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Research Article
Impact of Incorporating *Thymus vulgaris* as Leaves or Essential Oil in Damascus Goats Ration on Lactation Performance

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Abstract
Background and Objective: Medicinal plants and their Essential Oils (EO) are considered safe alternatives to antibiotics in animal feeding. So, this study aimed to evaluate the effect of thyme leaves or EO on the lactation performance of Damascus goats. **Materials and Methods:** Thirty-six multiparous lactating Damascus goats (4 years old, an average weight 35 kg) were divided into three groups (12 animals each) after a week of parturition. Animals in the control group (C) were fed Egyptian clover (*Trifolium alexandrinum*) plus concentrate feed mixture without additives at the ratio of 40:60, while the other experimental animals were fed control ration plus 1% thyme leaves of total DM (G1) or the equivalent as thyme essential oil (G2). **Results:** Data indicated that thyme addition in form of leaves or Essential Oil (EO) significantly increased the digestibility of DM, OM and CP, decreased the digestibility of CF and fibre fractions and improved TDN and DCP values. No significant effect of sampling time or thyme supplementation was observed on rumen fermentation parameters. The highest milk and FCM yields and the lowest blood concentrations of cholesterol and triglycerides were observed with thyme addition especially leaves form (G1). Moreover, decreases in total saturated fatty acids and increases in total unsaturated fatty acids concentrations in milk were observed with thyme addition. **Conclusion:** It could be concluded that the supplementation of lactating goat's ration especially with thyme leaves improved the digestibility, kids growth rate and produced the highest milk yield with more healthy aspects for consumers.

Key words: Thyme, essential oils, lactating goats, fatty acid profile, kids performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently the high concern of the eco-friendly and healthy means used in the improvement of animal production resulted in banning the use of antibiotics in animal feeding in 2006 by the European Union[1] due to their potential residues in milk and meat, meanwhile, their effects consequently on human health. So, there has been an increasing interest to search for natural alternatives for growth promoters and feeding antibiotics in ruminant nutrition such as medicinal plants and their essential oils[2-4]. Essential Oils (EO) are a mixture of volatile aromatic secondary metabolites that are extracted from plants by steam and/or water distillation[5]. Santos *et al.*[6] recorded an increase in milk fat content with feeding EO mixture to dairy cows. However, the inclusion of EO increased milk production decreased somatic cell count and did not affect the milk composition of dairy ewes[7].

Thyme is planted worldwide for many uses such as culinary, medical and cosmetic perennial purposes. Thyme is considered as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant[8]. Thymol, a phenolic monoterpene [(5-methyl-2-(1-methyl ethyl) phenol)] is one of the major compounds of thyme (*Thymus vulgaris*) and organo (*Origanum vulgaris*) essential oils[9]. The strong antimicrobial activity of thymol against a wide range of gram-positive and negative bacteria[10] is attributed to its ability in plasma membrane disruption of bacteria and the reduction in glucose uptake[5,11]. Salamataza *et al.*[12] suggested that thyme methanolic extract can affect fermentation efficiency in the rumen and could be used as a mitigating agent of methane. Ebrahimi *et al.*[13] found that the addition of thyme (*Thymus vulgaris*) oil to the diet of Brown Swiss Neonatal calves improved nutrient digestibility of DM and OM, with no significant effect on feed intake and feed conversion ratio. Also, no significant effects on DMI and feed efficiency with thyme oil supplementation were observed with feedlot Holstein calves[14] or with sheep[15]. While El-Naggar *et al.*[16] indicated an improvement in digestibility and feed efficiency of growing lambs with thyme oil supplementation. In contrast, there were no significant effects of thyme leaves addition at 3% of DM on nutrient digestibility of lambs[17]. Boutoial *et al.*[18] reported that using thyme leaves in rations of dairy goats significantly increased the milk contents of fat, protein and PUFA. In the same trend, El-Essawy *et al.*[19] recorded increases in milk TS, the total antioxidant capacity and concentration of USFA profile in the milk of Barki ewes fed the diet supplemented with thyme EO.

The objectives of this study were to investigate the effects of thyme addition as leaves or essential oil on digestibility, nutritive value, milk production and its composition, growth performance of kids, ruminal fermentation characteristics and blood metabolites of Damascus lactating goats.

MATERIALS AND METHODS

Study area: The field trial of the current study was carried out at Sheep and Goats Unit, Agriculture Experimental and Research Station, Faculty of Agriculture, Cairo University through the period from January to Mach, 2019. While the lab analysis extended to August 2020.

Ethical approval: The protocol of the present study was accepted by the Institutional Animal Care and Use Committee, Cairo University (IACUC) (Approval No. CU/II/F/22/21).

