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Physiological Responses, Oxidative Status and Productive Performance of Barki Sheep fed Dietary Addition with Quinoa Seeds and Exposed to Solar Radiation

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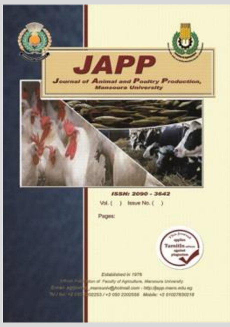

ABSTRACT

This study aimed to detect the effect of the dietary addition of quinoa seeds to ameliorate the drastic effects of oxidative stress in Barki male sheep exposed to environmental heat stress. Total of 30 Barki ram lambs were used in the present study. Animals in shaded and unshaded (animals exposed daily to direct solar radiation in the summer) groups were randomly divided into three similsr groups (5 males each). Animals of 1st group were fed a basal diet without quinoa seeds supplementation, while, those of 2nd and 3rd groups were fed diets containing 5 and 10 % quinoa seeds, respectively. Results cleared that the diet contained (10%) quinoa seeds modulated significantly (P<0.05) the increase in the thermo-respiratory responses and the observed anemia, dyslipidemia, and systemic inflammation, including blood leukocytosis, the increase in serum proinflammatory cytokines and heat shock protein-70 concentrations. Furthermore, it improved significantly the gain of body-weight and kidney functions, enhanced the blood antioxidant defense system (total antioxidant capacity, and catalase and superoxide dismutase activities) in the unshaded sheep, and protected them from oxidative stress. In conclusion, feeding on diet containing 10% of quinoa seeds was suitable and safe for sheep nutrition to efficiently alleviate the cruel effects of environmental heat stress in Barki sheep.

***Keywords*:** Heat stress, sheep, inflammation, oxidative stress, heat shock proteins.

INTRODUCTION

Heat stress (HS) is the most important topic in the global climatic change issue. HS harmfully affects animal health, and productive and reproductive performances. HS has induced oxidative stress that can be revealed in most body organs. Extreme production of reactive oxygen species (ROS) over the cellular antioxidant capacity resulted in oxidative stress that lead to the damage of cellular proteins, lipids, and DNA molecules. The cellular defense systems include both enzymatic and non-enzymatic compounds that upon damage and activate the repair mechanisms (Slimen *et al*., 2014). Moreover, the induced oxidative stress during heat stress caused proteins and DNA damage, that is an vital concern to enhance the intracellular heat stress protein (particularly HSP-70) production to assist protein refolding and sustain the structural function of the cellular protein (Slimen *et al*., 2016; Archana *et al*., 2017). Likewise, oxidative stress can trigger the inflammatory response which produces more free radicals that can lead to extra oxidative stress, creating a cycle. Inflammatory markers including IL-1β, IL-6, and TNF-α are serious cytokines that are interlinked with the production of nitric oxide and thus promote inflammatory routes involved in inflammation, thus inhibition of TNF-α and IL-6 are regarded as a treatment strategy of inflammations (Hussain *et al*., 2016; Inbaraj *et al*., 2016; Lauridsen, 2019).

Quinoa (*Chenopodium quinoa*) is a halophytic plant that tolerate harsh environmental conditions such as salty/acid soils and cold/ hot climates. It has received much interest due to its high nutritive and biological values (Maestro-Gaitán *et al*., 2022). Quinoa seeds are gluten-free and contain high-quality protein, carbohydrates, omega-3 fatty acids, and important minerals, so quinoa is considered a "super food". Also, it contains numerous phytochemicals including polysaccharides, polyphenols such as flavonoids and phenolic acids that have been suggested to be able to modulate the inflammatory processes (Yao *et al*., 2014; Mahdavi *et al*., 2023). Quinoa protein is a good precursor of bioactive peptides which have a wide range of biological activities and exhibit beneficial features as well as antioxidants, antimicrobial, anti-inflammatory and immunoregulatory, so it offers health-promoting qualities (Lin *et al*., 2019; Ng and Wang, 2021; Chaudhary *et al*., 2023).

Therefore, this study aimed to assess the alleviative effects of two doses of *Chenopodium quinoa* willd seeds on the oxidative stress and inflammations in Barki sheep exposed to the environmental heat stress.

MATERIALS AND METHODS

Biochemical analysis of quinoa seeds:

Seeds of quinoa (*Chenopodium quinoa*) were provided by the research project "Climate Smart Agriculture Entrepreneurship Development of Quinoa Value in Egypt. The quinoa seeds were analyzed according to AOAC (1990). The biochemical analysis cleared that quinoa seeds contained 67.5% carbohydrates, 16.0% proteins, 7.5% fat, 3.0% fibers, and 3.5% ash.

Animals:

Growing Barki ram lambs (*Ovis aries*), aged 8-10 months and weighing 19.17 ± 0.2 kg, were housed in animal yards in the Ras Sedr station of the Desert Research Center under veterinary care according to the program of the

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Ministry of Agriculture; and the design of this study was accepted by the Ain Shams University Research Ethics Committee (6/2014).

Animals drank fresh tap water and were fed on standard ruminant concentrate fed mixture in pelleted form (based on 2.5% of body weight) and wheat straw as roughage. The feed ingredients of the experimental diets are shown in Table 1.

Assessment of environmental conditions:

The environmental conditions were assessed daily in the summer months (July and August). The means of ambient temperature, humidity, and temperature-humidity index (THI) values are shown in Table (2).

THI wre calculated by the following equation:

$$\mathbf{THI = Ta - [(0.31 - 0.31RH) \times (Ta - 14.4)]}.$$

Where: Ta = Ambient temperature (°C); RH = Relative Humidity = Humidity (%) / 100.

