.0498
Week8	38.83^{a}	38.03^{b}	38.35^{b}	38.23^{b}	0.15	0.0025
Week9	39.12^{a}	38.10^{b}	38.43^{b}	38.16^{b}	0.14	0.0002
Respiration Rate (breaths/min)						
Week1	133.125^{a}	112.5^{b}	100.62^{c}	101.25^{c}	2.43	<0.0001
Week2	136.62^{a}	106.37^{b}	102.50^{b}	102.87^{b}	2.64	<0.0001
Week3	136.50^{a}	103.62^{b}	103.37^{b}	104.75^{b}	2.65	<0.0001
Week4	136.25^{a}	103.87^{b}	103.12^{b}	100^{b}	2.7	<0.0001
Week5	135.37^{a}	103.87^{b}	103.87^{b}	100.37^{b}	2.62	<0.0001
Week6	136.75^{a}	103.63^{b}	103.13^{b}	101.87^{b}	2.77	<0.0001
Week7	138.625^{a}	106.00^{b}	102.88^{b}	101.75^{b}	2.8	<0.0001
Week8	139.00^{a}	103.75^{b}	106.25^{b}	103.5^{b}	2.75	<0.0001
Week9	139.00^{a}	102.75^{b}	104.87^{b}	103.13^{b}	2.82	<0.0001

a, b, c Means within a row without a common superscript letter differ at $p < 0.05$.
[1] AOS0, AOS50, AOS100 and AOS150 = 0, 50, 100 and 150 mg AOS/kg DM diet, respectively.

diets. In contrast, Perenlei et al. (2014) elucidated that dietary supplementation with astaxanthin-rich yeast did not substantially influence the broiler's growth performance. However, numerous prior reports have revealed that the dietary inclusion of natural pigments has moderate effects on animal growth performance (Wang et al., 2006; Abdelnour et al., 2020). Our consequences align with (Wan et al., 2017), who indicated that dietary pig enriched with 50, 100, or 200 mg/kg diet AOS significantly improved growth performance. El-Deek et al. (2011) concluded that adding *Ascophyllum nodosum* (*A. nodosum*, 0.05% of feed) to the broiler diet tended to reduce the negative effect of prolonged HS on the growth performance, representing that this kind of feed additive can be applied to enhance the birds' welfare during HS events in the poultry sector. The impact of natural bioactive material derived from brown algae on growth performance has been studied in animals (Ellamie et al., 2020; Mohyuddin et al., 2021). However, the protective roles of those compounds in mitigating the detrimental influences of global warming on animal health as anti-heat stress agents are ambiguous.

3.2. Pro-inflammatory cytokinesis

Table 4 revealed significant ($P < 0.01$) changes in pro-inflammatory cytokinesis as affected by HS and dietary AOS inclusion. Rabbits fed with AOS at levels of 100 or 150 mg/kg diet showed a significant decline in TNF-α, IL-1β and myeloperoxidase levels during heat stress conditions. The AOS50 treatment exhibited intermediate values for TNF-α, IL-1β and myeloperoxidase levels with a significant difference compared with the control value. All dietary-supplemented groups successfully ($P < 0.01$) restored the levels of IFNγ in rabbits exposed to high temperatures. Compared to the control, the rabbit that received a diet enriched with AOS showed an elevation in nitric oxide levels ($P < 0.01$). Both AOS100 and AOS150 treatments showed a significant increase ($P < 0.01$) in plasma lysosome activity, while AOS50 recorded intermediate values with significance concerning the control value. Heat-stressed rabbits showed a significant reduction in the lysosome activity and nitric oxide levels and a significant increment in other pro-inflammatory indices (Table 4). High environmental temperature significantly elevated the plasma levels of inflammatory cytokines in pigs (Ju et al., 2014) and rabbits (Abdelnour et al., 2020; Sheiha et al., 2020). HS also affects the balance of pro-inflammatory cytokines and anti-inflammatory responses (Mohyuddin et al., 2021).

Using natural components to neutralize and restore the dysregulation of cytokine-induced by HS could be an innovative and effective strategy for improving the well-being of animals during elevated

Table 4
Effect of supplemental dietary AOS on pro-inflammatory cytokines of growing rabbits under heat stress.

