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Effects of *Acacia mearnsii* De Wild. extract and monensin on intake, digestibility, and ruminal variables of lambs

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ABSTRACT - This study evaluated the effects of tannin extract of *Acacia mearnsii* De Wild. or monensin on intake, digestibility, nitrogen balance, and ruminal variables of lambs. Five rumen cannulated lambs (40.3 ± 2.8 kg) were used in a 5 × 5 Latin square design, with periods of 21 days each. The treatments were: control, without additive; Tan-0.60, Tan-1.20, and Tan-1.80 for the doses of 0.60, 1.20, and 1.80 g kg^{-1} body weight (BW) of tannin extract, respectively; and ionophore (monensin) at 0.75 mg kg^{-1} of BW. Tannins reduced the digestibility of dry matter (DM) and the greatest effects were observed for Tan-1.80. Tannins also increased or tended to increase the fecal excretion of DM, and the greatest effects were observed for Tan-1.20 and Tan-1.80. Tannins increased the fecal excretion of N, decreased the amount of N in urine, but did not affect N retained. Furthermore, tannins reduced the concentration of valerate and the acetate:propionate ratio and increased propionate without affecting the amount of total volatile fatty acids, and the greatest effects were observed for Tan-1.80. The use of ionophore only increased the elimination of N in the urine. Thus, monensin does not affect nitrogen retention, and tannin impairs digestibility, but increases propionate production.

Keywords: antimicrobials, polyphenols, rumen fermentation, ruminants, tannins

1. Introduction

Ionophores like monensin have been widely used as growth promoters in livestock production because they inhibit gram-positive bacteria that produce acetate, thereby increasing energy efficiency while gram-negative strains, many of which produce succinate, are not as sensitive (Bergen and Bates, 1984). However, gram-positive bacteria may develop resistance to ionophores, reducing efficiency, and causing resistance to antibiotics used in humans (Robinson et al., 2017). Thus, livestock nutritionists are often looking for natural compounds, such as tannins. Previous work investigating the use of tannins in livestock production has been promising (Vasta et al., 2019).

Tannins are secondary plant metabolites that act as a defense mechanism (Beart et al., 1985). The antimicrobial effect of tannins is mainly due to polyphenolic compounds that react and bind to the bacterial cell wall and extracellular enzymes, inhibiting nutrient transport through the cell wall retarding microbial growth (McSweeney et al., 2001).

Among the tannin producing plants, *Acacia mearnsii* De Wild. is a multi-purpose Australian shrub legume species with one of the highest tannin concentrations (Grigoletti Júnior et al., 2003). The tannin extract of *Acacia mearnsii* De Wild. is a brown powder, with high water solubility, astringent flavor, and reported to contain 694 g kg^{-1} dry matter (DM) of total tannins in commercial product (Orlandi et al., 2015).

Further, supplying *Acacia mearnsii* De Wild. extract to steers reduces the digestibility of the diet and ruminal ammonia nitrogen ($N\text{-}NH_3$) concentration and increases N retention and aminoacid flow to the duodenum (Orlandi et al., 2015).

Our hypothesis was that the tannin extract of *Acacia mearnsii* De Wild. alters rumen fermentation variables and may be an alternative to replacing monensin as a feed additive. Thus, the objective of this study was to evaluate the effects of tannin extract of *Acacia mearnsii* De Wild. and monensin on intake, digestibility, nitrogen balance, and ruminal variables of lambs.

2. Material and Methods

The study was carried out in Campo Grande, MS, Brazil (20°30'20" S, 54°37'06" W), according to the institutional committee on animal use (case number 639/2014).

This study used five crossbred castrated lambs (½ Santa Inês + ½ Suffolk; body weight [BW] = 40.3 ± 2.8 kg), with permanent rumen cannula. Lambs were housed in individual metal cages, suitable for *in vivo* digestibility studies, which were equipped with feeder, water drinker, and urine collectors.

