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The Effect of Feeding *Tribulus terrestris* Plant Powder on Growth Performance, Digestibility, Rumen and Blood Parameters of Iranian Arabic Lambs

Research Article

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ABSTRACT

The effect of feeding *Tribulus terrestris* plant powder on performance, digestibility, protozoa morphology, blood and rumen parameters in Iranian Arabic lambs were studied. Eighteen 3 months old Iranian Arabic male lambs with average weight of 16 (±2) kg were assigned to a completely randomized design with 3 treatments and 6 replicates for 60 days. Experimental treatments were control ration and rations with 15 and 30 g *Tribulus terrestris* plant powder per kg dry matter. The treatments with *Tribulus terrestris* had the higher protein intake compared to control (P<0.05). The natural detergent fiber digestibility, amount of rumen ammonia nitrogen, the *Diplodinium*, *Ophryoscolex* and the total protozoa population in rations containing *Tribulus terrestris* was lower than control treatment (P<0.05). As ammonia nitrogen amount was 12.98, 9.87 and 9.68 mg/dL for control, 15 and 30 g/kg dry matter (DM), respectively. But the *Entodinium* and *Eudiplodinium* population were not different (P>0.05). The content of cholesterol, triglyceride, low density lipoprotein, urea and glucose of blood and liver enzymes of lambs treated with experimental rations were lower than control group; and the high density lipoprotein was higher (P<0.05). The content of low density lipoprotein was 12, 9 and 8 mg/dL for control, 15 and 30 g/kg DM, respectively. According to this current study, effect of feeding *Tribulus terrestris* plant powder to fattening lambs had significant effect on digestibility, rumen fermentation and blood parameters of Iranian Arabic fattening lambs.

KEY WORDS blood parameters, digestibility, lamb, *Tribulus terrestris*.

INTRODUCTION

One aspect of rumen fermentation control is to limit methane production. The medicinal plants have been used to change the fermentation pattern in the rumen (Khiaosa-Ard and Zebeli, 2013). Many plants produce secondary metabolites which have antimicrobial effects (Negi, 2012) and decrease degradation of protein, amino acid and starch in rumen (Benchaar and Chouinard, 2009). Therefore, scientists are oriented toward using medicinal plants and their extracts to manipulate and regulate rumen fermentation; improve the rumen ecology, and enhance the use of nutrients in the livestock (Patra and Saxena, 2011; Valenzuela-Grijalva *et al.* 2017). The *Tribulus terrestris* is a one-year-procumbent plant growing in tropical areas including America, Mexico and the Mediterranean areas (Adaikan *et al.* 2000; Gauthaman and Ganesan, 2008). The *Tribulus terrestris* is used in traditional medicine of Chinese, Iran, Iraq, India, Bulgaria and South Africa. Soft fruits with sharp thorns of this plant are the most effective part that is used as a medicine (Ahmed *et al.* 2009). Leaves and stems of *Tribulus terrestris* contain steroids, glycosides, flavon-

oids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, potassium, nitrates, polyphenols, minerals, aspartic acid, glutamic acid and steroid saponins (Adaikan *et al.* 2000). This plant has antimicrobial and antibacterial effects, antioxidant activity, and the ability to remove oxygen free radicals and damaging factors and decrease the blood cholesterol (Ody, 2000; Guo *et al.* 2007; Choudhury *et al.* 2015). Due to different components such as carbohydrates, glycosides, and vitamin A, the *Tribulus terrestris* affects the metabolism and weight (Hejazi and Hosseini, 2016).

Kianbakht and Jahaiani (2003) reported that due to the activity of the *Tribulus terrestris* plant against both gram-positive and gram-negative bacteria could act for the maintenance of animal or human health. *Tribulus terrestris* herb improved sperm motility and count (Moghaddam *et al.* 2013) due to presence of 55% furostanol saponins or protodioscin (Kistanova and Zlattev, 2006).

