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Effects of feed restriction and supplementary garlic oil on blood metabolites in ewes

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Abstract

The effects of garlic oil (GO) on serum fatty acids, lipid profiles and energy-related blood metabolites in feed-restricted fat-tailed ewes were investigated. For this purpose, twenty-eight dry, non-pregnant Makuei ewes (about three years of age; 45.00 ± 2.20 kg of body weight) were assigned randomly to four experimental groups including group 1 receiving basal diet as total mixed ration (TMR) without GO supplementation (control group), group 2 receiving 100% basal diet supplemented with GO (10.00 mg kg^{-1} BW), group 3 receiving 70.00% basal diet without GO supplementation and group 4 receiving 70.00% basal diet supplemented with GO (10.00 mg kg^{-1} BW). The main experimental period started eight weeks after performing adaptation and dietary allocations. Feed restriction reduced serum glucose levels along with higher serum levels of non-esterified fatty acids, triacylglycerols, β-hydroxybutyrate, low-density lipoprotein cholesterol, total cholesterol and very low-density of lipoprotein. Following feed restriction, the serum palmitic and oleic acids concentrations were increased. Garlic oil supplementation had a desirable effect on feed-restricted animals through lowering serum BHB and palmitic and oleic acids concentration and increasing the high-density lipoprotein cholesterol levels in the serum. These findings demonstrated that GO had the potential to reduce body-fat mobilization, thereby lowering the risk factors for disorders associated with negative energy balance in underfed ewes in the periparturient period.

Introduction

Poor pasture grazing, particularly in long drought periods, often results in failing to fulfil animal's requirements, causing negative energy balance (NEB) and harmful effects on animal production and health. After parturition, production of colostrum or milk, particularly in twin bearing ewes, increases energy requirements; but, low feed intake capacity restricts the animal energy intake and makes NEB a common phenomenon in this period.[1] Adipose tissue plays a critical role in maintaining energy homeostasis in early lactating ruminants.[1] Consequently, animals tend to satisfy the energy requirements through lipid mobilization from adipose tissues and experience extensive physiological changes in lipid metabolism.[1] However, very high and uncontrolled NEB can cause extensive adipose tissue lipolysis and increase the risk of energy-related disorders.[1] Accordingly, control of body reserve mobilization is one of the most important targets for research and development of new strategies to alleviate NEB. Natural compounds like plant essential oils have been recently considered for their positive effects on animal growth, health and performance.[2] Studies about the effects of plant derived active substances on rumen fermentations and improved feed efficiency can be considered as an interesting and potential way to control the metabolic consequences of NEB; but, there is not a detailed information about the effects of plant active chemicals on NEB in ewe.[3,4]

Owing to the active sulfur and other phenolic compounds, garlic (*Allium sativum*) has been widely used for its beneficial effects. Garlic and its components have been reported to be effective in improving propionate to acetate ratio, reducing methane emission and affecting glucose metabolism.[5-7] Furthermore, the lipid lowering effects of garlic have been extensively studied in humans

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and non-ruminant animals.[8,9] Recent studies regarding the mechanisms and effects of garlic on reduction of tissue lipolysis may provide a new perspective on control of NEB.[10,11] To the best of our knowledge, the literature is poor concerning the effects of garlic and its constituents on the blood lipid profile associated with energy metabolism. Studies on lambs and adult male sheep have demonstrated that garlic supplementation is not effective on non-esterified fatty acids (NEFA), blood β-hydroxy-butyrate (BHB), triglycerides and cholesterol.[5,6] In this regard, Zhu *et al.,* have obtained similar results.[12] However, they found that the NEFA concentration was lower in dairy goats fed with garlic oil (GO). Other researchers have indicated that garlic supplementation has a tendency to lower adipose tissue mobilization and could result in improved energy balance in sheep.[10] Accordingly, the present study aimed to investigate the effects of garlic essential oil supplementation on fatty acid profiles and blood energy-related metabolites in feed-restricted fat-tailed ewes.

