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Energy and tannin extract supplementation for dairy cows on annual winter pastures

Suplementação com extrato tanífero e energia para vacas leiteiras em pasto anual de inverno

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Abstract

Energy supplementation can increase the consumption of metabolizable energy and substrate for microbial growth, while condensed tannins aid in increasing the duodenal flow of metabolizable proteins. The objective of this study was to evaluate the effects of energy supplementation and the inclusion of tannin extract (TE) from *Acacia mearnsii* (Weibull Black, Tanac S. A., Montenegro, Brazil) on the production performance of dairy cows grazing on winter pastures. Nine multiparous Holstein cows in mid lactation were distributed in a 3 × 3 Latin square experimental design over three periods of 28 days (21 adaptation and 7 sampling). The treatments were: without supplementation (WS), supplementation with 4 kg of corn grain (CG), and corn grain + 80 g of tannin extract (TE). The dry matter (DM) intake from pastures was similar among treatments, but the consumption of DM of the supplement was higher in the CG treatment than that in the TE treatment. The total DM intake was higher for the supplemented animals (17.3 kg·day^{-1}) than that for the unsupplemented animals (14.9 kg·day^{-1}) and in the TE treatment (17.7 kg·day^{-1}) than in the CG treatment (16.7 kg day^{-1}). Milk production increased from the unsupplemented to the supplemented animals (20.9 to 23.5 kg, respectively), while the content of urea N in the milk decreased (12.6 to 10.5 mg·100 mL^{-1}, respectively). There were no differences in milk production or content of milk urea N between the CG and TE treatments. Energy supplementation is a tool for improving the nutritional profile and the performance of dairy cows in mid lactation grazing on annual winter pastures, while tannin extract aids in improving the energy balance.
Key words: Energy balance. Nutritional profile. Milk production. Condensed tannin.

Resumo

A suplementação energética visa aumentar o consumo de energia metabolizável e de substrato para o crescimento microbiano, enquanto os taninos condensados são capazes de aumentar o fluxo duodenal de proteína metabolizável de origem alimentar. Objetivou-se avaliar o efeito da suplementação energética e da inclusão de extrato tanífero de *Acacia mearnssi* (Weibull Black, Tanac S. A., Montenegro, Brasil) no desempenho produtivo de vacas leiteiras em pasto de inverno. Foram utilizadas nove vacas multíparas da raça Holandês no terço médio de lactação, distribuídas num delineamento experimental em Quadrado latino 3 × 3, com três períodos de 28 dias (21 de adaptação e 7 de coleta). Os tratamentos foram sem suplementação (SS), suplementação com 4 kg de grão de milho (GM) e grão de milho + 80

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g de extrato tanífero (ET). O consumo de MS de pasto foi similar entre os tratamentos, mas o consumo de MS do suplemento foi superior nos animais do tratamento GM em comparação àqueles do tratamento ET. O consumo de MS total foi superior nos animais suplementados (17,3 kg dia^{-1}) em comparação aos não suplementados (14,9 kg dia^{-1}) e nos do tratamento ET (17,7 kg dia^{-1}) em comparação aos do tratamento GM (16,7 kg dia^{-1}). A produção de leite aumentou (de 20,9 para 23,5 kg) e o teor de N ureico no leite reduziu de 12,6 para 10,5 mg 100 mL^{-1} nos animais suplementados em comparação aos não suplementados, mas não houve diferença entre os tratamentos GM e ET. A suplementação energética é uma ferramenta para a melhoria do perfil nutricional e do desempenho de vacas leiteiras no terço médio de lactação em pastos anuais de inverno, enquanto o extrato tanífero contribui para a melhoria do balanço energético.

Palavras-chave: Balanço energético. Perfil nutricional. Produção leiteira. Tanino condensado.

Introduction

The consumption of metabolizable energy is the main factor limiting milk production on pasture systems (PEYRAUD; DELAGARDE, 2013), and energy supplementation is a tool to ameliorate this deficiency. However, other factors, such as the supply of amino acids, become limiting factors in milk production for dairy cows with high yield potentials (>35 kg milk·day^{-1} and 650 kg live weight, LW) (HILLS et al., 2015).