The severity of heat stress was estimated by THI values according to Marai *et al*. (2002).

THI values of <27.8 (absence of HS); 27.8-28.9 (moderate HS); 29.0-30.0 (severe HS); and >30.0 (very severe HS).

Table 1. Feed ingredients of experimental diets

Ingredients %	Groups		
	Basal diet	Quinoa seeds 5%	Quinoa seeds 10%
Undecorticated cotton seed	50	45	40
Wheat bran	18	18	18
Yellow corn	15	15	15
Quinoa seed	0	5	10
Rice polish	11	11	11
Molasses	3	3	3
Lime stone	2	2	2
Common salt	1	1	1
Total	100	100	100

Table 2. Means of ambient temperature (AT), relative humidity (RH) and temperature humidity index (THI) throughout the experimental period.

Month	AT (ºC)		RH (%)		THI	
	Min.	Max.	Min.	Max.	Min.	Max.
July	25.00	37.45	19.77	38.22	22.97	31.71
August	25.14	39.45	20.25	40.90	23.17	33.25
Overall mean	25.07	38.45	20.01	39.56	23.07	32.48

Experimental plan and treatment schedule:

This study was conducted in Ras Sedr station of the Desert Research Center. After acclimatization period as 10 days, thirty Barki ram lambs were randomly divided into 6 groups; 3 shaded and 3 unshaded groups. In the shaded groups, animals lived in shaded yards and were fed on normal diet containing 0, 5, or 10% quinoa seeds for successive 60 days (July and August), the animals in unshaded groups were exposed to the daily direct solar radiation for 8 hours (7 a.m. : 3 p.m.) and fed on normal diet containing 0, 5, or 10% quinoa seeds during the same period.

Blood sampling:

At the end of experimental period blood samples of jugular vein were collected from each animal before feeding. The blood samples were collected into clean centrifuge tubes with or without anticoagulant. EDTA was added to blood samples for performing the complete blood picture analysis by HA-VET coulter (Clinding, Belgium), and optaining plasma. The blood samples wihout EDTA was left to coagulate at room temperature and then centrifuged to optain serum. plasma and serum were collected and preserved at –80°C until biochemical analysis.

Measurements:

The thermal responses of animal such as skin, coat, and rectal temperatures (ST, CT, and RT), in addition to respiration rate (RR), were measured at 2 p.m. weekly. The animals were weighed before feeding at the start of the experiment and weekly to follow the change in body-weight.

Serum total protein, albumin, total cholesterol, triacylglycerol, urea, creatinine, glucose, and malondialdehyde (MDA) concentrations as well as serum activity of alanine (ALT) and aspartate (AST) aminotransferases, lactate dehydrogenase (LDH), and superoxide dismutase (SOD), and plasma catalase (CAT) activity and total antioxidant capacity (TAC) were determined by commercial kits from Spectrum Diagnostics (Egypt) and Bio-diagnostic (Egypt). Serum tumor necrosis factor (TNF)-α, interleukin (IL)-6, and heat shock protein (HSP)-70 concentrations were quantitatively determined by ELISA kits from Cusabio Biotech (USA).

Statistical analysis:

All data were analyzed by a two-way analysis procedure (ANOVA) using the General Linear Models (GLM) procedure of SAS software (SAS, 2004). As follows:

$$Y_{ijk} = \mu + Q_i + S_j + Q*S_{ij} + e_{ijk}$$

Where:

Y_{ij} = any observation of j^{th} animal within i^{th} groups.

μ = overall mean.

Q_i = effect of i^{th} quinoa treatment (i: 1-3).

S_j = effect of j^{th} shading (j: 1-2).

$Q*S_{ij}$ = effect of interaction between quinoa treatment and shading

e_{ij} = experimental error.

The significant differences in the mean values between groups were assessed via Duncan's multiple range test (Duncan, 1955), the effects were considered significant at a level of $P < 0.05$.

Results and discussions:

Thermo-respiratory responses

The results indicated that the direct solar radiation under (ambient temrature: 39.56 ºC, relative humidity: 20.01%, and THI: 32.48) significantly ($P<0.05$) increased all thermo-respiratory responses (CT, ST, RT, and RR) of the unshaded animals compared to the shaded group (Table 3).

Under the heat stress, ruminants as homoeothermic increased avenues of heat loss to maintain euthermia (Sejian *et al*., 2017). The direct responses of animals to heat stress are the increase in pulsation, respiration, so, the thermo-respiratory responses reflected both the environmental heat stress and the internal heat load on the body of animals (Al-Dawood, 2017; Wijffels *et al*., 2021).

On the other hand, feeding animals with a diet containing 10 % quinoa seeds modulated significantly the increase in thermo-respiratory responses of the unshaded sheep, except CT that reflected the surrounding environmental conditions (Table 3). These results clear that the higher percentage of quinoa seeds in the feed lowerered the thermos-respiratory responses in unshaded sheep. These results agree with AL-Zafry and Medan (2012), who indicated that antioxidants supplementation under heat stress conditions alter directly the thermal set point by reducing prostaglandin output, particularly prostaglandin E series, whose turnover rises by heat stress, and has a direct effect on the hypothalamic thermoregulation. Therefore, diets containing quinoa seeds have an alleviating effect on heat stressed animals by regulating the prostaglandin output, where quinoa seeds riche in flavonoids and vitamins (Pathan and Siddiqui, 2022).

The interaction between direct solar radiation and quinoa seeds treatments on CT and ST were insignificant, while it was significant (P<0.05) on RT and RR. This trend indicated that diets containig quinoa seeds alleviated the harmful effect of direct solar radiation in Barki sheep.