Items[1]	AOS level[2]				Pooled SEM	*P*-value
	AOS0	AOS50	AOS100	AOS150		
TNF-α (pg/mg)	116.0^{a}	64.33^{b}	46.33^{c}	50.67^{c}	3.69	<0.0001
IFN Y (pg/mL)	78.0^{a}	9.67^{b}	60.33^{b}	58.00^{b}	1.75	0.0001
Interleukin1β (IL-1β) (pg/mL)	99.33^{a}	91.00^{b}	80.33^{c}	80.67^{c}	1.80	0.0002
Myeloperoxidase (ng/mL)	8.55^{a}	3.06^{b}	1.36^{c}	1.083^{c}	0.28	<0.0001
Lysosome (ng/mL)	1.34^{c}	1.77^{b}	2.07^{a}	2.17^{a}	0.05	<0.0001
Nitric oxide (Umol/L)	33.0^{b}	56.7^{a}	57.7^{a}	50.3^{a}	2.18	0.0001

a, b, c Means within a row without a common superscript letter differ at $p < 0.05$.
[1] Tumor necrosis factor -α = TNF-α; Interferon gamma (IFNγ).
[2] AOS0, AOS50, AOS100 and AOS150 = 0, 50, 100 and 150 mg AOS/kg DM diet, respectively.

temperatures in the upcoming eras. In the present investigation, AOS dietary supplementation (50, 100 and 150 mg/kg diet) induced a significant reduction in the inflammatory cytokines such as TNF-α, IFNγ, IL-1β and myeloperoxidase in heat-stressed rabbits. While considerable elevation was recorded in all treated groups regarding blood lysosome activity and nitric oxide in comparison with the HS group. TNF-α is a pro-inflammatory cytokine that plays a significant part in various cellular actions such as proliferation, differentiation, cell survival, and death (Parameswaran and Patial, 2010). TNF-α is secreted by inflammatory cells, which inflammation-related stressors may complicate. Several authors indicated that HS was reported to activate inflammatory signalling like TNF-α, IL-1β, and IFNγ in heat-stressed rabbits (Abdelnour et al., 2020; Madkour et al., 2020; Saadaoui et al., 2021).

This significant pro-inflammatory cytokine release during heat stroke might be associated with a progressive inflammation response (Madkour et al., 2020). Inclusively, the dietary AOS added during environmental HS produced a substantial boost in the immune response, regulated the redox status and pro-inflammatory pathway, and was efficiently employed to cope with the undesirable impacts of environmental HS. There were doubts about the fruitful commercialization of biological compounds derived from marine algae as a new bio-feed supplement applied in the livestock industry (Saadaoui et al., 2021).

Moreover, serum inflammatory cytokines (interleukins and TNF-α) response in mice was augmented in the HS group as related to the control group (Mohyuddin et al., 2021). Our data exhibited that AOS has a considerable anti-inflammatory competence which constrains the synthesis of inflammatory cytokines encouraged by environmental HS. Results suggested that AOS successfully decreased the negative impact of HS by repressing the high synthesis of TNF-α, IL-1β, and IFNγ in the plasma of rabbits. In previous work, dietary prodigiosin supplementation reinstated the changed pro-inflammatory synthesis in rabbits reared under high temperatures (Abdelnour et al., 2020). The supplement of AOS (0.2%) displayed dramatic immunostimulatory properties by bringing IL-1β, IL-10, and IFNγ mRNA expression in cecal tonsils of no challenged birds (Yan et al., 2011). Furthermore, our findings showed that the oxidative damage induced by HS is ascribable to the elevation of TNF-α, IL-1β, and IFNγ levels, elevating the activity of myeloperoxidase as it is released into extracellular spaces during inflammation and the activation of neutrophils (Rosales, 2018).

