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Original article

Effect of garlic oil on lamb fattening performance, rumen fermentation and blood parameters

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Summary. The objective of this study was to determine the effects of garlic essential oil (GEO) on performance, ruminal fermentation and blood parameters of Kivircik lambs. Forty-eight 2.5-3 month old Kivircik male lambs were allocated into four treatment groups. Diets were supplemented with GEO at the 0.0, 0.4, 0.8 and 1.2 g/kg DM. The growth trial lasted for 63 days. The supplementation of GEO significantly decreased the total weight gain (TWG) and average daily weight gain (ADWG), whereas it has no effect on the final body weight (FBW) daily feed intake (DFI) and feed conversion ratio (FCR). The supplementation of GEO to the lamb diets had also a significant impact on rumen fermentation and blood parameters. The addition of GEO to the lamb diets increased non-esterified fatty acid production while supplementation significantly reduced the levels of glucose, urea, protein, triglyceride, insulin and cholesterol. It can be said that the decrease of glucose, urea, triglyceride, insulin and cholesterol is important in terms of animal health when the blood parameters are taken into consideration. As conclusion, GEO supplementation up to 0.8 g/kg DM can be recommended for growing lambs to manipulate rumen and blood parameters without compromising important growth parameters.

Key words: Blood parameters, feed intake, garlic oil, lamb fattening performance, rumen fermentation

Introduction

It has been reported that pathogenic micro-organisms develop resistance to antibiotics used in animal feeding (1-3). The fact that pathogenic micro-organisms which develop resistance to antibiotics a negative effect on human health have been subject of discussion in recent years (4). This situation has caused concern and has banned the ionospheres group antibiotics, which stimulates the development of animal nutrition (5). Prohibition of antibiotic use in animal nutrition as a feed additive has led investigators to new, natural and environmentally friendly resources that do not create resistance to pathogenic microorganisms and do not leave residues in the product. In this context, researchers have focused on the use of antimicrobial and digestive stimulant properties of essential oils and their active components isolated from aromatic plants.

(6-8). In this context, garlic plant has come forward with respect to both itself and essential oil components (9). Garlic plants are reported to have a large number of active metabolites which have antimicrobial and antioxidant properties (10-15). In addition, these metabolites have the potential to reduce oxidative stress and cholesterol risk in humans (16, 17) and prevent cancer (11, 18). It has been suggested that garlic essential oils will be used to manipulate rumen microbial fermentation due to the antimicrobial effects of secondary metabolites such as allicin, diallyl sulphide, diallyl disulphide and allyl mercaptan (10, 19, 20). The secondary metabolites of allicin and diallyl sulphides of GEO have strong antimicrobial activity against gram positive and negative bacteria. These secondary metabolites can easily penetrate into the cell by damaging the cell membrane and disrupts the ion balance between intracellular and extracellular cells (21). In addition, the secondary metabolite of organosulfide of GEO which also antimicrobial effect, break down the structure of the cell membrane, combining cell membrane proteins (21). It is reasonable to hypothesize that the supplementation of GEO may possibly improve the lamb performance due to improvement in rumen and blood parameters. Although there are a lot of *in vitro* studies about the effect of GEO on digestion feedstuffs (22, 23), regulation of rumen environment (24-26) and methane production (24,25), there are limited *in vivo* experiments about the effect of GEO on growth performance (27-28), rumen fermentation (29-32) and blood parameters (28, 29). Therefore the objective of this study was to determine the effects of GEO on performance, ruminal fermentation and blood parameters of Kivircik lambs.

Materials and methods

Animals and experimental treatments

Forty-eight 2.5-3 month old Kivircik male lambs (21.7±0.05 kg initial body weight: IBW) were allocated randomly into four treatment groups given in Table 1. Four iso-nitrogenous and iso-caloric *diets were formulated* from practical ingredients to meet the nutrient requirements of lambs *according* to *NRC (33)*.

