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The impact of subjecting Turki-Qashqai goats to elevated thermo-humidity index under different nutritional systems on their performance, blood metabolites, and oxidative status

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ABSTRACT

Due to scarce research on how exposure to hot climates and different feeding schedules affects the oxidative status of nomadic animals a study was conducted with 66, 3 to 4 years old female Turki-Qashqai goats, weighing an average of 41.50±4.75 kg (mean ± standard error), and milk yield of 1.18±0.31 kg in a factorial experiment with a complete randomized design as a basic design. The goats were randomly divided into three groups: 1- natural pasture fed (**M**), 2- natural pasture in addition to 500 g of concentrate per day (**MS**), and 3- natural pasture hay along with 500 g of concentrate (**AS**). The experimental phase commenced on April 20th and concluded on June 20th. The collected data underwent statistical analysis using the mixed procedure of SAS. The body condition score was similar across all experimental groups during spring, but a notable decline was observed in summer within the pasture group. During the summer season, feed intake experienced a significant decrease compared to spring. Seasonal variations had a notable impact on milk production and fat-corrected milk (**FCM**) levels, as anticipated. The spring season exhibited the highest values in both aspects. The concentration of ROMs was affected by the season, diet, and the interaction of them. GPx and SOD showed increased activity in summer when compared to spring. Moreover, α-tocopherol levels were higher in spring than in summer. The results from this study suggest that seasonal variations have a more pronounced impact on oxidative status markers in dairy goats than nutritional factors.

Key words: Metabolic study; nomadic livestock; nutrition; oxidative stress; rural livestock

INTRODUCTION

Introduction

Reactive oxygen metabolites (**ROM**) are generated during normal cellular metabolism and in response to external stressors like uncomfortable conditions, radiation, and toxins. Elevated levels of ROMs can cause harm to proteins, lipids, and DNA within cells, resulting in oxidative stress and inflammation, which may contribute to the onset of certain conditions that antioxidants can counteract (Ponnampalam *et al.*, 2022). Oxidative stress refers to the disparity between the production of reactive oxygen species (**ROS**) and the body's antioxidant defense, leading to damage to different cellular components and affecting cellular functions like proliferation or apoptosis (Strycharz-Dudziak *et al.*, 2020).

Glutathione peroxidase (**GPx**) and superoxide dismutase (**SOD**) are essential enzymes responsible for scavenging ROMs within the body, forming part of the specialized inhibitory system in

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organisms (Strycharz-Dudziak *et al.*, 2020). It is worth noting that these enzymes can be altered in activity by energy balance and nutrition level and stress. Studies have shown that in dairy cows, a deficiency in dietary antioxidants or an increase in ROMs leading to oxidative stress can result in compromised health (Mikulková *et al.*, 2020). It is noteworthy that various factors such as energy balance, nutritional status (Tsiplakou *et al.*, 2018), and stress, particularly heat stress (Das *et al.*, 2016; Habeeb *et al.*, 2023), can impact the activity of these enzymes.

Livestock in the nomadic regions of southern Iran are traditionally raised on pastures for the majority of the year. During the last months of pregnancy and early lactation, these animals are supplemented with feed, primarily barley grains, in the form of partially mixed ration (**PMR**) (Shakour and Rezaei, 2010). The warm and Mediterranean climate in this area, coupled with hot summers, creates an environment that may induce oxidative stress in livestock. Goats grazing on pastures are particularly susceptible to oxidative stress due to factors such as heat stress, exposure to UV rays, and inadequate nutrition (Ponnampalam *et al.*, 2022). Elevated temperatures and intense sunlight can lead to increased production of free radicals in the body, resulting in oxidative damage of cells and tissues (Idris, 2020). This oxidative stress can manifest in reduced immune function, impaired reproductive performance, and overall poor health in goats (Ponnampalam *et al.*, 2022). Furthermore, the economic implications of oxidative stress on herd productivity and profitability can impact pastoralists and nomads (Idris, 2020; Ponnampalam *et al.*, 2022). Furthermore, it has been suggested that the involvement of ROMs and antioxidants extends to diverse physiological mechanisms, including the synthesis of milk (Darbaz *et al.*, 2019).

