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Effect of diets containing oak kernel on the rumen fermentation and digestibility, blood metabolites and liver enzymes in Khuzestani buffalo

TAHEREH MOHAMMADABADI*, MOJTABA SHEIKH AZADI and MEHDI BABAEI

University of Khuzestan, P.O. Box 63517-73637, Mollasani, Ahvaz, Iran

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ABSTRACT

This study was conducted to investigate the effect of different levels of oak kernel on rumen digestibility and fermentation, blood metabolites and liver enzymes of Khuzestani buffalo. Sixteen male buffaloes, one year old were fed with experimental diets containing different levels of oak kernel (0, 15.8%, 31.58% and 47.37% equal to 0, 1.5, 3 and 4.5% tannin, respectively) in a completely randomized design for 35 days. In end of experiment, dry matter intake, digestibility, rumination behaviour, rumen and blood parameters were measured. The results showed that oak kernel increased dry matter intake and digestibility of dry matter and organic matter but the digestibility of protein, NDF and ADF was decreased. Incorporation of 47.37% oak kernel in the diet caused significant decrease in ammonia-N and protozoa population of buffalo rumen, but *p*H and total fatty acids were not affected. The rumination activity in diet containing 47.37% oak kernel was the lowest. With the increasing oak kernel in the buffalo diet, blood cholesterol level decreased, but there were no significant changes in blood glucose, urea, triglycerides, alkaline phosphatase, aspartate and alanin amino transferase enzymes between the experimental treatments. Therefore, even if the digestibility of ADF and CP decreased by adding of oak kernel but the using of oak kernel up to 47.37% in buffalo diet had no negative effect on the rumen fermentation characteristics, digestibility and blood metabolites, hence it may be used in buffalo's diet.

Keywords: Blood metabolites, Buffalo, Digestibility, Fermentation, Oak kernel

One of feed items that can be used as an alternative to livestock in autumn and winter is oak kernel (Rajablo 2009). The main part of the oak kernel is starch (about 55%). Therefore it can be used as a source of energy, and as a substitute for concentrates in ruminant's nutrition (Kilic *et al.* 2010).

The approximate analysis of oak kernel shows that the chemical composition of oak fruits is similar to cereals (Ozcan 2006). The use of oak kernel is restricted due to the presence of anti-nutritional agents (8.8% tannins which 57% is hydrolysable) (Jafari *et al.* 2018).

Researchers reported that oak consumption probably reduces protein degradation in the rumen and growth of proteolytic bacteria. Studies have shown that tannins prevent digestion of lignocellulosic material, and the binding of microbes to feed particles, and consequently reduce digestibility (Rivera-Méndez *et al.* 2017).

Information on use of oak kernel tannin in buffalo nutrition is not sufficient; hence this study was conducted to investigate the effect of oak kernel on the digestibility, rumen fermentation characteristics and blood metabolites of Khuzestani buffalo.

*Corresponding author e-mail: mohammadabadi@asnrukh.ac.ir

MATERIALS AND METHODS

Sixteen male khuzestani buffaloes (average weight 290 kg) in the research center of Safiabd, Dezful, Iran were selected. Animals were randomly divided into 4 groups with 4 replications and placed in individual pens places. Drinking water and daily feed were provided separately. Daily meals were administered two times in the morning (08:00 h) and afternoon (16:00 h) to the livestock. The buffaloes diets were formulated on the weight of the animals and according to the dietary requirements (NRC 2000). Treatments consisted of control (without oak) and three levels of oak kernel (15.8, 31.58 and 47.37% equal to 1.5, 3 and 4.5% tannin, respectively). The chemical analysis of oak kernel is as follows: fat 5%, protein 4.8%, carbohydrate 64%, and total tannin 8.5%. Total tannins (TT) were conducted in three replicates as described by Makkar (2000).

Each treatment was randomly assigned to each group of animals for 35 days. All experimental diets were isoenergetic and iso-nitrogenous (Table 1).