The experimental design was a 5 × 5 Latin square, with five treatments and five periods of 21 days each. The animals received chopped alfalfa hay (*Medicago sativa*) restricted to 30 g kg^{-1} of body weight [BW] (3% of BW) per day (DM basis), fed twice a day at 07:00 and 17:00 h, and had free access to water and mineral supplement (Table 1). The amount of hay offered was intended to meet the requirement for growth (140 g day^{-1}) of eight-month-old lambs (40 kg) with late maturity (NRC, 2007). At the beginning of each period, animals were weighed after a 16-h fasting from feed to adjust diet intake. The guarantee levels of the mineral supplement were: 150 g kg^{-1} of calcium, 90 g kg^{-1} of phosphorus, 72 g kg^{-1} of sodium, 50 g kg^{-1} of sulfur, 900 mg kg^{-1} of fluorine, 20 mg kg^{-1} of cobalt, 250 mg kg^{-1} of copper, 28 mg kg^{-1} of iodine, 600 mg kg^{-1} of manganese, 9 mg kg^{-1} of selenium, and 1,800 mg kg^{-1} of zinc.

Treatments were supplied daily during the morning feeding and were diluted in 200 mL of distilled water, which was infused through the rumen cannula. Treatments were designed as follows: control, distilled water only; Tan-0.60, Tan-1.20, and Tan-1.80 for the doses of 0.60, 1.20, and 1.80 g kg^{-1} of BW of tannin extract, respectively; and ionophore, monensin at a dose of 0.75 mg kg^{-1} of BW (positive control). The source of condensed tannins (CT) was the commercial soluble extract obtained from the bark of *Acacia mearnsii* De Wild. (black wattle), reported by manufacturer to contain 72.5% CT (725 g kg^{-1} DM of tannin extract; Tanfood, Tanac S.A., Montenegro, RS, Brazil). The maximum tolerable CT amount in the diet was reported to be 55 g kg^{-1} diet DM (Min et al., 2003; Vitti et al., 2005), which

Table 1 - Chemical composition of alfalfa hay (*Medicago sativa*) and tannin extract (*Acacia mearnsii* De Wild.)

Ingredient	DM (g kg^{-1})	Chemical composition (g kg^{-1} of DM)									
		OM	Ash	CP	EE	NDFap	NFC	TDN	LIG	Total phenols	CT
Alfalfa hay	859.7	899.9	100.1	189.5	18.4	457.8	234.2	576.8	97.4	12.5[1]	0.3[1]
Tannin extract	930.0	973.7	26.3	22.0	1.3	-	-	-	-	750.0[2]	725.0[2]

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFap - neutral detergent fiber corrected for ash and protein; NFC - non-fibrous carbohydrates ((100 – (Ash + CP + NDFap + EE)) (Sniffen et al., 1992); TDN - total digestible nutrients; LIG - lignin; CT - condensed tannins.
[1] Approximate values described by Nozella (2001).
[2] Commercial product (Tanfood, Tanac S.A., Montenegro, RS, Brazil).

would correspond to 2.30 g kg^{-1} BW of tannin extract. In the current study, assuming the CT reported by manufacturer, the maximum intake was 1.80 g kg^{-1} BW, which does not surpass the limit suggested. Based on the estimated hay intake of 1.2 kg DM per day for lambs weighing 40.3 kg, the tannin extract infusion was equivalent to approximately 14.6 (Tan-0.60), 29.2 (Tan-1.20), and 43.8 (Tan-1.80) g CT kg^{-1} diet DM. The ionophore dose was established according to values recommended by Bretschneider et al. (2008).

Each experimental period had three phases. The first phase was the adaptation of the animals to diets, lasting ten days (d 1 - d 10); the second was a six-day data collection (d 11 - d 16), aimed at collecting samples for evaluation of intake, digestibility, and nitrogen balance (offered hay, orts, feces, and urine); and the third, from day 20 to 21, ruminal fluid was collected for measurement of pH and concentration of ammonia nitrogen ($N\text{-}NH_3$) and volatile fatty acids (VFA).

At the beginning of each experimental period, a parasitological examination of fecal egg counts per gram (EPG) was performed, and it was not necessary to treat the animals with anthelmintic during the study.

Although alfalfa hay intake was restricted to 3.0% of BW, when there were refusals, they were collected before the morning treatment, weighed, and stored to later calculate feed intake. Total fecal and urine collections were performed. The fecal collection was done using individual collection bags, that were fitted to the animal. Feces were collected, weighed, and sampled (10% of the total excreted after homogenization) twice a day after treatments were provided. After sampling, feces were stored in a freezer (−20 °C) for posterior analysis.

Urine was collected in buckets containing 100 mL of 5% sulfuric acid after being filtered through nylon mesh. Urine volume was measured, and 10% of the total volume was sampled. All samples were properly identified and frozen (−20 °C), obtaining one composite sample per animal per period.

