



Tannin-based product in feedlot diet as a strategy to reduce enteric methane emissions of Nelore cattle finished under tropical conditions

Elaine Magnani,[†] Thiago H. Silva,^{†,‡} Leandro Sakamoto,[†] Marcelo Q. Manella,[‡] Fabio. M. G. N. Dias,^{||} Maria E. Mercadante,^{†,‡} Darren Henry,^{§,||} Juliana O. S. Marcatto,^{||} Eduardo M. Paula,^{†,1,||} and Renata H. Branco[†]

[†]Institute of Animal Science, Beef Cattle Research Center, Sertãozinho, SP 14160-970, Brazil

[‡]Division of Research and Development, Silva Team Brasil, Estância Velha, RS 93600-000, Brazil

^{||}Division of Research and Development, JBS Friboi, São Paulo, SP 05118-100, Brazil

[§]Department of Animal and Dairy Sciences, University of Georgia, Tifton, GA 31793-89557, USA

¹Department of Global Climate Change and Agriculture, Embrapa Environment, Jaguariuna, SP 13820, Brazil

[†]Corresponding author: emarostegandepaula@gmail.com

ABSTRACT

A total of 120 Nelore bulls, [initial body weight (BW) = 307 ± 11.6 kg and 12 mo of age] were allocated into 12 collective pens (10 bulls per pen) in a commercial feedlot to evaluate the effects of a specific blend of tannin and saponins on enteric methane (CH₄) emissions. The study was a completely randomized design, in which pens were considered the experimental units (*N* = 6 pens per treatment) and were randomly allocated into one of two treatments: 1) Control (CON), a basal diet with monensin supplementation (25 mg/kg dry matter [DM]; Rumensin, Elanco Animal Health, Greenfield, IN, USA), or 2) Control + a specific blend of tannin and saponins (TAN; 0.7 g/kg DM; composed of quebracho and chestnut tannin extracts along with carriers from cereals rich in saponins; SilvaFeed BX, Silvateam, San Michele Mondovi, CN, Italy). After the adaptation period (20 d), the experiment was divided into two phases: growing phase (21 to 53 d; total of 33 d) and fattening phase (54 to 139 d; total of 86 d). Enteric methane emissions were estimated using the sulfur hexafluoride (SF₆) tracer gas technique. Interactions between treatment and period (growing vs. fattening) were detected for daily CH₄ emissions, in which animals fed TAN reduced CH₄ emissions by 17.3% during the fattening period compared to bulls fed CON (*P* = 0.05). In addition, bulls fed TAN had lower CH₄ emissions expressed by dry matter intake (DMI) during the fattening period compared to bulls fed CON (*P* = 0.06). The findings presented herein indicate that a specific blend of tannin and saponins can be used as a strategy to reduce enteric CH₄ emissions and its intensity of Nelore bulls finished in feedlot systems under tropical conditions.

LAY SUMMARY

This study evaluated the effects of a specific blend of tannin and saponins on enteric methane (CH₄) emissions of finishing Nelore bulls under tropical conditions. Nelore bulls were randomly allocated into 1 of 2 treatment groups [a basal diet with monensin supplementation (**CON**), and CON + a specific blend of tannin and saponins (**TAN**)]. Bulls fed TAN had lower CH₄ emissions during the fattening period compared to bulls fed CON; however, there were no effects of TAN on growth performance outcomes. The findings indicate that a specific blend of tannin and saponins can be used as a strategy to reduce enteric CH₄ emissions by Nelore bulls finished in feedlot systems with no impact on growth performance.

Key words: beef cattle, feed additives, greenhouse gases, sustainability

INTRODUCTION

Ruminants contribute to greenhouse gas (GHG) emissions (e.g., CH₄), which is a negative component of the production system since CH₄ emissions from enteric fermentation are the major source of GHG in animal production (Kinley et al., 2020; Honan et al., 2021; Arndt et al., 2022; Beck et al., 2022). Furthermore, from the perspective of animal productivity, CH₄ represents a loss of 2% to 12% of their dietary gross energy (Hristov et al., 2013). Given that CH₄ is a potent GHG (e.g., 28 times more potent than CO₂), it increases public concern about sustainable food production systems

and has resulted in community pressure on the livestock sector (Honan et al., 2021). Despite greater CH₄ potency, it has a short atmospheric life span (e.g., 12.5 yr) compared to CO₂, and its mitigation can quickly reduce the global warming rate and contribute to avoiding the Earth's temperature increase by 2050 (Jayanegara et al., 2012; Herremans et al., 2020; Congio et al., 2021; Orzuna-orzuna et al., 2021; Arndt et al., 2022); therefore, this can reduce enteric methane from beef cattle and play an important role to meet the Paris Agreement of limiting global warming well below 2 °C (Reisinger and Clark, 2018).

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According to two recently published articles, meta-analyses evaluating several strategies to mitigate methane emissions in Latin America (Congio et al., 2021) and Europe and Africa (Arndt et al., 2022), and also in another comprehensive review (Honan et al., 2021), the use of feed additives as rumen modifiers, such as ionophores and plant secondary compounds (e.g., tannins and saponins), are nutritional strategies that can effectively reduce CH₄ emission from enteric fermentation without compromising animals' growth performance.

