

Effect of Supplementing Sheep Diet with *Glycyrrhiza glabra* and *Urtica dioica* Powder on Growth Performance, Rumen Bacterial Community and some Blood Biochemical Constituents

Research Article

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ABSTRACT

In this study, nine male Dallagh sheep (initial weight 35 ± 2 kg) were used in a replicated 3×3 latin square design experiment with three treatments and three 21-day periods (14 d adaptation, 7 d collection). Treatments were control (without addition) and two treatment diets containing 10% dry matter *Glycyrrhiza glabra* (licorice) and *Urtica dioica* (nettle) powder. The dry matter intake, average daily gain, feed conversion ratio were determined. Rumen fluid was obtained at 0, 4 and 8 hours after feeding. Also, blood samples were collected 2 h after feeding. Diet containing 10% licorice significantly increased feed conversion ratio versus control ($P < 0.05$). In licorice treatment, lactic acid bacteria in rumen fluid were lower than nettle 8 hours after morning feeding. Sheep fed licorice had higher rumen protozoa count than control before morning feeding ($P < 0.05$). Dietary supplementations had no effect on the weight gain, dry matter intake, and total count of bacteria, coliforms, rumen pH, blood glucose, total protein, triglyceride, cholesterol, and albumin. In conclusion, licorice dry powder decreased lactic acid bacteria, increased feed conversion ratio, and had no significant effect on other studied factors. Also, nettle had no effect on performance, blood metabolite and rumen parameters.

KEY WORDS blood metabolites, licorice, nettle, rumen parameters, sheep.

INTRODUCTION

In livestock production systems, antibiotics are commonly used to prevent disease and metabolic disorders, as well as improve feed efficiency. However, in the recent years, public concern over the routine use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic resistant bacteria which affect human health. Consequently, considerable effort has been devoted toward developing alternatives to antibiotics. Plants offer a unique opportunity in this regard (Wallace, 2004), as many plants produce secondary metabolites, such as saponins, tannins and essential oils which have antimicrobial properties. These compounds

have been shown to modulate ruminal fermentation to improve nutrient utilization and production efficiency in ruminants (Benchaar *et al.* 2008).

The *Glycyrrhiza glabra*, commonly known as Licorice, is one of the important traditional medicinal plants that its growth is widely spread around the world, including Iran and has been used for medicinal purposes for at least 4000 years. It is a very sweet, moist, soothing herb; it grows upward about 2 meters in height. The roots are long, cylindrical, thick and multi-branched. The root of this plant has several useful pharmacological properties, such as anti-inflammatory, antiviral, antimicrobial, and anticancer activities as well as it has immunomodulatory, hepatoprotective

tive and cardioprotective effects (Gupta *et al.* 2008; Sedighinia *et al.* 2012). Licorice produces a sweet saponin glycyrrhizic acid (GA), that is widely used in the confectionery and pharmaceutical industries in various countries (Jalilzadeh *et al.* 2015). Dried licorice leaves with a proportion of 15% can minimize the negative effect of low-protein diets on feed conversion ratio (FCR) in lambs (Zamiri *et al.* 2015). Jiang *et al.* (2012) indicated that 0.3 g/kg GA had a positive influence in improving lipid deposition in channel catfish without negative effect on growth; the beneficial effect may be due to promoting lipolysis.

Stinging nettle or nettle (*Urtica dioica*) is an annual or perennial herbaceous plant (Korpe *et al.* 2013). Nettle naturally grows extensively within the northern regions of Iran in the borders of field, roads, and forests. In traditional medicine, *Urtica dioica* is used to treat allergies, kidney stones, burns, anemia, rashes, internal bleeding, diabetes, etc. (Salehzadeh *et al.* 2014). The primary use of stinging nettle herb among a sampling of traditional herbalists in the US, Australia, Canada, New Zealand, and the United Kingdom is as a tonic or nutritive agent, and an alternative for arthritic conditions. The other common uses are as an astringent to tone mucosa (reduce secretions, slow blood loss), a diuretic for general water retention as well as kidney stones, and an anti-inflammatory/anti-allergy (Upton, 2013). Stinging nettle extract contains a number of bioactive compounds, including histamine, serotonin, moroidin, and other phenolic compounds and flavonoids (Gulcin *et al.* 2004; Dar *et al.* 2013) and has long been used as an alternative anti-inflammatory therapy for arthritis (Humphries and Reynolds, 2014). In addition, antimicrobial effects of stinging nettle extracts have been demonstrated (Gulcin *et al.* 2004; Modarresi-Chahardehi *et al.* 2012; Korpe *et al.* 2013). Dietary supplementation with 0.5% of pennyroyal or 0.05% of nettle and interaction between them, significantly decreased the performance of laying hens (Nobakht *et al.* 2011).