At the end of the experiment, hay, tannin extract, refusals, and fecal samples (after defrosting at room temperature) were dried in a forced-ventilation oven at 55 °C for 72 h and ground to 1.0 mm. Samples were later used for chemical analysis.

Dietary nutrient intake was calculated by the difference between the amount offered and refusals. Apparent digestibility coefficients were obtained through the collection of feces, and feed intake and excretion [(ingested nutrient − excreted nutrient) / ingested nutrient]. Nitrogen retained was obtained by the difference between N ingested and excreted in feces and urine.

Approximately 200 mL of rumen fluid was taken at 07:00 h (time 0; before treatments were offered), then at 2, 4, 6, 8, 10, and 12 h after the provision of the morning diet and infusion of treatments. Immediately after each collection, the ruminal pH of the samples was measured using a digital pH meter (FE20 FiveEasy®, Mettler Toledo, Brazil). Subsequently, the samples were filtered through double-layered gauze, and about 50 mL of it was acidified with 1 mL of sulfuric acid (pre-diluted at 50:50 ratios, for sulfuric acid and distilled water, respectively), and then frozen (−20 °C) for further $N\text{-}NH_3$. For analysis of VFA concentration, 4 mL of ruminal fluid were acidified with 1 mL of metaphosphoric acid (pre-diluted at 25:75 ratios, for metaphosphoric acid and distilled water, respectively) and stored at −20°C for further analysis.

Samples of feces, feed, and feed refusals were analyzed according to AOAC (1990) as follows: DM, method 967.03; ash, method 942.05; crude protein (CP), method 981.10; and ether extract (EE), method 920.29. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using a detergent solution (Van Soest et al., 1991; without the addition of sodium sulfite and alpha-amylase) in a fiber extractor (Tecnal, TE-149; Tecnal, Piracicaba, SP, Brazil).

The NDF from hay, feces, and feed refusals was corrected for ash and protein to obtain the NDFap. Non-fibrous carbohydrates were calculated according to Sniffen et al. (1992) using NFC = 100 − (CP + Ash + NDFap + EE). Total digestible nutrients (TDN) were estimated based on the digestibility test performed, wherein TDN = digestible CP + digestible NDFap + 2.25 * digestible EE + digestible NFC (Sniffen et al., 1992).

The N-NH_3 analysis used the supernatant of ruminal fluid samples thawed at 4 °C and distillation with 2N KOH according to Ribeiro et al. (2011). The concentration of VFA was determined by gas chromatography (Shimadzu GC-2010, Kyoto, Japan) according to the methodology described by Erwin et al. (1961).

All data were analyzed using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4) with the Kenward-Roger approximation to determine the denominator degrees of freedom for the fixed effects test. For the analysis of intake, digestibility, and nitrogen balance, the statistical models contained treatment as a fixed effect and animal and period as random effects. The statistical model used was:

$$Yijk = \mu + Ti + Pj + Ak + eijk,$$

in which *Yijk* = observation of the effect of treatment *i* in period *j*, of animal *k*; μ is the overall mean; *Ti* = effect of treatment *i*, wherein *i* = 1 (control), 2 (Tan-0.60), 3 (Tan-1.20), 4 (Tan-1.80), and 5 (ionophore); *Pj* = effect of period *j* (*j* = five periods); *Ak* = effect of animal *k* (*k* = five animals); and *eijk* = random error associated with each observation.

However, pH, N-NH_3, and VFA data were analyzed as repeated measures, and the models contained treatment, time, and interaction as fixed effects and animal and period as random effects. The statistical model used was:

$$Yijk = \mu + Ti + Hj + Ak + Pj + (TH)ij + eijk,$$

in which *Yijk* = observation of the effect of treatment *i* per collection time (for pH, N-NH_3, and VFA) *j* in animal *k*; μ = overall mean; *Ti* = effect of treatment (*i* = 1 (control), 2 (Tan-0.60), 3 (Tan-1.20), 4 (Tan-1.80), and 5 (ionophore)); *Hj* = effect of collection times for ruminal parameters (*j* = 1, ..., 13); *Ak* = animal effect (*k* = 1, ..., 5); *Pj* = the period effect (*j* = 1, ..., 5); (*TH*)*ij* = interaction between treatment *i* and time *j*; and *eijk* = random error associated with each observation.

