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The effect of alfalfa silage treated with Ecosyl and acetic acid on microbial protein synthesis and blood parameters in Moghani male sheep

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Abstract The purpose of this study was to investigate the effect of processed alfalfa silage with acetic acid and Ecosyl on feed intake, microbial protein synthesis and blood parameters in four Moghani male sheep (90±2 kg BW) using a change-over design consisting of four 14-day periods. The experimental treatments were 1) alfalfa silage without additive as control, 2) alfalfa silage treated with Ecosyl, 3) alfalfa silage treated with 5% acetic acid, and 4) alfalfa silage treated with acetic acid and Ecosyl. The results showed that feed intake was not affected by treatments. The amounts of excreted purine derivatives were 12.29, 7.02, 12.03 and 8.64 g/d for alfalfa silage (control), alfalfa silage treated with acetic acid or Ecosyl and their combination. Also, the adsorbed purine derivatives values were 13.46, 7.19, 13.14 and 9.12 g/d for control, alfalfa silage treated with acetic acid or Ecosyl and their combination. Untreated alfalfa silage, treated with either acetic acid, Ecosyl or their combination resulted in 9.79, 5.23, 9.56 and 6.63 g/d of microbial protein synthesis, respectively. The excreted purine and adsorbed purine derivatives and microbial protein synthesis were reduced in alfalfa silage treated with acetic acid and Ecosyl and their combination ($P<0.05$). There were no significant differences in blood metabolites among the treatments, except for cholesterol values. Alfalfa silage treated with acetic acid alone did not change the nutritional values; however, alfalfa silage treated with acetic acid and Ecosyl improved the nutritional parameters in sheep.

Keywords: alfalfa silage, acetic acid, Ecosyl, purine derivatives

Introduction

The traditional forage preparation has been used as the main method of preserving forage feeds. However, postponing forage harvest until maturity to obtain more dry matter will reduce its digestibility. In addition, adverse weather conditions can lead to the loss of nutrients and overall decline in the nutritional value of dried forage feeds (McDonald et al., 2010). Use of natural fermentation to preserve the forages that called silage is one of the methods less dependent on weather conditions and used by the farmers (Kung, JR et al., 2018). Silage is forage with high humidity that is maintained by anaerobic fermentation (Khorvash et al., 2014). Silage quality is affected by creating anaerobic conditions, preventing or reducing inappropriate fermentation processes, and stimulating the growth of lactic acid-producing bacteria to produce hydrogen ions for lowering the pH. Creating anaerobic condition in the shortest time is essential to prevent plant respiration and overheating the ensiled feed. Lowering the pH, prevent the protein breakdown in ensiled forages and therefore resulted in lower non-protein nitrogen content in the final silage (McDonald et al., 2010). Predominating of lactic acid-producing bacteria can be efficiently done if enough water-soluble carbohydrates exist (McDonald et al., 2010). However,

