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The Effect of Yerba Mate (*Ilex Paraguariensis*) Supplementation on Nutrient Degradability in Dairy Cows: An *In sacco* and *In vitro* Study

Ellen Hartemink[1,2], Daniela Giorgio[3], Ravneet Kaur[1], Adriana Di Trana[3], and Pietro Celi[1,*]

[1] Faculty of Veterinary Science, The University of Sydney, PMB 4003, Narellan, NSW 2567, Australia

ABSTRACT: This study was carried out to investigate the effects of Yerba Mate (YM) supplementation on nutrients' degradation, *in vitro* dry matter disappearance, gas production and rumen ammonia concentration. Three rumen-fistulated Holstein Friesian cows were used for the *in situ* incubations and provided rumen liquor for *in vitro* incubations. The inclusion of YM in a control diet (pasture+pellets) affected some *in sacco* degradation parameters. YM supplementation decreased the effective degradability and degradation rate of pasture crude protein (CP), and it seems to slow down the degradation of pasture neutral detergent fiber. A significant increase of degradation of pasture acid detergent fiber (ADF) was detected after YM inclusion in the control diet. YM supplementation reduced *in vitro* gas production of pasture and ammonia concentration of pellets. The addition of YM in ruminant diet could decrease ammonia production and increase protein availability for productive purposes. The moderate presence of tannins in YM could have affected the degradation kinetics of pasture CP and ADF and the ammonia production of pellets. (**Key Words:** Dairy Cows, Degradability, *In vitro*, *In sacco*, Yerba Mate, Tannins)

INTRODUCTION

The non-conventional vegetables resources represent all those foods that have not been traditionally used in animal feeding or refer to food for human consumption and or are not normally used in commercially produced rations for livestock (Devendra et al., 1992). The use of herbs as natural additives in livestock nutrition, as an alternative to other chemical compounds, is becoming a new goal in livestock production (Makkar et al., 2007). While the use of plants containing secondary compounds in ruminant nutrition has been extensively investigated in ruminant production (Wanapat et al., 2012), several studies have focused on green tea byproducts because of their antioxidant and tannin content. For example, grazing on *Hedysarum coronarium* has been shown to have a positive impact on the productivity of meat and dairy sheep (Bonanno et al., 2007a) and goats (Bonanno et al., 2007b).

Yerba Mate (YM; *Ilex paraguariensis*), from the *Aquifoliaceae* plant family, is a native South American tree used for tea production (Heck and de Mejia, 2007). Within the last two decades, there has been an increased interest in this herb for its use in human health. The antioxidant, antimicrobial, antiobesity, anti-inflammation, anti-diabetic and cardiovascular properties of YM have been reviewed by Heck and de Mejia (2007). Mate shows central nervous system stimulant properties attributed to its content of methylxanthine alkaloids such as caffeine and is also known to contain compounds with antioxidant properties, such as phenolic acids and tannins that are the most abundant compounds in the leaves (Bastos et al., 2007; Heck and de Mejia, 2007). The leaves of YM contain also a significant amount of saponins, 10 to 15 mg/g dry weight total saponins. The composition of YM also includes amino acids, minerals (P, Fe, and Ca) and vitamins (C, B1, and B2; Heck and de Mejia, 2007). In ruminant health and production, the study of redox homeostasis is contributing

* Corresponding Author: Pietro Celi. Tel: +61-2-9351-1782, Fax: +61-2-4655-2374, E-mail: pietro.celi@sydney.edu.au
[2] Department of Farm Animal Health, Faculty of Veterinary Science, Utrecht University, Yalelaan 7, 3584CL Utrecht, the Netherlands.
[3] Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali (SAFE), Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, Potenza, 85100, Italy.
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to the understanding of important metabolic pathways. Indeed, oxidative stress seems to play a central role in the regulation of the metabolic activity of some organs and productivity in farm animals (Celi, 2011) and therefore it might be beneficial to supplement ruminants with antioxidants. YM has higher polyphenol concentration than green tea which correlates to its higher antioxidant capacity and its higher inhibition of free radicals than green tea. Green tea byproduct is widely used as protein supplement in lactating cow diets (Kondo et al., 2004), however research on nutritional properties of YM has had a late start and strongly lags behind the abundant literature on green tea and other plants or herbs (Makkar et al., 2007). Preliminary observations have shown that YM could be recommended as a natural novel feed supplement with the potential for improving feed intake and wool growth in lambs (Po et al., 2012a) and milk production in dairy cows (Celi and Raadsma, 2010). The supplementation of YM during the peri-partum period has positive effects on milk fat, protein and total solid concentration (Po et al., 2012b) while its administration in growing calves can influence lipid metabolism (Celi and Robinson, 2010). As the effects of YM supplementation on productive perfomances seems not to be mediated by changes in oxidative status it could be argued that the positive effects of YM supplementation on milk yield and wool growth, might have been due to increased efficiency of feed utilization owing to the presence of tannins which increased protein availability post-ruminally. Indeed, YM is rich in hydrolysable tannins (Heck and de Mejia, 2007) and has very little condensed tannins. Therefore the aim of this study was to investigate the effect of YM supplementation on rumen function by means of *in vitro* and *in sacco* techniques. The hypothesis of the present study was that the supplementation of YM in cows'diet will improve the nutrients' degradation, *in vitro* dry matter (DM) disappearance, gas production and rumen ammonia concentration.