There are limited reports available on the effects of feeding licorice and nettle in sheep. Therefore, this study was carried out to evaluate the effects of supplementation of these plants to sheep diet on performance, blood metabolites, and rumen parameters.

MATERIALS AND METHODS

Animals, treatments and management

The current research was carried out at the experimental farm of the Faculty of Agriculture, Gonbad Kavous University, Gonbad Kavous, Iran. Nine male Dallagh sheep (initial body weight 35 ± 2 kg, age 11-13 month) were used in a replicated 3×3 latin square design experiment with three treatments and three 21-day periods. Each experimental

period consisted of 14 days of adaptation to treatments and 7 days of sampling. The three treatments were control (without addition) and two treated diets containing 10% dry matter licorice (*Glycyrrhiza glabra*) and nettle (*Urtica dioica*) dry powder. The dosage of plants was based on literature data (Humphries and Reynolds, 2014; Zamiri *et al.* 2015). Licorice and nettle were collected from mountains around Minoodasht city, Golestan state, in the North of Iran. Roots of licorice and aerial part of nettle were shade-dried and ground through a 1 mm screen.

The ingredients and nutrient composition of the three diet treatments (dry matter basis) are given in Table 1. The ingredients to be investigated were: barley grain, ground, corn silage, licorice dry power, nettle dry powder, wheat straw, wheat bran, salt, and mineral-vitamin premix. The ingredients of three treatments were identical except that in experimental treatments, 10% of corn silage was replaced by licorice and nettle dry powder.

The neutral detergent fibre (NDF) content of licorice and nettle diet were the same (51%) and were slightly higher than of control diet. Protein content of licorice treatment was the highest (11.5%) that followed by nettle (10.5%) and control (10%) diet.

Diets were fed as total mixed rations (TMR) and formulated according to NRC (2007) recommendations. The TMR diets were offered in equal amounts twice daily at 08:00 and 16:00 h for *ad libitum* intake (10% refusals). The feed offered and refusals of each sheep were weighed daily to determine feed intake. The animals were weighed in the morning prior to feeding at the beginning and the end of each period, and the average daily gain (ADG) was determined by dividing weight gain (final LW– initial LW) by the number of days. FCR was calculated as the ratio between dry matter intake (DMI) and ADG (g of DMI/g of LW gain). The sheep were housed in individual pens (2 m \times 1.5 m) bedded with straw in a sheltered barn and had free access to water at all time. Throughout the experiments, animals were processed according to guide for the care and use of agricultural animals in research and teaching (Federation of Animal Science Societies, 2010).

Samples and procedures

Ruminal digesta was collected by aspiration using a stomach tube at 0, 4, and 8 h after the morning feeding on the last day of each period. The first 20 mL of samples were discarded to ensure they were not polluted with saliva and then squeezed through four layers of cheesecloth to obtain ruminal fluid. Rumen pH was measured immediately with a portable pH meter (Metrohm 691, Switzerland).

For ammonia N determination, 5 mL of each sample were acidified with 5 mL of 0.2 N HCl and frozen at -20 °C until laboratory analysis.

