









# Short-term use of monensin and tannins as feed additives on digestibility and methanogenesis in cattle

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**ABSTRACT** - The objective was to assess the effects short-term use of monensin and *Acacia mearnsii* tannins as feed additives on nutrient intake, digestibility, and CH<sub>4</sub> production in cattle. Six rumen-cannulated Holstein cows were distributed in two 3×3 Latin square experimental design, and each experimental period lasted 21 days. The basal diet was composed of corn silage and concentrate in a 50:50 dry matter (DM) basis proportion. Treatments were control, monensin (18 mg kg<sup>-1</sup> of DM), and tannin-rich extract from *Acacia mearnsii* (total tannins equivalent to 6 g kg<sup>-1</sup> of DM). Nutrient intake and apparent digestibility coefficients were not affected by the addition of monensin or tannins to diets. However, tannins showed a tendency to reduce crude protein digestibility. Monensin decreased CH<sub>4</sub> emission by 25.6% (g kg<sup>-1</sup> of body weight) compared with the control treatment. Monensin is more effective than *Acacia mearnsii* tannins in reducing CH<sub>4</sub> emissions in the short term, considering a diet of the same roughage:concentrate proportion for cattle.

**Keywords:** animal nutrition, gas production, methane, ruminants

## Introduction

Methane gas and its environmental effects, particularly on the greenhouse effect, have been increasingly studied, and strategies for emission reduction are increasingly sought (Guan et al., 2006; Wanapat et al., 2015). Methane is a byproduct of the ruminant digestive process and, depending on the components of the diets, its production might represent an energy loss of feed intake up to 2-12% (Johnson and Johnson, 1995).

Feed additives such as monensin are widely used to improve feed efficiency of ruminants; however, the use of this ionophore was banned by many countries, and alternatives have been studied (Wanapat et al., 2015), among them the use of *Acacia mearnsii* tannins (Carulla et al., 2005; Grainger et al., 2009). The extraction of tannins from *Acacia mearnsii* occurs on an industrial scale in Brazil, because they are widely used in leather tanning, effluent treatment, and in the food sector. Polyphenolic compounds are the main active substances of tannins, which can be classified into hydrolyzable (HT) and condensed (CT), depending on the molecule structural arrangement and the reactivity. Goel and Makkar (2012) highlighted that tannins have great potential to reduce CH<sub>4</sub> production; however, more research on

cows is needed to know the best dosage without decreasing organic matter digestibility and animal production.

Studies carried out with monensin or tannins on enteric CH<sub>4</sub> mitigation demonstrated that additive effectiveness depends on the source and its dietary levels (Oliveira et al., 2005) and species and physiological state of the animals (Makkar, 2003a). According to Johnson and Johnson (1995), after a short-term use (30 days), CH<sub>4</sub> production levels return to those observed before monensin administration, probably due to the ability of the microbiota to adapt to the ionophore. Rumen microbes can adapt to tanniniferous diets by increasing the proportion of tannin-resistant bacteria in the rumen, therefore, mitigating the inhibitory effects of these secondary plant compounds (Smith et al., 2005). Staerfl et al. (2012) showed that *Acacia mearnsii* tannin extract might be useful to mitigate enteric CH<sub>4</sub> formation in maize silage-based diets in the long term (nine months). Therefore, it is important to evaluate its short-term effectiveness.

The hypothesis of this study is that the inclusion of monensin or low dose tannin extracts from *Acacia mearnsii* as feed additives in the short term can reduce methanogenesis without altering digestibility. The objective was to compare low-dose tannins to the known monensin effect on intake, digestibility, and methanogenesis in cattle.

## Material and Methods

The trial was conducted in Pirassununga, state of São Paulo, southeastern Brazil (21°59'45"S, 47°25'37" W, and 625 m above sea level). All procedures involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines (case no. 8580120514).

Six rumen-cannulated dry Holstein cows (average body weight [BW] = 784±87 kg) were randomly allocated in individual stalls with sand bed, fans, and *ad libitum* access to feed and water. The feed was offered twice daily at 08:00 and 16:00 h as a total mixed ration in a 50:50 (dry matter [DM] basis) roughage to concentrate ratio. The basal diet was formulated to meet NRC (2001) nutrient requirements recommended for dry cows (Table 1). Before the beginning of the experiment, the animals were fed only corn silage.