In a previous work, Ribeiro-Filho et al. (2007) found no effect of supplementation amount (2 or 4 kg of milled corn) on the milk production of Holstein cows with an average yield potential (≈20 kg milk·day^{-1}) grazing on annual ryegrass (*Lolium multiflorum*) pasture. The authors attributed this lack of response to supplementation to the quality of the forage and to the productive potential of the animals, which obtained all the nutrients required to meet their nutritional requirements at the lowest level of supplementation. However, a comparison of the responses to energy supplementation of dairy cows with average yield potential and the responses of animals fed only on annual winter pasture deserves further study.

Well-fertilized winter pastures, such as oat (*Avena sativa*) and annual ryegrass, are known to have high degradable protein levels in the rumen, which often exceed the demand of the rumen flora, causing increases in the excretion of N urea into the milk and urine. This results in a reduction in the efficiency of the use of feed N (COLMENERO; BRODERICK, 2006) and also negative environmental impacts (DIJKSTRA et al., 2013). Energy supplementation thereby increases the fermentable energy supply in the rumen, which may affect microbial growth (BACH et al., 2005), as well as the demand of N by microorganisms, reducing ruminal N losses. In contrast, the use of fodder with secondary components, such as condensed tannins (MIN et al., 2003), has proven to be a viable alternative for reducing the degradability of ruminal N and improving use efficiency.

Tannins are polyphenols with the ability to form complexes with proteins mainly by reducing their degradation in the rumen (PATRA; SAXENA, 2011). Therefore, tannins have been investigated as a food additive with the purpose of increasing the intestinal metabolizable protein flow. Ávila et al. (2015) studied Holstein steers receiving a diet consisting of corn silage (70%) and protein concentrate (30%). They observed that the inclusion of 15 g of *Acacia mearnsii* tannin extract per kg of DM increased the duodenal flow of non-ammonia N without affecting the duodenal flow of microbial protein, and reduced the urinary nitrogen excretion. However, the effects of including *Acacia mearnsii* tannin extract in the diet of dairy cows ingesting winter pasture have not yet been studied sufficiently.

The objective of this study was to evaluate the effects of energy supplementation and dietary inclusion of *Acacia mearnsii* tannin extract on the yield performance of dairy cows grazing on winter pasture. The hypothesis tested was that energy supplementation plus the tannin extract improves the energy and protein nutritional status of the animals, resulting in improvements in animal performance and reductions in milk urea N concentrations.

Material and Methods

Treatments and experimental design

The treatments evaluated were: oat pasture + ryegrass without supplementation (WS); oats + ryegrass with supplementation of 4 kg of corn grain (CG); and oat + ryegrass with supplementation of 4 kg corn grain plus 80 g of *Acacia mearnsii* tannin extract (Weibull Black, Tanac S. A., Montenegro, Brazil) (TE). The tannin extract was characterized by maintaining total tannins at a concentration of 694 g·kg^{-1} DM (KOZLOSKI et al., 2012). The cows were distributed in a 3 × 3 Latin square design for three periods of 28 days (21 for adaptation and 7 for collection).

Site, animals, and experimental area

The experiment was conducted in Lages, SC, Brazil (27°47'S, 50°18'W; altitude 920 m), during the spring of 2013. Nine Holstein cows were divided into three homogeneous groups according to milk production (23.4 ± 4.2 kg), number of births (3.8 ± 1.2), days in milk (150 ± 68 days), and body weight (619 ± 48 kg).

The experimental area consisted of 6.5 ha of ryegrass (*Lolium multiflorum* cv. Barjumbo) + oat (*Avena sativa* cv. FAPA2), sown in April 2013. Four of the 6.5 ha were allocated to the adaptation periods and 2.25 ha were allocated for the collection periods. The animals were kept in the same paddock during the adaptation period, and separated by treatments during the collection periods. A 0.25 ha plot was allocated for each treatment per period. The strip grazing method was used, with a herbage allowance of 35 kg DM per day.

Animal measurements

Forage intake was measured as the difference between the biomass before and after grazing (LANTINGA et al., 2004) in each of the last 5 days of each experimental period. Milk production was measured in two daily milkings (7 a.m. and 4 p.m.). The samples for determining the milk composition were collected in two milkings during the last 4 days of each experimental period. Body weight was measured at the beginning of the experiment and at the end, and also at the beginning of each trial period. A total of 10 mL of blood from the jugular vein was collected in tubes without anticoagulant on the last day of each experimental period. The tubes were then centrifuged, and the plasma was cooled and stored at –80ºC for later analysis of non-esterified fatty acids (NEFA).