The impact of exposure to hot environments and varying rations on the oxidative status of nomadic animals, particularly goats, was investigated by the authors of this study. The primary goal of the research was to examine how various feeding methods during the summer season impact the oxidative status of lactating goats in nomadic settings, and compare them to the same feeding practices and rations during the spring. To achieve this objective, the study assessed ROMs and α-tocopherol concentration, along with the activity of GPx and SOD, in nomadic goats that rely on pasture for their nutrition. Furthermore, the researchers analyzed the essential metabolites of the goats under investigation to evaluate their metabolic condition.

MATERIALS AND METHODS

Animals, management and place of experiment: The investigation was carried out in Qirokarzin county, situated in the southern area of Fars province, Iran. The area's geographical coordinates are approximately 28.3459° north latitude and 52.9660° east longitude, with an elevation of 645 meters above sea level. The climate in this area is characterized by harsh summers, warm spring and autumn seasons, and moderate winters. The annual precipitation rate, which mainly falls during late autumn and winter, is less than 300 mm, as documented in the meteorological yearbook of Iran for the years 2014-2016. An electronic recording station (Latem Spa-LSI) owned by SabzBavaran-e-Nouandish Co. was utilized throughout the study to record the average ambient temperature and average relative humidity at hourly intervals. The experimental phase commenced on April 20th and concluded on June 20th. The highest and lowest ambient temperatures, as well as the highest and lowest relative humidity, recorded in April were 38°C, 21°C, 47%, and 21%, respectively. In June, these values were 49°C, 33°C, 31%, and 8%, respectively. The temperature humidity index (**THI**) was determined using the formula proposed by Ingraham *et al.* (1979). The formula was

$$THI = (1.8 \times AT + 32) - (0.55 - 0.55 \times RH) \times [(1.8 \times AT + 32) - 58]$$

The ambient temperature, denoted as AT in degrees Celsius, and the relative humidity, denoted as RH as a fraction of the unit, were used to calculate the mean daily THI, daily minimum THI, and daily maximum THI during the study period.

In this study, a total of 66 female goats belonging to Turki-Qashqai breed were selected. These goats had a mixed black and dark gray color and were of an average age range of 3 to 4 years. The live weight of the goats was recorded to be 41.50±4.75 kg (mean ± standard error). The daily milk production of the goats was measured to be 1.18±0.31 kg. Additionally, the body condition score of the goats was determined to be 2.25±0.25.

All the goats used in the study were approved by a veterinarian in terms of their health. The goats underwent a specific protocol in early September to guarantee the synchronization of their estrus. Cosequently, the goats gave birth in the middle of February. As a result, the goats were in days-in-milk (**DIM**) for 85 ± 7 and 120 ± 7 days in the months of April and June, respectively. At the beginning of June the goats were randomly divided into three groups. The first group was assigned to graze on natural pasture only (**M**). The second group was given a daily supplement of 500 grams

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of commercial concentrate in the form of partialy mixed ration (**PMR**) along with access to natural pasture (**MS**). The concentrate consisted of 260 grams of barley grains and 240 grams of milled alfalfa leaves. The third group was also allowed to access to the hay of the same natural pasture freely (*ad libitum*) but was also provided with 500 grams of the same concentrate (**AS**). Each group consisted of an equal number of goats, resulting in a total of 22 repetitions per group (n=22). The M and MS groups were granted access to graze on a sprawling 120-hectare natural pasture, which had been divided into several sections. Each section of the pasture was further divided into two equal parts, ensuring that both groups had access to similar plant composition.

Throughout the experimental phase, the goats were alternated between two sections of the pasture for 8 hours daily, divided into two feeding times (from 05:00 to 9:00 and 16:00 to 20:00). Prior to sampling, the goats were given a 14-day adjustment period to acclimate to the experimental settings, grazing routine, and stable surroundings. During the grazing period, the livestock in the M and MS groups were able to consume pasture forage obtained from the identical natural pasture. On the other hand, the AS group had unrestricted access to the same forage in harvested form. The concentrate feed was given to the MS and AS groups in two equal portions, once in the morning (4:00) and once in the evening (21:00) during milking times. All the animals were provided with unlimited access to water, salt licking blocks, mineral materials (manganese sulfate (277.20 mg/kg), copper sulfate (141.48 mg/kg), selenium (0.99 mg/kg), zinc sulfate (792 mg/kg), ferrous carbonate (372.60 mg/kg), calcium (5.54 mg/kg)), and were manually milked.

