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Effect of tannins and monensin on feeding behaviour, feed intake, digestive parameters and microbial efficiency of nellore cows

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ABSTRACT

This study aimed to evaluate the associative effect of monensin and tannins on intake, feeding behaviour, digestibility, rumen kinetics, microbial protein synthesis and nitrogen balance. In a 2×4 factorial arrangement, 8 rumen cannulated Nellore cows were distributed in 2 contemporary 4×4 Latin squares and received 8 diets that differed in the level of tannins (0.00, 0.75, 1.50 and 2.25% DM) and presence of monensin. Monensin was daily administered to each cow in one square (about 32 mg/kg DM). No interaction between monensin and tannins was observed ($p > .05$). Tannins linearly reduced feed intake, but linearly increased daily eating time ($p < .05$), although these did not alter the number of meals. Monensin increased CP digestibility by 6% ($p = .0387$) while tannins linearly reduced digestibility of DM, CP, OM and TDN, whereas the reduction was quadratic for ADF and NDF. Tannins linearly reduced the rumen disappearance rate by linearly reducing both passage and digestion rates. Tannins also linearly reduced urinary urea, though neither additive affected microbial protein synthesis. Monensin reduced the proportion of N excreted in faeces, whereas tannins linearly increased faecal N and linearly reduced both urinary and retained N. Monensin and tannins have shown independent effects on feeding behaviour, feed intake, digestive parameters, microbial protein synthesis and N balance, but they did not improve nutrient usage, although monensin alone has shown to have potential to promote N utilisation. Tannins may play an important role in reducing the excretion of N in urine.

HIGHLIGHTS

- Tannins reduce the efficiency of nutrient usage in cattle.
- Tannins change the pathway of the excretion of the feeding nitrogen.
- The emission of N_2O from the urine may be reduced by the use of tannins in cattle feeding.

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Introduction

Dry matter intake (DMI) is fundamentally important in nutrition by establishing the amount of nutrients for health and production. Many factors may affect DMI, either by rumen fill or by metabolic-feedback; therefore, feeds of low digestibility place constraints on DMI because of slow clearance from the rumen (NRC 2016).

Feed additives, such as monensin and tannins may affect feeding behaviour, and consequently alter DMI. Monensin increases the feed energy efficiency by promoting the production of propionate (Duffield et al., 2008a). This may alter the feeding behaviour and reduce DMI by a metabolic-feedback effect. The meta-analysis of Duffield et al. (2012) shows that monensin reduces DMI and improves average daily gain through improvement of feed efficiency. But other studies, such as of Hamilton et al. (2010), Mullins et al. (2012) and Perna Junior et al. (2017) found no effect, suggesting that the effect may vary among studies. Monensin also reduces rumen protein digestion with a consequent reduction of rumen ammonia production

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by inhibiting the rumen deamination reactions (Russel and Strobel 1989; Rangel et al. 2008).

Tannins have the ability to form complexes with proteins and, to a lesser degree, with carbohydrates and minerals (Addisu 2016). High tannin concentrations reduce intake and digestibility of nutrients, while low to moderate concentrations may improve the digestive efficiency of protein (Frutos et al. 2004). Gerlach et al. (2018a) did not observe any effect of tannins on the digestibility of the concentrate OM when the tannin content was 1%, but it reduced drastically when the content was 3% (−21%) and 5% (−28%). The effect of tannins on reducing DMI is generally by rumen physical filling and is attributed to the fact that tannins depress fibre digestion by forming complexes with lignocellulose and hence prevent microbial digestion (Piñeiro-Vázquez et al. 2015), either by direct inhibition of cellulolytic microorganisms or fibrolytic enzymatic activity or both (Patra and Saxena 2011).

The complexes formed with proteins render them inaccessible to rumen degradation and favour post-rumen release, thereby reducing ammonia concentration in the rumen (Nigrant et al. 2017). Using diets of up to 6% of tannins, Ahnert et al. (2015) found a linear reduction in urinary N and a linear increase in faecal N, and, regardless the level of tannins in diets, N retention was higher with tannins than without. The urinary purine derivatives declined linearly when tannins were increased up to 6% in the study of Dickhoefer et al. (2016).

There appear to be no studies which evaluated the possibility of interaction between monensin and tannins in ruminants. Although they have different mechanisms of action, these additives separately reduce rumen protein degradability (Russel and Strobel 1989; Ruiz et al. 2001; Piñeiro-Vázquez et al. 2015; Addisu 2016). Some authors such as Seo et al. (2010) report that the high rate of rumen protein degradation reduces the efficiency of protein utilisation because there is generally no simultaneous availability (synchronisation) of enough energy for microbial protein synthesis.

Given the above, the hypothesis tested in this study was that the combined use of monensin and tannins would improve the synchronisation of nutrient usage and microbial protein synthesis on Nellore cows. Specifically, the aim was to evaluate the interaction effect of monensin and tannins of *A. mearnsii* on feeding behaviour, DMI, digestibility, rumen kinetics, synthesis and efficiency of microbial protein synthesis, and N balance of Nellore cows.