MATERIALS AND METHODS

Location, animals and experimental design

The study was conducted at the Corstorphine Dairy Farm, Faculty of Veterinary Science, University of Sydney (Camden campus). Three rumen-fistulated Holstein Friesian cows, homogeneous for age (6 years), milk production (37.3±4.7 L/d) and liveweight (625±35 kg), were used for the *in situ* incubations and provided rumen liquor for *in vitro* incubations. All cows grazed kikuyu grass (Pennisetum clandestinum), oversown with short rotation ryegrass (Lolium multiflorum), and perennial ryegrass (Lolium perenne) and white clover (Trifolium repens). The cows had access to pasture between the two milkings and were grazed in accordance with the best practice of using pasture on offer and leaf stage as the criterion to flag time to graze (Fulkerson and Donaghy, 2001) and were fed 4.5 kg of pellets (Elite Dairy, Weston Animal Nutrition, Enfield, NSW, Australia) at each milking. Cows had free access to drinking water.

Two *in vitro* and *in sacco* incubations were performed. The first incubation was performed with cows fed a control diet (pasture+pellets) as described above. After the first incubation period, all cows were fed the control diet supplemented daily with 500 g/cow/d YM pellets for two weeks as adaptation period to YM supplement. The YM used in this study was organically grown in Paraguay and packaged in 500 g packs by RIO ITAMBEY S.A. (Paraguay) for human consumption. The content of the packs were pelleted immediately before the beginning of the experiment and stored in feed bins. After two weeks of feeding the YM pellets, the second *in vitro* and *in sacco* incubation was performed. Condensed tannin (CT) content was determined in YM pellets by the method of Terril et al. (1992). Total and free CT concentration in YM was 0.35% and 0.14% DM, respectively.

Pasture, pellets and YM pellets samples were used for *in vitro* and *in sacco* incubations. Pasture samples were harvested at normal grazing height (5 cm from ground level) at the Corstorphine Dairy Farm. Pellets samples (Elite Dairy, Weston Animal Nutrition, Enfield, NSW, Australia) were collected directly from the bunker in the farm. The chemical composition of feed used in this study is reported in Table 1.

In situ incubations

The nutrient degradation kinetics of each feed was determined in each cow. All feed samples were dried in a fan-forced oven at 60°C for 48 hours (Dulphy et al., 1999) and ground through a 2 mm sieve (Madsen and Hvelplund, 1994). Nylon bags (10×20 cm, 50 μm pores; Ankom Technology, NY, USA) were filled with 5 to 7 g DM of each feed in duplicate, sealed and dried for 24 hours at

Table 1. Chemical composition of sample feed

	Pasture*	Pellets	YM Pellets
DM (% as feed)	15.20	90.12	90.90
CP (%)	22.95	18.14	13.89
NDF (%)	56.56	16.75	19.28
ADF (%)	26.69	6.46	12.19
Cellulose (%)	24.10	7.50	7.28
WSC (%)	5.86	4.86	9.51
in vitro DmD (%)	88.26	83.16	77.18
ME (MJ/kg DM)	13.00	12.14	11.12

YM, Yerba Mate; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water soluble carbohydrates; DmD, dry matter disappearance, ME; metabolisable energy.
* Mean composition of pasture during the experimental period.

60°C. Sample bags were incubated in the rumen of each cow and removed after 2, 6, 12, 24, 48, and 72 hours of incubation. The 0 hours bags were not placed in the rumen. After removal from the rumen, the bags were immediately placed in an insulated box with ice to stop the fermentative activity. All the bags were rinsed under cold water, washed for 30 minutes on the cold rinse cycle in a washing machine and oven-dried at 60°C for DM and chemical analysis.

