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Efficiency Of Thyme-Celery Mixture (TCM) In Reducing Physiological Stress Of Pregnant Ewes Under Two Different Housing Systems

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Abstract

This study set out to look at the efficacy of using thyme - celery mixture (TCM) supplementation in mitigating stress and improving the performance and behavior of pregnant ewes housed under two different conditions. Forty (40) pregnant ewes were randomly distributed into four groups; two groups were housed in the semi-shaded house (SS), including one group that was provided with TCM supplemented in diet (SST), and the other received no supplementation (SSNT). Similar to the first two groups were housed in a fully shaded house (FS), including one group that was provided with the same TCM supplementation (FST) and the other that received no supplementation (FSNT). Ewe's performance including weekly feed intake was monitored during the late stage of pregnancy, stress indicators were measured in blood. Besides, feeding behavior was recorded. As a result full shaded houses succeed to protect pregnant ewes from environmental changes despite the decreased feed intake. TCM supplementation didn't achieve success in mitigating the physiological stress of pregnancy in both housing conditions but resulted in a significant increase in the feed intake of semi-shaded housed ewes.

KEYWORDS
Behaviour, Housing, Pregnant ewes, Stress, Thyme-Celery Mixture.

INTRODUCTION

Sheep plays an important role in the rural economy providing a good source of income to the shepherds with its versatility for manure, wool, milk, meat, and skins as well. All livestock houses represent a compromise between cost and animal performance, defined as productivity, well-being, and health (Webster, 1994).

Animal Houses have a critical role in improving livestock welfare, health, and productivity by protecting animals by providing enough protection from heat- or cold-stress and feeding (Berge, 1997; Brosh *et al*., 1998; Caroprese, 2008). Moreover, it may influence a person's eating, drinking, standing, and sleeping habits of an animal (Sevi *et al*., 2009; Caroprese *et al*., 2009). Shelter or shed management aids in microenvironment enrichment that positively affects animal physiology and behavior (Young, 2003). Moreover, it helps in mitigating environmental stressors so ensuring animal survival, and sustaining productivity (Nienaber and Hahn, 2007; Everett-Hincks *et al*., 2014).

Sheep are Animals that are social and non-aggressive that are more liable to fear, frustration, or anxiety from simple husbandry operations (Lynch *et al*., 1992; Fitzpatrick *et al*., 2006). Intensive sheep production systems are known to induce stress that may impact the animals' physiological and production responses (Miranda-De la Lama *et al*., 2010).

Like many other phytogenic feed additives, thyme and celery promote an increase in performance by causing positive changes in the ruminal microbiota, improving the immune response, and enhancing the morphological and histological modifications of the gastrointestinal tract and antioxidant activity (Kumar *et al*., 2014).

Behavior is considered the first line of defense of animals in response to environmental change, it could give information on animals' preferences, requirements, and internal states (Engeldal *et al*., 2013). cause of oxidative stress is a reduction in enzymatic and non-enzymatic antioxidants disrupting the normal function of biomolecules like nucleic acids, proteins, and cell membrane phospholipids (Singh *et al*., 1999) that may have a deleterious impact on physiological processes over time and growth performance (Rushen and de Passill, 1992).

Pregnancy sustainability and increased milk yield after parturition are vital goals for animal breeders that guarantee the normal and healthy growth of newborn offspring (Eid *et al*., 2021). Recently, there are growing interest in the inclusion of natural feed additives to enhance feed utilization and animal performance. Medicinal plants had a wide consideration as feed additives that improve farm animals' anti-inflammatory (Calsamiglia *et al*., 2007), antioxidant status, ameliorate physiological stress of pregnancy (Grosso *et al*., 2010), enhance ruminal fermentation, nutrient digestion, feed utilization (Salem *et al*., 2016), animal performance (Khattab *et al*., 2020) and animal health (Salem *et al*., 2017) as well, they cause of oxidative stress alternatives for growth promoters in ruminant's nutrition (Vakili *et al*., 2013).