Table 3. Effect of diet containing quinoa seeds on coat, skin and rectal temperatures (°C) as well as the respiration rate (breath/minute) in sheep sheepexposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation Unexposed	Exposed	±SEM	Overall mean	±SEM
Coat temperature (ºC)	0	40.92	47.98		44.45	
	5	41.56	49.28	1.17^{NS}	45.42	0.83^{NS}
	10	42.94	49.86		46.40	
Overall mean		41.80^{B}	49.04^{A}	0.67*		
Skin temperature (ºC)	0	38.38	40.16		39.27^{a}	
	5	37.58	39.32	0.43^{NS}	38.45^{ab}	0.30*
	10	37.72	38.42		38.07^{b}	
Overall mean		37.89^{B}	39.30^{A}	0.25*		
Rectal temperature (ºC)	0	39.18	40.50		39.84^{a}	
	5	39.46	40.16	0.15*	39.81^{a}	0.11*
	10	39.24	39.46		39.35^{b}	
Overall mean		39.29^{B}	40.04^{A}	0.09*		
Respiration rate (breath/minute)	0	56.40	72.40		64.40^{a}	
	5	57.20	66.00	2.04*	61.60^{ab}	1.44*
	10	55.60	60.00		57.80^{b}	
Overall mean		56.40^{B}	66.13^{A}	1.18*		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.

Body weight and body weight gain

Results in Table 4 indicated that, although the environmental heat stress had insignificant effect on final body weight, it caused significantly (P<0.05) higher gain in body weight of shaded than in unshaded sheep by 33.84% . Really, the environmental heat stress increased the water consumption but decreased the feed intake that adversely affected the animal productivity and growth rate (Júnior *et al*., 2014and Sejian *et al*., 2017).

Table 4. Effect of diet containing quinoa seeds on the changes in body weight in sheep exposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation Unexposed	Exposed	±SEM	Overall mean	±SEM
Initial body weight (kg)	0	19.30	19.24		19.27	
	5	19.46	18.30	1.65^{NS}	18.88	1.16^{NS}
	10	19.36	19.38		19.37	
Overall mean		19.37	18.97	0.95^{NS}		
Final body weight (kg)	0	24.76	21.88		23.32	
	5	24.96	21.52	1.72^{NS}	23.24	1.21^{NS}
	10	25.86	25.08		25.47	
Overall mean		25.19	22.82	0.99^{NS}		
Total body gain (kg)	0	5.46	2.64		4.05^{b}	
	5	5.50	3.22	0.56^{NS}	4.36^{b}	0.40*
	10	6.05	5.70		6.10^{a}	
Overall mean		5.82^{A}	3.85^{B}	0.32*		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.

The diet containing 10% quinoa seeds significantly improved total body gain by 50.61 and 39.9% as compared to sheep received 0 and 5% quinoa seeds, respectively (Table 4). This trend may suggest a beneficial effect of quinoa seeds on gain of sheep under heat stress condition by improving animals' appetite. Also, results referred to lowering thermo-respiratory response in the unshaded sheep received quinoa seeds revealed that they were less stressed. In addition, quinoa protein has high digestibility and it is a good amino acid source (Ng and Wang, 2021; Elbaz *et al*., 2022). The organic value of quinoa is parallel to beef, and greater than some cereals such as wheat, corn, and rice (Gordillo-Bastidas *et al.*, 2016; Pathan and Siddiqui, 2022). Quinoa, as a phytoecdysteroid, exhibits many health-promoting effects and has been described to enhance protein synthesis and muscle growth (Gorelick-Feldman *et al*., 2008; Anthony *et al*., 2015). In this respect, Ng and Wang (2021) reported that 50 g of quinoa protein can provide the daily need of amino acids and it may be a good nutrient source for body recovery.

However, the results indicated that the effect of interaction between direct solar radiation and quinoa seeds treatments on body weight and gain was insignificant.

Blood metabolites

Results illustrated in Tables 5 and 6 show that there was no significant differences concentration of serum total proteins, albumin, and globulin as well as albumin/globulin ratio, and also, glucose, urea, cholesterol, and triglycerides between shaded and unshaded sheep.

Although the quinoa seeds treatments had insignificant affect on the serum glucose and protein concentrations, it caused a significant dose-dependent regulatory effect on the metabolism of proteins. Where diet containing 10% quinoa seeds caused a significantly increase (P<0.05) in serum globulin concentration by 35.4% at the expense of albumin concentration which decreased by 11% compared to control group; that led to a decrease significantly the albumin /globulin ratio by 32.5% compared to sheep didn't receive quinoa seeds (Table 5). Quinoa can be considered as a healthy/functional food (Ng and Wang, 2021). Also,the diet containing 10% quinoa seeds significantly (P<0.05) decreased both serum urea concentrations by 11.7 % as compared to the control group (Table 6).

Table 5. Effect of diet containing quinoa seeds on serum proteins and the albumin/globulin ratio in body weight in sheep exposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation Unexposed	Exposed	±SEM	Overall mean	±SEM
Total protein (g/dl)	0	5.70	5.02		5.36	
	5	5.42	5.30	0.27^{NS}	5.36	0.19^{NS}
	10	5.78	5.70		5.74	
Overall mean		5.63	5.34	0.15^{NS}		
Albumin (g/dl)	0	3.42	3.12		3.27^{a}	
	5	2.98	3.02	0.16^{NS}	3.00^{ab}	0.11^{NS}
	10	2.90	2.92		2.91^{b}	
Overall mean		3.10	3.02	0.09^{NS}		
Globulin (g/dl)	0	2.28	1.90		2.09^{b}	
	5	2.44	2.28	0.25^{NS}	2.36^{ab}	0.18*
	10	2.88	2.78		2.83^{a}	
Overall mean		2.53	2.30	0.14^{NS}		
Albumin/Globulin ratio	0	1.56	1.65		1.60^{a}	
	5	1.36	1.36	0.18^{NS}	1.36^{ab}	0.12*
	10	1.07	1.08		1.08^{b}	
Overall mean		1.33	1.36	0.10^{NS}		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.