In this scenario, the ionophore monensin has been widely used in feedlot diets to increase propionate and reduce ammonia and CH₄ formation in the rumen. Hence, greater animal growth and improved feed efficiency have been reported (Beauchemin et al., 2008; Vyas et al., 2018). Tannin extracts have been investigated by several authors as a feed additive for beef cattle to improve animals' performance and reduce CH₄ emissions. Fitri et al. (2022) in a meta-analysis observed a reduction in CH₄ (L/kg of DMI) and, in a review by Honan et al. (2021), the authors observed that tannins can reduce up to 54% of CH₄ emissions in "in vivo" studies and that saponins can reduce up to 26% CH₄ emission in "in vitro" studies. Despite several beef studies published in the past two decades with tannins, to our knowledge, studies evaluating the tannins supplementation in diets containing monensin in a feedlot system in tropical regions as a strategy to reduce methane are scarce.

Therefore, we hypothesized that a specific blend of tannin and saponins product in feedlot diets would reduce enteric CH₄ emissions without compromising animal growth performance compared to control diets. The objective of the present study was to evaluate the effects of a specific blend of tannin and saponins supplementation on growth performance and enteric methane emissions of Nellore bulls finished in a feedlot system under tropical conditions.

MATERIALS AND METHODS

Animal care and handling followed protocols approved by the Animal Use Ethics Committee of the Institute of Animal Science (Protocol Number: 249-19).

Experimental Location, Design, and Diets Composition

One hundred twenty Nellore bulls, with initial body weight (BW) of 307 ± 11.6 kg and 12 mo-old, were allocated into 12 collective pens equipped with water and feed troughs (10 bulls per pen and 16.3 m²/head) in a commercial feedlot located in Guaiçara, SP, Brazil. Prior to the beginning of the trial, all bulls were weighed (after 16 h of feed and water withdrawal), vaccinated against seven strains of *Clostridium sp.* (Fortress 7; Zoetis, Campinas, SP, Brazil) and against respiratory disease pathogens (Bovi-Shield Gold; Zoetis), dewormed (ivermectin 1%; Ivomec, Boehringer Ingelheim, Campinas, SP, Brazil), and received individual visual ear tags. The study was a completely randomized design, in which the pens served as the experimental units ($N = 6$ pens per treatment) and were randomly allocated into one of two treatment groups: 1) Control (CON), a basal diet with monensin supplementation (25 mg/kg DM, Rumensin, Elanco Animal Health); and 2) Control + a specific blend of tannin and saponins (TAN; 0.7 g/kg DM; composed of quebracho and chestnut tannin extracts along with carriers from cereals rich in saponins; SilvaFeed BX, Silvateam).

The study was carried out using a series of three step-up energy level diets: adaptation, growing, and fattening phases. The adaptation protocol consisted of "ad libitum" intake of the following diet: 28% of ground corn, 16% of cottonseed cake, 11.5% of dried distiller grain, 10% of cottonseed meal, 5.35% of peanut meal, 5% of fresh citrus pulp, 3% of whole barley, 3.52% of mineral premix, 0.2% of urea, 0.25% of slow released nonprotein nitrogen, 0.2% of water, 10% sugarcane silage, and 7% of sugarcane bagasse (DM basis). After the adaptation protocol, the experiment was divided into two phases: growing phase (21 to 53 d; total of 33 d) and fattening phase (54 to 139 d; total of 86 d), totaling 139 d of study. The ingredient compositions of growing and fattening period diets are shown in Table 1. Regardless of treatment, all animals received the same step-up diets during the adaptation period and the same growing and fattening diets during the experimental period.

Measurement of Enteric Methane Emissions

Enteric methane emissions (CH₄, g/d) were estimated using the sulfur hexafluoride (SF₆) tracer gas technique (Johnson et al., 1994) that was adapted to the local conditions following the recommendations of Berndt et al. (2014). Four

Table 1. Ingredients and chemical composition of the basal diets (% DM)

Ingredients	Feedlot period	
	Growing ¹	Fattening ²
Ground corn	39.0	40.0
Rehydrated corn	–	10.0
Whole cottonseed	16.0	13.0
Sugarcane bagasse	10.0	8.56
Distillers' grains	8.76	7.01
Cottonseed meal	8.04	5.98
Citrus pulp, wet	5.98	4.02
Peanut meal	5.02	5.05
Brewers' wet grains	2.99	2.99
Vitamin-mineral premix ³	3.51	2.78
Water	0.31	0.21
Slow-released nonprotein nitrogen	0.21	0.21
Urea	0.21	0.21
Chemical Composition		
Dry matter, % as fed	51.8	57.1
Crude protein	19.4	17.7
Fat	4.16	4.16
Neutral detergent fiber	31.8	29.0
Nonfiber carbohydrate	39.1	43.8
Ash	6.29	6.00
NEm, Mcal/kg ⁴	1.68	1.76
NEg, Mcal/kg ⁴	1.20	1.30
Total Digestible Nutrients ⁴	73.7	76.5

¹Growing period 21 to 53 d.

²Fattening period 54 to 139 d.

