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Tannin extract of *Acacia mearnsii* for lactating ewes

Extrato tanífero de *Acacia mearnsii* para ovelhas em lactação

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Abstract

Condensed tannins can reduce protein degradation in the rumen by increasing the duodenal flow of metabolizable proteins and consequently improving animal performance. The objective of this study was to evaluate the effect of the tannin extract of *Acacia mearnsii* on lactating ewes receiving a total mixed ration (TMR) containing two concentrations of crude protein. The experimental treatments consisted of a TMR containing 16.4% or 22.3% of crude protein in dry matter either with or without the tannin extract of *Acacia mearnsii* at 20 g kg^{-1} dry matter. The extract concentration that guaranteed the daily intake of approximately 15 g of the extract per animal was determined. Eight lactating ewes of the Texel × Lacaune breed in the second third of lactation were distributed in a 4 × 4 Latin square experimental design, with four periods of 19 days, including 14 days of adaptation and 5 days of sample collection. Urinary nitrogen excretion was increased in the animals fed a diet containing a relatively higher protein concentration compared to those receiving a diet with a lower protein concentration. However, milk production and the concentration of milk solids did not vary significantly among treatments. The tannin extract of *Acacia mearnsii* decreased urinary nitrogen excretion, and consequently reduced the environmental impact of the production system, but did not significantly improve animal performance.
Key words: Nitrogen excretion. Milk production. Tannins.

Resumo

Os taninos condensados podem reduzir a degradação proteica no rúmen, aumentando o fluxo duodenal de proteína metabolizável com melhorias no desempenho animal. Objetivou-se avaliar o efeito do extrato tanífero de *Acacia mearnsii* em ovelhas lactantes recebendo ração totalmente misturada (RTM) com dois níveis de proteína bruta. Os tratamentos experimentais constituíram-se de RTM com 16,4 e 22,3% de PB na MS, com ou sem a adição de extrato tanífero de *Acacia mearnsii* na proporção de 20 g kg^{-1} na matéria seca. A quantidade de extrato foi calculada para permitir a ingestão de aproximadamente 15 g dia^{-1} por animal. Foram utilizadas oito ovelhas cruza da raça Texel × Lacaune no terço médio de lactação distribuídas em delineamento experimental em Quadrado latino 4 × 4, com quatro períodos de 19 dias, sendo 14 de adaptação e cinco de coleta. A excreção urinária de N aumentou nos animais recebendo a dieta com maior nível de proteína em comparação aos que receberam a dieta com menor

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nível de proteína. Entretanto, a produção de leite e de sólidos não variou entre os tratamentos. A suplementação com extrato tanífero de *Acacia mearnsii* reduziu a excreção urinária de N, o que reduz o impacto ambiental do sistema, mas não foi suficiente para melhorar o desempenho animal.
Palavras-chave: Excreção de nitrogênio. Produção de leite. Tanino.

Introduction

The use of protein sources with low ruminal degradability is an alternative approach to increase the intake of metabolizable proteins and the nutritional efficiency of animals with high productive potential; this is because highly digestible proteins in the rumen increase the urinary nitrogen (N) excretion without increasing the amount of metabolizable proteins available to the animal.

However, the primary sources of non-digestible proteins are expensive and little available in the Brazilian market. In these situations, the use of condensed tannins is justified because tannins form complexes with proteins, reducing ruminal proteolysis, increasing the levels of metabolizable proteins in the intestine (MEZZOMO et al., 2011) and increasing the efficiency of use of N by decreasing urinary N excretion.

The lower urinary excretion of N also helps reduce the environmental impact of animal production systems because urinary N is rapidly transformed into nitrous oxide, which is a greenhouse gas. The decrease in the emission of greenhouse gases is relevant because agricultural activities produce 10-12% of the total anthropogenic emission of these gases (ECKARD et al., 2010). In addition, the amount of N excreted may exceed the capacity of absorption of this nutrient by soil/plant systems. Therefore, the strategies used to reduce N_2O emissions should focus on improving the cyclic efficiency of N in the soil-plant-animal system. In this respect, fecal N is less volatile than urinary N and more rapidly nitrated to NO_3 (DE KLEIN; LEDGARD, 2005).

