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Original Article

Effect of *Thymus vulgaris* or Peppermint on Lactating Sanjabi Ewe Performance, Milk Composition, Lamb Growing and Relevant Blood Metabolites

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Abstract

Milk production and composition, lamb growth, blood hematological profile and relevant blood metabolites were assayed in Sanjabi ewes and lambs as affected by supplementing basal diet by either thyme (*Thymus vulgaris* L.) (THY) or Peppermint (PEP). Twenty-one pregnant Sanjabi ewes of similar weight (BW around 50 Kg) and age (45-48 months) were equally divided into three dietary treatments. Group (1) served as a control group received a basal diet (BD) with no feed supplement. Groups (2) THY and (3) PEP received 10 g/ewe/day THY or PEP, respectively. Lactation period and milk yield were higher ($P<0.05$) in ewes of THY supplement followed by that of PEP supplement than that of basal diet only, respectively. Whereas, weaning weight (WW) and average daily gain (ADG) of lambs were improved ($P<0.05$) by the addition of THY or PEP to the basal diet. The addition of THY and PEP increased ($P<0.05$) WBC's and lymphocytes counts of these lactating ewes, while, monocytes count was higher ($P<0.05$) in ewes fed basal diet only. No significant effects were noticed on eosinophils, RBC's count and Hb concentration in ewes fed the supplemented diets. RBC's, WBC's, lymphocytes counts and Hb concentration of suckling lambs were improved ($P<0.05$) by adding THY or PEP to the basal diet of ewes. The addition of THY or PEP to the basal diet increased ($P<0.05$) blood serum; total protein and globulin concentration of these ewes compared to those of the control group. However, no significant differences were observed in albumin, glucose, triglycerides and urea concentrations. Total serum cholesterol was decreased ($P<0.05$) by the addition of THY and PEP. Concerning suckling lambs, the addition of THY or PEP to the basal diet increased ($P<0.05$) total protein and globulin concentration, while, it decreased albumin: globulin ratio. No significant effects were noticed in albumin, glucose, triglycerides and cholesterol of suckling lambs blood serum. It is concluded that THY and PEP diet supplements could be used as natural growth promoters in ewe diets to improve milk yield and composition and lamb growth.

Keywords: Sanjabi ewes, *Thymus vulgaris* powder, Peppermint, Supplements

Introduction

The production of sheep milk is very important to the production of the lamb because it directly affects the growth of young growth, especially in the first few weeks of life [1,2]. Various attempts have been manipulated to maximize the productivity of the animals in different species, including antibiotics, hormones, chemical growth promoters, enzymes, and minerals [3]. Feed additives such as antibiotics "monensin and lasalocid" are used to optimize diet formulation and diminish losses of energy. Despite their economic benefits, growth-promoting antibiotics have been prohibited in most countries of the world because milk and meat residue are associated with many health hazards, particularly the development of multi-drug resistance [4].

Medicinal plants are important in terms of various aspects and applications [5-9]. They are used in animal nutrition to improve its performance, feed efficiency, nutrient utilization, overall animal health and the quality of

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livestock products. These effects are related to its ability to improve oxidative stability [10]. The use of herbal galactagogues is known to have a beneficial effect on milk production [1]. Galbat *et al* (2014) indicated that seeds from herbal plants enhancing the effect of hormonal alert by increasing prolactin and therefore release the somatotropin hormones which consequently increase the level of glucose through udder tissue activation and this leads to improvement the lactating animal productivity [11]. Zeid and Ahmed (2004) recorded that the BW tends to increase by feeding some medicinal herbs (Chamomile and thyme) in Zaraibi doe rations and Zaraibi goats [12]. Vakili *et al*. (2013) reported that feeding thyme does not change the plasma glucose, cholesterol, triglyceride, urea nitrogen, B-hydroxybutyrate, alanine aminotransferase and aspartate aminotransferase concentrations [13]. Abeer *et al* (2019) reported that Total solids (TS), solid not fat (SNF) and lactose contents were increased ($P<0.01$) with thyme [14]. Ismail *et al*., (2004) reported that Peppermint (PEP) might be useful galactagogues for lactating goats [15].