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No attempt has been may have a deleterious impact on physiological processes over time and housing microclimate and thyme-celery mixture (TCM) on pregnant ewe performance, physiology, and behavior. So the current study aims to look into the impact of microclimatic housing conditions in Full-shaded (FS) versus semi- shaded (SS) houses as well as using TCM in reducing possible physiological stress in late pregnancy using performance, physiological and behavioral indicators.

MATERIALS AND METHODS

The present investigation was conducted in the sheep experiment unit, Sids Agriculture Research Station, Beni-Suef Governorate, belonging to Animal Production Research Institute, Ministry of Agriculture through a period from October 2021 to February 2022.

Ethical approval

The study was approved by the Institutional animal care and use committee of Beni-Suef University (BSU-IACUC), Egypt.

Experimental design

Animals' accommodation

A total number of 40 pregnant ewes with an average age of 2 years old and 48.0±1.5 kg average body weight, were used to examine the impact of thyme, celery, and their mixture (TCM) on performance, behavior, and some stress indicators at different environmental conditions.

Animals were accommodated in two types of housing: Housing 1(Semi-shaded SS); a semi-shaded, roofed with a concrete slab 40 cm thickness and 5 meters in height, and covered with three rows of rice straw bales as thermal insulation and a natural dirt land towards the north and south. Housing 2 (Fully-shaded FS); a full-shaded, roofed with a layer of tin, 5 meters in height, and a natural floor in the west and east.

Animals were randomly and equally made consisting of 4 groups (n=10) as follows: Gp1: semi-shaded non-treated (SSNT); Gp2: semi-shaded treated (SS T); Gp3: full shaded non-treated (FS NT); Gp4: full shaded treated (FS T).

All ewes were fed about 500 grams 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 with one side of the house in addition to 600-gram rice straw all period of pregnancy. Water was available all day from a common water trough. Dried Thyme leaves and celery seeds (from Turkey) were brought from HARAZ herbal store, Cairo, Egypt. Ten (10) g. of thyme and ten (10) g. of celery/head/day are used as a TCM supplementation in feed according to the dosage recommended by Eid *et al*., (2021).

Sampling

Blood samples from ewes were obtained by puncture of jugular vein one month before parturition, divided into 2 clean and dry centrifuge tubes, one of them containing sodium fluoride for plasma separation and glucose measurement, the other was used for serum separation without anticoagulant for determination of total protein "TP", malondialdehyde "MDA" and reduced hepatic glutathione "GSH". After collection, blood in the second tube was left standing for about 15 minutes till complete clotting then cooled at 4 ºC for 4-6 hours in a refrigerator, after that centrifuged at 3000 rpm for 10-15 minutes for separation of serum separation after which they were pipetted and kept in clean labeled Eppendorf tubes and frozen at (-20 ºC) in the deep freezer till assayed.

Analysis and assay

Microclimatic conditions in each housing

The temperature and humidity levels were measured in each housing using a digital thermo-hygrometer. The average temperature and relative humidity in semi-shaded housing were 27.1±2.7 ºC and 67.7±4.8%, respectively. However, in fully-shaded housing, they were 27.9±2.2 ºC and 60.7±2.1%, respectively.

Behavioral observations

Feeding behavior was videotaped every two weeks for 30 minutes during late pregnancy. The scan method of observation was adopted in this study according to the method described by Giger-Reverdin *et al.* (2012).

Animals were allowed to eat then the total feeding duration for each animal was also recorded in minutes and expressed as TFD.

The time between each feeding for each animal was recorded in minutes and expressed as feeding bout duration (FBD) then the animals were classified according to the duration of FB and expressed as a percentage.

Calculation of feed intake

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

Feed intake "FI" (g) = feed allowed (g) - feed remained (g)

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

Determination of biochemical blood parameters

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

Statistical analysis

Data were presented as mean ± standard error of mean and analyzed by a one-way ANOVA test using SPSS (SPSS, 2011). Duncan was used to determining significance. Probability values less than 0.05 ($P<0.05$) were considered significant. Differences among Feeding Bout Duration (FBD) % were analyzed using the procedure for nonparametric models using Kruskal–Wallis one-way analysis of variance.