ensiling alfalfa forage is difficult because of its low content of water-soluble carbohydrates and high buffering capacity, which do not supply adequate fermentation and delay the lactic acid production and pH decline within the ensiled forage (Chen et al., 2014; Tamminga et al., 1991). Therefore, these forages exhibit a better response to silage additives and preservatives (Chen et al., 2014). The use of various chemicals and biological additives are being developed to improve the silage process. Silage additives help to achieve optimal fermentation, inhibit and restrict undesirable fermentation, or improve the nutritional quality of the silage (Adesogan and Salawu, 2004; Adesogan et al., 2007). The effect of silage processing using a material, such as acids on intake was modeled by manipulating the fill and roughage factors. These factors adjust the effect that the NDF content of each feed (Nadeau et al., 1996). Increasing the prevalence of bacterial diseases in the last decade, as well as increasing bacterial resistance to the use of conventional antibiotics, make the need to use a method that is complementary or even a substitute for antibiotics. Probiotics, organic acids, enzymes, herbs and extracts are among the most important compounds that have been introduced as substitutes for antibiotics (Cheng et al., 2014). The main reason for using organic acids in animal nutrition is improving the utilization of dietary nutrients in the gastrointestinal tract of dairy cows. Moreover, organic acids can reduce the toxic metabolites, colonization of pathogens and endocrine changes in the cow's body during heat stress (Ali et al., 2013). Ke et al. (2017) indicated that including malic or citric acids at the ensiling of alfalfa effectively improved silage fermentation quality, limited proteolysis, improved fatty acid composition of the ensiled forage, and could provide animals with additional feed additives proven to promote animal performance. The difference among the results of studies on the effects of probiotics may be due to the type of probiotics, amount of feed intake, management level, administration route of probiotics and environmental conditions (Agarwal et al., 2002). The studies indicated that probiotics are effective through increased concentration of globulin, the number and the cytotoxic activity of neutrophils and the reduced harmful microflora in the digestive system (Galvao et al., 2005). Adding urea and sulfuric acid to alfalfa silage showed that the addition of these additives resulted in preventing growth of fungi and molds, reducing proteolysis process and improve the efficiency of utilization of alfalfa silage (Delaware and Danesh-Mesgaran, 2003). McDonald et al. (1991) did not observe any significant effects from microbial additives when used for preparing grass silage with 15 percent dry matter. However, some studies focused on microbial additive reported positive effects on fermentation conditions, rapid decrease in pH and reduction of proteolysis process (Ke et al., 2017). The biological changes of forages ensiled with different additives during the ensiling process have been studied by many researchers. Nadeau et al. (1996) reported that after ensiling alfalfa or orchard grass by using cellulase enzyme of formic acid as additives, the mean NDF concentration of treated silages was 19% lower than that of untreated silages; the effect was greater for orchard grass than for alfalfa. Zielińska et al. (2015) reported that adding microbial additives led to increased intake of soluble carbohydrates and reducing the number of soluble carbohydrates in the final alfalfa silage. The application of bacterial inoculants as probiotics in alfalfa ensiling resulted in a significant reduction of the total number of undesirable microorganisms (Fabiszewska et al., 2019), and increased the immune system of ruminants by promoting the health and stimulate the growth of their hosts (Weinberg et al., 2004). Researchers reported that the addition of organic acid to fermented alfalfa reduces its pH and thus improves silage conditions (Delaware and Danesh-Mesgaran, 2003; Chen et al., 2014; Ke et al., 2017). The alfalfa silage treated with formic acid had lower pH and non-protein nitrogen content than untreated alfalfa silage (Ogunade et al., 2018; Verbic et al., 1999; Silva et al., 2016). The aim of this study was to evaluate the effects of treating alfalfa silage with acetic acid and Ecosyl on feed intake, microbial protein synthesis and blood parameters of Moghani sheep.

Materials and methods

Experimental animals, diets and samples collection

This experiment was performed at the university of Mohaghegh Ardabili, Iran Ardabil (Latitude: 38.253736°; Longitude: 48.299990°; Elevation: 4423 ft) and lasted from May to Aug 2018. For this experiment, four adult Moghani male sheep with a mean age of 2 years and an average body weight of 90 kg were used in a changeover design during four 14-day periods. Each sheep was housed in a metabolic cage (0.8 × 1.05 m) and each site was equipped with a separate manger and scavenger. Urine and feces were collected daily using containers beneath the cages (Church and Pond, 1988). The daily ration according to Table 1 was completely mixed (TMR) in both morning and evening (9:00 and 17:00). Access to water was free. Experimental diets were for-

Table 1. The composition of the experimental diet[1] fed to male sheep

Ingredient	% of DM
Alfalfa silage	15.46
Chopped alfalfa hay	35.18
Barley grain milled	12.15
Salt	0.28
Soybean meal	5.47
Wheat straw	14.75
Wheat bran	5.16
Sodium bicarbonate	0.55
Corn grain	10.45
Vitamin[2], mineral[3] premix	0.55
Chemical composition	
Dry matter (%)	73.12
Metabolizable energy (Mcal/kg)	2.22
Crude protein (%)	16.55
Ether extract (%)	2.96
Ash (%)	7.80
NDF (%)	40.70
ADF (%)	7.81
Calcium (%)	0.75
Phosphorus (%)	0.32

[1]The ingredients used to formulate the diets were obtained from SRNS software; Cannas et al., 2004; Tedeschi et al., 2010, USA.
[2]Mineral premix (g/kg of diet): calcium biphosphate, 20g; sodium chloride, 2.6g; potassium chloride, 5g; magnesium sulphate, 2g; ferrous sulphate, 0.9g; zinc sulphate, 0.06g; cupric sulphate, 0.02g; manganese sulphate, 0.03g; sodium selenate, 0.02g; cobalt chloride, 0.05g; potassium iodide, 0.004g.
[3]Vitamin premix (IU or mg/kg of diet): vitamin A, 25,000 IU; vitamin D3, 20,000 IU; vitamin E, 200mg; vitamin K3, 20mg; thiamin, 40mg; riboflavin, 50mg; calcium pantothenate, 100mg; pyridoxineHCl, 40mg; cyanocobalamin, 0.2mg; biotin, 6mg; folicacid, 20mg; niacin, 200mg; inositol, 1,000mg; vitamin C, 2,000mg; choline, 2,000mg.

mulated using SRNS software based on 90 kg sheep nutritional requirements and according to the chemical composition of the available feedstuffs (SRNS; Cannas et al., 2004; Tedeschi et al., 2010). Daily feed intake was measured from the amount of feed left from the daily feed (Church and Pond, 1988).

