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Effects of Two Sources of Tannins on Performance, Nitrogen Utilization and Efficiency of Microbial Nitrogen Synthesis in Dairy Goats

Research Article

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ABSTRACT

The aim of this study was to evaluate the effects of tannic acid (TA) and pistachio by-product tannin extract (PBE) on N utilization and efficiency of microbial nitrogen synthesis (EMNS) in Saanen dairy goats. The experimental design was a change over design with three treatments and three periods using six mid lactation multiparous dairy goats. Tannic acid solution and PBE were added to fresh alfalfa at ensiling to get the final concentration of about 1% tannin as dry matter (DM) basis. Dietary treatments were as follows: 1) untreated alfalfa silage (AS), 2) AS treated with TA (AS+TA) and 3) AS treated with PBE (AS+PBE). Intake of DM, organic matter (OM) and N and also milk yield and composition (as percent or as g/d) were not affected by tannin additions. A considerable shift occurred in the pattern of N excretion in goats fed PBE diet compared to control silage diet. Urinary N losses had a tendency ($P<0.10$) to lower (17.4 *vs*. 19.2 g/d) and fecal N losses was higher for goats fed AS + PBE compared with those fed the AS silage diet (14.6 *vs*. 12.6 g/d). As a proportion of N intake or as g N loss per d, retained N was higher ($P<0.05$) in goats fed AS + PBE compared to those fed the control diet. Microbial nitrogen synthesis (MN) was not affected by tannin additions. However, a trend ($P<0.10$) for less efficiency of microbial nitrogen synthesis (EMN) was observed in tannin fed goats compared to those fed the control diet. Although, tannins added to AS may exert some beneficial effects on N utilization and also environmental N outputs, they may interfere with rumen fermentation lead to decrease in EMN in goats especially those fed with PBE.

KEY WORDS dairy goats, nitrogen utilization, pistachio by-product extract, tannic acid.

INTRODUCTION

Extensive alfalfa protein degradation to non protein nitrogen (NPN) during ensiling and in the rumen (Muck *et al.* 2003) will lead to inefficient utilization of N by ruminants and increase environmental N excretion (Deaville *et al.* 2010). It also causes the need of expensive supplemental protein in silage based diets (Givens and Rulquin, 2004). Considerable attention has recently been focused on using natural plant extract containing polyphenolic compounds (tannins) in order to decrease protein degradation. Tannins are plant secondary metabolites that can bind with macromolecules such as protein and carbohydrates, hence retard rate and extent of their degradations (McSweeny *et al.* 2001; Frutos *et al.* 2004). One of the sources of tannins which produce in Iran with an annual production of about 765500 tonnes is pistachio by-product (PB). PB is obtained after de-hulling of fresh pistachio (*Pistacia vera*) and contains a relatively high amounts of phenolic compounds (9.06-14.57% of DM) and tannins (4.97-8.67% of DM)

depending on extraction methods, particle size of samples, extraction time and type of solvent (Mokhtarpour *et al*. 2014). Previous studies demonstrated that addition of tannins or tannin rich extract to legumes or grasses can exert some beneficial effects on protein degradability and N utilization both *in vitro* and *in vivo* (Tabacco *et al*. 2006; Deaville *et al*. 2010; Mokhtarpour *et al*. 2015). Recently, it has been reported that treating soybean meal by PB extract (BPE) in the diets of Holstein bulls (Jolazadeh *et al*. 2015) and also incorporation of PBE in the diets of lambs (Rajaei-Sharifaadi and Naserian, 2014) decreased ruminal degradation of protein, increased retained N and resulted in better animal performance. We hypothesized that addition of PBE to alfalfa silage not only may decrease protein degradability but also may improve milk production and N retention of dairy goats. Furthermore, since tannin concentration in PBE was expressed as tannic acid equivalent (Makkar, 2000) and tannins in PBE were mostly hydrolysable, tannic acid was also used as a source of commercial hydrolysable tannin for treating fresh alfalfa to compare these two sources of tannins. Therefore, the purpose of this study was to determine whether addition of TA and PBE to fresh alfalfa at ensiling can affect Saanen dairy goat's performance, nitrogen balance and microbial nitrogen synthesis.