Materials and Methods

Animals. According to the Ethics Committee rules,[13] sampling, caring and handling of the animals were confirmed by the Research Affairs of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. A total of twenty-eight dry, non-pregnant Makuei ewes (about three years of age; 45.00 ± 2.20 kg of body weight) with an approximate body condition score of 3.00 (3.00 ± 0.25) on a 1 to 5 scale were selected. The animals were maintained in a well-ventilated shed having individual feeding and partitioning. The experimental animals were fed twice daily (08:00 and 17:00 hr) with a fixed diet for 21 days and then, their diet was gradually changed to experimental diets.

Experimental design and treatments. Ewes were randomly assigned to either *ad libitum* access to feed, or feed restriction to 70.00% of the feed intake baseline and allocated to four groups including control (basal diet with no GO supplementation; CON), 100% basal diet supplemented with 10.00 mg kg^{-1} BW of GO (CON-GO), 70.00% basal diet with no GO supplementation (RES) and 70.00% basal diet supplemented with 10.00 mg kg^{-1} BW of GO (RES-GO). The doses of GO were calculated based on our previous work.[3,6] Garlic oil extraction was carried out by the conventional method described by Clevenger.[14] The basal diet was given as total mixed ration (TMR) containing 2.01 Mcal of metabolizable energy kg^{-1} of dry matter (DM) with 2.00% of feed refusals (Table 1).[15] For eight weeks, the animals in RES and RES-GO groups were restricted feed to receive 70.00% of baseline intake in CON group. This feeding level was in line with that of traditionally pasture-grazed feeds.

Biochemical analysis. General clinical signs and health factors were daily monitored and daily feed intake was measured. Before the morning feeding, on two consecutive days at the end of the experiment blood samples were obtained through jugular vein via 10.00 mL vacuum tubes without anticoagulants containing potassium oxalate and sodium fluoride. After clotting, the blood samples were centrifuged at 2,500 *g* for 10 min at 4.00 ˚C for sera collection. Serum glucose, BHB, NEFA, total cholesterol (TCH), triacylglycerols (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), very low-density lipoprotein (VLDL-C), albumin, total protein and fatty acid profiles were examined as explained below. The NEFA and BHB were analyzed using a NEFA kit and a D-3-hydroxybutyrate kit (Randox Laboratories, Ardmore, UK), respectively. In addition, concentrations of TCH, TG, LDL-C, AST, HDL-C, total protein, albumin and glucose were measured using an auto-analyzer spectro-photometer (Model RA-1000; Technicon Instruments Corporation, Tarrytown, USA) and commercial kits (Pars Azmoon, Tehran, Iran) and the VLDL-C concentrations were calculated according to the following equations: VLDL-C= TG/5.[16] A Finnigan gas chromatograph (Thermo Finnigan, Somerset, USA) equipped with an mass spectrometry (MS) detector was used to analyze the GO components, as explained formerly.[3] All the chemicals, solvents and reagents used in lipid extraction and preparation of fatty acid methyl esters (FAME) were of analytical grade and solvents were redistilled before use. As described previously, to avoid fatty acid oxidation, lipid extraction was carried out three times with chloroform/methanol (2/1 v/v) to a final volume of 100 mL administered under the argon gas blanket.[17] After each extraction step, the flasks were centrifuged (1,800 *g* for 10 min) and the organic fraction was separated and injected into a 100-mL volumetric flask. Afterward, using a rotary evaporator (Büchi, Flawil, Switzerland), they were treated with anhydrous Na-sulfate to be dry and then, vaporized at 40.00 ˚C under vacuum. Fatty acid methyl esters were prepared through mild methanolysis/methylation via methanolic hydrochloride acid, according to the method explained by Ichihara and Fukubayashi.[18] Hexagon was used as a solvent to extract the FAME and the solution was dried with anhydrous Na-sulfate (Merck, Darmstadt, Germany). Nonadecanoic acid was utilized as an internal standard and gas chromatography (GC) analysis was conducted with a gas chromatograph (Varian CP-3800; Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (FID). The samples (1.00 µL) were injected in split mode, 50:1, into a capillary column for FAME (100 m × 250 µm × 0.20 µm), (CP-Sil 88; Chrompack, Middelburg, The Netherlands). The detector and injector temperatures were set at 250.00 ˚C. Nitrogen with a constant flow of 1.00 mL per min was used as a carrier gas. The oven temperature was 70.00 ˚C for 1 min and then, it was increased 5.00 ˚C per min to 100˚C and kept for 2 min.