Each treatment group was consisted of 12 lambs housed in individual compartments (1x1.5 m) during the trial period. Diets were supplemented with GEO at the 0.0, 0.4, 0.8 and 1.2 g/kg DM. Garlic essential oil (Catalogue no: W250309) purchased from a commercial firm (Sigma-Aldrich) was added to diets by spraying. The prepared rations were put into 25 kg airtight nylon bags and used during the trial period. The growth trial of lambs was started and the lambs were fed with experimental diets following one week adaptation period. The experimental diets were given the lambs with a free access to water. During the fattening period, LWG and FI of the lambs were

Table 1. Chemical composition of lamb fattening diets used in the current experiment

	Diets			
Ingredients, (g/kg DM)	**I**	**II**	**III**	**IV**
Barley grain	330.0	329.6	329.2	328.8
Wheat grain	200.0	200.0	200.0	200.0
Maize grain	177.0	177.0	177.0	177.0
Sunflower seed meal	270.0	270.0	270.0	270.0
Lime stone	12.0	12.0	12.0	12.0
Salt	10.0	10.0	10.0	10.0
Min-Vit Mixture*	1.0	1.0	1.0	1.0
Garlic oil	0.0	0.4	0.8	1.2
Nutrient contents, Dry matter basis)				
Organic matter, %	96.79	96.79	96.79	96.80
Crude Protein, %	18.49	18.48	18.48	18.47
Ether extract, %	2.13	2.17	2.21	2.25
Crude ash, %	3.21	3.21	3.21	3.20
Crude Fibre, %	12.73	12.73	12.72	12.72
Nitrogen Free Extract, %	56.98	57.46	57.43	57.40
Neutral detergent fibre (NDF), %	23.87	23.85	23.17	22.96
Acid detergent fibre (ADF), %	11.74	11.70	11.45	11.15
ME, cal/kg DM	2809	2810	2812	2813

**Per kg min-vit mixture containing150 mg Zn $SO_4 7H_2 O$, 80 mg $MnSO_4 H O$, 200 mg MgO, 5 mg $CoSO_4 7H_2 O$, 1 mg KIO_3, 4000 IU vitamin A, 1000 IU vitamin D ve 20 IU vitamin E*

determined by the weighing on 21-d intervals. The lambs were weighed on an empty stomach. The growth trial lasted for 63 days. At the end of the experiment, blood samples were taken from *Vena jugularis* of the lambs. Rumen fluid samples were taken from rumen of the lambs by rumen probe. All procedures performed on lambs in the current study were in consistent with ethical standards indicated in directive 2010/63/EU and the experimental protocols were approved by the Animal Experimentation Ethics Committee of University of Uludag, Faculty of Agriculture (Protocol No: 2012/08-02).

Chemical analysis

The dry matter, crude ash, crude protein and crude oil of experimental diets were analyzed according to the methods reported in AOAC (34). NDF and ADF contents of diets were identified by Van Soest *et al*. (35). The pH of the rumen fluid was determined by digital pH meter (Sartorius PB-20, Goettingen, Germany) and ammonia nitrogen (NH_3-N) by Kjeldahl method (36). The VFA contents of rumen fluid were analyzed by gas chromatography (37). Lactic acid analysis was determined using a spectrophotometer (Shimatzu GC 15 A) (38). Blood glucose, urea, protein, cholesterol and triglycerides were analyzed by ARCHITECT 1600 brand (SN: 1600239) with photometric method and with ABBOTT kits. Insulin was determined by the ECLIA method and cobas e411 (SN: 0712-15) in the analyzer, while the un-esterified fatty acid was determined by the enzyme-linked immunosorbent assay.

Statistical analysis

Data were subjected to variance analysis (ANOVA) to determine the effect of GEO on the growth performance, rumen fermentation and blood parameters. The differences among treatments were determined by Duncan multiple comparison test using the IBM SPSS Statistics 20 (IBM Cooperation, Somers, NY, USA) (IBM Cooperation, Somers, NY, USA).