Sampling and laboratory procedures: Sampling and laboratory procedures were carried out to examine the chemical composition and nutritional value of pasture fodder and concentrate samples. The collection of samples took place during a five-day period in April and June. Subsequently, these samples were dispatched to the SabzBavaran-e-Nouandish laboratory in Qir city. The crude protein (**CP**) content was analyzed using method 930.15 according to the Kjeldahl method (AOAC, 2005), while the ether extract was determined by method 984.13 through Soxhlet extraction with diethyl ether (AOAC, 2005). Ash content was measured by igniting at 600°C for 2 hours (AOAC, 2005, method 920.39) using electric furnace (Shimaz co., Tehran, Iran). ADF was determined using the cetyl-trimethyl-ammonium bromide H_2SO_4 (CTAB) and 1N method (AOAC, 2005, method 973.18). The NDF content was assessed by heat-stable α-amylase and sodium sulfite (Van Soest *et al*., 1991). (Table 1).

Table 1- Chemical composition of the experimental diets

	Pasture			
	Spring	**Summer**	**Hay***	**Concentrate****
Dry matter (DM; g/kg of feed)	340	426	861	892
Organic matter (g/kg of DM)	878	881	922	954
Crude Protein (g/kg of DM)	169	151	110	163
Ether extract (g/kg of DM)	30.8	22.3	29.5	29.8
NDF[1] (g/kg of DM)	398	431	479	301
Milk Forage Unit[2] (kg of DM)	0.80	0.75	0.61	1.12

[1] NDF = Neutral Detergent Fiber
[2] Calculated according to INRA (1998)
* The hay was collected from the identical pasture and left to dry naturally in the open air.
** The concentrate consisted of 260 grams of barley grains and 240 grams of milled alfalfa leaves.

Blood analysis: During the experiment in April and June, the animals were weighed weekly, and their body condition score was determined following the methodology outlined by Santucci *et al*. (1991). In order to estimate the pasture feed consumption for the animals of M and MS groups, the Campbell (1966) method was employed. This involved randomly selecting five districts and pairing them with five similar districts. One of each pair was enclosed by a cage with dimensions of 2×2 meters, while the other was marked with nails. The difference in fodder mass before and after grazing was used to calculate the livestock's fodder consumption, which was expressed in grams of dry matter per day. Feed intake within the AS group was established by computing the variance between the feed consumed and the feed left over.

During the months of April and June, blood samples were collected from all animals every two weeks at 5:00 hours using two vacuum tubes (Vacutainer) containing lithium heparin and potassium ethylenediamine tetraacetic acid (to determine the α-tocopherol content) from the jugular vein. The blood samples were promptly placed on ice upon preparation. After one hour, the hemoglobin (Hb) levels were evaluated using a

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commercial kit provided by Antibodies (Cat#ABIN6956464) in Limerick, PA, USA. Subsequently, the plasma was separated by subjecting the samples to centrifugation at 1400 g for 15 minutes at a temperature of 4°C. Following this, 0.5 ml of blood was also centrifuged at 1400 g for 15 minutes and underwent four washes with 3 ml of 0.9% sodium chloride solution. Upon completion of the fourth wash, the red blood cells were lysed by adding 2 ml of double distilled water. ROMs were measured in serum by a colorimetric assay kit (d-ROMs test, Diacron International, Grosseto, Italy) in accordance with the manufacturer's guidelines. The results were measured in Carr units (Carratelli Units). Plasma α-tocopherol content was determined using HPLC, as described by McMurray and Blanchflower (1979). To determine the activity of blood GPx, a kinetic method was employed with the assistance of a commercial kit (SKU#RS504; RANSEL by Randox laboratories Ltd., Antrim, United Kingdom), following the manufacturer's instructions. The activity of SOD in lysates of red blood cells was determined using a kinetic method and a commercial kit (SKU#SD125; RANSOD by Randox laboratories Ltd., Antrim, United Kingdom), as instructed by the manufacturer.