Then, the column temperature was increased with a rate of 10.00 ˚C per min to 175 ˚C and maintained for 35 min. Eventually, the temperature was increased 4.00 ˚C per min to 225 ˚C and kept for another 35 min. The individual peaks of the FAME were specified based on a FAME standard mix (GLC 463; Nu-Chek Prep Inc., Elysian, USA).

Statistical analysis. In the present study, a completely randomized design was employed. Using a general linear model procedure of SAS (version 9.1.3; SAS Institute, Cary, USA), the data were analyzed by the least squares analysis of variance. Using Tuckey, LSmeans were adjusted and compared to the PDIFF option. The findings were showed as corresponding SEM and LSmeans. The results were regarded as significant at $p < 0.05$.

Results

Table 1 presents the ingredients and chemical compositions of basal diet and Table 2 shows the essential composition of GO. Trisulfide di-2-propenyl and diallyl disulfide were the main oil components. The NEFA concentration as an index of nutritional status increased in the RES group compared to the CON group (NEFA values: 0.66 *vs*. 0.33, respectively; Table 3).

Blood glucose concentration with restricted feeding was significantly reduced ($p < 0.05$). Concentrations of BHB, NEFA and TG were higher in feed-restricted animals compared to those fed *ad libitum*. However, in the feed-restricted groups, BHB values in group supplemented with GO (RES-GO group) were lower than those of the restricted fed groups without GO supplementation (RES group, $p < 0.05$; Table 3).

Table 1. Ingredients and chemical composition of experimental basal diet.

Ingredient	DM (%)
Alfalfa hay	45.00
Corn silage	15.00
Wheat straw	23.50
Wheat bran	10.00
Barley grain	5.00
Mineral-vitamin mixture	1.00
Salt	0.50
Chemical composition	
CP (%)	11.20
EE (%)	2.10
NDF (%)	56.00
Ash (%)	7.30
Calcium (%)	0.51
Phosphorus (%)	0.26
ME (Mcal kg^{-1} DM)	2.01
BCS change per day*	0.0024

DM: Dry matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ME: Metabolizable energy; BCS: Body condition score. * Calculated by the Small Ruminant Nutrition System (SRNS; version 1.9.4468) Software.

Table 2. Chemical composition of the garlic oil.

Components	Area (%)	Apex RT
Diallylsulfide	0.13	5.13
3,4-Dimethylthiophene	0.06	5.57
1,3-Dithiane	6.09	6.03
Disulfide, methyl 1-propenyl	0.67	6.30
Disulfide, methyl 1-propenyl	1.34	6.58
Propane, 2,2-bis(ethylthio)	0.13	7.20
Dimethyl trisulfide	1.76	7.50
Diallyl disulfide	37.30	11.74
Trisulfide, methyl 2-propenyl	16.98	13.76
1-Propene, 1-(methylthio)-, (Z)-	0.14	14.17
3-Vinyl-4H-1,2-dithiin	2.10	15.99
Trisulfide, di-2-propenyl	29.6	16.65
Disulfide, dipropyl	0.21	19.71
1-Propene, 3,3'-thiobis-	0.72	19.99
Disulfide, methyl 2-propenyl	0.30	21.63
Tetrasulfide, di-2-propenyl	1.39	26.56

RT: Retention time

Blood glucose concentration with restricted feeding was significantly reduced ($p < 0.05$). Concentrations of BHB, NEFA and TG were higher in feed-restricted animals compared to those fed *ad libitum*. However, in the feed-restricted groups, BHB values in group supplemented with GO (RES-GO group) were lower than those of the restricted fed groups without GO supplementation (RES group, $p < 0.05$; Table 3).

