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Influence of inclusion of low doses of tannin blends in beef cattle dietary supplements on *in vitro* and *in situ* digestibility of nutrients in some feedstuffs

Influência da inclusão de baixas doses de taninos em suplementos para bovinos de corte sobre a digestibilidade *in vitro* e *in situ* dos nutrientes de alguns alimentos

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Highlights

Tannins have minimal effect on in vitro digestion of ordinary feedstuffs.
Tannins reduce key ruminal potentially degradable fractions of cottonseed meal.
Tannins increase the ruminal undegradable fraction of soybean and cottonseed meal.
Tannins do not affect the ruminal digestion of the fibrous components in roughages.

Abstract

The objective of this study was to evaluate the effects of the daily supplementation with a low dosage of a blend of condensed and hydrolysable tannins on the in vitro and in situ digestibility of protein and fibrous feedstuffs. In situ ruminal incubation assays were conducted on seven protein and five roughage feedstuffs with and without tannin supplementation (1 g kg^{-1} DM intake). From these same cattle, rumen

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fluid was collected for in vitro incubations of the same feedstuffs. In vitro assays we evaluated the gas production (GP) up to 24 h and, sequentially, digestibility of dry matter (IVDMD), crude protein (IVCPD), fiber (IVNDFD), and ammonia nitrogen (NH_3-N). For in situ assays, the disappearance curves were modeled and then the potentially degradable (B), digestion rate (k_d), and undegradable (U) fractions were estimated. Of all the variables studied, only IVCPD of soybean meal showed effect ($P<0.05$) with the addition of dose of tannins. The supplementation of tannin affected only B of the DM and CP of the cottonseed meal. The k_d of DM and CP increased with the supplementation of the tannin blend only for Marandu (dry season), soybean and cottonseed meal. Additionally, the k_d of the DM of corn silage was reduced. The U of CP was affected by the increase of tannin supplementation for soybean and cottonseed meal. Daily supplementation with a low dose of tannin blend for grazing cattle affects for grazing cattle affects the ruminal digestibility of some of the evaluated feedstuffs, which are soybean and cottonseed meal.

Key words: Feed additives. Grazing cattle. Rumen degradability. Tannins.

Resumo

O objetivo com este estudo foi avaliar os efeitos da inclusão de uma mistura de taninos condensados e hidrolisáveis em suplementos sobre a digestibilidade in vitro e in situ de alguns alimentos proteicos e volumosos. Sete alimentos proteicos e cinco volumosos foram avaliados in situ no rúmen de bovinos de corte em pastejo recebendo suplementos com e sem taninos (dose de taninos = 1g kg^{-1} MS). Destes mesmos animais, foi coletado líquido ruminal para incubação in vitro dos alimentos. Nos ensaios in vitro avaliou-se a produção de gás (PG) até 24 h e, sequencialmente, a digestibilidade da matéria seca (DMS), proteína bruta (DPB), fibra (DFDN) e nitrogênio amoniacal (N-NH_3). Para os ensaios in situ, as curvas de desaparecimento foram modeladas e, em seguida, as frações potencialmente degradáveis (B), taxa de digestão (k_d) e indigestível (U) foram estimadas. De todas as variáveis estudadas, apenas a DPB do farelo de soja apresentou efeito ($P<0,05$) com a adição de taninos. A suplementação com taninos afetou apenas a fração B da MS e PB do farelo de algodão. O kd da MS e PB aumentaram com a inclusão de taninos para o capim Marandu (estação seca), farelo de soja e farelo de algodão. Adicionalmente, o kd da MS da silagem de milho foi reduzido. A fração indisgestível (U) da PB do farelo de soja e farelo de algodão foram afetadas pela suplementação com taninos. A inclusão de baixas doses de um *blend* de taninos em suplementos para bovinos em pasto afeta a digestibilidade ruminal de alguns dos alimentos avaliados, notadamente em farelo de soja e farelo de algodão.

Palavras-chave: Aditivo alimentar. Bovinos em pastejo. Degradabilidade ruminal. Taninos.

Introduction

Moderate amounts (2.0 to 4.0 g DM) of tannins extracted from certain tree species have been reported to have beneficial effects on protein metabolism in ruminants. They decrease rumen degradation of dietary protein and increase absorption of amino acids in the small intestine (Aerts et al., 1999). However, the effect of tannin types may compromise microbial digestion of fibrous materials. Therefore, the addition of tannins as a dietary supplement has been mainly studied to increase the supply of metabolizable protein to grazing ruminants without negatively affecting ruminal fiber digestion (Martello et al., 2020; Cidrini et al., 2022).

The effect of tannin types (hydrolyzed and condensed) on rumen function differ. Condensed tannins can be associated with antinutrional factors that reduce the palatability and digestibility of the feed, interfere with dry matter intake and complex with dietary components such as protein (Schofield et al., 2001), other polymers such as cellulose, hemicellulose and pectin, and minerals (McSweeney et al., 2001). Hydrolysable tannins, being easily hydrolysable, are considered toxic to animals. However, like the condensed tannins, the hydrolysable tannins can complex with proteins (McSweeney et al., 2001).

The use of tannins in grazing cattle diets, which inherently consume complex profiles of fibrous polymers, may decrease the ruminal digestibility of crude protein in feedstuffs while still reducing ammoniacal nitrogen. In this situation, tannins may reduce dry matter intake and fiber digestibility because most fibrolytic bacteria exclusively use ammonia as a substrate for their growth and activity, requiring, therefore, adjustments in diet formulation. The objective of this study was to evaluate the effects of the inclusion of a blend of condensed and hydrolysable tannins in the daily supplement for grazing beef cattle on the *in vitro* and *in situ* digestibility of some nutritional components of proteins and roughage feedstuffs commonly fed to beef cattle.

Material and Methods

This experiment was carried out at the Beef Cattle Research Sector from and Animal Nutrition Laboratory of Universidade Federal de Mato Grosso, Cuiabá, Brazil. The experimental protocol adhered to the Ethical Principles for Animal Research and was approved by the institutional Committee for Ethics in the Use of Animals (protocol number 23108.207702/2017-76).