³Provided (per kg of DM): 242.5 g of calcium; 18 g of phosphorus; 70 g of sodium; 17 g of magnesium; 23 g of sulfur; 14 mg of chromium; 1700.0 mg of zinc; 455 mg of copper; 1210.0 mg of manganese; 38 mg of iodine; 20 mg of cobalt; 14 mg of selenium; 83400.0 IU of vitamin A; 16680.0 IU of vitamin D; 170.0 IU of vitamin E; 900 mg of monensin/kg premix (Rumensin, Elanco).

⁴Estimated using tabular feed values NASEM (2016).

bulls randomly chosen from each pen, totaling 48 bulls (24 treatment replicates per treatment; $N = 6$ pens per treatment with 4 bulls each pen), were fitted with gas collection halters 10 d before methane sampling to allow the bulls' adaptation. Methane sampling started between 0700 and 0800 hours on five consecutive days of two periods—growing and fattening [from July 12 to 16, 2021 (days 39 to 43 of growing phase) and from September 06 to 11, 2021 (days 95 to 99 of fattening phase)]. The cylinders were exchanged every 24 h.

About 250 permeation tubes (brass capsules), containing SF₆ were kept in an oven at 39 °C and calibrated by weekly weighing on an analytical scale for 8 wk before the beginning of the sampling period. This procedure was performed to ensure constant and linear emission of SF₆. One hundred twenty capsules with an average constant release rate of 4.598 ± 0.32 and 2.460 ± 0.15 mg SF₆/d, were selected and orally administered 5 d before the beginning of the gas sampling period by using an oral bolus gun. These capsules settle and release SF₆ gas in the reticulo-rumen where they remain safe for the bulls. The eructated gases contained both methane and SF₆ were collected into evacuated canisters, and the ratio of methane to SF₆ in the eructated gases were used to estimate daily methane production.

The gases expelled by the bulls from the mouth and nostrils were captured in a controlled and continuous manner through a stainless-steel capillary tube according to Johnson et al. (1994). The device was protected by a flexible hose fixed to the halter and connected to the collection cylinder. The latter was subjected to vacuum (0 atm) and attached to the bulls' back. The pressures (initial and final) of the cylinders were monitored daily to ensure the sample quality. The background levels of SF₆ and methane were measured by suspending canisters daily at strategic points in the paddock. At the end of each sampling period, the sampled gases were submitted to the Environmental Biogeochemistry laboratory (Embrapa Meio Ambiente, Jaguariúna, SP, Brazil) and subsequently analyzed using a Hewlett Packard gas chromatograph (model 6890, Agilent Inc., Wilmington, DE, USA) using a flame ionization detector for CH₄ and an electron capture detector for SF₆. The amount of enteric methane was estimated as a function of SF₆ concentrations, relating the results to the known rate of tracer gas released by the capsule deposited in the rumen. Correction for sampled environmental concentrations and molecular weights was performed according to Berndt et al. (2014).

The daily methane emission (CH₄, g/d) of each bull was gathered as the mean of emissions estimated by the five daily samples in the growing and fattening periods. In addition, methane emission intensity was also expressed per kilogram of DMI (CH₄/DMI, g/kg/d).

Performance

Bulls were fed four times daily at 0700, 1000, 1300, and 1600 hours. Diets were mixed in a mixer feeder wagon and checked for residual feed between each dietary mix to avoid cross-contamination. The amount of feed provided daily was adjusted using a trough score with adaptations according to Pritchard (1998), to maintain a minimum of orts and assure an "ad libitum" intake. Dry matter intake (DMI) was obtained using data from the growth period and the fattening period, totaling 119 d, for each pen by weighing the food supplied daily, and weighing the orts. Ingredients, orts, and total diet were sampled weekly to determine the nutritional composition and stored at -18°C until laboratory analysis.

At the beginning (day 0) and end of the experimental period (day 139; adaptation + growing + fattening periods), the bulls were individually weighed after 16 h of fasting from solid food, intermediate weighing took place on days 20 and 53, without fasting, 4.0% of BW was discounted (Stock et al., 1983).

The average daily gain (ADG) was estimated by the linear regression coefficient of weights on days in test (DIT) according to the equation: $y_i = \alpha + \beta \times \text{DIT}_i + \epsilon_i$, where y_i is the weight of the animal in the i th observation; α is the intercept representing the initial weight of the bull; β is the linear regression coefficient representing ADG; DIT_i is the day in test in the i th observation; ϵ_i is the random error associated with each observation.

After the experimental period, the bulls were transported to a commercial abattoir (JBS/FRIBOI) located in the city of Lins, SP, Brazil, and harvested. All procedures were performed according to the Sanitary and Industrial Inspection Regulation for Animal Origin Products of Humanitarian Slaughter Guidelines as required by Brazilian law (Brasil, 2000). Hot carcass weight was assessed by un-chilled weight of the carcass after slaughter and the removal of the head, hide, intestinal tract, and internal organs.