Quinoa's high-quality protein may be suitable to satisfy the requirements of chronic kidney disease patients. Since high-quality protein produces less wastes in blood, so quinoa may reduce the kidneys' burden (Dakhili *et al*., 2019). Also, the administration of quinoa seeds has an antioxidant effect on the induced renal oxidative stress in rats (Al Shammari, 2019).

Table 6. Effect of diet containing quinoa seeds on serum glucose, urea, cholesterol, and triacylglycerol concentrations in sheep exposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation			Overall mean	±SEM
		Unexposed	Exposed	±SEM		
Glucose (mg/dl)	0	37.90	37.28		37.59	
	5	36.42	44.58	5.25^{NS}	40.50	3.71^{NS}
	10	40.84	39.04		39.94	
Overall mean		38.38	40.30	3.03^{NS}		
Urea (mg/dl)	0	82.08	84.40		83.24^{a}	
	5	86.14	82.76	5.64^{NS}	84.45^{a}	3.99*
	10	72.98	74.02		73.50^{b}	
Overall mean		80.40	80.39	3.25^{NS}		
Cholesterol (mg/dl)	0	92.12	94.88		93.50^{a}	
	5	75.44	84.12	7.84^{NS}	79.78^{ab}	5.54*
	10	73.10	74.18		73.64^{b}	
Overall mean		80.22	84.39	4.52^{NS}		
Triacylglycerol (mg/dl)	0	44.52	51.86		48.19^{a}	
	5	39.86	45.74	4.32^{NS}	42.80^{ab}	3.05*
	10	31.54	39.04		35.29^{b}	
Overall mean		38.64	45.54	2.49^{NS}		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.

Moreover, the quinoa seeds treatments may enhance lipid metabolism, the results in Table 6 revealed a significant (P<0.05) decrease in serum cholesterol and triacylglycerol concentrations and this trend is dose-dependant. Many reports recommended that the consumption of quinoa seeds improves lipid profile by lowering blood cholesterol and triacylglycerol concentrations (Navarro-Perez *et al*., 2017; Alghamdi, 2018; Noratto *et al*., 2019).

In this respect,Takao *et al*. (2005) demonstrated that quinoa protein fraction inhibited cholesterol-synthesizing enzyme expression, along with stimulated cholesterol-catabolizing enzyme expression, resulting in lower serum cholesterol. Meanwhile, quinoa seeds are a noble source of dietary fiber. Dietary fiber diminishes intestinal absorption of cholesterol and binds to biliary acid which increase the catabolism of cholesterol. Moreover, produced short-chain fatty acids via fiber fermentation in the colon contributed to decreasing the liver synthesis of cholesterol (Obaroakpo *et al*., 2020 and Pathan and Siddiqui, 2022). Tanwar *et al*. (2019) reported that the major quinoa oils are the ω-3 and ω-6 fatty acids which have health promoting effects. This trend may suggested the beneficial effect of quinoa seeds to prevent metabolic disorders and the risk of dyslipidemia in sheep under heat stress conditions.

The results indicated that the interaction between direct solar radiation and quinoa seeds treatments on blood metabolites were insignificant.

Antioxidant parameters:

The results in Table 7 illustrated that the direct solar rdiation significantly (P<0.05) increased serum activity of LDH as well as the MDA concentrationof the unshaded animals by 29.5 and 25 % respectively compared with the shaded group. These results agree with Badakhshan and Mirmahmoudi (2016) and Patel *et al*. (2016) who indicated that LDH is abundant in liver and muscle cells, which plays a vital role in cellular respiration,when these tissues were damaged, they released more LDH into the bloodstream. Heat stress is responsible for inducing oxidative stress in livestock (Slimen *et al*., 2014; Rathwa *et al*., 2017). Free radicals cause peroxidation of polyunsaturated fatty acids, leading to cell membrane destruction. The MDA was suggested to be the major product of lipid peroxidation (Slimen *et al*., 2016).

Table 7. Effect of diet containing quinoa seeds on the blood antioxidant parameters in sheep exposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation			Overall mean	±SEM
		Unexposed	Exposed	±SEM		
Lactate dehydrogenase (U/l)	0	692.40	880.60		755.00^{a}	
	5	562.40	753.80	52.84^{NS}	658.10^{ab}	37.11*
	10	520.80	582.80		551.80^{b}	
Overall mean		570.86^{B}	739.06^{A}	30.30*		
Malondialdehyde (nmol/ml)	0	36.60	48.40		42.50^{a}	
	5	34.20	38.80	3.20^{NS}	36.50^{ab}	2.26*
	10	28.20	36.60		32.40^{b}	
Overall mean		33.00^{B}	41.26^{A}	1.84*		
Superoxide dismutase (U/l)	0	147.20	133.40		140.30^{b}	
	5	190.40	147.40	14.89^{NS}	168.90^{b}	10.53*
	10	234.60	197.60		216.10^{a}	
Overall mean		190.73^{A}	159.46^{B}	8.60*		
Catalase (U/l)	0	538.40	512.40		525.40^{b}	
	5	564.40	529.80	24.27^{NS}	547.10^{ab}	17.16*
	10	612.60	565.00		588.80^{a}	
Overall mean		571.80^{A}	535.73^{B}	14.01*		
Total antioxidant capacity (mmol/l)	0	2.40	1.06		1.73^{c}	
	5	2.72	1.78	0.18^{NS}	2.25^{b}	0.12*
	10	3.10	2.46		2.78^{a}	
Overall mean		2.74^{A}	1.76^{B}	0.10*		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.0