Results

Effect of garlic essential oil on the fattening performance of Kivircik lambs

The effect of GEO on the fattening performance is shown in Table 2. The supplementation of 1.2 g/kg DM of GEO significantly decreased TWG and ADWG, whereas it has no effect on FBW, DFI and FCR. The TWG and ADWG ranged from 20.17 to 23.10 kg, 320.0 to 366.70 g respectively ($P<0.05$). The TWG of lambs fed with diet 4 was significantly lower than those obtained for lambs fed with diets I and II.

As can be seen from Figure 1 and 2 the mean decreases in TWG and ADWG of lambs were 2.51 kg and 39.97 g per g GEO supplementation.

Table 2. Effect of garlic essential oil on fattening performance of lambs

	Diets				sem	*P*
Growth performance	**I**	**II**	**III**	**IV**		
IBW , kg	21.75	21.80	21.80	21.78	1.197	1.000
FBW , kg	44.85	44.25	43.00	41.95	1.402	0.474
TWG, kg	23.10[a]	22.45[a]	21.20[ab]	20.17[b]	0.511	0.001
ADWG, g/day	366.70[a]	356.20[a]	336.40[ab]	320.00[b]	0.008	0.001
DFI, kg DM/day	1.476	1.446	1.427	1.384	0.046	0.565
FCR	4.042	4.080	4.270	4.321	0.150	0.480

[ab]Row means with common superscript do not differ (P> 0.05), sem: standard error mean

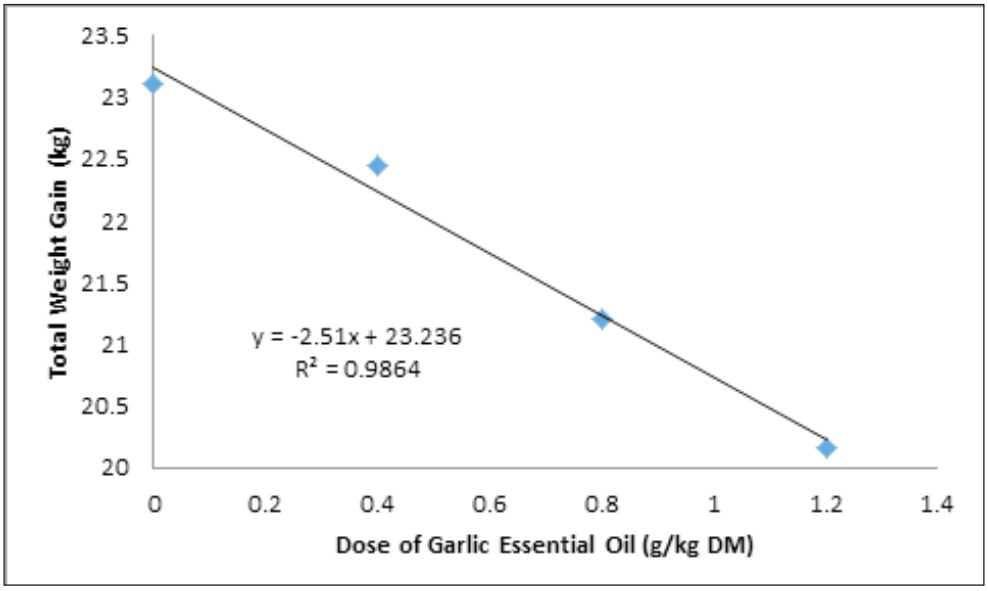

Figure 1. The relationship between dose of garlic essential oil and total weight gain

