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The Effect of Medicinal Plants Mixture and Organic Acid on Growth Performance, Blood Parameters, Immune System Status, Carcass Characteristics and Nutrient Digestibility in Moghani Male Lambs

Research Article

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ABSTRACT

This study was performed to investigate the effect of medicinal plants and organic acid on growth performance, blood parameters, immune system, carcass characteristics and nutrient digestibility in Moghani lambs. For this, 30 Moghani male lambs (28±2 kg average weight) were used in 6 treatments and 5 replications as a completely randomized design in factorial (2*3*3). experimental treatments include: basic diet (control), basic diet + 2 g organic acid, basic diet + 2% medicinal plants mix, basic diet + 4% medicinal plants mix, basic diet + 2% medicinal plants mix + 2 g organic acid, and basic diet + 4% medicinal plants mix + 2 g organic acid. The results showed that the main effect of organic acid and medicinal plants and the interaction between them on dry matter intake, feed conversion ratio and carcass characteristics were not significant. The daily weight gain was higher in the group receiving 2 g of organic acid than the 0 g and it was higher in the group receiving 2 and 4% of the medicinal plants mix than the group receiving 0%. The groups receiving 2 and 4% medicinal plants mix had the lowest blood cholesterol, triglycerides, and urea. The serum total protein increased compared to group receiving 0%. The groups receiving 4% medicinal plants mix + 2 g organic acid had the highest level of total antioxidant activity and the lowest concentration of malondialdehyde. The activity of superoxide dismutase enzyme was improved by receiving of 2% medicinal plants mix + 2 g organic acid. The apparent digestibility of nutrients was improved by receiving 4% medicinal plants mix + 2 g organic acid.

KEY WORDS blood parameters, immune system, lamb, medicinal plant, organic acid.

INTRODUCTION

The world's population is growing exponentially and is estimated to reach 9 billion by 2050 (Matzuk and Lamb, 2002). With this population growth, the powerful technology to supply food is necessary and the agricultural industry as the most central industry plays a very important role. in this regard, animal husbandry as one of the main branches of agriculture in the supply of animal protein are very important (Rashidi *et al.* 2008). The most important sources of meat are beef, sheep and goats. Due to the climatic, cultural and religious conditions in Iran, fattening lamb is one of the main sources of protein, so that the meat produced by sheep and goats is about 57% of red meat produced in the country.

Because of the ban on the use of growth-promoting antibiotics by the European Union in 2006 for livestock and poultry (Melendez *et al.* 2006), it is therefore essential to identify alternatives to growth-promoting antibiotics in the food industry. Suitable alternatives include Medicinal plant,

plant essential oils, organic acids, probiotics, prebiotics, enzymes, etc. (Wezyk *et al.* 2000). Numerous studies in Africa, Asia, Europe, Latin America, and North America show that medicinal plants are commonly used to treat livestock diseases (Yinegar *et al.* 2007) as well as They can be used as precursors for the synthesis of beneficial drugs (Farjani *et al.* 2014). The industry has stimulated interest in secondary compounds of plants or plant extracts such as essential oils as potential alternatives to antibiotic growth in livestock production (Thakare, 2004; Westendarp, 2005). These compounds exhibit selective antimicrobial activity and can prevent protein breakdown in the rumen, thus potentially increasing the supply of amino acids in the intestines of host animal (Wallace, 2004). The plant compounds, when added as feed components, have a wide range of effects on digestibility and blood parameters in ruminants. They can affect other blood parameters in sheep by maintaining plasma glucose concentration (Raghuvansi *et al.* 2007), while some other plants can increase plasma glucose concentrations in calves (Mohammed *et al*. 2004). Most medical plants are safe and affordable and have no problems with drug resistance.

These plants are used as digestive stimulants, antidiarrhea, disinfectant, anti-inflammatory, anti-parasitic, appetite stimulant and growth in animal. They also act as antibacterial, antioxidant, anticancer, antifungal, analgesic, and insecticidal and antioxidant agents (Tipu *et al.* 2006). Plants can help livestock nutrient requirements and stimulate the endocrine system and nutrient metabolism (Wenk, 2003). In addition to medicinal plants, the use of organic acids has recently been recommended in animal nutrition to improve performance.

The organic acids are known as an organic carboxylic acid with R-COOH structure, including fatty acids and amino acids that have antimicrobial activities similar to antibiotics and cause significant benefits in livestock (Partanen, 2001; Øverland *et al.* 2007). The most common organic acids are acetic, benzoic, butyric, citric, formic, lactic, malic, propionic, and tartaric acids. The effects of organic acids on increasing of growth have been confirmed, especially in young animals (Tung and Pettigrew, 2006). Many organic acids with positive effects on livestock performance are also known as food preservatives (Chaveerach *et al.* 2002). Bacterial depletion is associated with the feeding of organic acids, especially to non-acid-producing species such as Escherichia coli, *Salmonella* and *Campylobacter*, and due to the broad effects, they are effective on livestock performance, including improved protein and energy utilization, reduced production ammonia and other microbial metabolites reducing the growth and regulation and secretion of immune mediators.