The term used for repeated measurement was time and the subject was animal × period. The Toeplitz covariance structure was selected for the analysis of the ruminal fluid acetate:propionate ratio and the first-order autoregressive covariance structure was selected for all other analyzes. The covariance structures were selected according to the lower value in the table of Akaike information. Means were separated using the PDIFF function, and all results were reported as LSMEANS followed by standard error of the mean (SEM). Significance was defined as $P<0.05$ and tendency when $P>0.05$ and <0.10.

3. Results

There was no significant difference ($P>0.10$) between treatments for DM and organic matter (OM) intake offered to animals using tannin or ionophore compared to control animals (Table 2).

Tannin infusion reduced ($P<0.01$) the digestibility (except for EE) of DM and the other chemical constituents. The greatest effects were observed for Tan-1.80-treated animals, compared with control and ionophore-treated animals. In contrast, Tan-1.80-treated animals had greater ($P<0.05$) digestibility of EE compared with control and ionophore-treated animals (Table 2).

Regarding the amount of fecal nitrogen per day, the animals that received tannin had greater ($P<0.01$) excretion compared with control and ionophore-treated animals. In contrast, the ionophore-treated animals had greater ($P<0.01$) urinary N losses than the others. As a result, tannin did not affect ($P>0.10$) the amount of N retained compared with control and ionophore (Table 3).

No effects of treatment × time or treatment were detected ($P>0.10$) for ruminal fluid pH (Figure 1). Effects of treatment × time were detected ($P<0.01$) and effects of treatment tended to be detected ($P<0.10$) for the concentration of N-NH_3 in the ruminal fluid. The tannin-treated animals had a greater

concentration of N-NH_3 up to 8 h after infusion; however, it reduced at 10 h, and did not differ at 12 h after infusion, compared with control and ionophore-treated animals (Figure 1).

When VFA production was analyzed in mmol L^{-1}, no effects of treatment × time and treatment were detected (P>0.10) for acetate and total VFA (Figure 2). Effects of treatment (P<0.01), but not treatment × time (P>0.10), were detected for propionate concentration, which was greater for Tan-1.20 and Tan-1.80-treated animals than for the others (Figure 2). Effects of treatment × time were detected (P<0.01) and effects of treatment tended to be detected (P<0.10) for the concentration of butyrate, and Tan-1.20-treated animals had a greater concentration of this, only at 4, 6, and 8 h than the others (Figure 2). Effects of treatment (P<0.01), but not treatment × time (P>0.10), were detected for the concentration of valerate, and Tan-1.20-treated animals had the highest and Tan-1.80-treated animals had the lower concentration than the control-treated animals (Figure 2). Effects of treatment (P<0.01), but not treatment × time (P>0.10), were detected for the acetate:propionate ratio, and the Tan-1.80-treated animals had a lower value than the control-treated animals (Figure 2).

Table 2 - Dry matter (DM) and organic matter (OM) intake of hay, apparent digestibility coefficient of DM, and digestible nutrient content of the diet offered to lambs