MATERIALS AND METHODS

Preparation of alfalfa silages

It has been reported that more than 70% of TT in PB can be extracted by water (Mokhtarpour *et al*. 2014) and thus eliminating costly solvents such as acetone, methanol or ethanol which is commonly used for tannin extraction (Makkar, 2000). Preparations of PB extract and alfalfa silages for this experiment are described in our previous study (Mokhtarpour *et al*. 2105). Briefly, sun-dried PB was collected from pistachio de-hulling factory in Feizabad (Khorasan-e-Razavi Province, Iran), ground to pass a 2-mm screen and then was soaked in water with a ratio of 1:5 (w/v) at room temperature for 12 h. The contents were filtered through cheesecloth and then filtrated extract was sprayed on chopped alfalfa (~ 25% DM) with a ratio of 500 ml/kg DM to get the final concentration of about 1% tannin as tannic acid equivalent on DM basis (AS+PBE). To the second silo, tannic acid was first dissolved in the same amount of water and then sprayed on alfalfa to get the final concentration of about 1% tannin. The same amount of water was also applied to the third silo as control silage. All silos were wilted for 20 h to increase DM content and then were ensiled for 60 d.

Animals, experimental diets and management

Six multiparous Saanen dairy goats (110±13 days in milk, 1.42±0.29 kg milk production) were used in a change over design with three treatments and three periods. Animals were kept in individual cage to facilitate separate collection of feces and urine. Guidelines for the care and use of animals were approved by the Animal Care Committee of the Ferdowsi University of Mashhad. Dietary treatments were: 1) untreated alfalfa silage (AS), 2) AS treated with TA (AS+TA) and 3) AS treated with PBE (AS+PBE). Each experimental period lasted 21 d with the first 14 d used for diet adaptation and 7 d for sample collection. Experimental diets were formulated to meet the nutrient requirements of lactating goats using the Small Ruminant Nutrition System (SRNS; version 1.9.4468; Tedeschi *et al*. 2010). Diets were fed as a total mixed ration (TMR) with forage to concentrate ratio of 50:50. Diets were offered twice daily *ad libitum* at 08:00 and 16:00 h and goats had free access to water. Chemical composition, phenolic compounds and crude protein (CP) fractions of silages and the ingredients and chemical composition of the diets are presented in Tables 1 and 2, respectively.

Sampling and data collection

Feeds and orts were collected before the morning feeding and weighed daily during the measurement period and were composited for each goat. Dry matter intake was calculated by difference between total amount of DM offered and refused. Complete fecal collection of each goat was done through the 5-day collection periods and then dried in an oven. Daily dried samples were ground and later composited for each 5-day periods. Composite samples of the TMR, feed refusal and feces were dried in an oven, then ground to pass through a 2-mm screen and stored for later analysis. Urine from each goat was collected daily in plastic vessels containing 100 mL 4 N H_2SO_4 to maintain the pH level below 3 to prevent bacterial destruction of total purine derivatives (TPD) (Chen and Gomes, 1995). Urine was weighed once a day and mixed well and 10% of daily aliquot was pooled over the 5-day collection period per animal and stored at −20°C for later TPD (i.e., allantoin, uric acid and xanthine hypoxanthine) analysis and N content.

Goats were milked twice daily at 07:00 and 15:00 h and individual milk yields were recorded at each milking. A daily composite milk sample from the morning and afternoon milking was taken during the collection period and fresh subsamples were analyzed daily for chemical composition. Animals were weighed on day 22 before feeding, and then the diet given to each cow was changed.

Chemical analyses

Dry matter content of silages, feeds, orts and feces was determined by drying in an oven at 100 ˚C to a constant weight (method 934.01). Ash (method 942.05), and acid detergent fiber (ADF) (method 973.18) were determined

according to AOAC (2005) procedures. Crude protein (Kjeldahl N×6.25) was determined by the block digestion method using copper catalyst and steam distillation into boric acid (method 2001.11) on 2100 Kjeltec distillation unit as described in AOAC (2005). Neutral detergent fiber (NDF) was determined by the method of Van Soest *et al.* (1991). The sodium sulphite and α-amylase were not used and both NDF and ADF were expressed exclusive of residual ash. Crude protein fractions were determined by Licitra *et al.* (1996) procedure. Tannin assay was conducted according to Makkar (2000) procedure. Briefly, after drying samples in an oven at 40 ˚C to constant weight in order to minimize changes in tannin content and activity, they were ground to pass a 2 mm sieve and then 0.5 mm sieve. Approximately, 200 mg samples were extracted in four replicates in 70% aqueous acetone (v/v) by using an ultrasonic bath for 20 min. After centrifugation (3000 g/min, 4 ˚C, 10 min), the supernatant was collected and kept in refrigerator (4 ˚C). Total phenolic compounds (TP) and total tannins (TT) were determined by Folin-Ciocalteu reagent using tannic acid (Merck GmbH, Darmstadt, Germany) as a standard (Makkar, 2000). The values of TP and TT were expressed as tannic acid equivalent.