Feedstuffs

Incubated feeds comprised protein feedstuffs: corn dried distillers' grain (DDG), corn wet distillers' grains (WDG), xylose-treated soybean meal (soy pass®), soybean meal, sunflower meal, cottonseed meal, cottonseed cake, and roughage feedstuffs: Marandu grass (*Urochloa brizantha* cv. Marandu) rainy season, Marandu grass (dry season), Tanzânia grass (*Panicum maximum* cv. Tanzânia - rainy season), corn silage and 'Tyfton-85' bermudagrass (*Cynodon* sp) hay (Table 1). Marandu grass (rainy and dry season) and Tanzânia grass were not evaluated for in vitro incubations, except for cottonseed cake.

Table 1
Chemical composition of the feedstuffs used in vitro and in situ assays

Feedstuffs	Composition (% DM basis)[3]									
	DM	OM[4]	CP[5]	EE[6]	aNDF[7]	NDF_{ap}[8]	NDIN[9]	iNDF[10]	ADF[11]	ADIN[12]
Protein										
Corn DDG[1]	92.34	98.41	35.63	6.37	50.02	49.23	3.03	5.68	9.92	0.96
Corn WDG[2]	30.24	98.65	32.96	6.49	47.18	46.29	1.56	4.80	11.82	0.45
Soy pass®	90.95	93.25	49.93	2.42	16.12	15.42	4.61	2.43	5.01	0.43
Soybean meal	91.08	93.06	49.25	2.36	15.53	14.66	1.97	1.91	7.04	0.32
Sunflower meal	91.52	93.88	34.11	1.79	45.48	44.54	0.76	33.58	27.11	0.36
Cottonseed meal	91.46	94.17	40.30	0.81	37.48	36.58	1.42	13.91	17.43	1.09
Cottonseed cake	93.08	94.83	29.00	1.212	38.64	38.22	0.62	25.43	13.51	0.90
Roughage										
Marandu grass (rainy season)	29.85	92.50	7.30	-	66.98	66.07	1.03	21.42	-	-
Marandu grass (dry season)	37.57	84.17	4.16	-	74.35	73.48	0.54	32.44	-	-
Tanzânia grass	29.47	91.35	7.28	-	76.33	75.46	1.67	24.63	-	-
Corn silagem	26.68	89.75	6.99	-	48.34	47.43	0.52	23.69	23.01	0.24
Tyfton-85 hay	91.14	92.75	11.09	-	73.85	72.97	1.50	27.78	-	-

[1]DDG, dried distillers grains; [2]WDG, wet distillers grains; [3]DM, dry matter; [4]OM, organic matter; [5]CP, crude protein; [6]EE, ether extract; [7]aNDF, neutral detergent fiber assayed with a heat stable amylase; [8]NDFap, neutral detergent fiber corrected for residual ash and protein; [9]NDIN, neutral detergent insoluble nitrogen; [10]iNDF, indigestible neutral detergent fiber; [11]ADF, acid detergent fiber; [12]ADIN, acid detergent insoluble nitrogen.

The roughage feedstuffs and corn WDG, which had high moisture content were pre-dried in force-air oven at 55 ºC for 72 h. Subsequently, the feedstuffs of in vitro incubation were ground using a 1 mm porosity sieve (Goering & Van Soest, 1970), in Willey mill (Model 4; Thomas Scientific, Swedesboro, NJ); and for in situ incubation the protein and roughage feedstuffs were ground to a 2 and 3 mm porosity sieves, respectively.

Animals and treatments

Four rumen-cannulated Nellore bulls, with an average body weight of 578±37 kg and 27 months of age were used in this study. Prior to the experiment, the animals were weighed and randomly allocated into four individual paddocks, each of which was 0.25 ha and contained *Urochloa brizantha* cv. Marandu. The paddocks were equipped with water troughs and individual feedbunks.

The treatments consisted of either the inclusion or exclusion of tannins in the supplements offered to the donor animal of ruminal inoculum. Two of the four animals received supplementation with a blend of commercial tannins composed of 70% of condensed tannin (Quebracho extract - *Schinopsi lorentzii*) and hydrolysable tannin (*Castanea* spp.; Silvafeed-Bypro®, Silvateam-Inudor S.A., Argentina) to provide a daily intake of 1g kg^{-1} of total DM intake. The other two animals received supplementation without additives (Table 2).

Table 2
Ingredients and nutrients composition of the supplement and forage fed to rumen fluid donors

Ingredients	Supplements		*Urochloa brizantha* cv. Marandu
	Without Tannin	With Tannin	
Ingredient Composition (% DM basis)			
Ground corn	68.00	68.00	-
Soybean meal	30.00	30.00	-
Mineral premix[1]	2.00	2.00	-
Tannin	-	0.1	-
Chemical Composition (% DM basis)			
Dry matter	88.29	88.35	33.55
Organic matter	94.74	94.75	92.54
Crude protein	22.87	22.35	6.54
NDFap[2]	-	-	68.92
Ether extract	1.66	1.56	1.27

[1]Mineral premix contained (g d^{-1}): calcium 14.0 g; phosphorus 11.0 g; sodium 7.0 g; magnesium 9.0 g; sulpur 13.5 g; potassium 54.0 g; cobalto 0.9 mg; copper 90.0 mg; iodine 4.5 mg; manganese 180.0 mg; selenium 0.9 mg; zinc 270.0 mg and iron 450.0 mg. [2]NDFap, neutral detergent fiber corrected for ash and protein.

The animals were allowed to adapt to the experimental diet for 14 days. The supplements consisted of ground corn, soybean meal and mineral mixture with or without the tannin (Table 2). The supplements were delivered daily at 10h00, at 0.81% of the initial body weight.

In vitro gas production and digestibility

To measure in vitro gas production and digestibility, pre-dried feedstuffs were weighed (0.5 g) and transferred into 120 mL glass vials.

The McDougall buffer solution (McDougall, 1948) was prepared one day prior to start of the incubation and was maintained in a water bath at 39 ºC. On the day of incubation, the reducing solution using sodium sulfite anhydrous (Na_2SO_3) and resazurine 0.1 (w/v) solution were added the buffer solution and kept continuously purged under free-oxygen CO_2 for 40 min.