Plasma metabolites including glucose, triglycerides, total cholesterol, total protein, and albumin were quantified utilizing commercial kits (Pars Azmoun, Tehran, Iran) in accordance with the manufacturer's guidelines. The concentration of non-esterified fatty acids (**NEFA**) was assessed with a commercial kit (SKU#FA115 by Randox laboratories Ltd., Antrim, United Kingdom). Simultaneously, 30 ml of animal milk samples were collected during morning and evening milking sessions, proportionate to the total milk volume of each session after thorough mixing. The fat, crude protein, and lactose content of the milk samples were determined using the Milko-Scan machine (Foss Electric, Hillerod, Denmark).

Statistical analysis: The normal distribution of the data was assessed by employing the UNIVARIATE procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC, USA). The collected data underwent statistical analysis using the mixed procedure of SAS under the complete randomized design with livestock considered as a repeated factor. The Tukey test was used to identify specific locations where differences in means were observed. The data was summarized as (Mean ± SE), and significance was determined at $P \leq 0.05$, while a tendency towards significance was noted at $0.05 < P > 0.1$.

RESULTS

As expected, the THI values showed a higher value in the summer season (91.3±2.8) compared to the spring season (62.9±3.4) ($P<0.01$; data not presented). Group M's experimental animals exhibited a stable live weight over the summer months, even though they consumed less feed than in the spring season.

Table 2 The impact of different variables, namely the season (spring and summer) and experimental diets, on various parameters such as live weight (**LW**), body condition score (**BCS**), and feed intake (**FI**) in Turki-Qashqai goats under nomadic conditions.

	Season						SEM	Significance		
	Spring			Summer						
Diets	**M**	**MS**	**AS**	**M**	**MS**	**AS**		**Feed (F)**	**Season (S)**	**F × S**
LW (kg)	43.9	46.8	44.3	44.2	50.4	45.3	1.23		*	
BCS	2.14	2.12	2.26	1.88^{b}	2.21^{a}	2.30^{a}	0.09	**	*	*
FI (g of DM/Day)	620^{b}	900^{ab}	1309^{a}	440^{b}	780^{ab}	1219^{a}	129.11	*	*	*

The experimental diets included natural pasture alone (M), natural pasture supplemented with 500 grams per day of concentrate consisting of 260 grams of barley garins and 240 grams of ground alfalfa leaves (MS), and natural pasture fodder combined with 500 grams of concentrate (AS).
Significant differences at the 5% level are indicated by lowercase letters in each row in season.
The symbol "*" denotes statistical difference at the 5% level, "**" denotes statistical difference at the 1% level.

In contrast, the other groups did not display any alterations in terms of feed intake or live weight. (Table 2).

During the spring season, goats displayed similar body condition score index values across all experimental groups. However, in summer, the M group showed significantly lower values compared to the other groups ($P<0.05$). Additionally, the body condition score measurements for the M group were significantly higher during the spring season than during the summer season ($P<0.01$). On the contrary, food consumption decreased significantly during the summer season compared to the spring season ($P<0.05$).

It is worth noting that the M group experienced a notable 28% reduction in food consumption, as illustrated in Table 2.

Although other groups experienced a decrease in food intake during the summer, this decline was not statistically significant. The levels of ROM in the bloodstream were influenced by various factors

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such as the season, dites, and the interaction between these two variables (P<0.05).

Table 3 The effects of different variables, such as the season (spring and summer) and experimental diets, on oxidative stress markers in Turki-Qashqai goats under nomadic conditions.

	Season						SEM	Significance		
	Spring			Summer						
Diets	M	MS	AS	M	MS	AS		Feed (F)	Season (S)	F × S
ROMs (Carr Unit)	134^{a}	127^{ab}	95^{b}	156^{b}	197^{a}	168^{b}	14.16	*	*	*
GPx (U/g Hb)	220	183	193	289	348	321	36.52		**	
SOD (U/g Hb)	1041	1637	1198	1638	2158	2664	120.03		**	
α-tocopherol (µmol/L)	9.04	9.84	10.43	7.97	7.20	7.62	0.67		**	

The experimental diets consisted of natural pasture alone (M), natural pasture supplemented with 500 grams per day of concentrate containing 260 grams of barley garins and 240 grams of ground alfalfa leaves (MS), and natural pasture fodder combined with 500 grams of concentrate (AS).
Significant differences at the 5% level are indicated by lowercase letters in each row in season.
The symbol "*" denotes statistical difference at the 5% level, "**" denotes statistical difference at the 1% level.
ROMs are known as reactive oxygen metabolites, while GPx refers to glutathione peroxidase, and SOD stands for superoxide dismutase.