RESULTS

Feeding behavior and feed intake

As revealed in Table 1, neither the housing nor the TCM induced a significant effect on the total feeding duration (TFD) or

the feeding bout duration (FBD) of pregnant sheep.

Speaking on how housing type affects TCM on feed intake, it was noticed from results in Fig. 1, that the average weekly FI of pregnant sheep housed in FS house was significantly increased (P<0.01). Additionally, TCM supplementation significantly (P<0.01) increased the FI of SS-housed sheep.

Results in Table 2, demonstrated that FBD1, FBD2, FBD3, FBD4, and TFD are positively correlated with each other. On the contrary, FI is adversely associated with FBD1, FBD2, FBD3, FBD4, and TFD.

Biochemical parameters

As noticed in Table 3, SS house significantly (P<0.05) decreased GSH. Moreover, TCM supplementation was found to cause a significant (P<0.05) decrease in TP and an increase (P<0.01) in MDA in SS house. As well, levels of blood glucose and GSH are significantly lowered (P<0.01) with TCM-supplemented FS-housed sheep.

Table 1. The effect of thyme and celery mixture (TCM) on feeding behavior of pregnant ewes under different housing conditions.

Feeding behavior	Housing type	Semi-shaded (SS) house		Full-shaded (FS) house		Significance		
		SS-NT	SST	FSNT	FS-T	Housing effect	Treatment effect (SS house)	Treatment effect (FS house)
TFD (min)		28.60±0.64	30.68±0.86	28.79±0.75	25.95±2.15	NS	NS	NS
FBD (% of animals / FBD in min)	Less than 7.5 min	74.4	65.72	72.81	71.5	NS	NS	NS
	7.5-15 min.	61.51	75.07	67.67	47.77	NS	NS	NS
	16-22.5 min.	40.24	68.14	56.26	37.04	NS	NS	NS
	Over 22.5 min.	43.41	52.88	49.99	33.29	NS	NS	NS

Data are expressed as mean±standard error;
NS indicates non-significance; SSNT: Semi-shaded non-treated; SST: Semi-shaded treated; FSNT: Full shaded treated; FST: Full shaded non treated; TFD: Total feeding duration; FBD: Feeding bout duration; min: minutes

Table 2. Correlation between feeding behavior and feeding intake

		FBD 1	FBD 2	FBD 3	FBD 4	TFD	FI
FBD 1	Pearson Correlation	1	0.586*	0.424	0.479	0.025	-0.502-
	Sig. (2-tailed)		0.028	0.131	0.083	0.933	0.498
	N	14	14	14	14	14	4
FBD 2	Pearson Correlation	0.586*	1	0.901**	0.883**	0.287	-0.912-
	Sig. (2-tailed)	0.028		0	0	0.32	0.088
	N	14	14	14	14	14	4
FBD 3	Pearson Correlation	0.424	0.901**	1	0.881**	0.316	-0.938-
	Sig. (2-tailed)	0.131	0		0	0.272	0.062
	N	14	14	14	14	14	4
FBD 4	Pearson Correlation	0.479	0.883**	0.881**	1	0.447	-0.945-
	Sig. (2-tailed)	0.083	0	0		0.109	0.055
	N	14	14	14	14	14	4
TFD	Pearson Correlation	0.025	0.287	0.316	0.447	1	-0.668-
	Sig. (2-tailed)	0.933	0.32	0.272	0.109		0.332
	N	14	14	14	14	14	4
FI	Pearson Correlation	-0.502-	-0.912-	-0.938-	-0.945-	-0.668-	1
	Sig. (2-tailed)	0.498	0.088	0.062	0.055	0.332	
	N	4	4	4	4	4	4

**: The 0.01 level of significance for correlation (2-tailed); *:The 0.05 level of significance for correlation (2-tailed); FBD: Feeding bout duration; FBD 1: Less than 7.5 min.; FBD 2: 7.5-15 min.; FBD 3: 16-22.5 min.; FBD 4: Over 22.5 min.; TFD: Total feeding duration (minutes); FI: Feed intake (kg)

Table 3. The effect of thyme and celery mixture (TCM) on blood parameters of pregnant ewes under different housing conditions.

