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Changing the grazing session from morning to afternoon or including tannins in the diet was effective in decreasing the urinary nitrogen of dairy cows fed a total mixed ration and herbage

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ABSTRACT

Our aim was to evaluate whether increasing soluble carbohydrates in the herbage by changing the time of the grazing session or including *Acacia mearnsii* tannin in the diet would affect intake, digestion, N partitioning, and productive performance of dairy cows fed a diet combining ryegrass herbage with partial total mixed ration (PMR). We hypothesized that both strategies could reduce the concentration of NH_3-N in the rumen, reducing urinary N excretion. Nine Holstein cows were used in a triplicate 3 × 3 Latin square experiment with 3 experimental periods of 22 d. The cows were fed a fixed amount of PMR [60% of the predicted individual dry matter intake (DMI)], and an unrestricted amount of herbage in 1 grazing session of 5 h/d. The treatments were (1) morning grazing session and afternoon PMR meal (AM); (2) morning PMR meal and afternoon grazing session (PM); and (3) morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of PMR dry matter (TAN). Milk production was not affected by treatments. Although the protein concentration was lower for TAN than for PM, no differences were detected for the yield of any component between treatments. The concentration of individual or grouped fatty acids in milk fat was not affected by treatments, except for 16:1 *cis*-9 and Δ^9-desaturase ratios 14:1/14:0 and 16:1/16:0, which were lower for TAN. Treatments did not affect total DMI, but PM tended to increase herbage DMI and reduce dry matter and crude protein digestibilities. Treatments did not affect cow eating and ruminating behavior except for the proportion of time spent eating PMR, which was higher for PM and TAN. Although no relevant effects of treatments on ruminal fermentation, purine derivatives excretion in urine, or N excretion in milk were detected, both PM and TAN decreased the total N excreted in urine by an average of 8% compared with AM. In conclusion, changing the grazing session from the morning to the afternoon and including tannins in the diet were effective in decreasing the excretion of urinary N but did not change the productive performance of dairy cows fed PMR and ryegrass herbage.

Key words: *Lolium multiflorum*, milk production, N excretion, tannin, time of grazing

INTRODUCTION

Pasture-based dairy systems have multiple advantages from economic, environmental, and animal welfare viewpoints; moreover, milk from grass-fed cows contains compounds beneficial to human health, which contributes to consumer acceptance (Moscovici Joubran et al., 2021). However, 2 clear limitations exist for these systems, one related to DMI constraints and the other related to low efficiency of N use (Bargo et al., 2003; Mendoza et al., 2016a; Pastorini et al., 2019).

Concerning N use, N components of pastures are highly degradable (Repetto et al., 2005; Fulkerson et al., 2007; Cajarville et al., 2015) and are absorbed, metabolized, and excreted as urea in urine. In certain situations, especially linked to excessive N fertilization, a surplus of excretions can have negative productive, economic, and environmental implications (Calsamiglia et al., 2010). Increasing the proportion of urinary N increases NH_3 and nitrous oxide emissions to the atmosphere or nitrate leached to groundwater (Dijkstra et al., 2013). Therefore, improving N utilization by cows or shifting N excretion from urine to feces are both relevant challenges in the modern dairy industry.

A potential way to improve the efficiency of N utilization by ruminants is to increase the concentration of readily fermented carbohydrates (i.e., starch and sugars) in the diet and increase these types of carbohydrates in relation to N in the rumen (Hall and Huntington, 2008; Hoekstra and Schulte, 2008), which would lead to higher

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microbial protein synthesis (Berthiaume et al., 2010). However, some studies have demonstrated that feeding high-N fresh forages and supplementing them with fermentable carbohydrates at a different time (e.g., grazing pastures and providing cereals separately once or twice a day) was not effective for improving N utilization in sheep or cattle (García et al., 2000; Aguerre et al., 2013; Britos et al., 2018). Even when considering differences between studies in the methods used to quantify microbial protein synthesis and its efficiency, the results are consistent in showing that the supplementation of pastures with cereals, a typical situation in grazing systems of temperate regions, does not improve the use of pasture N. Bargo et al. (2002a,b), working with dairy cows, compared a TMR (17% CP, composed of corn silage, dry corn, a commercial concentrate, and alfalfa haylage), a combination of high CP pasture (26.3%) combined with the TMR (i.e., partial TMR, **PMR**), and pasture supplemented with concentrate (15% CP, composed of corn and wheat middlings). Those authors observed that PMR with herbage reduced the ruminal NH_3 concentration and improved dietary N utilization. Even so, the efficiency of N use decreases as the level of dietary N increases (Mulligan et al., 2004; Colmenero and Broderick, 2006). The concentration of N in herbage cannot be handled as in a TMR; thus, when the proportion of pasture in the diet is high, it is difficult to manage the amount of N ingested (Chilibroste et al., 2005). Therefore, improving N management and utilization in pasture-based dairy systems remains an important task.

One strategy to improve N use could be changing the grazing session from the morning to the afternoon because the soluble carbohydrate concentration in herbage increases in the afternoon (Gregorini, 2012; Cajarville et al., 2015). Although some studies using this management approach failed to find differences in DMI or milk production (Chen et al., 2017), some authors observed that this strategy could be effective in improving the ruminal uptake of N in grazing beef (Gregorini et al., 2008), nonlactating (Ueda et al., 2016), low-producing dairy cattle fed only herbage (Vibart et al., 2017; Chen et al., 2017), and even dairy cows consuming forages cut in the afternoon and ensiled as baleage (Brito et al., 2008). To our knowledge, no studies have investigated the change in the grazing session in medium-or high-yielding dairy cattle fed herbage and PMR.

Another potential strategy to decrease urinary N excretion is reducing ruminal protein degradability by including tannins in the diet. Tannins are polyphenols with the ability to form complexes with proteins, decreasing their ruminal degradation (Patra and Saxena, 2011). The tannin extract of *Acacia mearnsii* is commercially available, and previous studies reported that, at doses up to 18 g/kg of DM, it improved AA flow to the small intestine and decreased urinary N excretion without affecting OM digestibility in trials with nonlactating ruminants (Ávila et al., 2015; Orlandi et al., 2015). In contrast, results with dairy cows have been inconsistent. When dairy cows were supplemented with tannin extract of *Acacia mearnsii* from 10.9 to 19.1 g/kg of diet DM, decreased urinary N excretion were observed, albeit in addition to negative effects on feed intake and milk yield (Grainger et al., 2009). However, milk yield did not change with inclusions below 11.0 g/kg of diet DM (Griffiths et al., 2013; Alves et al., 2017), but neither digestibility nor urinary N excretion was evaluated in these studies.