The organic acids have several side effects, including decreased digestive pH, increased pancreatic secretion, and trophic effects on the gastrointestinal mucosa. By lowering the pH in the stomach, they optimize the conditions for pepsin activity and increase the digestion of nitrogen, phosphorus, and minerals (Dibner and Buttin, 2002). According to above, medicinal plants and organic acids are types of additives that are easily available and it is necessary to evaluate their effects on the nutrition of the country's livestock as food additives. So, the aim of the present study was to investigate the effect of a mixture of medicinal plants and organic acid on growth performance, blood parameters, immune system, nutrient digestibility and carcass characteristics in male lambs..

MATERIALS AND METHODS

Raw materials

The medicinal plants were including Echinacea purpurea, Mint, Thyme and Eucalyptus, which is prepared by Paya Tejarat Setareh Sabz Company located in Tehran, Iran. The organic acid used in this study was AND CID 1 made by AND GROUP Spain, has eight key acids including: formic acid, propionic acid, lactic acid, citric acid, acetic acid, phosphoric acid, butyric acid and other Short and medium chain fatty acids.

The place and the time of study

This study was conducted in the sheep farm of Mohaghegh Ardabili University, Iran. It lasted from early August 2019 to mid-November 2019 for 12 weeks, including 2 weeks of adaptation and 10 weeks of experimental period.

Animals and experimental treatments

This experiment was performed by 30 male Moghani lambs with an average initial weight of 28 ± 2.2 kg in a completely randomized design in the form of factorial (2*2*2) with six treatments and five repeat for 12 weeks (two weeks adaptation period and 10 week experimental period). During the adaptation period, the animals were given the required vaccines and antiparasite drugs. Lambs had been fed a basic diet (Table 1) formulated using sheep CNCPS software (version 21.0.10) and based on the nutritional requirement of a 28 kg lamb according to NRC (2007) and according to the chemical composition of available food. The treatments were including: 1) control (basal diet), 2) basal diet + 2 g organic acid/day/head, 3) basal diet + 2% medicinal plants mix/kg DM, 4) basal diet + 4% medicinal plants mix/kg DM, 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head, 6) Treatment containing basal diet + 4% medicinal plants mix/kg DM + 2 g organic acid/day/head.

Experimental diets were given to lambs at two times in the morning and in the afternoon. At the same time, clean and healthy water was always provided freely to the lambs.

The performance characteristics

The feed intake was recorded daily based on the feed given and the remaining feed of the previous day. The daily feed intake was adjusted so that the remaining feed in the stall was about 10% of the feed given to the livestock each day. The weight gain was measured on the first day and then every two weeks before the morning meal. The feed conversion ratio was calculated using the average ratio of feed intake per weight gain. Measurement of apparent nutrient digestibility was also measured using the AOAC (2000) method.

The blood parameters

The blood samples of lambs were obtained on days 35 and 70, 3 hours after feeding in the morning. The obtained serum and plasma were stored at -20 °C until laboratory tests. Concentrations of glucose, creatine, urea, total protein, cholesterol and triglycerides were measured using Pars Azmoun kits by Alpha Classic-AT plus system. Immune system parameters including glutathione peroxidase, superoxide dismutase, malondialdehyde and total antioxidant activity were measured using the related kits by ELISA spectrophotometer.

The carcass characteristics

After slaughtering the lambs, the weight of the head, legs, testicles, liver, lungs, kidneys and heart were measured. After complete emptying of the viscera, the weight of the hot carcass was determined and to determine the weight of the cold carcass, the carcasses were kept at 4 °C for 24 hours and weighed again. Subcutaneous fat thickness was measured with a caliper. The carcasses were divided into different parts including thigh, head, row, trunk and neck and weighed to examine the characteristics of the carcass.

Statistical analysis

The data were analyzed using a completely randomized design in factorial and GLM procedure of SAS software (SAS, 2003). Duncan's multiple range test ($P<0.05$) was used to compare the mean.

$$Y_{ij}= \mu + b\ (Xi-\bar{X}) + A_i + B_j + AB_{ij} + e_{ij}$$

Where:

Yij: observed value.

μ: mean.

$b\ (Xi-\bar{X})$: covariate factor.

A_i: organic acid.

B_j: medicinal plants.

AB_{ij}: interaction of organic acid and medicinal plants.

e_{ij}: experimental error.

RESULTS AND DISCUSSION

The results of experimental treatments on the dry matter intake and daily weight gain in different periods are shown in Table 2 and the results of experimental treatments on total dry matter intake, total weight gain and feed conversion ratio are shown in Table 3. Based on these results, the main effect of organic acid, the main effect of medicinal plants mixture and the interaction between them did not have a significant effect on the dry matter intake, but the amount of dry matter intake in the second period increased significantly compared to the first period. The results of the main effect of organic acid showed that 2 g organic acid caused a significant increase in daily weight gain ($P<0.05$). The daily weight gain was significantly increased in 4% medicinal plants mixture compared to the others ($P<0.05$). The periodic weight gain and overall weight gain were significantly increased in the group receiving 4% medicinal plants mixture, but did not make a significant difference in feed intake and feed conversion ratio.