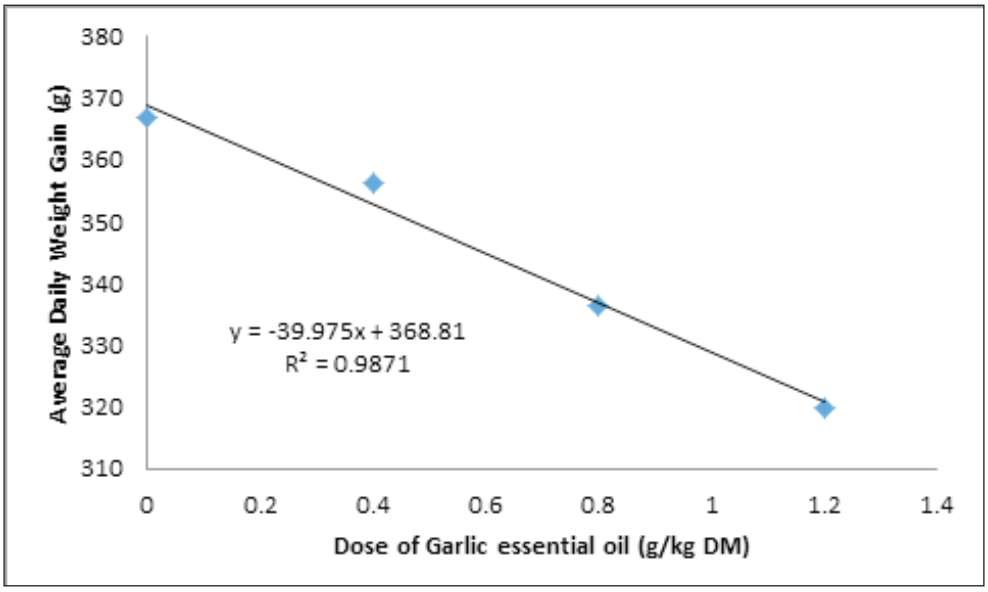

Figure 2. The relationship between dose of garlic essential oil and average daily weight gain

Effect of garlic essential oil on rumen fermentation parameters

The effect of GEO supplementation on the rumen fermentation parameters is given in Table 3. The rumen pH of lamb fed with diets containing garlic oil varied between 5.86 and 6.56, and the addition of GEO increased the rumen pH significantly ($p < 0.05$).

The highest increase in pH was determined in the 4th group with the addition of 1.2 g/kg DM garlic oil. As can be seen from Table 3, TVFA production significantly decreased with increasing level of GEO

Table 3. Effects of different doses of garlic oil on rumen fermentation

	Diets					
Fermentation Parameters	**I**	**II**	**III**	**IV**	**sem**	**P**
pH	5.86[c]	6.25[b]	6.34[b]	6.56[a]	0.040	0.000
Ammonia, mg/100 mL	31.97[a]	29.59[b]	23.99[c]	19.36[d]	0.538	0.001
Total VFA, mmol/L	128.93[a]	119.79[b]	108.60[c]	103.49[d]	1.303	0.000
Individual VFA (mmol/L**)**						
Acetic acid	64.02[a]	60.32[b]	53.97[c]	48.94[d]	0.698	0.000
Propionic acid	40.99[a]	37.39[b]	33.67[c]	33.66[c]	0.410	0.000
Butyric acid	15.76[a]	14.69[ab]	14.29[b]	14.16[b]	0.331	0.006
Other VFA	8.21[a]	7.51[b]	6.69[c]	6.58[c]	0.181	0.000
Lactic acid	3.01[a]	2.38[b]	2.77[b]	2.24[b]	0.112	0.000
AA/PA ratio	1.56[a]	1.61[a]	1.56[b]	1.45[b]	0.022	0.000

[ab]Row means with common superscript do not differ (P> 0.05), sem: standard error mean