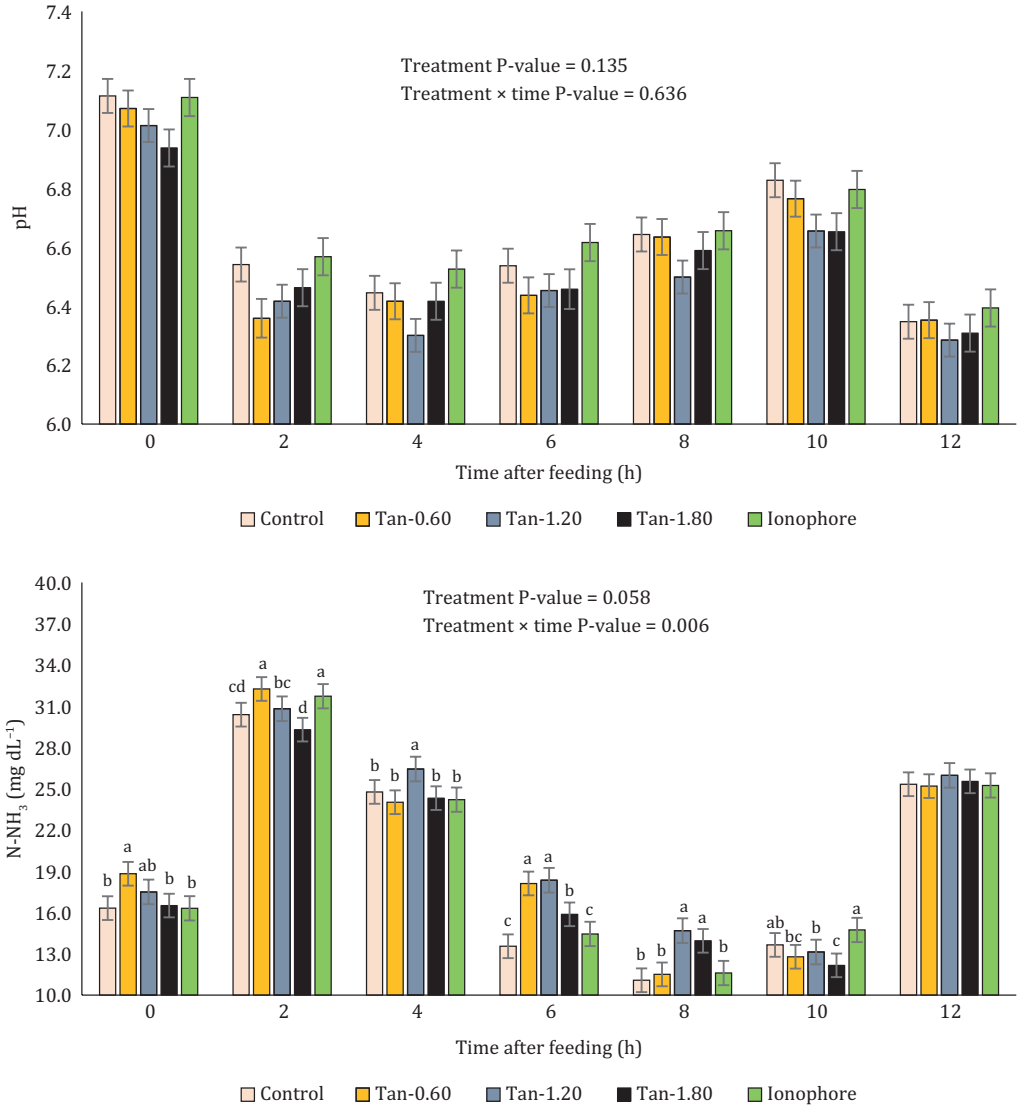
	Treatment[1]					SEM	P-value
	Control	Tan-0.60	Tan-1.20	Tan-1.80	Ionophore		
Intake (g day^{-1})							
DM	1205	1189	1213	1256	1169	37.3	0.174
OM	1084	1070	1092	1130	1051	32.9	0.142
Intake (g kg^{-1} of BW)							
DM	28	28	28	27	28	0.38	0.636
OM	25	25	25	25	25	0.35	0.644
Digestibility							
DM (fraction 0-1)	0.608a	0.588ab	0.565b	0.531c	0.600a	0.12	<0.001
Digestible amount (g kg^{-1} of DM)							
OM	637a	615a	576b	560b	632a	11.7	<0.001
CP	747a	713b	672c	625d	752a	14.3	<0.001
NDFap	523a	490bc	463dc	434d	501ab	17.1	<0.001
ADF	516a	456b	382c	325d	507ab	26.4	<0.001
EE	153c	185bc	224ab	255a	171bc	27.2	0.042
NFC	786a	789a	753b	740b	799a	13.8	<0.001
TDN	576a	557a	523b	508b	571a	10.9	<0.001

DM - dry matter; OM - organic matter; CP - crude protein; NDFap - ash- and crude protein-free neutral detergent fiber; ADF - acid detergent fiber; EE - ether extract; NFC - non-fibrous carbohydrates; TDN - total digestible nutrient; BW - body weight; SEM - standard error of the mean.
[1] Control, no additives; Tan-0.60, Tan-1.20, and Tan-1.80 represent 0.60, 1.20, and 1.80 g kg^{-1} of BW of tannin extract, respectively; and ionophore, 0.75 mg of monensin kg^{-1} of BW.
a-c - Differ from each other (P≤0.05) or tend to differ (P≤0.10).