**Table 1 - Ingredients and chemical composition (DM basis) of the basal diet**

Item	Basal diet
Ingredient (g kg <sup>-1</sup> of DM)	
Corn silage	500
Dry ground corn grain	347
Soybean meal	122
White salt	5
Dicalcium phosphate	1
Limestone	5
Vitamin and mineral premix <sup>1</sup>	20
Chemical composition (g kg <sup>-1</sup> of DM)	
Dry matter (g kg <sup>-1</sup> )	531
Ash	76
Ether extract	35
Crude protein	120
Neutral detergent fiber	271
Acid detergent fiber	144
Non-fibrous carbohydrates	498
Total digestible nutrients	799

<sup>1</sup> Composition of vitamin and mineral premix per kilogram of product: 200 g of Ca; 60 g of P; 20 g of S; 20 g of Mg; 70 g of Na; 15 mg of Co; 700 mg of Cu; 700 mg of Fe; 40 mg of I; 1,600 mg of Mn; 19 mg of Se; 2,500 mg of Zn; 200,000 IU of vitamin A; 50,000 IU of vitamin D3; 1,500 IU of vitamin E.

The experimental design was two 3×3 Latin squares with three treatments, and each experimental period of 21 days. The experimental diet was supplemented with the following feed additives: control (no additives), monensin (addition of 300 mg of sodium monensin per animal per day – equivalent to 18 mg kg<sup>-1</sup> of DM; Rumensin® 200, Elanco Animal Health, Brazil), or tannin (addition of 100 g tannin extract per animal per day – total tannins equivalent to 6 g kg<sup>-1</sup> of DM; tannin-rich extract obtained from Acacia-black, *Acacia mearnsii*; Veronese & Cia Ltda, Caxias do Sul, Brazil). The total phenol concentrations (895 g kg<sup>-1</sup> of extract) were determined by the Folin-Ciocalteu reagent method (Makkar, 2003b), and total tannins were estimated according to Makkar et al. (1993) as the difference in total phenol concentration before and after treatment with insoluble polyvinylpyrrolidone (873 g kg<sup>-1</sup> in tannic acid equivalents). The CT concentrations were determined according to Makkar (2003b) by the butanol-HCl method (339 g kg<sup>-1</sup> in leucocyanidin equivalents). The ionophore monensin was selected as strategy to decrease CH<sub>4</sub> emission to be a feasible feed additive commonly used in beef and dairy production (Guan et al., 2006). The additives were hand-mixed with concentrate mixture first, then with corn silage to obtain the total mixed diet.

The monensin dose was chosen based on the review of Beauchemin et al. (2008). According to these authors, who evaluated the same additive of this study, doses of 15 mg kg<sup>-1</sup> of DM do not affect the CH<sub>4</sub> production, but doses of 15 to 20 mg kg<sup>-1</sup> of DM can reduce the total CH<sub>4</sub> production in dairy cattle. Grainger et al. (2009), using 9 g kg<sup>-1</sup> DM of *Acacia mearnsii* CT extract, observed reduction in feed intake. Perna Junior (2018) used different levels of total tannins (0, 5, 10, and 15 g kg<sup>-1</sup> of DM), the same additive of this study, and found linear reduction for neutral detergent fiber (NDF), organic matter (OM), and crude protein (CP) digestibility. Therefore, to minimize the negative effect on DM intake (DMI) and digestibility, a lower level of supplementation was used in this study (total tannins equivalent to 6 g kg<sup>-1</sup> of DM or CT equivalent to 2 g kg<sup>-1</sup> of DM). Soltan et al. (2017) reported that many phenolic compounds act against methanogens and may decrease CH<sub>4</sub> emission without affecting ruminal nutrient degradability or total digestibility. Additionally, a study with feedlot Holstein steers fed diets containing low HT or CT extract (6 g kg<sup>-1</sup> of DM) did not show any difference in tannin source on growth and DMI, but inclusion of tannins or its combination increased animal performance compared with the control treatment (Rivera-Méndez et al., 2017). Given this information, we did not use a purified source of CT as the basis for this study.

All feeders were examined every morning at 08.00 h. From the 15th to the 21st day of each period, the feed refusal of each cow was weighed and discounted from the offered in the bunker. The amount of diet offered was adjusted daily, allowing for a minimum of 5% and maximum of 10% of refusal throughout the experiment. This was multiplied by DM content of feed.

The total tract apparent digestibility of diet and its fractions were determined by chromium oxide (Cr<sub>2</sub>O<sub>3</sub>) marker as described in Bateman (1970). Briefly, chromium oxide (7.5 g) was provided through ruminal cannula at 08.00 and 16.00 h from day 5 until day 14. Fecal samples (200 g) were collected directly from the rectum of each cow twice daily from the 10th to 14th day. Digestibility was performed during this period so as not to interfere with CH<sub>4</sub> collection. The samples were placed in plastic bags and stored in a freezer at -20 °C for chemical analysis. The basal diet used in this trial was also sampled for further analysis.