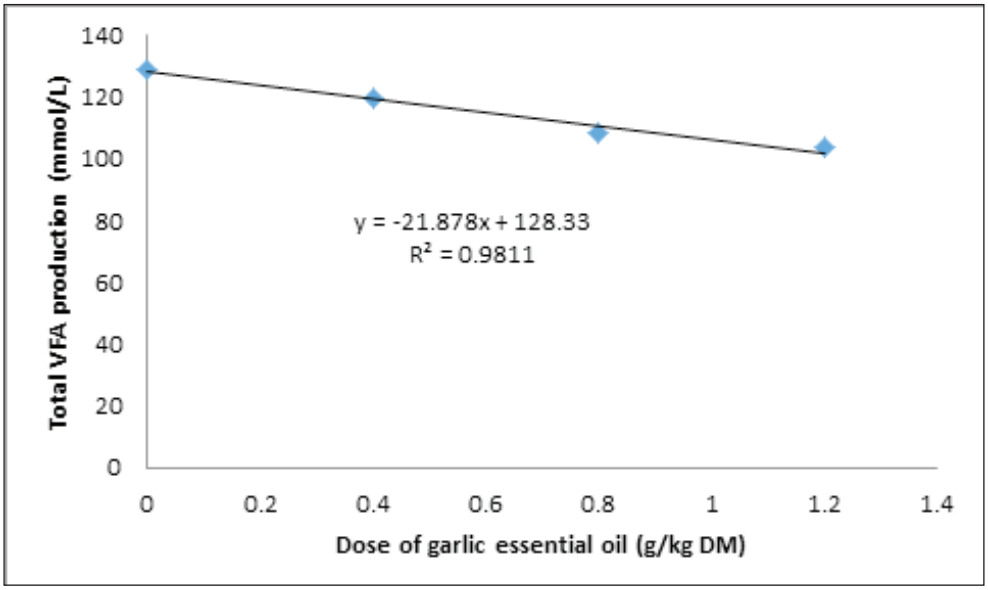

Figure 3. The relationship between dose of garlic essential oil and total VFA production

supplementation (P<0.05). Total volatile fatty acids ranged from 103.49 to 128.93 mmol/L. The TVFA production obtained in the lambs fed with diet 4 was significantly lower than the others (P<0.05). The relationship between dose of GEO and TVFA production was given in Figure 3. The mean decreases in TVFA of rumen fluid were 21.87 mmol per g GEO supplementation.

As can be seen from Table 3, all of the individual VFA (acetic, propionic, butyric and other fatty acid) production decreased with supplementation of GEO (P<0.05). The supplementation of GEO linearly decreased the ammonia concentration (Figure 4). The mean decreases in ammonia concentration of rumen fluid were 10.85 mg per g GEO supplementation.

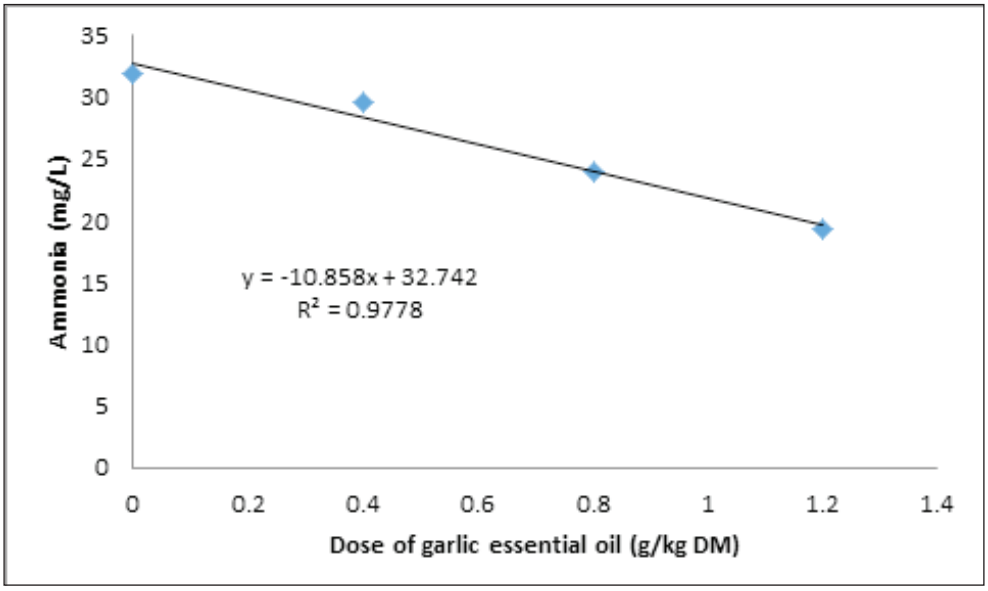

Figure 4. The relationship between dose of garlic essential oil and ammonia concentration