Table 3 - Nitrogen balance of lambs fed diets with varying levels of tannin extract from *Acacia mearnsii* De Wild. and monensin

Variable	Tratment[1]					SEM	P-value
	Control	Tan-0.60	Tan-1.20	Tan-1.80	Ionophore		
N balance (g day^{-1})							
N intake	35	36	37	37	36	1.86	0.502
Fecal N	9d	10c	12b	13a	8d	0.61	<0.001
Urinary N	15bc	16ab	14c	15bc	18a	0.96	0.005
Retained N	11	9	10	8	8	0.96	0.140

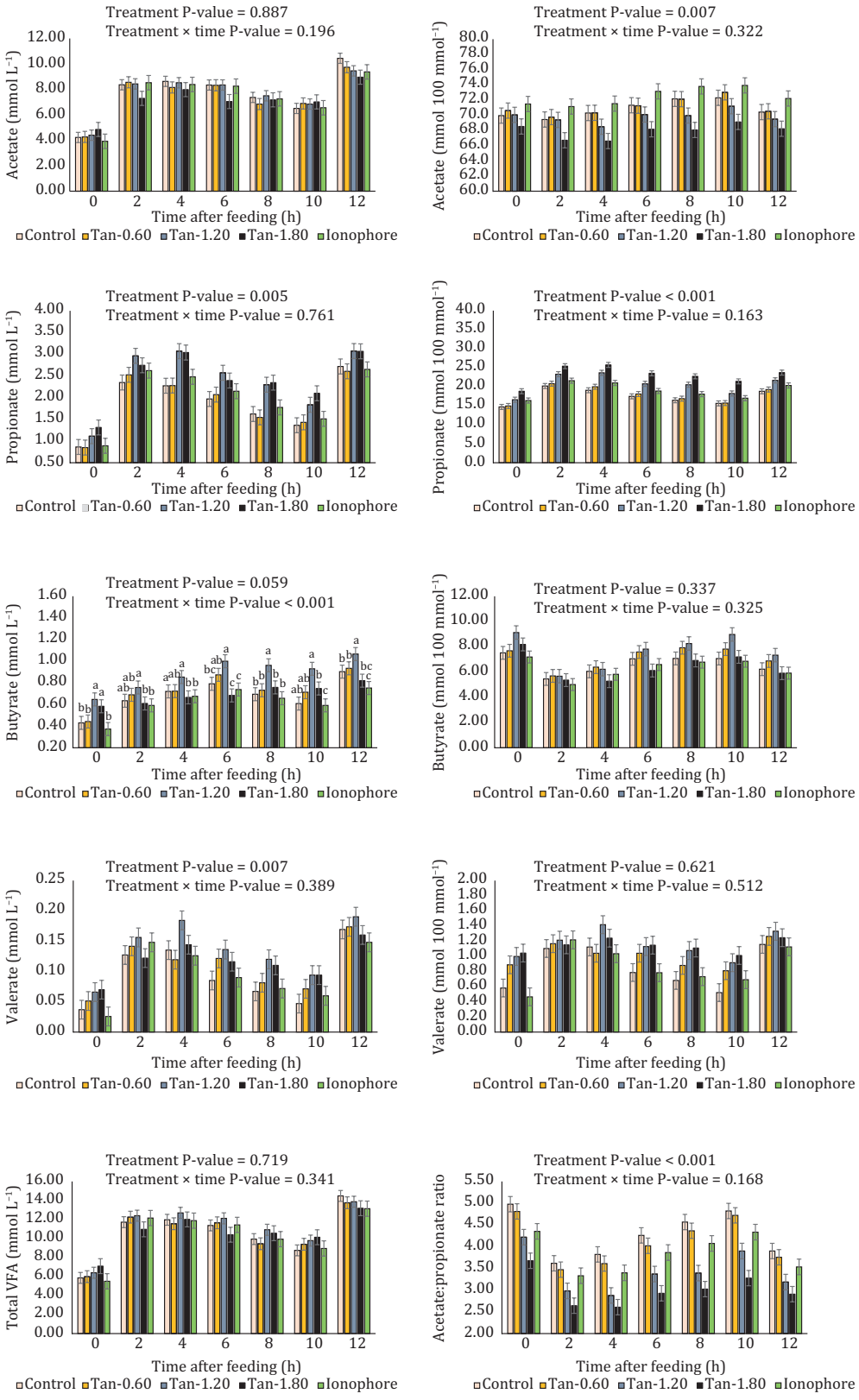
BW - body weight; SEM - standard error of the mean.
[1] Control, no additives; Tan-0.60, Tan-1.20, and Tan-1.80 represents 0.60, 1.20, and 1.80 g kg^{-1} of BW of tannin extract, respectively; and ionophore, 0.75 mg of monensin kg^{-1} of BW.
a-c - Differ from each other (P≤0.05) or tend to differ (P≤0.10).