The reason of more weight gain by consuming the same food can be due to the effect of medicinal plants in increasing the ratio of propionate to acetate, which is involved in energy metabolism (Chaves *et al.* 2008). The effect of medicinal plants and their essential oils on dry matter intake can vary depending on the type of medicinal plant and diet, dietary interaction or adaptation of the rumen microbial population to plants and essential oils (Yang *et al.* 2010a; Yang *et al.* 2010b; Geraci *et al.* 2012). In addition, it has been reported that dry matter intake can be affected by a number of dietary or managerial factors such as body weight, animal growth stage, physical and chemical properties of feed, or rumen fermentation metabolites (Yang *et al.* 2010b). The findings of the present study for dry matter intake were consistent with previous research on growing lambs (Chaves *et al.* 2008), sheep (Distel *et al.* 2007) and cattle (Benchaar *et al*. 2007).

According to Seifzadeh *et al.* (2017), adding 3% of a mixture of medicinal plants including pennyroyal, mint, eucalyptus, garlic, cumin, thyme and thyme didn't have a significant effect on feed intake in calves. The effect of organic acids in livestock can be very different due to various factors such as the type of acid and its pKa, the amount of intake, feed composition, buffering capacity, feed taste, etc. (Decuypere and Dierick, 2003). According to Nematpour *et al.* (2014), fattening calves fed diets containing barley grains treated with lactic acid and citric acid did not make a significant difference in daily dry matter intake compared to the control group.

Table 1 Diet ingredient and chemical composition

Ingredient	Percent	Chemical composition	
Alfalfa	45	Dry matter (%)	87.39
Barley	33	Metabolizable energy (Mcal/kg)	2.3
Corn	10.5	Organic matter (%)	92.8
Molasses	5	Crude protein (%)	14.5
Wheat bran	5	Crude fat (%)	2.9
Calcium carbonate	0.2	NDF (%)	33.2
Sodium bicarbonate	0.5	Ash (%)	7.5
Salt	0.5	Calcium (%)	0.73
Vitamin-mineral premix[1]	0.3	Phosphorus (%)	0.32

[1] Contains: vitamin A: 500000 U; Vitamin D3: 100000 U; Vitamin E: 100 mg; Magnesium: 19000 mg; Sodium: 60000 mg; Phosphorus: 90000 mg; Selenium: 1 mg; Manganese: 2000 mg; Iron: 3000 mg; Copper: 300 mg; Calcium: 180000 mg; Zinc: 3000 mg; Cobalt: 100 mg and Iodine: 100 mg.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 The effect of experimental treatments on the dry matter intake and daily weight of lambs

Treatments[1]		Dry matter intake (kg/d)	Daily body weight gain (g/d)
Organic acid[2]	0	42.85	180.76[b]
	2	45.98	195[a]
	SEM	1.48	4.182
	P-value	0.1475	0.0241
Medicinal plant[3]	0	43.43	174.36[c]
	2	44.11	186.86[b]
	4	45.71	202.43[a]
	SEM	1.81	5.122
	P-value	0.6650	0.0029
Organic acid × Medicinal plant	1	42.546	168
	2	44.314	180.71
	3	43.333	188.43
	4	42.670	185.86
	5	44.894	185.29
	6	48.743	219
	SEM	2.564	7.24
	P-value	0.6167	0.0609
Period	1	42.155[b]	188.38
	2	46.680[a]	187.38
	SEM	1.102	5.16
	P-value	<0.0001	0.9071
Treat × period		0.2030	0.8430

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results of the main effect of organic acid, the main effect of the medicinal plants mixture and the interaction between them on the blood parameters of lambs are presented in Table 4.

The results of the main effect of the mixture of medicinal plants on some blood parameters were significant. The level of 4% medicinal plants mixture caused a significant increase in the concentration of total protein and a significant decrease in the concentration of urea, cholesterol and triglycerides. The results related to the effect of period on blood parameters showed that the concentration of urea and creatine decreased and total protein increased significantly in the second period compared to the first period (P<0.05).

In a study by Soltan (2009), it was reported that the addition of eucalyptol oil, peppermint oil and crystalline menthol to the diet of Holstein calves had no significant effect on blood glucose concentration. Biricik *et al.* (2016) concluded that the carvacrol and thymol did not have effect on serum glucose concentrations of Merino sheep.

According to the results of Mohammadi *et al.* (2017) it was found that serum glucose concentration in Dalagh sheep was not affected by adding 110 mg of peppermint and peppermint essential oil to the basal diet. The results of Galbat *et al.* (2014) showed that 250 mg/kg_{BW} medicinal plant in goat diet did not cause a significant difference in blood creatine concentration, which indicates a healthy

effect of medicinal plant mixtures. In line with the results of this study regarding the effect of medicinal plants on serum urea concentration, Odhaib *et al.* (2018) reported that adding 1% black seed, 1% rosemary leaf and a combination of 1% black seed and 1% rosemary leaf caused Significant decrease in serum urea concentration in Dorper lamb.