On the other hand, the results indicated that the environmental heat stress significantly (P<0.05) decreased the SOD and CAT activities as well as the blood TAC by 35.8% (Table 7) as compared to shaded group. The decrease in blood TAC and the activities of blood antioxidant enzymes may be clarified by the consumption of blood antioxidants to detoxify the produced free radicals during heat stress and to preserve the redox's steady state (Slimen *et al*., 2016). The enzymatic antioxidants such as SOD and CAT are responsible for scavenging the intracellular and extracellular ROS and avoiding lipid peroxidation of cellular plasma membrane (Kumar *et al*., 2011). The SOD catalytically converts the $O_2^{\bullet-}$ to oxygen and hydrogen peroxide in the presence of copper, zinc, or manganese as metal ion cofactors. However, the CAT converts hydrogen peroxide to water and oxygen (Stone and Yang, 2006). These antioxidant enzymes prevent lipid peroxidation and maintain the cell membrane structure and function (Slimen *et al*., 2016; Ellamie *et al*., 2020).

Then again, quinoa seeds tratments (especially 10%) significantly moderated the elevation in serum markers for tissue damage in the unshaded group and upturn the blood TAC and both of SOD and CAT activities by 60.7, 54 and 12.1% respectively (Table 7).

Quinoa seeds are rich in phenolic acids and flavonoids which are stronger antioxidants (Tang *et al*., 2015; Lin *et al*., 2019). The hydroxyl groups of polyphenols possess antioxidant activity via scavenging free radicals, chelating ability to suppress ROS output by either inhibition of enzymes involved in their creation or up regulation of antioxidant defenses (Hussain *et al*., 2016). Where, the gastrointestinal digestion of quinoa flavonoids resulted in doubling its antioxidant capacity (Balakrishnan and Schneider, 2020). Many studies suggested that some quinoa peptides are antioxidants that act as electron donors to neutralize free radicals, exhibit free radical scavenging activity and inhibition of lipid peroxidation (Ren *et al*., 2017; Lin *et al*., 2019; Guo *et al*., 2023). Quinoa seeds may show gastro protective potential by inhibiting lipid peroxidation and increase of gastric pH, gastric mucus secretion, and endogenous antioxidant enzymes (Mariod andSalama, 2020).

The quinoa seeds powder showed significant hepatoprotective and antioxidant activity against CCL4-induced liver toxicity by restoring the level of liver antioxidant enzymes toward the normal levels and decreasing the elevated level of MDA due to the high concentration of phytochemical compounds particularly flavonoids and phenolic acids (Saxena *et al*., 2017; Al-Qabba *et al*., 2020). Also, quinoa seeds contain a high content of minerals in bioavailable forms including calcium, magnesium, iron, copper and zinc, and vitamins (C, B6, and B5) as well as thiamine, folic acid, and the ω-3 and ω-6 fatty acids. This trend suggest a good antioxidant effect of quinoa seeds (Gordillo-Bastidas *et al*., 2016; Schoenlechner, 2017; Tanwar *et al*., 2019; Angeli *et al*., 2020; Pathan and Siddiqui, 2022). The interaction between direct solar radiation and quinoa seeds treatments on antioxidant parameters were insignificant.

Hematological parameters:

The results in Table 8 revealed that hemoglobin (Hb) concentration, red blood cells count (RBCs) and hematocrit were significantly decreased (P<0.05) in the unshaded lambs by 7.02, 6.74 and 3.75%, respectively as compared to shaded lambs. However, there was no significant differences of Wintrobe indices (MCV, MCH and MCHC), that indicat a normochromic normocytic anemia in the unshaded animals. This anemia may result from neither appetite loss, or releasing of proinflammatory cytokines that caus intestinal injury (Mittal *et al*., 2014). On the other hand, the environmental heat stress significantly (P<0.05) increased the counts of blood total leucocytes by 35.6% compared to the shaded group (Table 8). Inbaraj *et al*. (2016) reported that HS caused splanchnic tissue hypoxia leading to lower integrity of the intestinal epithelium that may lead to endotoxemia by enabling pass of bacteria and its endotoxins into the portal and systemic blood circulation, and suggested that animals under heat stress suffered from blood leukocytosis that could be related to an active infection.

Table 8. Effect of diet containing quinoa seeds on the hematological parameters in sheep exposed or unexposed to solar radiation.