It was observed that during the summer season, both the MS and AS groups exhibited elevated ROM levels compared to the levels recorded during the spring season.
However, there was no significant statistical difference in ROM levels for the M group between the months of April and June. The activity levels of GPx and SOD were affected by the season (P<0.01; Table 3), with higher levels observed during the summer season compared to the spring season. Furthermore, as illustrated in Table 3, the circulatory α-tocopherol exhibited a significant higher values during the spring season in comparison to summer (P<0.01).
The concentration of blood glucose levels in goats from the M and MS groups was higher during the summer compared to the spring (P<0.001; Table 4). Specifically, in April, goats consuming the AS diet had higher plasma glucose levels than those in the M and MS groups (P<0.001).
Conversely, during June, the blood glucose levels of goats in the M group were higher than those in the MS group (P<0.05).

Table 4 The effects of different variables, such as the season (spring and summer) and experimental diets, on metabolic profile of Turki-Qashqai goats under nomadic conditions.

	Season						SEM	Significance		
	Spring			Summer						
Diets	M	MS	AS	M	MS	AS		Feed (F)	Season (S)	F × S
Glucose (mmol/L)	3.23^{b}	3.45^{b}	4.28^{a}	5.25^{a}	4.49^{b}	4.51^{b}	0.21	*	***	***
Triglycerides (mmol/L)	0.16	0.19	0.17	0.21	0.27	0.19	0.02		***	***
Cholesterol (mmol/L)	0.15	0.19	0.18	1.55	1.78	1.61	0.07			
NEFA (mmol/L)	0.41	0.28	0.26	0.17	0.24	0.21	0.08		*	
Total protein (g/L)	70.4	73.1	72.7	70.8	74.3	73.7	2.08			
Albumin (g/L)	26.4^{a}	29.1^{b}	29.8^{b}	26.0^{a}	28.2^{b}	28.7^{b}	0.94	*	*	
Globulin (g/L)	44.8	44.3	42.1	44.9	46.8	45.4	2.18			

The experimental diets consisted of natural pasture alone (M), natural pasture supplemented with 500 grams per day of concentrate containing 260 grams of barley garins and 240 grams of ground alfalfa leaves (MS), and natural pasture fodder combined with 500 grams of concentrate (AS).
Significant differences at the 5% level are indicated by lowercase letters and differences at the 1% level are indicated by uppercase letters in each row.
The symbol "*" denotes statistical difference at the 5% level, and "***" denotes statistical difference at the 0.1% level.
NEFA stands for Non-Esterified Fatty Acids

The triglyceride levels in the experimental goats were also higher in the hot season than in the moderate season (P<0.001), while the NEFA plasma concentration showed the opposite trend (P<0.05; Table 4). Additionally, the albumin level was found to be higher in the spring compared to the summer (P<0.05), with higher levels observed in the MS and AS groups compared to the M group (P<0.05; Table 4).
The study did not find any significant effects of season, ration, or their interactions on the plasma levels of cholesterol, total protein, and globulin in the observed animals (Table 4).The daily amount of milk produced and the 4% fat-corrected milk (**FCM**) showed significant changes throughout the seasons and were affected by both the season and the interaction of feed and season (Table 5).
The FCM index underwent a relatively modest decline during the summer, with more significant reductions seen in M and MS group livestock. In June, milk had a higher fat percentage compared to April (P<0.05), while lactose levels were

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significantly lower in June than in April (P<0.05) according to Table 5.

Table 5 The influence of different variables, such as season (spring and summer) and experimental diets, on specific quantitative and qualitative characteristics of milk in Turki-Qashqai goats under nomadic circumstances.