Material and methods

Ethical issue and place of experimentation

The experiment followed the guidelines established in accordance with the ethical principles of animal experimentation of the Commission of Ethics in the Use of Animals of the College of Animal Science and Food Engineering of the University of Sao Paulo (USP) under the protocol number CEUA 3080240518. It was carried out at the Animal Nutrition and Production Department of the College of Veterinary Medicine and Animal Science of USP in Brazil.

Treatments, experimental design and feeding management

Eight Nellore cows, non-pregnant and non-lactating, carrying rumen cannula and having a mean body weigth of 582 kg (± 96), were kept in a roofed shed in individual pen with free access to sand bedding. They were distributed in 2 contemporary 4×4 Latin square design in a 2×4 factorial arrangement where they received experimental diets that differed in the levels of tannin inclusion (0.00, 0.75, 1.50 and 2.25% of DM) and the inclusion or not of monensin (Rumensin® 200, Elanco Animal Health, Brazil), which was administered daily to each cow in one square (300 mg (30 PPM), about 32 mg/kg DM), i.e. monensin was only administered in one square, but not in both. Caulin was added as the tannin level decreased from 2.25 to 0.00%, to equalise the DM in all treatments.

The tannins, from a commercial extract, were obtained from the bark of *A. mearnsii* (Seta Natur® – Seta Acacia Tannin Extract). The concentration of total phenols (84.40%) was determined by the Folin-Ciocalteau method (Makkar 2003b) and total tannins (82.30% tannic acid equivalent) were estimated by the difference in total phenol concentration before and after treatment with insoluble polyvinylpolypyrrolidone (Makkar et al. 1993). The concentration of condensed tannins (32.3% leucocyanidine equivalent) was determined by the HCl-butanol method (Makkar 2003b).

The feed was offered at 8 a.m. and 4 p.m. in the form of total mixed ration with a ratio of 50% of corn silage and 50% of concentrate. The feed consumption was *ad libitum*. The proportions of ingredients and the chemical composition of the diets are shown in Table 1.

Experimental period

The experiment was carried out in 4 periods of 28 days each, but the last two days of each period the cows

Table 1. Proportions of ingredients and estimated chemical composition of experimental diets.

	Tannin level			
Ingredients (% DM)	0.00%	0.75%	1.50%	2.25%
Corn silage	50.00	50.00	50.00	50.00
Dry ground corn grain	32.36	32.36	32.36	32.36
Soybean meal	12.40	12.40	12.40	12.40
White salt	0.50	0.50	0.50	0.50
Mineral mixture[a]	2.00	2.00	2.00	2.00
Tannin extract[b]	–	0.91	1.82	2.74
Caulin	2.74	1.82	0.91	–
Chemical composition of the diet for all tannin levels				
DM[c] (%)	60.35			
CP[c] (%DM)	14.43			
RDP[d] (%CP)	65.30			
RUP[d] (%CP)	34.70			
NDF[c] (%DM)	28.06			
NDFe[d] (%DM)	24.47			
ADF[c] (%DM)	15.41			
NFC[c] (%DM)	47.59			
Starch[d] (%DM)	42.58			
Ash[c] (%DM)	6.73			
Ca[c] (%DM)	0.69			
P[c] (%DM)	0.40			
EE[c] (%DM)	3.19			
TDN[d] (%DM)	74.10			
E_L[d] (Mcal/kg DM)	1.50			

DM: Dry matter; CP: Crude protein; RDP: Ruminally degraded protein; RUP: Ruminally undegraded rotein; NDF: Neutral detergent fibre; NDFe: Effective neutral detergent fibre; ADF: Acid detergent fibre; NFC: Non-fibre carbohydrates; Ca: Calcium; P: Phosphorus; EE: Ether extract; TDN: Total digestible nutrients; E_L: Net energy for lactation.
[a]Mineral mixture, quantity per kg of product: 140 g of calcium, 80 g of phosphorus, 10 g of sulphur, 129 g of sodium, 80 mg of cobalt, 1400 mg of copper, 800 mg of fluorine, 80 mg of iodine, 1 g of manganese, 20 mg of selenium, 3.50 g of zinc.
[b]Extract of *Acacia mearnsii* with 82.30% of total tannins, of which 32.30% condensed tannins.
[c]Determined through chemical analysis.
[d]Estimated by the Spartan Dairy Ration Evaluator/Balancer software, version 3.0.3.

spent in pasture. The first 15 days were for diet adaptation. Thereafter, evaluations were recorded at the following times: the feeding behaviour on day 16; the feed intake, digestibility and rumen degradability between days 17 and 21; the passage rate between days 23 and 25; the urinary parameters on day 24, and finally, the rumen volume and the rumen disappearance rate on days 25 and 26.

Feeding behaviour and feed intake

The feeding behaviour (performed according to Maekawa et al. (2002)) was assessed for 24 hours through observation every 5 minutes. Each parameter observed was considered to be executed during the entire interval period (5 minutes) between observations and was called activity. In this study are presented data concerning the Eating, Ruminating and Masticating parameters, reporting the total number of events (NE) of eating, ruminating or masticating as well as the total time per day the cows spent eating, ruminating or masticating. An event was considered to be two or more consecutive activities interrupted by a different activity than the current one. The data concerning the masticating parameter were considered as the sum of the respective data concerning eating and ruminating parameters.