Items	Tr. (%)	Solar radiation Unexposed	Exposed	±SEM	Overall mean	±SEM
Red blood cells ($\times 10^6$/mm^3)	0	8.00	7.38		7.69^b	
	5	8.36	7.60	0.28^{NS}	7.98^{ab}	0.20*
	10	8.56	8.26		8.41^a	
Overall mean		8.30^A	7.74^B	0.16*		
Hemoglobin (g/dl)	0	9.34	8.40		8.87^b	
	5	9.58	8.72	0.29^{NS}	9.15^{ab}	0.21*
	10	9.70	9.50		9.60^a	
Overall mean		9.54^A	8.87^B	0.17*		
Hematocrit (%)	0	27.92	26.84		27.38	
	5	28.74	27.20	0.62^{NS}	27.97	0.44^{NS}
	10	28.76	28.16		28.46	
Overall mean		28.47^A	27.40^B	0.36*		
Total leucocytes ($\times 10^3$/mm^3)	0	6.30	8.86		7.58^a	
	5	5.52	7.76	0.50^{NS}	6.64^{ab}	0.35*
	10	5.54	6.90		6.22^b	
Overall mean		5.78^B	7.84^A	0.29*		
Lymphocytes ($\times 10^3$/mm^3)	0	3.68	5.64		4.66^a	
	5	3.28	4.48	0.36^{NS}	3.88^b	0.25*
	10	3.26	4.36		3.81^b	
Overall mean		3.40^B	4.82^A	0.21*		
Neutrophils ($\times 10^3$/mm^3)	0	2.12	2.64		2.38	
	5	1.78	2.81	0.22^{NS}	2.29	0.15^{NS}
	10	1.81	2.13		1.97	
Overall mean		1.90^b	2.53^a	0.13*		

Means bearing different superscripts in terms of capital litters in the same row or small litters in the same column are significantly different (P<0.05) NS= non-significant, * Significant at P<0.05.

According to quinoa seeds tratments, results indicated that the Hb concentration and RBCs were increased (P<0.05) by 8.2 and 9.4% respectively in the lambs fed on a diet containing 10% quinoa seeds by lambs fed on a control diet (0% quinoa seeds). Quinoa seeds may alleviat anemia due to their high content of iron, folic acid, and vitamins B and C (Angeli *et al*., 2020; Pathan and Siddiqui, 2022). Also, diet containing 10%quinoa seeds significantly modulated the elevation in blood total leucocytes counts. Panche *et al*. (2016) discussed the ability of flavonoids to decrease the leukocytosis which may be related to the decrease in total serum complement and the protective mechanism against inflammation. That may related to the potential antioxidant activity of quinoa seeds (Tang *et al*., 2015 and Ren *et al*., 2023).

The interaction between direct solar radiation and quinoa seeds treatments on hematological parameters were insignificant.

Immunological responses:

Results in Figures 1, 2, and 3 indicated that the direct solar radiation induced inflammatory response by a significant (P<0.05) increase in the serum concentrations of TNF-α, IL -6 and HSP-70 by 81.7, 29.3, and 50.9 % respectively in unshaded sheep compared to the shaded sheep. These results agree with Ellamie *et al*. (2020), who indicated that the environmental heat stress induced excessive release of the proinflammatory cytokines in the serum of Barki sheep.

Chauhan *et al*., (2021) reported that the health status and the immune response of animals are influenced by oxidative stress, also HS has been described to cause oxidative stress.

The oxidative damage of the internal organs may lead to enhanced systemic inflammation and the production of HSPs (De-Punder and Pruimboom, 2015). The level of TNF-α could be a dependable immunological marker to reflect immunological responses during HS exposure, also pro-inflammatory cytokines, are Potential immunological indicators to reflect the HS impact on the immune response in ruminants (Rashamol *et al*. 2019).

On the other hand, the diet containin quinoa seeds coused a significantly dose-dependent decrease in serum concentrations of TNF-α, IL -6, and HSP-70 by 13.1, 11.3 and 27.8% respectively (in dose 5%) and by 39.1, 35.4 and 45.3% respectively (in dose 10%).

Several studies explained that polyphenols are useful as support therapy of their anti-inflammatory associated with antioxidant activity, and inhibition of enzymes involved in the pro-inflammatory cytokines production in addition to activating the transcription factor that plays a main role in cellular protection against oxidative stress and inflammation (Cardozo *et al*., 2013; Hussain *et al*., 2016; Liu *et al*., 2020; Mahdavi *et al*., 2023). Quinoa seeds have been suggested as antioxidant, antidiabetic, antihypertensive, anti-obesity and anti-inflammatory (Tan *et al*., 2021; Ren *et al*., 2023).

Also, Chander *et al*. (2014) and Moura *et al*. (2018) reported that flavonoids were effective in reducing oxidative stress and decreasing the HSP-70 expression in induced-lead acetate neurotoxicity.

Furthermore, quinoa saponin may be associated with the inhibition of inflammatory mediators (TNF-α, IL-1β, IL-6, and nitric oxide) overproduction, so it can prevent and treat inflammation (Lim *et al*., 2019; El Hazzam *et al*., 2020; Mahdavi *et al*., 2023).

Ren *et al*. (2017) illusterated that the isolated amino acid (lunasin) from quinoa exhibits anti-inflammatory activity on macrophage cells where, inhibited the up regulation of NO, TNF- α and IL-6 stimulated by lipopolysaccharide (LPS). Also, Polysaccharide of quinoa was considered a natural antioxidant, and immune-regulating in drug application (Hu *et al*., 2017; Tan *et al*., 2021).

There was a significant (P<0.05) interaction between the direct solar radiation and quinoa seeds treatments on serum TNF-α, IL-6 and HSP-70 concentrations, indicated that diets contain quinoa seeds improved and regulated the immune response in heat stressed Barki sheep.