	Season						SEM	Significance		
	Spring			Summer						
Diets	M	MS	AS	M	MS	AS		Feed (F)	Season (S)	F × S
Milk yield (g/day)	1129^{b}	1432^{a}	1151^{b}	594^{b}	636^{ab}	1014^{a}	112.8	*	*	**
4%FCM† (g/day)	925^{b}	1089^{a}	922^{b}	752^{b}	755^{b}	989^{b}	71.3	*	*	*
Fat (%)	2.79	2.41	2.68	5.88	5.25	3.84	2.04		*	
Fat (g/day)	31.5	34.4	30.8	34.93	33.4	38.9	2.82			
Protein (%)	2.81	2.66	2.93	4.26	4.55	3.48	1.79		*	
Protein (g/day)	31.7	38.1	33.8	25.31	28.3	35.2	3.26		*	
Lactose (%)	4.44	3.78	4.51	5.99	6.34	4.86	2.89		*	
Lactose (g/day)	50.3	54.2	51.7	35.58	40.1	49.3	4.57		*	

The experimental diets included natural pasture alone (M), natural pasture supplemented with 500 grams per day of concentrate containing 260 grams of barley garins and 240 grams of ground alfalfa leaves (MS), and natural pasture fodder combined with 500 grams of concentrate (AS).
†FCM= Fat corrected milk for 4%. FCM=0.4 × milk yield + 15 × fat yield
Significant differences at the 5% level are indicated by lowercase letters in each row.
The symbol "*" denotes statistical difference at the 5% level, "**" denotes statistical difference at the 1%.

DISCUSSION

There are multiple methods available to determine if livestock is experiencing heat stress. Several common approaches include behavioral observations, physiological indicators, monitoring respiratory rate, assessing heat tolerance index, and evaluating feed intake and productivity (Idris *et al.*, 2021). The temperature range that is considered critical for goats in housing conditions usually falls between 25 and 30°C. However, when the ambient temperatures go beyond 30°C, heat stress becomes a concern for these animals (Gupta and Mondal, 2021). According to a specific study, the THI values recorded during the summer indicated that the goats experienced a moderate level of heat stress (Srivastava *et al.*, 2021). Interestingly, the live weight of livestock in the MS and AS groups showed a significant increase during the summer, while the M group did not exhibit any change in this aspect. These findings are consistent with the observations published by Schmidely *et al.* (1999). The body condition score was similar across all experimental groups in the spring, but there was a significant decrease in this parameter for the M group during the summer, which can be attributed to several factors. Firstly, the M group only had access to natural pasture, which likely required more energy for grazing compared to the other groups. Secondly, the quality of the pasture and feed consumption were lower in the summer compared to the spring. Therefore, it appears that the goats in the M group may have been inefficient in utilizing energy. Furthermore, it is well documented that heat stress can have a negative impact on rumen function and reduce microbial activity in the digestive system. The rumen plays a crucial role in digesting feed and producing enzymes that aid in nutrient absorption, including lactose. Disruption in rumen function can lead to some consequences such as a decrease in lactose production in ruminant livestocks (Kim *et al.*, 2022). In elevated THI, BCS noted in MS and AS groups was comparable and superior to the values recorded for the M group, which may be due to the superior quality diet provided to MS and AS animals, enabling them to maintain fat reserves.

Triglyceride levels displayed a linear linkage with the lactation stage, as evidenced by spikes observed at the conclusion of the lactation period. During the initial stage of lactation, when milk production is at its highest point, dairy goats depend on utilizing triglycerides stored in adipose tissue as an energy source (El-Tarabani *et al.*, 2018). This utilization aids in meeting the escalated energy requirements of lactation. Elevated levels of plasma triglycerides can serve as an indication that the goat possesses ample energy reserves to sustain milk production (McNamara, 1991). Conversely, the pattern observed for NEFA levels was contrary to that of triglyceride levels. This discovery can be clarified by the concept proposed by Mazur *et al.* (1987) that indicated the increased re-esterification of fatty acids linked with higher triglyceride concentrations, resulting in decreased NEFA concentration. The elevated levels of mobilized NEFA observed in April may be attributed to the enhanced milk production by the animals during this period, resulting in a more significant physiological lipomobilization.