Preparation of alfalfa silage and experimental treatments

The fresh fodder of alfalfa was mowed during second cutting at the early bloom stage of maturity from farms in Parsabad city, Ardabil province and was chopped then ensiled. Nylon was used for the silo that the capacity of nylon bags was for 17 kg silo with dimensions of 0.027 cubic meters. The alfalfa was cut to 10-15 cm in size and hand-pressed to remove air and the nylons were closed and ensiled for 40 days in a sealed place. The probiotic used in this study (Ecosyl Products, Ltd., Stokesley, England) was obtained from Niko Tak company in Tehran, Tehran Province, as well as distillated vinegar (Purity: 5%; $C_2H_4O_2$) from the grocery shop. The experimental treatments consisted of 1) alfalfa silage without additive as control, 2) alfalfa silage treated with Ecosyl, 3) alfalfa silage treated with 5% acetic acid, and 4) alfalfa silage treated with acetic acid and Ecosyl (1:1). For alfalfa silage without any additives was added distilled water (400 mL/kg forage) in order to create the same conditions as other treatments. Ecosyl was used at a rate of 0.25 mg/kg of forage equivalent to 1×10^5 unit's colony forming per kg of forage. Each gram of Ecosyl was dissolved in 1600 mL of distilled water and sprayed uniformly on all forage surfaces. Compounds of Ecosyl included dried *Lactobacillus plantarum*, sugar, dried mushrooms, potassium diphosphate, monosodium phosphate, glycine, sodium erythorbate (preservative), magnesium sulfate, sodium aluminosilicate. Acetic acid (CH_3COOH) (5% distillate vinegar) was applied in 50 mL/kg alfalfa and sprayed uniformly.

Measurement of feed, blood and urine samples

The total urine was collected within 4 days of sampling over a 24-hour period in plastic containers fitted below the outlet of metal tray of each metabolic cage. Containers were covered with screen to avoid contamination by hair, feces and ration. Collector funnels coupled to hoses were used, which conducted urine to plastic containers with 100 mL of 20% H_2SO_4. The urine volume was quantified at the end of each 24-h period, and subsamples were filtered through cheesecloth layers and immediately diluted with 40 mL of 0.036 N H_2SO_4 solution (Valadares et al., 1999). At the end of the collection period, an aliquot of 20 mL of total urine output per animal was sampled. Urine samples were composited per period for each animal, and proportionally to the 3 sampling days. Composite samples were stored at −20 °C for later laboratory analyzes (Valadares et al., 1999). On the last day of each period, 3 h after feeding, the blood were taken from the jugular vein either by venipuncture and inserted into the test tubes containing anticoagulant (EDTA). The samples were then centrifuged at 2500 rpm (700×g) for 15 minutes to separate the plasma. Isolated plasma was poured into 3 mL microtubes. The samples were stored at -20 °C until laboratory analysis. Blood parameters including glucose, cholesterol, triglyceride, urea nitrogen and total protein were measured using standard kits (Pars Test Company). The chemical constituents of the consumed feed, residual feed and feces samples were measured by conventional methods (AOAC, 2000). Neutral detergent fi-

ber was measured by the method of Van Soest et al. (1991).

Measurement of urinary purine derivatives and estimation of microbial protein synthesis

Purine derivatives including allantoin, uric acid, and xanthine-hypoxanthine were measured using photometric methods following Chen and Gomes (1995) guidelines.

Statistical analysis

This experiment was conducted in the form of a change over design over four 14-day periods. After data collection, statistical analysis was performed using SAS software and mixed procedure (2001). The means were compared using the Tukey test. The statistical model used was:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk}$$

where: Y_{ijk} is the individual observation, μ is the overall mean, T_i is the effect of the ration, P_j is the period effect, C_k is animal effect and e_{ijk} is test error effect. The normalization of the data was evaluated using SAS software (2001). Statistical significance between groups was computed by analysis of variance and $P<0.05$ was considered significant.

