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ANIMAL PRODUCTION

Acacia mearnsii tannin extract as a feed additive: impact on feed intake, digestibility and nitrogen excretion by sheep fed a tropical grass-based diet

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ABSTRACT: *The aim of this study was to evaluate the effect of the dietary inclusion of Acacia mearnsii tannin extract (TA) on nutrients intake and digestibility, and nitrogen (N) retention by sheep given a tropical grass-based diet. The trial was conducted with six castrated male sheep in a cross-over design in two 21-days experimental periods. The sheep were housed in metabolic cages and offered Tifton 85 hay (Cynodon dactylon) ad libitum plus concentrate at a rate of 12 g of dry matter (DM)/kg body weight (BW). The treatments were concentrate without (Control) or with 10 g TA/kg DM (Tannin). Concentration of TA in the diet was 3.8 g/kg DM and did not affect the feed intake or apparent digestibility. The TA decreased the true digestibility of n compounds (P<0.05) whereas did not impact the N retention, microbial N flow to the small intestine or the efficiency of rumen microbial protein synthesis. In conclusion, a low dietary concentration of TA did not impact the nutrients supply and N use by sheep fed with a tropical grass-based diet.*
Key words: *Cynodon dactylon, rumen microbial protein synthesis, tannins.*

Extrato tanífero de *Acacia mearnsii* como aditivo alimentar: impacto sobre o consumo, digestibilidade e excreção de nitrogênio em ovinos alimentados com uma dieta a base de gramínea tropical

RESUMO: *O objetivo do presente estudo foi avaliar o efeito da inclusão alimentar de extrato tanífero de Acacia mearnsii (TA) sobre o consumo, a digestibilidade e a retenção de nitrogênio (N) em ovinos alimentados com uma dieta a base de gramínea tropical. O experimento foi conduzido com seis ovinos machos castrados, em delineamento em reversão simples, com dois períodos experimentais de 21 dias cada. Os animais foram alojados em gaiolas metabólicas e alimentadas ad libitum com feno de Tifton 85 (Cynodon dactylon) mais concentrado oferecido a uma taxa de 12 g de matéria seca (MS)/kg de peso corporal. Os tratamentos foram: concentrado sem (Controle) ou com 10 g de TA/kg MS (Tanino). A concentração de TA na dieta foi de 3,8 g/kg MS e não afetou o consumo e nem a digestibilidade aparente dos nutrientes. O TA diminuiu a digestibilidade verdadeira do N (P<0,05), mas não afetou a retenção de N, o fluxo microbiano de N para o intestino delgado ou a eficiência de síntese de proteína microbiana no rúmen. Em conclusão, a inclusão de uma baixa dose de TA/kg MS na dieta não afetou a oferta de nutrientes nem o uso de N em ovinos alimentados com uma dieta baseada em uma gramínea tropical.*
Palavras-chave: *Cynodon dactylon, síntese de proteína microbiana ruminal, taninos.*

INTRODUCTION

Tannins are plant polyphenol compounds with the capacity to form complexes with proteins and carbohydrates reducing their degradation in the rumen (MCSWEENEY et al., 2001). Because of those properties, tannins can modulate the rumen microbial population and/or activity toward a reduction of both methane emissions from rumen and the urinary excretion of urea N, this last one contributing for nitrous oxide emissions to atmosphere (MAKKAR, 2003; BHATTA et al., 2009). The TA is an industrial source of tannin that has been investigated in the last years as a feed additive for ruminants. In previous studies of our group, the TA showed the potential to decrease the excretion of urinary N by cattle and sheep (ÁVILA et al., 2015; ORLANDI et al., 2015; ORLANDI et al., 2020). However, when the TA was included in the diet at a rate equal or above 10 g/kg DM, it reduced the OM digestibility in sheep fed with high-quality forage or concentrate based diets (CARULLA et al., 2005; KOZLOSKI et al., 2012;

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GERLACH et al., 2018). The impact of TA in diets based on tropical grasses, which usually contain more fiber and less soluble N than temperate grasses, was not clearly established. In fact, the inclusion of 7.0 g TA/kg DM reduced the enteric emissions of methane but, in turn, increased the concentration of non-esterified fatty acids in blood of dairy cows grazing a pearl millet (ALVES et al, 2017), an evidence of a negative impact in the energetic status of dairy cows. However, there is not consistent information on the impact of a low dose of TA on digestion processes in ruminants fed tropical grass-based diets.