	Semi-shaded (SS) house		Full-shaded (FS) house		Significance		
	SS-NT	SST	FSNT	FS-T	Housing effect	Treatment effect (SS house)	Treatment effect (FS house)
Glucose (mg/dl)	53.14±3.20^{ab}	59.29±1.85^{a}	58.79±1.90^{a}	47.86±2.57^{b}	NS	NS	P <0.05
Total protein (g/dl)	6.95±0.74^{b}	9.86±0.67^{a}	6.94±0.71^{b}	6.48±0.85^{b}	NS	P <0.05	NS
MDA (µmol/m)	0.18±0.02^{b}	0.33±0.01^{a}	0.18±0.03^{b}	0.17±0.01^{b}	NS	P<0.01	NS
GSH (µmol/ml)	0.13±0.03^{b}	0.11±0.01^{b}	0.21±0.04^{a}	0.08±0.01^{b}	P<0.05	NS	P<0.01

Data are expressed as mean±standard error.
MDA:Malondialdehyde; GSH: Reduced glutathione; SSNT: Semi-shaded non treated; SST:Semi-shaded treated; FSNT:Full shaded treated; FST: Full shaded non-treated.
Values followed by different superscript lettes (a, b) within a raw show significant changes between means.

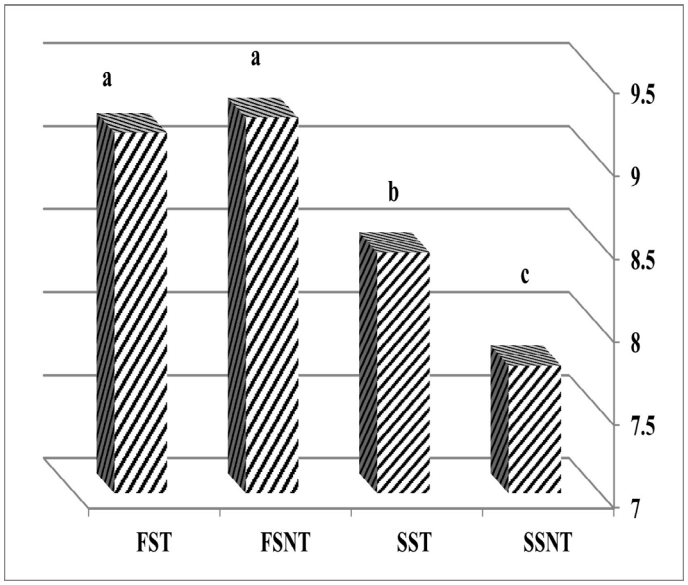

Fig 1. The effect of thyme and celery mixture (TCM) on the feed intake (FI) of pregnant ewes under different housing conditions.
SSNT: Semi-shaded non-treated; SST: Semi-shaded treated; FSNT: Full shaded treated; FST: Full shaded non treated
Different superscript litters (a, b, c) within a raw show significant variations between means.

DISCUSSION

Housing is considered an important barrier that aids in protecting the animal against adverse environmental conditions as well it helps in improving welfare, performance, and behavior. Moreover, the use of organic feed additives in ruminant feed became a way to improve performance and minimize stress.

The obtained results indicated that neither the housing nor the TCM induce a significant effect on the total feeding duration (TFD) or the feeding bout duration (FBD) of pregnant sheep. Casamassima *et al*. (2001) Recorded no significant alteration in feeding behavior due to housing conditions that agreed with the results in the current study. As opposed to that, Sevi *et al*. (2009) and Caroprese *et al*. (2009) stated that Housing can impact an animal's feeding habits of sheep. Baraz *et al*. (2021) Noticed that thyme didn't affect eating duration in cattle. The lack of significant difference in the microclimate between the two houses may explain the lack of significant difference in the feeding behavior.