Rumen fluid was obtained separately from each animal at 06h00, filtered through cheesecloth with a pore size of 250 μm and stored in insulated thermos without leaving empty spaces. Then, 40 mL of butter solution was added into each vial, followed by 10 mL of rumen fluid, resulting in a rumen fluid: buffer ratio of 1:4 (v/v). The vials were immediately sealed with rubber caps and aluminum rings and maintained in water bath (Dubnoff Agi. Orbital SL-158 Solab) at 39 °C in constant agitation. The volume of gas production was recorded at 6, 12, and 24 h of incubation, using the semiautomatic reading technique described by Theodorou et al. (1994).

The in vitro study was performed over four consecutive days and for each in vitro incubation (run), we prepared four glass vials per treatment (referring to two replicates per inoculum of each animal) to measure gas production over time and to evaluate in vitro digestibility. Four blanks (only ruminal fluid and buffer solution) per treatment were used per run.

To evaluate in vitro digestibility of the dry matter (IVDMD), crude protein (IVCPD), and fiber (IVNDFD) in 24 h of incubation, the vials removed from the water bath were immediately placed in an ice bath to stop microbial fermentation. After opening the vials, the contents were filtered in a crucible with porosity of 2 mm to obtain residual DM as well as to CP and aNDF analysis.

The in vitro ammoniacal nitrogen content (NH_3-N) was measured from an aliquot of 2 mL of each vial removed after 24 h incubation, then 0.05 mL of H_2SO_4 (1:1; v/v) was added (Batista et al., 2016), centrifuged at 10,000 rpm for 10 min at a temperature of 4 ºC, and stored at -20 ºC for further analysis by the method of colorimetric reaction catalyzed by indophenol (method INCT-CA no. N-006/1).

In situ degradability

In situ degradability was assessed by weighing feedstuffs into non-woven textile filter bags (NWT - 100 g m^2), to provide about 20 mg cm^2 of bag area (Nocek, 1988), in quadruplicate for each tannin × feedstuffs × time. The bags were introduced into the rumen in reverse sequence at 96, 72, 48, 24, 12, 8, 4 and 2 h until their joint removal at time zero.

After removal from the rumen, bags were washed with cold tap water, and frozen (-30 ºC) for 24 h to help remove microbial attachment to feed particles. Once thawed, the bags were washed with cold tap water in a laundry machine by applying three washing-cycles of 20 min each. Zero-time values were measured by washing four bags per ingredient as described above without previous incubation in the rumen. Afterwards, bags were pre-dried in forced-air oven at 55 ºC during 72 h and weighed to determine the residual DM as well as to CP and aNDF content.

Estimation of in situ digestion kinetics

To estimate the digestion kinetics of the protein (DM and CP) and roughage (DM and aNDF) feedstuffs, samples were incubated in the rumen of bulls fed supplements with and without tannin (tannin×feedstuffs arrangement). The disappearance (degradation) profiles of the arrangements were modeled using the decreasing exponential model (Mertens, 1977).

$$R_t = B \times (\exp(-k_d t)) + U \qquad (1)$$

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[8] Denotes the presence or absence of tannin in the supplement offered to bulls.

The variable R_t is the remaining residue (i.e., DM, CP and aNDF) after incubation of the feed sample in time *t*; B is the potentially degradable fraction (B_0-U), k_d is constant fractional digestion rate of the fraction B, and U is the undegradable fraction of DM (protein and roughage feedstuffs), CP (protein feedstuffs) or aNDF (roughage feedstuffs).

The general structure attributed to the disappearance (digestion) models was $Y_t = R_t + e_t$, for $Y_t \sim Normal(R_t, \sigma^2_{Y_t})$, and the nonlinear functions (R_t) used was Eq. (1). The error (e_t) was assumed follow an independent distribution $(e_t \sim Normal\,(0, \sigma^2_{Y_t}))$. A more complete description of the models can be given by:

$$Y_{ij} = f(\Theta_{ij}, t_{ijkl}) + e_{ijkl} \tag{2}$$

In this model, $Y_{ij} = R_t = f(\Theta_{ij}, t_{ijkl})$ is the expected value of residue (DM, CP and aNDF) at a given incubation time *t*, the greek letter Θ denotes the number of estimated parameters of the disappearance model (Eq.(1)) evaluated to described all set of disappearance profiles obtained by in situ incubation. The animal within run *animal(run)* (u_k *(ij)*) was considered a random experimental unit and the residue in the k-th *animal(run)* observed at the l-th time. Thus, there were *k*=1,⋯,*rk*(*rk*=16) experimental units *(animal (run))* being 8 units for each *i×j* (tannin×feedstuff) arrangement, with four replicates per *l*=1,⋯,*rl*(*rl*=8) time taken as repeated measurements for each *animal(run)* within each arrangement *i×j.* The tannin×feedstuff arrangements were considered fixed effects, resulting in the following treatments: roughage feedstuffs (2×5=10 for DM and aNDF) and protein feedstuffs (2×7=14 for DM, and 2×6=12 for CP).

The variances (covariance) were modeled according to the following expressions (Pinheiro & Bates, 2006):

$$\sigma^2_t = \sigma^2 \tag{3}$$

$$\sigma^2_t = \sigma^2 \left| f(\Theta_{ij}, t_{ijkl}) \right|^{2\rho} \tag{4}$$

$$\sigma^2_t = \sigma^2 \left| f(\Theta_{ij}, t_{ijkl}) \right|^{2\rho_c} \tag{5}$$

The common residual standard deviation of the population is represented by σ. The Eq. (3) corresponds to the homoscedastic assumption to mimic the variance over time for all non-linear models. The power of the mean variance (*varPower*) was tested by a dimensionless exponent ρ (Eq. (4)), which can also be adjusted for each tannin×feedstuffs arrangement, which denoted by letter c (ρ_c; Eq. (5) (Pinheiro & Bates, 2006).

In relation to the variance-covariance (var-covar) structure of random effects, we tested the diagonal matrix, and the general positive-definite var-covar matrix or unstructured matrix (Pinheiro & Bates, 2006). Furthermore, as in situ disappearance profiles were generated from time-repeated measurements in the same experimental unit, a continuous-time autoregressive variance (correlation function, corCAR1) was used as recommended by Pinheiro and Bates (2006).