The aim of the present study was to evaluate whether the dietary inclusion of a low dose of TA would decrease the voluntary feed intake, digestibility and N retention by sheep given a tropical grass-based diet.

MATERIALS AND METHODS

Animals, experimental design, treatments and diets

Six Santa Ines male sheep with 67 ± 6.5 (mean ± S.D.) kg BW were housed in metabolism pens for the trial. The experiment was conducted throughout two 21 days periods in a cross-over design. Each period consisted of 14 days for adaptation to the experimental diet followed by 7 days of sample and data collection. The treatments evaluated were: concentrate without (Control) or with 10 g TA/kg DM. The TA (Weibull Black, Tanac S.A., Montenegro, Brazil) was the same previously used and described by KOZLOSKI et al. (2012) and contained 716, 694 and 156 g/kg DM of total phenols, total tannins and condensed tannins, respectively. Diet was Tifton 85 hay (*Cynodon dactylon*) offered *ad libitum* plus concentrate offered at a rate of 12 g DM/kg BW. The concentrate was composed by cracked corn (0.36), wheat bran (0.36) and soybean meal (0.28). The chemical composition of hay and concentrate is shown in table 1. Hay and concentrate were offered in separated feeders in three daily meals at 8:00h, 12:00h and 17:00h. The animals had permanent access to water and a commercial mineral salt containing (g/kg): Ca: 120, P: 87, Na: 147, Mn: 1.3, Zn: 3.8, Fe: 1.8, Cu: 0.59, Co: 0.040, I: 0.080, Se: 0.015 and F: 0.87.

Sampling and data collection

All sampling and data collection were carried out from day 15 to 21 of each experimental period. Total feed, orts and feces were weighed and sampled daily. These samples were oven-dried at 55°C for at least 72 h and ground through a 1 mm screen for subsequent chemical analysis. Total urine was collected daily, in buckets containing 100 mL of sulfuric-acid (3.6 N). The total volume of urine was measured and a sample of 10 mL was taken, diluted to 50 mL with distilled water and stored frozen (–20°C). Samples of orts, feces and urine were pooled by animal and period for analysis.

Chemical analysis

Samples of feed, feces and orts were analyzed for DM content by oven-drying at 110 °C overnight. Ash was then determined by combustion at 600 °C for 3 h and OM by mass difference. Total

Table 1 - Chemical composition of experimental feeds.

Item	Tifton hay	Concentrate[1]
Dry matter (g/kg)	861	870
----------Composition (g/kg dry matter)----------		
Organic matter	939	955
Crude protein	58	244
Neutral detergent fiber	797	201
Acid detergent fiber	400	64
Acid detergent lignin	43	14
Non-fiber carbohydrate	92	465
Ether extract	15	65
Neutral detergent insoluble N (g/kg N)	419	92
Acid detergent insoluble N (g/kg N)	108	38

[1]The concentrate was the same for both treatments, except that in the Tannin treatment, nutrient concentrations were diluted by TA addition (i.e. 10 g TA/kg concentrate).

N was assayed by a Kjeldahl method (Method 984.13 of AOAC, 1997). The neutral (NDF) and acid (ADF) detergent fiber analyses were based on the procedures described by MERTENS (2002) and AOAC (1997), respectively, except that samples were weighed in polyester filter bags (porosity of 16 µm) and treated with neutral or acid detergent in an autoclave at 110 °C for 40 min (SENGER et al., 2008). For sulfuric-acid lignin analysis, the bags containing residual ADF were treated with sulfuric-acid (12 M) for 3 h (AOAC, 1997) and then ashed at 600 °C for 3 h. Analysis of neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) were performed according to LICITRA et al. (1996). Ether extract (EE) concentration was determined in a reflux system (Ankom XT15, Ankom Technology, USA) with ethyl ether at 90 °C for 60 minutes. In urine samples, total N was assayed by the Kjeldahl method and allantoin and uric acid concentrations were determined colorimetrically according to CHEN and GOMES (1995). Uric acid was determined using a commercial kit (BIOCLIN, MG, Brazil) after xanthine and hypoxanthine were converted to uric acid with xanthine oxidase.