It has been reported that protodioscin as an steroidal saponin in *Tribulus terrestris* extract, can be converted to dehydroepiandrosterone that increase muscle mass in broiler chicks (Adimoelja and Adaikan, 1997).

Using of *Tribulus terrestris* plant, reduced blood glucose (Hussain *et al.* 2009), and total cholesterol and LDL in diabetic mice. Flavonoids and saponins of *Tribulus terrestris* are responsible for reduce serum glucose, triglyceride and cholesterol in diabetic mice (Samani *et al.* 2016). Sahin (2009) revealed that daily feed intake and internal organ weights of broilers were not influenced with *Tribulus terrestris* powder but feed conversion ratio was lower and *Tribulus terrestris* powder positively influence growth performance and immune responses of broiler chicks. Also Cek *et al.* (2007) concluded beneficial effects of *Tribulus terrestris* on growth rate of fish. Information on *Tribulus terrestris* plant powder in the ruminant nutrition is rare; therefore this study was designed to investigate the dietary effect of of *Tribulus terrestris* on performance, digestibility and rumen fermentation, protozoa morphology and blood parameters in Iranian Arabic lambs.

MATERIALS AND METHODS

Animals and experimental rations

The samples of *Tribulus terrestris* plant were collected from the northeast of Iran in early May (whole plant including flower, fruits, leaves and stem). Sampling of plants was performed by plot method with dimensions of 10 × 10 meters and in each plot about 10 plants sampled. Then the equal amount of samples mixed, air-dried in the shadow and grounded.

Chemical composition of whole plant of *Tribulus terrestris* including crude protein (CP) (Kjeldahl method, Foss 2033, Sweden), dry matter (DM), ether extract (EE) and acid detergent fiber (ADF) were measured (AOAC, 1990). Natural detergent fiber (NDF) was determined according to Van Soest *et al.* (1991).

Eighteen three months old Iranian Arabic male lambs with average initial weight of 16 (±2) kg were assigned to a completely randomized design with 3 treatments and 6 replicates for 60 days. 10 days was for adaptation and 60 days was the main period of the experiment. Two weeks before the experiment, wool operations, vaccinations and anti-parasite drugs were performed.

All animals' management and sampling procedures conducted according to the care and use of agricultural animals in research and teaching guidelines (FASS, 2010). All procedures and guidelines involving animals were approved by the animal experiment committee at Agricultural Science and Natural Resources University of Khuzestan, Iran.

The basal ration was formulated based on NRC (2007) according to 150 g/day live weight gain. The ration composed of 60% concentrate and 40% forage (Table 1). The experimental treatments were included basal ration or control and basal ration with 15 and 30 g *Tribulus terrestris* plant powder per kg DM. As at first the plant powder fed to lambs then ration fed. The composition of *Tribulus terrestris* plant powder is given in Table 2. The animals were kept individually in metabolic cages. The daily feed was given to the animals in the morning (8:00 a.m.) and afternoon (16:00 p.m.). Feed intake was recorded daily and animals weighed weekly.

Sampling

For determination of nutrient digestibility, at the end of the experiment, feed and feces sampling was done for 5 days consecutiveand feed intake and excretion were recorded daily (Taherinia *et al.* 2015). Every morning samples of feed, orts, and faces were collected, oven-dried, grounded and analyzed for DM, organic matter (OM), ADF, NDF and CP.

Rumen fluid samples were taken 3 h after morning feeding by stomach tube. Samples pH was recorded immediately after collection by using a pH meter (Metrohm model, Swiss). The rumen fluid was strained through 4 layers muslin cloth and ammonia nitrogen amount was determined by using phenol-hypochlorite method (Broderick and Kang, 1980). About 10 mL rumen fluid was mixed with 10% formaldehyde by 50:50 ratio. Protozoa counting was performed using the inverter light microscope with a magnification of X 40 (NIS-Elements F 3.0) by the method described by Veira *et al.* (1983).