In order to determine the apparent digestibility of nutrients, during the last five days of the experiment, feed, feces, and residual feed per animal was collected. The samples were dried in an oven at 60°C for 48 h, milled, then NDF (nautral detergent fibre), ADF (acid detergent fibre) and dry matter were measured by standard methods

Table 1. Ingredient (%) and chemical composition of experimental diets

Ingredient	Treatment			
	1	2	3	4
Corn	25.00	4.00	4.00	0.00
Barely	13.00	19.00	8.00	0.00
Wheat bran	12.00	11.00	6.42	4.00
Oak kernel	0.00	15.80	31.58	47.35
Wheat straw	17.00	12.00	5.50	0.00
Alfalfa hay	18.00	24.00	37.50	49.00
Corn silage	14.00	13.00	6.00	0.00
Salt	0.50	0.50	0.50	0.50
Mineral and vitamin supplement	0.50	0.50	0.50	0.50
Net energy (Mcal/kg)	1.52	1.47	1.44	1.41
Crude protein (%)	10.58	10.61	10.30	10.40
Neutral detergent fibre (%)	37.76	42.76	43.95	45.96
Acid detergent fibre (%)	22.29	26.52	29.53	32.64
Ether extract (%)	2.55	2.54	3.19	3.76
Calcium (%)	0.48	0.57	0.72	0.85
Phosphorous (%)	0.44	0.41	0.33	0.26

1, 2, 3 and 4 were diets containing 0, 15.8, 31.58 and 47.37% oak kernel equal to 0, 1.5, 3 and 4.5% tannin, respectively.

(AOAC 1990) then digestibility of nutrients was measured.

Blood samples were collected 2 h after morning feeding in the tubes containing EDTA and centrifuged (3,000 rpm for 15 min). Then glucose, urea, cholesterol, triglyceride and liver enzymes were measured using Pars Azmon chemical kits and Auto Analyzer (BS200). Also for determination of ammonia nitrogen (phenol hypochlorite method) and *p*H (*p*H meter), rumen fluid was taken, 3 h after morning meal, by using stomach tube.

For rumen protozoa count, the rumen fluid fixed by using a stabilizing solution of 10% formaldehyde. Methylene blue coloring and counting were performed with optical microscopy (Yanez 2004).

The estimation of total volatile fatty acids in this experiment was carried out according to Getachew *et al.* (2002). The activities of rumination, chewing, resting and eating were recorded every 5 min with in 24 h. Statistical analysis of the obtained data was done in a completely randomized design using General Linear Models (GLM) procedure in SAS software, using the following statistical model:

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

where Y_{ij}, observation; μ, general mean, T_i, effect of experimental treatments and e_{ijk}, standard error term. The means were compared using Duncan's multiple range tests at 5% level of significance.

RESULTS AND DISCUSSION

The results of this experiment showed that the dry matter intake of buffaloes (Table 2) increased with increasing of the oak kernel in the diet (P<0.05). In agreement of this study, Rivera-Méndeza *et al.* (2017) reported tannin supplementation tended to increase dry matter intake in steers during the finishing feed lot phase. But Henke *et al.* (2017) reported quebracho tannin extract did not have any effect on feed intake of dairy cows and intake of organic matter (Al-Kindi *et al.* 2016). Shakeri and Fazaeli (2005) reported that the 30% dried pistachio byproducts in the diet of lamb's decreased feed intake

Table 2. Effect of different levels of oak kernel on dry matter intake, nutrient digestibility and rumen parameters in buffaloes

Parameter	Treatment				SEM	P value
	1	2	3	4		
Dry matter intake (g/d)	7125[b]	8250[ab]	8350[ab]	8500[a]	314.49	0.03
Digestibility						
Dry matter	78.33[b]	81.18[a]	83.32[a]	85.05[a]	1.62	0.04
Crude protein	43.9	30.12	26.8	23.31	4.71	0.03
Organic matter	72.24[b]	76.32[ab]	79.64[ab]	83.51[a]	2.51	0.03
NDF	55.7	52.11	48	45.21	4.09	0.2
ADF	44	39.1	38.2	33.1	2.2	0.012
Rumen parameters						
*p*H	7.07	6.77	6.67	6.63	0.05	0.05
Ammonia-N (mg/dL)	16.62[a]	13.23[b]	8.28[b]	6.81[b]	0.62	0.02
VFA (mmol/L)	75.1	77.1	76	78	0.3	0.4
Protozoa (Cell/mL rumen fluid*10^4	6.2[a]	5.1[b]	4.8[b]	3.4[b]	0.27	0.02

1, 2, 3 and 4 were diets containing 0, 15.8, 31.58 and 47.37% oak kernel equal to 0, 1.5, 3 and 4.5% tannin, respectively.

SEM, standard error of the averages; There are significant differences in the numbers in each row with non-identical alphanumeric characters (P<0.05).

Increasing oak kernel level in diet cause to increase dry matter and organic matter digestibility and the highest dry matter digestibility was for treatment containing 4.5% oak tannin. But the digestibility of protein, NDF and ADF was decreased (P<0.05).