Calculations

The content of non-fiber carbohydrates (g/kg) was estimated using the equation of VAN SOEST et al. (1991) as follows: OM – [(NDF – (NDIN x 6.25)) + (N x 6,25) + EE]. The apparent digestibility of feed fractions was calculated as follows: [intake (g/d) − fecal excretion (g/d)]/intake (g/d). The true digestibility of OM was estimated considering that neutral detergent soluble fractions of the feces are from endogenous origin and only the NDF fraction of feces originated from feed (VAN SOEST, 1994) as follows: [OM intake (g/d) − fecal NDF (g/d)]/OM intake (g/d). The true digestibility of N compounds was estimated considering that neutral detergent soluble N of the feces were from endogenous origin and only the NDIN fraction of feces originated from feed (VAN SOEST, 1994) as follows: [N intake (g/d) − fecal NDIN (g/d)]/N intake (g/d). The amount (g/d) of retained N was calculated as: N intake (g/d) − fecal N (g/d) − urinary N (g/d). The uric acid values in urine were the sum of uric acid, xanthine and hypoxanthine and, the total purine derivatives (PD) as the sum of uric acid and allantoin. The amount of absorbed purines (X, mmol/d) corresponding to the amount of PD excreted (Y, mmol/d, considering 158 mg/mmol of allantoin and 168 mg/mmol of uric acid), was calculated from the relationship derived by CHEN and GOMES (1995): $Y=0.84X + (0.150\ BW^{0.75}e^{-0.25X})$. The calculation of X based on the value of Y was made using the iterative process of Newton-Raphson as: $X(n+1) = Xn – [((0.84X + (0.150\ BW^{0.75}e^{-0.25X})) - Y)/(0.84 - (0.038\ LW^{0.75}e^{-0.25X}))]$. The supply of microbial N (Nm) was estimated as: $Nm\ (g/d) = 70X/(0.116 \times 0.83 \times 1000) = 0.727X$ assuming a digestibility of the microbial purines of 0.83, a N content in the purines of 70 mg/mmol and a ratio of purine N/Nm of 0.116 (CHEN & GOMES, 1995).

Statistical analysis

Data were averaged by animal, treatment and period for analysis. Analysis was performed with the MIXED procedure of SAS (Version 9.4, SAS Institute, Cary, NC, USA) using the following model: $Y_{ij} = \mu + T_i + P_j + e_{ij}$, where Y is the dependent variable, µ is the overall mean, T is the fixed effect of treatment, P is the random effect of the period and e is the residual error. Significant difference was declared when $P \leq 0.05$.

RESULTS AND DISCUSSION

The proportion of concentrate in the diet was 0.38 for both treatments (data not shown), therefore, the intake of TA in TA treatment averaged 7.0 g/d and represented 3.8 g/kg of DM intake. The intake and digestibility of both OM and NDF, as well as the digestible OM intake were not affected by treatments (Table 2). Tannins might negatively impact feed intake due to decrease feed palatability and/or OM degradation by rumen bacteria (PATRA & SAXENA, 2011) and the negative impact of relatively high dietary concentrations of TA (i.e. above 10 g/kg DM) on OM and fiber digestibility in sheep has been well documented (CARULLA et al., 2005; KOZLOSKI et al., 2012; GERLACH et al., 2018). The level of TA used in the present experiment was considerably lower than those reported above and was not high enough to negatively impact the feed intake and digestibility, even with a high fiber diet (570 NDF/kg DM). However, the potential of this low dose of TA on decreasing methane emissions needs to be further evaluated.