In June, it was observed that higher THI led to an increase in ROMs by 0.50 unit. Specifically, during the summer season, the MS group experienced a rise of 0.59 in ROMs, while the AS group saw an increase of 0.85. This rise in ROM levels can be attributed to the simultaneous increase in THI

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during this season (Gupta and Mondal, 2021). Additionally, higher ROMs in summer grazing goats is due to a variety of factors. Firstly, goats grazing in summer are exposed to increased environmental stressors such as higher temperatures, solar radiation, and other stress-inducing factors. This exposure to heat stress and UV radiation leads to the production of ROS in the body, resulting in elevated ROMs levels (Chauhan *et al*., 2023). Secondly, grazing goats may face a higher level of oxidative stress during summer due to environmental factors, which can overwhelm their natural antioxidant defense mechanisms. This imbalance between ROS production and antioxidant capacity contributes to higher ROMs levels in summer grazing goats (Giorgio *et al*., 2020). Moreover, the higher production level observed in the MS and AS groups may have played a role in increasing the concentration of ROMs (Das *et al*., 2016; Idris *et al*., 2021).

Thermal stress can greatly impact the health, productivity, and general welfare of livestock. Several indicators can help identify thermal stress in these animals which have been mentioned by Idris *et al*. (2021). Firstly, an increased respiration rate is a common sign of thermal stress. Livestock may pant excessively as a means to regulate their body temperature. Secondly, animals experiencing thermal stress may consume more water than usual in order to stay hydrated and cool down (Das *et al*., 2016). Thirdly, heat stress can lead to a reduced feed intake in livestock, which can negatively impact their growth and productivity (Das *et al*., 2016; Habeeb *et al*., 2023). Additionally, animals may display signs of agitation or restlessness as they attempt to find ways to cool down and seek relief from the heat (Idris, 2020). Moreover, dairy cows may experience a decrease in milk production during periods of thermal stress (Das *et al*., 2016; Habeeb *et al*., 2023). Lastly, livestock with light skin or hair may be prone to sunburn (Idris, 2020). Heat stress also led to an increase in the activity of GPx and SOD, which were 0.60 and 0.64 times higher, respectively, compared to the values recorded in spring. It is important to note that the rise in the activity of these antioxidant enzymes coincided with an elevation in the concentration of plasma ROMs. The present findings showing the relationship between GPx and SOD activity with the concentration of ROMs ($r=0.61$ and $r=0.48$, respectively) emphasize the crucial role of these enzymes in effectively monitoring and neutralizing free radicals. Antioxidant enzymes such as SOD and GPx play an active role in eliminating excessive ROMs caused by oxidative stress (Bizoń *et al*., 2023). Yang *et al*. (2010) reported a significant increase in reactive oxygen species production, as well as antioxidant enzymes including SOD, catalase, and GPx, and lipid peroxidation in response to heat stress. Furthermore, Bhusari *et al*. (2008) discovered that chronic heat stress induces hepatic SOD and catalase, indicating a higher presence of ROMs in response to stress. The activation of antioxidant enzymes is prompted by heat exposure. During June, the level of circulatory α-tocopherol, the primary element of vitamin E, was decreased in comparison to April, aligning with the findings of Asadian *et al*. (1995) in sheep. The decrease in vitamin E levels during summer, when an increase in ROMs concentration was observed, could potentially be attributed to its direct interaction with various free radicals (Machlin and Bendich, 1987). The inverse relationship ($r=-0.52$) observed between ROMs and circulatory α-tocopherol provides additional support to this association. The reduction in plasma α-tocopherol concentrations from April to June may also be attributed to its decline as plants mature (Steinshamn and Leiber, 2023). Moreover, a significant reduction in plasma albumin concentration was observed during the summer. Considering that albumin plays a crucial role in the antioxidant system and acts as a scavenger of free radicals (Halliwell, 1988), the decrease in albumin levels during summer can be justified. It is possible that the experimental animals underwent oxidative stress, as evidenced by the simultaneous decrease in α-tocopherol levels and increase in ROMs concentration.