Pasture and supplement measurements

The herbage mass above ground level was estimated using a rising plate meter (Farmworks®, F200 model, New Zealand), which was calibrated by accounting for the amount of DM present in the plate diameter (0.1 m²). Samples from 15 points (five per treatment), from a range of sward height across the total pasture area, were cut in each experimental period for calibration. The total herbage mass was cut at the soil level and oven dried with forced ventilation at 60°C for 48 h for each site. Regression equations were developed to estimate the herbage mass (kg DM ha^{-1}) as a function of the compressed height (cm). At the end of the experiment, the equations were recalculated using an equation to estimate the herbage mass before grazing (R^2 = 0.89) and another for estimating herbage mass after grazing (R^2 = 0.79). The average compressed height of each paddock was calculated from at least 100 readings.

The morphological composition was determined by manually separating the fractions of leaves, stems, and dead material of the ryegrass and oat plants. In all evaluation periods, twenty fistfuls of grass, with an approximate diameter of 10 cm each, were collected by cutting with scissors to ground level before grazing. A subsample was used to determine the morphological composition and another to perform chemical bromatological analyses. The latter sub-sample was cut to the average height of the tiller after grazing, which was measured with a graduated scale for 300 tillers per paddock. The upper fraction, along with samples of the offered supplement and supplement leftovers, were dried in an oven with forced ventilation at 60°C for 48 h and then stored for further analysis. The height of the extended tillers was measured before and after grazing in the last 4 days of each experimental period.

Laboratory analysis and nutritional value

The chemical composition of the forage samples was determined for samples dried in an oven with forced ventilation at 60°C for 48 h and milled to the size of 1.0 mm. The total DM was determined by drying at 105°C until reaching a constant weight, and mineral matter (MM) was determined by burning in a muffle furnace at 550°C for 4 h. The total N content was determined by the Kjeldahl method (method 984.13, AOAC, 1997). The neutral detergent fiber content (NDF) was determined without using sodium sulfite, following Mertens (2002). The acid detergent fiber content (ADF) was determined according to the AOAC method (method 973.18) (AOAC, 1997), without using asbestos. The NEFA analysis was performed from blood plasma, using commercial kits (Wako NEFA-HR, Wako Chemicals EUA®, Richmond, EUA and BHBA: Ranbut, Randox® Laboratories Ltd, UK) according to the method described by Ballou et al. (2009). The net energy for lactation (NE_l) and the true protein values digestible in the intestine were calculated from the chemical composition of the forage samples and the supplements, using the equations proposed by INRA (2007). These calculations were performed by taking into account that the microbial synthesis in the rumen is limited by energy (PDIE), but also by considering that microbial synthesis in the rumen is limited by nitrogen (PDIN).

Statistical analysis

Data were subjected to analysis of variance using the PROC MIXED (SAS Institute, 1999). The variables linked to milk production, milk composition, and blood components were analyzed accounting for the fixed effects of the treatment, the random effects of the animals, and time (n = 9). The fixed effects of treatment and the random effects of the lot and period (n = 3) were taken into account for the characteristics of pasture and consumption. Treatments were compared using orthogonal contrast for treatment WS × the mean of the supplemented treatments (CG and TE), and treatment CG × treatment TE.

Results and Discussion

The average pre-grazing herbage mass, irrespective of the treatments, was about 2600 kg $DM \cdot ha^{-1}$ with an average height of 26.7 cm and approximately 50% leaf blades (Table 1). The average levels of crude protein (CP) and NDF of the consumed pasture were 173 and 540 $g \cdot kg^{-1}$ DM, respectively. The average concentrations of NE_l and metabolizable protein in the pasture of the three treatments were 6.63 $MJ \cdot kg^{-1}$ DM and 92 $g \cdot kg^{-1}$ DM, respectively (Table 1). The herbage allowance was 35.3 kg·DM $cow \cdot day^{-1}$ (Table 2).