One of the reasons for increasing digestibility of dry matter in diets containing oak kernel may be attributed to the higher amounts of non-structural carbohydrates (NSC) in these diets. Ghasemi *et al.* (2010) also indicated that replacement of pistachio hulls (7.7% phenolic compounds) up to 50% in Baluchi sheep's, increased the digestibility of dry matter and organic matter, due to the higher content of NSC in these diets in compared to alfalfa diet.

The recent results showed the protein, NDF and ADF digestibility was reduced by oak kernel. Besharati *et al.* (2008) reported that raisin residue as a tannin source in Ghezel sheep reduces the protein digestibility. But Henke *et al.* (2017) reported quebracho tannin extract did not influence N digestibility. Tannins reduce the fibre digestion through the formation of a complex with lignocellulose, binding to microorganisms and extracellular enzymes of microorganisms.

Mohammadabadi and Jolazadeh (2017) reported that NDF and ADF digestibility after 24 and 48 h by rumen bacteria was not affected by inclusion of subabul leaves (containing tannin) in goat's diet. Aguerre *et al*. (2016) reported that quebracho-chestnut tannin extracts reduced digestibility of dry matter, organic matter, crude protein and NDF.

The ammonia nitrogen concentration (NH_3-N) and protozoa population significantly decreased by increasing oak kernel level in the buffalo diet (P<0.05). Bhatta *et al.* (2012) reported tannin sources reduce the ammonia nitrogen content in the rumen.

Mohammadabadi and Chaji (2012) reported the NH_3-N in media culture decreased by processing sunflower meal with 30 g/kg DM oak fruit tannin. Also quebracho-chestnut tannin extracts reduced ruminal NH_3-N concentration (Aguerre *et al.* 2016). Sunflower meal treated with pistachio hull extract decreased *in vitro* ruminal NH_3-N and pH (Jolazadeh and Mohammadabadi 2017). In general, the reduction in the NH_3-N indicates that tannin of oak kernel protects the protein against rumen microbial digestion.

With the increase of oak kernel levels in the diet, the *p*H did not change. But Maldar *et al.* (2010) reported that the consumption of oak leaves and Jolazadeh and Mohammadabadi (2016) concluded sunflower meal treated with pistachio hull tannin decreased rumen *p*H.

Considering that with the increase of oak level in the diet, the cell wall content (especially NDF) decreased and by decreasing the rumen stimulation; salivary secretion and pH was reduced. Also one of the causes of decrease of rumen *p*H with increasing oak level in the diet is high carbohydrates of oak kernel.

The increase of oak kernel levels in the diet caused to decrease the protozoa population. Also incorporation of oak a corn to the diets decreased total protozoa (Aghamohamadi *et al.* 2014).

One of the reasons for the decrease in the protozoa population can be reduction of rumen *p*H in the diet. Probably other reasons for reduction in rumen protozoa population can be the presence of high amounts of starch in these diets and reducing the growth of proteolytic bacteria by tannins (Maldar *et al*. 2010).

The amount of total volatile fatty acid was not affected by the oak kernel tannin (P>0.05). In contrary to the current experiment, tannins extract of pistachio hull decreased ruminal VFA concentration (Jolazadeh and Mohammadabadi 2017).

Quebracho-chestnut tannin extracts reduced ruminal total branched-chain volatile fatty acid concentration (Aguerre *et al.* 2016). Tannin inclusion did not affect rumen total volatile fatty acid concentration and total protozoa populations (Aboagye *et al.* 2018).

The rumination time in diet containing 15.8% oak kernel was higher than other treatments and decreased linearly with the increase oak kernel in the diets (P<0.05). The highest resting time was observed in diet containing 47.37% oak kernel. The total chewing and eating time/dry matter, linearly decreased with increasing oak kernel in the diet (P<0.05) (Table 3).

Table 3. Effect of different levels of oak kernel on feed intake behavior in buffaloes

Parameter	Treatment				SEM	P value
	1	2	3	4		
Eating (min/day)	385.0	317.5	280.0	264.0	34.20	0.40
Resting (min/day)	670.0[b]	685.0[b]	717.5[a]	745.0[a]	31.70	0.02
Rumination (min/day)	437.7[a]	442.5[a]	415.0[a]	385.0[b]	45.85	0.03
Chewing	822.7[a]	760.0[a]	695.0[b]	649.1[b]	31.70	0.03
Activity for dry matter						
Eating	45.1[a]	44.1[a]	33.1[b]	30.0[c]	0.30	0.03
Rumination	60.0	50.0	47.0	47.0	5.10	0.40
Chewing	105.0[a]	94.1[b]	80.0[c]	77.0[d]	0.20	0.03

1, 2, 3 and 4 were diets containing 0, 15.8, 31.58 and 47.37% oak kernel equal to 0, 1.5, 3 and 4.5% tannin, respectively. SEM, standard error of the averages. Figures with non-identical alphanumeric characters in each row differ sigificantly (P<0.05) from each other.