Table 1 Ingredients and nutrient composition of experimental diets (DM basis)

Ingredients (%)	Treatments		
	Control ¹	Licorice ²	Nettle ³
Barley grain, ground	18	18	18
Corn silage	37	27	27
Licorice dry power	-	10	-
Nettle dry powder	-	-	10
Wheat straw	18	18	18
Wheat bran	26	26	26
Salt	0.5	0.5	0.5
Mineral-vitamin premix ⁴	0.5	0.5	0.5
Composition			
Crude protein (%)	10	11.5	10.5
Metabolizable energy (Mcal/kg)	2.3	2.25	2.2
Neutral detergent fiber (%)	50	51	51
Ash (%)	10	12	12
Calcium (%)	0.75	0.77	0.79
Phosphorus (%)	0.53	0.54	0.56

¹ Diet without additive.

² Diet containing 10% *Glycyrrhiza glabra* powder.

³ Diet containing 10% *Urtica dioica* powder.

⁴ Contained: Mg: 21 g/kg; Zn: 0.3 g/kg; Mn: 2.2 g/kg; Fe: 3 g/kg; Cu: 0.3 g/kg; Se: 0.001 g/kg; Co: 0.1 g/kg; I: 0.12 g/kg; Ca: 195 g/kg; P: 80 g/kg; vitamin A: 600 IU/g; vitamin D: 200 IU/g and vitamin E: 2.5 IU/g.

Ruminal ammonia-N concentration was determined using the spectrophotometry (Libra S12, Biochrom Ltd., Cambridge, London) by the phenol hypochlorite method of Broderick and Kang (1980).

Briefly, with the absorbance and results, a regression equation to calculate NH₃-N concentration was performed: NH₃-N (mg/dL) = absorbance - (a) / b; b = equation R² drawn from the standard. Serial 10-fold dilutions of strained ruminal fluid were prepared and used as inoculum on plate count agar (PCA), modified rogosa and sharp agar (MRSA) and violent red bile agar (VRBA) medium for total viable bacteria, lactic acid bacteria and coliforms count, respectively (Harley and Prescott, 2002).

The number of protozoa was counted in the rumen fluid using a Neubauer haemocytometer under a light microscope according to the method described by Cedrola *et al.* (2015).

A volume of 0.5 mL of rumen fluid was mixed with 4.5 mL methylgreen formalin salt (MFS) solution and kept at room temperature until counting. Each sample was counted twice and if the average of the duplicates differed by more than 10%, the counts were repeated.

Jugular blood samples were collected in vacutainers without clot activator at the end of each period (2 h after the morning feeding). After the clot formation, samples were centrifuged (Denley, BS400, England) at 2000 × g for 15 min, the supernatant serum was transferred to labeled plastic tubes, and stored at -20 °C over a period of one month. Glucose, total protein, albumin, triglyceride and cholesterol were measured using the kit package (Darmanfaraz Company, Isfahan, Iran) by spectrophotometry (Libra S12, Biochrom Ltd., Cambridge, London).

Statistical analysis

All data were statistically analyzed according to a replicated 3 × 3 latin square design using the general linear model (GLM) procedure of SPSS software (version 18) according to model below:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_l + c_{ykl} + e_{ijk}$$

Where:

y_{ijkl} : represents the observation on animal k given treatment i at period j in squared l .

α_i : fixed effect of the i^{th} treatment ($i=1$ to 3).

β_j : fixed effect of the j^{th} period ($j=1$ to 3).

γ_l : fixed effect of the l^{th} square ($l=1$ to 3).

c_{ykl} : random effect of animal within square ($k=1$ to 9).

e_{ijk} : random error associated with each observation.

Significant differences between means of treatments were assessed by the tukey test, and the differences among treatments were declared significant at $P < 0.05$. The initial weight of sheep was used as covariate for weight gain data analysis.

RESULTS AND DISCUSSION

Feed intake, daily gain and FCR

Data on dry matter intake and performance are presented in Table 2. Dry matter intake and body weight gain were not influenced by supplementation of the diet with licorice and nettle. Whereas, the FCR value, expressed as feed: gain ratio (DMI to average daily gain ratio), was higher ($P < 0.05$)

in licorice group than in the control group.

Blood biochemical constituents

The effects of the herb treatments on serum metabolites are shown in Table 3. There were no significant differences in the plasma concentrations of total protein, albumin, glucose, triglyceride and cholesterol.