Table 1. Pre-grazing characteristics for mixed pastures of oat (*Avena sativa*) and annual ryegrass (*Lolium multiflorum* Lam.) grazed upon by dairy cows without supplementation (WS) or supplemented with corn grain (CG) or corn grain + 80 g of the tannin extract of *Acacia mearnsii* (TE).

Item	Treatment				Contrast (P value)	
	WS	CG	TE	CV[1]	WS×S[2]	CG×TE
Herbage mass (kg DM·ha^{-1})	2636	2693	2461	14.1	0.712	0.251
Sward height (cm)						
Rising plate meter	27.1	27.7	25.3	14.6	0.712	0.251
Extended tiller	32.8	32.3	31.2	13.6	0.611	0.605
Sheath	15.4	17.2	14.5	8.8	0.460	0.014
Leaf blade	17.4	15.1	16.7	24.0	0.419	0.440
Morphological composition (g·kg^{-1} DM)						
Leaf blade	461	453	525	14.9	0.387	0.098
Culm + sheath	380	417	369	11.3	0.493	0.078
Dead material	66	45	40	68.5	0.166	0.782
Chemical composition (g·kg^{-1} DM)						
Dry matter (g·kg^{-1} green)	14.8	14.3	15.5	13.1	0.906	0.258
Organic matter	910	919	920	0.2	0.256	0.938
Crude protein	168	185	165	1.0	0.406	0.069
Neutral detergent fiber	539	550	532	3.6	0.809	0.118
Acid detergent fiber	252	255	253	7.5	0.781	0.867
Nutritional value						
NE_L. MJ·kg^{-1} MS[3]	6.57	6.70	6.62	2.7	0.342	0.469
PDIN. g·kg^{-1} MS[4]	105	115	103	9.5	0.412	0.068
PDIE. g·kg^{-1} MS[5]	91.8	92.1	92.0	1.2	0.590	0.889

[1]Coefficient of variation; [2]Comparison between unsupplemented and supplemented animals (mean of the CG and TE treatments); [3]Net energy for lactation estimated according to INRA (2007); [4]True protein digestible in the intestine, considering that the microbial synthesis in the rumen is limited by nitrogen (INRA, 2007); [5]True protein digestible in the intestine considering that the microbial synthesis in the rumen is limited by energy (INRA, 2007).

The forage characteristics and the management practices precluded the main objective of this study because the supply of forage had a curvilinear effect on consumption (PEYRAUD et al., 1996), where levels above 35 kg DM·day^{-1} had little effect on the individual DM consumption, with a significant reduction in productivity per area (PÉREZ-PRIETRO; DELAGARDE, 2013). The intake rate was greatest when the grass height exceeded 20 cm (DELAGARDE et al., 2011) and the proportion of leaves only significantly affected the intake rate values when their values fell below 37% of the total DM (GUZATTI, 2013). The observed leaf removal ratio also indicated that there was a quantitative restriction on consumption, after a reduction of approximately 42% of the height from the input point and after approximately 41% of the original forage mass was eaten. Overall, the consumption ability of dairy cows was enhanced when the residual height of winter pastures was greater than or equal to 50% of the initial height (DELAGARDE et al., 2006). In this experiment, the average residual height was approximately 58% of the initial height.

Table 2. Grazing management, post-pasture characteristics, and post-grazing characteristics of mixed pastures of oat (*Avena sativa*) and annual ryegrass (*Lolium multiflorum* Lam.) grazed upon by dairy cows without supplementation (WS), supplemented with corn grain (CG) or corn grain + 80 g of the *Acacia mearnsii* tannin extract (TE).

Item	Treatment				Contrast (P value)	
	WS	CG	TE	CV[1]	WS×S[2]	CG×TE
Herbage mass (kg DM·ha^{-1})	1550	1585	1471	5.4	0.544	0.042
Sward height (cm)						
Rising plate meter	15.6	16.0	14.7	11.9	0.542	0.042
Extended tiller	18.3	18.8	18.9	15.6	0.654	0.983
Sheath	9.9	9.5	10.1	23.5	0.959	0.632
Leaf blade	8.4	9.3	8.8	16.6	0.359	0.482
Herbage allowance (kg DM·day^{-1})						
Total DM	35.3	35.3	35.3	0.4	0.321	0.831
Green material	29.6	30.7	31.6	3.7	0.030	0.194
Leaf blade	16.2	16.0	18.5	14.6	0.364	0.096
Offered area (m²·cow·day^{-1})	137	144	147	19.1	0.503	0.841

[1]Coefficient of variation; [2]Comparison between unsupplemented and supplemented animals (mean of the CG and TE treatments).