Results

There was a significant difference between the percentages of dry matter, crude protein, ash and ADF between treatments, but the percentage of fat and NDF did not show significant differences between treatments (Table 1). The results showed that feed intake was not affected by the experimental treatments and there was no significant difference between treatments (Table 2).

According to the results of Tables 3 and 4, the amounts of excreted and adsorbed purine derivatives and microbial protein synthesis were between alfalfa silage (control) and alfalfa silage treated with acetic acid and Ecosyl and their combination for excreted purine derivatives (mean values of 12.29, 7.02, 12.03 and 8.64 g/d, respectively; $P=0.05$), and absorbed purine derivatives (mean values of 13.46, 7.19, 13.14 and 9.12 g/d, respectively; $P=0.05$), and microbial protein synthesis (mean values of 9.79, 5.23, 9.56 and 6.63 g/d, respectively; $P=0.05$).

In the present experiment, excreted and adsorbed purine derivatives and microbial protein synthesis were reduced in alfalfa silages processing with Ecosyl and the combination of acetic acid and Ecosyl. The decrease in microbial protein synthesis was due to the decreased excretion of the purine xanthine and hypoxanthine derivatives ($P<0.05$).

The results showed no significant differences in the levels of glucose, protein, triglyceride and urea nitrogen between the treatments as are presented in Table 5, but there was a significant difference in the levels of blood cholesterol between the treatments ($P<0.05$).

Table 2. Feed Intake (g/day)

Alfalfa silage	Feed Intake
Control	2125
Probiotic (Ecosyl)[1]	2401
Acetic acid [2]	2420
Acetic acid and Ecosyl combination	2392
P-value	0.40
SEM	139

[1] Ecosyl Products, Ltd., Stokesley, England.
[2] Acetic acid as distillation vinegar from the grocery shop of Ardabil, Iran.

Discussion

In alfalfa silages with 30% dry matter, inoculation of Ecosyl resulted in silages with more lactic acid and a lower pH than in untreated silage after 2 d of ensiling (Whiter et al., 2001). Researchers have been shown feed intake can be unaffected by processed alfalfa. Behgar et al. (2007) reported that dry matter intake in cows fed using sulfuric acid + formic acid for alfalfa silage was higher than cows fed by the acid-free alfalfa silage, but this difference was not statistically significant. The results of Delaware and Danesh-Mesgaran (2003) showed that dry matter intake of alfalfa silage treated with urea and sulfuric acid did not differ significantly between treatments.

Aksu et al. (2017) observed that the reducing in the load of undesirable microorganisms (yeast and mold etc.) without causing a decline in the number of lactobacilli can be provided significantly advantages in terms of improving the aerobic stability of alfalfa silages. Microbial inoculation increased initial counts of lactic acid bacteria from approximately 3.5 to 4.9 log10 cfu/g forage. Inoculation markedly improved fermentation as evident by lower silage pH ($P<0.01$), acetic acid ($P<0.05$) and NH3-N ($P<0.05$) contents (Kung, JR., 1991). Ghodousi et al. (2015) reported that dry matter intake in sheep fed banana (cut branches with leaf) silage with the waste dates was not affected by the experimental diets but the trend was linear with increasing silage level. Hossein-Abadi et al. (2013) indicated dry matter intake in calves fed bacterial probiotic in milk and starter feed was not significant. The addition of bacterial inoculant

Table 3. Purine derivatives (mmol/day)

Alfalfa silage	Xanthine	Hypoxanthine	Uric Acid
Control	2.05[a]	8.35	1.88[a]
Probiotic (Ecosyl)[1]	1.43[ab]	5.51	0.07[c]
Acetic acid [2]	2.07[b]	8.96	0.98[b]
Acetic acid and Ecosyl combination	1.21[b]	6.85	0.58[ab]
P-value	0.03	0.34	0.01
SEM	0.24	1.44	0.19

a,b: Within columns, mean values with common superscript (s) are not different (*P*>0.05; Tukey's test).
[1]Ecosyl Products, Ltd., Stokesley, England.
[2]Acetic acid as distillation vinegar from the grocery shop of Ardabil, Iran.