Microbial nitrogen synthesis (MNS) was estimated on urinary purine derivatives (PD) excretion based on the relationship derived by Chen and Gomes (1995). The amounts of allantoin, uric acid and xanthine plus hypoxanthine were determined by spectrophotometric method. The urinary PD excreted in a day was used in the iteration process to calculate the microbial nitrogen supply as described by Chen and Gomes (1995). Efficiency of microbial nitrogen synthesis (EMNS) was calculated as:

EMNS= MN (g/d) / DOMR

Where:
DOMR: apparently digested OM in the rumen (assuming that rumen digestion was 65 g/100 g OM of digestion in total tract.
DOMR= DOMI × 0.65
DOMI: digestible organic matter intake according to ARC (1984).

Milk samples were analyzed for fat, protein, lactose, solids-not fat, and total solids content by Milk-O-Scan 605 analyser (Foss Electric, Hillerød, Denmark). The gross energy content in milk was calculated according to Tyrrell and Reid (1965) as:

Milk energy content (MJ/kg)= 4.184 × 2.204 × [41.63 × fat (%) + 24.13 × protein (%) + 21.60 ×lactose (%) − 117.2] / 1000.

Statistical analyses

Mixed procedure of SAS (2001) was used to analyze data as a following model for a change over design:

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

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
T_i: fixed effect of treatment (i=1, 2, 3).
P_j: fixed effect of period (j= 1, 2, 3).
C_K: random effect of goat.
ε_{ijk}: random residual error.

Least squares means procedure (LSMEANS) was used to test the differences among means if a value of $P < 0.05$ was detected. Trends were discussed at $P < 0.10$. All results are reported as least squares means.

RESULTS AND DISCUSSION

The concentration of total tannins in untreated and treated silages were, 0.5 and 1.2% of DM, respectively. The pH values decreased in silages treated with TA and PBE (Table 1). A substantial decreased in ammonia N concentration occurred in PBE treated silage compared to control and TA silages (6.5 *vs*. 14.7 and 13.5 mg/dL, respectively). Soluble protein significantly decreased in AS + PBE compared to control (44.8 *vs*. 49.6). Intake of DM, OM and N and also milk yield and composition were not affected by addition of tannins (Table 3). Intake, losses and retention of N are shown in Table 4. Addition of PBE to alfalfa silage increased fecal N ($P<0.05$) and lowered urinary N excretions ($P<0.10$) when expressed as g N per day. However, as proportion of N intake, excretion of fecal N was not affected by addition of PBE, but urinary N excretion decreased significantly. A tendency for lower urinary N excretion ($P<0.10$) was also observed by addition of TA. Milk N as g per day or as percentage of N intake was not affected by addition of tannins. Retention of N was significantly higher in goats fed PBE compared to other treatments when expressed either as g per day or percentage of N intake. Microbial N synthesis was not affected by addition of tannins, however, EMNS had a tendency to decrease in goats fed PBE compared to those fed control diet. In spite of the same concentration of tannin in AS + TA and AS + PBE, addition of TA to the silage had no effect on any of the parameters mentioned above.

Lower pH value in AS + PBE may be due to a relatively low pH of 4.95 in PBE (data not shown). Another reason could be possibly due to organic acids production during fermentation of water soluble carbohydrates in PBE.