Following feed restriction, serum concentrations of LDL-C, VLDL-C and TCH and AST activity were significantly increased. Garlic oil supplementation was not effective on the parameters ($p > 0.05$) except for AST activities that were significantly reduced with GO supplementation ($p < 0.05$). Serum total protein and albumin remained constant ($p > 0.05$) in feed-restricted or normal ewes without or with GO supplementation.

Table 4 presents the changes in the serum fatty acid profiles of experimental ewes. In this lipid fraction, the stearic, linoleic, oleic and palmitic acids were the dominant fatty acids. Contradictory results from non-saturated and saturated fatty acid as well as a response to restricted feeding were seen with an increase in hexadecadienoic (C16:2), palmitic (C16:0) and oleic (18:1 cis-9) acids or a reduction in trans-10 C18:1, margaric (C17:0), arachidonic (20:4n-6), linoleic (18:2n-6), docosahexaenoic (22:6n-3) and eicosapentaenoic acids (22:5n-3). In feed-restricted ewes (Table 4), GO showed compensatory effects on some losses in serum n-3 fatty acids (22:6n-3). The high level of oleic and palmitic acids was seen in undernourished ewes. However, GO reduced the concentration of palmitate. No statistically significant changes were found in linolenic (18:3n-3) concentrations in the experimental groups. However, it seems that feed-restricted ewes had a tendency to have lower values ($p < 0.10$).

Table 3. Least square means for blood metabolites of normal or feed restricted ewes supplemented with garlic oil.

Parameters	Treatments				SEM	*p*-value
	CON	CON-GO	RES	RES-GO		
Glucose (mg dL^{-1})	55.96^{a}	55.88^{a}	52.17^{b}	52.19^{b}	0.57	**
BHB (mmol L^{-1})	0.34^{c}	0.33^{c}	0.81^{a}	0.68^{b}	0.014	***
NEFA (mmol L^{-1})	0.33^{b}	0.33^{b}	0.66^{a}	0.62^{a}	0.014	***
Cholesterol (mg dL^{-1})	53.97^{bc}	52.71^{c}	59.86^{a}	57.37^{ab}	1.41	*
HDL (mg dL^{-1})	34.48^{bc}	33.88^{bc}	36.04^{ab}	37.27^{a}	0.75	**
LDL (mg dL^{-1})	19.36^{b}	18.83^{ab}	22.08^{a}	21.25^{a}	0.47	*
VLDL-C (mg dL^{-1})	4.35^{b}	4.11^{b}	5.02^{a}	4.78^{a}	0.14	***
TG (mg dL^{-1})	21.76^{b}	20.58^{b}	25.12^{a}	23.92^{a}	0.33	***
AST (IU L^{-1})	78.81^{c}	76.25^{c}	89.04^{a}	81.80^{bc}	2.00	*
TP (mg dL^{-1})	7.01	6.93	6.97	7.03	0.17	NS
Albumin (mg dL^{-1})	3.65	3.64	3.66	3.74	0.17	NS

BHB: β-hydroxybutyrate; NEFA: Non-stratified fatty acids; HDL: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein cholesterol; VLDL: Very low-density lipoprotein; TG: Total triglycerides; AST: Aspartate aminotransferase; TP: Total proteins.
^{abc} Data in each row with different letters differ significantly ($p < 0.05$).
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: Non-significant.

Table 4. Serum fatty acid composition of normal or feed restricted ewes supplemented with garlic oil.