The models were created by assigning parameters representing each tannin×feedstuff arrangement and random effects in parameters. The best model was select using the information theory approach (Burnham & Anderson, 2004), based on the Akaike criterion corrected for small and finite samples of the r-th model (AICc$_r$). The differences among AICc$_r$ values (Δ_r), likelihood probabilities (w_r), and the evidence ratio or relative likelihood (ER_r) were computed as that suggested by Vieira et al. (2012).

Therefore, based on the estimated covariance matrix of $f(\Theta_{ij}, t_{ijkl})$ and parameters estimates from the data set, the reliable confidence intervals (95% CI) were estimated for $\hat{f}(\hat{\Theta}_{ij}, t_{ijkl})$:

$$95\%CI = \hat{f}(\hat{\Theta}_{ij}, t_{ijkl}) \pm t_{(1-\propto,\ df)} SE_{\hat{f}(\hat{\Theta}_{ij},t_{ijkl})} \quad (6)$$

The estimated $SE_{\hat{f}(\hat{\Theta}_{ij},t_{ijkl})}$ is the standard error of the estimate $SE_{\hat{f}(\hat{\Theta}_{ij},t_{ijkl})}$. $t_{(1-\propto,\ df)}$. is the critical value of the two-tailed distribution of Student's *t* test using the significance level α = 0.05 and the degrees of freedom used to calculate the estimate, $df = n - \theta$ which *n* represents the number of observations and θ corresponding to the number of parameters $\hat{f}(\hat{\Theta}_{ij}, t_{ijkl})$, including the parameters of the variance functions of Eqs. ((1) and (3) to (5)).

Chemical analysis

Feedstuffs samples previously ground by Willey mill type with 1-mm porosity screen sieve were analyzed for determination of the chemical composition as DM (INCT-CA method G-003/1), CP, aNDF (INCT - CA method F-001/1), organic matter (OM), ether extract (EE; INCT - CA method G-004/1), neutral detergent fiber corrected for residual ash and protein (NDFap; INCT-CA method M-002/1), acid detergent fiber (ADF, INCT-CA method F-003/1), neutral detergent insoluble nitrogen (NDIN; INCT-CA method N-004/1), acid detergent insoluble nitrogen (ADIN; INCT-CA method N-005/1), indigestible NDF (iNDF; INCT-CA method F-008/1) using F57 bags (Ankom Technology Corp., Macedon, NY, USA) according to the techniques described by Detmann et al. (2012) (Table 1).

The residue of all incubated feedstuffs was oven-dried at 105 °C for 16 h and weighed to determine IVDMD and ISDMD. Sequentially, the IVNDFD and ISNDFD of roughage feedstuffs were analyzed using a heat-stable α-amylase, omitting sodium sulfite (method INCT-CA no. F-002/1) kept at 100 °C for 1 h. For the protein feedstuffs, the IVCPD and ISCPD were estimated according to INCT-CA method no. N-001/1 (Detmann et al., 2012).

To determine the in vitro concentration of ammoniacal nitrogen (NH_3-N), 2 mL of each vial were sampled with an addition of 0.05 mL of 1:1 (v/v) sulfuric acid (Batista et al., 2016) centrifuged at 10.000 rpm for 10 min at a temperature of 4 ºC and stored at -20 ºC for further analysis by the method of colorimetric reaction catalyzed by indophenol (method INCT-CA no. N-006/1).

Experimental design and statistical analysis

In vitro gas production, the in vitro and in situ digestibilities of DM, CP and aNDF were evaluated considering a completely randomized design.

The in vitro variables were analyzed using the MIXED procedure of SAS (version 9.4). The inclusion or not of tannins was considered as fixed effect and animal as random effect. The LSMEANS option was used to obtain means for experimental treatments. In all procedures it was considered significant when P<0.05.

The combinations of the in situ disappearance model with the CAR1 and each variance functions (testing the homoscedasticity) were adjusted using the nlme function of the nlme package of R (Pinheiro & Bates, 2006). The tannin×feedstuff arrangements were considered a fixed effect and animal within run was random. The parameter estimates were evaluated considering a confidence interval of 95% as follows: comparisons between CI 95% values were done by the difference between the lower limit of the larger mean $(Ll_{\hat{\hat{X}}} = \hat{\hat{X}}_l - 95\%CI_{\hat{\hat{X}}l})$ and the upper limit of the smaller mean $(Ul_{\hat{\hat{X}}s} = \hat{\hat{X}}_s - 95\%CI_{\hat{\hat{X}}s})$. Therefore, if $Ll_{\hat{\hat{X}}l} > Ul_{\hat{\hat{X}}s} \therefore$ the parameter estimates for both treatments, without and with tannin, not equal.

Results and Discussion

Tannin supplementation provided by adapted ruminal inoculum of donor bulls did not affect in vitro gas production in 24 h for all feedstuffs (P>0.05), IVDMD of protein feedstuffs (Table 3), as well as the IVDMD and IVNDFD of roughage feedstuffs (Table 4). On the other hand, the IVCPD was reduced for soybean meal, in which inoculum with 1% tannin was 3.39% lower than that without tannin. (P<0.05; Table 3).

Table 3

Effects of tannin in the supplement for bovine inoculum donors on in vitro digestibility of dry matter (IVDMD [1]) and protein (IVCPD [1]) of protein feedstuffs