The impact of the season on milk yield and FCM was found to be significant, with the highest values recorded in April. The MS group showed the highest milk production levels during the spring season, indicating that a combination of green pasture and concentrate supplementation enabled the goats to achieve their maximum production potential. In June, there was a general decrease in milk production, although the M and pasture groups still exhibited notably higher values. These findings align with previous research, indicating that supplementing feed with concentrate can help mitigate the decline in milk production during mid-lactation. This effect was particularly evident in the AS group, where the relative milk production only decreased by 0.10 units. Heat stress has the potential to significantly reduce milk yield and FCM production in goats. The effects of heat stress on milk production in goats are multifaceted. For instance, it can result in a decrease in milk yield as the physiological strain of regulating body temperature in hot conditions can divert energy and resources away from milk synthesis. This ultimately leads to lower milk yields during periods

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of heat stress (Mehaba *et al.*, 2019). Furthermore, heat stress can also bring about changes in the composition of goat milk. Research indicates that heat-stressed goats may produce milk with lower protein content, and lactose levels. These alterations in milk composition can impact the overall quality of the milk and its commercial value. (Salama *et al.*, 2014).

Bava *et al.* (2001) and Goetsch *et al.* (2001) also observed that the dietary treatments did not have any impact on the concentration of milk fat. The higher milk fat content observed in summer compared to spring in this study may be attributed to the decrease in milk production as goats progress through their milking cycle. Additionally, The spike in milk fat production seen in summer may be linked to the increased NDF content present in the pasture during that season, which is crucial for stimulating rumen fermentation and maintaining milk fat levels in ruminants (Kim ert al., 2022; Zhou *et al.*, 2022; Habeeb *et al.*, 2023). The milk protein content did not show a significant difference between treatments, consistent with findings from previous studies (Kawas *et al.*, 1991; Bava *et al.*, 2001). However, there was a tendency for the protein content of spring milk to be significantly different from summer milk (P<0.06). The correlation between the increase in THI values and the decrease in milk lactose content aligns with the pattern observed in dairy cows (Giannone *et al.*, 2023). Lactose assumes a pivotal function as an osmotic agent in milk, exerting a substantial impact on the movement of water from the bloodstream into the milk. The synthesis of lactose takes place within the udder, where glucose from the blood is assimilated across the basal membrane of the mammary epithelial cells and subsequently transformed into lactose (Antanaitis *et al.*, 2024). Lactose biosynthesis may have been hindered due to a decrease in lactalbumin availability in June. The rise in plasma glucose levels at the same time, along with the inverse relationship between glucose levels and milk production (r=-0.53; P<0.04), lends credence to the theory of diminished glucose absorption by mammary glands (Habeeb *et al.*, 2023). Moreover, heat stress can also affect appetite and feed intake in dairy cows, resulting in an overall reduction in nutrient intake, including the precursors necessary for lactose synthesis. When combined with the metabolic strain caused by heat stress, this can contribute to a decrease in lactose production in dairy cows (Rhoads *et al.*, 2009).

CONCLUSION

During the summer months, lactating goats may have experienced various levels of oxidative stress, as indicated by the markers of metabolic profile and oxidative status in this study. It appears that seasonal influences have a stronger impact on certain enzymes in nomadic conditions compared to nutritional factors. In the experimental setting, incorporating a concentrate supplement helped maintain milk production and could be a beneficial strategy to counteract the decrease in the lactation period in dairy goats when faced with high THIs. Therefore, it is highly recommended for nomadic goat farmers to include a certain amount of concentrate mixture or suitable commercial concentrates in goat rations, especially for twin or multiple suckling she-goats during periods of oxidative stress.

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CONFLICT OF INTEREST

There was no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Amir Ahmadpour (A.A.) was responsible for formulating the project, creating the primary conceptual ideas, outlining the evidence, and overseeing most of the technical aspects. Mrs. Shiva Forouzanfar and Dr. Nazanin Sajjadi equally contributed to the sampling and execution of specific laboratory analyses. Dr. Mousa Zarrin (M.Z.) performed the numerical computations for the proposed experiment and independently verified the numerical findings of the trial. A.A. collaborated with others in composing the manuscript, while Prof. Ahmad Oryan and M.Z. thoroughly examined and revised it.

DATA AVAILABILITY

The datasets produced and analyzed in the present study are not accessible to the public, however, they can be obtained from the corresponding author upon reasonable request.

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ETHICAL CONSIDERATIONS

The Animal Care and Animal Handling rules and regulations, approved by the Animal Ethics Committee of Yasouj University, were strictly followed for all live-animal related procedures. Throughout the experiment, animals were consistently supervised by veterinary professionals, ensuring that all handling standards were adhered to.

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