According to the results of Shams Al-deen and Abdalwahad (2015), it was found that adding 600 mg/d of black seed and fenugreek seeds based on body weight in Awasi male lambs diet caused a significant reduction in blood Urea concentration. A significant decrease in blood urea concentration may be due to the fact that the addition of medicinal plants increases the production of ammonia in the rumen, which can increase urea recycling (Atta Elmnan *et al.* 2013). The formation of urea produces detoxification of ammonia in the rumen and ultimately reduces ammonia nitrogen in the blood (Abo El-Nor *et al.* 2007), which indicates a higher efficiency of protein metabolism.

Similarly, dietary supplements of various medicinal plants reduce the concentration of blood urea nitrogen in cows (Wanapat *et al.* 2013). Medical plants and their essential oils can play an important role in the group of hypercholesterolemia, which may be due to the presence of antioxidants, high levels of omega-3 fatty acids and coenzyme Q10, which can reduce the concentration of serum lipoproteins (Changizi-Ashtiyani *et al.* 2013).

The pectin of Medical plants may be another reason for lowering blood cholesterol. The mechanism of possible action of pectin in lowering serum cholesterol may be due to their binding to bile acids in the intestine and subsequent reduction of cholesterol absorption and excretion through the feces (Ezekwe *et al.* 2011). Significant reductions in cholesterol and triglycerides may be due to the fact that Medical plants contain compounds such as steroidal saponins, which act as precursors of sex hormones along with cholesterol, thereby lowering blood cholesterol (Tiran, 2003). Saponins are rumen indigestible, so when they reach into the small intestine they bind to bile acids and reduce the absorption of cholesterol in the small intestine (Rao and Sharma, 1987). On the other hand, Medical plants stimulate the conversion of cholesterol to bile salts, which is a direct result of increased bile secretion and decreased serum cholesterol (Bhat *et al.* 1985). Medicinal plants also contain unsaturated fatty acids such as Linolenic and Linoleic acids (Thomas, 2002) and Glucosinolates (Al-Doghachi *et al.* 2010). These compounds have an inhibitory role in the absorption of fatty acids by inhibiting the enzyme hydroxymethylglutaryl coenzyme A, which is important in the formation of cholesterol (Bulbul *et al.* 2009) and thereby reduce the concentration of cholesterol in the blood (Al-Doghachi *et al.* 2010). In confirmation of the results of this study, Safari *et al.* (2016) reported that lambs that received 10 and 15% of dried portulaca oleracea powder through the diet, had the lowest levels of triglycerides and blood cholesterol compared to the control group.

According to Barwary *et al.* (2019), the addition of 10 g/kg DM of Darmazu oak powder (tin oak) to the diet of Awasi ewes significantly reduced the cholesterol concentration compared to the control and other experimental treatments. Shams Al-din and Abdalvahed (2015) observed that the addition of 600 mg of black seed and fenugreek seeds based on body weight per day to the basic diet of male Awasi lambs significantly reduced cholesterol and triglycerides compared to the control group.

Salem *et al.* (2019) reported by adding 25 mg black seed oil, 11 mg of chamomile oil and 25 mg of oregano leaf oil kg to the basic diet of Friesian cows, the cholesterol and triglyceride concentrations were reduced. Flavonoids, glycoproteins, polypeptides and steroids in medicinal plants can well show fat-reducing properties (Changizi-Ashtiyani *et al.* 2013). In this regard, the use of garlic in the diet of fattening lambs reduced blood serum cholesterol (Chaves *et al.* 2008). Also, by adding 0.2% of peppermint extract in the diet of male Atabai lambs, a significant reduction in cholesterol and triglyceride levels and no effect on blood glucose was observed (Shahabi *et al.* 2012). The results of the study by Galbat *et al.* (2014) showed that the addition of 250 mg/kg BW of medical plants mixtures in goat diet increased blood protein, which could improve synthesis of ruminal microbial protein. Increasing blood protein concentrations in experimental treatments may be due to the fact that medicinal plants contain essential amino acids required for protein production (Ahmed *et al.* 2004) or may be due to increased crude protein digestibility which increases total protein in the blood (Kassab, 2007). It may also be due to the fact that herbal supplements have a positive effect on the secretion of thyroid hormones (Sanad, 2010). Other reasons for increasing serum protein may be due to medical plants contain saponins that reduce the activity of microorganisms in the rumen, which increases the indigestible protein in the rumen and their entry into the intestine (Thomas *et al.* 1994). Whole blood protein can be used as indicators to assess ruminant nutritional status and physiological changes (Kummer Snigh and Verma, 1981). According to the study of Shams Al-din and Abdolvahead (2015), the total protein concentration increases by adding 600 mg of black seed and fenugreek seeds to the basic diet of Awasi male lambs. Hassan *et al.* (2013) reported that by adding black cumin and garlic powder to the diet of buffalo calves, the total protein concentration increased and cholesterol was decrease. Salem *et al.* (2019) found that by adding 25 mg/kg BW of black seed oil to the diet of Friesian cows, the concentration of total protein in serum increased.