Variables	Supplements		SE [2]	p-*value*
	Without Tannin	With Tannin		
Corn DDG				
GP 6 [3]	28.5	26.0	2.12	0.220
GP 12 [3]	54.5	52.2	2.61	0.454
GP 24 [3]	81.5	78.7	3.17	0.489
IVDMD [1]	328.4	322.9	13.00	0.732
IVCPD [1]	596.1	604.2	8.27	0.138
Corn WDG				
GP 6 [3]	29.7	27.2	2.49	0.178
GP 12 [3]	55.9	54.4	2.78	0.502
GP 24 [3]	86.2	83.1	4.06	0.410
IVDMD [1]	364.2	359.2	20.82	0.649
IVCPD [1]	642.6	632.0	9.78	0.465
Soy pass®				
GP 6 [2]	39.2	36.3	2.28	0.297
GP 12 [3]	80.7	77.9	2.33	0.346
GP 24 [3]	125.5	124.1	2.97	0.732
IVDMD [1]	589.0	575.7	12.34	0.467
IVCPD [1]	573.8	531.1	22.39	0.065
Soybean meal				
GP 6 [3]	41.9	37.0	2.33	0.132
GP 12 [3]	89.7	83.4	2.72	0.137
GP 24 [3]	138.8	132.4	2.99	0.170
IVDMD [1]	7491.	700.9	21.26	0.132
IVCPD [1]	855.6	827.0	15.58	0.048
Sunflower meal				
GP 6 [3]	29.3	27.1	1.75	0.249
GP 12 [3]	53.0	52.0	1.36	0.611
GP 24 [3]	79.3	76.3	1.46	0.184
IVDMD [1]	530.9	526.9	11.01	0.789
IVCPD [1]	906.2	903.6	8.27	0.766
Cottonseed meal				
GP 6 [3]	32.1	30.4	2.04	0.564
GP 12 [3]	60.1	61.7	3.07	0.714
GP 24 [3]	81.1	86.3	3.28	0.295
IVDMD [1]	467.8	456.3	13.62	0.249
IVCPD [1]	768.5	760.0	16.72	0.510

[1] IVDMD and IVDMD (g kg^{-1} DM); [2] SE, standard error; [3] GP 6, GP 12, and GP 24, in vitro cumulative gas production in 6, 12, and 24 h, respectively (mL g^{-1} DM).

Table 4
Effects of tannin in the supplement for bovine inoculum donors on in vitro digestibility of dry matter (IVDMD [1]) and fiber (IVNDFD [1]) of roughage feedstuffs

Variables	Supplements		SE [2]	p-*value*
	Without Tannin	With Tannin		
Corn silage				
GP 6 [3]	20.0	19.5	1.46	0.590
GP 12 [3]	39.4	39.6	2.77	0.875
GP 24 [3]	78.7	76.2	4.59	0.247
IVDMD [1]	358.1	355.3	5.95	0.753
IVNDFD [1]	92.9	81.2	13.36	0.377
Tyfton-85 hay				
GP 6 [3]	14.8	13.7	0.98	0.382
GP 12 [3]	27.0	25.7	1.87	0.369
GP 24 [3]	44.9	40.4	3.05	0.059
IVDMD [1]	272.3	274.6	8.58	0.650
IVNDFD [1]	238.0	239.2	9.31	0.900

[1] IVDMD and IVDMD (g kg^{-1} DM); [2] SE, standard error; [3] GP 6, GP 12, and GP 24, in vitro cumulative gas production in 6, 12, and 24 h, respectively (mL g^{-1} DM).

The supplementation of tannins did not change (P>0.05) the in vitro ammoniacal nitrogen content (NH_3-N) of all the feedstuffs (Table 5). This is consistent with previous studies that have reported no significant effect of tannin supplementation on ammoniacal nitrogen content (Martello et al., 2020).The fitted models consisted of combinations of Eq. (1) with the variance functions described by equations Eq. (3) to Eq. (5), corCAR1, the random effects in the parameters, and variance-covariance matrix modeled for the random effects, were considered the fitted models, which summoned to 90 models tested for the in situ digestibility in each tannin×feedstuffs arrangement: ISDMD of protein and roughage feedstuffs, ISCPD, and ISNDFD protein and roughage feedstuffs, respectively. According to the selection of multimodels ranked by $AICc_r$. Table 6 shows the models selected for the nutrients' digestibility for these feedstuffs. It should be noted that only the ISDMD of both feedstuffs' types were unanimous, i.e., w_r <0.95.

Table 5
Effect of tannin on the in vitro ammoniacal nitrogen content (NH_3-N) in mg dL^{-1}

Feedstuffs	Supplements		SE [2]	p-*value*
	Without Tannin	With Tannin		
Protein feedstuffs				
Corn DDG	8.04	8.58	0.890	0.673
Corn WDG	8.58	8.58	0.671	0.998
Soy Pass®	10.76	10.18	1.031	0.691
Soybean meal	17.38	15.87	2.884	0.714
Sunflower meal	12.29	13.87	2.241	0.622
Cottonseed meal	15.42	14.32	1.570	0.622
Roughage feedstuffs				
Corn silage	6.39	5.94	0.820	0.699
Tyfton-85 hay	7.79	8.06	0.740	0.798
IVDMD [1]	272.3	274.6	8.58	0.650
IVNDFD [1]	238.0	239.2	9.31	0.900

[1] SE: standard error.

All the models selected for in situ digestion (Table 6) were constituted by a heterogeneous variance function, power of the mean, which has a mean scaling parameter for each tannin×feedstuffs arrangement (ρ_c; Eq. 5). Only the ISDMD of roughage presented an unstructured var-covar matrix (Symm), the other variables the best fit were with the diagonal var-covar matrix. Moreover, it can be noted that, only the best model selected for ISCPD for protein feedstuffs presented a continuous time autoregressive correlation function (corCAR1) (Table 6).

Table 6

Information criteria of the best-fitted models for in situ degradability of dry matter (ISDMD), crude protein (ISCPD), and fiber (ISNDFD) of protein and roughage feedstuffs

Feedstuffs	RE/v-c Matrix [1]	σ_t^2/corCAR [2]	$AICc_r$ [3]	Δ_r [3]	w_r [3]	ER_r [3]	Θ_r [3]
Protein							
ISDMD	B, U/*Diag*	*varPower #*	-9509.2	0.0	0.952	1.0	59
ISCPD	kd/*Diag*	*varPower*/corCAR1#	-8821.8	0.0	0.483	1.0	51
	B, kd/*Diag*	*varPower*/corCAR1	-8819.7	2.1	0.171	2.8	52
	kd, U/*Diag*	*varPower*/corCAR1	-8819.7	2.1	0.171	2.8	52
	B, kd/*Symm*	*varPower*/corCAR1	-8818.9	2.9	0.113	4.3	53
	B, kd, U/*Diag*	*varPower*/corCAR1	-8817.7	4.1	0.061	7.9	53
Roughage							
ISDMD	B, kd, U/*Symm*	*varPower #*	-3519.2	0.0	1.00	1.0	47
ISNDFD	B, kd/*Diag*	*varPower #*	-2930.3	0.0	0.517	1.0	43
	B, kd/*Diag*	*varPower*/corCAR1	-2928.2	2.1	0.177	2.9	44
	B, kd, U/*Diag*	*varPower*	-2928.2	2.1	0.177	2.9	44
	B, kd/*Symm*	*varPower*/corCAR1	-2926.8	3.5	0.088	5.9	45

[1] RE - random effects on decreasing exponential model parameters (Eq. (1)); and v-c Matrix - variance-corvariance matrix to random effects - diagonal variance matrix *(Diag)* and symmetric matrix *(Symm);*

[2] heterogeneous variance function (*varPower,* power of the means), more details of Eq. (6) - (8) are in the text; and correlation function for repeated measurements over time in the same experimental unit (corCAR1).