Fatty acid (g per 100 g FAME)	Treatments				SEM	*p*-value
	CON	CON-GO	RES	RES-GO		
12:0 (Lauric acid)	0.29	0.25	0.29	0.28	0.03	NS
14:0 (Myristic acid)	0.46	0.38	0.42	0.41	0.06	NS
15:0 (Pentadecylic acid)	0.73	0.74	0.68	0.72	0.06	NS
16:0 (Palmitic acid)	18.19^{b}	17.68^{b}	20.11^{a}	18.12^{b}	0.42	*
16:1 cis-9 (Palmitoleic acid)	0.53	0.49	0.45	0.52	0.05	NS
16:2 (Hexadecadienoic acid)	0.16^{c}	0.18^{b}	0.21^{a}	0.19^{b}	0.005	**
16:4n-3 (Hexadecatetraenoic acid)	0.21	0.22	0.19	0.22	0.08	NS
17:0 (Margaric acid)	0.84^{a}	0.67^{c}	0.72^{bc}	0.76^{b}	0.02	*
18:0 (Stearic acid)	22.13	21.49	22.67	22.87	0.85	NS
18:1 cis-9 (Oleic acid)	11.54^{b}	12.11^{b}	16.12^{a}	15.03	0.74	***
trans-11 C18:1 (Vaccenic acid)	0.87	0.88	0.74	0.82	0.21	NS
trans-10 C18:1	0.94^{a}	1.11a	0.69^{b}	0.72^{b}	0.09	*
18:2n-6 (Linoleic acid)	34.11^{ab}	35.49^{a}	30.12^{c}	32.16^{bc}	0.82	**
18:3n-3 (α-Linolenic acid)	1.19	1.46	0.97	1.14	0.15	NS
18:4n-3 (Stearidonic acid)	0.76	0.75	0.84	0.81	0.18	NS
20:00 (Arachidic acid)	0.35	0.42	0.39	0.37	0.01	NS
20:1cis (Gondoic acid)	0.28	0.33	0.36	0.33	0.04	NS
C20:3n-6 (Eicosatrienoic acid)	0.34	0.42	0.29	0.32	0.07	NS
20:4n-3 (Eicosatetraenoic acid)	0.11	0.13	0.12	0.11	0.04	NS
20:4n-6 (Arachidonic acid)	0.64^{ab}	0.72^{a}	0.45^{b}	0.58^{b}	0.08	**
20:5n-3 (EPA)	0.02	0.02	0.01	0.01	0.03	NS
22:5n-3 (DPA)	0.42^{a}	0.62^{a}	0.31^{b}	0.38^{b}	0.04	***
22:6n-3 (DHA)	0.65^{a}	0.62^{a}	0.26^{c}	0.48^{b}	0.025	***
24:0 (Lignoceric acid)	0.37	0.39	0.21	0.31	0.10	NS
SFA	43.36^{ab}	42.44^{b}	45.49^{a}	43.84^{ab}	0.87	*
MUFA	14.16^{b}	14.92^{b}	18.36^{a}	17.42^{a}	0.85	*
PUFA	38.61^{ab}	40.45^{a}	33.77^{c}	36.40^{b}	1.02	*

FAME: Fatty acid methyl esters; EPA: Eicosapentaenoic acid; DPA: Docosapentaenoic acid; DHA: Docosahexaenoic acid; SFA: Saturated Fatty acids; MUFA: Mono unsaturated fatty acids; PUFA: Poly unsaturated fatty acids.
Data in each row with different superscripts were statistically different ($p < 0.05$).
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS: Non-significant.

Discussion

Serum NEFA and BHB concentrations in the current study increased through feed restriction. In the feed-restricted animals, the increase in NEFA concentration with a concomitant decrease in glucose concentration showed fat mobilization from adipose tissues and a low energy status (Table 3). Hence, lower glucose concentration is an indicator of lower energy consumption. These findings were consistent with those of other studies on dairy cows and pregnant ewes in which negative energy balance was observed.[19,20] Nevertheless, it was indicated that in feed-restricted cows, an energy restriction of 50.00% in a short period did not increase BHB concentration;[21] the smaller increase in the NEFA concentration serving as a substrate for ketone body production might explain this issue.

The blood BHB values showing NEB in ewes were reported to be between 0.80 and 1.60 mmol L^{-1}.[22] In the current study, high serum TG and VLDL-C levels accompanied with borderline BHB values (0.81 mmol L^{-1}) indicated lower body-fat mobilization owing to possible adaptation of ewes to under nutrition as a consequence of the long-term study of probable adaptations in fatty acid metabolism in extra-adipose tissues. Ewes' significant ability to adapt to low-level nutrition has already been showed.[23] In this study, no significant effects of GO supplementation were found on blood energy-related metabolites in normal or restricted fed animals except for triacylglycerol and BHB being decreased significantly in ewes fed on restricted diet with GO supplementation (RES-GO group). Kamruzzaman *et al.,* have found low NEFA concentration and a greater blood glucose in sheep fed with garlic silage.[10] In another study, Zhu *et al.,* have demonstrated that treatment with GO decreases NEFA concentration and increases lipase activity in dairy goats.[12]