Table 3 The effect of experimental treatments on overall performance of lambs

Treatments[1]		Weight gain (kg/ overall period)	Dry matter intake (kg/overall period)	Feed conversion ratio
Organic acid[2]	0	12.65[b]	85.699	6.79
	2	13.65[a]	91.968	6.78
	SEM	0.293	2.961	0.207
	P-value	0.0241	0.1475	0.9713
Medicinal plant[3]	0	12.20[b]	86.86	7.13
	2	13.08[ab]	88.23	6.75
	4	14.17[a]	91.41	6.48
	SEM	0.358	3.627	0.254
	P-value	0.0029	0.6646	0.2062
Organic acid × Medicinal plant	1	11.76	85.09	7.24
	2	12.65	88.62	7.03
	3	13.19	86.66	6.58
	4	13.01	85.34	6.56
	5	12.97	89.79	6.92
	6	15.33	97.49	6.40
	SEM	0.5070	5.129	0.359
	P-value	0.0608	0.6171	0.6960

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 The effect of experimental treatments on blood parameters of lambs

Treatments[1]		Glucose (mg/dL)	Urea (mg/dL)	Creatine (mg/dL)	Total protein (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Organic acid[2]	0	77.08	40.65	0.87	76.40	37.68	15.62
	2	77.20	39.37	0.93	80.56	36.38	15.25
	SEM	1.26	0.881	0.033	2.32	1.45	0.468
	P-value	0.9482	0.3133	0.1862	0.2170	0.5315	0.5818
Medicinal plant[3]	0	75	43.55[a]	0.92	67.66[c]	40.82[a]	17.68[a]
	2	76.40	39.60[b]	0.86	75.62[b]	37.45[b]	15.55[b]
	4	80.25	36.87[c]	0.91	92.16[a]	32.82[c]	13.07[c]
	SEM	1.539	1.079	0.0403	2.841	1.773	0.574
	P-value	0.0783	0.0008	0.5293	<0.0001	0.0140	<0.0001
Organic acid × Medicinal plant	1	75.15	44.25	0.902	66.22	39.40	17.80
	2	74.85	42.85	0.940	69.10	42.25	17.57
	3	76.85	40.25	0.835	74.88	39.05	15.18
	4	79.25	37.45	0.863	88.10	34.60	13.89
	5	75.95	38.95	0.887	76.36	35.85	15.93
	6	80.80	36.30	0.963	96.22	31.05	12.26
	SEM	2.1772	1.5261	0.0570	4.0183	2.5080	0.8115
	P-value	0.8430	0.9966	0.8508	0.6883	0.3725	0.3535
Period	1	75.82	41.40[a]	1.004[a]	75.73[b]	37.183	15.047
	2	78.47	38.62[b]	0.7933[b]	81.23[a]	36.883	15.830
	SEM	1.1398	0.6458	0.0320	1.6608	1.0787	0.4654
	P-value	0.0756	<0.0001	<0.0001	<0.0001	0.6625	0.2424
Treat × period		0.9997	0.0148	0.2977	0.0005	0.2218	0.9498

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results of main effect of organic acid, main effect of medical plant mixtures, and the interaction between them on the immune system parameters of male lambs including superoxide dismutase, glutathione peroxidase, malondialdehyde and total antioxidant capacity are shown in Table 5. The results of the main effect of organic acid showed that 2 g organic acid significantly reduced the total antioxidant activity compared to control group (P<0.05).

The activity of superoxide dismutase enzyme increased significantly in the group of 4% medicinal plants mixture compared to the control and 2% medicinal plants mixture (P<0.05). The 4% medicinal plants mixture caused a significant decrease in the concentration of malondialdehyde and significant increase in total antioxidant activity compared to others (P<0.05).

According to the results of the interaction of organic acid and medicinal plants, the activity of superoxide dismutase, malondialdehyde level and total antioxidant activity were affected, so that the activity of superoxide dismutase in group of 4% medicinal plants was significantly more than the other experimental treatments (P<0.05). By increasing mixture medicinal plants and acid organic content, the malondialdehyde was significantly reduced compared to the control group (P<0.05). The glutathione peroxidase and superoxide dismutase are part of the body's first line of defense against free radicals (Harris, 1992), and total antioxidant capacity is also an indicator of anti-radical activity, enzymatic and non-enzymatic antioxidants. Improving the antioxidant status reduces the level of malondialdehyde, which is an indicator of lipid peroxidation in the body (Sen *et al.* 2010).

The lipid peroxidation can also be used as an indicator of reactive oxygen species damages (Kuhn and Borchert, 2002). Recently, the active ingredients of medicinal plants have been investigated as potential antioxidants (Halvorsen *et al.* 2002; Dragland *et al.* 2003; Wang *et al.* 2008a). The antioxidant activities of Echinacea (Gajalakshmi *et al.* 2012), Mint (Mairapetyan *et al.* 2016), Thyme (Tepe *et al.* 2005) and Eucalyptus (Iqbal *et al.* 2003), which are mixtures of medicinal plants used in this study, have been confirmed.