The ingested pasture quality can be qualitatively classified as average to good based on its energy value. This would allow an intake of up to 17 kg DM·day^{-1} for high potential productive cows in the absence of quantitative or structural restrictions (PEYRAUD; DELAGARDE, 2013). In addition, the protein content is within the range described in experiments using ryegrass pastures in the same experimental area (168 to 196 g·kg^{-1} DM) in previous years (MIGUEL et al., 2014; RIBEIRO-FILHO et al.,2007, 2009a).

The forage DM intake was similar between treatments (mean = 14.3 kg·day^{-1}), but the DM intake of the supplement was higher ($P < 0.05$) in the CG treatment compared to that for the TE treatment. The total DM intake was higher for the supplemented animals ($P < 0.001$) than for the unsupplemented animals and for the animals with the TE treatment than those with the CG treatment ($P < 0.05$). The substitution rate (kg DM of forage ingested minus / kg DM of supplement consumed) was 0.45 in the CG treatment and close to zero in the TE treatment, which indicated a higher total DM intake ($P < 0.05$) for animals fed with corn grain and tannin extract than that for animals that received grain without extract.

The substitution rate observed in the CG treatment is consistent with those observed in dairy cows grazed on pastures with high herbage allowance (BARGO et al., 2002). In other words, the low substitution rate observed in the TE treatment can be explained by the lower intake of supplement (MCEVOY et al., 2008) and/or by the action of condensed tannins in the rumen. The reduction of supplement ingestion resulting from the inclusion of *Acacia mearnsii* tannin extract has been previously reported by other authors (GRIFFITHS et al., 2013). Tannins have also been associated with a reduction in ruminal degradation of organic matter (OM) (ÁVILA et al., 2015; KOZLOSKI et al., 2012), and proteins and fibrous carbohydrates (ORLANDI et al., 2015). The fibrous carbohydrates that escape from ruminal fermentation have low post-ruminal digestibility, causing a reduction in the consumption of the digestible OM and of liquid energy per kg of ingested DM. Therefore, the lowest substitution rate recorded in the TE treatment may have been the result of a mechanism for maintaining the daily energy intake, once the foods with high OM digestibility, such as the pastures used in this study, have rumen fill with lower importance in regulating consumption (KETELAARS; TOLKAMP, 1992).

Milk production was higher ($P < 0.01$) in the supplemented animals than it was in the unsupplemented animals (+ 2.6 kg·day^{-1}), but it did not vary with the inclusion of tannin extract compared to the supplementation exclusively with the energy concentrate (Table 3). These results can be explained by the increase in liquid energy obtained exclusively from the pasture, which was not enough to meet the nutritional needs of dairy cows with 620 kg of LW producing 21 kg milk·day^{-1}, resulting in a negative energy balance and higher concentration of NEFA in the blood of animals that did not receive supplementation. These results corroborate the findings of Ribeiro-Filho et al. (2009b) who reported that dairy cows in pastures with abundant annual ryegrass produced the equivalent to the animals in this study, sustaining a positive energy balance. However, the cows used by the aforementioned authors were lighter (532 ± 56.5 kg) and the pasture used had higher CP contents and lower NDF contents than the ones in this study. In addition, the pasture used by Ribeiro-Filho et al. (2009a), had characteristics that probably contributed to a higher daily intake of pasture (16.6 kg DM) than those in this study.

Table 3. Dry matter intake, productive performance, and energy balance of dairy cows in mixed pastures of oat (*Avena sativa*) and annual ryegrass (*Lolium multiflorum* Lam.) without supplementation (WS) or supplemented with corn (CG) or corn grain + 80 g of the *Acacia mearnsii* tannin extract (TE).