Chemical Analyses

According to AOAC (1990), feed and ort samples were analyzed for DM (method 930.15), ash (method 942.05), and EE (method 2003.05), with OM calculated as the difference between DM and ash content. Total N ($6.25 \times N = CP$) content of feed samples was determined using the combustion method with a nitrogen analyzer, Dumatherm (Gerhardt GmbH & Co, Königswinter, Germany; method 990.13; AOAC, 2005). For NDF and ADF, samples were sequentially analyzed, and treated with alpha thermo-stable amylase without sodium sulfite according to Van Soest et al. (1991) and adapted for a Fiber Analyzer (TE 149; TECNAL, Piracicaba, SP, Brazil). Nonfiber carbohydrate (% of DM) was calculated according to NRC (2001): $\text{NFC} = 100 - (\% \text{NDF} + \% \text{CP} + \% \text{EE} + \% \text{ash})$.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Before the actual analysis, data were explored to seek disparate information and for normality of residuals by Shapiro–Wilk test (PROC UNIVARIATE). An individual observation was considered an outlier when standard deviations for the residual mean or to the model were greater than +3 or lesser than -3. To assess the effect of treatments, periods (growing and fattening) and their interactions on all variables related to CH₄ emission and its intensity, repeated-measure models were fitted by mixed linear models using the generalized linear mixed models (PROC GLIMMIX). For these repeated-measure models, several variance-covariance structures were tested and those with lowest Bayesian Information Criterion were applied to the models to account appropriately for within-pen residuals correlation among times evaluated. The fixed effects of treatment, period, and their interaction were included into all statistical models even in the absence of statistical significance. The random effects of pen within treatment were also included in the linear predictor for all models to recognize pen as the experimental unit.

The effects of additives on growth performance and carcass characteristics were fitted through generalized linear mixed

models (PROC GLIMMIX) including the fixed effects of treatments and random effects of pen within treatment to recognize the pen as the experimental unit. Least squares means were reported for all variables evaluated. For all analyses, differences detected at $P \leq 0.05$ were considered significant, and differences at $0.05 < P < 0.10$ were considered a tendency toward statistical significance.

RESULTS

The effect of treatments on enteric methane emissions is shown in Table 2. Interactions between treatment and period (growing vs. fattening) were detected for CH_4 emissions (g/d), in which animals fed TAN had lower emissions during the fattening period compared to CON, 152.6 vs. 184.6 g/d, respectively ($P = 0.05$). In addition, bulls fed TAN had lower CH_4 emissions expressed by DMI during the fattening period compared to CON ($P = 0.06$). Methane emission (g/d) for both treatments was greater during the fattening period compared to the growing period ($P = 0.002$). In this sense, CH_4 emission expressed by DMI, was greater during fattening period compared to the growing period as well ($P = 0.008$).

The results of growth performance and carcass characteristics are presented in Table 3. The tannin-based additive did not affect FBW, DMI, ADG, or feed efficiency ($P \geq 0.65$). Similarly, HCW was not affected by tannin-based additive compared to CON ($P > 0.05$).

DISCUSSION

GHG emissions from cattle have been concerning consumers as enteric methane (CH_4) emissions contribute to anthropogenic global warming (Reisinger and Clark, 2018). Indeed, agriculture contributes about 10% to 12% of GHG emissions, and livestock enteric fermentation along with CH_4 and

N_2O from manure management are responsible for 38% of these emissions (Smith et al., 2007; Beck et al., 2022). Further, enteric CH_4 has been a controversial topic due to its greater potency in the atmosphere compared to CO_2 (IPCC, 2013). Altogether, these circumstances have been driving the livestock industry to accelerate its sustainability goals concerning nutritional innovation strategies. In the last 10 yr, several studies have reported positive effects of tannin-based products supplementation on dairy and beef cattle by reducing CH_4 emissions and improving animal performance (Jayaneegara et al., 2012; Aboagye and Beauchemin, 2019; Min et al., 2020; Honan et al., 2021; Verma et al., 2021; Rossi et al., 2022). In the present study, we investigated the impact of a specific blend of tannin and saponins on growth performance and CH_4 enteric emissions of bulls finished in a feedlot system under tropical conditions. We observed a reduction in gross (g/d) and intensity (e.g., g of CH_4 /DMI) of CH_4 emissions of 17.3% and 15.5%, respectively, for bulls supplemented with TAN vs. CON, during the fattening period.

Tannins are phenolic compounds that accumulate in plant tissues and contribute to defense against herbivory (Clausen et al., 1992). They are synthesized by many plants and may be found mainly in roots, stems, bark, leaves, buds, and seeds, composing about 5% to 10% of tree leaves (Barbehenn and Peter Constabel, 2011). Tannins are structurally divided into two groups: hydrolyzable and condensed forms (Izawa et al., 2010). In tropical forages, condensed tannins are often one of several polyphenolic compounds, which may include hydrolyzable tannins (Waghorn, 2008). High condensed tannin contents may limit DM digestibility; otherwise, hydrolyzable tannins may provide nutrients for absorption in a time-dependent manner (Lowry et al., 1996). The mechanisms involved in CH_4 mitigation by tannins are not well-understood; however, two factors may be involved: 1) direct inhibition of methanogenic micro-organisms (Tavendale

Table 2. Least square means of the enteric methane emission of Nellore bulls receiving a diet supplemented with monensin (CON) vs. one supplemented with monensin and tannins (TAN)