The use of herbs such as *Thymus vulgaris* L. in the diet of the dairy animal also has a positive effect on milk production and feed conversion efficiency. Adding thyme (THY) to the diet can reduce serum cholesterol and total lipids [16,17].

The same author also reported that the use of THY in doe compatibility reduced infant mortality to zero during lactation compared with other herbs, suggesting that common thyme may play a role in improving children's immunity and performance [18]. Abdelhamid *et al* (2004) reported that by adding THY to goat diets, milk production and all measured components tend to increase.

This work was designed to evaluate the effect of THY and PEP galactagogues addition to lactating Sanjabi ewe diets; on milk yield and composition and relevant blood metabolites, as well as lambs growth [19].

Material and Methods

The experiment was conducted in the Animal Science Department, Kermanshah Agricultural and Natural Resources Research and Education (Mehregan farm located in Kermanshah province in the west of Iran).

Animals and Feeds

Twenty-one pregnant Sanjabi ewes with a body weight of around 50 Kg and 45-48 months of age were treated at late gestation, two weeks before lambing, were randomly divided into equal three dietary groups. The first group was fed the basal diet (BD) and served as a control group. The second group was fed the basal diet supplemented by 10 g /ewe/day *Thymus vulgaris* (THY), while the third group was fed the basal diet supplemented by 10 g /ewe/day Peppermint (PEP) Each the group was housed in a semi-roofed barn (5×4×4 m). The daily basal diet (BD) at the experimental start was 1.2 Kg concentrate and 0.5 Kg straw/ewe/day offered in two equal meals, at 09:00 and 15:00 hr. The ration was increased biweekly by 0.2 Kg concentrate and 0.3 Kg straw/ewe/day until weaning. The extra addition of 10 g /day of THY or PEP was added when lambs reached one month of age to ensure that the lambs consume a considerable amount of additives. Table 1 shows the composition of concentrate feed. The study was started two weeks before parturition and lasted until the end of lactation.

Milking Assessments

Starting from the end of the first week (after colostrum) lambs were isolated from their dams in separate pens after the second meal (15:00 hr) till the next day. At weekly intervals complete milking was done in the morning till stripping by hand, thus milk yield was recorded throughout the lactation period. Milk samples were obtained for analysis. Fat, protein and ash percentages were determined using the method described by Ling (1963), and lactose percentage by the procedure of Barnett and Abd AL-Tawab (1957). Total solids not fat were calculated.

Table 1 Formulation of concentrate feed mixture, and proximate analysis of the basal diet (BD)

Components	% Proximate analysis		
Barley grain	68.0	Dry matter% (DM)	87.55
Wheat bran	15.0	Organic matter% (DM)	95.55
Soybean meal	14.6	Components% of DM	
Calcium Carbonate	13.0	Ash%	6.45
Sodium Chloride	0.5	Crude protein%	13.60
Calcium Phosphate	0.5	Either extract%	4.66
Trace elements & Vitamins	0.1	Crude fiber%	7.40
	-	Nitrogen free extract (NFE)%	67.89
Total	100.0		

Lamb Growth

Lambs were weighed at birth, once a week, until weaning at 60 days of age. Daily weight gain was calculated.

Blood Samples Collection

The ewes in each diet treatment group were used for blood evaluation. At the beginning of the experiment, after lambing, at monthly intervals until weaning (60 days), blood samples were collected from the jugular vein of each ewe. Part of the blood was received the EDTA anticoagulation tube to determine hematology items. The other part of the blood was collected in a non-heparinized test tube and then centrifuged at 4000 rpm for 3 minutes. The clear serum was separated and stored at (-20 °C) for blood chemistry analysis. Three-day-old lambs use the same procedure, then every two weeks until weaning.