Table 4. Effect of garlic essential oil on blood parameters of Kivircik lambs

	Diets					
Parameters	**I**	**II**	**III**	**IV**	**sem**	**P**
Glucose, mg/100 mL	85.03^{a}	81.15^{b}	77.63^{c}	74.08^{d}	0.604	0.000
Urea, mg/100 mL	15.45^{a}	14.81^{a}	13.36^{b}	12.59^{c}	0.056	0.000
Protein, g/100 mL	8.98^{a}	7.79^{ab}	7.36^{bc}	6.61^{c}	0.209	0.000
Triglyceride, mg/100 mL	26.61^{a}	23.59^{ba}	25.27^{ab}	22.72^{c}	0.548	0.000
Insulin, µu/mL	27.19^{a}	20.59^{c}	22.05^{bc}	23.66^{b}	0.599	0.000
Cholesterol, mg/100 mL	63.08^{a}	58.68^{b}	56.22^{c}	52.97^{d}	0.557	0.000
Non-esterified fatty acid µmol/L	235.19^{c}	240.9^{b}	247.09^{a}	250.36^{a}	1.030	0.000

^{ab}Row means with common superscript do not differ (P> 0.05), sem: standard error mean

Ammonia concentration (NH_3N) ranged from 19.36 to 31.97 mg/100 mL. Ammonia concentration of rumen fluid obtained from lambs fed with diet 4 was significantly lower than the others.

Effect of Garlic Essential Oil on Blood Parameters of Kivircik Lambs

The effect of GEO on blood parameters of Kivircik lambs is presented in Table 4. The glucose, urea, protein, insulin and cholesterol serum concentration significantly decreased with increasing level of GOE supplementation whereas supplementation increased NEFA.

The level of blood glucose, urea, triglyceride and cholesterol varied between 74.08- 85.03, 12.59-15.45, 22.72 26.61, 52.97- 63.08 mg/100 mL respectively. Insulin varied between 20.59-27.19 µu/mL and NEFA values between 235.19-250.36 µmol/L. The level of blood glucose, urea, triglyceride, insulin and cholesterol obtained in the lambs fed with diet 4 was

significantly lower than the others. On the other hand the level of blood protein and NEFA varied between 6.61 and 8.98 g/100 mL and 235.19 and 250.36 μmol/L respectively. The level of blood protein and NEFA obtained in the lambs fed with diet 4 was significantly lower than those for diet I and II, but NEFA value higher than diet I and II.

Discussion

The supplementation of GEO had a significant effect on the growth performance, rumen fermentation and blood parameters. The effect of GEO depends on the inclusion level of GEO in the lamb diets. Chaves *et al*. (28) showed that supplementation of GEO at 0.2 g /kg DM did not affect the DMI, FBW, ADWG and FCR of lambs. In the current experiment supplementation of GEO at 0.4 and 0.8 g/kg DM) had no effect on DFI, FBW, ADWG and FCR of lambs whereas supplementation of GEO at 1.2 g/kg DM significantly decreased TWG and ADWG of lambs.

The supplementation of GEO linearly decreased the ammonia concentration (Figure 3). This result is consistent with finding of Anassori *et al*. (39) who also reported that supplementation of GEO significantly decreased the ammonia concentration. On the other hand this result is not consistent with finding of Chaves *et al*. (28) who reported that supplementation of GEO fid not decrease the ammonia concentration. The difference between two experiments is associated with dose level of GEO supplementation. The dose level of GEO used in the experiment carried out by Chaves *et al*. (28) was 0.2 g/kg DM which is lower than those used in the current experiment.

Reduced rumen ammonia nitrogen due to GEO supplementation is probably explained by the limitations on microorganisms involved in the degradation of proteins in rumen (25,28,32). It can be suggested that supplementation of GEO can increase the efficiency of protein utilization in the rumen decreasing the degradability of protein.

The addition of GEO to lamb rations also significantly decreased the acetic, propionic, butyric and lactic acid and acetic acid/propionic acid ratio ($P < 0.05$). The results of the study obtained in the current experiment were similar to some studies (24,32) whereas the results of the study obtained in the current experiment was not consistent with those obtained by Chaves *et al*. (28); Patra and Yu (40); Mbiriri *et al*. (25).