Table 4. Purine derivatives and microbial protein synthesis

Alfalfa silage	Absorbed purine derivatives (g/day)	Excreted purine derivatives (g/day)	Microbial protein production (g/day)	Microbial nitrogen production (g/day)
Control	13.46[a]	12.29[a]	9.79[a]	61.17[a]
Probiotic (Ecosyl)[1]	7.19[b]	7.02[b]	5.23[b]	32.66[b]
Acetic acid [2]	13.14[a]	12.03[a]	9.56[a]	59.72[a]
Acetic acid and Ecosyl combination	9.12[ab]	8.64[ab]	6.63[ab]	41.43[ab]
P-value	0.05	0.05	0.05	0.05
SEM	1.9	1.6	1.4	8.6

a,b: Within columns, mean values with common superscript (s) are not different (*P*>0.05; Tukey's test).
[1]Ecosyl Products, Ltd., Stokesley, England.
[2]Acetic acid as distillation vinegar from the grocery shop of Ardabil, Iran.

Table 5. The blood parameters male sheep (mg/dL)

Parameters	Experimental diet				*P*-value	SEM
	Control	Probiotic (Ecosyl)[1]	Acetic acid [2]	Acetic acid and Ecosyl combination		
Glucose	60.60	57.58	63.02	59.22	0.52	2.60
Protein (g/dL)	6.64	7.04	7.09	6.90	0.52	0.24
Triglyceride	19.46	17.64	18.21	18.39	0.38	2.90
Cholesterol	37.69[a]	37.53[a]	33.98[b]	35.51[ab]	0.03	0.90
Blood urea nitrogen	16.45	16.92	16.34	16.23	0.93	0.80

a,b: Within row, mean values with common superscript (s) are not different (*P*>0.05; Tukey's test).
[1]Ecosyl Products, Ltd., Stokesley, England.
[2]Acetic acid as distillation vinegar from the grocery shop of Ardabil, Iran.

resulted in better preservation and reduced the aerobic stability of silage (Sifeeldein et al., 2018). Also, these researchers suggested that, for well-preserved silage, the isolates may be useful as inoculants for silage making, and could play a major role in developing silage production (Sifeeldein et al., 2018). In similar results to the current experiment performed by Mohammadi Roodposhti and Dabiri (2012), Riddell et al. (2010) found that dry matter intake of calves fed bacterial probiotic in starter milk or starter diet was not affected compared to the control group. But contrasting the present experiment, the results of studies by Chaudhary et al. (2008) and Donovan et al. (2002) on calves showed that the addition of probiotics to calves diet significantly increased feed intake compared to the control group. The results of this experiment are consistent with the results of Behgar et al. (2007), Hossein-Abadi et al. (2013), Mohammadi Roodposhti and Dabiri (2012) and Riddell et al. (2010). Research led by Karmshahi et al. (2013) uric acid was not affected by camel thorn silage with the waste dates in experimental diets. Xanthine and hypoxanthine levels increased linearly with camel thorn silage using the waste dates. This might be due to the high levels of these compounds in the urine of sheep fed diets containing 14 and 21% camel thorn silage with the waste dates, increased excretion of purine derivatives with these diets, and better efficiency of microbial protein synthesis, respectively. Alfalfa haylage ensiled at high DM contents (in particular those with >50–55% DM) may present a tobacco smell that is associated with changes in the characteristics of the protein fractions, producing heat-damaged proteins as a result of the Mail-