Ruminal pH, NH₃-N and microbial population

Table 4 displays the effect of experimental treatments on rumen fluid pH, NH₃-N and protozoa. Rumen fluid pH did not differ among treatments before morning feeding, at 4 h and 8 h after morning feeding. No difference for NH₃-N was found among diets. The rumen protozoa was significantly lower in control compared to licorice treatment before morning feeding, but the differences were not significant among three treatments at 4 and 8 hours after morning feeding in this study.

Table 5 summarizes the data obtained from the effects of experimental treatments on rumen bacterial population. Total viable bacterial count and coliform bacteria had no significant difference before feeding, at 4 h and 8 h after feeding. Lactic acid bacteria count was influenced by treatments. Lactic acid bacteria count was significantly lower in licorice treatment compared with nettle treatment at 8 h after morning feeding. However, there was no significant difference among the treatments before morning feeding and at 4 h after feeding.

Literature is very scarce on the effect of licorice and nettle on performance, blood biochemical constituents and rumen microbial of ruminants so we must compare the results of this study with other medicinal plants and species.

Feed intake and performance

Two potential physiological mechanisms that could be associated with feed efficiency are immune function and glucocorticoid concentrations. Additionally, variation in glucocorticoid concentrations can lead to inefficient use of vital nutrients which would likely lead to poor growth and nutrient conversion (Foote *et al.* 2016). There is little information on the effect of licorice and nettle on DMI and growth performance in sheep, but the main active component of licorice was evaluated in some of different studies and other species. Glycyrrhizin is the main constituent of licorice root (*Glycyrrhiza glabra*), which when orally administered is hydrolyzed by the glucuronidase of intestinal bacteria into glycyrrhetic acid (GA), being absorbed into the blood. Many studies indicated that the role of GA was similar to that of cortisol (a glucocorticoid). Results of these studies have indicated that GA extended endogenous cortisol half-life, and increased the duration of action (Jiang *et al.* 2012). Increased cortisol concentrations stimulating

lipolysis and gluconeogenesis and increasing the energy available to support the animal in times of stress, or when there is a threat to an animal's homeostasis. In doing so, nutrients are diverted away from growth, and the animal is likely to be slower growing, as the metabolic needs of the immune system and the stress response need to be met before tissue accretion occurs (Knotte *et al.* 2010). It was expected that the licorice would increase FCR and decrease ADG. In this study, licorice significantly increased FCR and numerically decreased ADG. The lack of significant influence of these plants on DMI and ADG could have been due to the short duration of the periods (3 weeks) and longer duration studies are more appropriate for growth performance assessment. The data presented here is in disagreement with previous data that showed dietary glycyrrhetic acid (GA) did not significantly affect final body weight, weight gain, and feed conversion ratio juvenile channel catfish (Jiang *et al.* 2012). Similarly, production levels in terms of milk output were maintained when ryegrass silage was replaced by nettle in the diet of lactating dairy cows, in spite of a numerical reduction in feed intake (Humphries and Reynolds, 2014). Somasiri *et al.* (2015) showed that both plantain mix and chicory mix lambs had greater live weight gains and were heavier at slaughter and displayed greater carcass weights and dressed out percentages compared to the pasture mix lambs. In a study, lambs grazing chicory had increased performance and average daily gain and reduced gastrointestinal infection compared to those grazing *Cynodon dactylon* (Miller *et al.* 2011). In a study with sheep, weight gain, dry matter intake, feed conversion ratio were not affected by diet containing 10% *Matricaria chamomile* and *Cichorium intybus* dry powder (Ghasemifard *et al.* 2017). The findings of the present study for DMI was in line with previous researches in growing lambs (Chaves *et al.* 2008), sheep (Distel *et al.* 2007) and cattle (Benchaar *et al.* 2007; Geraci *et al.* 2012) which found no effect of essential oil (EO) of medicinal plant on DMI. The effects of EO of medicinal plant on DMI might vary with EO source, type of diet, diet interaction or adaptation of rumen microbial population to EO (Yang *et al.* 2010a, Yang *et al.* 2010b; Geraci *et al.* 2012). Furthermore, it has been reported that DMI can be affected by a number of dietary or management factors, such as body weight, animal growth stage, specific physical and chemical characteristics of diet or rumen fermentation metabolites (Allen, 2000; Yang *et al.* 2007; Yang *et al.* 2010b).