The DM, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) disappearances of feed were calculated from the loss of weight of the bag contents at the end of each incubation time. In sacco degradability data were fitted to the Ørskov and McDonald (1979) equation using NEWAY program (Chen, 1995):

$$p = a+b\ (1–e^{-ct})$$

where p is percentage of material in the bag which disappeared at time t; a, b and c are constants: a is the intercept of degradation curve at time zero, b is the asymptote of degradation curve and c is the rate constant for the degradation of b (h^{-1}). Effective degradability (Ed) was calculated at 0.05 outflow rate per hour using NEWAY program according to the equation reported by Ørskov and McDonald (1979):

$$Ed = a+(bc)/(k+c)$$

where a, b, and c are the constants from the Ørskov and McDonald (1979) equation above and k is the outflow rate.

***In vitro* incubation**

Feed samples were dried (48 hours at 60°C) and finely ground (1 mm sieve) for *in vitro* incubation. Special *in vitro* bags (F57; Ankom Technology, NY, USA) were filled with 0.5 g of each feed in duplicates, sealed and placed into bottles for *in vitro* incubation. The 0 hours bags were not placed into bottles. Ruminal fluid was strained through four layers of cheesecloth and mixed with a buffer (ANKOM Technology, USA). The bottles were filled with 25 mL of buffered ruminal fluid, bubbled with N-gas and closed with rubber stoppers. During the incubation, the bottles were held in a stove (39°C) with shaking plates.

Gas production was measured in all bottles after 2, 6, 12, 24, 48, and 72 hours of incubation using a water displacement technique as in Fedorah and Hrudey (1983). At each incubation time bottles were opened, bags were removed and ammonia samples were taken. All bags were washed with cold water, dried (48 hours at 60°C) and weighed for *in vitro* DM disappearance (IVDMD) calculation.

Chemical analysis

Samples of feed and residues in bags were analysed for DM by drying at 60°C for 48 hours in a forced air oven. Feed samples were analysed for ash after igniting in a muffle furnace at 600°C for 3 hours. The water soluble carbohydrate (WSC) content was determined using an autoanalyser according to the method used by Fulkerson et al. (2008). Nitrogen (N) content was determined by means of a Leco Fp-428 analyzer (Leco Corp., St. Joseph, MO, USA) according to Dumas method (Method 1.5R, Australian Fodder Industry Association Inc., 2009) and CP was calculated as N%×6.25.

NDF and ADF were analysed according to Van Soest (1963). NDF was assayed with a heat stable amylase in pellet samples and without a heat stable amylase in pasture and YM samples; both values were expressed inclusive of residual ash. ADF was expressed exclusive of residual ash. Cellulose was calculated as the difference between ADF and ADL.

Ammonia (NH_3) was measured by the method of Weatherburn (1967). The metabolisable energy (ME) of feed was calculated from IVDMD using Eq. (2) (Australian Agricultural and Ruminants, 1990)(SCA, 1990):

$$ME\ (MJ/kg\ DM) = (0.17×IVDMD)–2.0$$

Statistical analysis

The statistical analyses of the *in sacco* data were performed using the MIXED procedure of SYSTAT ver. 13 (SISTAT Software, Inc. Chicago, IL, USA). The effect of YM supplementation (No YM supplementation and YM supplementation) was fixed factor and the specific cow was considered a random factor. The *in vitro* data were performed using the General Linear Model procedure considering the effect of YM supplementation and incubation time. Significance was declared at $p<0.05$ and tendencies were declared at $0.05<p\leq0.10$.

RESULTS

The inclusion of YM in diet affected significantly some *in sacco* degradation parameters (Table 2). In pasture sample, the degradation rate (c) of NDF was lower after YM supplementation ($p = 0.05$), while the Y-intercept at time 0 (a), asymptote of degradation curve (b) and effective rumen degradability (Ed) values of pasture NDF were not affected by YM supplementation. The effective degradability (Ed) and the a value of pasture ADF were higher when cows were fed YM pellets, while the b value was lower after its supplementation ($p\leq0.05$). Moreover, the c value decreased after YM supplementation, although this difference was only a trend ($p = 0.07$). YM supplementation resulted in a decrease in the Ed ($p\leq0.01$) and c ($p = 0.01$)