Thyme leaves and essential oils: Thyme (*Thymus vulgaris*) leaves were obtained from Beni Mazar districts, Minia Governorate, Egypt. The fresh leaves were dried at shadow till drying, then it was saved in polyethylene bags. A hydro distillation method by Clevenger type apparatus was used to extract the essential oil from fresh thyme leaves as described by Vakili *et al.*[14]. Then the collected oil was dehydrated by using anhydrous sodium sulfate. Oil percentage was calculated (1.65%) and stored in the fridge until using. The bioactive components in the thyme oil were determined by Gas Chromatography-Mass Spectrometry (GC-MS) instrument (a trace GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ™EC Single Quadrupole Mass Spectrometer) at the Department of Medicinal and Aromatic Plants Research, National Research Center.

Thyme in the current study contained 42.70% Thymol, 2.93% Carvacrol, 17.41% p-Cymene, 15.62% γ-Terpinene, 4.25% α-Terpinene, 1.65% Myrcene, 2.01% Linalool, 3.30% α-Pinene, 1.91% β-Pinene, 3.72% β-Caryophyllene and 4.50% Limonene.

Experimental animals and rations: Thirty-six multiparous lactating Damascus goats, 4 years old and an average weight 35 kg, after 7 days of parturition were divided into three groups (12 animals each) according to the milk production then, the experimental groups were randomly assigned to receive one of the experimental rations. The experiment lasted for 80 days (30 days adaptation period, milk yield was recorded 4 times with 2 weeks interval, then followed by 4 days for digestion trial and collection of rumen liquor and blood samples). Kids were separated from their mothers and changes in body weight of mothers and kids were determined biweekly.

Table 1: Chemical composition of the concentrate feed mixture, clover hay and experimental ration

Items	Feedstuffs		*Experimental ration
	CH	CFM	
DM	91.75	90.97	91.28
Chemical composition (%) (DM basis)			
OM	88.47	94.83	92.29
Ash	11.53	5.17	7.71
CP	13.62	14.57	14.19
EE	1.49	2.58	2.14
CF	27.37	5.98	14.54
NFE	45.99	71.70	61.42
Fibre fraction (%)			
NDF	43.83	29.87	35.45
ADF	31.99	11.23	19.53
ADL	5.53	2.99	4.01
Cellulose	26.46	8.24	15.53
Hemi-cellulose	11.84	18.64	15.92

CH: Clover hay, CFM: Concentrate feed mixture and *Calculated

Animals in the control group (C) were fed Egyptian clover (*Trifolium alexandrinum*) plus Concentrate Feed Mixture (CFM) without additives, while the other experimental animals were fed control ration plus 1% thyme leaves of total DM (G1) or the equivalent as thyme essential oil (G2). The equivalent amount of thyme essential oil was based on oil% in thyme which was 1.65%. All animal groups were offered rations at 3% of LBW (60% CFM: 40% Egyptian clover) to cover the total requirements recommended by NRC[20]. Both thyme leaves (G1) and EO (G2) were mixed with a small amount of CFM daily just before feeding to ensure that animals consumed all amounts. The concentrate feed mixture consists of 68% yellow corn, 13% wheat bran, 17% soybean meal, 0.5% common salt and 1.5% limestone. The chemical compositions of the experimental ratio are shown in Table 1.

Feeding procedures: The concentrate feed mixtures and Egyptian clover were divided into two equal portions fed twice daily at 0800 and 1600 hrs. Freshwater was always available. Minerals blocks were available for all animals.

Milk sampling: Milk yield was recorded and milk samples were collected four times during the collection period for all animals by hand milking twice daily at 7 am and 5 pm. Whereas, one-tenth of the morning and the evening milk yields were mixed for each animal and stored at (-18°C) for further analysis.

Digestion trial and faeces sampling: After the collection period of milk, the digestion trial was performed using six animals from each group for three successive days using the bag technique to determine nutrient digestibility via the Acid Insoluble Ash (AIA) method as described by Lee and Hristov[21]. Nutrient's digestibility was calculated according to the following formula:

$$\text{Digestion coefficient} = 100\text{-}\left[100 \times \frac{\text{Indicator in feed (\%)}}{\text{Indicator in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}}\right]$$

A subsample (10%) of total collected faeces was sprayed with 10% sulfuric acid, then the drying process was done at 70°C for 24 hrs. The dried faeces were ground and stored for chemical analysis.

Rumen liquor sampling: Rumen liquor samples were collected at 0, 3 and 6 hrs. post-feeding by stomach tube from the six animals at the end of the faeces collection period. Samples were strained via two layers of cheesecloth, the ruminal liquor pH was determined immediately using a digital pH meter. Rumen fluid samples, after adding ortho-phosphoric acid, were stored using glass bottles at deep freeze (-18°C), then used for the analysis of Total Volatile Fatty Acids (TVFA's) and Ammonia Nitrogen (NH_3-N).

Blood sampling: Blood samples were taken from the jugular vein from the six animals on the last day of the experimental period before the morning feeding in tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant. Blood plasma was obtained by centrifugation of the blood samples at 4000 rpm for 20 min and kept at deep freeze (-18°C) for further analysis.