For example, due to the presence of phenolic compounds (thymol and carvacrol) in thyme, this plant has antioxidant, antimicrobial and antifungal effects that maintain health (Basilico and Basilico, 1999; Criag, 1999). Research has shown that thymol and carvacrol in thyme essential oil reduce lipid peroxidation in tissues (Nieto *et al.* 2011). It can act as dietary supplements; improve the body's condition in terms of Improving antioxidant, glutathione peroxidase and superoxide dismutase activity (Youdim and Deans, 2000; Tsai *et al.* 2007). According to the research of Fartashvand and Haji Sadeghi (2016), it was found that 1 g dried ginger root powder to the diet of Ghezel sheep increased the total serum antioxidant capacity and superoxide dismutase. The results of Hosoda *et al.* (2006) showed that the use of a mixture of medical plants including mint, valerian and cloves in the diet of fattening calves increased the total antioxidant capacity compared to the control group. El-Far *et al.* (2014) reported that 3 g of dry ginger powder reduced malondialdehyde and significantly increased total antioxidant capacity and superoxide dismutase of ewes. In another study Wang and Wang (2016) also found that 15 g/kg of goat feed Chinese plants increased superoxide dismutase activity and total antioxidant capacity and the amount of malondialdehyde decreased. Habibi *et al.* (2014) reported that ginger root powder and ginger essential oil increased the total antioxidant capacity and decreased serum malondialdehyde concentration of heat stressed chickens. According to the research of Wang *et al.* (2008b), it was found yellow jasmine essential oil increased the total antioxidant capacity and superoxide dismutase and reduced malondialdehyde in broiler. Raeisi *et al.* (2015) found that adding 400 ppm of thyme essential oil to broilers diet increased total antioxidant activity and reduced malondialdehyde compared to the control group. The effect of organic acids on the immune system is still largely unknown. The organic matter can help the immune system by improving digestion and absorption of nutrients, as well as reducing harmful bacteria that reduce subclinical infections in animals (Skrivanova and Marounek, 2007).

The results of the effect of feeding organic acid and mixtures of medicinal plants on the apparent digestibility of nutrients in male Moghani lambs are shown in Table 6. According to these results, the main effect of medicinal plants mixtures in lamb was significant, 2% of medicinal plants mixtures significantly increased the apparent digestibility of crude fat, NDF, and ADF. 4% medicinal plants mixture also significantly increased apparent digestibility of all the nutrients compared to 0% medicinal plants mixture and also the apparent digestibility of organic matter, crude protein, crude fat and NDF compared to 2% medicinal plants mixture (P<0.05). The results of organic acid and medicinal plants mixtures interactions showed that supplementation of the diet with 4% medicinal plants mixture + 2 g organic acid significantly increased the apparent digestibility of dry matter compared to control and 2 g of organic acid. The apparent digestibility of organic matter in this treatment was significantly increased compared to the others (P<0.05).

The digestibility of crude protein in group of 4% medicinal plant mixed + 2 g organic acid and 4% medicinal plant mixed was significantly increased compared to control and other experimental treatments (P<0.05).

Table 5 The effect of experimental treatments on the immune system parameters of lambs

Treatments[1]		Superoxide dismutase (U/g Hb)	Glutathione peroxidase (U/g Hb)	Malone dialdehyde (Nmol/m)	Total antioxidant capacity (mmol/L)
Organic acid[2]	0	1391.1	51.44	1.45	0.291^{a}
	2	1402.7	51.51	1.40	0.246^{b}
	SEM	76.02	0.967	0.052	0.009
	P-value	0.9364	0.9769	0.4777	0.0023
Medicinal plant[3]	0	1212.7^{b}	52.14	1.53^{a}	0.234^{b}
	2	1323.3^{b}	51.87	1.45^{ab}	0.275^{a}
	4	1659.2^{a}	50.57	1.30^{b}	0.297^{a}
	SEM	93.106	1.185	0.0640	0.0115
	P-value	0.006	0.6115	0.0531	0.0026
Organic acid × Medicinal plant	1	1356.2^{ab}	49.80	1.58^{a}	0.218^{b}
	2	1069.2^{b}	54.48	1.48^{ab}	0.250^{b}
	3	1129.6^{ab}	53.56	1.44^{ab}	0.268^{b}
	4	1696.4^{a}	51.16	1.34^{ab}	0.252^{b}
	5	1517^{ab}	50.18	1.46^{ab}	0.282^{ab}
	6	1622^{ab}	49.98	1.1^{b}	0.342^{ab}
	SEM	131.67	1.675	0.0872	0.0163^{a}
	P-value	0.0490	0.0639	0.7793	0.0700

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 The effect of experimental treatments on apparent digestibility of nutrients of lambs