Myeloperoxidase (MPO) is a vital component of the humoral innate immune system. Neutrophils release it principally to extend the defence system alongside invading pathogens or other stressors (Davies and Hawkins, 2020). Myeloperoxidase has been a valuable biomarker and diagnostic tool in several diseases and stressful environmental conditions (Aratani, 2018). Our results exhibited that the heat-stressed rabbits presented a substantial increase in the levels of MPO as compared with the treated groups. Supplementation diets with AOS significantly suppressed the level of MPO in rabbits exposed to HS. These results depicted that AOS applied defensive action against the inflammatory releasing via suppression of MPO resealing. Feeding heat-stressed rabbits with prodigiosin (isolated from *Serratia marcescens*; 100 and 150 mg/kg) as a natural antioxidant induced a significant ($P < 0.009$) increase in both nitric oxide and lysosome activity relative to the HS group (Abdelnour et al., 2020). As a green microalga, it has been indicated that the *Spirulina platensis* significantly decreased the TNF-alpha immunostaining and MPO in the inflamed paw from human neutrophils (Joventino et al., 2012). Previous publications confirmed that some extracts and active ingredients isolated from an inclusive diversity of algae have plausible anti-inflammatory actions alongside various stressors (Radhika et al., 2013; Aladaileh et al., 2020). AOS can employ an immunomodulatory agent by inducing greater expression of inducible nitric oxide synthase (iNOS) to augment the synthesis of nitric oxide (NO) (Iwamoto et al., 2005). AOS represents a potent stimulator for activating the macrophage. Authors anticipated that higher levels of NO might be associated with increased blood flow to the skin, thus helping an animal to increase heat dissipation and enhance heat loss from the body. The dietary inclusion of AOS may allow the animals to sustain their body homeostasis by triggering endogenous cellular defence machinery to cope with protein oxidation and inflammation induced by environmental HS. More investigations on various animal species should be explored with growing awareness relating marine algae and its derivatives as a reliable source of bio-feed supplements hired in the animal industry and welfare. In in vivo and in vitro trials, HS could activate the lysosomal-mitochondrial apoptotic in a cell line (Yi et al., 2017). AOS may offer potential pharmaceutical targets and approaches to restoring the disturbance occurrence related to the physiological pathways caused by heat stroke. Additionally, a higher level of lysosomal enzymes was observed after exposing humans to thermal stress, suggesting a deleterious impact on the lysosomal membranes (Mila-Kierzenkowska et al., 2012).

3.3. Redox status

Results presented in Table 5 revealed that the levels of CAT and GSH significantly ($P < 0.001$) while PC increased significantly ($P < 0.001$) reduced as a response to AOS inclusion in stressed rabbits. The plasma levels of CAT and GSH were significantly increased in AOS150, followed by ASO100 and AOS50 treatments, with a significant difference concerning those in the control group. Both TAC and MDA levels showed non-significant influence by a diet enriched with AOS during heat stress. AOS considerably ($P < 0.001$) reduced the protein carbonyl (PC) as a protein oxidation marker in heat-stressed rabbits. Generally, the present study indicated that the diet enriched with AOS reduced significantly ($P < 0.05$) the concentration of plasma PC and improved significantly ($P < 0.001$) the blood antioxidant defence system of the stressed rabbit (Table 5).

The environmental HS significantly enhanced the serum oxidative markers like protein carbonyl (PC) and malondialdehyde (MDA) while significantly decreasing the antioxidant defences for CAT and GSH in plasma rabbits with no significant impact on TAC value. The curtailment of enzyme antioxidants (SOD and CAT) activities trigged by environmental HS may be elucidated by the exploitation of these enzymes to neutralize the oxidative stress formed by the HS, and sustain the steady redox state (Abdelnour et al., 2019). Dietary inclusion of AOS significantly modulated the caused tissue impairment. It maintained the rabbits from the deleterious effects of oxidative stress in the environmental HS group via augmenting the SOD and CAT levels. A significantly modulated the caused tissue injury, improved the kidney functions,

Table 5
Effect of supplemental dietary AOS on redox status of growing rabbits under heat stress.

Items[1]	AOS level[2]				Pooled SME	*P*-value
	AOS0	AOS50	AOS100	AOS150		
Antioxidant status						
TAC (ng/mL)	0.243	0.244	0.242	0.257	0.012	0.77
GSH (ng/mL)	0.16^{c}	0.23^{b}	0.20^{b}	0.32^{a}	0.011	<0.0001
CAT (U/mL)	1.587^{c}	2.06^{b}	2.08^{b}	3.01^{a}	0.066	<0.0001
Oxidative stress						
MDA (nmol/mL)	0.317	0.273	0.263	0.23	0.017	0.0513
PC (nmol/mL)	3.16^{a}	2.07^{b}	2.32^{b}	2.16^{b}	0.104	0.0003

a, b, c Means within a row without a common superscript letter differ at $p < 0.05$.