Item	Treatments ¹		Pooled SEM ²	P-values		
	CON	TAN		Treatment	Period ³	Interaction
DMI ⁴ , kg/d						
Growing	9.85	9.87				
Fattening	10.63	10.48				
Average	10.24	10.18	0.317	0.88	<0.01	0.53
CH_4 , g/d						
Growing	158.7 ^{b,B}	145.2 ^{b,B}				
Fattening	184.6 ^{a,A}	152.6 ^{bb}				
Average	171.7 ^a	148.9 ^b	7.07	0.05	<0.01	0.05
CH_4 /DMI ⁴ , g/kg/d						
Growing	15.25 ^{d,B}	14.18 ^{d,B}				
Fattening	17.32 ^{c,A}	14.63 ^{d,B}				
Average	16.28 ^c	14.41 ^d	0.649	0.07	<0.01	0.06

Within a row, means without a common lowercase letter superscript differ (^{a,b} $P < 0.05$; ^{c,d} $0.05 < P < 0.10$).

Within a column, means without a common uppercase letter superscript differ (^{A,B} $P < 0.05$).

¹CON = basal diet + mineral added monensin (25 mg/kg DM; Rumensin, Elanco); TAN = basal diet + mineral added monensin (25 mg/kg DM; Rumensin, Elanco) + tannin-based product (7 g/kg DM; Silvafeed BX, Silvateam).

²Standard error of the mean.

³Growing period (21 to 53 d) and fattening period (54 to 139 d).

⁴DMI, dry matter intake calculated based on the 5 d period of methane collection for each period.

Table 3. Least squares means of the performance of Nellore bulls receiving a diet supplemented with monensin (CON) vs. one supplemented with monensin and tannins (TAN) during a 139 d finishing period

Item	Treatments ¹		Pooled SEM ²	P-value	
	CON	TAN			
N	6	6	–	–	
Body weight, kg	Initial	308	307	10.94	0.99
	Final	487	488	12.15	0.93
Growth performance and carcass characteristics					
	Dry matter intake, kg/d	9.77	9.70	0.275	0.87
	DMI, % of body weight	2.52	2.51	0.024	0.68
	Average daily gain, kg/d	1.31	1.32	0.0149	0.72
	Gain:Feed	0.134	0.137	0.0034	0.65
	Hot carcass weight, kg	287	286	8.55	0.99

¹CON = basal diet + mineral added monensin (25 mg/kg DM; Rumensin, Elanco); TAN = basal diet + mineral added monensin (25 mg/kg DM; Rumensin, Elanco) + tannin-based product (7 g/kg DM; Silvafeed BX, Silvateam).

²Standard error of the mean.

³Dry matter intake = DMI;

et al., 2005); and 2) reduction of substrates, mainly H₂, to produce CH₄ through the formation of fiber and/or protein-tannin complexes (Goel and Makkar, 2012).

DMI has been positively associated with CH₄ emission (Hristov and Melgar, 2020). Although in the present study DMI increased by about 7% between growing and fattening periods (data from methane collection periods), CH₄ emission only increased about 3% in bulls fed with TAN vs. CON between these periods. Several studies have reported the positive effects of tannin-based additives in decreasing CH₄ emissions by ruminants (Min et al., 2020; Orzuna-orzuna et al., 2021; Fitri et al., 2022; Perna Junior et al., 2022). Plant tannins have a recognized effect on the gut microbiota through their antibacterial activity (Tong et al., 2022), where this effect is hypothesized due to its interaction with specific substrates, such as protein and bacterial cell walls (Bae et al., 1993). This fact may lead the animal to a ruminal fermentation profile modulation and bacterial community diversity (Min et al., 2020). In an “in vitro” study carried out by Chen et al. (2016), the abundance of *Firmicutes* as well as its ratio with *Bacteriodes* populations (F:B) was correlated to reduced CH₄ production. Tannin addition in ruminant diets has been associated with increased *Firmicutes* and F:B ratio in the rumen (Min et al., 2014; Díaz Carrasco et al., 2017). Further, Díaz Carrasco et al. (2017) supplementing a blend of quebracho and chestnut tannins, detected a reduction in methanogenic micro-organisms, particularly those members of Genus *Mathanosphaera* from *Eurychaeota* phylum (archaea organisms). Complementing, the reduction of production and intensity of CH₄ by TAN treatment herein reported, was detected mainly during the fattening phase (after 53 d of study). Johnson and Johnson (1995) suggested that ruminal micro-organisms may possess the ability to adapt to conventional feed additives such as monensin, which these authors reported that CH₄ production per unit of diet returned to initial levels within 2 wk in cattle fed either grain or forage-monensin supplemented diets. Our evidence presented herein, therefore has suggested that long-term tannin supplementation may be effective in reducing enteric CH₄ emission from beef cattle in feedlot systems by modulating the micro-organisms involved in the ruminal fermentative process.

