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Role of thyme and celery mixture in improvement of behavior, performance, and immunity of lactating Ossimi ewes at two climate diverse housings

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ABSTRACT

Improvement of sheep health and productivity has become a global goal. An effective house must provide adequate space, shelter, and protection from climatic changes and stress. Some plants such as thyme (*Thymus vulgaris*) and celery (*Apium graveolens*) could alleviate stressors. Hence, this investigation was conducted to determine the role of thyme and celery seed mixture (TCM) in improving the behavior and performance of lactating Ossimi ewes at diverse housings during autumn and winter. Forty lactating ewes (2 years old and 48 ± 1.5 kg average body weight) were randomly equally distributed into two semi-shaded buildings (SSB) (20 ewes each). These were roofed with a concrete slab 40 cm thick and 5 m high which was covered with three rows of rice straw bales as thermal insulation. It had natural dirt areas to the north and south. Ten ewes were fed a basal diet, and the others received a basal diet containing TCM (10 gm thyme and 10 gm celery/head/day). Similarly, 20 ewes were treated in a fully-shaded building (FSB) which was roofed with a layer of tin 5 m high and had natural flooring to the west and east. These ewes were fed a basal diet + TCM for 1 month pre-lambing and 2 months post-lambing. Both behavior and weight were recorded bi-weekly. Blood samples were collected monthly to measure oxidative stress indicator reduced glutathione (GSH), malondialdehyde, total protein, glucose, gene expression of nuclear factor 2 linked to erythroid 2 (Nrf2), and interleukin 2. Cumulative feed intake was calculated, and milk samples were collected for 2 months after lambing until weaning to measure moisture, ash, total protein, lactose, non-solid fat (NSF), total solids, fat, and antioxidant capacity of milk (free radical and inhibition factor). The findings revealed that sheep exhibited better feeding behavior in the FSB and the oxidative stress indicator was lower than in the SSB. TCM enhanced feeding behavior and decreased the oxidative stress indicator (reduced GSH) in the SSB. Additionally, it increased total protein and dam body weight (at the 6th week) in the FSB and improved feed intake in both buildings. Thyme and celery have antioxidant capacities in milk. Hence, the present data suggest that adding TCM to the basal diet could reduce the stress of climatic changes on lactating ewes and improve animal behavior and immunity leading to enhanced production.

Introduction

Sheep is a low-price meat that is abundant in vitamins, minerals, and essential polyunsaturated fatty acids (Ponnampalam *et al*., 2016). Therefore, improving sheep health and productivity has become a global goal. Proper housing and management enhance sheep performance (Sejian *et al*., 2017), and an effective sheep house must provide adequate space, shelter, and protection from climatic changes that stress sheep and impair health and performance (Bhatta *et al*., 2005; Caroprese, 2008).

Closed-roof buildings protect animals from hot and cold weather more than semi-open buildings (Abozed *et al*., 2013). However, outdoor feeding has enhanced Awassi lambs' feed conversion ratios compared with indoor feeding (Serafettin, 2011). Though open-front buildings are best suited to dry climates (Caroprese, 2008), controlling climatic changes and air drafts, especially during autumn and winter is very difficult. During these seasons, most ewes are pregnant or in the lactation stage and therefore are under physiological stress. Thus, their protection from additional stressors caused by climate change is necessary. One suggested strategy to counter climate change's effects on sheep is to improve the animals' health and immunity.

Natural additives improve feed utilization and performance in lactating animals (Kholif and Olafadehan, 2021). Thymol is the primary component in thyme (*Thymus vulgaris*). and has a variety of antimicrobial activities, enhances ruminal fermentation, increases the level of ruminal volatile fatty acids (VFA), and decreases ruminal ammonia-N concentration (Walsh *et al*., 2003; Abd El Tawab *et al*., 2020; Khattab *et al*., 2020). Additionally, the primary celery (*Apium graveolens*) bioactive compounds are limonene, which has a broad spectrum of Gram-negative bacteria-fighting ability, and selinene (Dorman and Deans, 2000; Sowbhagya, 2014). Moreover, celery contains more phenolic chemicals than thyme (Akbarian-Tefaghi *et al*., 2018). Thus, they provide different actions when added at the same concentration to livestock feed (Shaaban *et al*., 2021). Furthermore, an amalgamation of several additives induced a better effect than one owing to the synergy that potentially influences immunity, performance, and production.

Nuclear factor erythroid 2-related (Nrf2) is a redox-sensitive factor transcriptional factor, that has antioxidant, anti-inflammatory, and cytoprotective properties. It maintains cellular defense mechanisms, is secreted in response to stress, and can activate some antioxidants such as glutathione peroxidase (Kensler *et al*., 2007; Bellezza *et al*., 2018; de la Vega *et al*., 2018). Moreover, interleukin-1 (IL-1) is associated with inflammation and innate immunity (Mantovani *et al*., 2019). There have been no previous studies examining the role of a thyme and celery mixture (TCM) in combating the stress effects of climatic changes in sheep. Therefore, this study aimed to evaluate the effectiveness of TCM on improving the performance of ewes that were pregnant and nursing in semi-shaded and fully shaded closed houses. Sheep performance (weight gain and milk content), blood antioxidant (reduced glutathione), Nrf2, oxidants (malondialdehyde), and immunity (interleukin-2) were measured in pregnant and lactating ewes.