[1] TAC = total antioxidant capacity; GSH = glutathione; CAT = catalase; MDA = Malondialdehyde; PC = Protein carbonyl.

[2] AOS0, AOS50, AOS100 and AOS150 = 0, 50, 100 and 150 mg AOS/kg DM diet; respectively.

improved the antioxidant system and maintained heat-stressed sheep from the oxidative stress (Yan et al., 2011). The ethanolic extract of the brown algae SAG has plausible hepatoprotective effects (Madkour et al., 2012). Yan et al. (2011) suggested that dietary supplementation of AOS (0.2%) can reduce *Salmonella* Sp. community in the intestinal, boosting the intestinal barrier function and growth performance of the broiler. Wan et al. (2017) revealed that AOS could be used effectively to promote the antioxidant defence system by improving serum catalase and glutathione activities. Several studies have shown that the alginate oligosaccharides caused a significant increase in the beneficial microbiota like *bifidobacteria* and *lactobacilli* while reducing the pathogens bacteria in rats (Wang et al., 2006) and broiler (Yang et al., 2016). This feature may reflect the ability of AOS to enhance the health status of the heat-stressed rabbits. The moderate solubility of algae polysaccharides in the water of the above-revealed certainly facilitates their application in animal feeding and other commercial uses.

3.4. Haematological and immunity status

Results of the erythrocytes indices are presented in Table 6. All experimental feed additives showed significant improvement in haemoglobin (Hb), red blood cells (RBCs), platelets, and MCHC compared to the control group. While other erythrocytes indices revealed no significant difference among all experimental groups. No significant effects for MCH values were detected among all experimental groups. The immunological response in the growing rabbit of different groups is summarized in Table 6. When exposed to HS, rabbits showed a significant decrease in WBCs, basophils and neutrophils counts ($P < 0.001$). At the same time, the AOS dietary inclusion significantly reduced this elevation induced by HS in rabbits (Table 6). The highest values of WBCs and neutrophils ($P < 0.001$) were recorded in all AOS groups compared with the control, while AOS150 did not exhibit any significant alteration among groups regarding basophils. AOS150 group showed a significant increase in the levels of IgG and IgM in the plasma.

Additionally, all AOS dietary inclusion in rabbit diets increased ($P < 0.001$) the IgG and IgM in rabbits (Except AOS50 for IgM). Regarding the IgM levels, no significant differences were detected between the control group and AOS50. The improvement in IgG and IgM production ($P < 0.001$) in rabbits exposed to HS indicates an enhancement of animal humoral immune response. Brown algae are wealthy in many functional polysaccharides such as fucoidans and alginates, which are recognized to have several biological properties involving anti-inflammatory, antioxidants, anti-viral, antimicrobial, anti-coagulant, and anti-tumor characteristics. Those compounds isolated from brown algae have been recently hired as effective additives or supplements in the food trade, pharmaceuticals, and last years in aquaculture and livestock areas (reviewed by (Ming et al., 2021)). Alginate and its derives are naturally occurring anionic polymers, utmost often separated from the brown algae. It is an acidic polysaccharide in the family of linear and non-repeating copolymers. Moreover, due to its minimum toxicity, biocompatibility, moderately low charge, and capacity to process a gel under moderate environments in divalent cations like Ca2+ (Yan et al., 2011; Wang et al., 2006), alginate could be used in several biomedical proposes.

Alginates are interesting molecules owing to their safety and unique feature for feeding humans and animals (Ming et al., 2021). Thus, the relationship between chemical structure and the promotional ability of AOS in molecular archerites in animals reared under environmental HS is unclear and needs further exploration. Haematological elements state animals' physiological responsiveness. Our results show that the AOS significantly increased ($P < 0.001$) the haematological indices (except for hematocrit and MCV) at all levels used in the current study. It was also described that rabbits exposed to HS presented higher blood leucocyte counts, which could be connected with an active infection or other stressors (Sheiha et al., 2020). Maintaining the normal ranges of the haematological indices could reflect the stable homeostasis status of animals. In this paper, the dietary AOS could effectively maintain the levels of leucocytes in rabbits representing good health and well-being. Interestingly, marine algae are recognized as an anti-coagulant activity (Kim and Wijesekara, 2011), thus enhancing the blood flow in the body and resulting in heat loss by convection. Based on the literature (Ming et al., 2021), AOS exhibited several biological activities like antioxidant, antimicrobial, immune regulation, and anti-inflammatory activities.