Table 1 Chemical composition and phenolic compounds of silages

Item	Treatment			SEM	P-value
	AS	AS + TA	AS + PBE		
Chemical composition, % of DM					
Dry matter	27.7	27.7	28.3	0.30	0.72
Crude protein	17.8	17.9	17.5	0.10	0.08
Neutral detergent fiber	42.8	42.7	43.7	0.39	0.61
Acid detergent fiber	31.5	27.2	29.3	0.95	0.20
Ash	9.9	10.5	10.0	0.15	0.31
Total phenolic compounds†	1.1^{b}	1.4^{ab}	1.7^{a}	0.11	0.04
Total tannins†	0.5^{b}	1.2^{a}	1.2^{a}	0.13	< 0.01
pH	5.44^{a}	5.09^{b}	4.68^{c}	0.139	< 0.01
NH_3-N (mg/dL)	14.7^{a}	13.5^{a}	6.5^{b}	1.30	
CP fractions, % of CP‡					
Soluble protein (A+B1)	49.6^{a}	47.3^{ab}	44.8^{b}	0.91	0.01
A	46.7	44.3	42.3	1.03	0.25
B1	3.0	2.9	2.5	0.59	0.96
B2	37.2	41.0	42.5	1.20	0.19
B3	8.2	7.7	8.2	0.45	0.90
C	4.9	4.0	4.5	0.24	0.37

AS: alfalfa silage (control); AS + TA: alfalfa silage with tannic acid and AS + PBE: alfalfa silage with pistachio by-product extract.
† Expressed as grams of tannic acid equivalent.
‡ A= non protein nitrogen; B1, B2 and B3= true protein based on decreasing solubility and C= acid detergent insoluble protein.
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.

The action of tannins in AS + PBE to bind proteins and reduced proteolysis might cause a decreased in NH_3-N and soluble protein concentrations (Table 1).

The lack of a significant effect on dry matter intake (DMI) as a result of feeding TA or PBE to dairy goats in our trial may have been due to the low concentration of TT in silages (Table 1) and in diets (Table 2). The similar milk yield and composition in goats fed treated or untreated silages (Table 3) can be due to similar DMI (Table 3). However, a slight increase in DMI and organic matter intake (OMI) (about 9%) was observed in goats fed AS + PBE. Dry matter intake in goats fed with PB at 32% of diet DM in replacement of alfalfa hay in the ration (2.12% dietary tannin) of dairy goats had no effect on DM intake (Sedighi-Vesagh *et al*. 2015). Use of PB extract as a source of tannin in treating alfalfa silage had no effect on DM intake of goats in early lactation (Mokhtarpour *et al*. 2015). Naserian *et al*. (2015) reported that DM intake of dairy goats was increased by increasing pistachio skins (soft external hulls) in replacement of wheat bran up to 21% of diet DM (0.45-1.9% dietary tannin). Higher DM intake in goats fed pistachio skins may be due to the positive effect of secondary metabolites on ruminal fermentation and higher nutrient digestibility observed in that study.

Similar to our results, feeding PB to dairy goats at 30 and 32% of diet DM containing 1.81 and 2.12% tannin respectively had no effect on milk yield and composition (Ghaffari *et al*. 2014; Sedighi-Vesagh *et al*. 2015). In contrast, Mokhtarpour *et al*. (2015) observed an increase in milk fat and protein yield by treating alfalfa silage with 1% PBE in goats at early lactation.

A linear increase in milk fat content also occurred by increasing pistachio skins in dairy goats ration, whereas concentrations of other milk components were not affected by experimental treatments (Naserian *et al*. 2015). They stated that the higher milk fat content in goats fed pistachio skins may be partly associated with an increased intake of ether extracts (EE) due to higher EE content in pistachio skins than wheat bran.

Regarding similar dietary ingredients and similar N intake, changes in N utilization among treatments can be attributed to the tannin added to the silages (Table 4). The higher loss of fecal N (g/d) in PBE fed goats suggests that already formed tannin–protein complexes may incompletely dissociate post ruminally. This reversibility depends on the affinity of tannins to bind proteins (Makkar, 2003) and the larger the molecular size of tannins, the higher the binding affinity (Ozdal *et al*. 2013). Thus, our results indicated that PB tannins may be generating some indigestible rumen escape protein. According to Butter (1999) an increment in fecal N excretion as a result of tannin addition could be due to protein bound to tannin, reduced digestive enzymes activity, impaired intestinal function and increase in secretion of endogenous proteins. Patra (2010) also reviewed that fecal N as percentage of N intake increased in sheep fed high foliage diets containing high concentrations of tannins. In spite of the same concentration of tannins in alfalfa silages treated with TA and PBE, lack of effect on N utilization pattern following feeding dairy goats with AS + TA revealed that the nature of tannin (chemical structures and molecular weight) can influence the animal response to dietary tannins.