Blood biochemical constituents

Cortisol increases blood glucose and proteins and decrease blood lipids (Guyton and Hall, 2006). According to similar role of GA to cortisol (Jiang *et al.* 2012), our expectations did not occur.

Table 2 Effect of licorice and nettle powder on performance of sheep

Item	Treatments			SEM	P-value
	Control ¹	Licorice ²	Nettle ³		
Initial weight (kg)	35.42	36.11	35.91	1.65	0.95
Final weight (kg)	38.62	38.37	38.05	1.74	0.97
Daily weight gain (g)	204.55	159.77	198.44	18.30	0.19
Daily dry matter intake (g)	1301.25	1408.15	1464.35	62.85	0.19
Feed conversion ratio	6.86 ^b	9.75 ^a	7.70 ^{ab}	0.74	0.03

¹ Diet without additive.² Diet containing 10% *Glycyrrhiza glabra* powder.³ Diet containing 10% *Urtica dioica* powder.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 3 Effect of licorice and nettle powder on some blood parameters of sheep

Item	Treatments			SEM	P-value
	Control ¹	Licorice ²	Nettle ³		
Total protein (g/dL)	9.21	9.52	6.68	1.16	0.19
Albumin (g/dL)	2.8	3.36	3.12	1.16	0.54
Glucose (mg/dL)	70.63	87.90	67.65	7.47	0.14
Triglycerides (mg/dL)	23.30	34.66	28.25	3.86	0.13
Cholesterol (mg/dL)	50.48	45.77	35.54	6.42	0.28

¹ Diet without additive.² Diet containing 10% *Glycyrrhiza glabra* powder.³ Diet containing 10% *Urtica dioica* powder.

SEM: standard error of the means.

Table 4 Effect of licorice and nettle powder on pH, NH₃-N and protozoa of rumen fluid

Item	Time	Treatments			P-value	SEM
		Control ¹	Licorice ²	Nettle ³		
pH	Before feeding	7.01	7.07	6.83	0.10	0.27
	4 h after feeding	6.82	6.84	6.64	0.10	0.30
	8 h after feeding	6.74	6.97	6.81	0.12	0.41
NH ₃ -N (mg/dL)	Before feeding	2.68	2.37	2.71	0.17	0.34
	4 h after feeding	2.36	2.61	2.72	0.13	0.18
	8 h after feeding	2.87	2.28	2.99	0.28	0.19
Protozoa ($\times 10^5$ /mL)	Before feeding	6.61 ^b	9.29 ^a	8.31 ^{ab}	0.63	0.02
	4 h after feeding	8.08	7.35	7.78	0.67	0.74
	8 h after feeding	6.88	8.16	6.98	0.57	0.23

¹ Diet without additive.² Diet containing 10% *Glycyrrhiza glabra* powder.³ Diet containing 10% *Urtica dioica* powder.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Total serum protein, albumin, triglyceride, and cholesterol did not significantly differ between licorice and control treatment. This finding is in disagreement with some studies that showed levels of plasma triglyceride and cholesterol were decreased in fish (Jiang *et al.* 2012) and rat (Kalaiarasi *et al.* 2009) fed dietary supplementation of GA. Variable responses to dietary GA (in these studies) and whole plant (in our study) supplementation may be due to differences in intestinal absorption and energy metabolism among species (Shibata *et al.* 2001) and / or differences in experimental conditions between studies. The results of glucose, total serum protein, albumin, triglyceride and cholesterol in this study were in agreement with some studies that declared 10% dry matter (DM) chamomile and chicory

dry powder had no effect on blood glucose and total protein in sheep (Ghasemifard *et al.* 2017). Moreover, 10 and 20 g/d *Satureja hortensis* dry powder had no significant effect on glucose, total protein, triglyceride, cholesterol and high-density lipoprotein (HDL) in kids (Payvastegan *et al.* 2015).