Table 2. Effect of Yerba Mate inclusion on *in situ* dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) and crude protein (CP) degradation parameters

		Control diet	Yerba mate	SEM	p value
Pasture					
DM	a	43.69	38.98	3.47	ns
	b	47.41	52.61	2.91	ns
	c	0.10	0.09	0.01	ns
	Ed %	75.50	72.90	1.01	ns
NDF	a	25.56	35.11	3.75	ns
	b	63.68	56.07	3.06	ns
	c	0.10	0.07	0.01	0.05
	Ed %	67.23	67.27	1.08	ns
ADF	a	10.95	30.25	3.55	0.02
	b	76.55	62.33	3.34	0.04
	c	0.08	0.06	0.01	0.07
	Ed %	59.07	62.73	0.89	0.04
CP	a	45.31	36.89	10.25	ns
	b	50.65	57.74	9.65	ns
	c	0.18	0.10	0.02	0.05
	Ed %	84.93	75.90	1.52	0.01
Pellets[1]					
DM	a	67.23	61.01	1.76	0.07
	b	26.53	31.05	2.44	ns
	c	0.08	0.11	0.01	ns
	Ed %	83.47	82.00	0.42	0.07
NDF	a	25.72	24.78	4.55	ns
	b	60.53	55.87	2.49	ns
	c	0.03	0.04	0.01	ns
	Ed %	47.40	50.03	1.16	ns
CP	a	59.69	48.50	2.17	0.02
	b	38.14	46.52	3.12	ns
	c	0.10	0.13	0.01	ns
	Ed %	84.63	82.43	0.67	ns

SEM, standard error of the mean; ns, not significant.
a, y-intercept at time 0; *b*, asymptote of the curve; *c*, degradation rate constant (h^{-1}); *Ed*, effective degradability;
[1] Because of the small amount of sample remaining after NDF analysis, degradability of ADF was not measured.

values of pasture CP while the *a* and *b* values were not affected. Overall, pasture DM degradation parameters were non affected by YM supplementation. Both pellet *a* value and effective degradability of pellet DM decreased after YM supplementation, although these differences were only trends (p = 0.07). YM supplementation also caused a decrease of the *a* value of pellet CP (p = 0.02) while it did not affect *b*, *c* and *Ed* values. Pasture NDF degradation parameters were non affected by YM supplementation.

A significant effect of YM inclusion on *in vitro* gas production (p = 0.05) and ammonia concentration (p = 0.02) was noted for pasture and pellets, respectively (Table 3). YM supplementation reduced gas production in pasture samples and decreased ammonia concentration in pellet ones. No effect of YM supplementation was detected on IVDMD.

As expected, a significant effect of incubation time was noted on IVDMD, gas production and ammonia concentration values (p≤0.05) (Table 4). The degradation of DM, the production of gas and the concentration of ammonia increased with increasing incubation time.

DISCUSSION

YM supplementation decreased the effective degradability and degradation rate of pasture CP. The digestion of dietary protein in the rumen can be attributed to the combined processes of solubilization and degradation. Solubilization can be defined as the release of protein from plant cells into the rumen environment during chewing and it is an important prerequisite for degradation (Mangan, 1982). Degradation is the catabolism of protein by microbial proteolysis resulting in the formation of peptides, amino acids and ammonia. Dietary protein degradation provides substrates for microbial protein synthesis in the rumen. The quantity of dietary and microbial protein flowing from the rumen is a major factor determining the productivity of ruminant livestock. It is generally agreed that tannins decrease ruminal protein degradation, mainly through the formation of tannin–protein complexes in the rumen and inhibition of the growth and activities of proteolytic bacterial populations (Patra and Saxena, 2011). Regarding the effect of hydrolysable tannins on rumen

Table 3. Effect of Yerba Mate inclusion on *in vitro* dry matter disappearance, gas production and ammonia concentration

	No Yerba Mate supplementation	Yerba Mate supplementation	SEM	p value
Pasture				
IVDMD (%)	60.51	57.21	6.06	ns
Gas production (mL/g DM)	59.06	28.88	10.25	0.05
Ammonia concentration (mmol/L)	0.22	0.20	0.03	ns
Pellets				
IVDMD (%)	64.34	62.46	4.69	ns
Gas production (mL/g DM)	60.80	87.12	18.32	ns
Ammonia concentration (mmol/L)	0.09	0.05	0.01	0.02

SEM, standard error of the mean; IVDMD, *in vitro* dry matter disappearance; ns, not significant.