The cows had a free access to feed 24 hours a day, but the management stradegy was to ensure leftovers of approximately 5%. During the 5 days of evaluation, the leftovers from each cow were collected and weighed for the quantification of intake which was obtained by the difference between the amount of feed supplied and the leftovers. On the same days, samples of silage and concentrate were collected to determine the content of DM, ash, CP, EE, calcium, phosphorus, NDF and ADF. The water intake was quantified with the use of individual automatic drinking fountains with water metres.

Evaluation of total apparent digestibility

The digestibilities of DM, CP, EE, non-fibrous carbohydrates (NFC), NDF, and ADF were determined by using the external marker, titanium dioxide (TiO_2) according to Titgemeyer et al. (2001), whereby TiO_2 was administered (15 g/cow.day) directly into the rumen during 5 days for adaptation and 5 days for faece collection. The apparent digestibility coefficients (ADC) were calculated based on the TiO_2 content of the diet and faeces according to Myers et al. (2004), using the following equations:

$$ADC_{DM} = 100 - \left(100 \times \frac{TiO_2\ (\%)\ \text{in the diet}}{TiO_2\ (\%)\ \text{in the faeces}}\right) \quad (1)$$

$$ADC_N = 100 - 100\left[\left(\frac{\%TiO_2 d}{\%TiO_2 f}\right) \times \left(\frac{\%Nf}{\%Nd}\right)\right] \quad (2)$$

where: ADC_{DM} = DM apparent digestibility coefficient; ADC_N = Nutrient apparent digestibility coefficient; % TiO_2d = Titanium dioxide content in diet; % TiO_2f = Titanium dioxide content in faeces; % Nd = Nutrient content in the diet; % Nf = Nutrient content in faeces.

The DM content of feed and faeces was determined by drying using a forced air oven at 65 °C for 72 hours according to AOAC (1995). All analyzes were corrected for the analytical DM content determined at 105 °C for 4 hours. The ash was obtained by calcination in a muffle furnace at 550 °C for 4 hours. The organic matter (OM) was obtained by the difference between 100 and ash (AOAC 1990). The CP was obtained by the

total N content (N × 6.25) using the micro-Kjeldahl technique (method 920.87; AOAC 1990). The EE was obtained by using the ANKOM XT15 Extractor® equipment (method Am 5-04; AOCS 2005). The NDF and ADF were obtained by the method of Van Soest et al. (1991). The diet NDF was obtained by using thermostable α-amylase. Calcium (Ca) was determined by titration (method 968.08, AOAC 1995) and phosphorus (P) by colorimetry (method 965.17; AOAC 1990). The NFC content was obtained by subtracting the amounts of CP, EE, ash and NDF (expressed in percentage of DM) from 100.

Evaluation of rumen kinetics

Rumen degradability

The determination of rumen degradability was performed according to Ørskov et al. (1980). Silage and concentrate samples were dried at 65 °C for 72 hours and milled with Willye-type mills with 2 mm sieves. Next, both portions were mixed in proportions of 50:50 (DM basis), then 9 g were put in 10 × 20 cm nylon bags of 50 μm porosity. These were incubated in rumen for 0, 3, 9, 24, 48, and 96 hours. After the removal they were washed with fresh water and then dried again and finally weighed. The DM disappearance was obtained by the difference between initial (before incubation) and final (after incubation) weights and obtained the percentage of degraded fraction. The zero-time bags were put in a thermostatic bath at 39 °C for 5 minutes and washed with fresh water. Subsequently, they were submitted to the same procedures adopted for the other time bags. The residues were analysed for CP and NDF in order to determine their rate of degradation.

The potential degradability of DM and CP was calculated according to the model of Ørskov and McDonald (1979) with the aid of SAS NLIN procedure (version 9.3).

$$p = a + b(1-e^{-ct}) \quad (3)$$

where: p = disappearance of nutritive component analysed at time "t"; a = intercept of the degradation curve when $t = 0$, corresponding to the water-soluble and completely degradable fraction of the analysed nutritive component leaving the nylon bag rapidly; b = degradation potential of the water insoluble fraction of the nutritive component analysed; c = rate of degradation per fermentative action of b; t = incubation time.

After the determination of the coefficients a, b and c, these were applied in the equation proposed by Ørskov and McDonald (1979) to calculate the real effective degradability (RED) (Equation 4).

$$\text{RED} = a + [(b \times c)/c] + kp \quad (4)$$

To determine the RED of NDF, *lag* time was introduced into the model according to McDonald (1981). The potential degradability (p) was calculated according to the following models, also with the aid of the SAS NLIN procedure (version 9.3).

$$p = \text{a if } t \leq lag \quad (5)$$

$$p = a + b[1-e^{-c\times(t-lag)}] \text{ if } t > lag \quad (6)$$

where: *lag* is the time at which the equation derived for a data set equals the actual potencially degradable fraction at zero time (Mertens 1993).

The RED of NDF was thereafter calculated using the following equation:

$$\text{RED} = [\text{b} \times \text{c} \times \text{e}^{(-kp \times \text{lag})}]/(\text{c} + \text{kp}) \quad (7)$$

Rumen passage rate

Twenty grams of chromium oxide (as indicator) were infused in rumen. Then, rumen content samples were collected at zero (0), 8, 10, 12, 24, 36 and 48 hours after the infusion. Next, were analysed for DM and chromium oxide content. The passage rate (h^{-1}) was calculated by using the model of Czerkawski (1986).

$$Y = a.e^{-kp\times t} \quad (8)$$

where: Y = indicator concentration in time (t); t = indicator sampling time (h); a = concentration of the indicator at initial time (t_0), assuming instant mixing to the rumen content (ppm); e = base of the neperian logarithm.