Supplementation of 20 g/d *Mentha spicata* and *Mentha pulegium* dry powder had also no significant effect on plasma glucose, triglyceride and total protein in calves (Ghahhari *et al.* 2016). Likewise, peppermint EO feeding did not affect significantly blood glucose, total protein and triglyceride in cattle (Hosoda *et al.* 2006), as well as 110 mg/d peppermint and pennyroyal essential oil had no effect on blood metabolites of sheep (Mohamadi *et al.* 2017).

Table 5 Effect of licorice and nettle powder on rumen bacteria (Log^{cfu/ml}) of sheep

Item	Time	Treatments			SEM	P-value
		Control ¹	Licorice ²	Nettle ³		
Total bacteria	Before feed	9.82	9.81	10.06	0.18	0.56
	4 hours after feed	9.81	9.91	9.91	0.14	0.85
	8 hours after feed	10.02	10.09	10.26	0.13	0.65
<i>Coliform</i> bacteria	Before feed	3.88	3.86	3.97	0.09	0.62
	4 hours after feed	3.95	3.88	4.10	0.08	0.20
	8 hours after feed	3.94	4.00	4.02	0.05	0.51
<i>Lactic acid</i> bacteria	Before feed	5.78	5.66	5.67	0.09	0.64
	4 hours after feed	5.78	5.66	5.72	0.08	0.65
	8 hours after feed	5.68 ^{ab}	5.55 ^b	5.73 ^a	0.04	0.02

¹ Diet without additive.² Diet containing 10% *Glycyrrhiza glabra* powder.³ Diet containing 10% *Urtica dioica* powder.

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Furthermore, 100 and 200 mg peppermint EO did not significantly change glucose, total protein, triglyceride and cholesterol of sheep (Ahmadi Naghdahi *et al.* 2014). It has been reported that concentration of some blood metabolites such as triglycerides can be influenced by EO supplementation via changing of feed intake (Yang *et al.* 2010a) and no change of blood metabolites in the present study may be contributed to lack of DMI alteration by dry powder.

Rumen pH, NH₃-N and protozoa

Many rumen microbes are able to depolymerize feed protein and peptides and deaminate amino acids into volatile fatty acid (VFA) and ammonia. Much (up to 50% of the total) of the rumen ammonia is produced by a group of bacteria called hyper ammonia-producing bacteria (HAP); (Cobellis *et al.* 2016). Protozoa represent almost half of the microbial biomass in the rumen. Their proteolytic activity and the active engulfment of bacteria make their presence in the rumen undesirable. However, they are also responsible for a significant proportion of the fibrolytic activity in the rumen, so the potential benefit of their absence needs to be placed in the context of the diet being used (Hart *et al.* 2008). Because licorice and nettle have antimicrobial property (Gulcin *et al.* 2004; Gupta *et al.* 2008; Modarresi-Chahardehi *et al.* 2012; Korpe *et al.* 2013), it was expected that these plants would also reduce protozoal count and rumen NH₃-N. Despite the antimicrobial effect of most medicinal plants or essential oils, they had variable impacts on ruminal ammonia and protozoa in the different studies. Results from different *in vitro* studies showed that the effects of EO and their main components on rumen NH₃-N concentration and protozoa are dose-dependent and that these compounds are more effective when used at high doses compared with at low doses (Vakili *et al.* 2013; Hart *et al.* 2008).

Similarly, supplementation with 5% and 10% DM nettle haylage did not affect ruminal ammonia in lactating dairy cows (Humphries and Reynolds, 2014). Moreover, sheep fed 10% DM chamomile and chicory dry powder showed no alteration in ruminal ammonia (Ghasemifard *et al.* 2017). In contrast, in a study it was reported that free thymol and sustained release thymol decreased plasma urea concentration and rumen ammonia at 5 and 7 hours after feeding (Zamani *et al.* 2015). Hosoda *et al.* (2006) found that peppermint feeding at 5% of the diet (DM basis) significantly increased rumen ammonia in Holstein steers. Also, this is in disagreement with the *in vitro* results of

Busquet *et al.* (2006) who observed that capsicum oil, carvacrol, carvone, cinnamaldehyde, cinnamon oil, clove bud oil, eugenol, fenugreek, and oregano oil resulted in a 30 to 50% reduction in ammonia N concentration.