Our objective was to evaluate the effect of changing the grazing session from morning to afternoon or including tannins in diets for cows that graze during the morning, on intake, digestion, N partitioning, milk composition, and milk production of dairy cows fed a diet combining ryegrass herbage (*Lolium multiflorum*) with PMR, a common feeding system in dairy farms of many regions of the Southern Hemisphere. We hypothesized that both strategies could reduce ruminal NH_3 concentration, which should be reflected in reduced excretion of urinary N of dairy cows.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

The experiment was carried out at the Experimental Station of the Veterinary Faculty (Facultad de Veterinaria, Universidad de la República, Uruguay), located in San José, Uruguay (34°40′S, 56°32′W), from September to November 2015 (spring). All procedures carried out during this experiment were previously approved by the Bioethics Committee of the Veterinary Faculty (protocol number: CEUAFVET-473). Nine multiparous Holstein dairy cows (experimental unit = cow), with permanent ruminal catheters (K227 Koler, chest drainage probes, 150-cm long, 13.5-mm outer diameter; Kine Estetic), were blocked into 3 squares balanced by BW, DIM, and milk yield, and within each square were randomly assigned to treatment sequences according to a replicated 3 × 3 Latin square design. After assignment, we verified that the squares were partially equilibrated in the succession of treatments. Cows produced 7,320 kg (SD = 1,044) of milk yield in the previous lactation, and at the start of period 1, cows had an average BW of 546 kg (SD = 34), milk production of 23 kg (SD = 2.6), and were 197 DIM (SD = 12). The experiment was conducted throughout 3 successive 22-d periods, with 14

Table 1. Chemical composition of partial TMR (PMR) and herbage (SD in parentheses) grazed in the morning (a.m.) or in the afternoon (p.m.; n = 3 herbage samples)

		Herbage	
Item	PMR[1]	a.m.	p.m.
DM, % of fresh matter	41.1 (1.6)	18.6 (2.1)	19.6 (2.5)
Chemical composition, % of DM			
OM	94.3 (0.2)	87.9 (1.1)	88.7 (1.2)
Ethanol-soluble carbohydrates (ESC)	3.7 (0.04)	6.9 (1.24)	8.9 (1.40)
NDF	27.1 (0.7)	45.9 (5.2)	43.4 (7.9)
ADF	16.6 (0.5)	27.1 (2.7)	26.3 (3.8)
ADL	1.3 (0.25)	3.7 (1.05)	3.6 (1.63)
NFC	47.1 (0.9)	26.9 (5.3)	31.4 (7.7)
Ether extract	3.8 (0.33)	2.1 (0.04)	1.8 (0.16)
CP	16.5 (0.1)	13.3 (1.4)	12.3 (1.0)
CP fractions, % of CP			
Soluble	48.1 (1.0)	21.5 (1.5)	21.3 (4.8)
NDIN	5.1 (0.40)	13.1 (0.3)	12.3 (2.8)
ADIN	1.4 (0.18)	2.9 (1.0)	3.0 (1.4)
ESC:CP ratio	0.22 (0.00)	0.53 (0.15)	0.73 (0.15)

[1]Composed of (DM basis) corn silage 60.0%, solvent-extracted soybean meal 19.5%, high-moisture corn grain silage 18.5%, urea 0.7%, sodium bicarbonate 0.4%, dicalcium phosphate 0.2%, calcium carbonate 0.2%, salt 0.2%, and 0.3% of a commercial product with (concentration per kg of DM) 12.5 g of Mn, 10 g of Mg, 5.0 g of Zn, 1.25 g of Cu, 0.75 g of Se, 17.5 mg of I, 1.8 mg of Co, 25,000 IU of vitamin A, 3,750 IU of vitamin D, and 188 IU of vitamin E.

d of treatment adaptation followed by 8 d of data and sample collection. The sample size was calculated using PROC POWER of SAS (version 9.1, SAS Institute Inc.) to determine the sample size required to detect a difference of 1 kg of milk produced, with a type I error (α) = 0.05 and a power of 80% (Festing and Altman, 2002), based on previous studies of the team and published information (Griffiths et al., 2013; Mendoza et al., 2016a,b; Ueda et al., 2016; Alves et al., 2017). The treatments tested within each Latin square consisted of a combination of PMR and 1 grazing session of 5 h, as follows: (1) morning grazing session and afternoon PMR meal (**AM**); (2) afternoon grazing session and morning PMR meal (**PM**); and (3) morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of PMR DM (**TAN**). Considering a proportion of 0.6 PMR in diet, the amount of tannin in PMR was chosen to reach a dietary concentration of 9 g of tannins/kg of DM, which is below the dose reported to have a negative effect on feed intake and digestibility (i.e., 11 g of tannins/kg of DM; Grainger et al., 2009). Tannin made from the bark of *Acacia mearnsii* (Weibull Black, Tanac S.A.) contained 716, 694, and 156 g/kg of DM of total phenols, total tannins, and condensed tannins, respectively (composition declared by the manufacturer).

Cows were milked twice a day from 0600 to 0700 h and from 1500 to 1600 h. Ryegrass pasture (*Lolium multiflorum*) was located about 150 m from the milking parlor and was offered for 5 h after the morning or afternoon milking (depending on treatment). Excluding the time at pasture, the cows were in a shaded pen where the PMR was provided and freely available. The PMR (Table 1) was formulated to meet the requirements of a cow with BW of 550 kg producing 28 kg/d of milk (NRC, 2001) and was offered daily in individual feeders located outdoors in the pen with fresh water, located between the pasture and the parlor. The amount of PMR was established individually at the beginning of the experiment as 60% of the total DMI, predicted according to BW, milk production, and week of lactation (18.7 ± 1.68 kg of DM/d; NRC, 2001).

The pasture area was divided into daily individual paddocks managed under strip grazing throughout the trial. The average (3 periods) herbage mass availability was 1,802 ± 413 kg of DM/ha (5 cm from ground). To obtain individual estimates of herbage intake, each cow was offered an individual paddock with fresh water and a daily herbage allowance of approximately 13 kg of DM per cow (Table 2). This quantity was defined for unlimited intake, following Pérez-Prieto and Delagarde (2013), for herbage allowances above 5 cm from the ground and considering that the main part of the diet was PMR.

Feed Analysis

Herbage and PMR samples were collected daily from d 15 to 22 of each experimental period. Approximately 300 g of PMR was taken daily from the individual feeders immediately before feeding. As refusals represented, on average, only 3.3% of the PMR DM offered, their

Table 2. Grazing conditions and effect of grazing session or tannin supplementation on intake and digestibility of nutrients in dairy cows fed partial TMR (PMR) and herbage

Item	Treatment[1]			SEM	*P*-value
	AM	PM	TAN		
Pregrazing herbage mass (kg of DM/ha)	1,868[ab]	1,740[b]	1,884[a]	243	0.04
Rising plate meter (cm)	15.1[a]	14.3[b]	15.2.[a]	1.20	0.03
Offered area (m^2)	71.1[b]	76.7[a]	70.9[b]	9.64	0.01
Herbage allowance (kg of DM/d per cow)	13.3	13.4	13.4	0.84	0.83
Postgrazing herbage mass (kg of DM/ha)	760[a]	653[b]	788[a]	97	<0.01
DMI (kg/d)					
Herbage	7.9	8.3	7.8	0.44	0.09
PMR	11.1[a]	10.9[b]	11.1[a]	0.47	0.03
Total	19.0	19.3	18.9	0.69	0.32
Nutrient intake (kg/d)					
OM	17.4	17.6	17.2	0.61	0.23
NDF	6.6	6.5	6.5	0.30	0.79
NFC	7.5[b]	8.0[a]	7.4[b]	0.32	<0.01
Ethanol-soluble carbohydrates (ESC)	0.96[b]	1.15[a]	0.95[b]	0.07	<0.01
Digestible OM	13.0	12.9	12.7	0.59	0.20
ESC:CP ratio	0.33[b]	0.41[a]	0.33[b]	0.028	<0.01
Digestibility (%)					
DM	72.6[a]	70.6[b]	71.4[ab]	1.46	0.05
OM	74.9[a]	73.0[b]	73.6[ab]	1.57	0.06
NDF	64.5	62.8	62.8	2.11	0.73
CP	71.7[a]	67.7[b]	69.6[ab]	0.93	0.01