Analytical procedures

Feeds and faeces analysis: Chemical analysis of feedstuffs and faeces samples was carried out according to AOAC[22]. The Nitrogen Free Extract (NFE) was calculated by difference. Fibre fractions were determined in feeds and faeces according to Van Soest *et al.*[23].

Rumen liquor analysis: Values of rumen pH were determined using Hanna digital pH meter. The concentration of Ammonia-Nitrogen (NH_3-N) in the rumen liquor was determined by the Kjeldahl distillation method[22]. Rumen total volatile fatty acids were estimated by steam distillation method as recommended by Wang *et al.*[24].

Blood plasma analysis: Blood plasma total protein and creatinine were measured as mentioned by Tietz[25]. Blood plasma albumin was determined by the method of Doumas *et al.*[26]. Blood plasma urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglycerides, total cholesterol and glucose were estimated according to Reed[27].

Milk analysis: Total solids, fat, protein and lactose of milk samples were analyzed by infrared spectrophotometer (Foss Matic 120 Milko-Scan, Foss Q3 183 Electric, Hillerød, Denmark) as described by AOAC[22]. Solids not fat content of milk was calculated by the difference between total solids and fat content. Milk fatty acids profile was determined via High-Pressure Liquid Chromatography (HPLC) according to AOAC[28].

Statistical analysis: The general linear model procedure of SAS[29] was used for data analysis. Data of nutrients digestibility, blood and milk were analyzed using one way ANOVA procedure by following the next model:

$$Y_{ij} = \mu + R_{ij} + E_{ij}$$

Where:

μ = Overall mean of Y_{ij}
R_{ij} = Treatment effect
E_{ij} = Experimental error

While two-way ANOVA procedure was used to analyse the data of pH, NH3-N and TVFA according to the following model:

$$Y_{ij} = \mu + L_i + T_j + [L \times T]_{ij} + E_{ij}$$

Where:

Y_{ij} = Experimental observation
μ = General mean
L_j = Effect of time
T_j = Effect of experimental rations
$[L \times T]$ = Interaction effect due to time and experimental rations

The differences among means were separated according to Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Nutrient's digestibility and nutritive value: Data concerns nutrients digestibility and nutritive value in Table 2 indicated significant increases ($p<0.05$) in digestibility of DM (73.09 and 72.14%), OM (76.10 and 74.62%) and CP (72.59 and 73.39%) with G1 and G2, respectively compared with C (70.48, 73.75 and 70.69%), in the same order. Mirzaei-Aghsaghali *et al.*[8] explained the digestion improvement with thyme EO addition by the positive effect of herbs and spices and their active compounds on activation of ruminal bacteria that are involved in the digestion process or by stimulation the secretion of bile salt, saliva or other digestive enzymes[19]. Also, this improvement could be due to the anti-microbial properties of the main active components identified in the thyme EO such as thymol, carvacrol, pinene, p-cymene and limonene, which inhibit some microbial activity and stimulate the digestion process[30,31].

Table 2: Effect of thyme leaves or essential oil addition on nutrients digestibility and nutritive value

	Experimental groups				
Items	C	G1	G2	±SE	p-value
Apparent digestibility (%)					
DM	70.48[b]	73.09[a]	72.14[ab]	0.48	0.026
OM	73.75[b]	76.10[a]	74.62[ab]	0.75	0.037
CP	70.69[b]	72.59[a]	73.39[a]	0.47	0.018
CF	65.89[a]	63.23[b]	61.56[c]	0.67	0.002
EE	72.76	72.48	71.22	0.38	0.227
NFE	76.35[b]	80.09[a]	78.11[b]	0.89	0.01
Fiber fractions					
NDF	62.43[a]	60.16[b]	59.48[b]	0.53	0.027
ADF	63.01	62.57	61.12	0.41	0.136
Nutritive values (%)					
TDN	70.01[b]	72.18[a]	70.77[ab]	0.71	0.099
DCP	10.03	10.3	10.41	0.29	0.891

Different alphabetic letters means in the same row with various superscripts are significantly different ($p<0.05$), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively, TDN: Total digestible nutrients and DCP: Digestible crude protein

In the same trend, El-Naggar *et al.*[16] and Ebrahimi *et al.*[13] reported increases in DM and OM digestibility when thyme EO was added to lambs or calves' rations, respectively. The same result was obtained by Mehrabadi *et al.*[32] with *in vitro* DM and OM degradability when thyme leaves were added by 5, 10 and 15% of concentrate DM. However, in other studies, neither addition of thyme leaves[33] nor thyme EO[15,19] had a significant effect on DM and OM digestibility. In the same context, no significant effect of thyme EO was detected on *in vitro* DM[34] or OM degradability[35]. The variations in the digestibility results with EO addition could be attributed to many factors such as dose, chemical composition, source of plant, extraction procedures of EO[14].