Disappearance rate of rumen solid mass

The disappearance rate (*kt*) was determined by rumen emptying. The rumen content was manually removed through the rumen cannula as described by Allen and Linton (2007). On the 25th day, the emptying was performed at 11 a.m., three hours after feed administration. On the 26th day, the emptying was at 8 a.m. prior to diet administration. During the removal, the liquid and solid phases were separated by using a 2 mm mesh sieve, then weighed. Samples of each phase were collected for DM determination. Afterwards, both phases were reconstituted and returned to the rumen. The rumen DM and *kt* were calculated based on the dry weight of each sample. The *kt* was estimated using the following equations:

$$kt(\%/h) = 100 \times [\text{DMI(kg)}/\text{Rumen content DM(kg)}]/24 \quad (9)$$

$$kt(\text{kg}/h) = \text{Rumen content DM(kg)} \times [kt(\%/h)/100] \quad (10)$$

The rumen digestion rate (*kd*) was determined by the difference between *kt* and *kp* as follows:

$$kd = kt - kp \quad (11)$$

Urinary parameters and nitrogen balance

To calculate the production of microbial protein, the urinary volume was determined through urine creatinine, according to Valadares et al. (1999).

On the 24th day, urine was collected every 6 hours, either during spontaneous urination or stimulation by vulva massage. At each collection time, 10 mL of urine were taken and diluted in 40 mL of 0.036 N sulphuric acid as a preservative in order to reduce the pH to below 3 to avoid losses of nitrogen (Vasconcelos et al. 2010) as well as bacterial destruction, conservation of purine derivatives and precipitation of uric acid.

Allantoin was determined according to the colorimetric method described by Chen and Gomes (1992). The uric acid was determined by colorimetric enzymatic reaction with Uricase and Peroxidase, through commercial kit (Bioclin® Ref K139).

The concentrations of urea and creatinine were determined by using commercial kits (Bioclin® Ref K047 and Bioclin® Ref K067, respectively), through the colorimetric enzymatic reaction and reaction with Alkaline Picrate in buffered medium, respectively.

The daily urinary creatinine excretion (CE) was estimated in relation to the animal body weight (BW) using the equation proposed by Chizzotti et al. (2004):

$$\text{CE}(\text{mg/kg BW/d}) = 32.27 - 0.01093 \times \text{BW}(R^2 = 0.70) \quad (12)$$

The daily urinary volume (L/cow) was determined by dividing the daily urinary creatinine excretion by the observed values of urinary creatinine concentration (mg/dL) of the spot samples. This volume was used to calculate the estimated daily excretion of urea, allantoin and uric acid.

The excretion of purine derivatives (PuD) over 24 hours was calculated by multiplying the daily urine volume by the concentration of PuD in the urine sample. The absorbed microbial purines (AP, mmol/day) were calculated from the excretion of PuD (mmol/day) as proposed by Verbic et al. (1990), by means of the following equation:

$$\text{PuD} = 0.85 \times \text{AP} + 0.385 \times \text{BW}^{0.75} \quad (13)$$

where: 0.85 = recovery of purines absorbed as urinary PuD; $0.385 \times \text{BW}^{0.75}$ = excretion of purines of endogenous origin per kg of metabolic weight per day.

The intestinal flow of microbial nitrogen compounds (micN, g N/day) was calculated in relation to AP, according to Chen and Gomes (1992):

$$\text{micN} = (70 \times \text{AP})/(0.83 \times 0.116 \times 1000) \quad (14)$$

where: micN = microbial nitrogen; 70 = N content in the purines (mg N/mmol); 0.83 = digestibility of microbial purines; 0.116 = ratio of purine N and total N of rumen microorganisms.

The efficiency of microbial N synthesis (EMNS) was calculated by the relationship between the production of micN (g) and the amount of OM digested.

The consumption of N was determined by dividing the consumption of CP by 6.25 obtaining the quantity (g) of N consumed. The same calculation was carried out with the CP values of faeces, obtaining the total faecal N. The concentration of urea N in urine was obtained by multiplying the concentration of urea by 0.466, corresponding to the N content in urea. The retained N was obtained by calculating ingested N minus excreted N (faeces + urine). The N balance, in percentage, was obtained by the relation of N contained in faeces, urine or retained by ingested N, according to Silva and Queiroz (2002).

Statistical analysis

The data were analysed by using the Statistical Analysis System (SAS 9.3, Institute Inc. 2013). First, they were evaluated in relation to the presence of discrepant information (outliers) and normality of the residues by the Shapiro-Wilk test. When the normality premises were not met, the data were transformed. The data were then submitted to analysis of variance which separated, as causes of variation, the monensin effect (also considered as the effect of the square), the tannin level effect, the interaction between monensin and tannin level, the period effect, and the animal effect within the square. The tannin level effect was evaluated by the use of orthogonal polynomials then separating the effects into linear, quadratic and quadratic deviations. A significance level of 5% was adopted.