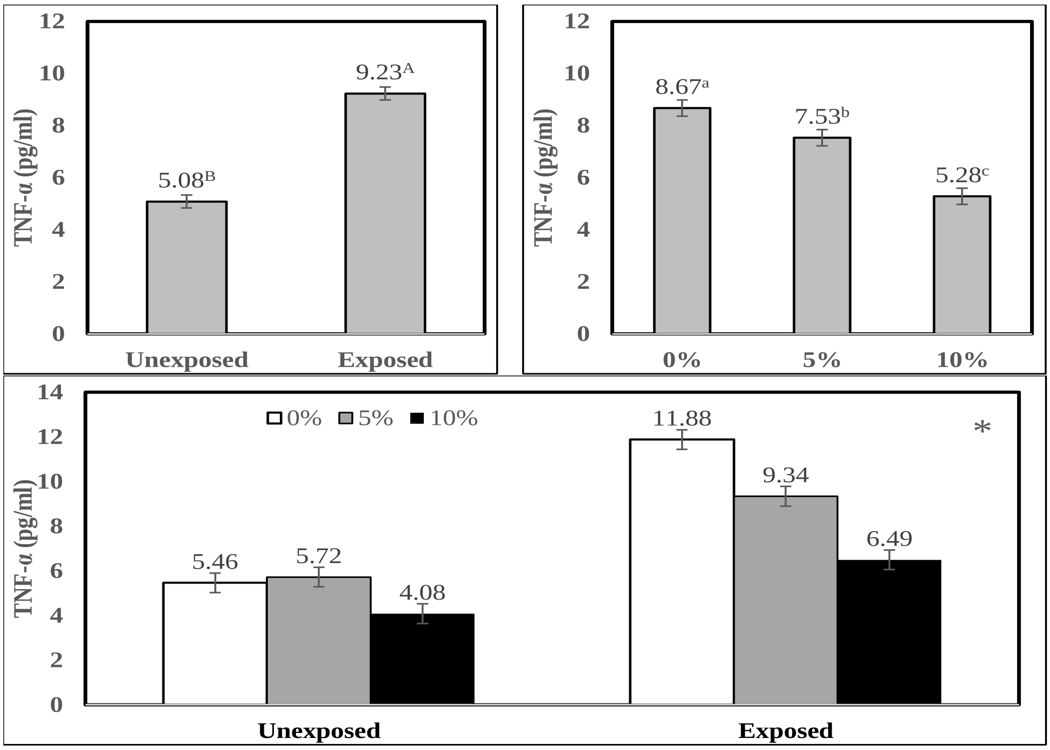

Figure 1. Effect of diet containing quinoa seeds on serum tumor necrosis factor-alpha (TNF-α) in sheep exposed or unexposed to solar radiation.

Means bearing different superscripts in terms of capital litters or small litters are significantly different (P<0.05), * Significant at P<0.05.

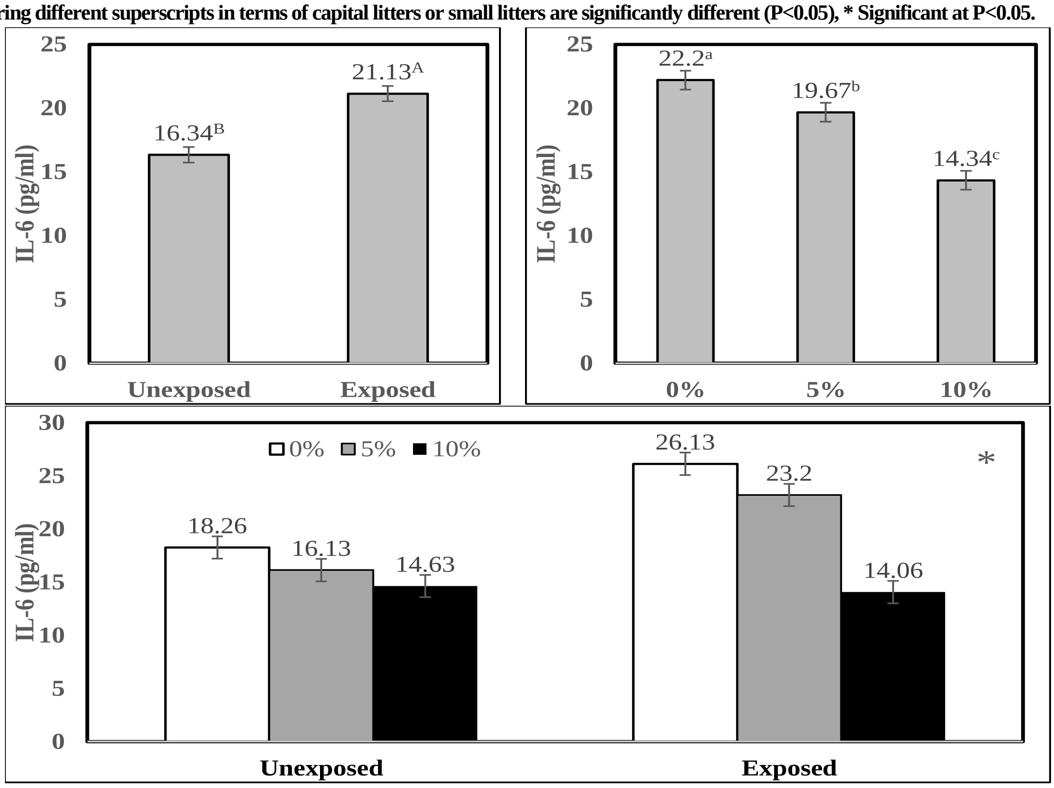

Figure 2. Effect of diet containing quinoa seeds on serum interleukin 6 (IL-6) in sheep exposed or unexposed to solar radiation.

Means bearing different superscripts in terms of capital litters or small litters are significantly different (P<0.05), * Significant at P<0.05.