The improvement in CP digestibility may be due to the anti-microbial effect of some phenolic compounds in EO such as thymol, which can modulate the microbial population in the rumen, which reduce the degradation of dietary protein, increases N escape and increase the protein flow to the lower gut, which result in improvement of nitrogen utilization[33,36].

In the same trend, significant[16,33] or insignificant increases[19] were recorded in CP digestibility either with thyme leaves or EO addition.

In contrast, a significant reduction in CF digestibility was observed with thyme addition especially with EO being 61.56% compared with control being 65.89%. The same result was obtained with NDF digestibility, with no significant effect on ADF digestibility because of thyme addition. The NDF digestibility was reduced by 3.6 and 4.7% with G1 and G2, respectively. The decrease in CF and fibre fraction digestibility may be attributed to the sensitivity of fibrolytic bacteria to phenolic compounds as thymol found in thyme EO[34]. Also, Cieslak *et al.*[37] indicated that, thymol may inhibit the growth of certain microorganisms in the rumen, which affects proteolysis and reduce fibre degradation.

In agreement with the previous results, Benchaar *et al.*[34] noted a significant decrease *in vitro* disappearance of NDF with thyme EO supply. The same results in CF and NDF digestibility were conducted by El-Essawy *et al.*[19] when ewes' ration supplemented with thyme EO, with no significant effect on ADF digestibility. On the other hand, increases in digestibility of CF[16], NDF and ADF[13] were reported with thyme EO supply. While Khattab *et al.*[38] observed no significant effect of thyme leaves addition on *in vitro* disappearance of NDF and ADF.

The highest TDN value (72.18%) was recorded with thyme leaves addition (G1), with no significant difference between G1 and G2 (70.77%). This improvement may be due to the increase in DM and OM digestibility with G1 and G2. Although DCP value was not significantly affected by thyme addition, there was an improvement especially with EO supply because of digestibility improvement of CP. The DCP values were 10.03, 10.30 and 10.41% for C, G1 and G2, respectively.

In agreement with our findings, TDN and DCP values were improved either with thyme leaves[33] or EO addition[16,19].

Rumen liquor parameters: Table 3 indicated no significant ($p<0.05$) effect of sampling time or thyme supplementation either in form of leaves or essential oil on rumen fermentation parameters. The lack of significant effect of essential oils on fermentation characteristics may be explained by the results of McIntosh *et al.*[39] that the active EO dose that can affect ruminal bacteria ranges from 35-360 mg L^{-1}, which cannot be achieved by *in vivo* trial. Also, Cardozo *et al.*[40] and Busquet *et al.*[41] reported many factors that may eliminate the effect of EO on rumen parameters such as inadequate dose, concentrate to roughage ratio and adaption time, as longer adaption time will result in more adaption of rumen microorganisms to EO.

The mean values of ruminal pH ranged from 6.25 for C and G1 to 6.28 for G2. Matching with our results, neither thyme leaves addition[17,36,38], nor essential oil[14,16,19] had a significant effect on ruminal pH value. However, Benchaar *et al.*[34] recorded a significant increase *in vitro* pH value with thyme EO supply.

Current data showed insignificant decreases in NH3-N concentrations either at 3 (26.73 and 26.12 mg/100 mL) and 6 hrs (24.65 and 24.04 mg/100 mL) post-feeding or the mean value (24.96 and 24.63 mg/100 mL) with thyme addition (G1 and G2), respectively compared with control (29.66, 25.28 and 26.17, in the same order). Molero *et al.*[42] indicated that, the active components in EO including thymol may reduce proteolysis and degradation of amino acids in the rumen because of reduction either inactivity of ammonia producing bacteria or preventing their growth[39]. The major mode of action of these active components in herbal plants was suggested by their reduction effect on adhesion sites of ruminal bacteria to feed particles, which reduce the deamination process and NH3 production[5,36]. In the same context, Evans and Martin[43] noted that thymol may affect the energy metabolism of *Selenomonas ruminantium* and *Streptococcus bovis*, the major deamination bacteria in the rumen.

Table 3: Effect of thyme leaves or essential oil addition on rumen parameters of lactating goats

Items	Sampling time (hrs)	Experimental groups			±SE	p-value
		C	G1	G2		
pH	0	6.73	6.60	6.68	0.15	0.953
	3	5.68	5.37	5.48	0.25	0.903
	6	6.35	6.77	6.69	0.11	0.267
	Mean	6.25	6.25	6.28	0.19	0.997
NH_3-N, mg/100 ml rumen liquor	0	23.58	23.50	23.72	0.41	0.982
	3	29.66	26.73	26.12	0.77	0.127
	6	25.28	24.65	24.04	0.45	0.601
	Mean	26.17	24.96	24.63	0.67	0.675
TVFA, mleq/100 ml rumen liquor	0	9.19	9.04	8.97	0.41	0.981
	3	12.28	10.89	10.54	0.63	0.561
	6	8.10	7.19	7.67	0.51	0.813
	Mean	9.86	9.04	9.06	0.55	0.83

[a,b,c]Means in the same row with various superscripts are significantly different ($p<0.05$), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively

Patra and Yu[44] showed that reducing the deamination of amino acids in the rumen may alter the digestion site of protein to the intestine. In the same line, Bodas *et al*.[45] reported that the decreases *in vitro* CP disappearance and NH3-N concentration may lead to an increase in the passage of dietary protein to the intestine and improve the nitrogen utilization by ruminants.