The objective of this study is to evaluate the effect of the tannin extract of *Acacia mearnsii* on lactating ewes receiving a total mixed ration (TMR) with two concentrations of crude protein (CP).

Materials and Methods

Animals and study design

Eight lactating ewes of the Texel × Lacaune breed in the first third of lactation were distributed in a 4 × 4 Latin square experimental design with four periods of 19 days, including 14 days of adaptation and 5 days of sample collection. The treatments consisted of a TMR with two concentrations of CP (16.4% or 22.3%) in dry matter (DM) either with or without the tannin extract of *Acacia mearnsii* at 20 g kg^{-1} DM. The composition of the diets according to contents and bromatological composition is shown in Table 1. The study animals were housed and the ration was offered twice daily (8:00 a.m. and 4:00 p.m.). Voluntary ration consumption was adjusted by offering an amount 20% higher than the amount consumed the previous day.

Sampling and evaluated parameters

The TMR samples offered were collected from days 14 to 18 in the morning and afternoon. Samples of forage and feces were collected starting on day 15 of each study period. The collected samples were oven dried at 60 °C for 72 hours, ground in a 1-mm sieve, and stored for further analysis. The total volume of urine produced daily by each animal was collected from days 15 to 19. Urine was collected in 100-mL volumetric flasks containing 20% sulfuric acid. Aliquots (1% of the total urine volume) were collected, filtered on gauze, and diluted with distilled water in 100-mL volumetric flasks. Urine samples were collected daily from each animal and study period and stored at -20 °C for future analysis.

Table 1. Contents and bromatological composition of the diets.

	Treatments	
	Low protein content	High protein content
Composition (g kg^{-1} DM)		
Corn silage	517	210
Pre-dried alfalfa	222	490
Soybean meal	245	284
Mineral mixture	16.0	16.0
Bromatological analysis (g kg^{-1} DM)		
Organic matter	917	889
Crude protein	161	212
Crude fat	35	31
NDF	437	452
ADF	202	196
Lignin	2.56	2.56

DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber. The protein concentrations were 160 g and 210 g crude protein kg^{-1} DM.

The TMR consumption was measured from days 14 to 19 in each study period by determining the difference between the amount of forage offered and the amount of forage not consumed. Digestible organic matter intake (DOMI) and the consumption of the organic matter (OM) components of the ration were calculated from the concentration of nutrient offered minus the concentration of nutrients present in the leftovers. The apparent digestibility of DM, OM, and OM components of the TMR was calculated by determining the difference between the amount ingested and the amount excreted in the feces. The true digestibility of OM (TDOM) was estimated according to Mulligan et al. (2001) considering that the excreted plant-derived OM corresponded to the neutral detergent fiber (NDF) excreted in the feces.

The daily milk production was quantified during two milkings. Milk samples were collected at each milking to determine the concentration of fat, protein, lactose, casein, somatic cell count, and urea N. Venous blood samples were collected on the last day of each study period to measure the concentrations of urea and non-esterified fatty acids.

Laboratory analysis

The DM concentration in the ration, leftovers, and feces was calculated after drying the samples in an oven at 105 °C for 20 hours, and the mineral matter was determined after burning the samples in a muffle oven at 550 °C for 4 hours. The total N content was determined using the Kjeldahl method (AOAC, 1995). NDF was determined as proposed by Mertens (2002) in an ANKOM fiber analyzer (ANKOM Technology, USA), where the samples were weighed in ANKOM F57 filter bags and treated with a neutral detergent solution. The concentrations of acid detergent fiber (ADF) and lignin were measured according to AOAC method No. 973.18 (1997). The crude fat content was determined in a reflux system (Soxtherm, Gerhardt, Germany) using ethyl ether for 4 hours.