At the end of each collection period, composite feed and feces samples were homogenized and dried in a forced-air oven at 55 °C for 72h. They were then ground in a mill (using a 1-mm sieve and placed in closed vessels) for subsequent determinations of the following: DM (method number 967.03; AOAC, 1990); ash (method number 923.03; AOAC, 1990); CP (method number 920.87; AOAC, 1990); ether extract (EE), determined gravimetrically after extraction using ether in a Soxhlet extractor (method number 920.85; AOAC, 1990); NDF assayed using heat stable amylase and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991), both expressed excluding residual ash contents (Mertens, 2002).

Based on the feedstuff chemical composition, the nutrient digestibility was calculated as described: Digestibility (%) = 100 - [100 × (Cr<sub>2</sub>O<sub>3</sub> on feed (%)) / Cr<sub>2</sub>O<sub>3</sub> on feces (%) × (Nutrient on feces (%) /

Nutrient on feed (%)]. Non-fibrous carbohydrates (NFC) were calculated as proposed by Sniffen et al. (1992):  $\text{NFC} = 100 - (\text{CP}\% + \text{EE}\% + \text{ash}\% + \text{NDF}\%)$ . Total digestible nutrients (TDN) were calculated by using the equation  $\text{TDN} = \text{digestible CP} + (2.25 \times \text{digestible EE}) + \text{digestible NDF} + \text{digestible NFC}$ , according to NRC (2001).

To determine enteric  $\text{CH}_4$ , the sulfur hexafluoride ( $\text{SF}_6$ ) tracer technique was used, as described by Johnson and Johnson (1995) and adapted in Brazil by Primavesi et al. (2004). Prior to the onset of methane collection,  $\text{SF}_6$  permeation capsules, previously identified, were loaded and calibrated with constant and known release rates of  $\text{SF}_6$  and then inserted in the rumen. The animals were adapted to the canisters during five days (10th to 14th) prior to collection, and then gas samples were collected over 24-h intervals for seven consecutive days, starting from the 15th experimental day. Concentrations of  $\text{CH}_4$  and  $\text{SF}_6$  were determined by gas chromatograph (HP6890, Agilent, Delaware, USA).

The  $\text{CH}_4$  emission was calculated by dividing the release rate of  $\text{SF}_6$  by the  $\text{SF}_6/\text{CH}_4$  concentration ratio in the canisters. The potential emission of  $\text{CH}_4$  was then expressed in several ways: grams per day ( $\text{g day}^{-1}$ ); grams per hour ( $\text{g h}^{-1}$ ); grams per kilogram of body weight ( $\text{g kg}^{-1}$  of BW); grams per kilogram of metabolic weight ( $\text{g kg}^{-1}$  of  $\text{BW}^{0.75}$ ); grams per kilogram of dry matter intake ( $\text{g kg}^{-1}$  of DMI); grams per kilogram of organic matter intake ( $\text{g kg}^{-1}$  of OMI); grams per kilogram of digestible organic matter ( $\text{g kg}^{-1}$  of DOM); percentage of gross energy lost as  $\text{CH}_4$  (% GE), which considered the gross energy intake as calculated from the organic matter intake; and percentage of digestible energy lost as  $\text{CH}_4$  (% DE). The conversion of  $\text{CH}_4$  from grams to energy unit was performed according to the conversion factor, stated by Holter and Young (1992), in which  $\text{CH}_4$  produces  $0.0556 \text{ Mcal g}^{-1}$  when it is burned.

Data were statistically analyzed using the SAS software (Statistical Analysis System, version 9.3). Before the actual analysis, the data were analyzed for the presence of disparate information ("outliers") and normality of residuals (Shapiro-Wilk). Individual observation was considered outlier when standard deviations in relation to mean was bigger than +3 or less than -3. When the normality assumption was not accepted, the logarithmic transformation or the square root was required. The data were analyzed according to the following model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + A_i(S_k) + \varepsilon_{ijkl}$$

in which  $Y_{ijkl}$  = dependent variable,  $\mu$  = general mean,  $T_i$  = treatment effect (fixed effect),  $P_j$  = period effect (random effect),  $S_k$  = square effect (random effect),  $A_i(S_k)$  = animal within square effect (random effect), and  $\varepsilon_{ijkl}$  = random error associated with each observation.

The experimental unit was the animal within period, wherein each animal received a different treatment in each period. Therefore, there were six observations per treatment, totaling 18 experimental units. Treatments were evaluated by the least significance difference (LSD) test using 0.05 significance level.