Table 2 Feed ingredients and chemical composition of experimental diets

Item	Treatment		
	AS	AS + TA	AS + PBE
Diet ingredients, %			
Alfalfa silage	50.0	50.0	50.0
Barley grain	26.0	26.0	26.0
Wheat bran	23.0	23.0	23.0
Limestone	0.35	0.35	0.35
Salt	0.15	0.15	0.15
Vitamin-mineral mix[†]	0.50	0.50	0.50
Chemical composition, % of DM			
Dry matter	41.8	42.5	42.8
Organic matter	91.8	92.0	91.3
Crude protein	14.2	14.2	14.2
Neutral detergent fiber	41.3	41.3	40.9
Acid detergent fiber	24.0	23.3	23.4
Non fiber carbohydrate[‡]	33.2	33.3	33.0
Ether extract	3.2	3.2	3.2
Total phenolic compounds[§]	0.68	1.28	1.37
Total tannins[§]	0.29	0.65	0.67

AS: alfalfa silage (control); AS + TA: alfalfa silage with tannic acid and AS + PBE: alfalfa silage with pistachio by-product extract.
[†] Contained (g/kg premix; DM basis): vitamin A: 330000 IU; vitamin D: 60000 IU; vitamin E: 1000 IU; Ca: 160 g; P: 85 g; Na: 63 g; Mg: 45 g; Zn: 2100 mg; Mn: 1500 mg; Cu: 535 mg; Se: 12 mg and I: 45 mg.
[‡] Non-fibre carbohydrates calculated as: 100 – (neutral detergent fiber+crude protein+ether extract+ash) (NRC 2001).
[§] Expressed as grams of tannic acid equivalent.

Table 3 Effect of treatment on dry matter intake, milk yield and composition

Item	Treatment			SEM	P-value
	AS	AS + TA	AS + PBE		
Intake, kg/d					
Dry matter	1.91	1.90	2.09	0.056	0.22
Organic matter	1.75	1.74	1.90	0.049	0.25
Milk production, kg/d	1.40	1.37	1.37	0.067	0.71
Milk Composition, %					
Fat	4.47	4.62	4.55	0.181	0.61
Protein	3.57	3.53	3.62	0.031	0.13
Lactose	4.38	4.39	4.39	0.049	0.88
Solid not fat	9.13	9.02	9.09	0.092	0.32
Total solids	12.72	12.80	12.81	0.221	0.68
Milk component yield, g/d					
Fat	62.80	64.68	62.97	4.628	0.43
Protein	49.90	48.60	51.37	1.090	0.47
Lactose	61.15	60.42	60.38	3.181	0.88
Milk energy, MJ/kg[†]	3.27	3.33	3.32	0.081	0.55
Milk energy output, MJ/d	4.59	4.62	4.58	0.284	0.86

AS: alfalfa silage (control); AS + TA: alfalfa silage with tannic acid and AS + PBE: alfalfa silage with pistachio by-product extract.
[†] Estimated according to the equation of Tyrrell and Reid (1965).
SEM: standard error of the means.

Similar to our results, Getachew *et al*. (2008a) reported that the pattern of N utilization by sheep was not affected by alfalfa hay supplemented with different levels of TA (3 to 9% DM).

However, inclusion of PBE shifted partitioning in N from urine to feces which is consistent with the results of the most studies on tannins effects (Deaville *et al*. 2010; Ahnert *et al*. 2015).

Reduction in urinary N excretion as a proportion of N intake ($P<0.05$) following PBE supply and also a trend ($P<0.10$) for lower urinary N losses (g/d) in goats fed AS + TA or AS + PBE can be attributed to the action of tannins in binding proteins and consequently lower ruminal degradation of N and ammonia-N losses (Patra and Saxena, 2011). This is confirmed by the strong protein protection from *in vitro* ruminal degradation of alfalfa hay by addition of TA and quebracho tannins (Getachew *et al*. 2008b).

Increased in retained N as a result of supplementing PBE to alfalfa silage is consistent with recent report for tannin action in N retention (Ahnert *et al*. 2015).