Statistical analysis

The data were subjected to analysis of variance using the Proc Mixed procedure of the SAS statistical package (SAS INSTITUTE, 2002). The model included the random effects of animal and study period, and the fixed effects of the concentrations of protein and tannin extract and interaction level of protein × tannin extract.

Results and Discussion

Effect of the protein content and tannin extract on the intake and digestibility of non-nitrogen compounds

There was no interaction effect of the CP content and tannin extract on any of the analyzed variables. The consumption of DM and NDF, expressed as g day^{-1}, was increased ($p<0.05$) as the CP concentration of the diets was increased (Table 2). The analysis of the results expressed as live weight (LW) indicated that the animals fed diets containing a higher protein concentration increased the intake of DM ($p<0.05$) and NDF ($p<0.01$) compared to those receiving diets with a lower protein concentration, whereas the animals fed the tannin extract decreased the intake of DM and NDF ($p<0.05$) in 2.5 and 1.0 g kg^{-1} LW, respectively, compared to those that did not receive the extract. The apparent digestibility of DM and OM was similar between the treatments. Nonetheless, the apparent digestibility of NDF and TDOM was increased as the CP content of the ration was increased, and the supplementation of the tannin extract did not significantly affect these parameters.

Table 2. Consumption and digestibility of non-nitrogenous substances and energetic value of diets containing two crude protein concentrations (164 g or 223 g kg^{-1} DM) either with or without the tannin extract of *Acacia mearnsii* at the concentration of 20 g kg^{-1} DM for lactating ewes.

Items	Treatments				rsd	*P-value*		
	Low protein		High protein					
	Without tannins	With tannins	Without tannins	With tannins		Protein	Tannins	P × T
Consumption (g/day)								
DM	1540	1369	1654	1569	168	0.037	0.092	0.545
OM	1408	1261	1463	1393	146	0.136	0.098	0.527
CP	254	234	372	347	41.4	<0.001	0.23	0.883
NDF	644	584	723	694	71.4	0.006	0.162	0.604
ADF	373	305	389	372	144	0.495	0.499	0.669
DM intake (% LW)	2.7	2.36	2.93	2.75	0.29	0.022	0.049	0.516
OM intake (g kg^{-1} LW$^{0.75}$)	67.9	59.9	71	67	6.81	0.088	0.056	0.49
NDF intake (% LW)	1.1	1.0	1.3	1.2	0.11	<0.001	0.049	0.447
Apparent digestibility								
DM	0.65	0.66	0.68	0.68	0.04	0.139	0.799	0.825
OM	0.68	0.69	0.7	0.7	0.04	0.228	0.82	0.685
CP	0.63	0.64	0.76	0.75	0.04	<0.001	0.935	0.587
NDF	0.55	0.54	0.63	0.62	0.05	0.002	0.54	0.856
ADF	0.6	0.47	0.36	0.32	0.45	0.303	0.678	0.828
True digestibility of OM	0.79	0.79	0.81	0.81	0.02	0.039	0.63	0.94
Digestible OM (g kg^{-1} DM)	624	632	624	623	31.4	0.736	0.753	0.74
ME (MJ kg^{-1} DM)	9.79	9.92	9.79	9.79	0.49	0.736	0.753	0.74

rsd, residual standard deviation; NDF, neutral detergent fiber; ADF, acid detergent fiber; OM, organic matter; ME, apparent metabolizable energy proposed by AFRC (1993); P × T, interaction effect of protein × tannin extract.

The effect of the tannin extract on OM intake and digestibility seems to be strongly correlated with the extract concentration in the ration. Kozloski et al. (2012) observed that OM intake and degradation were decreased, which affected the DOMI. Krueger et al. (2010) used a ration containing 14.9 g kg^{-1} DM of condensed tannins and found no significant difference in DM intake in steers, indicating that relatively low levels of condensed tannins had no detrimental effect on energy intake and animal performance. Similarly, Ávila (2013) observed that, although ruminal digestibility was reduced, OM intake and degradation were not affected by the tannin extract in the diet.