[3] $AICc_r$, Akaike's information criterion corrected for r-th model; Δ_r, difference between the $AICc_r$ values; w_r, likelihood probability of the r-th model; ER_r, evidence ratio of th r-th model; Θ_r, number of estimated parameters for r-th model.

#Model that provided the best fit to the data set.

Considering the 95%CI, there was influence of the inclusion of 1% tannins in bull's supplementation on the ISDMD parameters only for cottonseed meal, which the mean of the B parameter reduced 0.144 times in relation to that not supplemented with tannin (Table 7). It should be highlighted that the soy pass® and soybean meal, showed high degradability without the presence of tannin in the supplementation, for this reason the appearance of negative values in the mean and the lower limit of the 95% CI, tending to the minimum of U or the total rumen degradation these feedstuffs. Additionally, there was increase on ISDMD digestion rate [k_d (*/h*)] of 2.44 times for Marandu grass (dry season) and a decreased of 0.75 times for corn silage when 1% of tannin was added to the supplementation of bulls compared to those not supplemented with tannin (Table 7).

Table 7
Estimates of the means and 95% confidence intervals ($\hat{\bar{X}}$ ± 95%CI)[1] of the kinetic parameters of in situ dry matter degradability (ISDMD) of protein and roughage feedstuffs

Feedstuffs	B[2]		k_d[2]		U[2]	
	Without tannin	With tannin	Without tannin	With tannin	Without tannin	With tannin
Protein feedstuffs						
Corn DDG	0.65±0.04	0.61±0.04	0.016±0.005	0.021±0.005	0.20±0.05	0.25±0.04
Corn WDG	0.63±0.05	0.60±0.04	0.019±0.005	0.024±0.006	0.18±0.05	0.21±0.04
Soy pass®	0.63±0.03	0.59±0.03	0.019±0.005	0.027±0.005	-0.01±0.03	0.04±0.03
Soybean meal	0.60±0.02	0.59±0.02	0.024±0.005	0.035±0.006	-0.01±0.02	0.02±0.02
Sunflower meal	0.32±0.02	0.32±0.02	0.076±0.012	0.073±0.012	0.36±0.01	0.36±0.01
Cottonseed meal	0.40±0.03*	0.34±0.02*	0.015±0.005	0.025±0.006	0.31±0.03	0.37±0.03
Cottonseed cake	0.31±0.03	0.29±0.03	0.013±0.005	0.017±0.006	0.36±0.03	0.38±0.04
Roughage feedstuffs						
Marandu grass (rainy season)	0.55±0.10	0.45±0.07	0.012±0.010	0.027±0.011	0.25±0.09	0.33±0.06
Marandu grass (dry season)	0.38±0.09	0.34±0.08	0.016±0.011*	0.055±0.025*	0.46±0.09	0.51±0.08
Tanzânia grass	0.39±0.07	0.34±0.06	0.013±0.010	0.034±0.015	0.48±0.06	0.52±0.05
Corn silage	0.26±0.06	0.27±0.06	0.348±0.097*	0.088±0.023*	0.52±0.04	0.46±0.04
Tyfton-85 hay	0.44±0.06	0.38±0.06	0.015±0.010	0.031±0.011	0.35±0.06	0.40±0.05

[1] The comparison between confidence intervals is shown in Material and Methods section; [2] B (dimensionless; *dmls*), potentially degradable fraction in proportion; k_d (h^{-1}), fractional rate digestion of fraction B per hour; U (*dmls*), undegradable fraction in proportion * the feedstuffs that showed effect among treatments in parameter from 95%CI.

All ISCPD disappearance model parameters of the cottonseed meal were sensitive to tannins when compared to those not supplemented with tannin (decreased 0.44 times the parameter *B* and increased 2.1 and 2.6 times for k_d, and U parameters, respectively). The parameters k_d and were U of soybean meal were also affected by the presence of tannins, which increased 0.84 and 1.6 times, respectively. It should be noted that both the mean and the 95%CI (lower and upper values) for parameter U were estimated with negative values (Table 8), due to the high ruminal degradability of the CP in this feedstuff, the estimate of U tended to zero, similar behavior can be seen in the soy pass®.

Table 8
Estimates of the means and 95% confidence intervals ($\hat{X}$ ± 95%CI)[1] of the kinetic parameters of in situ crude protein degradability (ISCPD) of protein feedstuffs

Feedstuffs	B^2		$k_d{}^2$		U^2	
	Without tannin	With tannin	Without tannin	With tannin	Without tannin	With tannin
Corn DDG	0.65±0.12	0.51±0.09	0.009±0.003	0.018±0.008	0.22±0.12	0.39±0.09
Corn WDG	0.59±0.08	0.65±0.14	0.027±0.010	0.021±0.010	0.28±0.09	0.20±0.14
Soy pass®	1.00±0.14	1.12±0.39	0.010±0.003	0.009±0.005	-0.19±0.14	-0.33±0.40
Soybean meal	0.83±0.06	0.75±0.04	0.021±0.004*	0.040±0.006*	-0.07±0.06*	0.04±0.03*
Sunflower meal	0.42±0.03	0.45±0.03	0.732±0.117	0.832±0.122	0.11±0.01	0.12±0.01
Cottonseed meal	0.57±0.10*	0.32±0.03*	0.009±0.003*	0.026±0.007*	-0.09±0.10*	0.15±0.03*

[1] The comparison between confidence intervals is shown in MATERIALS AND METHODS section; [2] B (dimensionless; *dmls*), potentially degradable fraction in proportion; k_d (h^{-1}), fractional rate digestion of fraction B per hour; U (*dmls*).