Table 4. Effect of time on *in vitro* dry matter disappearance, gas production and ammonia concentration (mmol/L)

	Time						SEM	p value
	2	6	12	24	48	72		
Pasture								
IVDMD (%)	38.20^{e}	40.25^{e}	46.47^{d}	64.57^{c}	80.81^{b}	88.77^{a}	1.04	0.00
Gas production (mL/g DM)	-	5.65^{c}	12.45^{c}	25.13^{b}	39.73^{a}	48.55^{a}	2.96	0.00
NH_3 concentration (mmol/L)	0.07^{d}	0.10^{d}	0.15^{cd}	0.22^{bc}	0.28^{b}	0.38^{a}	0.02	0.00
Pellets								
IVDMD (%)	42.72^{d}	46.03^{d}	55.53^{c}	68.72^{b}	78.96^{a}	82.45^{a}	1.29	0.00
Gas production (mL/g DM)	1.65^{d}	13.65^{d}	32.33^{c}	51.70^{b}	68.10^{a}	81.40^{a}	3.33	0.00
NH_3 concentration (mmol/L)	-	0.05^{b}	0.05^{ab}	0.07^{ab}	0.11^{a}	-	0.02	0.05

SEM, standard error of the mean; IVDMD, *in vitro* dry matter disappearance; ns, not significant.
^{a,b,c,d,e} Means with different letters within the same row differ for the $p<0.05$.

degradation, data in literature are often contradictory. *In sacco* and *in vitro* studies have shown that hydrolysable tannins decrease rumen degradation of soya bean meal in sheep without detrimentally affecting its intestinal digestion (Hervás et al., 2000). Additionally, a significant reduction of ammonia was observed in the rumen fluid of sheep fed pasture supplemented with increasing levels of chestnut hydrolysable tannins (Pulina et al., 2010). On the contrary, Poncet and Remond (2002) found that the addition of chestnut hydrolysable tannins did not decrease the *in situ* degradability of pea seed and did not significantly affect the N digestion of sheep diet. It may be inferred that the individual plants tend to produce complex mixtures of tannins and not all tannins have the same feeding effects. Further research is required to achieve a better understanding of the roles and utility of hydrolysable tannins in livestock feeding. Regarding the effect of CT on rumen degradability, several studies reported some beneficial effects when feeding at low concentrations to ruminant. In general, it has been assumed that low to moderate concentrations of CT (2% to 4% DM) can exert beneficial effects on rumen metabolism while high dietary CT concentrations (>5.5% DM) can depress voluntary feed intake, digestive efficiency and animal productivity. However, at the same inclusion level, effects are not the same for all CT as they depend upon its chemical structure and molecular weight (Patra and Saxena, 2011). Even if in our study the content of CT in diet was low, the continued presence of tannins in rumen during the feeding adaptation period can have modified rumen microbial ecosystem involved in the digestion of the diet consumed by the animal. In our study, CT content of YM pellet was 3.54 g/kg DM of YM pellet and it could explain the reduction of protein degradation observed in this study. During YM supplementation, the presence of CT could have reduced the growth rate of proteolytic bacteria in the rumen and/or inhibited the activity of microbial proteases. An effect of CT on proteolytic activity of rumen bacteria was detected by Min et al. (2002) in sheep fed *Lotus corniculatus*. The decreased rate and extent of protein degradation in the rumen could increase dietary protein flow to the duodenum and, consequently, making feed protein available post-ruminally for production purposes. The moderate quantity of CT in *Hedysarum coronarium* (ranging from 0.8% to 5% for whole plant DM in Mediterranean environments) showed a positive effect on protein utilization at the intestinal level and, consequently, on milk casein synthesis (Bonanno et al., 2007b). According to Roy et al. (2004), the high milk protein secretion observed in Sulla-fed sheep can be attributable to the CT content that is able to regulate the mammary blood flow, increasing the partitioning of essential amino acids to the mammary gland. In a previous study, a positive effect of YM on wool growth in lambs has been reported (Po et al., 2012a), this result could be linked to the increase of absorption of essential amino acids. Wool is mainly protein, with a high sulphur content mainly as cystine. Wool growth is dependent upon absorption of essential ammino acid from the small intestine, specifically the availability of sulphur-containing amino acids (SAA; Reis, 1979). In sheep fed *Lotus corniculatus*, Wang et al. (1994) showed that CT reduced degradation of SAA in the rumen and increased the flux of cysteine to body synthetic reactions.