Control, no additives; Tan-0.60, Tan-1.20, and Tan-1.80 represent 0.60, 1.20, and 1.80 g kg^{-1} of BW of tannin extract; and ionophore, 0.75 mg of monensin kg^{-1} of BW.
a,b,c - Within each time, means with different letters differ (P≤0.05).
Vertical bars represent the standard error of the mean (SEM).

Figure 1 - Rumen pH and N-NH_3 (mg dL^{-1}) concentration following feeding.

When VFA production was analyzed in mmol 100 $mmol^{-1}$, the effects of treatment (P<0.01), but not treatment × time (P>0.10), were detected for acetate production, which was lower for Tan-1.20 and Tan-1.80-treated animals than control-treated animals (Figure 2). Effects of treatment (P<0.01), but not treatment × time (P>0.10), were detected for propionate production, which was higher for Tan-1.20 and Tan-1.80-treated animals than for control-treated animals (Figure 2). No effect of treatment × time and treatment were detected (P>0.10) for butyrate and valerate production, and the use of ionophore did not alter the production of any VFA evaluated in this study (Figure 2).



Control, no additives; Tan-0.60, Tan-1.20, and Tan-1.80 represent 0.60, 1.20, and 1.80 g kg^{-1} of BW of tannin extract; and ionophore, 0.75 mg of monensin kg^{-1} of BW.
a,b,c - Within each time, means with different letters differ (P≤0.05).
Vertical bars represent the standard error of the mean (SEM).

Figure 2 - Volatile fatty acids (VFA) concentration of rumen fluid following feeding.

4. Discussion

The supply of tannins reduced the digestibility of the DM and the nutrients (except for EE). Inclusion of tannins from the *Acacia mearnsii* extract decreased the protein (CP) and fibrous fractions (NDFap and ADF) with the greatest reduction occurring when tannins were included in the diet at 29.2 and 43.8 g CT kg^{-1} (Tan-1.2 and Tan-1.8). In other studies, the supply of tannins for cattle also reduced the digestibility of the diet (Orlandi et al., 2015; Piñeiro-Vázquez et al., 2018). Avila et al. (2020) observed a linear reduction in CP digestibility; however, DM, OM, and NDF digestibility were not affected when tannins of *A. mearnsii* were included in the diet of steers at levels of 0, 5, 10, 15, and 20 g kg^{-1} DM. Kozloski et al. (2012) evaluated the inclusion of tannins from *A. mearnsii* at levels of 20, 40, and 60 g kg^{-1} DM in the diet of wethers and reported a linear reduction in the digestibility of DM, OM, NDF, and CP. Our initial hypothesis was that tannins would improve CP digestibility by reducing ruminal protein degradation by protecting this nutrient from microbial action and increasing its availability for post-ruminal absorption. However, McSweeney et al. (2001) described that the use of CT can increase endogenous protein losses, and thus this effect may have reduced protein fraction digestibility in the present study.

The reduction in the digestibility of the fibrous fraction of the diet with the supply of CT shows that in the rumen, fibrolytic bacteria may also have their activity reduced due to the possible interaction with the added tannin, either through their binding with the substrate or by direct inhibition of the bacterial cell (McSweeney et al., 2001), or by complexation with extracellular enzymes released in the ruminal degradation of carbohydrates (Bae et al., 1993).

Tannin supply increased fecal N excretion, and this effect was also observed by Orlandi et al. (2015). At first glance, this seems to contradict the assumption that the addition of tannin would improve the utilization of the nitrogen fraction by animals receiving diets with rumen degradable protein source, due to its likely effect on the protection of this from ruminal degradation (Reed, 1995; Min et al., 2003). However, in ruminants, the true protein content in feces is very low, so the dietary contribution to nitrogen excretion is probably lower than that from the microbial mass (Van Soest, 1994). In this experiment, with the addition of CT in the diets, there was a higher daily amount of fecal nitrogen excreted when compared with the monensin and control groups. Fecal excretion of N increased with increasing levels of CT in the diet. Therefore, it is possible that the contribution of fecal metabolic N also increased, considering that there was no significant difference in the amount of N ingested in the different treatments. In addition, Van Soest (1994) described that large amounts of enzymes and other gastrointestinal secretions from animals cannot be considered to make up fecal protein since almost all potentially degradable fraction is absorbed. However, only more resistant materials such as residues of the microbial cell wall could remain and form part of the fecal nitrogen.