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

chemical composition was not considered for nutrient ingestion of PMR. Herbage sub-samples (~100 g of fresh matter) were taken from individual paddocks at the start of grazing (i.e., 0700 h for AM and TAN, and 1600 h for PM) using the hand clipping procedure (Cook, 1964) and were immediately frozen in liquid N. All samples were then stored at −20°C, and PMR was dried in a forced-air oven at 60°C for 48 h. Herbage was freeze-dried (Benchtop Pro, Vir-Tis) for carbohydrate analysis to detect the a.m. and p.m. composition. All samples were then ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co.) and composited by period and treatment for analysis of DM, ash, total N, and ether extract (AOAC, 1990; methods 934.01, 942.05, 955.04, and 920.39, respectively). Organic matter was calculated by mass difference. The NDF analysis was based on the procedures described by Mertens (2002), with heat-stable α-amylase and without sodium sulfite. The concentration of ADF was analyzed according to Van Soest et al. (1991), except that the samples were weighed in polyester filter bags (porosity of 16 μm) and treated with acid detergent in an autoclave (Prismatec Autoclaves) at 110°C for 40 min (Senger et al., 2008). For ADL analysis, the bags containing residual ADF were treated with 12 M H_2SO_4 for 3 h (AOAC, 1990; method 973.18). Analysis of soluble N, NPN, NDIN, and ADIN was performed according to Licitra et al. (1996). Ethanol-soluble carbohydrates (**ESC**) were extracted from feed samples according to Hall (2000) and analyzed according to DuBois et al. (1956). The concentration of NFC was calculated as suggested by NRC (2001) and corrected for NDIN as follows:

$$\text{NFC (\%)} = 100 - [\text{\% CP} + (\text{\% NDF} + \text{\% NDIN}) + \text{\% ether extract} + \text{\% ash}].$$

DM and Nutrient Intake

Refusals of PMR were individually weighed between d 15 and 19 of each experimental period. The herbage intake was estimated individually on the same days as the difference between the pre- and postgrazing herbage mass (Haydock and Shaw, 1975). The pregrazing herbage mass was assessed every day as the average value of 30 measurements made in each paddock with an electronic rising plate meter (Farmworks Electronic Plate Meter, Farmworks Ltd.). The plate meter was calibrated for each experimental period 2 d before intake measurements by cutting at 5 cm from the ground all the herbage mass within the plate area, in 3 contrasting sward heights in quadruplicate, which were averaged to build the regression. The general equation (3 periods) to estimate pregrazing herbage mass (kg of DM/ha) was 160 (SD = 37) × plate meter value (cm) − 548 (SD = 624) (n = 9, 3 heights/period, R^2 = 0.96). As

the postgrazing herbage mass was more heterogeneous, we decided to use direct cutting for its measurement instead of the plate meter. Therefore, after each grazing session and in each cow paddock, 2 transects (12 × 0.5 m) that covered 15 to 20% of the total grazed area were defined, and all the forage mass contained was cut with a mower (Toro CNB94, The Toro Company) at 5 cm from the ground and weighed. The cutting height was defined before starting the experiment, testing for different height cuts, and it was established considering the height at which all herbage was reached by the mower in pre- and postgrazing paddocks (final data in Table 1). Pre- and postgrazing herbage samples were taken separately, weighed, oven-dried at 105°C for 24 h, and weighed again. The intakes of DM, OM, CP, NDF, NFC, and ESC were calculated using data of pregrazing herbage, postgrazing herbage, and PMR chemical composition.

Behavior Recording

On d 15 and 18 of each experimental period, individual behavior patterns were recorded by 4 trained observers every 5 min for 16 h, beginning after the morning milking (0700 h), by instantaneous sampling of individual cows (Martin and Bateson, 1993). The behaviors were defined as "eating" (grasping and chewing PMR or herbage), "ruminating" (chewing regurgitated boluses of feed), and "other" (neither eating nor ruminating). The proportion of each event in each hour was calculated for 2 intervals: for the whole period (16 h) and for the 5 h after offering the PMR or herbage. The time for each activity after offering PMR and herbage was calculated by multiplying the proportion by 300 min (5 h).

Milk Production and Composition

Milk yield was recorded from d 15 to 19 of each experimental period. Individual milk samples were taken in the morning and afternoon milkings on d 15 and 17 and stored at 4°C with bronopol preservative; on d 17, additional milk samples were taken and stored at −20°C without preservatives. The milk samples stored with bronopol were analyzed for fat, CP, total casein, and lactose by infrared spectroscopy (NIRS Model 2000, Bentley Instruments Inc.). The FCM (3.5% fat) yield was calculated according to NRC (2001). The milk samples stored without preservatives were thawed at room temperature, and milk lipids were separated according to Feng et al. (2004). The fatty acids (**FA**) composition was analyzed by GC-MS using an Agilent 7890A GC System (Agilent Technologies Inc.) equipped with a 60-m column (250-μm i.d., 0.25-μm film thickness; ThermoFisher Scientific Inc.). Helium was used as the carrier gas, with a flow rate of 1.0 mL/min. The injector temperature (split ratio of 100:1) was set to 250°C. The initial column temperature (40°C) was held for 0.5 min, increased at 25°C/min to 175°C and held for 10 min, and then increased at 5°C/min to 210°C and held for 5 min. Finally, the column temperature was increased at a rate of 5°C per min to 230°C and held for 5 min. Fatty acids were identified by comparing their retention times with the following FAME standards: 37-component FAME mix (47885, Supelco), *trans*-11-octadienoic methyl ester (46905-U, Supelco), octadecadienoic acid conjugated methyl ester (05632, Sigma-Aldrich).

Digestion

Apparent total-tract nutrient digestibility was evaluated indirectly, estimating the total fecal output using the indigestible NDF (**iNDF**) as an internal marker (Huhtanen et al., 1994). Following Colmenero and Broderick (2006) and Mendoza et al. (2016b), on d 16, 17, and 19 of each period, spot fecal samples were collected from all cows twice a day at 1200 and 2100 h (5 h after the start of morning and afternoon meals). Fecal grab samples (~200 g of fresh matter) were collected directly from the rectum, dried at 60°C for 48 h, and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co.). A composite sample per cow and period was obtained by mixing equal DM amounts from each sample. The samples were then analyzed for DM, ash, NDF, and total N as described above. Fecal, herbage, and PMR samples were weighed (2 g) in a polyester filter bag (5 × 5 cm, 16 μm porosity) and incubated for 288 consecutive hours in the rumen of a cannulated steer, with an estimated intake of 10 kg of DM/d, grazing a Tifton (*Cynodon dactylon*) pasture and receiving supplementation with 2 kg of DM/d of a concentrate composed (DM basis) of cracked corn (36%), wheat bran (36%), and soybean meal (28%). After incubation in the rumen, the bags were rinsed with tap water for 45 min and dried in a forced-air oven at 60°C for 48 h, and the residues were analyzed for NDF as described above. The total fecal output (kg of DM/d) was calculated as follows:

$$\text{Total fecal output (kg of DM/d)} = [\text{herbage DMI (kg/d)} \times \text{iNDF in herbage (g/kg of DM)} + \text{PMR DMI (kg/d)} \times \text{iNDF PMR (g/kg of DM)}]/\text{iNDF in feces (g/kg of DM)}.$$

Apparent total-tract digestibility coefficients for DM, OM, NDF, and N were calculated as follows:

$$\text{Apparent total-tract digestibility} = [\text{intake (g/d)} - \text{fecal output (g/d)}]/\text{intake (g/d)}.$$

Ruminal Fermentation

On d 20 of each period, ruminal fluid samples were collected at 0 (prefeeding), 2, 4, 6, 9, 11, 13, 15, and 17 h relative to the beginning of the morning meal (i.e., 0700 h). This sampling scheme included 8 h from the beginning of the grazing session, and samples were taken every 2 h for all treatments. Ruminal pH was determined immediately after sample collection using a calibrated digital pH meter (EW-05991-36, Cole Parmer). Ruminal fluid was filtered through 2 layers of cheesecloth, and a 9-mL sample of ruminal fluid was preserved with 1 mL of 3.6 *M* H_2SO_4 for NH_3-N and ESC analysis. Another 1-mL sample was preserved with 1 mL of 0.1 *M* $HClO_4$ for VFA analysis. All samples of ruminal fluid were stored at −20°C until analysis. The samples of ruminal fluid acidified with H_2SO_4 were thawed at room temperature, centrifuged at 4,000 × *g* for 20 min, and analyzed for NH_3-N (Weatherburn, 1967) and total ESC (Dubois et al., 1956). For VFA analysis, only samples taken at h 0, 4, 9, and 13 were analyzed. Samples were thawed at room temperature, centrifuged (10,000 × *g* for 15 min at 4°C), and analyzed by HPLC (Dionex Ultimate 3000) as described by Adams et al. (1984) using an Acclaim Rezex Organic Acid H^+ column (8%; Phenomenex) of 7.8 × 300 mm, adjusted at 210 nm.