Materials and methods

The current study was conducted in the Faculty of Veterinary Medicine- Beni-Suef University, Egypt. The period of experiment was from October 2021 to February 2022. This study was included in a protocol

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approved by BSU-IACUC (022-306).

Experimental design

Animal accommodation

A total number of 40 lactating ewes with an average age of 2 years and 38.95±2.3 kg average weight, were randomly selected were used to ascertain the impact of thyme, celery, and their mixture (TCM) on performance, behavior, certain stress indicators, and milk components under different environmental conditions.

Animal accommodation was in two types of housing. Housing 1, a "semi-shaded building (SSB)," was semi-shaded, roofed with a concrete slab 40 cm thick, 5 m high, and covered with three rows of rice straw bales as thermal insulation. It had natural dirt land to the north and south. Housing 2, a "fully shaded building (FSB)" was fully shaded, roofed with a layer of tin 5 m high, and had a natural floor in the west and east.

Animals were randomly and equally assigned to four groups (n = 10) as follows;

Group 1: Semi-shaded building non-treated (SSB-NT)
Group 2: Semi-shaded building treated (SSB-T)
Group 3: Full-shaded building non-treated (FSB-NT)
Group 4: Full-shaded building treated (FSB-T)

The indoor temperature and humidity were assessed (at morning, afternoon and at evening) in each housing unit using a digital thermo-hygrometer. The average temperature and relative humidity were calculated to be 18.7±3.93ºC and 64.75±3.50%, respectively in SSB while, they were 21.63±1.53ºC and 65.50% ± 7.22% in order in FSB.

The study was conducted one month before lambing and for 2 months post lambing (October- December/ 2023).

All ewes were fed according to (NRC, 1985), were fed approximately 500 g of processed feed (10% yellow maize, 22% cotton seed cake, 44% wheat bran, 20% molasses, 2.5% ground limestone, and 1.5% common salt) provided in a fixed manager along one side of the house in addition to 600 g rice straw and green bar during all periods of lactation. Water was available all day from a common water trough. Dried thyme leaves and celery seeds (from Turkey) were delivered by the herbalist HARAZ, in Cairo, Egypt. A total of 10 g of thyme and 10 g of celery/head/day were used as a TCM supplement in feed according to the dosage recommended by Eid *et al*. (2021).

Sampling

Blood samples were obtained from ewes by simple vein puncture of the jugular vein, 2 months after parturition and were divided into three clean and dry centrifuge tubes. One tube contained sodium fluoride for plasma separation that was used for glucose measurement, and the second tube was processed for serum separation and used for measuring total protein (TP), malondialdehyde (MDA), and reduced hepatic glutathione (GSH). Serum samples were frozen at −20ºC until assayed. The third tube contained EDTA as an anticoagulant for the determination of Nrf2 and interleukin-1. Samples of milk were gathered 1 and 2 months after parturition (lambs were separated from their mothers for a total of 8 h). Ewes were milked by hand at 6:00 am. Milk sample analyses were conducted for moisture, ash, total protein, lactose, NSF, total solids, fat, and antioxidant capacity of milk (free radical and inhibition factor).

Analysis and assay of behavioral measurement

Feeding behavior (10 ewes / group) was videotaped every 2 weeks for 30 min twice daily (for two successive days) after parturition. The scan observational technique was adopted in this study according to the method described by Giger- Reverdin *et al*. (2012). The time of feeding behavior recording was fixed during the day (8:00 am and 5:00 pm), the animals were allowed to eat then behavior recording was started. The total feeding duration (TFD) / minute for each animal was calculated and expressed as TFD% (Shehata *et al*, 2022). The time between each feeding for each animal was also recorded in minutes and expressed as feeding bout duration (FBD) then the animals were classified according to FBD and expressed as percentages.

Calculation of feed intake

Feed was provided daily to animals in known quantities, and the feed intake was determined as follows:

Feed intake (FI) = feed allowed (g) − feed remained (g)

This was calculated daily, then the average weekly FI/group was recorded.

Body weight measurement

Ewes and their newly born lambs were weighed individually on the scale at a time of day to minimize variation and to obtain accurate weight (Fasted weight) in the morning (before feeding or drinking) at 2 weeks intervals. Then, weight gain was calculated by subtracting the current weight from the previous weight biweekly.

Determination of biochemical blood parameters

The glucose level in plasma samples was determined calorimetrically by the enzymatic reaction described by Trinder (1969). Total protein was analyzed calorimetrically using the Biuret reaction (Henry, 1964). Lipid peroxidation in the serum was estimated colorimetrically by measuring serum malondialdehyde content as described by Albro *et al.* (1986). Reduced hepatic glutathione content was performed according to Ellman (1959).