Lower eating times in treatment 4, is probably due to decrease of NDF or increase of non-structural carbohydrates (NSC). Linear decrease in rumination time and chewing was associated with increase in the level of pistachio by-products and decreasing the amount of NDF, reduction in feed size in the diets, and linear decrease in cell wall digestibility. The small size of the particles causes faster passage rate from the rumen and reduces the digestibility of fibre and dry matter, and reducing the particles size in the diets with the same fibre content reduces the chewing time (Krause and Combs, 2003).

There was no significant difference in blood glucose, urea, and triglyceride levels among experimental treatments (P>0.05). The lowest cholesterol was for diet containing 4.5% oak kernel tannin (Table 4). Sinclair *et al.* (2009)

Tabel 4. Effect of different levels of oak kernel on blood parameters in buffaloes (mg/dl)

Parameter	Treatment				SEM	P value
	1	2	3	4		
Glucose	73.5	77.5	79.5	83.1	8.27	0.20
Urea	31.6	26.3	24.7	19.2	5.8	0.40
Cholesterol	113.0[a]	87.0[b]	81.5[c]	62.0[d]	1.1	0.02
Triglyceride	14.1	12.0	11.1	9.4	3.7	0.30
Alanine amino-transferase	21.67	18.1	14.3	11.2	8.2	1.03
Aspartate amino transferase	188.5	176.0	165.5	153.5	17.8	1.30
Alkaline phosphatase	627.5	602.0	591.0	580.5	52.2	1.10

1, 2, 3 and 4 were diets containing 0, 15.8, 31.58 and 47.37% oak kernel equal to 0, 1.5, 3 and 4.5% tannin, respectively. SEM, standard error of the averages. Figures with non-identical alphanumeric characters in each row differ sigificantly (P<0.05) from each other.

reported that high levels of tannin in the diet, did not change blood glucose levels, but in lower levels, glucose increased, especially when the level of dietary protein was low.

The results of many experiments show that tannin caused a significant decrease in ammonia concentration due to decreasing protein degradation rate, followed by decreasing plasma urea nitrogen (Ben Salem *et al.* 2005). Sinclair *et al.* (2009) stated increasing of tannin levels, along with increase of blood urea concentrations.

The results showed that the use of different levels of oak kernel had significant effect on serum cholesterol. Flavonoids, phenolic compounds and tannins reduce blood lipids by increasing LDL receptors in the liver, as well as binding to apolipoprotein B, thereby increasing the ability of the liver to remove LDL, and decrease cholesterol. Also, lowering blood cholesterol levels is probably due to excessive excretion of bile by excessive secretion due to stimulation of phenols and tannins, which can reduce serum cholesterol and triglyceride levels (Clover and Vandermeer 1993). Animal studies indicate that secondary plant metabolites may decrease cholesterol by reducing the activity of lipogenic hepatic enzymes and cholesterogenetic enzymes such as glucose 6-phosphatase dehydrogenase and 3-hydroxy-3-methyl-glutaryl coenzyme A. Goats fed diets containing oak acorn had a lower plasma glucose, urea and triglyceride compared with those fed the control diet (Jafari *et al.* 2018).

The results of this study showed that alkaline phosphatase, aspartate and alanine aminotransferase were not affected significantly by the treatments (P>0.05). Aspartate aminotransferase (ALT) is used to evaluate the protein balance in cows and buffaloes, also synthesized in response to increased protein intake and tissue protein catabolism (Zurek *et al.* 1995). Jafari *et al.* (2018) reported animals fed oak acorn containing diets had higher plasma ALT than those fed the control diet.

Results of one study showed that the plant extract containing antioxidant compounds can enhance and protect the liver tissue and reduce liver transaminases by inhibiting the destructive effects of oxidative compounds, and can reduce the degradation of the liver tissue and thereby reduce the leakage of enzymes. The decrease in liver enzymes is probably due to the oxidative antistress effects of these plants in comparison with the effects of oxidative stress on liver tissue (El Damdash *et al.* 2005).

Thus, even if the digestibility of ADF and CP is decreased by oak kernel; incorporation of oak kernel up to 47.37% in buffalo diet had no negative effect on the fermentation characteristics, digestibility and blood metabolites, hence may be used in buffalo's diet.

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