Excretion of Urinary Purine Derivatives and N Partitioning

On d 16, 17, and 19 of each period, spot urine samples were collected from all cows twice a day (Chizzotti et al., 2008), at 1200 and 2100 h (5 h after the start of morning and afternoon meals) by manual stimulation of the vulva. A 10-mL sample of fresh urine was acidified with 1 mL of H_2SO_4 20% (vol/vol), diluted with 49 mL of distilled water, and stored at −20°C. The urine samples were later thawed at room temperature, and equal parts of each of the 6 samples were mixed to obtain a composite sample, which was used for analyses. A subsample of urine was filtered through a paper filter (7.5 μm porosity) and analyzed for creatinine by a colorimetric method using a commercial kit (Labtest). Total urine volume (L/d) was then calculated according to Valadares et al. (1999), but assuming a daily creatinine excretion of 21.9 mg/kg of BW, obtained from an experiment in which total urine collection was performed in herbage-fed lactating cows (Pacheco et al., 2007) as follows:

$$\text{Total urine volume (L/d)} = [\text{BW (kg)} \times 21.9]/\text{creatinine in urine (mg/L)}.$$

In addition, another subsample of urine was centrifuged (10,000 × *g* for 15 min at 4°C), and purine derivatives (**PD**; i.e., allantoin and uric acid) were analyzed using an HPLC (Dionex Ultimate 3000) procedure as described by Balcells et al. (1992) with an Acclaim C18 (Phenomenex) column of 205 nm, 5 μm, and 4.6 × 250 mm.

The concentration of N in urine and feces was determined by the Kjeldahl method (AOAC, 1990; method 955.04). The N excreted in manure was calculated as urinary N plus fecal N. The urine samples were also analyzed for urea-N by a colorimetric method using commercial kits (Bioclin).

On d 22 of each period, individual blood samples were collected into heparinized tubes from the coccygeal vein at 0, 3, 9, and 12 h relative to the beginning of the first meal (0700 h). After centrifugation (3,000 × *g* for 20 min at 20°C), plasma was separated and stored at −20°C. Plasma urea-N (**PUN**) was determined by colorimetric analysis using a commercial kit (Bioclin).

Milk CP and MUN concentrations were determined by infrared analysis (model 2000, Bentley Instruments Inc.). The excretion of N in milk N (g/d) was calculated as total milk protein (g/d) divided by 6.38.

Statistical Analysis

Data were analyzed using SAS software version 9.1 (SAS Institute Inc.). Data of variables with only one measurement during each period (i.e., intake, digestibility, N excretion, productive performance, and milk FA profile) were averaged per animal and period within treatments and analyzed using PROC MIXED with the following model (Kaps and Lamberson, 2004):

$$Y_{ijkl} = \mu + S_i + C_j(S_i) + P_k + T_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable (n = 9 observations); S_i is the random effect of square (i = 1 to 3); $C_j(S_i)$ is the random effect of cow nested in the square (j = 1 to 3); P_k is the random effect of the period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, PM, or TAN); and e_{ijkl} is the residual error. Data of variables with repeated measurements over time such as behavioral events, PUN, ruminal pH, NH_3-N, ESC, and VFA, were analyzed using the cow as subject for

the repeated measurement, in each period, using PROC MIXED with the following model:

$$Y_{ijklm} = \mu + S_i + C_j(S_i) + P_k + T_l + H_m + T_l \times H_m + e_{ijklm},$$

where Y_{ijklm} is the dependent variable; S_i is the random effect of square (i = 1 to 3); $C_j(S_i)$ is the random effect of cow nested in the square (j = 1 to 3); P_k is the random effect of the period (k = 1 to 3); T_l is the fixed effect of treatment (l = AM, PM, or TAN); H_m is the fixed effect of the hour of measurement; $T_l \times H_m$ is the fixed effect of the interaction of treatment and hour of measurement; and e_{ijklm} is the residual error. The covariance structure was first-order autoregressive [AR(1)] for evenly spaced data (i.e., behavioral events) and spatial power [SP(POW)] for unevenly spaced data (i.e., PUN, and ruminal pH, NH_3-N, ESC, and VFA). Treatment × period effect was tested in both models and removed after verifying that it was not significant. Differences among treatments were declared significant at $P \leq 0.05$, and trends were declared at $0.05 < P \leq 0.10$. Means were separated using the PDIFF option after a significant treatment effect was detected.

RESULTS

Total DMI was not affected by treatments, but the cows grazing during the afternoon ingested less PMR ($P = 0.03$) and tended ($P = 0.09$) to consume more herbage than cows in AM and TAN (Table 2). As a consequence, the percentage of herbage in the diet was higher in PM than in AM and TAN (43.2 vs. 41.3%, SEM = 1.55, $P = 0.03$; data not shown in the table). Similarly, in the PM treatment, the final diet had a higher forage percentage (herbage + corn silage) than the other treatments (77.3 vs. 76.6%, SEM = 0.62, $P = 0.03$). The intake of DM and nutrients was similar for all treatments, except for NFC and ESC intake, which in PM was 20% higher on average than in AM and TAN ($P < 0.01$). The average calculated dietary concentration of tannins in TAN was 8.9 ± 4 g/kg of DM.

Changing the grazing session from the morning to the afternoon reduced the apparent digestibility of DM ($P = 0.05$) and CP ($P < 0.01$) by 3 and 6%, respectively, compared with AM (Table 2). The addition of tannins to the PMR did not affect nutrient digestibility (Table 2).

The treatments did not affect grazing, eating, or ruminating activities recorded throughout 16 h of observation, but an interaction between treatment and hour was observed (Table 3). During the first 5 h relative to the start of feeding PMR or grazing, treatments did not affect behavior except for the proportion of time spent eating PMR, which was higher for PM ($P = 0.03$). However, an interaction between treatment and time was observed for these variables (Table 3; Figure 1). Cows in all treatments spent most of their time eating during the first 2 h after the PMR was offered, and this proportion was higher in TAN (1600 to 1800 h) and PM (0700 to 0900 h, Figure 1). After 2 h of PMR meal, most of the time was used for rumination activity, and eating activities were low. During the first hour after access to pasture, all the time was used for grazing (0700 to 0800 h for AM and TAN, and 1600 to 1700 h for PM); thereafter, this activity gradually decreased, increasing ruminating activity up to 4 h. At this time (1100 to 1200 h), cows in the AM and TAN treatments increased their grazing activity.

The production of milk and milk components was similar for all treatments (Table 4). Although milk protein concentration was lower ($P < 0.05$) for TAN compared with PM, no differences were detected for the yield of any component. The concentration of individual or grouped FA in milk fat was not affected by treatments, except that 16:1 *cis*-9 and the 14:1/14:0 and 16:1/16:0 Δ^9-desaturase ratios were lower for TAN ($P < 0.05$; Table 5).