## Results

The DMI was not affected ( $P > 0.05$ ) when monensin or tannins were added to the diet. Treatments did not affect ( $P > 0.05$ ) intake of different nutrients, total tract apparent digestibility, and GE intake. However, tannins tended to reduce digestibility of CP ( $P = 0.08$ ) by 2.8% compared with control (Table 2). Monensin decreased  $\text{CH}_4$  emission ( $P < 0.05$ ) when expressed in  $\text{g day}^{-1}$ ,  $\text{g kg}^{-1}$  of BW,  $\text{g kg}^{-1}$  of  $\text{BW}^{0.75}$ , and less energy was lost as  $\text{CH}_4$  ( $\text{MJ day}^{-1}$ ) compared with the control treatment (24.3, 25.6, 24.1, and 23.5%, respectively). Additionally, monensin tended to decrease ( $P = 0.09$ )  $\text{CH}_4$  production in relation to the digested OM by 25% compared with the control (Table 3).

## Discussion

The present experiment showed that the use of monensin at  $18 \text{ mg kg}^{-1}$  of DM does not influence changes in DMI and nutrient intake nor in digestibility, as shown by other studies (Oliveira et al., 2005; Fonseca et al., 2016). This fact is attributed to the action of monensin in altering the proportion of rumen microorganisms, and consequently, the proportion of short-chain fatty acids (SCFA) and byproducts

(i.e., H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>) formed from feed fermentation (Nagaraja and Taylor, 1987), but without significantly affecting the digestion, passage rate, and feed intake (Bretschneider et al., 2008). Similar results were reported by other authors in bulls (Fonseca et al., 2016) and Holstein cows in lactation (Eifert et al., 2005).

As observed for monensin, the inclusion of tannin did not affect the intake and apparent digestibility of nutrients in cows. Studies demonstrated that CT in high concentrations in ruminant diets binds fiber, metal ions, polysaccharides, and proteins, and mainly, form complexes that hinder the action of ruminal bacteria, with consequent decreases in ruminal digestibility of nutrients (Grainger et al., 2009).

**Table 2 - Effect of short-term use of monensin and *Acacia mearnsii* tannins on feed intake and total tract apparent digestibility in cattle**

Item	Treatment			SEM	P-value
	Control	Monensin	Tannin		
Dry matter intake					
kg day <sup>-1</sup>	18.02	16.89	17.03	0.44	0.338
g kg <sup>-1</sup> of BW <sup>0.75</sup>	113.07	105.36	106.36	2.96	0.193
Daily nutrient intake (kg day <sup>-1</sup> )					
Organic matter	16.85	16.01	16.47	0.39	0.529
Crude protein	2.15	2.03	2.09	0.05	0.488
Neutral detergent fiber	5.78	5.49	5.65	0.13	0.497
Acid detergent fiber	3.47	3.29	3.40	0.08	0.486
Ether extract	0.56	0.53	0.55	0.01	0.466
Gross energy (MJ day <sup>-1</sup> )	320.6	304.8	314.3	1.71	0.527
Total tract apparent digestibility (g g <sup>-1</sup> )					
Dry matter	0.816	0.833	0.798	0.101	0.307
Organic matter	0.823	0.841	0.805	0.099	0.255
Crude protein	0.796	0.820	0.774	0.090	0.082
Neutral detergent fiber	0.740	0.752	0.702	0.160	0.362
Acid detergent fiber	0.742	0.753	0.691	0.184	0.265
Ether extract	0.875	0.895	0.872	0.066	0.157
Gross energy	0.815	0.834	0.797	0.100	0.258

BW<sup>0.75</sup> - metabolic body weight; SEM - standard error of the mean.  
Within rows, values with different letters are statistically different (P<0.05).

**Table 3 - Effect of short-term use of monensin and *Acacia mearnsii* tannins on methane production in cattle**

CH <sub>4</sub> production <sup>1</sup>	Treatment			SEM	P-value
	Control	Monensin	Tannin		
g day <sup>-1</sup>	373.9a	282.9b	334.0ab	17.87	0.045
g kg <sup>-1</sup> BW	0.43a	0.32b	0.38ab	0.01	0.037
g kg <sup>-1</sup> BW <sup>0.75</sup>	2.32a	1.76b	2.08ab	0.09	0.038
g kg <sup>-1</sup> DMI	20.69	16.81	19.93	0.96	0.200
g kg <sup>-1</sup> OMI	22.07	17.74	20.54	0.98	0.182
g kg <sup>-1</sup> DOM	38.92	29.17	38.69	2.03	0.099
% GE	6.43	5.22	6.18	0.30	0.209
% DE	11.76	8.82	12.00	0.62	0.084
MJ day <sup>-1</sup>	20.59a	15.75b	18.38ab	0.23	0.045

SEM - standard error of the mean.