Milk analysis

Total solids, protein, and ash

The total solids, protein, and ash of the milk sample were determined according to AOAC (2009). Fat content in milk samples was estimated using Gerber's method as described by Bradley *et al*. (1992).

Fat-corrected milk yield (FCM)

Fat-corrected milk (4% FCM) was calculated using the following equation according to Gaines (1928): FCM = 0.4 M + 15

Where: M = milk yield (g/d), F = fat yield (amount of fat = M × fat %).

Solids-not-fat

Solids-not-fat (SNF) content was calculated by difference (Total solid % − fat %).

Lactose

Lactose content was calculated by difference (SNF% − (Protein % + Ash %))

Determination of radical scavenging activity in milk

The activity of free radical scavengers in milk samples was measured using DPPH (1, 1 diphenyl 2, picryl hydroxyl) assay (Brand-Williams *et al*., 1995). A total of 2 mL of DPPH solution (0.2 mM) prepared methanol was combined in a milk sample (100 µl) and then incubated at room temperature for 30 min. After the incubation period, 1 mL of chloroform was added to the mixture and centrifuged at 3000 × g for 5 min. The absorbance of the solution was then measured at 517 nm. A total of 100 mM of DPPH without any milk was used as a blank. The inhibition of DPPH (free radical scavenging %) percentage was determined using the following equation: Free radical scavenging %= (Absorbance of control-Absorbance of sample)/(Absorbance of control)×100

The actual decrease in absorption was inferred using the tested compounds and compared with the positive control. Measurements were conducted in triplicate, expressed the inhibition of coloration as a percentage, and obtained the inhibition concentration of 50% (IC50) from the inhibition curve where there is a positive relationship between free radical scavenging percentage and antioxidant activity.

Gene expression

The mRNA expression levels of Nrf-2, TNF-α, and IL-1β

Total RNA was isolated from the blood samples using the RNeasy mini extraction kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Spectrophotometry was used to assess the integrity and purity of the isolated RNA since the 260:280 ratios were between 1.8 and 2.0 (Khalaf *et al*., 2019). Next, using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), complementary DNA (cDNA) was created (Hassanen *et al*., 2023) following the manufacturer's instructions. The Ovis aries sequences available in GenBank were used to create the primer sets for quantifying the mRNA levels of specific genes (Table 1). The primers were designed using the primer3 tool (Ko *et al*., 2009). Real-time PCR analysis was used to determine the relative expression of the chosen genes using SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, Cat number: 4309155). ABI Prism StepOnePlus Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions (Bashandy *et al*., 2022). PCR reactions were carried out twice for each sample. The expression levels of interleukin-1 beta (IL-1β), nuclear erythroid factor 2 (nrf-2), and tumor necrosis factor-alpha (TNF-α), were normalized to the housekeeping gene (gapdh). The DDCt technique was used to analyze gene expression data (Livak and Schmittgen, 2001).

Statistical analysis

Data were shown as a mean ± standard error and analyzed using a one-way analysis of variance (ANOVA) test using SPSS, ver. 20 (IBM Corp., Armonk, NY, USA). The least significant difference test was used to determine significance. Probability values less than 0.05 ($P < 0.05$) were considered significant. Differences among FBD % were analyzed using the Kruskal-Wallis one-way ANOVA procedure for nonparametric models in SPSS.

Results

Table 2 shows the effect of housing and TCM on the feeding behavior and feed intake of ewes after parturition. Housing 2 (FSB-NT) caused a significant increase in FBD % periods compared to Housing 1(SSB-NT).

TCM increased FBD % significantly ($P < 0.05$) in housing 1 (SSB-T) during the first period (less than 7.5 min) while it was not significant during other periods in comparison with SSB-NT. However, FBD % was increased significantly ($P < 0.05$) by TCM in FSB-T compared to FSB-NT. Additionally, TCM increased FI significantly (P < 0.05 in both treated housings.

Table 3 declare the effect of housing and TCM on the body weight of ewes after parturition. Data showed that housing-induced non-significant increase in the dams' weight throughout the experiment post-parturition. TCM increased the dams' weight (($P < 0.05$) in housing 2 (FSB) at the sixth week.

Figure 1 illustrates the housing and TCM did not significantly alter the birth weight of the infant lamb males and females.

Table 4 shows the effect of housing and TCM on blood parameters of ewes after parturition. After the first month of the experiment, neither housing nor TCM had significant effects on blood glucose, total protein, and MDA. FSB induced a significant ($P < 0.05$) increase in GSH level compared to SSB. TCM increased significantly ($P < 0.05$) blood level of GSH in H1 (SSB-NT). Two months after parturition, no significant effect of housing on glucose, total protein, and MDA was observed while FSB significantly ($P < 0.05$) increased GSH. Additionally, TCM increased the total protein level significantly ($P < 0.05$) in FSB. TCM had no discernible impact on glucose or MDA and GSH levels.