In the same trend, significant[16,17,35,46] and insignificant[19,32,38] decreases in the ruminal concentration of NH_3-N were reported with thyme addition. However, Benchaar *et al*.[34], Vakili *et al*.[14], Ahmed *et al*.[47] and Ribeiro *et al*.[15] recorded no significant effect.

Although the ruminal concentration of Total Volatile Fatty Acids (TVFA's) was not affected by thyme supplementation, there were insignificant decreases with G1 and G2 being 9.04 and 9.06 mL eq/100 mL compared with control being 9.68 mL eq/100 mL. Castillejos *et al*.[48] suggested that, the reduction in TVFA' s concentration with EO supply may be due to the anti-microbial effect of thymol, which inhibits rumen microorganisms and fermentation process. This anti-microbial activity of thymol may be attributed to the hydroxyl group in its structure, which reduce the membrane integrity of bacterial cells and reduce glucose uptake[49-51].

Also, a reduction *in vitro* TVFA' s concentration was observed when thyme EO was added by 30, 300 and 600 ppm[35] or 100 mL L^{-1} [46]. On the other hand, TVFA's concentrations were not significantly changed with thyme leaves or EO supply in many studies[14,19,32,34].

Blood parameters: However, Table 4 showed no significant differences among groups in blood concentrations of Total Protein (TP), Albumin (ALB) and Globulin (GLO), there were enhancement in TP (7.35 and 7.39 g dL^{-1}) and ALB (4.27 and 4.36 g dL^{-1}) values with thyme addition (G1 and G2), respectively compared to 6.93 and 3.81 g dL^{-1} in control. This result may be attributed to the improvement in CP digestibility with thyme groups.

In the same trend, no significant effect was detected in blood TP, ALB with the addition of either thyme EO[19] or thyme leaves[17]. Also, Ebrahimi *et al*.[13] did not find a significant difference in serum TP among dairy calves supplemented either with 1- or 2-ml thyme oil and control group. In contrast, Soltan[52] recorded increases in blood TP concentration with EO supplementation at a level of 187 or 281 mg/day.

In the current study, there were decreases (p<0.05) in concentrations of Blood Urea Nitrogen (BUN) with thyme leaves (G1) being 22.36 mg dL^{-1} or EO (G2) supply being 21.09 mg dL^{-1} compared with control being 24.25 mg dL^{-1}. The high correlation between BUN concentration and NH3-N concentration in the rumen[53,54], may explain the reduction in BUN concentration in the current study by the inhibition effect of EO on ruminal bacteria that is responsible for ammonia production such as *Peptostreptococcus anaerobius* and *Clostridium astyklandy*[11].

This reduction in BUN concentration is in accordance with results obtained by El-Essawy *et al*.[19], who recorded insignificant decreases in BUN concentration with the addition of thyme EO to ewes' ration. On the other hand, Vakili *et al*.[14], Biricik *et al*.[55] and Khamisabadi *et al*.[17] found no significant effect. While Seirafy and Sobhanirad[56] reported a significant increase in BUN concentration when 5 mL/day thyme oil was added to Holstein suckling calves.

The present data indicated that blood creatinine and glucose concentrations were not affected by thyme addition. Also, Vakili *et al*.[14] and Khamisabadi *et al*.[17] agree with the previous result. However, a significant increase in glucose concentration was observed by Ebrahimi *et al*.[13] with thyme EO supply.

Table 4: Effect of thyme leaves or essential oil addition on blood parameters of lactating goats

	Experimental groups				
Items	C	G1	G2	±SE	p-value
Total proteins (g dL^{-1})	6.93	7.35	7.39	0.10	0.119
Albumin (g dL^{-1})	3.81	4.27	4.36	0.12	0.13
Globulin (g dL^{-1})	3.12	3.08	3.03	0.02	0.131
Urea-N (mg dL^{-1})	24.25[a]	22.36[ab]	21.09[b]	0.96	0.106
Creatinine (mg dL^{-1})	0.82	0.89	0.91	0.03	0.492
AST (IU L^{-1})	86.18[b]	87.05[ab]	88.24[a]	0.39	0.065
ALT (IU L^{-1})	12.41[b]	13.15[ab]	13.73[a]	0.29	0.018
Glucose (mg dL^{-1})	52.19	53.54	52.87	0.34	0.305
Total cholesterol (mg dL^{-1})	105.72[a]	92.17[c]	94.51[b]	2.09	0.000
Total triglycerides (mg dL^{-1})	19.08[a]	15.14[c]	15.65[b]	0.24	0.000