Treatments[1]		Dry matter (%)	Organic matter (%)	Crude protein (%)	Crude fat (%)	NDF (%)	ADF (%)
Organic acid[2]	0	63.14	65.32	70.88	67.60	63.50	57.71
	2	63.59	65.96	71.55	68.31	63.69	57.79
	SEM	0.352	0.447	0.279	0.382	0.228	0.363
	P-value	0.3715	0.3220	0.1047	0.2037	0.5690	0.8814
Medicinal plant[3]	0	62.36^{b}	64.10^{b}	69.52^{b}	65.06^{c}	62.42^{c}	56.16^{b}
	2	63.22^{ab}	65.21^{b}	70.67^{b}	68.04^{b}	63.45^{b}	58.15^{a}
	4	64.52^{a}	67.59^{a}	73.44^{a}	70.76^{a}	64.91^{a}	58.93^{a}
	SEM	0.432	0.548	0.342	0.468	0.279	0.444
	P-value	0.0060	0.0005	<0.0001	<0.0001	<0.0001	0.0006
Organic acid × Medicinal plant	1	62.17^{b}	63.84^{b}	69.63^{b}	64.95^{c}	62.59^{bc}	56.37^{b}
	2	62.55^{b}	64.37^{b}	69.41^{b}	65.17^{c}	62.25^{c}	55.96^{b}
	3	63.6^{ab}	65.22^{ab}	70.23^{b}	67.82^{bc}	64.04^{b}	58.64^{ab}
	4	63.67^{ab}	66.89^{ab}	72.78^{a}	70.04^{ab}	63.87^{bc}	58.11^{ab}
	5	62.83^{ab}	64.81^{b}	70.72^{b}	68.27^{b}	62.86^{bc}	57.65^{ab}
	6	65.4^{a}	68.30^{a}	74.11^{a}	71.49^{a}	65.95^{a}	59.75^{a}
	SEM	0.6111	0.7656	0.4656	0.6623	0.3959	0.6280
	P-value	0.0154	0.0032	<0.0001	<0.0001	<0.0001	0.0025

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
NDF: neutral detergent fiber and ADF: acid detergent fiber.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Crude fat digestibility was significantly increased by adding 4% medicinal plants mixture + 2 g organic acid to the basal diet compared to the control and experimental treatments (P<0.05). The groups of 4% medicinal plants mixture and 2% medicinal plants mixture + 2 g of organic acid also had a significant effect on the digestibility of crude fat compared to the control treatment and the others (P<0.05).

Supplementation of basal diet with 4% medicinal plants mixture + 2 g organic acid significantly increased the apparent digestibility of NDF compared to control and other experimental treatments (P<0.05). Apparent digestibility of ADF was significantly increased in 4% medicinal plants mixture+2 g organic acid group compared to control and 2 g organic acid groups (P<0.05).

Digestibility is different in animals and this ability is also affected by the amount and composition of the diet (Van Soest, 1994) and generally increases with increasing feed intake and the use of nutrient supplements (Rajabi Aliabadi *et al.* 2017). Recently, there has been a new interest in using medicinal plants as dietary supplements in the diet of ruminants. Whereas, Significant increase was observed in dry matter, crude protein, crude fiber and crude fat digestion according to the results of Al-Ashry *et al.* (2006) in calves of buffalo and Abo El-Nor *et al.* (2007) in dairy buffaloes. In addition, some studies have shown that medicinal plants increase ruminal activity and nutrient digestibility (El-Saadany *et al.* 1996; Mohamed and El-Saidy, 2004). Hassan *et al.* (2013) observed that the apparent digestibility of dry matter, crude protein and crude fiber in calves of buffalo fed diets containing 2 g black seed or 2 g garlic powder or a mixture of both was significantly higher than the control group. Also, the digestibility of crude fat in the treatment of 2 g garlic powder had an increase compared to the control treatment. They attributed the improvement in nutrient digestibility to the effect of additives in maintaining proper liver function (Borek, 2001), increasing the activity of pancreatic lipase and amylase enzymes (Rao and Sharma, 1987), and modulating the number and species of rumen microorganisms (Gupta *et al.* 2005).

According to the study of Odhaib *et al.* (2018), lambs fed a diet containing 1% black seed based on dry concentrate had a significant increase in crude fat digestibility compared to other experimental treatments. Rongzhen *et al.* (2019) observed that the apparent digestibility of dry matter and crude protein was increased by supplementing diets with garlic powder in lambs. However, the apparent digestibility of lipids, NDF and ADF was not affected by garlic supplementation. Merkhan *et al.* (2019) concluded that the 1% Darmazu oak (tin oak) did not change the apparent digestibility of dry matter and organic matter in Awasi ewes. Regarding the different results obtained from the use of medicinal plants and their effect on the apparent digestibility of nutrients, it can be said that medicinal plants and their essential oils are different in terms of source, chemical structure and activity, thus having different effects on ruminal function, fermentation, and digestibility of animals (Hamidi *et al.* 2013). Jamali *et al.* (2017) reported that the addition of 3 g or 4.5 g of organic acid to the Holstein calf diet had no effect on the digestibility of dry matter, organic matter, ADF, and NDF. According to the results of Laki *et al.* (2016), processing barley grains with lactic acid and citric acid, rolled barley and water had no effect on the apparent digestibility of nutrients in Holstein cows. The processing barley grains with lactic acid reduced the effective ruminal digestibility of barley grain dry matter in dairy cows, in a study by Iqbal *et al.* (2009). But soaking barley grains in lactic acid combined with heating barley to 55° C improved digestibility and increased milk production in mid-lactating cows in another study by Iqbal *et al.* (2012).

Nematpour *et al.* (2014) concluded that processing barley grains with organic acids could increase the digestibility of dry matter, organic matter, crude protein and cell wall in fattening calves consuming barley grains processed with lactic acid.