At the end of the experiment, blood samples were collected from the jugular vein in the tubes containing anticoagulant agent (EDTA 10%), 4 h after the morning feeding (Talebzadeh *et al.* 2012).

Table 1 Ingredients and chemical composition of basal ration[1]

Ingredients	(% of DM)
Alfalfa	30.0
Sugarcane top	7.0
Wheat straw	3.0
Barley	53.0
Wheat bran	6.0
Mineral and vitamins supplement[2]	0.5
Bicarbonate	0.3
Salt	0.2
Total	100
Chemical compositions	
Dry matter (DM)	92.3
Ether extract	2.7
Neutral detergent fiber (NDF) (%)	7.39
Acid detergent fiber (ADF) (%)	7.25
Organic matter (OM) (%)	4.95
Ash (%)	4.6
Crude protein (CP) (%)	13.7
Metabolizable energy (Mcal/kg)	2.5

[1] The *Tribulus terrestris* plant powder was add as 0, 15 and 30g/kg DM to the basal ration.

Table 2 Chemical composition of *Tribulus terrestris*

Chemical compositions	(% of DM)
Dry matter	92.02
Crude protein	20.1
Ether extract	11.5
Natural detergent fiber	53.1
Acid detergent fiber	44.4

Collected samples were centrifuged for 3000 × g at 4 ˚C for 15 min (Hermel, Germany) and plasma separated. The content of glucose, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), blood urea nitrogen (BUN) and liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)] was measured using quantitative diagnostic kit of Pars Azmon Company and using an auto-analyzer (Mindry, BS200, China).

Statistical analysis

The obtained data were analyzed as a completely randomized design using General Linear models (GLM) procedure in SAS software (SAS, 2002) which is based on the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

Where:
Y_{ij}: observation.
μ: general mean.
T_i: effect of treatment.
e_{ijk}: standard error term.

The means were compared using Duncan's multiple range tests at 5% level of significance.

RESULTS AND DISCUSSION

The treatment containing 15 g *Tribulus terrestris* had the highest protein intake compared to the other treatments ($P<0.05$). But feed intake, feed conversion ratio and daily weight gain were not affected by *Tribulus terrestris* ($P>0.05$) (Table 3).

According to Table 4, there was no important difference in the digestibility of DM, CP, OM and ADF among experimental rations ($P>0.05$). The NDF digestibility, amount of ammonia nitrogen, the *Diplodinium*, *Ophryoscolex* and the total protozoa population in the rations containing *Tribulus terrestris* plant powder was significantly lower than the control group ($P<0.05$). But there was no difference in the *Entodinium* and *Eudiplodinium* number among treatments ($P>0.05$).

The content of blood cholesterol, triglyceride, LDL, BUN, creatinine and glucose of lambs treated with experimental rations were lower than control group; and the HDLwas higher ($P<0.05$) (Table 5).

Table 3 Nutrient intake and performance in lambs fed with *Tribulus terrestris* (g DM/day)

Item	Control	15 g	30 g	SEM	P-value
Dry matter intake	639.3	665.8	667.4	16.8	0.28
Organic matter intake	597.2	604.4	597.9	8.4	0.80
Crude protein intake	84.1 [b]	87.5 [a]	87.4 [a]	0.8	0.003
Daily weight gain (g)	118.9	130.0	148.3	13.1	0.34
Feed conversion ratio	6.53	6.56	5.96	0.7	0.78

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 4 Digestibility of nutrients, rumen parameters and protozoa morphology in lamb fed with *Tribulus terrestris*