Treatment did not affect the ruminal concentrations of NH_3-N, ESC, or pH. Interactions between treatment and time were detected for these variables ($P < 0.01$; Table 6). Independently of treatment, the minimal value of pH was observed at 4 h and the maximal NH_3-N concentration 2 h after PMR supply. The maximal ESC concentration was detected between 2 and 8 h after PMR supply for all treatments (Figure 2). The concentration of VFA and the molar proportions of acetate, butyrate, and propionate are presented in Table 6. The total concentration of VFA in the rumen was not affected by treatment. An interaction between treatment and hour was detected for the molar proportion of acetate (which was lower at h 4 for TAN and higher at h 13 for AM with respect to the first meal) and butyrate (which was higher at h 4 for TAN and at h 13 for PM with respect to the first meal; data not shown, $P < 0.01$).

Treatment did not affect the estimated excretions of allantoin, uric acid, or total PD (Table 7). Moreover, treatment did not affect the intake of N or the amount of N excreted in milk and manure (Table 8). Treatment did not affect PUN (Table 8), but there was an interaction between treatment and time, as this variable was higher at h 3 relative to the first meal for PM ($P < 0.01$; Figure 3). The cows in PM treatment excreted more grams of N in feces than cows in the AM treatment. In contrast, tannin inclusion increased the grams

Table 3. Effect of grazing session or tannin supplementation on behavioral events of dairy cows during the first 16 h after the initial feeding (0700 h) and in the first 5 h relative to the start of feeding partial TMR (PMR) or grazing

	Treatment (Trt)[1]				*P*-value		
Behavioral events	AM	PM	TAN	SEM	Trt	H	Trt × H
Proportion of total observations in 16 h							
Eating	0.29	0.30	0.30	0.015	0.34	<0.01	<0.01
Ruminating	0.34	0.34	0.35	0.022	0.95	<0.01	<0.01
Other[2]	0.37	0.35	0.35	0.027	0.54	<0.01	<0.01
During feeding PMR: proportion of total observations in 5 h							
Eating	0.28 (84)[3]	0.34 (102)	0.31 (93)	0.015 (4.5)	0.03	<0.01	0.03
Ruminating	0.38 (114)	0.34 (102)	0.38 (114)	0.044 (13.2)	0.56	<0.01	0.01
Other	0.34 (102)	0.32 (96)	0.30 (90)	0.047 (14.1)	0.72	<0.01	0.02
During grazing: proportion of total observations in 5 h							
Eating	0.63 (189)	0.63 (189)	0.65 (195)	0.044 (12.0)	0.75	<0.01	<0.01
Ruminating	0.26 (78)	0.29 (87)	0.26 (78)	0.035 (10.5)	0.41	<0.01	<0.01
Other	0.11 (33)	0.07 (21)	0.09 (27)	0.019 (5.7)	0.29	<0.01	0.79

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

[2]Not eating or ruminating.

[3]Values in parentheses represent minutes calculated according to the number of observations in 5 h.

of fecal NDIN and ADIN excreted ($P < 0.05$). Cows in the PM and TAN treatments excreted less N and urea-N in urine than cows in AM ($P \geq 0.03$), and the urinary N to manure N ratio was reduced for the PM and TAN treatments.

DISCUSSION

Although no changes in total DMI were observed, PM treatment increased the proportion of herbage in the diet, which would be positive considering the benefits of pasture and the difficulties observed in increasing pasture intake with respect to PMR in mixed diets reported by others (Vibart et al., 2008; Mendoza et al., 2016a; Pastorini et al., 2019). The higher NDF, ADF, and ADL concentrations in herbage compared with PMR could explain, in part, the reduced digestibility of PM related to a higher herbage intake. However, the small change in the proportion of pasture in the diet seems insufficient to explain the reduction observed in digestibility. Also, the higher herbage proportion could have led to a higher rate of passage. Although recent experiments questioned the change of passage rate with the change of forage source in TMR diets (Wang et al., 2018), a higher rate of passage could also explain the reduction in digestibility (Colucci et al., 1982). An increased proportion of herbage in cows grazing during the afternoon period is consistent with the higher ESC concentration observed in the p.m. herbage. Other authors also observed similar results associated with higher soluble carbohydrate concentrations (Hernández-Ortega et al., 2014; Ueda et al., 2016) or with a higher DM concentration of herbage (Ueda et al., 2016). It is noteworthy, however, that cows in the AM treatment ingested more DM and spent less time eating during the afternoon, although the feedstuff was PMR. Therefore, the changes observed in intake and ingestion activity seem to be more related to diurnal behavioral patterns, as described by DeVries et al. (2003), rather than to changes in herbage composition. The mean quantities consumed per hour in each intake session for PMR calculated from the proportion and time were 8.2, 6.7, and 7.2 kg of DM/h for AM, PM, and TAN, respectively. Considering that the intake rate of PMR was nearly 3 times greater than that of herbage, a few hours after starting a session, the total DM eaten was much greater after feeding PMR compared with after grazing. Therefore, earlier ruminal repletion and satiety can explain this behavior. Considerable grazing activity was still observed at the end of the grazing period (5 h from the beginning) in AM and TAN. We could not find a clear explanation for this behavior, but extending pasture allocation beyond 5 h would probably yield different results.

The absence of a negative effect of tannins on feed intake and digestibility is consistent with the dose used

(15.0 g/kg of DM of PMR, which resulted in 8.9 g/kg of DMI), which was chosen based on results published by Grainger et al. (2009), as mentioned previously.

In respect to digestibility results, it is necessary to point out the limitation of using only 2 time points for fecal collection, considering the results of Morris et al.