The statistical model used was described according to the equation below:

$$Y_{ijkl} = \mu + L_i + M_j + L_i * M_j + P_k + A_l(S_j) + e_{ijkl}$$

Where: Y_{ijkl} = observed value; μ = overall mean; L_i = Tannin level effect (fixed effect); M_j = Effect of

monensin (fixed effect); $L_i{}^*M_j$ = Interaction between the tannin level (i) and monensin (j) (fixed effect); P_k = Period effect (random effect); $A_l(S_j)$ = Effect of animal within the square (random effect); e_{ijkl} = Random error associated with each observation.

Results

Feeding behaviour, feed intake and total apparent digestibility

There was no interaction between monensin and tannins and no effect of monensin on feeding behaviour ($p > .05$). The tannins linearly increased ($p < .05$) the number of events (NE) of rumination and mastication as well as the total eating time (TET), but no effect was observed on the number of daily meals (Table 2). Tannins linearly increased the time to ruminate 1 kg of NDF, but they had a quadratic increasing effect on the time to eat or masticate the same quantity of NDF.

There was no effect of monensin ($p > .05$) on feed intake, while the tannins linearly reduced DMI and water consumption ($p < .05$) (Table 3).

Monensin increased CP digestibility. Tannins linearly reduced the digestibility of DM, CP, NFC, OM and TDN, but for NDF and ADF the reduction was quadratic (Table 3).

Rumen kinetics

There was neither effect of monensin nor interaction ($p > .05$) on **a**, **b**, **c**, *lag* parameters, or consequently, on the degradability and undigested fractions of DM, NDF or CP (Table 4).

The tannins had no effect on the '*a*' parameter but linearly reduced ($p < .05$) parameters *b*, *c* and *lag*. They also linearly reduced the RED and potential degradability (PD) of DM and NDF. Despite the linear reduction of the PD of CP, the RED was not altered.

Table 2. Feeding behaviour of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p*-Value		
Variables	0	30	0%	0.75%	1.5%	2.25%	SEM	M	TL	M*TL
Ruminating										
NE	13.75	14.06	13.38	13.25	13.88	15.13	0.50	NS	0.0242^{L}	NS
TRT (min)	427.7	391.3	408.8	406.3	406.3	416.3	14.3	NS	NS	NS
NDF (min/kg)	171.2	150.7	153.3	157.6	157.0	175.9	0.04	NS	0.0059^{L}	NS
Eating										
NE (meals)	8.31	9.06	8.50	8.00	8.63	9.63	0.42	NS	NS	NS
TET (min)	182.2	211.3	192.5	178.8	201.9	215.0	8.18	NS	0.0263^{L}	NS
NDF (min/kg)	73.00	82.05	72.49	69.48	78.44	89.70	3.53	NS	0.0386^{Q}	NS
Masticating										
NE	22.06	23.13	21.88	21.25	22.50	24.75	0.66	NS	0.0247^{L}	NS
TMT (min)	610.3	602.5	601.3	585.0	608.1	631.3	16.1	NS	NS	NS
NDF (min/kg)	244.2	232.8	225.8	227.1	235.4	265.6	7.16	NS	0.0384^{Q}	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; Q: Quadratic; NS: Non-significant.NE: Number of events; TRT: Total ruminating time; TET: Total eating time; TMT: Total masticating time; NDF: Neutral detergent fibre.

Table 3. Feed intake and total apparent digestibility of DM and its fractions of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p*-value		
Variables	0	30	0.0%	0.75%	1.5%	2.25%	SEM	M	TL	M*TL
DM intake (kg/d)	9.34	9.49	9.80	9.59	9.56	8.71	0.275	NS	0.0034^{L}	NS
Water intake										
Litres/d	31.67	24.00	29.25	28.58	27.75	25.75	1.596	NS	0.0120^{L}	NS
Litres /kg DM	3.31	2.54	2.99	2.96	2.84	2.93	0.111	0.0533	NS	NS
Digestibility (%)										
DM	68.21	70.96	72.29	70.92	69.02	66.12	0.862	NS	$<.0001^{L}$	NS
CP	65.10	69.07	72.97	69.02	65.41	60.93	1.100	0.0387	0.0010^{L}	NS
NDF	56.24	55.93	61.78	58.78	56.77	47.02	1.655	NS	0.0199^{Q}	NS
ADF	41.12	42.64	54.91	46.84	43.85	21.91	3.032	NS	0.0336^{Q}	NS
EE	73.84	74.00	73.96	73.66	74.96	73.10	1.433	NS	NS	NS
NFC	81.40	85.67	85.96	84.71	81.57	81.88	0.819	0.0534	0.0063^{L}	NS
OM	71.03	73.73	76.24	74.09	71.36	67.82	0.928	NS	$<.0001^{L}$	NS
TDN	69.24	71.76	74.10	72.08	69.59	66.22	0.897	NS	$<.0001^{L}$	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; Q: Quadratic; NS: Non-significant; DM: Dry matter; CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; EE: Etheric extract; NFC: Non-fibrous carbohydrates; OM: Organic matter; TDN: Total digestible nutrients.