The mechanism of changing lipid profiles by garlic or garlic compounds has not been fully investigated. In general, garlic can decrease the hepatic activities of cholesterogenic and lipogenic enzymes such as fatty acid synthase, malic enzyme, β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase and glucose 6-phosphate dehydrogenase, offering a possible explanation for the garlic lowering effect on serum TG and cholesterol.[24] Yeh and Liu, and Duehlmeier *et al.,* have indicated that changes in BHB values in the blood of sheep might be dependent on the rate of ketogenesis and lipolysis rather than the utilization rate of ketone body.[24,25] Therefore, in the present study, GO could have reduced the catabolism of free fatty acids in feed-restricted ewes, which in turn resulted in animals' energy status improvement and lower plasma BHB concentrations. Moreover, the compensatory GO effects on ewes' energy status may be owing to the garlic effects on rumen fermentation characteristics. Previous studies have demonstrated that feed restriction in ruminants significantly reduces ruminal volatile fatty acids (VFA) concentration and its absorption across the reticulorumen.[26] The effects of GO and raw garlic have already been reported on rumen fermentation efficacy and increase in concentrations of propionate to the acetate ratio in sheep.[3] Also, the effects of GO supplementation on BHB value in feed-restricted ewe may be expounded by elevation in insulin response resulting in hypoglycemia and hypolipidemia along with improvements in the rumen propionate proportion induced by garlic supplementation.[3,27] Propionate is a potent insulin secretagogue and one of the major glucogenic precursors.[28] Insulin plays a crucial role in the metabolism of lipids through inhibition of lipolysis (reduction of NEFA) or ketogenesis and stimulation of lipogenesis.[29] Furthermore, the effects of GO on restricted fed animals are numerically more significant than those on the control group. These findings were consistent with those obtained by Cullen *et al.* demonstrating better feed conversion with depressed feed intake in pigs with garlic diets.[30] These findings show that garlic can change feed utilization efficacy and lipid metabolism more drastically in animals having low energy status compared to normal fed animals. Non-esterified fatty acids mobilized from fat resources are transferred to the liver, where re-esterification to TG occurs.[31] Therefore, TG are packed in VLDL-C to be exported from the liver.[32] Very low-density lipoproteins are the major triacylglycerol carriers in blood.[33] In the present study, higher concentrations of VLDL-C and TG in feed-restricted ewes might be associated with the high level of lipoproteins secretion by the liver in response to under feeding. The LDL-C and HDL-C are the main components of phospholipids and cholesterol. The variations in VLDL-C metabolism and concentration may be related to the changes in metabolism of triglycerides.[34] Feed restriction rises the concentration of LDL-C and cholesterol as a product of VLDL-C catabolism.[34] It has been demonstrated that GO s-components are the potent monooxygenase inhibitors preventing cholesterol synthesis.[24,35] In addition, Mohammadi and Abbasi Oshaghi have indicated that garlic extract could reduce cholesterol and triacylglycerol and increase HDL-C levels through stimulating liver X receptor's expression in the mice intestine.[36] Furthermore, decreased VLDL-C secretion referred as reduced microsomal triacylglycerol transfer protein gene expression in rats received garlic supplementation.[37]

Tanaka *et al.,* have indicated that an increase in malate dehydrogenase and AST activities in the malate–aspartate shuttle needs translocation of cytosolic NADH into mitochondria.[38] In addition, these authors found that in the liver of restricted fed ewes, phosphoenolpyruvate carboxykinase changes in gluconeogenesis and higher fructose were assumed as responses to compensate for

lower energy absorption.[38] Therefore, in this study, activity of serum AST in the feed-restricted ewes was higher than the one in normal feeding groups. In this regard, with a lower nutrition plan in the ewes, GO supplementation exerted a suppressing effect on AST activity. Thus, it might show a tendency for decreased blood lipids and ketone in GO-treated ewes.