Determination of Blood Relevant Metabolites Concentrations

In this project, the protocol of animal behavior of Razi University has been observed.
The serum total protein concentration was determined by the Biuret method using kits of (Chemelex, Barcelona) (Oyewo and Fakunle PB 2013). Special kits from (Chemelex, Barcelona) were also used for the determination of; albumin, globulin, urea and glucose concentrations Albumin: globulin ratio (A/G) [20] was calculated. Serum total cholesterol concentration was assayed spectrophotometrically using kits from (Bio Mereuxas, France). Serum triglycerides concentration was determined by the triglyceride-GPO method using kits from (Biolabosa, France) after hydrolysis with lipase [21].

Determination of bBlood Hematological Items

The determination of hematological items, white blood cells (WBC's) total and differential counts, red blood cells (RBC's) count and hemoglobin (Hb) concentration were determined according to Wintrobe, 2008 [22].

In this project, the protocol of animal behavior of Razi University has been observed.

Statistical Analysis

We used a completely random statistical design and the statistical software package SPSS for Windows (2013), standard version, to perform statistical analysis on the collected data through analysis of variance (ANOVA).

Results

Milk Production and Composition

Ewes fed a basal diet (BD) supplemented with THY had the longest (P<0.05) lactation period and the greatest milk yield compared to those fed either the basal diet or the basal diet supplemented with PEP (Table 2). The use of THY and PEP as natural feed additives increased (P<0.05) total protein (TP), solids non-fat (SNF) and ash (A) percentages compared to the control group. However, no significant effects were noticed in lactose (L) and total solids (TS) as a result of THY and PEP supplementation.

Lambs Growth Performance

Table (3) presents the means ± SE of lamb birth weight (LBW), weaning weight (WW) and average daily gain (ADG). Addition of *Thymus vulgaris* (THY) or Peppermint (PEP). PEP to the basal diet improved (P<0.05) WW and ADG of lambs compared to the non-supplemented control diet.
Blood serum metabolites
Tables (4 and 5) show the concentrations of relevant blood serum metabolites of these ewes and their lambs. The addition of PEP and THY to the basal diet increased (P<0.05) serum total protein and globulin of both ewes and lambs.
However, no significant differences were noticed in albumin, glucose, urea and triglycerides values of ewes and lambs serum among all treatments.

Table 2 Milk production and composition of lactating Sanjabi ewes as affected by dietary treatments (Means ± SE) (n= 7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
Lactation period (d)	68.0±3.7 c	92.0±3.8 a	83.0±2.0 b
Total milk yield (Kg)	326.3±20.1 c	490.9±39.8 a	349.9±28.7 b
Fat% (F)	5.41±0.47	5.20±0.48	4.87±0.33
Total protein% (TP)	4.84±0.11 b	5.45±0.13 a	5.47±0.12 a
Lactose% (L)	4.60±0.16	4.97±0.08	4.20±0.10
Total solids% (TS)	15.12±0.44	15.60±0.51	15.52±0.34
Solids not-fat% (SNF)	9.71±0.21b	10.40±0.16 a	10.65±0.11 a
Ash% (A)	0.88±0.02b	0.98±0.01 a	0.98±0.02 a

a-c Means with different superscripts in the same row are significantly different at P<0.05.

Furthermore, serum the cholesterol of ewes was decreased significantly (P<0.05) by the addition of PEP and THY while an insignificant increase was found in lamb blood serum. Albumin: globulin ratio of ewes was increased (P<0.05), opposite to \ the trend observed in lambs, by supplementing the basal diet with these additives.

Hematological Profile

Tables 6 and 7 show that the addition of PEP and THY to the basal diet increased (P<0.05) total WBC's and lymphocytes counts of ewes and lambs. On the other hand, ewes fed the unsupplemented basal diet had the highest (P<0.05) monocytes count compared to the supplemented groups.

Table 3 Growth performance of suckling Sanjabi lambs as affected by dietary treatments (Means ± SE) (n= 7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
Birth weight (Kg)	4.59±0.19	4.66±0.21	4.77±0.25
Weaning weight (Kg)	15.64±0.81 b	20.14±0.8	20.29±1.15 a
Average daily gain (Kg)	0.185±0.01 b	0.258±0.01 a	0.259±0.02 a

a-b Means with different superscripts in the same row are significantly different at P<0.05.