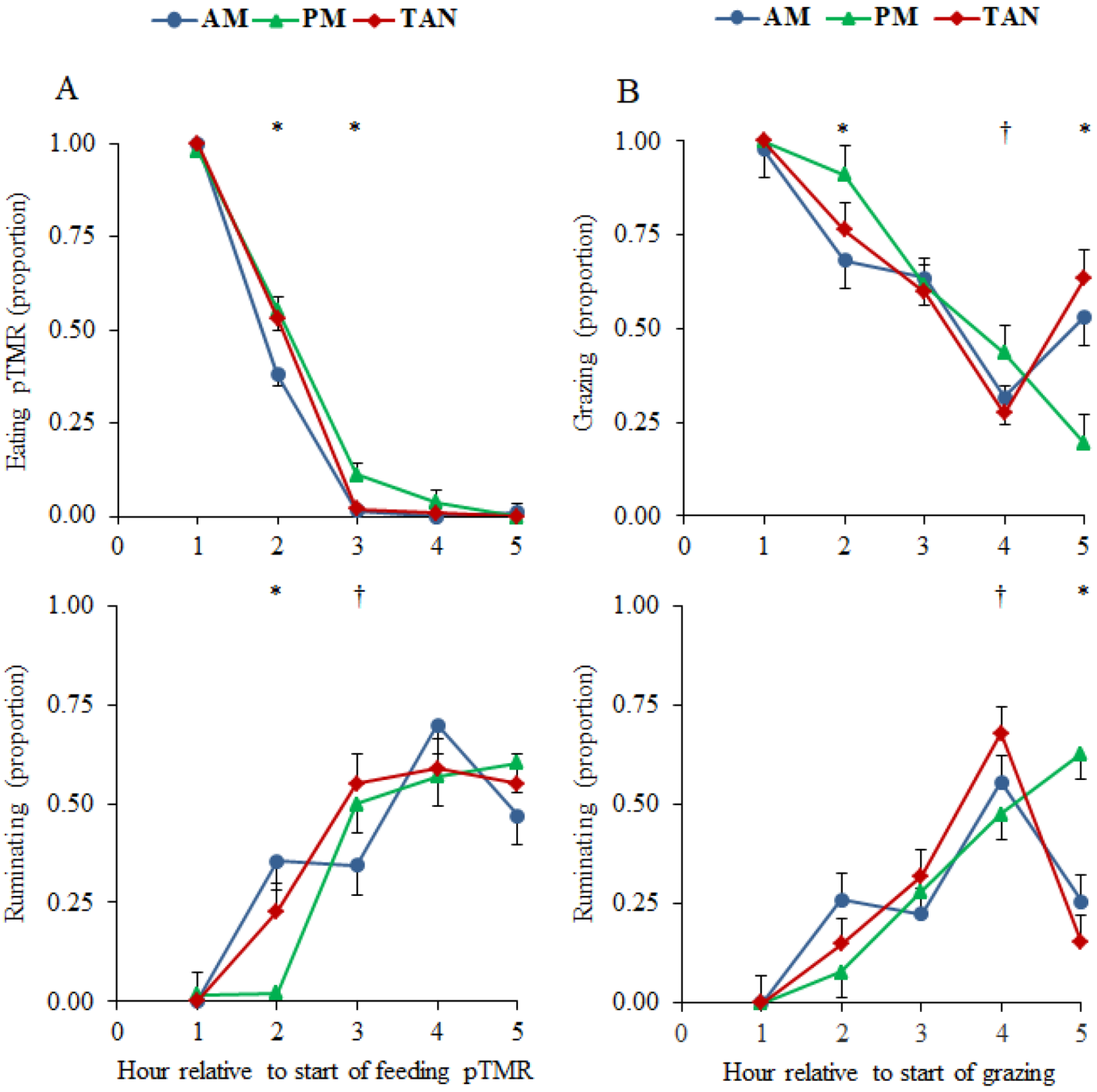

Figure 1. Effect of grazing session or tannin supplementation on behavioral events in the first 5 h relative to the start of the meal when feeding a partial TMR (PMR; A) or grazing (B). The values are expressed as a proportion of the total observations in each hour. Symbols at each hour indicate at least one difference ($*P \leq 0.05$) or tendency ($\dagger P \leq 0.10 > 0.05$) among the treatments in the interaction analysis. AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM. Error bars represent SEM.

Table 4. Effect of grazing session or tannin supplementation on milk yield and composition of dairy cows fed partial TMR (PMR) and herbage

	Treatment[1]				
Item	AM	PM	TAN	SEM	*P*-value
Milk (kg/d)	21.7	21.4	21.6	2.12	0.86
3.5% FCM[2] (kg/d)	23.6	24.2	23.8	1.89	0.63
Fat (kg/d)	0.88	0.92	0.89	0.062	0.40
Fat (%)	4.11	4.35	4.13	0.196	0.21
Protein (kg/d)	0.76	0.75	0.74	0.062	0.55
Protein (%)	3.51[ab]	3.54[a]	3.43[b]	0.095	0.03
Casein (kg/d)	0.57	0.56	0.55	0.044	0.41
Casein (%)	2.65	2.66	2.56	0.095	0.07
Lactose (kg/d)	1.02	1.02	1.02	0.107	0.99
Lactose (%)	4.70	4.75	4.72	0.089	0.41
Total solids (kg/d)	2.65	2.69	2.65	0.226	0.72
Total solids (%)	12.3	12.6	12.3	0.279	0.09

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

[2]3.5% FCM (kg/d) = 0.4324 × milk yield (kg/d) + 16.218 × fat yield (kg/d).

Table 5. Effect of grazing session or tannin supplementation on the milk fatty acid (FA) profile and components of dairy cows fed partial TMR (PMR) and herbage

	Treatment[1]				
FA concentration (g/100 g of total FA)	AM	PM	TAN	SEM	*P*-value
Selected individual FA					
4:0	1.41	1.54	1.15	0.322	0.66
6:0	1.38	1.41	1.13	0.249	0.66
8:0	0.98	1.02	0.88	0.157	0.80
10:0	2.33	2.70	2.45	0.418	0.81
12:0	3.15	3.51	3.06	0.341	0.60
14:0	12.4	12.8	12.4	0.79	0.91
14:1 *cis*-9	1.26	1.30	1.09	0.147	0.42
15:0	0.94	1.01	0.93	0.065	0.50
16:0	38.2	36.3	35.0	1.79	0.08
16:1 *cis*-9	2.35[a]	2.34[a]	1.86[b]	0.177	<0.01
18:0	8.86	8.83	10.69	1.373	0.19
18:1 *cis*-9 (oleic acid)	21.4	21.9	23.8	1.14	0.31
18:1 *trans*-9	0.24	0.21	0.27	0.054	0.31
18:1 *trans*-11 (vaccenic acid)	1.60	1.63	1.85	0.506	0.75
18:2 *cis*-9,*cis*-12	1.26	1.38	1.20	0.163	0.66
18:2 *cis*-9,*trans*-11 (rumenic acid)	0.70	0.78	0.65	0.181	0.67
18:3 *cis*-9,*cis*-12,*cis*-15 (linolenic acid)	0.32	0.30	0.36	0.066	0.54
Summation by origin					
De novo (4:0–15:0)	22.7	24.1	22.1	2.15	0.78
Mixed origin (16:0 + 16:1)	40.5	38.6	36.9	1.96	0.06
Preformed (>17:0)	35.1	35.7	39.5	2.92	0.28
Summation by saturation					
SFA	70.3	69.7	68.4	1.53	0.65
MUFA	27.3	27.8	29.2	1.27	0.52
PUFA	2.25	2.46	2.20	0.308	0.72
Saturated:unsaturated ratio	2.43	2.37	2.21	0.159	0.55
n-6:n-3 ratio	4.55	4.16	4.42	0.328	0.35
Δ^9-desaturase ratio					
14:1/14:0	0.103[a]	0.101[a]	0.086[b]	0.0068	0.01
16:1/16:0	0.062[a]	0.064[a]	0.053[b]	0.0042	0.01
18:1/18:0	2.92	2.83	2.59	0.244	0.33
18:2 *cis*-9,*trans*-11/18:1 *trans*-11	0.39	0.42	0.47	0.104	0.82

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

Table 6. Effect of grazing session or tannin supplementation on ruminal pH, ammonia-N (NH_3-N), ethanol-soluble carbohydrates (ESC), and VFA of dairy cows fed partial TMR (PMR) and herbage

	Treatment (Trt)[1]				*P*-value		
Item	AM	PM	TAN	SEM	Trt	H	Trt × H
pH	6.33	6.34	6.29	0.04	0.69	<0.01	<0.01
NH_3-N (mg/dL)	10.6	10.8	11.4	2.27	0.62	<0.01	<0.01
ESC (mg/dL)	61.0	58.9	59.6	2.68	0.74	<0.01	<0.01
Total VFA (m*M*)	100.3	105.7	108.1	14.7	0.68	0.25	0.66
VFA (mol/100 mol)							
Acetate	65.2[a]	64.5[a]	62.2[b]	1.89	<0.01	<0.01	0.02
Propionate	20.0[ab]	18.6[b]	21.2[a]	0.73	0.02	0.36	0.06
Butyrate	14.8	16.8	16.7	1.58	0.11	<0.01	<0.01

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

(2018). Those authors observed an overestimation of fecal iNDF when using few spot samples, with results varying with the diet type. Although overestimation may have occurred in our experiment, we can expect a similar effect for all treatments because the diets were similar.