The celery and thyme supplementation resulted in significant upregulation of Nrf-2, and downregulation of IL-1 and TNF-α, and mRNAs in both the SST and FST groups. These results indicated that such treatment provided potential protection against housing stress in treated ewes compared with the non-treated ewe groups (Fig 2).

Table 5 shows the effects of housing and TCM on milk samples of

Table 1. The primer sequences for the target genes.

Gene	Sense primer	Antisense primer	Accession number	Annealing temp (°C)	Amplicon size (bp)
il1β	ACAGATGAAGAGCTGCACCC	GAACACCACTTCTCGGCTCA	NM_001009465.2	58	379
tnf-α	GGTGAGAGGCTGCTAAGTGA	TCCCTTGTCCTGAGGGAGAG	EF446377.1	58	170
nrf-2	CCAGAACCAAAGTGGACCGA	GTATCGTCACAGTGTCGGGG	AY369137.1	58	333
gapdh	TTATGACCACTGTCCACGCC	TCAGATCCACAACGGACACG	NM_001190390.1	58	216

Table 2. The effect of housing, thyme and celery mixture on feeding behavior and feed intake of ewes after parturition.

		Housing 1 (SSB)		Housing 2 (FSB)	
TFD		No Treatment (SSB-NT)	TCM treated (SBB-T)	No Treatment (FSB-NT)	TCM treated (FSB-T)
		30.57±0.47	29.56±0.70	27.80±2.37	27.10±1.53
FBD "% of animals/ FBD in min"	Less than 7.5 min	76.28^{b}	87.65^{a}	91.46^{a}	96.67^{a}
	7.5-15	59.45^{c}	59.22^{c}	67.00^{b}	81.73^{a}
	16-22.5	43.33^{c}	42.20^{c}	64.49^{b}	88.33^{a}
	Over 22.5	25.67^{c}	26.57^{c}	49.05^{b}	54.44^{a}
Feed intake		15.71^{b}±0.30	23.14^{a}±0.38	15.8^{b}±0.27	22.17^{a}±0.16

Results are expressed as means±standard error, (n=10). Different superscript letters in row indicate significant at P<0.05. Rows with no superscript letters indicate no significant alterations. SSB-NT: semi-shaded non treated thyme and celery mixture; SSB-T: semi-shaded treated thyme and celery mixture; FSB-NT: full shaded non treated thyme and celery mixture; FSB-T: full shaded non-treated thyme and celery mixture; TFD: total feeding duration; FBD: feeding bout duration.

ewes after parturition. One month after parturition, neither housings nor TCM altered the antioxidant activity and chemical makeup of ewe milk. Similarly, the two houses induced no modifications to the milk's content during the second month. Milk from ewes kept in the FSB house and fed a ration containing TCM had significantly($P<0.05$) higher ash, total protein, lactose, NSF, and total solids content than those fed a non-treated diet.

TCM increased the antioxidant content of ewe's milk in both houses during the second month post-parturition. Free radicals in ewe' milk fed on TCM in both the SSB and FSB were significantly (($P < 0.05$)) lower than that of ewes fed on the traditional diet (Table 5). On the contrary, inhibition factors in milk from ewes who were fed TCM were higher than those of non-treated groups ($P < 0.05$).

Table 3. The effect of housing, thyme and celery mixture on ewe weight(kg) after parturition.

Duration/ week	Housing 1 (SSB)		Housing 2 (FSB)	
	No Treatment (SSB-NT)	Treated (SBB-T)	No Treatment (FSB-NT)	Treated (FSB-T)
Second	40.60±1.39	36.00±1.45	39.50±1.71	42.00±1.32
Fourth	37.50±2.09	37.30±1.56	38.53±1.39	39.70±1.64
Sixth	40.88±1.35^{a}	38.40±1.19^{a}	40.60±1.37^{a}	45.78±1.53^{b}
Eighth	45.00±2.53	40.32±1.83	40.62±1.35	42.89±1.70

Results are expressed as means±standard error (n=10). Different superscript letters in row indicates significant at P<0.05.
Rows with no superscript letters indicates no significant alterations.
SSB-NT: semi-shaded non treated thyme and celery mixture; SSB-T: semi-shaded treated thyme and celery mixture; FSB-NT: full shaded non treated thyme and celery mixture; FSB-T: full shaded non-treated thyme and celery mixture.

Table 4. The effect of housing, thyme and celery mixture on blood parameters of ewes after parturition.