Previous studies have reported detrimental effects of tannin on ruminal protein and fiber digestibility through tannin-nutrient complexes formation and suppressed effects on cellulolytic bacteria (McSweeney et al., 2001; Carulla et al., 2005; Goel and Makkar, 2012; Min et al., 2014). Notwithstanding, Fitri et al. (2022), who evaluated acacia and quebracho extracts in a meta-analytic study detected lower acetic acid proportion as well as lower acetate-to-propionate ratio at a constant total VFA concentration in ruminal fluid from large and small ruminants. Similar results were reported by Carulla et al. (2005) who supplemented sheep with 41 g *Acacia mearnsii* extract (containing 0.615g/g condensed tannins)/kg dietary DM. Consequently, these authors did not detect differences in energy retention and utilization between tannin-fed and control animals. In addition, through the perspective of the energy dynamic in ruminants, decreasing energy losses by ruminal gas production would mathematically increase the efficiency of conversion of digestible energy to metabolizable energy (Johnson and Johnson, 1995). However, as mentioned by Beauchemin et al. (2020), a severe reduction in enteric CH₄ emissions (e.g. over 50%) may be necessary to detect increases in animal performance. The authors explained that digestible energy surplus due to reductions in CH₄ emissions, may not be enough to substantially increase metabolizable energy and net energy retention.

In our study, there was no adverse effect of TAN supplementation on growth performance and carcass characteristics when compared to CON treatment. Orzuna-orzuna et al. (2021) corroborated this in their study on the effect of dietary tannin supplementation on growth performance of beef cattle through a meta-analysis and reported no effect of tannins on weight gain, feed intake, and feed efficiency. Further, Congio et al. (2021), who performed a meta-analysis on enteric methane mitigation strategies and performance of confined beef cattle production in the Latin American and Caribbean region, did not detect effect of tannins plus saponins on DMI and ADG. Notably, Rivera-Méndez et al. (2017) fed a combination of quebracho and chestnut tannins and found an improvement on animal performance and feed efficiency of Holstein steers during the finishing phase. Similar to Tavendale et al.

(2005), our study found that tannins may have acted directly by inhibiting methanogenic microbes; however, this phenomenon did not translate into greater energy retention and better growth performance of Nelore bulls finished in feedlot systems under tropical conditions.

CONCLUSION

In the present study, a specific blend of tannin and saponins was effective to reduce enteric CH₄ emissions by 17.3% or 88.76 kg of CO₂ equivalent compared to control diet with no detrimental effect on growth performance. Tannin blends can be used as a strategy to reduce enteric CH₄ emissions and its intensity from Nelore bulls finished in feedlot systems under tropical conditions. Our findings indicated that quebracho and chestnut extracts (tannin plus saponin product) is a viable nutritional strategy to aid tropical countries to meet the Paris Agreement of limiting global warming well below 2 °C, and to improve sustainable beef production.

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Conflict of interest statement. None declared.