Item	Control	15 g	30 g	SEM	P-value
Digestibility					
Crude protein (%)	58.6	57.5	55.8	1.7	0.05
Acid detergent fiber (%)	50.2	44.0	40.3	3.3	0.08
Neutral detergent fiber (%)	56.2[a]	53.2[b]	49.7 [b]	1.9	< 0.01
Dry matter (%)	64.9	63.7	62.7	1.6	0.64
Organic matter (%)	50.6	58.8	56.9	1.7	0.09
Rumen parameters					
pH	6.43	6.40	6.38	0.03	0.41
Ammonia nitrogen (mg/100 mL)	12.9[a]	9.8 [b]	9.6[b]	0.6	< 0.01
Protozoa count (10^4/mL)					
Entodinium	24.7	28.3	31.0	1.7	0.10
Diplodinium	8.0[a]	6.0[b]	5.7 [b]	0.5	0.04
Eudiplodinium	7.7	8.0	7.3	0.8	0.17
Ophryoscolex	9.0[a]	0.0[b]	0.0[b]	0.3	0.03
Total protozoa	49.3[a]	42.6[b]	44.6[b]	2.4	0.02

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 5 Blood metabolites in lambs fed with *Tribulus terrestris* (mg/dL)

Treatment	LDL	HDL	Cholesterol	TG	Cratinine	BUN	Glucose
Control	12.0[a]	17.6[a]	38.3[a]	15.7[a]	0.9[a]	35.6[a]	73.0[a]
15 g	9.0[b]	21.9[a]	32.7[b]	12.0[b]	0.7[b]	31.6[b]	61.7[b]
30 g	8.0[b]	22.4[a]	32.6[b]	9.0[b]	0.6[b]	28.4[b]	52.3[b]
SEM	0.8	1.9	1.3	0.8	0.02	0.8	0.9
P-value	0.03	0.04	0.04	< 0.01	< 0.01	< 0.01	< 0.01

LDL: low density lipoprotein; HDL: high density lipoprotein; TG: triglycerides and BUN: blood urea nitrogen.
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 concentration of liver enzymes of ALT, AST and ALP (Table 6) decreased by addition of *Tribulus terrestris* plant to ration in comparison with control (P<0.05).

Due to the information on the using of *Tribulus terrestris* plant powder in the ruminant nutrition is rare, therefore other plants with similar metabolites were investigated. The current results showed that feed intake, feed conversion ratio and daily weight gain were not affected by *Tribulus terrestris*. In accordance with results of recent study, the use of myrtle plant powder containing tannin and quercytin; as an additive to sheep ration, didn't affect the feed intake, weight gain and feed conversion ratio (Salehpour *et al.* 2018). Payvastegan *et al.* (2015) reported that savory herb powder containing phenolic compounds had no significant effect on the dry matter intake and daily weight gain of growing lambs.

The effects of medicinal plants components on feed intake in ruminants are influenced by the composition, type and amount and animal resistance (Cobellis *et al.* 2016). Also, it has been demonstrated that the *Tribulus terrestris* extract containing carbohydrates, glycosides, and fat soluble vitamins that influence metabolism and increases appetite and weight of hyperlipidemic rats (Guo *et al.* 2007).

Researches revealed that feed intake can be influenced by interaction between medicinal plants with ration components (Benchaar and Chouinard, 2009). In the current study we no observed any effect on feed intake that indicated *Tribulus terrestris* had not any effect on palatability.

Also, no notable influence on weight gain, dry matter intake and feed conversion ratio was remarked by using savory essence in West Azarbaijan goats (Talatapeh *et al.* 2014).

Table 6 Liver enzymes in lambs fed with *Tribulus terrestris* (ng/mL)

Treatment	AST	ALT	ALP
Control	22.6[a]	105.3[a]	63.7[a]
15 g	10.0[b]	77.7[b]	11.7[b]
30 g	13.3[b]	95.7[b]	2.0[b]
SEM	2.4	12.4	11.3
P-value	0.02	0.03	0.02

AST: aspartate transaminase; ALT: alanine transaminase and ALP: alkaline phosphatase.
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 secondary compounds of plants change the rumen microbial population and can increase fermentation efficiency and improve nutrient utilization (Cobellis *et al.* 2016). In the present study, there is not any change on the performance that's way maybe the secondary compounds of *Tibullus terrestris* plant had not any significant effect on rumen microbial population, digestibility and fermentation and consequently the animal performance (Benchaar and Chouinard, 2009; Kumar *et al.* 2014). Saeedi *et al.* (2014) observed improve of daily weight gain and the feed conversion ratio in dairy calves fed 0.4 and 0.8% fennel powder containing quercetin.