lard reaction (Kung, JR et al., 2018). The mean total purine derivatives in the sheep increased linearly with increasing levels of camel thorn silage with the waste dates in the experimental diets. Ghodousi et al. (2015) reported that the mean excreted allantoin in sheep increased linearly with increasing levels of banana (cut branches with leaf) silage with the waste date in experimental diets so that sheep fed diets 14% of this silages had higher urinary excretion of allantoin. Shakeri et al. (2011) indicated the excretion of allantoin in Holstein male calves fed with pistachio by-product silage was affected by the experimental diets and the observed difference was significant. These researchers attributed the increase in allantoin to increased feed intake and microbial protein synthesis. Also, it would be because the inoculation increasing proteolysis as indicated by a reduction in true protein and neutral detergent insoluble protein and an increase in non-protein nitrogen. Ruminal degradability of DM, crude protein and neutral detergent fiber of alfalfa silage were not affected by inoculation (Rizk et al., 2005). With increasing feed intake, growth and proliferation of rumen microorganisms have increased due to the availability of energy for microorganisms, which resulted in increased allantoin and ultimately increased microbial protein synthesis. The nucleic acids of the rumen microbes are degraded when they enter the gut, and the purine and pyrimidine nucleosides are decomposed by the xanthine oxidase enzyme into purine derivatives (Vakil-Faraji et al., 2009). Ammonia in the rumen is a key intermediate in the degradation of microbes as well as the main substrate for microbial protein production. The dietary protein is converted to microbial protein in the rumen by microbial enzymes. Rumen bacteria need three nitrogen sources to produce microbial protein, including ammonia nitrogen, amino acid nitrogen, and peptide nitrogen (Chaudhary et al., 2008; Guo et al., 2018). The microbial protein produced contains ribonucleic acid that breaks down after being transferred to the intestine. Purine nucleosides and free purines are absorbed through the intestine. These compounds are excreted in the urine by the activity of xanthine oxidase to purine base derivatives such as allantoin, uric acid, xanthine, and hypoxanthine broken down by the kidneys and in the urine. About 90% of these derivatives are allantoin, so it can be estimated that the amount of microbial protein entering the small intestine can be estimated (Balcells et al., 1992). Chen et al. (1990) reported that more excretion of purine derivatives in the urine indicates that greater amounts of microbial nucleic acids enter the small intestine. The higher excretion of purine derivatives with higher levels of silage diets is probably due to more growth of microorganisms and production of more microbial protein due to soluble carbohydrates and efficiency of feed nitrogen utilization. The synchronization of dietary energy and protein increases the flow of microbial protein to the duodenum and the efficiency of microbial protein production (Balcells et al., 1992). Chen and Orskov (2004) showed that purine derivatives excreted in the urine are absorbed by microbial purines and that their purine tissues are derived from the animal. Zamani et al. (2007) found that the excreted, absorbed, and microbial nitrogen purines fed to the duodenum were affected by the diet containing urea and sulfur supplementation and had the highest share among the purine allantoin derivatives and urea and sulfur nutrition at 0.2 level. Sulfur and 0.5% urea caused the highest excretion of allantoin. Uric acid, xanthine and hypoxanthine increased the levels of urea and sulfur, respectively, and increased the excretion of these derivatives. Microbial nitrogen entered into the duodenum as well as microbial nitrogen. For the organic matter eaten and the rumen fermented organic matter also increased with increasing levels of urea and sulfur. Purines found in ruminant feeds are low, and these low purines also decompose in the rumen during bacterial fermentation, so the nucleic acids that leave the rumen are mostly of microbial origin. The results of the study of Whiter et al. (2001) agree with previous research that shows that microbial inoculation (Ecosyl) can improve the fermentation of alfalfa silage. In another investigation, on the contrary with the results of Sadeghi et al. (2012) that showed homo-fermentative Ecosyl inoculants did not improve the fermentation characteristics and nutritive value of low dry matter corn silage. This may be due to the type of forage and the amount of moisture it contains. Absorbed and excreted in the urine after purification of purine derivatives, in allantoin ruminants, the most important product of the purine catabolism and the major derivative excreted in urine (Yu et al., 2002).

Changes in blood parameters are an important indicator of metabolic changes and their pathways such that Ghodousi et al. (2015) reported that the blood glucose level of sheep was increased linearly and significantly (P=0.07) by increasing the level of banana (cut branches with leaf) silage with the waste date. In ruminants, one of the causes of increased blood glucose concentration can be due to increased propionate in the rumen (Van Soest, 1994). The protein, cholesterol and nitrogen urea levels of sheep blood did not change with the addition of banana (cut branches with leaf) silage with the waste dates in the diet (Ghodousi et al., 2015). The most protein-rich diet is the one with the lowest blood urea nitrogen (Li et al., 2016). Blood triglyceride levels increased

linearly with increasing levels of banana (cut branches with the leaf) silage in experimental diets (Ghodousi et al., 2015). Hajalizadeh et al. (2013) reported that the blood glucose, protein and triglyceride levels of animals were not altered by feeding different levels of silage residue obtained from pistachio peel. Blood cholesterol levels in sheep increased by 14% and then decreased, but blood urea nitrogen decreased linearly. Hossein-Abadi et al. (2013) reported the addition of probiotics to the diet of calves (milk or feed) decreased blood glucose. Riddell et al. (2010) also found that probiotic supplementation had no significant effect on total blood protein concentration.

Conclusion

Total excretion of purine derivatives and adsorbed purine derivatives in urine and microbial protein synthesis were reduced in alfalfa silage treated with acetic acid and Ecosyl and their combination. Findings showed that there were also no significant differences in all of the haematological parameters between the treatments, except for cholesterol values. Generally, alfalfa silage treated with Ecosyl alone could not change its nutritional value. However, alfalfa silage treated with acetic acid and Ecosyl improved the nutritional parameters of sheep.

Competing Interests

There is no conflict of interest for publication of this article.

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