Table 4. Degradability of DM, NDF and CP of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p-value*		
Variables	0	30	0.00%	0.75%	1.50%	2.25%	SEM	M	TL	M*TL
DM										
a (%)	32.84	32.53	32.39	32.87	32.41	33.08	0.155	NS	NS	NS
b (%)	53.29	52.90	55.15	52.77	52.79	51.65	0.500	NS	0.0183^{L}	NS
c (h^{-1})	0.043	0.043	0.051	0.046	0.041	0.035	0.002	NS	0.0010^{L}	NS
RED (%)	62.67	61.65	64.00	62.25	61.37	61.03	0.761	NS	0.0816^{L}	NS
PD (%)	86.13	85.58	87.54	85.64	85.52	84.73	0.409	NS	0.0268^{L}	NS
Und (%)	13.87	14.42	12.46	14.36	14.48	15.27	0.409	NS	0.0268^{L}	NS
NDF										
b (%)	65.12	65.05	69.75	66.99	62.93	60.67	1.092	NS	0.0008^{L}	NS
c (h^{-1})	0.030	0.026	0.034	0.025	0.028	0.023	0.002	NS	0.0399^{L}	NS
lag (h)	2.64	3.63	2.59	2.95	2.96	4.04	0.288	NS	0.0301^{L}	NS
RED (%)	27.14	25.17	31.64	24.98	24.88	23.12	1.411	NS	0.0087^{L}	NS
PD (%)	65.12	65.05	69.95	66.99	62.93	60.67	1.092	NS	0.0008^{L}	NS
Und (%)	34.88	34.95	30.25	33.02	37.07	39.33	1.092	NS	0.0008^{L}	NS
CP										
a (%)	33.83	34.52	32.83	34.94	34.05	34.87	0.498	NS	NS	NS
b (%)	60.29	60.54	63.90	59.95	58.74	59.07	0.741	NS	0.0208^{L}	NS
c (h^{-1})	0.042	0.039	0.046	0.045	0.041	0.032	0.002	NS	0.0114^{L}	NS
RED (%)	66.89	65.97	67.93	67.57	66.07	64.16	0.913	NS	NS	NS
PD (%)	94.12	94.99	96.74	94.89	92.80	93.79	0.517	NS	0.0095^{L}	NS
Und (%)	5.88	5.007	3.26	5.11	7.20	6.21	0.517	NS	0.0095^{L}	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; NS: Non-significant; *a*: Water-soluble and completely degradable fraction of the analysed nutritive component leaving the nylon bag rapidly; *b*: Potentially degradable fraction; *c*: Rate of degradation of *b*; *lag*: time at which the equation derived for a data set equals the actual potentially degradable fraction at zero time; RED: Real effective degradability; PD: Potential degradability ($a + b$); Und: Undigested fraction (100-PD).

Table 5. Rumen kinetic of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p-value*		
Variables	0	30	0%	0.75%	1.5%	2.25%	SEM	M	TL	M*TL
Rumen DM (%)	12.92	13.49	12.92	13.04	13.46	13.40	0.211	NS	NS	NS
Rumen liquid mass (kg)	35.69	34.94	33.34	34.77	36.89	36.26	0.979	NS	0.0085^{L}	NS
Rumen solid mass (kg)	5.36	5.45	5.01	5.23	5.72	5.67	0.203	NS	0.0117^{L}	NS
Total rumen mass (kg)	41.05	40.39	38.34	40.00	42.61	41.93	1.164	NS	0.0072^{L}	NS
kt (%/h)	7.69	7.45	8.65	7.84	7.10	6.684	0.235	NS	0.0001^{L}	NS
kp (%/h)	3.45	3.50	3.82	3.73	3.38	2.99	0.153	NS	0.0122^{L}	NS
kd (%/h)	4.24	3.94	4.84	4.11	3.72	3.70	0.226	NS	0.0316^{L}	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; NS: Non-significant; *kt*: Disappearance rate of rumen solid mass ($kp + kd$); *kd*: Rate of digestion in the rumen ($kt - kp$); *kp*: Passage rate of undigested residues ($kt - kd$).

The tannins linearly increased the rumen undigested fractions of DM, NDF or CP.

There was neither interaction between monensin and tannins nor effect of monensin ($p > .05$) on the solid, liquid, total rumen mass, kt, kp or kd as well as on the DM content of the total rumen mass (Table 5). The tannins also did not affect the DM content of the rumen mass, but linearly increased ($p < .05$) the liquid, solid and total mass. Tannins linearly reduced kt by linearly reduce kp and kd.

Microbial protein, efficiency of microbial nitrogen synthesis and nitrogen balance

No interaction was observed between monensin and tannins nor with monensin alone ($p > .05$) on the urinary volume (L/day), urinary compounds (mmol/day), synthesis or efficiency of microbial nitrogen synthesis (EMNS). Further, no effect of tannins was observed on urinary volume, microbial protein synthesis or EMNS, but a linear decreasing effect was observed on urinary urea (g/day) ($p < 05$) and uric acid (mmol/day) (Table 6).

Monensin reduced the proportion of N excreted in faeces ($p < 05$) (Table 7). Tannins linearly reduced N ingestion. Regarding the ingested N, tannins linearly reduced the amount and the proportion of N excreted in urine, but linearly increased the amount and the proportion of N excreted in faeces and also linearly reduced the amount and the proportion of N retained.