According to the current results, digestibility of DM, OM, CP and ADF was not influenced but the NDF digestibility deceased in the rations containing *Tribulus terrestris* plant powder. But it was reported that using of *Silybum marinum* plant containing flavonoids and tannin increased NDF digestibility (Mojadam *et al.* 2015).

According to studies, leaves and stems of *Tribulus terrestris* contain tannins, and polyphenols that the plants tannins decrease fiber digestibility through binding with lingocellulose and microorganisms and or direct inhibition of microorganisms (McSweeney *et al.* 2001). Study of Kumar *et al.* (2014) exhibited that use of pure phenol compounds reduced the NDF digestibility; due to effect on fibrolytic bacteria. Active compounds of plants, the concentration, doze, forage type, feeding method, forage to concentrate ratio and the concentrate composition affect animal response to plant active compounds (Kumar *et al.* 2014).

The low impact of medicinal plants on DM digestibility has been attributed to the used level that may not be able to change the rumen microbial activity (Fereidounpour *et al.* 2014).

Tribulus terrestris plant powder reduced the ammonia nitrogen concentration but not rumen pH. The addition of savory essence containing flavonoids inhibits the conversion peptide to amino acid and ammonia (Ghasemifard *et al.* 2017). The reason for reduction in ammonia nitrogen concentration may be because of inhibitory effect of some compounds such as tannin on the proteolytic activity of rumen microbes, or anti-protozoa effect of these compounds (Talebzadeh *et al.* 2012).

Saponins inhibit protozoa and subsequently decrease ammonia nitrogen concentration (Bartos *et al.* 2016).

Possibly negative effect of active ingredients of medicinal plants on high ammonia producing bacteria can cause a reduction in rumen ammonia and pH (Busquet *et al.* 2006). But in accordance with the current results, fennel powder did not have any considerable effect on rumen pH (Yari Haj Ata'lou *et al.* 2018).

According to this study, the rations containing *Tribulus terrestris* plant powder reduced *Ophryoscolex*, *Diplodinium* and total protozoa population. The flavonoid compounds in medicinal plants due to anti protozoa effects reduce the protozoa species (Patra and Saxena, 2011). Also, the phenolic structure of secondary metabolites cause to breakdown the cell membrane, inactivate enzymes, and reduce the substrate and metal ions required for microorganism metabolism (Bartos *et al.* 2016).

Researchers reported that the rumen protozoa population in sheep and cattle fed with plant essences were not influenced (Ghasemifard *et al.* 2017). Different factors such as the type and physiological condition of animal, the using period and preparation method of the plant compounds, and base ration influence results different experiments (Abarghouei and Roozbahan, 2013). Similar with current study, the grape extract containing tannin and saponin reduced the total protozoa, *Ophryoscolex* and *Diplodinium* (Abarghouei and Roozbahan, 2013). Also, Nourian Sarvar *et al.* (2015) revealed that high levels of eucalyptus essence caused to decrease *Ophryoscolex* and *Diplodinium.*

The current result showed the blood glucose, BUN, triglycerides, cholesterol and LDL decreased with the addition *Tribulus terrestris* powder and HDL increased. The prescription of *Tribulus terrestris* plant extract in diabetic mice considerably decreased the serum triglycerides, cholesterol and LDL; and its methanol extract reduced the blood glucose in diabetic mice (Ahmed *et al.* 2009).

Saponin of *Tribulus terrestris* plant can reduce glucose and inhibit gluconeogenesis. In addition, oral prescription of saponin of *Tribulus terrestris* plant in rat, may lead to delayed glucose uptake and reduce blood glucose by inhibiting of alpha glucosidase of the small intestine (Gandhi *et al.* 2013).