The results of the effect of organic acid and medicinal plants mixtures on the carcass characteristics of fattening lambs are presented in Table 7. According to this table, none of the carcass characteristics including the weight of hot carcass, cold carcass, liver, lung, heart, kidney, testes, head and legs not affected by the main effect of organic acid, the main effect of medicinal plants mixture and the interaction of them. In the study of Biricik *et al.* (2016), diets with different levels of carvacrol or thymol had no effect on the percentage of carcass, hot carcass, cold carcass, viscera, head weight, quadrupeds, heart, lungs, liver, testis, kidneys, spleen, and ventricular fat of Merino sheep. This result was consistent with the findings of Chaves *et al.* (2008) and Bampidis *et al.* (2005). Hashemi *et al.* (2016) by adding 25% of chicory forage as an alternative to alfalfa did not observe a significant difference in carcass characteristics including warm carcass weight, carcass percentage and head weight of Qashqai Turkish lambs. In a study, Mirzaei Alamouti *et al.* (2017) observed that by adding monensin and plant extract to the diet of Afshari lambs, there was no significant difference in hot and cold carcass weight, hot and cold carcass percentage, liver weight of lambs. Leea *et al.* (2015) concluded that substituting 30 or 50 g of medicinal plant by-products per kilogram of Hanwoo calf feed had no effect on carcass characteristics including carcass weight, carcass percentage, and back fat thickness.

Table 7 The effect of experimental treatments on carcass characteristics of lambs

Treatments[1]		Hot carcass (kg)	Cold carcass (kg)	Liver (kg)	Lung (kg)	Heart (kg)	Kidney (kg)	Testicles (kg)	Head (kg)	Legs (kg)
Organic acid[2]	0	21.29	21.16	0.715	0.521	0.213	0.134	0.472	2.216	1.021
	2	22.06	21.91	0.727	0.502	0.206	0.127	0.462	2.261	1.009
	SEM	0.758	0.759	0.0217	0.0207	0.0068	0.0035	0.0261	0.0688	1.0374
	P-value	0.4833	0.4898	0.7000	0.5232	0.5423	0.1483	0.7889	0.6417	0.8127
Medicinal plant[3]	0	21.14	21	0.763	0.545	0.223	0.131	0.520	2.358	1.051
	2	21.42	21.27	0.727	0.496	0.203	0.130	0.419	2.110	0.946
	4	22.46	22.34	0.673	0.493	0.202	0.131	0.460	2.247	10.047
	SEM	0.928	0.930	0.0266	0.0254	0.0084	0.0042	0.0319	0.0842	0.0458
	P-value	0.5753	0.5673	0.0754	0.2854	0.1530	0.9817	0.1011	0.1364	0.2075
Organic acid × Medicinal plant	1	21.07	20.94	0.802	0.585	0.238	0.138	0.527	2.382	1.028
	2	21.21	21.06	0.725	0.506	0.209	0.124	0.514	3.334	1.074
	3	21.05	20.90	0.666	0.483	0.196	0.133	0.418	1.950	0.940
	4	21.76	21.64	0.678	0.496	0.204	0.132	0.470	2.315	1.096
	5	21.79	21.64	0.788	0.510	0.210	0.127	0.421	2.271	0.953
	6	23.17	23.04	0.669	0.491	0.201	0.130	0.450	2.180	0.999
	SEM	1313	1.315	0.037	0.035	0.011	0.006	0.045	0.119	0.064
	P-value	0.8900	0.8888	0.0429	0.3348	0.2114	0.6033	0.9667	0.1496	0.5217

[1] The treatments were including: 1) control (basal diet); 2) basal diet + 2 g organic acid/day/head; 3) basal diet + 2% medicinal plants mix/kg DM; 4) basal diet + 4% medicinal plants mix/kg DM; 5) basal diet + 2% medicinal plants mix/kg DM + 2 g organic acid/day/head and 6) treatment containing basal diet + 4% medicinal plants mix/kg DM +2 g organic acid/day/head.
[2] Organic acid: 0, 2 g organic acid/day/head.
[3] Medicinal plant: 2%, 4% medicinal plants mix/kg DM.
NDF: neutral detergent fiber and ADF: acid detergent fiber.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

CONCLUSION

According to the results, the tested levels of medicinal plants mixture and organic acid had no effect on feed intake, feed conversion ratio and carcass characteristics of male Moghani lambs. Also, the use of 2 g organic acid caused a significant increase in daily weight. On the other wise, 2% medicinal plants mixture caused a significant increase in daily weight gain. The use of 4% medicinal plants mixture + 2 g organic acid in the diet also significantly increased the apparent digestibility of nutrients and the 2% medicinal plants mixture significantly increased the apparent digestibility of crude protein. The consumption of 2% and 4% medicinal plants mixture caused a significant increase in blood total protein and a significant decrease in the blood concentration of cholesterol, triglycerides and urea of lambs. Also, 4% medicinal plants mixture significantly increased the activity of superoxide dismutase enzyme. 4% medicinal plants mixture + 2 g organic acid significantly increase the total antioxidant activity and decrease the malondialdehyde concentration.

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