Discussion

Feed intake and feeding behaviour

The lack of interaction between monensin and tannins on feed intake may indicate the independence of these additives on these parameters. The evaluation of

Table 6. Urinary parameters, synthesis and efficiency of microbial protein synthesis of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p-value*		
Variables	0	30	0.0%	0.75%	1.50%	2.25%	SEM	M	TL	M*TL
Urinary volume										
Litres/d	10.7	7.54	9.38	9.50	9.19	8.32	0.578	NS	NS	NS
Urinary compounds										
Urea (g/d)	137.2	138.3	154.7	143.7	132.9	119.6	9.258	NS	0.0051^{L}	NS
Al (mmol/d)	127.3	129.0	130.7	130.1	126.7	125.1	4.149	NS	NS	NS
UA (mmol/d)	19.38	20.45	22.00	19.59	20.15	17.92	1.200	NS	0.0092^{L}	NS
PuD (mmol/d)	146.6	149.6	152.7	149.9	146.7	143.0	4.608	NS	NS	NS
Synthesis of micN										
g/d	83.91	86.81	89.08	86.92	84.00	81.44	3.878	NS	NS	NS
EMNS (g/kg DOM)	14.86	14.10	13.94	13.71	14.02	16.25	0.687	NS	NS	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; NS: Non-significant; Al: Allantoin; UA: Uric acid; PuD: Purine derivatives; micN: Microbial nitrogen; EMNS: Efficiency of microbial nitrogen synthesis; DOM: Digested organic matter.

Table 7. Nitrogen balance of Nellore cows fed monensin (ppm) and tannins of *A. mearnsii*.

	Monensin (M)		Tannin Level (TL)					*p-value*		
Variables	0	30	0.0%	0.75%	1.5%	2.25%	SEM	M	TL	M*TL
N ingested (g/d)	205.7	209.9	217.1	211.7	211.5	190.8	6.260	NS	0.0043^{L}	NS
N excreted (g/d)										
Faeces	68.60	62.36	55.99	62.85	70.85	72.23	2.328	NS	<.0001^{L}	NS
Urine	63.91	64.45	72.10	66.96	61.94	55.74	4.314	NS	0.0051^{L}	NS
N retained (g/d)	73.17	83.09	89.00	81.88	78.69	62.95	5.683	NS	0.0027^{L}	NS
N balance (% of N ingested)										
Faeces	33.86	30.03	26.26	30.08	33.54	37.90	1.065	0.0390	<.0001^{L}	0.0828
Urine	31.58	30.43	34.58	31.26	29.04	29.12	1.960	NS	0.0276^{L}	NS
Retained	34.57	39.54	39.15	38.66	37.42	32.98	2.201	NS	0.0228^{L}	NS

SEM: Standard error of mean; M: Monensin; TL: Tannin level; M*TL: Interaction between monensin and tannins; L: Linear; NS: Non-significant; N: Nitrogen; N retained: N ingested − N excreted (faeces + urine).

this study found no evidence of possible interaction between monensin and tannins in ruminat feeding.

The meta-analyzes of Duffield et al. (2008b) and Duffield et al. (2012), which evaluated the effects of monensin, have shown a reduction of DMI in dairy and beef cattle, but the studies of Hamilton et al. (2010), Mullins et al. (2012), Perna Junior et al. (2017) as well as the present study, found no effect of monensin on DMI (Table 3). These differences may suggest that the effect might depend on the study. The lack of effect of monensin on water consumption was also observed in pre- and postpartum periods of dairy cows in the study of Mullins et al. (2012).

The linear reduction of DMI caused by tannins corroborates the meta-analysis of Jayanegara and Palupi (2010). The reduction was also observed by Aguerre et al. (2016), Grainger et al. (2009) and Dschaak et al. (2011). Patra and Saxena (2011) stated that concentrations of tannins above 50 g/kg DM may negatively affect DMI while low concentrations usually have no effect. However, taking into account the highest level of tannins in the present study (22.5 g/kg DM), it is lower than the level mentioned by these authors, although the DMI was reduced. The effect of tannins may depend on the type of tannins consumed or the chemical structure and molecular weight and not only on the amount ingested (Makkar 2003; Frutos et al. 2004). The linear reduction of water consumption by tannins was somewhat surprising, since it was thought that the tannin astringent effect would cause more water intake. Perna Junior (2018) found no effect on water consumption when included tannins of *A. mearnsii* in Holstein and Nellore cows' diet, but it may have been due to the highest level of tannins, which was 1.50%.

Mullins et al. (2012), who reported on feeding behaviour of transition dairy cows fed monensin, only observed a reduction of the inter-meal interval in prepartum period, but in the present study, no effect was observed (Table 2), and this may be the reason why monensin did not alter DMI.

The linear increase in the number of ruminating and masticating events caused by tannins (Table 2) is considered positive because mastication or rumination elicits saliva production for rumen pH. The linear increase of total eating time (TET) associated with the reduction of DMI may be due to the astringent effect of tannins, which caused a reduced consumption rate whenever the cows engaged in consumption activity. This consequently reduced the amount of DM

consumed per meal and increased the time to eat or ruminate the same amount of NDF. Therefore, these effects corroborate with Lamy et al. (2011), Patra and Saxena (2011), and Addisu (2016) in asserting that tannins decrease feed palatability and consequently reduce the amount of feed ingested.