LITERATURE CITED

- Aboagye, I. A., and K. A. Beauchemin. 2019. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: a review. *Animal*. 9:1–18. doi:10.3390/ani9110856
- AOAC. 1990. *Official methods of analysis*. 15th ed. (K. Helrich, editor). Washington DC: Association of Official Analytical Chemists, Inc.
- AOAC. 2005. *Official method of Analysis*. 18th Edition, Association of Officiating Analytical Chemists, Arlington, VA, USA.
- Arndt, C., A. N. Hristov, W. J. Price, S. C. McClelland, A. M. Pelaez, S. F. Cueva, J. Oh, J. Dijkstra, A. Bannink, A. R. Bayat, et al. 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. *Proc. Natl. Acad. Sci. U. S. A.* 119:1–10. doi:10.1073/pnas.2111294119
- Bae, H. D., T. A. McAllister, J. Yanke, K. J. Cheng, and A. D. Muir. 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. *Appl. Environ. Microbiol.* 59:2132–2138. doi:10.1128/aem.59.7.2132-2138.1993
- Barbehenn, R. V., and C. Peter Constabel. 2011. Tannins in plant-herbivore interactions. *Phytochem.* 72:1551–1565. doi:10.1016/j.phytochem.2011.01.040
- Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: a review. *Aust. J. Exp. Agric.* 48:21–27. doi:10.1071/ea07199
- Beauchemin, K. A., E. M. Ungerfeld, R. J. Eckard, and M. Wang. 2020. Review: fifty years of research on rumen methanogenesis: lessons learned and future challenges for mitigation. *Animal*. 14:S2–S16. doi:10.1017/S1751731119003100
- Beck, M. R., L. R. Thompson, T. N. Campbell, K. A. Stackhouse-Lawson, and S. L. Archibeque. 2022. Implied climate warming contributions of enteric methane emissions are dependent on the estimate source and accounting methodology. *Appl. Anim. Sc.* 38:639–647. doi:10.15232/aas.2022-02344
- Berndt, A., T. M. Boland, M. H. Deighton, J. I. Gere, C. Grainger, R. S. Hegarty, A. D. Iwaasa, J. P. Koolgaard, K. R. Lassey, D. Luo, R. J. Martin, C. Martin, P. J. Moate, G. Molano, C. Pinares-Patiño, B. E. Ribaux, N. M. Swainson, G. C. Waghorn, and S. R. O. Williams. 2014. Guidelines for use of sulphur hexafluoride (SF₆) tracer technique to measure enteric methane emissions from ruminants. In: M. G. Lambert, editor. *New Zealand Agricultural Greenhouse Gas Research Centre*, New Zealand.
- Brasil. 2000. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa 3, de 17 de janeiro de 2000. Aprova o regulamento técnico de métodos de insensibilização para o abate humanitário de animais de açougue. *Diário Oficial da União* 3–10 (in Portuguese).
- Carulla, J. E., M. Kreuzer, A. Machmüller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Aust. J. Agric. Res.* 56:961–970. doi:10.1071/ar05022
- Chen, S., H. Cheng, K. N. Wyckoff, and Q. He. 2016. Linkages of Firmicutes and Bacteroidetes populations to methanogenic process performance. *J. Ind. Microbiol. Biotechnol.* 43:771–781. doi:10.1007/s10295-016-1760-8
- Clausen, T. P., P. B. Reichardt, J. P. Bryant, and F. Provenza. 1992. Condensed Tannins in Plant Defense: A Perspective on Classical Theories. In: R.W. Hemingway, and P. E. Laks, editors. *Plant Polyphenols*. Basic Life Sciences, vol 59. Boston, MA: Springer. doi:10.1007/978-1-4615-3476-1_36
- Congio, G. F. de S., A. Bannink, O. L. Mayorga Mogollón, G. Jaurena, H. Gonda, J. I. Gere, M. E. Cerón-Cucchi, A. Ortiz-Chura, M. P. Tieri, O. Hernández, et al. 2021. Enteric methane mitigation strategies for ruminant livestock systems in the Latin America and Caribbean region: A meta-analysis. *J. Clean Prod.* 312:127693. doi:10.1016/j.jclepro.2021.127693
- Díaz Carrasco, J. M., C. Cabral, L. M. Redondo, N. D. Pin Viso, D. Colombatto, M. D. Farber, and M. E. Fernández Miyakawa. 2017. Impact of Chestnut and Quebracho Tannins on Rumen Microbiota of Bovines. *Biomed Res. Int.* 2017:1–11. doi:10.1155/2017/9610810
- Fitri, A., Y. R. Yanza, A. Jayanegara, R. Ridwan, W. D. Astuti, K. A. Sarwono, R. Fidriyanto, R. Rohmatussolihat, Y. Widyastuti, and T. Obitsu. 2022. Divergence effects between dietary Acacia and Quebracho tannin extracts on nutrient utilization, performance, and methane emission of ruminants: A meta-analysis. *Anim. Sci. J.* 93:e13765. doi:10.1111/asj.13765
- Goel, G., and H. P. S. Makkar. 2012. Methane mitigation from ruminants using tannins and saponins. *Trop. Anim. Health Prod.* 44:729–739. doi:10.1007/s11250-011-9966-2
- Herremans, S., F. Vanwindekens, V. Decruyenaere, Y. Beckers, and E. Froidmont. 2020. Effect of dietary tannins on milk yield and composition, nitrogen partitioning and nitrogen use efficiency of lactating dairy cows: A meta-analysis. *J. Anim. Physiol. Anim. Nutr. (Berl)* 104:1209–1218. doi:10.1111/jpn.13341
- Honan, M., X. Feng, J. M. Tricarico, and E. Kebreab. 2021. Feed additives as a strategic approach to reduce enteric methane production in cattle: Modes of action, effectiveness and safety. *Anim. Prod. Sci* 62:1303–1317. doi:10.1071/AN20295
- Hristov, A. N., and A. Melgar. 2020. Short communication: Relationship of dry matter intake with enteric methane emission measured with the GreenFeed system in dairy cows receiving a diet without or with 3-nitrooxypropanol. *Animal*. 14:s484–s490. doi:10.1017/S1751731120001731
- Hristov, A. N., J. Oh, G. Waghorn, P. J. Gerber, B. Henderson, H. P. S. Makkar, and J. Dijkstra. 2013. SPECIAL TOPICS-Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. *J. Anim. Sci.* 91:5070–5094. doi:10.2527/jas.2013-6584
- IPCC. 2013. Summary for policymakers. In: T. F. Stocker, D. Qin, G. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, editors. *Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. United Kingdom and New York, NY, USA: Cambridge University Press, Cambridge, p. 1535 doi:10.1515/ci-2021-0407