		Housing 1 (SSB)		Housing 2 (FSB)	
		No Treatment (SSB-NT)	Treated (SBB-T)	No Treatment (FSB-NT)	Treated (FSB-T)
First month	Glucose (mg/dl)	73.30±3.08	74.40±3.80	81.11±1.67	81.40±1.91
	TP (g/dl)	8.1 2±0.33	7.51±0.25	8.36±0.43	7.46±0.47
	MDA (µmol/ml)	0.15±0.02	0.24±0.04	0.21±0.03	0.17±0.07
	GSH (µmol/ml)	0.10±0.01^{b}	0.16±0.03^{a}	0.15±0.01^{a}	0.13±0.01^{a}
Second month	Glucose (mg/dl)	68.67±1.21	68.30±2.09	66.44±2.81	68.80±2.36
	TP (g/dl)	8.16±0.29^{b}	7.46±0.17^{b}	7.26±0.17^{b}	8.79 ±0.58^{a}
	MDA (µmol/ml)	0.14±0.01	0.13±0.02	0.12±0.01	0.11±0.03
	GSH (µmol/ml"	0.12±0.02^{b}	0.09 ±0.01^{b}	0.41±0.01^{a}	0.31±0.06^{a}

Results are expressed as means±standard error (n=10). Different superscript letters in row indicates significant at P<0.05.
Rows with no superscript letters indicates no significant alterations. SSB-NT: semi-shaded non treated thyme and celery mixture; SSB-T: semi-shaded treated thyme and celery mixture; FSB-NT: full shaded non treated thyme and celery mixture; FSB-T: full shaded non-treated thyme and celery mixture.

Table 5. The effect of housing, thyme and celery mixture on antioxidant activity and chemical composition of milk.

		Housing 1 (SSB)		Housing 2 (FSB)	
		No Treatment (SSB-NT)	Treated (SBB-T)	No Treatment (FSB-NT)	Treated (FSB-T)
First month	Moisture%	86.99±0.94	86.38±1.23	84.83±0.76	84.53±0.52
	Ash	0.77±0.07	0.74±0.03	0.74±0.04	0.75±0.03
	Total protein	3.46±0.33	3.34±0.13	3.33±0.19	3.36±0.13
	Lactose	5.00±0.48	4.83±0.18	4.82±0.27	4.85±0.19
	NSF	9.22±0.88	8.91±0.33	8.89±0.50	8.95±0.35
	Total Solids	14.89±0.66	14.06±0.32	13.94±0.35	14.35±0.39
	Fat	5.67±0.47	5.15±0.26	5.05±0.19	5.41±0.26
	Radical Scavenging	62.45±0.79	63.49±0.59	61.96±0.41	62.39±1.28
	Inhibition	37.55±0.79	36.51±0.59	38.04±0.41	37.62±1.28
Second month	Moisture	85.76±0.53	84.88±0.65	85.13±0.57	84.90±0.38
	Total protein	3.36±0.26^{b}	3.72±0.21^{b}	3.01±0.08^{b}	3.77±0.09^{a}
	Lactose	4.85±0.37^{b}	5.38±0.31^{b}	4.35±0.12^{b}	5.44±0.12^{a}
	NSF	8.95±0.69^{b}	9.94±0.57^{b}	8.02±0.22^{b}	10.05±0.23^{a}
	Total Solids	13.83±0.36^{b}	14.75±0.54^{b}	13.61±0.17^{b}	15.14±0.32^{a}
	Fat	4.88±0.41	4.82±0.24	5.59±0.16	5.09±0.47
	Radical Scavenging%	66.45±0.39^{a}	58.01±1.17^{b}	66.00±0.65^{a}	59.57±0.83^{b}
	Inhibition%	33.55±0.39^{b}	41.9±1.17^{a}	34.01±0.65^{b}	40.44±0.83^{a}

Results are expressed as means±standard error (n=10). Different superscript letters in row indicates significant at P<0.05.
Rows with no superscript letters indicates no significant alterations. SSB-NT: semi-shaded non treated thyme and celery mixture; SSB-T: semi-shaded treated thyme and celery mixture; FSB-NT: full shaded non treated thyme and celery mixture; FSB-T: full shaded non-treated thyme and celery mixture.

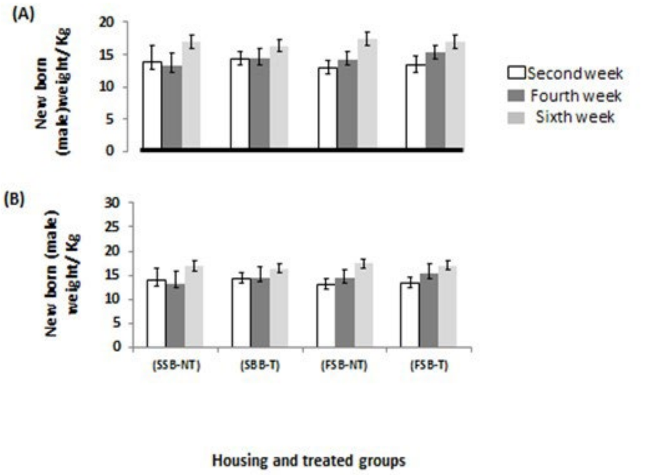

Fig. 1. The effect of housing, thyme and celery mixture on lambs' weight.
Results are expressed as means ± standard error (n=8). (A) weigh(kg)of newlyborn(male) lambs. (B) weight(kg) of newborn (female) lambs. No superscript letters on bars indicates no significant difference (P<0.05).
SSB-NT: semi-shaded non treated; SSB-T: semi-shaded treated; FSB-NT: full shaded non treated; FSB-T: full shaded non-treated.