Digestibility of nutrients

Oliveira et al. (2007) observed an increased protein digestibility and a reduction of faecal N in animals receiving monensin. This study corroborates with these authors, because monensin increased CP digestibility (by 6%) and reduced the proportion of faecal N (% of N ingested) (Table 7), which indicated improvement of N utilisation efficiency. This effect was not observed with tannins, where the CP digestibility linearly decreased, and as a consequence, the faecal N linearly increased. An increase in faecal N was also observed by Gerlach et al. (2018b), who included tannins in Holstein diets. Ahnert et al. (2015) also observed a linear increase of faecal N in diets with up to 6% of tannins. The reduction of CP digestibility may have been due to formation of protein-tannin complexes, which may have caused reduction of protein solubility and hence increased faecal N. Tannins may depress the fibre digestion either by direct inhibition of cellulolytic microorganisms or by inhibition of fibrolytic enzymatic activity or both (Patra and Saxena 2011). This may be the cause of digestibility reduction in the present study (Table 3).

Rumen degradability and rumen kinetics

The lack of interaction between monensin and tannins on rumen kinetics may indicate independence of these additives. The lack of effect of monensin on rumen kinetics (Tables 4 and 5) was also observed by Perna Junior et al. (2017) in Holstein cows.

The linear reduction of the RED of DM and NDF (Table 4) was also observed by Perna Junior (2018), although only on the effective degradability of NDF and CP, but not on DM. Despite the linear reduction of CP digestibility and linear increase in faecal excretion of CP (Tables 3 and 7), tannins were not able to alter the RED of CP. The linear reduction of RED and PD and the linear increase of undigested rumen fractions of DM caused by tannins were not observed by Perna Junior (2018). The linear reduction of the disappearance rate was due to the linear reduction of kp and kd (Table 5). This may have influenced the reduction of DM intake (discussed above) by increasing the rumen retention time. Perna Junior (2018) observed no effect on the kp, although he did observe a linear reduction of *kt* and *kd*. These differences may be due to the tannin content used by this author, which was probably not enough to alter these parameters. Feeding up to 4% of quebracho extract for goats, Al-Kindi et al. (2017) also did not observe changes in the kp, but that difference may be due to the different type of tannins used.

Although the tannins have been shown to reduce DM intake, their effect on the reduction of *kp* could be seen as beneficial since the long duration of feed particles in the rumen would minimise the effect of low *kd* and improve nutrient digestibility.

Urinary parameters, microbial protein and nitrogen balance

Unlike monensin, tannins had a substantial effect on inhibiting rumen protein degradation and reduced the rate of urea production. This effect was observed by the linear reduction of urinary urea in addition to the linear reduction on uric acid. This shows the potential of tannins to reduce rumen microbial activity. Dickhoefer et al. (2016) found a linear decline on urinary excretion, not only of uric acid but of allantoin as well. The difference may be due to the level of tannins, since these authors included tannins up to 6%. The lack of effect of tannins on micN or EMNS was also observed by Aguerre et al. (2016) and Mokhtarpour et al. (2017), although they found a reduction on the concentration of rumen ammonia, but Gerlach et al. (2018b) found reduction of EMNS.

Oliveira et al. (2007), using monensin, observed reduction of faecal N. This effect was also observed in the present study, and along with the increased protein digestibility, it may be thought that monensin was important on feed N utilisation.

In the studies of Mokhtarpour et al. (2017), Aguerre et al. (2016), Ahnert et al. (2015), Gerlach et al. (2018b) and Perna Junior (2018) the different levels of tannins linearly reduced the urinary N and linearly increased the faecal N. This effect was also observed in this study (Table 7), but there was a reduction of N retention. The change in N excretion from urine to faeces is a widely known tannin effect (Theodoridou et al. 2010). There was no effect on the RED of CP, but it is also widely known that tannin-protein complexes increase the supply of rumen undegradable protein (RUP) in the duodenum that leads to reduction of N losses through urine. Perna Junior (2018) and Jayanegara and Palupi (2010) observed no effect on N

retention, but in the study of Ahnert et al. (2015) the N retention increased. These results show the effect of tannins on the inhibition of protein degradation due to the formation of complexes which, besides hindering protein degradation, may hinder post-rumen enzymatic action culminating in increased faecal N.

The pathway change of N excretion caused by tannins (from urine to faeces) might have an environmental benefit, since the faecal N has a lower N_2O emission factor (0.15%) when compared to the urinary N (0.26%) (Sordi et al. 2014).

Conclusions

Monensin and tannins have shown independent effects on feeding behaviour, feed intake, nutrient digestibility, rumen kinetics, synthesis and efficiency of microbial protein synthesis, but they did not improve nutrient usage or microbial protein synthesis. Monensin has shown to have potential to promote N usage by increasing digestibility of CP and reduce faecal N, although not affecting urinary N. Tannins may play an important role in reducing the excretion of N in urine.

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Ethical Approval

The protocol used in this experiment was approved by the Committee of Ethics in the Use of Animals of the College of Animal Science and Food Engineering of the University of Sao Paulo.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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