Our hypothesis that changing the grazing session from morning to afternoon and including tannins in the diet could improve the productive performance of dairy cows was not confirmed in the present experiment. This lack of improvement can be explained by at least 3 factors: (1) the dose of tannin was not insufficient (already discussed), (2) due to the low CP concentration of the herbage, there was not a considerable excess of degradable N, or (3) the cows were in a stage of lactation when nutrients are oriented toward weight gain and not milk production (NRC, 2001). Despite the higher herbage proportion in the diet of cows in PM, we did not observe positive changes in milk FA, similarly to other studies by our team (Mendoza et al., 2016a; Pastorini et al., 2019). However, the additional herbage intake of this treatment was low (only 5 and 6% higher compared with AM and TAN, respectively), and we have no information about FA concentrations of the feedstuffs used. Also, although the percentage of solids increased in PM, total solids yields were similar among treatments. Regarding the TAN treatment, tannins consumed in our experiment were close to 169 g/d per cow. Other studies also failed to confirm the theoretical positive effect of *Acacia mearnsii* tannins on productive performance of dairy cows, using both higher and lower doses per animal [e.g., Grainger et al. (2009), working with 163 to 326 g/d per cow; Griffiths et al. (2013), working with 111 to 444 g/d per cow; Alves et al. (2017), working with 80 g/d per cow]. However, *Acacia mearnsii* tannins were reported to impair ruminal biohydrogenation (Khiaosa-Ard et al., 2009), which can affect the FA profile in milk. In the present study, the proportion of most relevant individual FA in milk fat remained unchanged after dietary tannin inclusion. The exception was the Δ^9-desaturase ratio for 14- and 16-carbon FA and the proportion of 16:1 *cis*-9 in milk fat, which were lower in TAN. This could be related to phenolic compounds associated with the hydrolyzable fraction of tannins that could be absorbed in the digestive tract (Makkar, 2003), which might affect (by modulating the substrate availability to the mammary gland) the activity of Δ^9-desaturase (Toral et al., 2013; Buccioni et al. 2015). This finding is interesting because clear effects of tannins on the milk FA profile have been reported only with tannin addition as high as 30 g/kg of diet DM (Dschaak et al., 2011; Henke et al., 2017), which, in turn, could have negative effects on intake.

The interactions between treatments and time detected for ruminal pH, NH_3-N, ESC concentrations, and molar proportions of VFA seem to have been a consequence of the different timings on pasture and PMR of PM compared with AM and TAN. The lower pH and NH_3-N and ESC peaks were similar for all treatments with respect to the time after the beginning of PMR meal. Feeding PMR in either the morning or afternoon resulted in greater N-NH_3 peaks than grazing pasture, which could be related to a higher intake rate and the higher CP concentration of PMR compared with herbage. The higher molar proportion of propionate and a lower molar proportion of acetate in TAN compared with AM could be related to selective suppression of cellulolytic bacteria by tannins (Bhatta et al., 2009; Hassanat and Benchaar, 2013), although fiber total-tract digestibility was not affected in the present study.

Before commenting on N use results, we need to point out some characteristics of the feedstuffs used in this study. First, although in a vegetative stage, the pasture had a lower N concentration than we expected (2% of N on a DM basis), which was probably related to low N fertilization. In contrast, PMR had a high

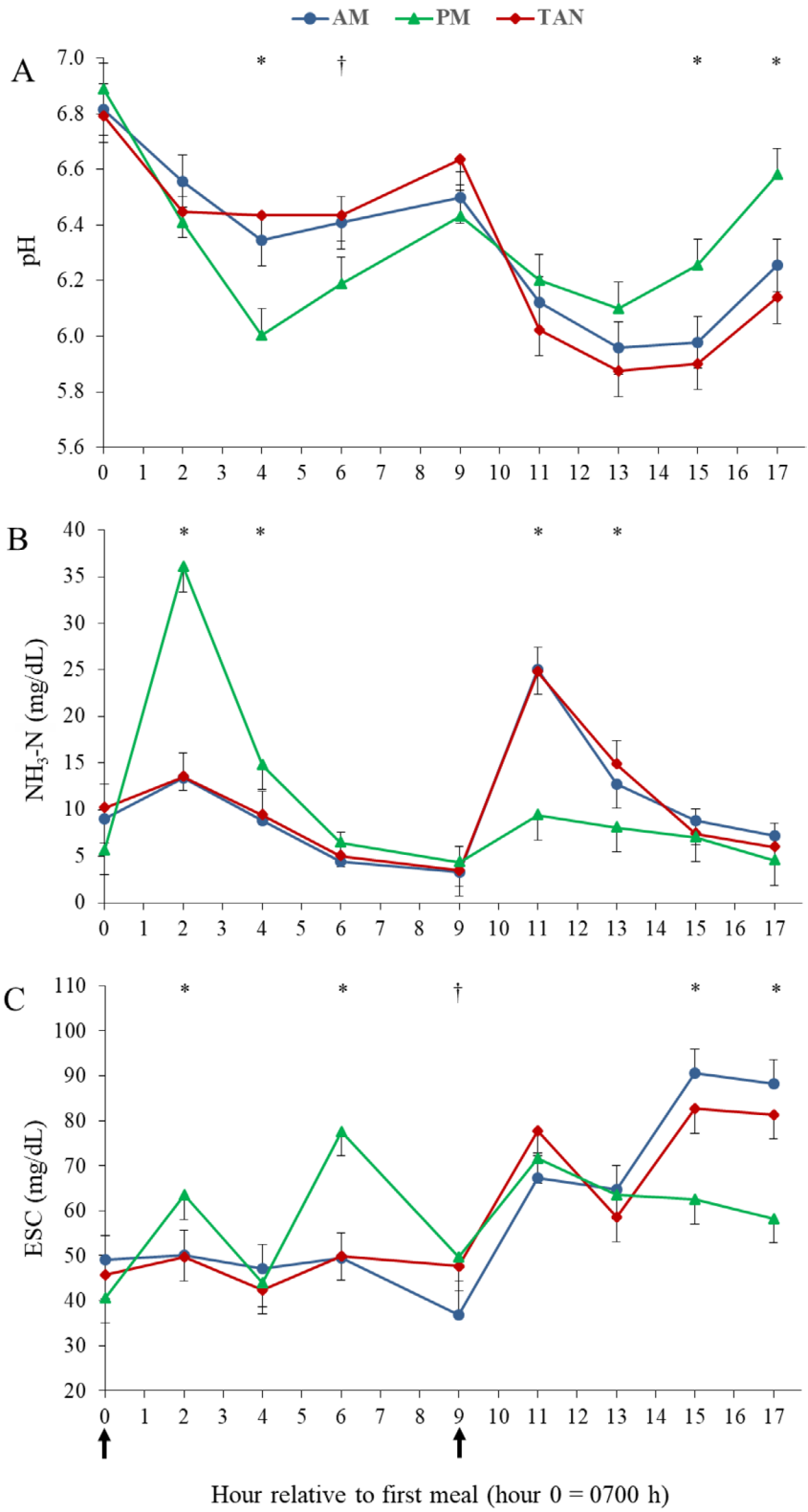

Figure 2. Effect of grazing session or tannin supplementation on ruminal pH (A) and concentration of ammonia-N (NH_3-N; B) and ethanol-soluble carbohydrates (ESC; C). Symbols at each hour indicate at least one difference ($^*P \leq 0.05$) or tendency ($\dagger P \leq 0.10 > 0.05$) among the treatments in the interaction analysis. AM = morning grazing session and afternoon partial (p)TMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM. The arrows indicate the meals. Error bars represent SEM.