Different alphabetic letters means in the same row with various superscripts are significantly different (p<0.05), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively, ALT: Alanine aminotransferase and AST: Aspartate aminotransferase

Current results revealed that the highest ($p<0.05$) concentrations of AST and ALT were recoded with thyme EO addition (G2) being 88.24 and13.73 IU L^{-1}, with no significant difference between G1 (87.05 and 13.15 IU L^{-1}) and G2 compared with control (86.18 and 12.41 IU L^{-1}), in the same order.

In the same context, Seirafy and Sobhanirad[56] noted increases in AST and ALT concentrations with thyme EO supply. While in the opposite to current findings, no significant effect was detected by Vakili *et al.*[14], Khamisabadi *et al.*[17] and El-Essawy *et al.*[19].

The lowest ($p<0.05$) Concentrations of Total Cholesterol (CHOL) being 92.17 mg dL^{-1} and Total Triglycerides (TRIG) being 15.14 mg dL^{-1} were observed with thyme leaves-supplemented goats (G1), followed by those supplemented with thyme EO (G2) being 94.51 and 15.65 mg dL^{-1} compared with the control group being 105.72 and 19.08 mg dL^{-1}, respectively. Michel *et al.*[57] explained these reductions in blood CHOL and TRIG concentrations by the hypolipidemic and hypercholesterolemia properties of aromatic plants and their EO. Also, it was indicated that the product of herbal plants metabolism may inhibit the production of the enzyme (3-hydroxy-3-methylglutaryl CO. A) that controls the cholesterol synthesis[58].

In matching with our results, Morsy *et al.*[59], Khamisabadi *et al.*[17], Seirafy and Sobhanirad[56] and El-Essawy *et al.*[19] reported decreases in blood CHOL and TRIG concentrations with thyme leaves or EO addition. In contrast, concentrations of CHOL and TRIG were not affected by the addition of thyme EO in the study conducted by Vakili *et al.*[14].

Milk yield, composition and feed efficiency: Data of Dry Matter Intake (DMI), milk yield and composition, feed efficiency in Table 5 showed an insignificant ($p<0.05$) increase in DMI with thyme leaves supplementation (1215 g/day), while DMI tended to be decreased insignificantly ($p<0.05$) with thyme EO supply (1135 g/day) compared with control (1175 g/day). The depression in total DMI with EO supply may have resulted from palatability problems, which require an encapsulation of EO to overcome that problem[5]. Moreover, Ribeiro *et al.*[15] cleared that mainly composed of EO may be affected by sensory properties such as odour, flavour and acceptability.

In the same trend, Khamisabadi *et al.*[17] recorded an increase in DMI when lambs' ration was supplemented with thyme leaves. While decreases in DMI were observed with blend EO addition to dairy cows' ration[6,60,61]. In contrast, Seirafy and Sobhanirad[56] reported an increase in total DMI with thyme EO addition to Holstein suckling calves. However, no effect of thyme leaves or EO addition on DMI was detected by Vakili *et al.*[14], El-Naggar *et al.*[16], Mohamed *et al.*[33], Ebrahimi *et al.*[13], El-Essawy *et al.*[19] and Ribeiro *et al.*[15]. The variation in DMI with EO supply may be attributed to many factors such as EO source, diet type, the adaption of rumen microbes to EO[3,62]. Furthermore, the chemical and physical characteristics of the diet can affect DMI[3,63].

In the current study, the highest ($p<0.05$) milk and 4% Fat Corrected Milk (FCM) yields were observed with thyme leaves addition (G1) being 1170 and 1133 g/h/day, respectively, with no significant difference between G1 and G2 (1125 and 1059 g/h/day) compared with control (935 and 939 g/h/day), in the same order. The increase in milk yield with thyme addition maybe because of the improvement in DM and OM digestibility. In this context, many theories explain the increase in milk production with thyme addition. One of these theories indicated that the increase in milk production with EO supply may be attributed to the change of ruminal fermentation pattern, through reducing acetate: Propionate ratio[5,64] or due to reduction in methane production[11]. Also, it may be because of stimulating endocrine

Table 5: Effect of thyme leaves or essential oil addition on dry matter intake, milk yield and composition and feed efficiency