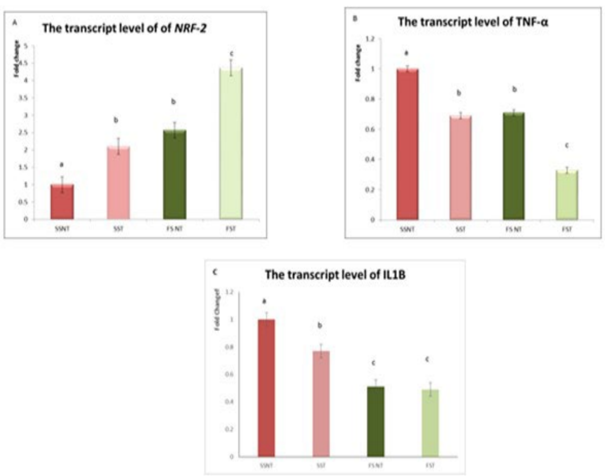

Fig. 2. Bar charts representing the transcript levels of (a) *nrf2*; (b) *tnf-α* ; (c) *iliβ* genes. Values are presented as mean ± SD, (n= 7) and different superscript letters indicated the significant difference among groups at P < 0.05.

Discussion

These results indicate the efficacy of TCM in the mitigation of climate change-driven stress in SSB during the autumn and early winter seasons. Additionally, it improved the behavior and performance of Ossimi ewes in the fully shaded house (FSB).

The observed results indicated that housing did not alter TFD while, housing 2(FSB) improved feeding bouts duration. In the early studies, Casamassima *et al.* (2001) recorded no significant changes in the feeding behavior of early lactating Comisana ewes resulting from housing conditions (indoor and outdoor daytime environment). Additionally, Baraz *et al.* (2021) observed that thyme didn't affect the time cattle spent feeding. On the other hand, Sevi *et al.* (2009), and Caroprese *et al.* (2009) stated that housing can reduce the feeding behavior of sheep. Though climate changes could alter the function of the rumen and diet digestibility in ruminants, sheep are livestock that can somewhat adapt to climate changes (Sejian *et al.*, 2017).

The observed data indicated that TCM improved FI in both housing units. Similar results were observed by Burrow and Dillon (1997); Kamruzzaman *et al.* (2011); Kumar *et al.* (2014) and Kalyan *et al.* (2015). Shaaban *et al.* (2021) also reported that TCM improved sheep FI. Furthermore, Khamisabadi *et al.* (2016) found that the use of T. vulgaris in the diet of male Sanjabi lambs improved dry matter intake. Unlike our data, Calsamiglia *et al.* (2007) found that FI decreased in cattle because of thyme palatability. on the contrary, thyme oil was reported to not affect the feed consumption of lambs (Akbarian-Tefaghi *et al.*, 2018; Yurtseven *et al.*, 2018). The reported increase in FI with TCM is potentially due to the improvement of nutrient digestibility. The improved nutrient digestibility includes a decreased transit digestion window and the minimization of the outflow rate from the rumen which then allows increased consumption (Olafadehan, 2013; Olafadehan and Okunade 2018). Moreover, the secondary metabolites of thyme and celery can stimulate saliva secretion (Frankič *et al.*, 2009) and enhance nutritional absorption (Elghandour *et al.*, 2015; Kholif *et al.*, 2015), in addition to improving the available escaping protein for digestion in the lower colon and decrease the ruminal proteolysis of dietary proteins (Olafadehan *et al.*, 2014; 2020).

The outcomes of the current study show that housing did not affect ewe weight after parturition. Data also revealed that TCM increased ewe weight during the 6th week after parturition in the FSB which concurs with Zeid and Ahmed (2004), who found that body weight was increased by including medicinal herbs (chamomile and thyme) in Zaraibidoe rations (Abdelhamid *et al.*, 2011). Early reports indicated a TCM improvement effect on the dams' weight (Shaat *et al.*, 2004; Olafadehan and Adewumi, 2009; Kumar *et al.*, 2014; Abd El Tawab *et al.*, 2020; Ebeid *et al.*, 2020; Khattab *et al.*, 2020).

The enhanced weight gains among ewes with TCM treatments are the results of the reported increases in FI, and nutrient utilization and absorption of ewes on these diets (Kumar *et al.*, 2014; Ebeid *et al.*, 2020). In the FSB, TCM improved ewe performance because this house provided adequate protection to ewes from the stress of climate change. All the ewes' changes to body weight 3 months after lambing may be attributable to compensatory growth that occurred during the experimental period. These changes and assessments agree with previous reports (Shaat *et al.*, 2004). Improvement in the nutrition of ewes post-lambing could be another reason for the weight gain.