The intake, apparent digestibility, fecal and urinary excretion and retention of N were also similar in both treatments (Table 3). In contrast, a reduction of N true digestibility was observed in TA treatment (P=0.009; Table 3). Tannins usually decrease both ruminal protein degradation and N losses via urine, and increase the excretion of fecal N, which effects were largely reported in studies where TA was included in the diet of ruminants at levels above 9

Table 2 - Intake and total tract digestibility of dry matter (DM), organic matter (OM) and neutral detergent fiber (NDF) in sheep fed a tropical pasture (0.62) plus concentrate (0.38) without (Control) or with 10 g/kg DM of tannin extract from *Acacia mearnsii* (Tannin).

	Treatments			
Item	Control	Tannin	SEM	p-value
Total intake (g/d)				
DM	1814	1828	57.5	0.866
OM	1714	1727	54.3	0.869
NDF	1032	1045	32.2	0.780
Digestible OM	1315	1337	48.4	0.763
Apparent digestibility				
DM	0.75	0.76	0.012	0.628
OM	0.77	0.77	0.013	0.717
NDF	0.72	0.72	0.016	0.815
OM true digestibility[1]	0.83	0.83	0.010	0.860

[1][OM intake (g/d) – fecal NDF (g/d)]/OM intake (g/d).

g/kg DM (KOZLOSKI et al., 2012; ÁVILA et al., 2015; ORLANDI et al., 2015). In the present study, no effect of TA on N partition was observed, whereas, even at a low dose, the TA increased the proportion of fecal N originated from feed (i.e. fecal NDIN) and decreased the true digestibility of N compounds, what is an expected effect of tannins (MAKKAR et al., 1995). This result is positive, because in the soil, fecal NDIN degrades much more slowly than fecal endogenous N (POWELL et al., 2009), which may be beneficial for pastures and crops in the long term (MAKKAR, 2003).

The Nm flux to the small intestine and the efficiency of rumen microbial protein synthesis (ERMPS) were not affected by TA (Table 3). Tannins were also reported to exert a positive effect on the

Table 3 - Nitrogen intake, excretion and digestibility, microbial N (Nm) flux to the small intestine and efficiency of rumen microbial protein synthesis (ERMPS) in male sheep fed a tropical pasture (0.62) plus concentrate (0.38) without (Control) or with 10 g/kg DM of tannin extract from *Acacia mearnsii* (TA).

Item	Treatments		SEM	p-value
	Control	TA		
Intake (g/d)	38.2	38.2	1.34	0.980
Digestibility				
Apparent	0.82	0.82	0.008	0.760
True[1]	0.97	0.95	0.002	0.009
Excretion (g/d)				
Fecal	6.9	6.7	0.43	0.832
Urine	21.8	21.0	0.95	0.549
Fecal endogenous N[2]	0.81	0.76	0.009	0.004
Retention (g/d)	9.5	10.5	0.64	0.302
Nm (g/d)	21.2	22.0	1.44	0.726
ERMPS[3]	16.3	16.5	1.22	0.888

[1][N intake (g/d) – fecal neutral detergent insoluble N (g/d)]/N intake (g/d).
[2][fecal N (g/d) – fecal neutral detergent insoluble N (g/d)]/fecal N (g/d).
[3]g rumen Nm/kg digestible OM intake.

ERMPS and on metabolizable protein flux to the small intestine (MAKKAR, 2003; AVILA et al., 20015; ORLANDI et al., 2015). In the present study, both the flux of microbial N to small intestine and the ERMPS were not affected indicating that the TA did not impact the rumen OM digestibility.

In conclusion, it is possible to include 10 g TA/kg concentrate DM, supplemented at a rate of 12 g/kg BW to sheep fed a tropical grass-based diet without any negative impact on nutrients supply.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All experimental procedures followed the guidelines of the Animal Care and Ethical Committee of the Universidade Federal de Santa Maria (Nº 008/2014).

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Tiago Orlandi performed trial conduction throughout all steps. Claudio Antonio Pozo contributed with data analysis and prepared the draft of the manuscript. Mariana Patrícia Mezzomo performed sampling collection and laboratory analysis. Gilberto Vilmar Kozloski conceived, designed and supervised the experiment, performed data analysis and contributed with manuscript writing and editing. All authors critically revised the manuscript and approved the final version.

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