	Experimental groups				
Items	C	G1	G2	±SE	p-value
DMI (g)	1175	1215	1135	-	-
Milk yield (g/h/day)	935^{b}	1170^{a}	1125^{a}	38.32	0.010
4% FCM (g/h/day)	939^{b}	1133^{a}	1059^{ab}	38.75	0.030
Milk composition (%)					
Fat	4.03	3.79	3.61	0.13	0.494
Protein	3.26	3.51	3.60	0.12	0.533
Lactose	4.77	4.68	4.78	0.15	0.965
TS	12.98	12.93	12.93	0.18	0.992
SNF	8.95	9.14	9.32	0.11	0.490
Ash	0.92	0.95	0.94	0.03	0.901
Feed efficiency					
Milk yield/DMI (g g^{-1})	0.80^{b}	0.96^{a}	0.99^{a}	0.03	0.009

Different alphabetic letters means in the same row with various superscripts are significantly different ($p<0.05$), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively glands in animals to release some hormones such as growth hormone[65], which stimulate milk production by elevating the conversion rate of feed nutrients into milk[66]. Furthermore, the antioxidant property of the thymol may inhibit the lipid peroxidation of the free radicals, which protect the mammary secretory cells and keep them healthy[67] which lead to more milk production.

In parallel to the previous results, Mohamed *et al.*[33] recorded significant increases in milk and FCM yields when dairy goats fed ration supplemented with thyme leaves. Also, higher milk yield was recorded with EO addition to rations of dairy cows[61,68] or Chios dairy ewes[7]. Furthermore, Smeti *et al.*[69] demonstrated higher milk yield with goats supplemented with rosemary leaves than those supplemented with rosemary EO. On the other hand, EO addition had no significant effect on milk yield of lactating goats[59], lactating ewes[19] and dairy cows[6,60,70,71].

Although milk composition was not affected either by thyme leaves or EO supplementation, there were insignificant decreases in fat contents (3.79 and 3.61%) and increases in protein contents (3.51 and 3.60%) with thyme (G1 and G2), respectively compared to 4.03 and 3.26% for control, in the same order. The previous result could be a reflection of the decrease in CF and the increase in CP digestibility (Table 2) and the improvement in blood total protein (Table 4) with thyme addition. Moreover, the reduction in NH_3-N concentration in the rumen (Table 3) may be an indicator of improving the synthesis of microbial protein, which reflects on higher milk protein content[63].

In matching with current findings, Mohamed *et al.*[33] and El-Essawy *et al.*[19] found that milk fat content was decreased with thyme leaves or EO supply. Also, in coincidence with present results, Morsy *et al.*[59] and El-Essawy *et al.*[19] reported an increase in milk protein with EO addition. On contrary, no change in milk fat content[60,61] and protein content[6,71] was detected with EO supply. However, Giannenas *et al.*[7] and Flores *et al.*[70] did not record any effect of EO addition on milk composition.

Feed efficiency (milk yield, g/DMI, g) was significantly ($p<0.05$) improved with thyme leaves and EO addition by 20 and 24%, respectively compared with control.

In agreement with our data, feed efficiency was improved by 16.2% when dairy goats fed ration supplemented with thyme leaves[33]. Also, it was reported that the supplementation of blend EO improved feed efficiency of Chios dairy ewes[7] and dairy cows[61,68,71]. However, El-Essawy *et al.*[19] found that the addition of thyme EO to ration of lactating ewes did not affect feed efficiency.

Milk fatty acids profile: The milk fatty acids profile in Table 6 indicated significant and insignificant decreases in C6:0 to C15:1, C17:0, C18:0 and 20:0 with thyme addition. However, C16:0, C16:1 and C18:1 to C18:3 were increased with both thyme leaves and EO supply. As a coincidence to the previous results, concentrations of Total Saturated Fatty Acids (TSFA) were decreased (69.55 and 69.52%), while concentrations of Total Unsaturated Fatty Acids (TUFA) being (30.45 and 30.48%) and Total Poly Unsaturated Fatty Acids (TPUFA) being

Table 6: Effect of thyme leaves or essential oil addition on milk fatty acids profile

	Experimental groups				
Items	C	G1	G2	±SE	p-value
Caproic acid (C6:0)	1.29	1.21	1.15	0.04	0.110
Caprylic acid (C8:0)	3.04[a]	2.67[b]	2.88[ab]	0.16	0.029
Capric acid (C10:0)	11.24[a]	10.14[b]	10.02[b]	0.20	0.001
Lauric acid (C12:0)	5.96	5.78	5.82	0.14	0.879
Myristic acid (C14:0)	12.13[a]	11.32[b]	11.51[ab]	0.32	0.069
Myristoleic acid (C14:1)	0.57	0.35	0.43	0.08	0.625
Pentadecanic acid (C15:0)	1.35	1.24	1.21	0.07	0.770
Ginkgolic acid (C15:1)	0.14	0.11	0.10	0.02	0.623
Palmitic acid (C16:0)	24.53	27.08	26.50	0.71	0.350
Palmitoleic acid (C16:1)	1.82[b]	2.19[ab]	2.35[a]	0.19	0.035
Heptadecanoic acid (C17:0)	0.22	0.19	0.15	0.04	0.841
Stearic acid (C18:0)	11.43	9.76	10.02	0.47	0.331
Oleic acid (C18:1)	23.15	24.81	24.62	0.37	0.124
Linoleic acid (C18:2)	1.49	1.71	1.68	0.05	0.214
Linolenic acid (C18:3)	1.21	1.28	1.30	0.05	0.788
Arachidic acid (C20:0)	0.43	0.25	0.26	0.07	0.528
TSFA	71.62	69.55	69.52	0.82	0.547
TUFA	28.38[b]	30.45[a]	30.48[a]	0.45	0.065
TPUFA	2.70[b]	2.99[a]	2.98[a]	0.06	0.024