In the current study, feed restriction led to increased proportions of hexadecadienoic, oleic, and palmitic acids. Body fat mobilization occurs mainly in the form of free fatty acids when energy balance is negative. The palmitic, stearic and oleic acids have been explained to be the amplest fatty acids in the ruminant's adipose tissues.[39] Therefore, NEFA profiles may directly reflect the fatty acid composition of adipose tissue during periods of negative energy balance. Accordingly, the higher levels of oleic and palmitic acids concentration seen in feed-restricted ewes could be associated with the NEB-induced lipolysis and/or increases in their synthesis from stearic acid. These variations in serum fatty acid profile were consistent with the findings obtained by Neil Douglas *et al.,* indicating that oleic and palmitic acids were increased within negative energy balance and following parturition.[40] In this respect, Rowe *et al.,* have reported the potential of palmitic acid to raise LDL-C.[39] High palmitic acid levels are not favorable. In the present study, GO supplementation reduced serum palmitic acid in feed-restricted ewes; this could be the potential pathway for beneficial effects of GO, as research results showed inflammatory effects for palmitate. Previous studies had also indicated an increase in unsaturated fatty acids in avian and the effects of garlic supplementation in reducing palmitic acid and saturated fatty acids. [41] Garlic oil supplementation did not alter the levels of linolenic, myristic and stearic acids. Feed restriction led to a lower concentration of linoleic, trans-10 C18:1, arachidonic, docosahexaenoic and eicosapentaenoic acids. Nevertheless, GO appeared to have a beneficial effect by compensating for some of these losses. The effects were more considerable on serum concentration of docosahexaenoic acids. The underlying mechanism is vague. However, considerable researches have depicted some pathways. The adenosine monophosphate activated protein kinase (AMPK), a cellular energy indicator, has been indicated to react to different conditions reducing cellular energy levels like starvation (especially glucose). Nearly all sulfur-containing compounds in the garlic are also implicated in the increase of AMPK activation.[42] Anti-lipogenic effects of garlic seem to be owing to an increase in AMPK activity. The AMPK activation is a factor decreasing the expression of multiple active genes in adipogenesis.[43] The AMPK represses CoA carboxylase-1 (ACC-1), which in turn, results in a decrease in malonyl-CoA. Malonyl-CoA inhibits carnitine acyltransferase (CPT-1), which is the enzyme responsible for the transport of fatty acids into mitochondria for oxidation.[44] The inhibition of linoleic acid or other unsaturated fatty acids oxidation is extremely sensitive to malonyl-CoA.[45]

In the rumen, this could be important for conserving the essential fatty acid in which absorption of the diet unsaturated fatty acids is restricted by rumen biohydrogenation. Garlic oil has been demonstrated to have anti-bacterial activity.[46] Thus, it seems that garlic might increase the bioavailability of essential fatty acids for ruminants.

In a study conducted by He *et al.,* supplementation of GO to the fat cell media reduced the amount of oleic acid (C18:1), cellular stearic acid (C18:0), myristoleic acid (C14:1), myristic acid (C14:0), palmitic acid (C16:0) and C16:1. Their study indicated that GO enhanced the activity of glycerol-3-phosphate dehydrogenase, leading to the observed changes in fatty acid profiles.[47]

To the best of the author's knowledge, among ruminants, data regarding the effects of plant essential oils on blood lipid profile are extremely limited. Further researches are necessary to investigate the mechanism by which GO changes lipid metabolism in sheep.

In conclusion, this study found that GO supplementation at a dose of 10.00 mg kg^{-1} reduced lipolysis and altered lipid profile in feed-restricted ewes. The BHB values were lower in feed-restricted ewes with GO supplementation. However, BHB levels in RES-GO group were not decreased to the levels of that in the control group. Moreover, GO showed compensatory effects on some losses in serum n-3 fatty acids. Thus, during sub-optimal feeding conditions, supplementing GO might be an effective strategy to modulate negative energy balance.

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Conflict of interest

The authors declare that there is no conflict of interest.

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