The findings of this investigation showed that the increase in the average weekly FI of pregnant sheep housed in the FS house didn't meet with results obtained by Bøe *et al*. (1991), who found no significant differences in roughage intake due to housing conditions. Additionally, Van *et al*. (2007) and Villeneuve *et al*. (2009) reported that the type of housing does not affect performance. In the contrast, calf housing that is naturally ventilated during winter has been shown to have a positive effect on young calf development and feed consumption as previously declared by Razzaque *et al*. (2009).

The increased FI of SS-housed sheep was similar to the noticed by Khattab *et al*. (2020) who declared that incorporating thyme–celery treatment improved performance, nutrient digestibility, and feed intake (Shaaban *et al*., 2021) in lambs. Moreover, it resulted in heavier ewes as previously announced by Ebeid *et al*. (2020) and Khattab *et al*. (2020). As opposed to that, it was clear that TCM didn't alter the FI of pregnant ewes housed in the fully shaded house on FI of pregnant sheep. Akbarian-Tefaghi *et al*. (2018) and Baraz *et al*. (2021) reported that dry matter intake wasn't affected by the treatment in calves or cattle respectively.

The observed data declared that the decreased GSH in SS-housed ewes disagreed with Maraba *et al*. (2018) who reported that glutathione peroxidase was not significantly affected by housing conditions. The decreased GSH level may be an indicator of stress that the sheep were subjected to due to the lack of insulation from inclement weather during the study.

Additionally, the decreased GSH level in FS-housed sheep in the current research were more or less similar to results obtained by Kolarovic *et al*. (2009) who reported GSH levels with Celery supplementation. On the other hand, this is not following the results obtained by Eid *et al*. (2021) who declared that Celery and Thyme increased GSH during late pregnancy. Additionally, El-Zaher *et al*. (2021) noticed an increase in serum GSH in oestrus ewes supplemented with thyme and/or celery.

This study illustrated that The reduced serum TP in SS-housed ewes was previously recorded by Abeer *et al*. (2019) and Kalaitsidis *et al*. (2021) with thyme supplementation in the feed. On the contrary, Shaaban *et al*. (2021) found that TC M didn't significantly affect TP in lambs. The altered TP levels are potentially due to altered liver metabolism by the physiological stress of late pregnancy.

The obtained data indicated that The increased MDA levels didn't run with results published by Eid *et al*. (2021) who declared that TCM non-significantly affects MDA. El-Zaher *et al*. (2021) declared that administration of thyme and/or celery throughout ewes' estrous cycle enhanced immune response via decrement of serum MDA.

The findings of this investigation showed that decreased glucose levels in the current study with TCM are in harmony with results obtained by Akbarian-Tefaghi *et al*. (2018). Shaaban *et al*. (2021) stated that the Mixture didn't significantly affect glucose levels in lambs. Moreover, Kalaitsidis *et al*. (2021) find that thyme did not affect glucose levels. The lowered glucose levels revealed the negative impact (Casamassima *et al*., 2001) of TCM on pregnant sheep. Glucose is a significant molecule in an animal's metabolism. It is a crucial energy source for the maintenance of many tissues stress is accompanied by high insulin levels resulting in lower glucose levels (Abbas *et al*., 2020). A positive relationship exists relating blood sugar levels to propionate production as more than half of the blood glucose in ruminants is synthesized from propionate in the liver (Huntington *et al*., 2006). The greater molar ratio of acetate-to-propionate may be the reason for the lower blood glucose concentration in calves fed thyme (Akbarian-Tefaghi *et al*., 2018).

Factors like variations in parts of plants, seasons, conditions of the environment, and agronomic practices (fertilization, irrigation, cultivation method, and harvesting method) may account for the disparity in results (Rożek *et al*., 2016; Al-Asmari *et al*., 2017).

CONCLUSION

The current study revealed that full-shaded houses succeed to protect pregnant ewes from environmental changes that are indicated by a lack of negative effects on blood parameters. Moreover, it resulted in improved performance through a significant increase in feed intake. Unfortunately, TCM increased physiological stress of late pregnancy at both houses but resulted in an increased feed intake in SS-housed ewes without affecting feeding behavior. Further study is required to learn more about the part of TCM on the behavior and performance of post-parturient ewes and their lambs under different housing conditions.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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