The findings of this study revealed that housing type and TCM did not alter newborn weight (male or female) or weight through the first 3 weeks of life. These findings concur with Goddard *et al.* (1998), Kalyan *et al.* (2015), and Shaaban *et al.* (2021), who found that TCM did not enhance lamb weight. Moreover, Smeti *et al.* (2015) reported that rosemary essential oils did not affect lambs' birth weight. However, Khattab *et al.* (2020) observed that TCM improved feeding usage and weight increase of lambs, and Hassan Khamisabadi and Fazaeli (2021) observed weaning age weight gain. Furthermore, a thermocol-insulated cold-protected shed was reported to improve weight gain in lambs (Kalyan *et al.*, 2015). The difference between our data and that of previous studies could be credited to the ages of the animals used in the other studies, as well as the stage of production and form of the tested plants (e.g., essential oils, extracts, or whole plants).

Increased blood glucose concentrations associated with thyme treatment were not expected because it did not affect propionate and increased acetate production. Since more than half of the blood glucose in ruminants is synthesized from propionate in the liver (Huntington *et al.*, 2006), these results reveal that TCM did not impact glucose levels. This data is not in harmony with Shaaban *et al.* (2021) who stated that the mixture did not significantly affect glucose levels in lambs. In addition, Kalaitsidis *et al.* (2021) found that thyme did not affect glucose levels in dairy sheep. In addition, Akbarian-Tefaghi (2018) found that glucose levels decreasedin dairy calves fed on a diet containing TCM. It is noteworthy that increased glucose concentrations indicate less body fat mobilization and improved energy status of ewes (McGuffey *et al.*, 2001; Olafadehan *et al.*, 2014). More than half of ruminants' blood glucose is synthesized from liver propionates. Thus, enhanced organic matter digestibility and total VFA, and the increased FI in our study are suspected reasons for the increased serum glucose levels (Huntington *et al.*, 2006). In the current study, although FI increased, the ewes' blood glucose levels were low. This could be explained by the increased level of lactose in milk.

Factors like variations in plant parts, seasons, environmental conditions, and agronomic practices (e.g., fertilization, irrigation, cultivation methods, and harvesting methods) may cause the different results (Rożek *et al.*, 2016; Al-Asmari *et al.*, 2017).

This study showed that housing and TCM did not affect TP after the first month post-parturition. The result is in harmony with Hosten (1990) who found that serum metabolites were within the normal ranges for healthy sheep. Additionally, Shaaban *et al.* (2021) found that TCM didn't significantly affect TP in lambs while TCM increased TP levels at the second month after parturition. Boyd (1984) found that thyme and celery treatments had minimal effects on serum TP suggesting the unaffected nutritional status of ewe's parallel marginal protein catabolism and proper kidney condition.

The observed data indicated an increase in reduced GSH in ewes at the 1st and 2nd months after parturition in the FSB. This data is not agreeable with Marabaa *et al.* (2018) who reported that glutathione peroxidase was not substantially impacted by housing conditions. In this study, the increase in reduced GSH levels in the FSB may be an indicator of reduced stress on sheep as a result of the cold weather during winter. The marked increase in blood antioxidants may be due to the strong antioxidative effect that enhances the production of CAT, SOD, and GPx as essential parts of the antioxidant defense system in cells (Dhama *et al*., 2015).

The obtained data indicated that housing and TCM have altered MDA levels. This result is parallel with Eid *et al*. (2021) who found that TCM was not significantly affected MDA. On the other hand, thymol and carvacrol intake caused an increase in antioxidant enzyme activity and a decrease in MDA levels in the serum and liver of broilers, thereby reducing the risk of an inflammatory response (Hashemipour *et al*., 2013). Blood MDA is among the most utilized markers of oxidative stress and subsequent products of lipid peroxidation. The antioxidant activity of spices and herbs often originated from the phenolic compounds inherent in herbal essential oils, and the primary chemical elements in essential oils extracted from thyme, eucalyptus, and celery are thymol, citronellal (and citronellol), and phthalides, respectively (Benchaar *et al*., 2008).

Changes in animal immune function have been linked to environmental factors including ambient temperature. Stressed ewes have previously displayed a compromised cellular immune response (Caroprese *et al*., 2014). Boosting cellular immune response by protection from climate changes can reduce the negative effects of heat stress on ewe's immune status (Bouroutzika *et al*., 2020). Thyme and celery supplements can help ewes exposed to climate changes maintain their immunological response. This study investigated the influence of exposure to climate changes and thyme and celery supplementation on the regulation of Nrf-2, IL-1β, and TNF-α gene expression pathways involved in controlling the immune responses of ewes.