The assumption is that the increase in fecal N may be derived, at least in part, from the increase in microbial protein production, and this increase could result from higher net turnover rates as a consequence of higher saliva flow in animals that consume tannins (Van Soest, 1994). Furthermore, the effect of the higher proportion of metabolic nitrogen participating in total fecal excretion with the addition of tannins (Norton, 1999) could also return to another cause, such as a possible increase in the flaking of the gastrointestinal tract membranes due to the ability of CT to complex, when in high-dose, even with mucosal constituent proteins, which would favor increased fecal nitrogen losses.

We also should assume that some of the nitrogen that passes into the CT-complexed small intestine may come from endogenous protein components (McSweeney et al., 2001) and that the complex may not be satisfactorily broken down in this part of the gastrointestinal tract. Nevertheless, Perez-Maldonado and Norton (1996) found that sheep and goats receiving CT had higher fecal nitrogen excretion (14%), but tannins did not affect post-ruminal digestion of this nutrient, since animals receiving CT from Desmodium (1%) or Calliandra (2.3%) absorbed more N per kilogram of digested organic matter than the control diet (pangola grass only). Dawson and Boling (1983) also observed an increase in fecal excretion of sheep receiving tannin-containing diets, which had about 14% more fecal DM production

than animals in the control treatment. These authors also verified that there may be increased nitrogen recycling and therefore less excretion of nitrogen in the urine.

The results obtained for N retained, considered as excretions through the fecal and urinary tract, indicate that the tannin was not efficient in improving the use of N ingested.

The use of ionophore increased the elimination of N in the urine but did not improve the amount of N retained. Ionophores usually improve the use of dietary N as a result of reduced DM intake and, consequently, a reduction in N intake and lower rumen clearance (McGuffey et al., 2001); however, these effects were not observed in this study.

The tannin-treated animals had greater N-NH_3 concentration for up to 8 h and lower concentration 10 h after the additive infusion than the control animals. However, although the tannin supply increased N-NH_3 production, it did not reflect in greater urinary N losses, as the amount of N lost in urine was even lower in animals receiving tannin.

We observed lower N-NH_3 production 10 h after feeding in tannin-fed animals, which could be due to its possible inhibiting effect on proteolytic bacteria (Yang and Russel, 1993) and reducing ruminal deamination either by its complexation with enzymes (Bae et al., 1993) or even with dietary protein, making substrate unavailable to microorganisms (McSweeney et al., 2001; Orlandi et al., 2015).

The addition of tannin reduced the production of acetate, valerate, and the acetate:propionate ratio and, in turn, increased the production of propionate and butyrate, without changing the total production of VFA. This was a beneficial effect because, especially the increased proportion of propionate reflects greater energy efficiency of the diet because propionate is a precursor of glucose and has a higher energy value. Increased propionate production and reduced acetate:propionate ratio have also been described by other studies using tannins (Hassanat and Benchaar 2013; Dickhoefer et al. 2016; Piñeiro-Vázquez et al., 2018). The specific mechanism of rumen tannins, which results in altered VFA production, is not yet fully understood and varies according to the type of CT, its structure, source, concentration, and molecular weight (Patra et al., 2017; Pinheiro-Vazquez et al., 2018). However, tannins can alter the microbial population by inhibiting the development of gram-positive bacteria and protozoan and favoring the growth of gram-negative bacteria (Perna Junior, et al., 2017; Piñeiro-Vázquez et al., 2018) or by protecting certain nutrients of microbial action due to their complexation with them (McSweeney et al., 2001; Orlandi et al., 2015), thereby altering the proportion of certain microorganisms that are substrates dependent. As the type of VFA produced is dependent on the type of microorganisms that colonize the rumen, the change in the microbial population may be responsible for the effects in VFA production in the present study.

5. Conclusions

Monensin did not affect the variables studied. Tannins impaired digestibility and nitrogen utilization, but increased propionate production and thus increased the energy efficiency of the diet. The highest effects were observed for the highest tannin dose (1.80 g kg^{-1} of BW) provided to lambs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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