Table 7. Effect of grazing session or tannin supplementation on excretion of urinary purine derivatives (PD) of dairy cows fed partial TMR (PMR) and herbage

	Treatment[1]				
Item	AM	PM	TAN	SEM	*P*-value
Creatinine (mmol/L)	5.1	5.3	5.2	0.62	0.67
PD excretion (mmol/d)					
Allantoin	265	267	232	19.4	0.12
Uric acid	22.9	23.3	20.0	1.93	0.16
Total	288	290	252	20.3	0.12
PD:creatinine[2]	2.7	2.7	2.3	0.17	0.07

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

[2]PD and creatinine concentrations (mmol/L).

concentration of soluble protein. These characteristics may have limited the expression of differences between treatments. Although the PM treatment increased the intake of ESC and NFC, we could not demonstrate a clear in vivo positive effect on microbial uptake of NH_3-N from the rumen, because we observed no differences in urinary excretion of PD. This could be related to the fact that herbage represented only part of the diet (i.e., ≤43%), and the differences in ESC ingested, although significant, were ≤195 g/d. Also, the estima-

Table 8. Effect of grazing session or tannin supplementation on nitrogen (N) partitioning of dairy cows fed partial TMR (PMR) and herbage

	Treatment[1]				
Item (g/d, unless noted)	AM	PM	TAN	SEM	*P*-value
Intake					
N	463	452	459	20.6	0.21
Digestible N	333[a]	306[b]	319[ab]	17.5	<0.01
Soluble N	179	175	178	7.3	0.16
Potentially degradable N[2]	275	269	272	15.5	0.27
ADIN	9.0	9.0	9.0	1.02	0.84
Plasma urea-N (mg/dL)	13.2	14.0	13.3	1.56	0.47
MUN (mg/dL)	20.9	22.6	18.9	2.25	0.29
Milk N excretion	119	118	116	9.70	0.55
Milk N:N intake	0.26	0.26	0.25	0.018	0.54
Fecal excretion					
DM (kg/d)	5.2	5.6	5.4	0.27	0.07
N	130[b]	146[a]	139[ab]	4.87	0.02
Fecal N:N intake	0.28[b]	0.32[a]	0.30[ab]	0.009	0.01
NDIN	35.6[b]	37.6[b]	46.4[a]	4.06	<0.01
ADIN	6.3[b]	7.3[b]	13.9[a]	1.36	<0.01
Urine excretion					
Volume (L/d)	22.4	20.5	21.6	2.28	0.16
N	176[a]	163[b]	162[b]	13.6	0.03
Urine N:N intake	0.38	0.36	0.35	0.021	0.14
Urea-N	101[a]	87[b]	87[b]	15.4	0.01
Urea-N:urine N	0.57	0.52	0.53	0.058	0.10
Manure N excretion[3]	306	308	301	18.6	0.63
Urine N:manure N	0.58[a]	0.52[b]	0.54[b]	0.019	<0.01
Urine N:creatinine[4]	14.5[a]	13.5[b]	13.1[b]	1.05	0.02
N balance[5]	36.3	25.3	42.1	7.91	0.30

[a,b]Within a row, means with different superscripts are different ($P \leq 0.05$).

[1]AM = morning grazing session and afternoon PMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM.

[2]Potentially degradable N (g/d) = [total N (g/d) – soluble N (g/d)] – ADIN (g/d).

[3]Manure N excretion (g/d) = urinary N excretion (g/d) + fecal N excretion (g/d).

[4]Urine N and creatinine concentration in g/L.

[5]N intake (g/d) – [milk N (g/d) + urine N (g/d) + fecal N (g/d)].

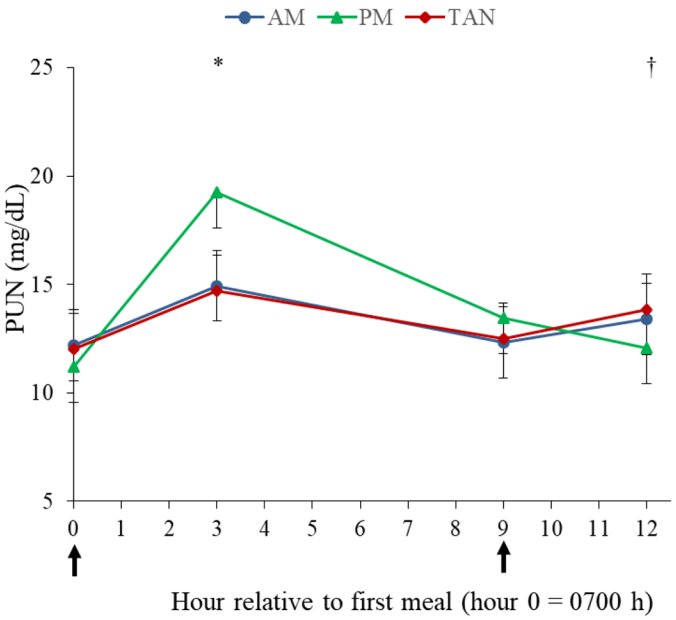

Figure 3. Effect of grazing session or tannin supplementation on the concentration of plasma urea-N (PUN). Symbols at each hour indicate at least one difference ($*P \leq 0.05$) or tendency ($\dagger P \leq 0.10 > 0.05$) among the treatments in the interaction analysis. AM = morning grazing session and afternoon partial (p)TMR meal; PM = morning PMR meal and afternoon grazing session; TAN = morning grazing session and afternoon PMR meal supplemented with 15.0 g of tannins/kg of DM. The arrows indicate the meals. Error bars represent SEM.

tions of urine production and urinary excretion of PD were carried out by indirect methods, which could have prevented us from capturing differences. Additionally, the PM grazing session reduced the excretion of N and urea-N in urine by 7 and 14% and increased the excretion of N in feces by 12%. After recommendations by Lee et al. (2019), we must consider the inaccuracies associated with the use of only 2 time points in the spot sampling as a possible explanation of this result. However, the reduction of N excretion in urine is consistent with a lower digestibility of N compounds observed in PM.

Dietary tannin inclusion is expected to reduce the degradation of protein and the concentration of NH_3-N in the rumen, which should be reflected in reduced excretion of urinary N in dairy cows. The reduction in urinary N and urinary urea-N excretions without a change in fecal N excretion in TAN could be the consequence of a reduced ruminal degradability of N compounds, although neither ruminal NH_3 nor PUN and MUN results can confirm this hypothesis. A reduction in urinary N was previously reported in studies where tannins were included in the diet of nonlactating ruminants (Ávila et al., 2015; Orlandi et al., 2015) or dairy cows (Grainger et al., 2009). In several of those experiments, however, the reductions in urinary N were accompanied by increased excretion of fecal N, which was not observed in the present experiment. Milk N did not increase, suggesting that N was probably redirected to tissue deposits, which we could not confirm by the estimated N balance. We must note that the values of N balance obtained in the present study should be interpreted with caution because of inaccuracies associated with herbage and fecal sampling protocols and marker recovery in feces, according to Hristov et al. (2019). The increased fecal ADIN excretion observed in TAN should have come from tannin-protein complexes formed in the rumen, considering that the intake of ADIN was similar among treatments. These complexes could have both been incompletely dissociated through the gastrointestinal tract or dissociated in the abomasum and complexed again with proteins in the small intestine (Patra and Saxena, 2011), increasing the fecal excretion of ADIN. This finding would be beneficial for pastures and crops in the long term, as fecal N bound to fiber has been reported to degrade slowly in soil (Powell et al., 2009).

Overall, our results showed that PM and TAN reduced, on average, the excretion of N and urea-N in urine by 8 and 14%, respectively. In addition, PM and TAN reduced the urine N:manure N ratio by 10 and 7%, respectively. Even considering the possible inaccuracies related to the techniques used for the measurements, from an environmental point of view, this change in N partitioning from urine to feces is positive, as fecal N is less volatile than urinary N (Powell et al., 2011), thus decreasing N_2O emissions from manure (Dijkstra et al., 2013).

CONCLUSIONS

Changing the grazing session from the morning to the afternoon and including 8.9 g of tannins from *Acacia mearnsii* per kg of DMI in mid-lactation dairy cows fed a PMR combined with ryegrass herbage did not lead to changes in total DMI, milk production and composition, ruminal fermentation, or estimated microbial protein synthesis. However, both treatments were effective in decreasing the excretion of urinary N without affecting the production compared with grazing during the morning without tannin. The afternoon grazing session increased the proportion of herbage intake in the whole diet, probably associated with the higher concentration of DM and soluble carbohydrates. Even considering the small differences observed in the present study, the change in grazing session from the morning to the afternoon is a potential strategy for increasing herbage intake by dairy cows.

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