Table 4 Concentrations of relevant metabolites in blood serum of lactating Sanjabi ewes as affected by dietary treatments (Means ±SE) (n= 7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
Total protein (gm/dl)	8.14±0.50 b	9.95±0.75 a	9.85±0.55 a
Albumin (gm/dl)	3.63±0.39	4.36±0.36	4.85±0.35
Globulin (gm/dl)	4.62±0.32 b	5.79±0.30 a	5.89±0.31 a
Albumin: Globulin (A:G) ratio	0.79±0.03 b	0.84±0.03 a	0.82±0.04 ab
Glucose (mg/dl)	68.8±4.04	73.43±1.22	70.61±6.04
Urea (mg/dl)	64.37±6.28	66.73±9.62	69.66±7.61
Triglycerides (mg/dl)	43.28±5.48	44.26±4.42	470.11±5.42
Cholesterol (mg/dl)	89.30±5.12 a	62.56±6.89 b	63.00±6.00 b

a-b Means with different superscripts in the same row are significantly different at P<0.05.

Table 5 Concentrations of relevant metabolites in blood serum of suckling Sanjabi lambs as affected by dietary treatments (Means ±SE) (n= 7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
Total protein (gm/dl)	6.60±0.86 b	9.57±0.68 a	10.14±0.78 a
Albumin (gm/dl)	3.11±0.12	3.03±0.21	3.44±0.17
Globulin (gm/dl)	3.49±0.81 b	6.54±0.62 a	6.70±0.72 a
Albumin: Globulin (A:G) ratio	0.89±0.18 a	0.46±0.05 b	0.51±0.07 b
Glucose (mg/dl)	139.89±7.3	125.90±7.2	125.97±10.3
Urea (mg/dl)	37.57±2.29 a	30.36±4.14 b	23.59±1.93 c
Triglycerides (mg/dl)	45.21±5.23	52.44±10.01	68.56±8.52
Cholesterol (mg/dl)	43.76±5.41	52.57±6.43	59.16±9.4 6

a-b Means with different superscripts in the same row are significantly different at P<0.05.

Table 6 Hematological profile of lactating Sanjabi ewes as affected by dietary treatments (Means ± SE)(n= 7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
WBC's (103/mm3)	83.1±3.3 b	96.9±3.3 a	91.1±3.3 b
Lymphocytes (103/mm3)	28.58±1.2 b	37.50±2.7 a	34.70±1.5 a
Monocytes (103/mm3)	1.91±0.4 a	0.97±0.0	1.2±0.2 b
Neutrophils (103/mm3)	52.26±2.1	58.43±4.8	55.20±1.6
RBC's (106/mm3)	3045.7±178.3	3171.0±110.7	3214.3±111.7
Hb (g/dl)	9.14±0.59	9.23±0.34	9.74±0.29

a-b Means with different superscripts in the same row are significantly different at P<0.05.

Table7 Hematological profile of suckling Sanjabi lambs as affected by dietary treatments (Means ± SE) (n=7/group)

	Dietary treatments		
Items	Control	THY powder	PEP powder
WBC's (103/mm3)	95.20±1.1 b	98.30±1.1 a	98.90±1.1 a
Lymphocytes (103/mm3)	26.09±1.8 b	34.46±1.6 a	36.21±3.3 a
Monocytes (103/mm3)	1.74±0.5	1.90±0.4	1.77±0.4
Neutrophils (103/mm3)	66.70±1.9 a	61.53±2.7 b	59.93±3.5 b
Eosinophils (103/mm3)	0.96±0.3	1.32±0.3	0.98±0.4
RBC's (106/mm3)	3550.0±54.6 b	3756.2±24.1	3814.3±86.4 a
Hb (g/dl)	10.60±0.1 b	11.10±0.1 a	11.20±0.1 a

a-b Means with different superscripts in the same row are significantly different at P<0.05.

Neutrophils count of both ewes and lambs were not affected by these additives. RBC's count and Hb the concentration of ewes was not affected by these additives, on the contrary, both were significantly increased in lambs.