A change in the pattern of the ruminal fermentation of OM in the presence of tannins may be partially explained by their effect on the activity of cellulolytic bacteria. Beelen et al. (2006) found that these bacterial species were inhibited by soluble condensed tannins in the rumen, limiting forage fiber degradation. Similarly, Ávila et al. (2015) reported that condensed tannins of *Acacia mearnsii* at 15 g kg^{-1} DM reduced fiber degradability by ruminal microorganisms.

The lower intake of NDF and metabolizable energy and reduced NDF degradation in diets with a lower CP content may be due to a decrease in the ruminal concentration of N-NH_3 because the growth of bacteria that ferment fibrous carbohydrates depends on ammonia N (KOZLOSKI, 2011). However, the lower intake of NDF did not significantly affect the productive performance of the animals, and the energy balance remained positive in all treatments.

Effect of the protein content and tannin extract on the use of nitrogen compounds and yield and chemical composition of milk.

The N consumption was increased ($p<0.001$) as the CP content was increased; however, N consumption was not significantly affected by the tannin extract (Table 3). The excretion of urine N and total N was increased ($p<0.001$) as the CP level was increased. In contrast, urine N excretion was decreased ($p<0.05$) whereas total N excretion was not significantly affected by the extract. The apparent digestibility of N was increased ($p<0.001$) as the CP concentration was increased but was not significantly affected by the extract (Table 2). The amount of N excreted in feces and milk was similar in animals from different treatments.

Table 3. Nitrogen intake and excretion in lactating ewes fed two crude protein concentrations (164 or 223 g kg^{-1} DM) either with or without the tannin extract of *Acacia mearnsii* at a concentration of 20 g kg^{-1} DM.

Items	Treatments				*rsd*	*P-value*		
	Low protein		High protein					
	Without tannins	With tannins	Without tannins	With tannins		Proteins	Tannins	P × T
N intake (g day^{-1})	40.6	37.5	59.4	55.5	6.62	<0.001	0.230	0.883
N excreted in feces (g day^{-1})	14.4	13.1	13.8	13.7	2.55	0.959	0.546	0.577
N excreted in urine (g day^{-1})	31.8	25.5	44.6	41.6	5.09	<0.001	0.048	0.436
N excreted in milk (g day^{-1})	11.6	11.0	11.1	11.2	1.31	0.799	0.610	0.488
Total N excreted (g day^{-1})	57.9	49.6	69.6	66.7	7.60	<0.001	0.101	0.410
N balance (g day^{-1})	-17.3	-12.1	-10.1	-11.1	5.92	0.109	0.425	0.224

rsd = residual standard deviation; P × T, interaction effect of protein and tannin extract.

Milk production and fat and protein levels were similar between treatments. The concentration of urea N in milk was increased (P <0.001) as the CP content was increased in the diet but was not significantly affected by the tannin extract. The energy balance was positive in all animals and was significantly higher (p<0.05) in ewes fed a diet containing a higher protein concentration than those receiving a diet with a lower protein concentration (Table 4).

Table 4. Milk production, milk composition, and energy balance of lactating ewes fed diets containing two protein concentrations (164 or 223 g kg^{-1} DM) either with or without the tannin extract of *Acacia mearnsii* at a concentration of 20 g kg^{-1} DM.