- Izawa, K., Y. Amino, M. Kohmura, Y. Ueda, and M. Kuroda. 2010. Human-environment interactions - taste. *Comp. Nat. Prod. II: Chem. Biol.* 4:631–671. doi:10.1016/b978-008045382-8.00108-8
- Jayanegara, A., F. Leiber, and M. Kreuzer. 2012. Meta-analysis of the relationship between dietary tannin level and methane formation in ruminants from in vivo and in vitro experiments. *J. Anim. Physiol. Anim. Nutr. (Berl)* 96:365–375. doi:10.1111/j.1439-0396.2011.01172.x
- Johnson, K., M. Huyler, H. Westberg, B. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a SF₆ tracer technique. *Environ. Sci. Technol.* 28:359–362. doi:10.1021/es00051a025
- Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. *J. Anim. Sci.* 73:2483–2492. doi:10.2527/1995.7382483x
- Kinley, R. D., G. Martinez-Fernandez, M. K. Matthews, R. de Nys, M. Magnusson, and N. W. Tomkins. 2020. Mitigating the carbon footprint and improving productivity of ruminant livestock agriculture using a red seaweed. *J. Clean Prod.* 259:120836. doi:10.1016/j.jclepro.2020.120836
- Lowry, J. B., C. S. McSweeney, and B. Palmer. 1996. Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. *Australian J. Agric. Res.* 47:829–842. doi:10.1071/AR9960829
- McSweeney, C. S., B. Palmer, D. M. McNeill, and D. O. Krause. 2001. Microbial interactions with tannins: nutritional consequences for ruminants. *Anim. Feed Sci. Technol.* 91:83–93. doi:10.1016/S0377-8401(01)00232-2
- Min, B. R., S. Solaiman, H. M. Waldrip, D. Parker, R. W. Todd, and D. Brauer. 2020. Dietary mitigation of enteric methane emissions from ruminants: a review of plant tannin mitigation options. *Anim. Nutr.* 6:231–246. doi:10.1016/j.aninu.2020.05.002
- Min, B. R., C. Wright, P. Ho, J. -S. Eun, N. Gurung, and R. Shange. 2014. The Effect of Phytochemical tannins-containing diet on rumen fermentation characteristics and microbial diversity dynamics in goats using 16S rDNA amplicon pyrosequencing. *Agric. Food Anal. Bacteriol.* 4:195–211.
- NRC. 2001. *Requirements of Dairy Cattle Seventh Revised Edition, 2001*. Seventh. (N. R. Council, editor.). Washington D.C: The National Academies Press.
- National Academies of Sciences, Engineering, and Medicine. 2016. *Nutrient Requirements of Beef Cattle: Eighth Revised Edition*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/19014>.
- Orzuna-orzuna, J. F., G. Dorantes-iturbide, A. Lara-bueno, L. A. Miranda-romero, and P. Abel. 2021. Effects of dietary tannins' supplementation on growth performance, rumen fermentation, and enteric methane emissions in beef cattle: a meta-analysis. *Sustain.* 13:1–27. doi:10.3390/su13137410
- Perna Junior, F., R. Galbiatti Sandoval Nogueira, R. Ferreira Carvalho, E. Cuellar Orlandi Cassiano, and P. H. Mazza Rodrigues. 2022. Use of tannin extract as a strategy to reduce methane in Nellore and Holstein cattle and its effect on intake, digestibility, microbial efficiency and ruminal fermentation. *J. Anim. Physiol. Anim. Nutr. (Berl)* 107:89–102. doi:10.1111/jpn.13702
- Pritchard, R. H. 1998. Bunk Management-Observations from research. In: Plains Nutrition Council Spring Conference. Texas A&M Research and Extension, Amarillo, TX, USA. Publication number AREC 98-24, p. 68–75.
- Reisinger, A., and H. Clark. 2018. How much do direct livestock emissions actually contribute to global warming? *Glob. Chang. Biol.* 24:1749–1761. doi:10.1111/gcb.13975
- Rivera-Méndez, C., A. Plascencia, N. Torrentera, and R. A. Zinn. 2017. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. *J. Appl. Anim. Res.* 45:199–203. doi:10.1080/09712119.2016.1141776
- Rossi, C. A. S., S. Grossi, M. Dell'anno, R. Compiani, and L. Rossi. 2022. Effect of a blend of essential oils, bioflavonoids and tannins on in vitro methane production and in vivo production efficiency in dairy cows. *Animals.* 12:728. doi:10.3390/ani12060728
- Smith, P., Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, et al. 2007. Agriculture. In: B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, L. A. Meyer, editors. *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. United Kingdom and New York, NY, USA: Cambridge University Press, Cambridge. p. 498–540.
- Tavendale, M. H., L. P. Meagher, D. Pacheco, N. Walker, G. T. Attwood, and S. Sivakumaran. 2005. Methane production from in vitro rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Anim. Feed Sci. Technol.* 123-124 Pa:403–419. doi:10.1016/j.anifeeds.2005.04.037
- Stock, R., T. Klopfenstein, D. Brink, S. Lowry, D. Rock, and S. Abrams. 1983. Impact of weighing procedures and variation in protein degradation rate on measured performance of growing lambs and cattle. *J. Anim. Sci.* 57:1276.
- Tong, Z., W. He, X. Fan, and A. Guo. 2022. Biological function of plant tannin and its application in animal health. *Front. Vet. Sci.* 8:1–7. doi:10.3389/fvets.2021.803657
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2
- Verma, S., F. Taube, and C. S. Malisch. 2021. Examining the variables leading to apparent incongruity between antimethanogenic potential of tannins and their observed effects in ruminants—a review. *Sustainability (Switzerland)*. 13:1–24. doi:10.3390/su13052743
- Vyas, D., A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindemann, and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets. *J. Anim. Sci.* 96:2923–2938. doi:10.1093/jas/sky174
- Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—progress and challenges. *Anim. Feed Sci. Technol.* 147:116–139. doi:10.1016/j.anifeeds.2007.09.013