Also saponin suppres glucose transfer from gastric to small intestine and inhibition the transfer of glucose across the small intestine and prevent hepatic gluconeogenesis (Roghani *et al.* 2010).

Ghasemifard *et al.* (2017) revealed that 10% chamomile and chicory had no effect on blood metabolites of Dallagh sheep. Hussain *et al.* (2009) concluded that treating diabetic mice with *Tribulus terrestris* extract resulted in significant reduction in triglycerides, total cholesterol and LDL compared with the untreated mice. Administration of *Tribulus terrestris* is effective in reducing of total cholesterol, triglycerides, LDL and increasing of HDL in diabetic rats (Raghavendra *et al.* 2011).

In one study, it has been defined that the *Tribulus terrestris* extract can lead to reduce hyperlipidemia in rabbits fed with high-fat diet, and it also reduced the damage to the endothelium cells (Huang *et al.* 2008). The polysaccharides, flavonoids, glycoproteins, polypeptides, steroids, alkaloids and pectin in medicinal plants can well explain the hypoglycemic and hypolipidemic properties of some medicinal plants such as *Tribulus terrestris* (Yan *et al.* 1996). In fact, flavonoids reduce the risk of heart diseases by inhibiting the LDL oxidation and reduce cholesterol. Medicinal plants and their extracts play an important role in reducing of cholesterol and blood lipids by production of bile acid degrader enzymes, and the pH reduction in the intestinal duct (Kianbakht and Jahaiani, 2003).

The results of many experiments illustrated that tannin decrease the rumen ammonia concentration due to the lower protein degradation and subsequently reduce plasma urea nitrogen. The use of *Silybum marinum* containing flavonoids leads to reduce of blood glucose and BUN (Mojadam *et al.* 2015). The reduction of BUN by plant essences can be a reflection of the effect of active compounds on rumen microorganisms, reduction of deamination and urea production in the liver. This can increase feed efficiency by reducing the amount of consumed energy in the excretion of nitrogen metabolites (Kholif *et al.* 2012). One of the notable points in the present study was the nitrate compounds in *Tribulus terrestris* (Adaikan *et al.* 2000). Lack of increase in BUN and ruminal ammonia may indirectly show that the nitrate of *Tribulus terrestris* had not negative effect in this study.

The concentration of ALT, AST and ALP was decreased by addition of *Tribulus terrestris* plant to ration. It was declared that *Tribulus terrestris* extract reduced liver enzymes in mouse (Hejazi and Hosseini, 2016). Also, in another study, *Tribulus terrestris* extract had antioxidant activity, strengthens and protects liver cells (Lakshmi *et al.* 2012). Therefore, *Tribulus terrestris* extract having strong antioxidant compounds, has been illustrated to reduce liver transaminases by inhibiting the destructive effects of oxidative compounds (Kannan *et al.* 2015). Researchers have also indicated that *Tribulus terrestris* can increase levels and activity of non-enzymatic antioxidants and antioxidant enzymes in the body and reduce lipid peroxidation and oxidative stress (Kamboj *et al.* 2011). In the present study, the reduction of liver enzymes in *Tribulus terrestris* treatments is probably due to the anti-oxidative effects of this plant on liver tissue. Also, due to the anti-apoptosis effects of saponins of the *Tribulus terrestris* plant, it probably caused to greater survival of liver cells and has resulted in reduction in the serum levels of liver enzymes (Huang *et al.* 2008).

CONCLUSION

According to the current study, adding *Tribulus terrestris* plant powder (15 and 30 g per kg DM ration) had not the negative effects on digestibility, rumen fermentation and blood parameters. As digestibility of rations no changed and amount of ammonia nitrogen, protozoa population, the blood cholesterol, glucose and liver enzymes of lambs were lower; and the HDL was higher by *Tribulus terrestris*. Therefore, it can be used as a feed additive in diets of lambs, but it needs to do more studies on the effect of a higher inclusion level of this plant on growth performance, digestibility and carcass traits of lambs.

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