Interleukins regulate cytokine production and stress modulation. They have both local and systemic effects and locally they influence the cellular immune response. They also alter neuroendocrine secretion, metabolism, and behavior on a systemic level (Borghetti *et al*., 2009).

Animal adaptation to environmental stress is mediated by a complex network of genes (Mwacharo *et al*., 2017). Studies examining the regulation of gene expression are becoming increasingly important in investigating thermo-tolerance genes involved in adaptation to environmental stress (Kim *et al*., 2016).

According to current findings, oxidative stress brought on by heat stress may encourage the production of excess reactive oxygen species, which can result in oxidative injuries that induce protein and DNA/RNA damage and membrane lipid peroxidation (Mujahid *et al*., 2007). Activation of the physiological thermoregulation processes causes immunological changes in heat-stressed nursing ewes exposed to climate changes (Sevi *et al*., 2001). The current study showed that in pregnant ewes raised in stressful housing, exposure to climate changes encouraged macrophages to generate and secrete pro-inflammatory mediators such as IL-1β and TNF-α. The inflammatory response in an animal's body is mediated by these inflammatory cytokines. The growth of mature T cells, B cells, and thymocytes is aided by IL-1β. Anorexia, exhaustion, and other symptoms are brought on by high IL-1β (Zhang *et al*., 2017). This may be connected to the observation that the mRNA levels of IL-1β also increase after heat stress treatment, as was seen in mice (Xu *et al*., 2015). TNF- α, another significant pro-inflammatory cytokine, was also upregulated in the SSNT group in the current investigation. TNF-α is crucial for cell proliferation, differentiation, regulation of inflammation, and many other cellular immunological responses. These outcomes were consistent with prior research that showed significant increases in IL-1β and TNF-α in mice exposed to heat (Lee *et al*., 2015). The effects of celery and thymol supplementation on the mRNA expression of inflammatory markers, such as TNF-α and IL-1β, as well as the oxidative stress marker Nrf-2, were examined in this study. While Nrf-2 showed a considerably elevated expression in both the SST and FST groups, the mRNA expression of IL-1β and TNF-α was significantly downregulated in the FST group. According to these findings, thymol and celery supplementation reduced the oxidative damage and inflammatory response in ewes exposed to the negative effects of stressful housing.

The present results indicate that, after one month of parturition, neither housings nor TCM altered the antioxidant activity and chemical composition of the ewes' milk. However, TCM improved the chemical makeup of the milk in the FHB house during the second month. These findings were consistent with the previous results of Morsy *et al*. (2018); and Tassoul and Shaver (2009) with dairy cattle. Additionally, milk fat tended to decrease in association with the supply of thyme essential oil. Conversely, Kung *et al*. (2008); Santos *et al*. (2010); and Cannas *et al*. (2003) recorded a rise in the milk yield and fat from dairy cows given thyme essential oils. Total solids (TS), SNF, and lactose contents of milk. In agreement with the present work, Morsy *et al*. (2018) found elevated levels of milk lactose, TS, and SNF associated with thyme essential oil supply. This could be explained by its phenolic structure. Additionally, increased milk protein resulted from improved feed protein utilization with thyme and clove essential oils, and essential oils are rich sources of organic antioxidants, including phenolic compounds (Zheng and Wang, 2001).

Furthermore, TCM increased the antioxidant and inhibition factors while decreasing the free radical's content of ewe milk in both houses 2 months post-parturition. In previous studies, milk antioxidants were affected by the feeding of basal diets containing medicinal plants. Total antioxidants capacity (TAC) was positively affected by clove, anise, and thyme essential oil which resulted in more nutritious milk with higher levels of TAC than control milk. Additionally, eugenol was reported as a preventive measure for 24 different genera of bacteria, including animal and plant pathogens. Moreover, thymol has an active antiseptic property and strong antimicrobial activities. These characteristics are due to their phenolic structure and ability to disrupt cell wall membranes (Dorman and Deans, 2000; Trombetta *et al*., 2005). Mastromatteo *et al*. (2010) observed that antioxidants are found in the structure of thyme essential oil and were transferred to milk. Moreover, thyme essential oil has anti-toxigenic properties (Ultee and Smid, 2001).

Although milk composition parameters and antioxidants were increased by TCM, the weight of lambs was not significantly increased. This may be urged to lamb's body may lose some weight when they are resisting climate changes,

Further studies are required to investigate the efficacy of TCM on the mitigation of heat stress during the summer season, especially in semi-shaded or open-front buildings.

Conclusion

Sheep exhibit better feeding in FSB than SSB and the oxidative stress indicator is lower than that of sheep in the SSB. TCM enhances feeding behavior and decreases the oxidative stress indicator (reduced GSH) in the SSB. Additionally, it increases total protein level and dam body weight in the FSB animals and improves FI in both buildings. Therefore, the present data suggests that the addition of TCM to the basal diet could reduce the stress of climatic changes on lactating ewes and improve the performance and production of animals.

Conflict of interest

The authors declare that they have no conflict of interest.

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