Our result on rumen protozoa was in agreement with some researchers (Ghasemifard *et al.* 2017; Mohamadi *et al.* 2017) who reported that 10% DM chamomile and chicory dry powder and 110 mg/d peppermint and pennyroyal essential oil had no effect on rumen protozoa in sheep, respectively. Conversely, Wanapat *et al.* (2013) reported that lemongrass supplementation at 100 g/d alone or plus peppermint powder at 10 g/d with or without garlic powder at 40 g/d decreased rumen protozoa. Busquet *et al.* (2005) showed *in vivo* that 200 g peppermint powder decreased rumen protozoa.

Various results have been reported for the effects of medicinal plant and essential oil on ruminal pH. The findings of the present study for ruminal pH were in line with some studies. Supplementation with 5% and 10% DM nettle haylage did not alter rumen mean pH in lactating dairy cows (Humphries and Reynolds, 2014). Peppermint feeding at 5% DM (Hosoda *et al.* 2006) and 10% DM chamomile and chicory dry powder (Ghasemifard *et al.* 2017) had no effect

on rumen pH in steers and sheep respectively. Moreover, 100 and 200 mg peppermint EO feeding did not affect rumen pH in sheep (Ahmadi Naghdahi *et al.* 2014). In contrast with our study, peppermint feeding (200 g/day) decreased rumen pH and protozoa count in steers (Ando *et al.* 2003). Similarly, 20 g/d dry powder and 200 mg/d essential oil of *Satureja hortensis* decreased rumen pH in sheep (Payvastegan *et al.* 2015). Meyer *et al.* (2009) reported that variable effects of EO on rumen pH can result from different diets and levels of EO as when EO was added to diets with higher roughage/concentrate ratio, rumen pH increased.

Rumen bacteria

The diversity structure of rumen microbiome plays a key role in ensuring stability of the rumen ecosystem and increasing its ability to adapt to a wide range of dietary and management strategies. In fact, ruminal microbiome provides a huge gene pool that can cope with dietary changes by alternating metabolic pathways and stabilize a new equilibrium within the ecosystem. Its activity directly affects ruminant performance, health, and welfare, but it is still poorly understood (Cobellis *et al.* 2016). Information on effects of feeding licorice and nettle on rumen bacteria is also scarce. According to antibacterial effect of licorice and nettle as mentioned above, we expected that these plants reduce rumen bacteria but total bacteria and coliform bacteria (gram-negative bacteria) did not affect and only lactic acid bacteria (gram-positive bacteria) significantly decreased 8 hours after feeding in licorice compared with nettle treatment. This could be possibly due to that gram-positive bacteria appeared to be more susceptible to inhibition by plant essential oil compounds than did gram-negative bacteria. The lack of an effect of essential oils has been related to the presence of an outer membrane on gram-negative organisms, which endows them with a hydrophilic surface that acts as a strong impermeability barrier. Furthermore, many essential oils having dose-dependent effects on bacteria, protozoa, and fungi (Wanapat *et al.* 2013).

In another study 10% DM chamomile dry powder decreased rumen coliforms at 4 after feeding (Ghasemifard *et al.* 2017). Moreover, *Coliforms* of rumen fluid significantly decreased at 4 h and increased at 8 h after morning feeding following peppermint and pennyroyal essential oil supplementation, respectively (Mohamadi *et al.* 2017). The observed changes in rumen pH suggest potential benefits of feeding licorice for reducing rumen acidosis. Factors such as EO type, diet property, species, and geographical situation affect variability and bacterial count including lactic acid bacteria of rumen (Dehority, 2003). Considering a large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacte-

rial activity is not attributable to one specific mechanism but that there are several targets in the cell (Brenes and Roura, 2010).

CONCLUSION

Although licorice dry powder decreased lactic acid bacteria and increased feed conversion rate, it did not significantly affect the other studied factors. The present study showed that nettle had no effect on performance, blood metabolite and rumen parameters. In general, results suggested that the use of licorice and nettle as a feed additive and their commercial use may be approved by experiments including longer duration and larger sample size.

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