Item	Treatment				Contrast (P value)	
	WS	CG	TE	CV[1]	WS×S[2]	CG×TE
Dry matter intake (kg day^{-1})						
Forage	14.9	13.4	14.7	19.5	0.501	0.367
Supplement	0.00	3.33	3.02	6.6	<0.001	0.013
Total	14.9	16.7	17.7	6.0	<0.001	0.044
Milk production (kg·day^{-1})	20.9	23.7	23.4	11.7	0.001	0.739
4% FCM production (kg·day^{-1})[3]	17.9	20.1	19.4	13.6	0.009	0.340
Milk composition						
Fat (%)	3.07	3.04	2.94	174	0.550	0.502
Protein (%)	3.38	3.39	3.37	53.8	0.928	0.639
Casein (%)	2.61	2.63	2.61	55.6	0.765	0.606
Urea nitrogen (mg 100 mL^{-1})	12.6	10.4	10.6	19.5	0.002	0.854
Fat production (g·day^{-1})	635	710	670	19.5	0.095	0.273
Protein production (g·day^{-1})	698	800	785	12.6	0.001	0.572
Live weight (kg)	621	622	622	2.4	0.817	0.852
Consumed NE_L (MJ·day^{-1})[4]	96.9	113	120	5.6	<0.001	0.036
Consumed MP (kg·day^{-1})[5]	1.33	1.52	1.58	65.0	0.001	0.194
Energy balance (MJ·day^{-1})[6]	-2.69	6.57	15.5	131	0.014	0.042
Protein balance (kg·day^{-1})[7]	-0.07	0.00	0.13	8550	0.074	0.142
NEFA (mmol·L^{-1})[8]	0.37	0.29	0.27	24.1	<0.001	0.698

[1]Coefficient of variation; [2]Comparison between unsupplemented and supplemented animals (mean of the CG and TE treatments); [3]Milk production corrected to 4% fat; [4]Net energy for lactation; [5]Metabolizable protein; [6]Calculated as the difference between NE_L consumption minus the requirements for NE_L; [7]Calculated as the difference between MP consumption minus the MP requirements; [8]Non-esterified fatty acids in the blood.

Supplement supply caused a NE_l increase of approximately 20 $MJ \cdot day^{-1}$, leading to increased milk and protein production. The best diet balance in the supplementation treatments resulted in the reduction in blood contents of NEFA as well as in the reduction in the milk urea nitrogen (MUN) concentration. The existence of negative correlations between NEFA concentration in the blood and the energy balance of dairy cows (REIST et al., 2002), and between MUN and the efficiency of converting the consumed protein into true milk protein (NOUSIAINEN et al., 2004), are well documented in the literature.

The lack of effects of tannin extract on milk production and on the efficiency of using N may be explained by the protein contents of the pasture (≈ 160 $g \cdot kg^{-1}$ DM) and yield potential of the animal. The protein balance (consumption of metabolizable protein – requirement of metabolizable protein) did not differ between treatments, and had near-zero values (Table 3). These results confirm that it is not necessary to provide protein supplementation to dairy cows in winter pastures with protein contents of approximately 160 $g \cdot kg^{-1}$ DM and productive potential between 20 and 25 kg of milk per day. The protein contents of the pasture are directly related to its use efficiency, as ruminal nitrogen loss depends on the CP content of the diet. The increase in the CP contents of the diet is associated with the concentrations of ammonia in the rumen and of urea in the blood, and with the increase of urea excretion into the urine and milk (COLMENERO; BRODERICK, 2006). In the present study, the higher values of true digestible protein considering that the microbial synthesis in the rumen was limited more by PDIN of the pasture than by PDIE (Table 1) indicated that the quantity of the rumen degradable protein intake in animals without supplementation was greater than the availability of energy for microbial growth (INRA, 2007). The excess intake of degradable nitrogen would cause urea nitrogen losses in milk and urine. Therefore, a search for a balance between the PDIE and PDIN contents is recommended (FAVERDIN et al., 2003). The use of protein supplementation in the conditions tested in this experiment would likely result in lower efficiency in using consumed nitrogen, with no responses in milk production compared to energy supplementation.

Conclusions

Energy supplementation is a tool for improving the nutritional profile of dairy cows in oat and ryegrass pastures, resulting in an increase in the total consumption of DM, an increase in productive performance, and reduction in nitrogen urea content of the milk. Including tannin extract from *Acacia mearnsii* did not improve the productive performance of these animals, but it did help reduce the substitution rate and improve the energy balance of cows in mid-lactation. Future studies should be conducted to evaluate the effects of tannin extract on animals of higher production potential, and on the profile of milk fatty acids.

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