Different alphabetic letters means in the same row with various superscripts are significantly different ($p<0.05$), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively, TSFA: Total saturated fatty acids, TUFA: Total unsaturated fatty acids and TPUFA: Total polyunsaturated fatty acids

Table 7: Effect of thyme leaves or essential oil addition on kids' performance

	Experimental groups				
Items	C	G1	G2	±SE	p-value
Birth BW (kg)	3.07	3.22	3.60	0.15	0.365
Final BW (kg)	9.32[b]	11.58[a]	11.22[a]	0.41	0.030
Total BW gain (kg)	6.25[b]	8.36[a]	7.62[a]	0.38	0.022
Average daily gain (g)	83.33[b]	111.47[a]	101.61[a]	4.95	0.008

Different alphabetic letters means in the same row with various superscripts are significantly different (p<0.05), C: (Control group) goats fed CFM+clover hay, G1 and G2: Control group supplemented with thyme leaves or essential oil, respectively, average daily gain: Total BW gain, g/75 days

(2.99 and 2.98%) were increased either with thyme leaves or EO addition, respectively compared with control being 71.62, 28.38 and 2.70%, in the same order.

The decrease in TSFA and the increase in TUFA of milk with thyme supplementation may be attributed to the high anti-microbial properties of some phenolic compounds in thyme such as thymol, eugenol and carvacrol, because of the hydroxyl group in their structure[49,72] which negatively affect both gram-negative and gram-positive bacteria[73] via disturbance in the cytoplasmic membrane[10]. These compounds can inhibit the rumen microorganisms such as bacteria[74], which are involved in the biohydrogenation process of unsaturated fatty acids in the rumen[33]. Furthermore, Tholstrup *et al.*[75] indicated that, the reduction in saturated fatty acids is correlated with less blood cholesterol, as reported in the present study (Table 4), which is considered as an indicator for more quality and healthy milk to consumers.

In the same trend, milk fatty acids profile either of lactating goats or ewes was improved because of EO supply[19,59] or due to thyme leaves addition[33].

Kids' performance: Table 7 revealed no significant difference in birth Body Weight (BW) of kids among different groups. The average values of birth BW were 3.07 kg for C, 3.22 kg for G1 and 3.60 kg for G2. However, final BW (11.58 and 11.22 kg), total BW gain (8.36 and 7.62 kg) and Average Daily Gain (ADG) (111.47 and 101.61 g) were significantly (p<0.05) increased for kids of G1 and G2, respectively compared with those of control group being 9.32 kg, 6.25 kg and 83.33 g, in the same order. These results may be attributed to the improvement in energy metabolism with thyme addition because of the thymol effect on decreasing acetate and decreasing propionate production without affecting total VFA[33]. Furthermore, residues of EO in milk may improve the appetite and weight of kids due to the digestion stimulant, antioxidant and antiseptic properties of thymol[19]. Moreover, a positive correlation between the growth rate of kids and milk production of mothers was reported by Smeti *et al.*[69], which matches with higher milk production with thyme addition in the current study.

In the same context, significant increases in final BW and ADG of kids and lambs were observed either with dairy goats supplemented with thyme leaves[33] or with lactating ewes supplemented with thyme EO[19], in the same order. Moreover, Smeti *et al.*[69] demonstrated that kids of dairy goats who received rosemary leaves had higher final BW and ADG than those who received rosemary EO.

CONCLUSION

It could be concluded that both thyme forms (leaves or EO) improved digestibility and lactation performance of Damascus goats with no significant difference between the two forms. However, the supplementation of lactating goat's ration especially with thyme leaves recorded the best values of DM and OM digestibility, TDN and kids' growth rate, the lowest concentrations of blood total cholesterol and triglycerides and the highest milk production with more healthy aspects for consumers.

SIGNIFICANCE STATEMENT

This study discovered that incorporating thyme (*Thymus vulgaris*) especially in form of leaves could be used successfully as a natural feed additive that can be beneficial for lactating goats' performance. Furthermore, it has the potential to produce functional milk with a low concentration of total saturated fatty acids and a high concentration of total unsaturated fatty acids. This study will help the researchers to investigate more natural alternatives for antibiotics.

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