With the current study, using AOS as an innovative bio-feed additive for combating the harmful influences of HS may reflect the safety, applicability, efficiency, and suitability for animal nutrition and anti-heat stress agent via boosting the antioxidant defence system and regulating pro-inflammatory and immune responses in rabbits. Further investigation is needed to elucidate the roles of these bioactive compounds in the gastrointestinal tract and microbial community of animals. Additionally, it is imperative to develop safe, new, and more effective functional feed additives and antibiotic alternatives to certify the health and welfare of poultry and livestock.

Table 6
Effect of different levels of supplemental dietary AOS on erythrocyte's indices and immunity profile (leucocyte indices and immunoglobins G and M) of growing rabbits under heat stress.

Items[1]	AOS level[2]				Pooled SME	*P*-value
	AOS0	AOS50	AOS100	AOS150		
Erythrocyte's indices						
RBCs (10^6/mL)	3.36^{b}	4.34^{a}	4.70^{a}	4.65^{a}	0.255	0.0197
Haemoglobin (pg)	10.07^{b}	11.56^{a}	12.53^{a}	13.01^{a}	0.442	0.0071
Platelets (MCL)	849.0^{b}	1023.6^{a}	1041.6^{a}	1057.6^{a}	35.04	0.0097
Hematocrit (%)	37.42	37.24	37.15	37.17	0.272	0.8915
MCV (pg)	94.257	95.683	96.003	95.81	3.746	0.9862
MCH (pg)	28.87^{b}	28.74^{b}	30.87^{ab}	32.01^{a}	0.699	0.0283
MCHC (pg)	25.01^{b}	30.91^{a}	31.18^{a}	32.87^{a}	1.565	0.0335
Leucocytes						
White blood cells (WBCs;10^3)	8.19^{a}	5.25^{b}	5.03^{b}	4.47^{b}	0.276	<0.0001
Basophils (%)	0.27^{a}	0.21^{bc}	0.19^{c}	0.24a^{b}	0.014	0.0196
Eosinophils (%)	0.41	0.38	0.37	0.32	0.029	0.3208
Lymphocytes (%)	82.62	81.2^{6}	80.22	84.91	1.12	0.0805
Neutrophils (%)	14.33^{a}	11.67^{b}	11.33^{b}	12.33^{b}	0.33	0.0009
Immunoglubins						
Ig G (ng/mL)	60.3^{c}	71.0^{b}	86.0^{a}	88.0^{a}	2.26	<0.0001
Ig M (ng/mL)	45.3^{c}	50.0^{cb}	54.3^{b}	70.0^{a}	1.84	<0.0001

a, b, c Means within a row without a common superscript letter differ at $p < 0.05$.
[1] RBCs: red blood cells (10^6); MCV: mean corpuscular volume (%); MCH: mean corpuscular haemoglobin (%); MCHC: mean corpuscular haemoglobin concentration.
[2] AOS0, AOS50, AOS100 and AOS150 = 0, 50, 100 and 150 mg AOS/kg DM diet, respectively.

4. Conclusions

The current research demonstrated that the dietary inclusion of AOS (100 or 150 mg/kg diet) was safe and effective in protecting growing rabbits from the detrimental impacts of environmental HS via boosting the immune indices, enhancing the antioxidant defence system, and regulating the inflammatory pathways. Opportunities for using aqua-feeds in animal feeding have presented the most promising results in a reasonable period; however, further studies are urgently needed in the upcoming periods.

Ethics statement

The animal study was reviewed and approved by the Department of Animal Production, Faculty of Agriculture, Zagazig University, Egypt.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Data availability statement

All the data produced and analyzed in the present study are in the manuscript as tables. The corresponding author will respond to the requests concerning the raw data and reasonable accommodations will be provided.

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