The in situ digestion kinetics of roughage feedstuffs did not change with the supplementation of tannins. Unlike what was observed with the U, for the ISCPD of some protein feedstuffs, the estimates for this parameter in the ISNDFD of roughage feedstuffs did not show negative values (Table 9), due to the lower digestibility of this nutrient in relation to CP.

Table 9
Estimates of the means and 95% confidence intervals ($\hat{X}$ ± 95%CI)[1] of the kinetic parameters of in situ neutral detergent degradability (ISNDFD) of roughage feedstuffs

Feedstuffs	B^2		$k_d{}^2$		U^2	
	Without tannin	With tannin	Without tannin	With tannin	Without tannin	With tannin
Marandu grass (rainy season)	0.80±0.17	0.72±0.12	0.010±0.006	0.016±0.007	0.28±0.17	0.32±0.12
Marandu grass (dry season)	0.52±0.11	0.49±0.09	0.010±0.006	0.021±0.008	0.49±0.11	0.51±0.09
Tanzânia grass	0.52±0.10	0.44±0.07	0.011±0.006	0.023±0.013	0.53±0.09	0.58±0.07
Corn silage	0.45±0.13	0.43±0.07	0.008±0.006	0.012±0.006	0.48±0.12	0.51±0.07
Tyfton-85 hay	0.59±0.08	0.55±0.07	0.011±0.006	0.015±0.007	0.38±0.08	0.39±0.07

[1] The comparison between confidence intervals is shown in MATERIALS AND METHODS section; 2 B (dimensionless; dmls), potentially degradable fraction in proportion; k_d (h^{-1}), fractional rate digestion of fraction B per hour; U (dmls), undegradable fraction in proportion.

Protein degradation is affected by both condensed and hydrolysable tannins, as indicated in in vitro assays by Getachew et al. (2008). Taking this into account, it is expected that both can provide to ruminants improvements in the use of dietary N due to the capacity of these polyphenols to form bonds with dietary proteins and to prevent degradation of soluble proteins in rumen. However, it is important to note that the protein-tannin bonds are reversible in the acidic environment of the abomasum, making the proteins available for enzymatic digestion in the small intestine (Arowolo & He, 2018).

By affecting the digestion both protein and carbohydrates, tannins might affect the digestibility of dry matter Van Hoven (1984). In addition to the possible bonds between tannins and proteins and tannins and polysaccharides of dietary origin, these polyphenols also can directly affect ruminal microorganisms, which they interact with the cell wall and extracellular enzymes of multiple bacteria strains by inhibiting the enzymatic action responsible for the transport of nutrients into the cell (McSweeney et al., 2001).

Rivera-Méndez et al. (2016) and Aboagye et al. (2018) studied sources of tannins and low (2 g kg^{-1} DM) and high doses of condensed and hydrolyzed tannins in the feeding of steers, evaluating, among other things, their effects on the efficiency of the use of N. Based on this study, we used an even lower dose of one blend (1 g kg^{-1} DM) these tannins in the daily supplementation of bulls in grazing. Through in vitro and in situ assays we verified whether the tannins ingested and maintained in the rumen fluid would provide slight restriction on the degradability of some nutritional components such as dry matter, protein and fiber, of feedstuffs that often integrate into the diet of supplemented grazing beef cattle.

The reduction of the metabolic activity of ruminal bacteria in the presence of tannins in the ruminal environment can occur when there are interactions between tannins and microbial enzymes of external action and bacterial cell wall components (Mangan, 1988; Jones et al., 1994). From the perspective of digestion kinetics, this deleterious effect of tannins can be measured indirectly by digestion extent and the rate of gas production with in vitro assays, since the gas produced through fermentation is proportional to the microbial metabolism. Conversely, fermentation lag time measured in in vitro assays tends to increase due to a decrease in microbial production associated with feeding tannins. The fact that we did not observe effects in the in vitro gas production up to 24 h, may be related to the very low dose used for daily supplementation (1g kg^{-1} DM), being insufficient to specifically affect the degradation of fibrous and non-fibrous carbohydrates of protein and roughage feedstuffs.

It is possible that with the strategy of daily supplementation of tannins to grazing ruminants, the supply of metabolizable protein be increased, as long as it does not compromise the growth of ruminal microorganisms, consequently, the microbial protein flow towards the small intestine. The greater amount of dietary protein that reaches the small intestine, without compromising the amount of microbial protein flow might provide better efficiency of the use of N in grazing beef cattle receiving strategic supplementation (McMahon et al., 2000; Min et al., 2003). Bearing this in mind, we expected that tannin would affect in a subtle way the degradation of protein (IVCPD) in protein feedstuffs, but this effect only was observed in the soybean meal protein. This led us to consider that, just as with the digestion of carbohydrates, the dosage of tannin blend was very low (1g kg^{-1} DM intake) to provide some restriction in the degradation of protein by the rumen microorganisms of most of the protein feedstuffs we evaluated.

The main nutritional effect of tannins is their binding affinity for proteins that are precipitated, but these polyphenols can also limit the digestion of fibrous components not only by their ability to bind to enzymes, which are inherently proteins, but also to fibrolytic microorganisms substrates, e.g.: cellulase and cellulose, respectively (Jones et al., 1994; Silanikove et al., 2001). This effect on fibrous organic matter digestion could be more evident in high doses of tannin in daily supplementation of cattle or for other grazing ruminants (Rivera-Méndez et al., 2016). Working with ewes, Hervás et al. (2003) observed a reduction in intake with the highest dose of quebracho tannin (166 g kg^{-1} of DM). Conversely, low to moderate doses can be nutritionally beneficial to ruminants, since they do not compromise the digestion of fiber, as well the dry matter intake (Frutos et al., 2000). In our in vitro study, the lack of effect of bull ruminal inoculum supplemented with the tannin blend on fiber digestion (IVNDFD) of roughage feedstuffs, most likely was consequence of very low doses (Frutos et al., 2000) that was insufficient for decreasing fiber digestion. However, it is worth noting that we did not measure the feed intake, since the animals were grazing.