The decrease in the concentrations of individual VFA and TVFA can be attributed to the limitation on overall ruminal fermentation due to antimicrobial effect of GEO (19,21,32). Decrease in acetic acid concentration in rumen fluid was significantly higher than that for propionic acid concentration, which resulted in decrease in AA/PP ratio. The fermentation of carbohydrate results in VFA which is the main energy sources of ruminant (41). The reduction in VFA production is not desirable and impairs the growth performance of ruminant animals. The reduction in VFA due to GEO supplementation up to 0.8 g/kg DM is tolerable in terms of TDWG and ADWG of lambs. However GEO supplementation after this point had a detrimental effect on those parameters previously mentioned.

Several researchers also reported that rumen pH was increased with the addition of garlic oil (22,24, 30). The increase of rumen pH due to supplementation of GEO to lamb feed rations is related to the decrease in the individual VFA and lactic acid production in rumen due to antimicrobial effect of GEO in rumen (19,21,22).

Supplementation of lamb diets with GEO significantly decreased the glucose level. This result is not consistent with findings of Chaves *et al*., (28), Anassori *et al*., (39) and Blanch *et al*. (32) who reported that the supplementation of GEO did not significantly affect blood glucose levels. The blood glucose level obtained in the study was lower than that determined by Chaves *et al*. (28) but higher than that reported by Anassori *et al*. (39) and Blanch *et al*. (32). Decreased blood glucose levels with the addition of GEO can be attributed to the decrease in the concentration of propionic acid, which is involved in the synthesis of glucose due to increased dose of garlic oil (Table 3) (42).

Blood protein and urea level obtained in the current experiment is consistent with finding of Anassori *et al*. (39). The decrease of blood urea nitrogen due to increased dose of GEO can be explained by the decrease of rumen ammonia formed in the rumen by showing antimicrobial effect (21,39). A decrease in the

concentration of rumen ammonia is due to lowering the ammonia level in blood and lowering the urea conversion rate of the liver (39,43,44).

Blood triglyceride level obtained in the study was lower than that determined by Chaves *et al*. (28) and Anassori *et al*. (39) who used garlic oil and lower than that determined by Pirmohammadi *et al*. (45) who used garlic in their experiment. The decrease in blood triglyceride level due to the increase in the dose of garlic oil can be explained by the decrease in the blood glucose level used as an energy source in animals and the increase in the rate of esterified fatty acid (45).

The insulin level obtained in the current experiment was lower than that determined by Blanch *et al*. (32). A decrease in insulin level due to an increase in the dose of GEO can be explained by a decrease in the amount of glucose that triggers insulin release to the blood (39,46). It can also be said that the use of esterified fatty acid as an energy source may result in decrease in insulin level (45).

Supplementation of garlic oil to fattening lamb diets decreased the blood cholesterol level significantly ($P < 0.05$). Some other researchers also reported that supplementation of garlic oil and garlic to fattening lamb diets significantly decreased blood cholesterol level (39,45,47) Reduction of blood cholesterol levels is associated with organosulfide compound in GEO which limited the enzyme responsible for cholesterol synthesis (19,24, 39, 47,48, 49).

Conclusion

The supplementation of GEO to the lamb diets had a significant impact on rumen fermentation, blood parameters and weight gain whereas GEO supplementation had no effect on daily average feed consumption and feed conversion ratio. Rumen fermentation parameters except pH were negatively affected by garlic oil supplementation. However, the addition of garlic oil to the lamb diets increased non-esterified fatty acid production from the blood parameters, while supplementation significantly reduced the levels of glucose, urea, protein, triglyceride, insulin and cholesterol. It can be said that the decrease of glucose, urea, triglyceride, insulin and cholesterol is important in terms of animal health when the blood parameters are taken into consideration. As conclusion, GEO supplementation up to 0.8 g/kg DM can be recommended for growing lambs to manipulate rumen and blood parameters without compromising important growth parameters.

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