Discussion

Milk Production and Composition

This study showed that the addition of either THY or PEP to the basal ration of Sanjabi ewes improved milk production and composition in terms of milk yield, total protein, ash and solids not-fat percentages. On the other hand, it extended the lactation period. THY had a significantly better effect compared to PEP treatment.

Concerning PEP, the increase in milk yield and lactation period may be attributed to the galactopoietic effect of the active components that PEP may contain [23]. El-Hawy (2018) discovered that the positive effect of galactagogues on milk production may be due to the decrease in circulating biogenic amines such as, histamine, tryptamine and tyramine in the blood which is known to cause excessive release of catecholamines in mobile pool leading to the suspension of milk secretion as well as causing indigestion by inhibiting the ruminal mobility and absorption [24].

The composition and properties of PEP have been fairly investigated by Loolaie *et al*. (2017) [25], Ibrahim et al (2019) [26], Nayak *et al* (2020) [27] and Van Vuuren *et al* (2020) [28]. These researchers reported that PEP oils have; anti-bacterial, anti-fungal, anti-inflammatory and antihistaminic effects. PEP particularly its oils showed antibacterial activity against 5 strains of Listeria monocytogenes, which may enhance the performance of the animal [29].

Proximate analysis showed that PEP contains 23.5-33.2% carbohydrate, 20-27% proteins and 34.5- 38.7% lipids which is consistent with the results of some research [30, 31].

It has been reported that most of the PEP properties are, mainly, attributed to thymoquinone, the main constituent of PEP volatile oil [32] and nigellimine N-oxide, as isoquinoline alkaloid [33].

PEP increases mammary gland development in rats thus milk yield is increased at different stages of lactation. Therefore, PEP appears to be a potential multipurpose feed additive and may be promising in improving sheep and lamb's performance [34].

Thymus vulgaris supplementation increased milk yield and lactation period of Sanjabi ewes. Both agricultural and medical literature was searched for studies that examined THY effects on milk production and composition. However, one article by Boutoial *et al*. (2013) [35] crossed our search, where they reported that THY improved daily milk yield of goats compared to other medicinal plants, over the control treatment. However, the lack of information about the use of THY as a feed additive to sheep, rations make it difficult to understand the mechanism through which milk yield and lactation period increased. The present results of the increase in milk yield and lactation period resulting from THY supplementation are, probably, due to the remarkable vasodilator action of Thymus vulgaris essential oil, particularly bisabolol and bisabolol oxides, [36] with the fact that vasodilator action increases the blood flow in blood vessels which normally supplies the mammary glands by all components required for milk production.

Several researchers found that the use of PEP and THY as feed additives increased milk yield and maintained a relatively high level of persistency [19].

Lamb Growth Performance

The addition of either THY or PEP to the basal diet (BD) improved significantly lamb growth performance in terms of WW and ADG. These favorable effects of THY and PEP could be related to their high nutritive biological value.

Previous studies in this regards reported that 15 amino acids make-up the proteins of PEP [25]. Moreover, PEP contains a mixture of essential fatty acids particularly, linolenic, oleic and linoleic acids that are necessary for good body growth [25]. One more factor that may explain the favorable effects of using PEP as a feed

additive is its stimulating effect on the animal digestive system. It has been reported that PEP has also choleric effects which produce a definite increase in bile flow [26]. This flow is an emulsifying agent that activates the pancreatic lipase that aids fat digestion and fat-soluble vitamin absorption [31].

Concerning THY, it has been reported that its essential oils have an anti-ulcer effect [30], this maintains normal proper structure and function of the digestive tract thus improving digestion and absorption of nutrients that may explain enhancements of lambs' growth. Unfortunately, the scarcities of researchers are noted in this field.

The present findings are consistent with the previous findings of Emami *et al* (2012) [37] who reported that feeding herbs, of which one of them was PEP, improved nutrient.

Conclusion

It could be concluded that THY and PEP as feed additives may have a beneficial effect on sheep performance and may replace traditional growth promoters. THY and PEP are characterized by being natural edible promoters, which have no side effect on animals and consequently on humans. However, further detailed studies must be performed on using different levels of THY and PEP to evaluate the suitable level that can be used in sheep rations.

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