In tropical regions, the strategy of daily tannin supplementation with the purpose of forming tannin-protein bonds may be advantageous for animals grazing in the rainy season. The justification would be the increase in the supply of rumen-undegraded protein (RUP), if the requirement for rumen-degradable protein (RDP) is met before, mainly by the crude protein from the pasture (Santos et al., 2000; Gilani et al., 2005). Therefore, this strategy could avoid the unwanted degradation of dietary true protein and the triggering of excessive production of NH_3-N. The absence or inexpressive bond between the proteins of the evaluated feedstuffs and tannins present in low concentrations in the ruminal inoculum, of the animals used supplied with the tannins blend, were insufficient to reduce production of NH_3 in in vitro assays until 24h, in relation to the same feedstuffs incubated in animals without supplementation of the tannin blend.

Concerning the in situ assays, the simultaneous analyzes for each nutritional component (DM, CP and NDF) of the tannin×feedstuff arrangements provided a more appropriate basis for comparing of nutrient disappearance profiles in situ. Furthermore, the package nlme (Pinheiro & Bates, 2006) of R, for nonlinear mixed effect model procedures allowed us to build a more complete and complex modeling approach by including a variance function (Eqs. 6-8) to account for the heterogeneity of variance over disappearance profile, as well as the correlation function (corCAR1) which allowed us to account for correlations between the measurements taken overtime. Therefore, each model was built as: exponential model (Eq. 1) + variance function (Eqs 6, 7 or 8) + correlation function (with or without corCAR1) + random effect for combination of parameters (B; k_d; U; B, k_d; B, U; k_d; U; or B, k_d, U) + variance and covariance matrix of random effects (Symm or Diag) (Pinheiro & Bates, 2006). This warranted a more reliable adjustment of nutrient disappearance profiles along with a better mechanistic understanding of the digestibility models (Vieira et al., 2012).

The difference observed in the in situ ruminal degradability of cottonseed (B, k_d and U) and soybean (k_d and U) meals, in bulls with or without supplementation of tannins blend may be related to the intrinsic characteristic of the protein structure of this feedstuff, i.e, size, open and flexibility of the protein structure, hydrophobic amino acids content and proline content (McMahon et al., 2000). The globular proteins, such as those from cottonseeds and soybean (Perez-Maldonado et al., 1995) can also influence the affinity for the condensed tannins.

Higher affinities are observed in less compacted proteins due the greater accessibility to backbone peptides these macromolecules (Mehansho et al., 1987). Furthermore, the storage of proteins of high molecular weight of the cottonseed are amorphous (Youle & Huang, 1979) a characteristic that could possibly contribute to increase the affinity for tannin.

The negative values observed in the estimates of the means and 95%CI of the U parameter of protein feedstuffs (ISCPD) are related to the high ruminal degradability observed for these feedstuffs. In this case, we can consider that there is a tendency for the undegradable fraction in the rumen (U) of the protein to have been close to zero after 96 h of in situ incubation, moreover, the in situ disappearance profiles did not show well-defined asymptotic values, even with 96 h of in situ incubation. In this case, the first-order exponential model (asymptotic or monomolecular), such as the one we use Eq. (1) (Mertens, 1977) could estimate the non-biological value (numerical artifact) of the asymptote (U). In this situation, which is common for highly fermentable substrates as concentrated feedstuffs, these values of parameters could be interpreted with as greater ruminal degradation (B) of the substrates and consequently the extremely low of indigestible residue (U).

Therefore, we observed that to evaluate the effect of the tannin blend on the protein degradation of common protein feedstuffs in beef cattle supplementations under grazing conditions, the cottonseed and soybeans meals the in situ assays showed a greater sensitivity to the presence of tannins in the supplementation these bulls when comparing with the in vitro assays. Hence, there is an indication that the animal effect contained in in situ assays (i.e., ruminal motility and rumination), might be important to observe some effect of tannin on nutrient degradability in rumen.

The in situ disappearance profiles of the NDF (ISNDFD) of roughage feedstuffs showed well-defined asymptotic behavior in 96 h of incubation in the rumen. The common lower degradability of the fiber of roughage feedstuffs compared to the protein in protein feedstuffs is the probable reason for this shape of the disappearance profile of fiber degradation. This is also the reason the fiber asymptotic value (U) estimated of fiber for each evaluated roughage feedstuff was positive.

Therefore, at low doses of a tannins blend supplementation offered to grazing beef cattle minimally affected the roughage feedstuffs tested, being insufficient to compromise the in vitro e in situ ruminal digestibility of the fiber, either by not complexing with fibrolytic enzymes and carbohydrates from the plant cell wall or causing toxicity to microorganisms. Nevertheless, two main protein feedstuffs that are usually part of cattle supplementation, soybean and cottonseed meal, indicated that a low dose of tannins blend might affect the kinetics parameters of ruminal digestion of the protein in situ assays. Probably the quebracho condensed tannins and the hydrolysable of *Castanea* spp have greater affinities for the proteins of these two protein feedstuffs than the others tested and, even in low contents, these tannins are capable of interfering in the kinetic parameters of the protein digestion of both protein feedstuffs. However, the tannins blend dose of these tannins in low concentration does not affect the NH_3-N content in in vitro digestion tests.

Conclusion

Overall, the tannins blend at 1g kg^{-1} (DM basis) in the supplementation of grazing beef cattle may provide some benefits, since the ruminal digestion of protein from soybean and cottonseed meal among other protein feedstuffs reduces the potentially digestible fraction (*B*; cottonseed meal only), and increases the indigestible fraction (*U*) and digestion rate (k_d) in the rumen, while the fiber digestion of the roughage feedstuffs does not appear to be affected.

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Declarations

Ethics Approval

The trial was performed at the Faculdade de Agronomia e Zootecnia, Universidade Federal de Mato Grosso (UFMT; Cuiabá, Mato Grosso, Brazil), and it followed humane animal care and handling procedures based on UFMT guidelines (Protocol 23108.207702/2017-76).

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