The inclusion of YM in diet seems to slow down the degradation of pasture NDF. The low *c* value observed after YM supplementation indicates that microbial population was either slow to colonise or slow to degrade the fiber fraction of pasture. Previous study indicates that tannins in diets may depress fiber digestion by complexing with lignocellulose thus preventing microbial digestion or by directly inhibiting cellulolytic microorganisms and activities of fibrolytic enzymes or both (McSweeney et al., 2001). It has been suggested that high concentrations of free CT can react with other sources of protein after chewing by animals, such as enzymes secreted by rumen bacteria, and so inhibit rumen carbohydrate fermentation (Barry and Manley, 1986).

A significant increase of degradation of pasture ADF

was detected after supplementation of dairy cows' diet with YM. ADF is a portion of the plant fiber that includes cellulose, lignin and cutin while NDF also includes hemicellulose. The different behavior of fiber fractions in the rumen observed during YM supplementation could be related to the reduction in the activity of hemicellulases in the presence of tannins (Waghorn, 1996). Indeed, with respect to fibrolytic enzymes, tannins more easily inhibit the activity of hemicellulases than cellulases (Waghorn, 1996). This is possibly due to the fact that the latter are associated with bacterial cell walls while the hemicellulases are extracellular and therefore more sensitive (van Soest, 1994). In our study, YM supplementation could have affected bacterial populations in the rumen in favor of bacterial cellulolytic, with a consequent increase in ADF degradation. Dietary carbohydrates are the main fermentation substrates for volatile fatty acids (VFAs) production in the rumen; they are then degraded to their constituent hexoses and pentoses before being fermented to VFAs via pyruvate (France and Dijkstra, 2005). The improvement in ADF degradation observed in this study might increase total volatile fatty acid concentration in the rumen and, consequently, the amount of energy available for production purposes. Further studies are necessary to confirm this hypothesis.

YM supplementation reduced *in vitro* gas production of pasture (Table 3). Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. Hence, the extent of gas production reflects the efficiency and/or extent of degradability of the feed. The positive correlation between gas production and quantitative production of VFAs, which are a major source of energy for ruminants, is strongly affected by the chemical composition of dietary substrate. However, this relationship could be impaired in presence of secondary metabolites (tannins, saponins etc.). It has been reported that CT and phenolic compounds affect gas productions during *in vitro* ruminal incubation (Makkar et al., 1995). In our study, the reduction of gas production after YM supplementation (Table 3) could be explained by the strong negative correlation between tannins and *in vitro* gas production in diets containing tannins (Monforte-Briceño et al., 2005). Additionally, it has been reported that tannins may decrease VFA production by inhibiting the activity of hemicellulases (Waghorn, 1996).

In this study, YM supplementation reduced *in vitro* ammonia concentration of pellets while a not significant reduction (from 0.22 to 0.20 mmol/L in ammonia concentration) was observed for pasture. Much of the ammonia liberated in the rumen, together with some free amino acids, is incorporated into microbial protein in the rumen (Ørskov, 1982). Ammonia that is not utilized diffuses through the rumen wall and is transported to the liver where it is converted to urea. Some of the urea returns to the rumen in saliva or via the bloodstream, but the majority is removed from the blood by the kidneys and is excreted in the urine (Webb and Bergman, 1991). Reducing excess ruminal ammonia concentration is desirable because it minimizes N losses to the environment and may also prevent reproductive problems in cows that are associated with high level of plasma urea nitrogen concentration (Ferguson, 1996). Reductions in ammonia concentration have been observed *in vitro* (Gemeda and Hassen, 2015) and *in vivo* (Carulla et al., 2005) studies when supplementing tanniferous forages. In the present study, the lower ammonia concentration observed for pellet after YM supplementation could be due to the formation of tannin-protein complexes leading to lowered degradation of protein in the rumen (Patra and Saxena, 2011). The less efficient ammonia reduction observed in pasture samples compared to pellets could be attributed to the different interaction between the available substrate, pasture and pellet, and the microbial environment rumen (Wallace, 1996) which is made *in vitro* (Wallace, 1996).

CONCLUSION

In our investigation, the supplementation of YM to dairy cows seems to affect fiber and protein degradation in the rumen. The addition of YM in ruminant diet could decrease ammonia production and increase protein availability for productive purposes. The observed effect of YM on some degradation kinetics of nutrients could be due to the moderate presence of tannins.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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