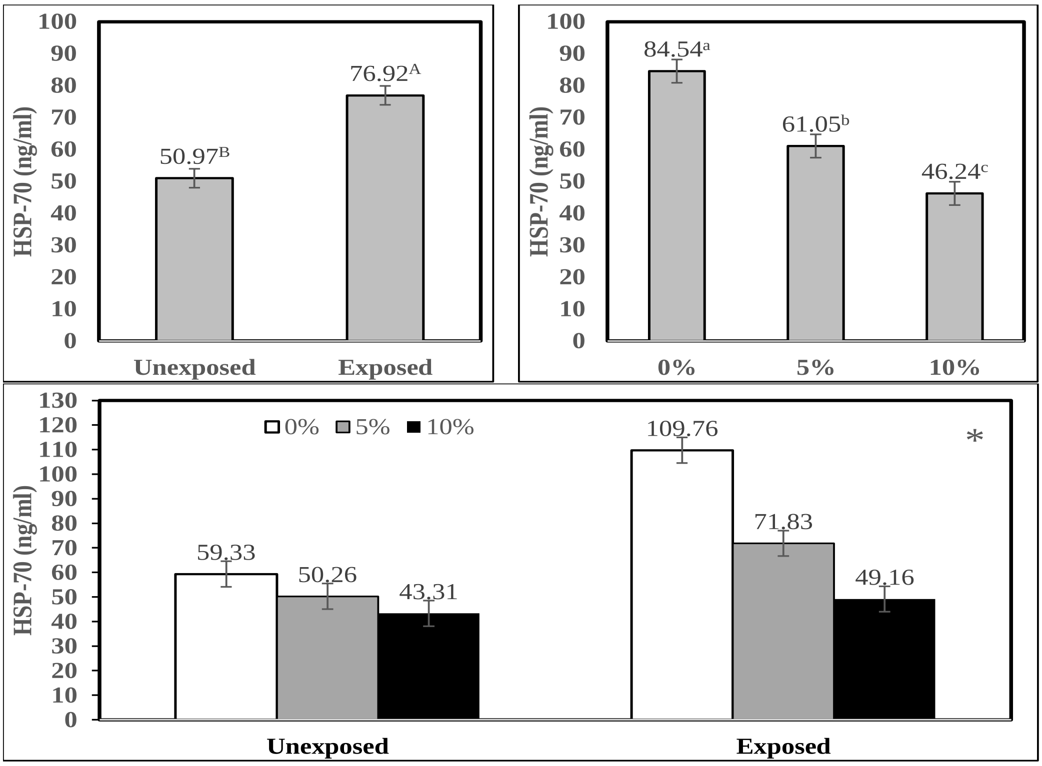

Figure 3. Effect of diet containing quinoa seeds on serum heat shock protein (HSP)-70 in sheep exposed or unexposed to solar radiation.

Means bearing different superscripts in terms of capital litters or small litters are significantly different (P<0.05), * Significant at P<0.05.

CONCLUSION

This study demonstrated that quinoa seeds showed suitable antioxidant activity with regard to their bioactivity via increase antioxidant enzymes (SOD, CAT); decrease of lipid peroxidation; and inflammatory mediators' expression. Thus, the diet containing quinoa seeds was suitable for sheep nutrition, and alleviated the cruel effects of the environmental HS by regulating the thermo-respiratory response, improving the antioxidant defense system and inflammatory responses that really reflected on the animal productive performance.

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Competing interests.

The author emphasizes that, about the manuscript, there is no any real or potential conflict of interest in any financial, personal or other relationships with other people or organizations within three years of starting the submitted work that could inappropriately influence, or be perceived to influence, their work.

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الاستجابات الفسيولوجية والحالة التأكسدية والأداء الإنتاجي لأغنام البرقي المغذاه على بذور الكينوا والمعرضة للاشعاع الشمسى المباشر

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الملخص

هدفت هذه الدراسة إلى الكشف عن تأثير إضافة بذور الكينوا لتخفيف تأثير الإجهاد التأكسدي في ذكور أغنام البارقي المعرضة للإجهاد الحراري البيئي. تم استخدام 30 ذكر من أغنام البرقي وتم تقسيمهم في المجموعة المظللة وغير المظللة (المعرضة يوميا للإشعاع الشمسي المباشر خلال موسم الصيف) إلى ثلاث مجموعات متساوية (5 ذكور لكل معاملة). تم تغذية الحيوانات فى المجموعة الأولى دون اضافة بذور الكينوا، في حين تم تغذية الحيوانات في المجموعتين الثانية والثالثة بوجبات تحتوي على 5 و10٪ من بذور الكينوا. وأظهرت النتائج أن النظام الغذائي الذي يحتوي على بذور الكينوا (10٪) خفض بشكل معنوى (P<0.05) الزيادة في الاستجابات التنفسية الحرارية فى الحيوانات المعرضة للاشعاع الشمسى مقارنة بالحيوانات التى لاتتناول بذور الكنوا، كما عدل الخلل في مستويات الكوليستيرول والدهون الثلاثية وفقر الدم، والالتهابات الجهازية المتمثلة فى زيادة عدد كريات الدم البيضاء، وزيادة السيتوكينات المسببة للالتهابات وتركيز بروتين الصدمة الحرارية 70 في الدم. علاوة على ذلك حسن بشكل معنوى (P<0.05) الزيادة فى وزن الجسم وعزز نظام الدفاع المضاد للأكسدة متمثلا فى زيادة نشاط الأنزيمات المضادة للأكسدة، والفاعلية الكلية المضادة للأكسدة بالدم في الأغنام غير المظللة ،مما ادى الى حماية الحيوانات من الاجهاد التأكسدى. وختاما فإن تغذية الأغنام بالنظام الغذائي الذي يحتوي على 10٪ من بذور الكينوا كانت مناسبة و آمنة لتغذية الاغنام، كماساهمت بكفاءة في تخفيف التأثيرات الضارة للإجهاد الحراري البيئى في أغنام البرقي.