Items	Treatments				*rsd*	*P-value*		
	Low protein		High protein					
	Without tannins	With tannins	Without tannins	With tannins		Proteins	Tannins	P × T
Milk production (g day^{-1})	985	955	990	1002	106	0.561	0.846	0.638
CMP (g/day^{-1})	983	939	980	981	95.9	0.640	0.607	0.577
Composition (%)								
Fat	7.69	7.43	7.30	7.29	0.53	0.245	0.548	0.580
Protein	5.72	5.62	5.83	5.80	0.20	0.098	0.459	0.649
Lactose	4.49	4.45	4.42	4.47	0.12	0.625	0.876	0.402
Solids	18.7	18.3	18.4	18.42	0.62	0.653	0.453	0.445
Casein	4.59	4.49	4.66	4.64	0.16	0.111	0.400	0.562
Urea (mg dL^{-1})	31.8	30.1	36.3	36.4	1.70	<0.001	0.288	0.223
SCC (*1000)	23.3	11.7	309	456	603	0.158	0.789	0.749
Casein (mg dL^{-1})	0.34	0.33	0.32	0.32	0.02	0.142	0.468	0.305
Yield (g day^{-1})								
Fat	73.7	69.7	71.9	72.04	7.49	0.930	0.551	0.526
Protein	74.5	70.2	71.2	71.80	8.37	0.799	0.610	0.488
Lactose	0.60	0.57	0.53	0.55	0.09	0.195	0.827	0.557
Solids	0.44	0.42	0.43	0.43	0.04	0.762	0.572	0.533
ME content (MJ/day)	15.1	13.5	16.2	15.37	1.69	0.049	0.105	0.640
Energy balance (MJ/day)	5.47	4.04	6.61	5.70	1.49	0.036	0.084	0.679

rsd, residual standard deviation; CMP, milk production corrected for fat and protein; SCC, somatic cell count; ME, metabolizable energy; P × T = interaction effect of protein × tannin extract.

The tannin extract significantly reduced urinary N excretion independently of the CP level in the ration but did not significantly change milk production and chemical composition. In this respect, Carulla et al. (2005) reported that there are environmental benefits from a lower urinary: fecal N ratio and lower emission of enteric methane in sheep fed ryegrass and the tannin extract of *Acacia mearnsii* at 2.5% of DM; moreover, the tannin extract did not significantly affect animal growth. With respect to the effect of the CP content in the diet, the increased urinary N excretion and higher urea N concentration in milk, without improvement in the productive performance of the animals, are expected. The increase in the urea N concentration as the CP level was increased without substantial changes in milk production and fat and protein levels were observed by Colmenero and Broderick

(2006) in dairy cows.These authors found that the urinary N excretion was on average 12% lower in the animals receiving the tannin extract compared to those that did not receive the extract. The comparison of the animals fed a diet containing the extract and a low protein concentration and those receiving a diet with a high protein concentration and without the extract indicated that urinary N excretion was reduced by 43% in the former group. However, Grainger et al. (2009) found that urinary N excretion was decreased by 40-43% in lactating cows fed two diets containing 0, 163, or 244 g of tannin per day. Moreover, Kozloski et al. (2012) observed that the intake, degradation, and urinary excretion of N were decreased by supplementing a tannin extract to sheep at 20-60 g kg^{-1} DM.

The lack of effect of condensed tannins on the yield and chemical composition of milk suggests that the diets without the tannin extract contained a concentration of amino acids sufficient to ensure the synthesis of milk proteins in the study animals. Therefore, all diets met the nutritional requirements of the animals. Although it has been shown that the tannin extract of *Acacia mearnsii* can increase duodenal flow (KOZLOSKI et al., 2012; ÁVILA, 2013) and change the amino acid profile (ORLANDI, 2013) in the small intestine of ruminants, a possible increase in duodenal flow may not be reflected in increased protein synthesis in the mammary gland and increased milk production. This result is because the circulating amino acids that exceed the productive capacity of the animals can be deaminated, and N can be excreted in the urine in the form of urea. In this respect, it has been shown that the tannin extract had no significant effect on the yield and chemical composition of milk in dairy cows (ALVES et al., 2017). In contrast, cattle fed diets containing soybean meal or canola meal combined with 1.5% of the tannin extract of *Acacia mearnsii* presented an increase in the intestinal flow of α-amino N and non-ammonia non-microbial N, whereas the intestinal flow of microbial N and the efficiency of microbial protein synthesis were not affected (ÁVILA, 2013). These results indicate the importance of genetic merit and the stage of lactation to identify increases in animal production by tannin extracts.

Conclusion

Supplementing the tannin extract of *Acacia mearnsii* to the ration of lactating ewes did not significantly affect the yield and chemical composition of milk.

The presence of the extract in the rations decreased urinary N excretion regardless of the dietary concentration of CP. The productive response to the use of this extract should be tested in animals with high production potential.

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