Table 4 Effect of treatment on nitrogen utilization and microbial nitrogen synthesis

Item	Treatment			SEM	P-value
	AS	AS + TA	AS + PBE		
N intake, g/d	43.5	43.4	47.3	1.30	0.23
N losses					
Feces	12.6^{b}	12.7^{b}	14.6^{a}	0.35	0.02
Urine	19.2	17.5	17.4	0.29	0.08
Milk	7.8	7.6	8.1	0.16	0.47
Retained	4.0^{b}	5.6^{ab}	7.2^{a}	0.48	0.04
N losses and retention as percentage of N intake (%)					
Feces	28.8	29.3	30.9	0.66	0.47
Urine	44.3^{a}	41.0^{ab}	37.2^{b}	1.14	0.04
Milk	18.1	17.9	17.1	0.38	0.52
Retained	8.9^{b}	12.8^{ab}	15.1^{a}	0.92	0.03
Microbial N, g/d	22.7	21.9	20.9	0.87	0.76
Efficiency of Microbial N, g N/kg DOMR†	25.8	23.4	22.5	0.63	0.07

AS: alfalfa silage (control); AS + TA: alfalfa silage with tannic acid and AS + PBE: alfalfa silage with pistachio by-product extract.
† DOMR= apparently digested organic matter in the rumen (65% of apparently digested organic matter in total tract) according to ARC (1984).
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.

In contrast, it was noted a decline in urinary N excretion counteracting the elevated fecal N excretion in goats (Al-Dobaib *et al*. 2009) and sheep (Deaville *et al*. 2010) fed a diet supplemented with hydrolysable (HT) or condensed tannin (CT) which resulted in similar retained N at both experiments. However, effect of tannins on N retention is still contradictory.

Researches carried out in the last decade have highlighted the risk of large losses of N compounds to the environment. A shift in N excretion from urine to feces in goats fed AS + PBE can exert beneficial effects environmentally as urinary N is mainly in the form of urea, which is rapidly convert into ammonia and then to volatile nitrous oxides (green house gases) (Patra and Saxena, 2011). Moreover, nitrate produced by oxidation of ammonia (ammonium) causes water pollution (Eckard *et al*. 2010). Therefore, lower urinary N content means less volatile ammonia and nitrous oxides losses into the environment. In our study, MNS was not affected by treatments, however numerically higher OM intake in goats fed AS + PBE compared with untreated silage (1.90 *vs*. 1.75 kg/d) may lead to a tendency (P<0.10) for decrease in MNS efficiency. Furthermore, free phenolic compounds in crude PBE may negatively affect the activity of ruminal microorganisms and / or interfere with their enzyme secretion (McSweeny *et al*. 2001). Ghasemi *et al*. (2012) reported that substitution of PB at 25% of DM (1.92% dietary tannin) with alfalfa hay in sheep ration had no effect on MNS and efficiency of MNS. However, they observed a significant decrease in both MNS and efficiency of MNS at 50% of DM PB (3.07% tannin). Inclusion of PEG to the goats fed a high tannin containing diet increased urinary excretion of allatoin, thus MNS which was likely the result of improved N bioavailability in the rumen (Salem *et al*. 2005).

Al-Dobaib *et al*. (2009) reported that MNS decreased in goats fed alfalfa hay treated with 3% quebracho tannin, however, supplementing with 1 and 2% tannin slightly increased MNS. They also stated that efficiency of MNS significantly enhanced at all levels of tannin (1, 2 and 3%). Makkar (2003) concluded that low levels of tannins can decrease the rate of digestion of feeds, hence, synchronizing the release of various nutrients resulting in higher microbial protein efficiency.

However, studies are required to know the levels of tannins in order to have this positive response. Different responses of tannins among different studies can be ascribed to tannin concentration, type of tannin, chemical structure and type of diet besides other factors such as animal species and physiological state of the animal (Makkar, 2003; Patra and Saxena, 2011).

CONCLUSION

Presence of TA and PBE at 0.7% DM in diet had no effect on milk yield and composition. Addition of PB tannin extract increased fecal N losses and reduced urinary N and also increased retained N. This shift from urine to feces may have some beneficial effects on environment in the case of reducing N pollution. However, a tendency to lower EMN as a result of PBE addition may indicate that PB tannins can bind strongly with proteins which would need to be counteracted with a competitive agent such as PEG. However, at the same level of tannin, TA had no significant effect on pattern of N utilization, MNS and EMNS in dairy goats compared to PBE indicating the different biological effect of tannins. However, it is suggested to identify the effect of PB on N utilization and EMNS by using purified PB tannins.

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