<sup>1</sup> Methane emission in grams per day (g day<sup>-1</sup>), grams per kilogram of body weight (g kg<sup>-1</sup> BW), grams per kilogram of metabolic weight (g kg<sup>-1</sup> BW<sup>0.75</sup>), grams per kilogram of DM intake (g kg<sup>-1</sup> DMI), grams per kilogram of organic matter intake (g kg<sup>-1</sup> OMI), grams per kilogram of digestible organic matter (g kg<sup>-1</sup> DOM), percentage of gross energy lost as methane (% GE), percentage of digestible energy lost as methane (% DE).

Within rows, values with different letters are statistically different (P<0.05).

Additionally, CT decreased total apparent digestibility and increased excretion of nutrients in feces (Carulla et al., 2005; Abdalla et al., 2007; Tiemann et al., 2008). However, in lower doses, tannins can act beneficially on feed digestion and rumen metabolism (Waghorn and Shelton, 1997), without altering intake and apparent digestibility of nutrients (Beauchemin et al., 2007). In the present study, even using a small amount of tannins, there was a tendency to reduce digestibility of CP by 2.8% compared with the control.

Formation of tannin-nutrient complexes, mainly with protein, may improve synchronization of available protein and energy for microbial protein production by improving the efficiency of synthesis (Makkar, 2003a). Furthermore, tannin-protein complexes of low pH (1.0 to 3.0), found in the abomasum of ruminants, are almost completely undone (Leinmüller et al., 1991), and protein are released and digested by gut proteases (Sliwinski et al., 2002). Thus, the tannin amount used in the present study was not sufficient to alter the intake and apparent digestibility of nutrients. Studies with tannin inclusion similar to the present study, for cows (Beauchemin et al., 2007) and lambs (Sliwinski et al., 2002), did not report alteration in intake and digestibility. However, studies that reported decrease of intake and/or digestibility offered greater amount of inclusion, just as in cows (Grainger et al., 2009) and in sheep (Carulla et al., 2005; Abdalla et al., 2007).

The dietary inclusion of monensin decreased CH<sub>4</sub> production, when expressed in g day<sup>-1</sup>, g kg<sup>-1</sup> of BW, and g kg<sup>-1</sup> of BW<sup>0.75</sup>, in relation to control by 24.3, 25.6, and 24.1%, respectively. Additionally, tannin-fed cows exhibited intermediate values of CH<sub>4</sub> production. According to Soltan et al. (2018), CH<sub>4</sub> emission is expressed in different ways, but determined CH<sub>4</sub> emission relative to the digested OM may become more persuading than determining CH<sub>4</sub> relative to the unit of the animal's daily production. In the present study, monensin, besides reducing CH<sub>4</sub> in relation to animal weight, showed tendency to reduce CH<sub>4</sub> by digested OM.

Monensin inhibits the growth of some gram-positive bacteria, such as *Eubacterium*, *Lactobacillus*, and *Streptococcus*, which produce acetic acid and other compounds such as H<sub>2</sub> (Chen and Wolin, 1979), and improves the growth of gram-negative bacteria, which produce propionic acid. Furthermore, there are indications that monensin has direct effect on ciliated protozoa by inhibiting its development and reducing the consequent production of H<sub>2</sub> (Hino, 1981; Russell and Strobel, 1989). Thus, the formation of ruminal CH<sub>4</sub> is lower due to the lack of H<sub>2</sub>, the primary substrate for this synthesis (Buddle et al., 2011). Fonseca et al. (2016) found a decrease on daily CH<sub>4</sub> production in bulls when feeding monensin (22 mg kg<sup>-1</sup> of DM) at similar levels of the current study.

Studies suggest that tannins could act in two ways on methanogenesis: first, directly on methanogenic *archaea* by reducing CH<sub>4</sub> formation (Tavendale et al., 2005); second, by indirectly reducing the primary substrate for the CH<sub>4</sub> formation, mainly H<sub>2</sub> (Goel and Makkar, 2012). In the second case, tannins might form complexes with the feed (i.e., protein and fiber), preventing the action of bacteria, mainly cellulolytic (Bento et al., 2005), and consequently reducing the formation of acetic acid and other products such as H<sub>2</sub> and NH<sub>3</sub> (Tiemann et al., 2008). Carulla et al. (2005) fed sheep diets supplemented with *Acacia mearnsii* CT at dose of 25 g kg<sup>-1</sup> of DM and reported reduction in daily CH<sub>4</sub> production (L day<sup>-1</sup>) by 7 and 12% reduction when CH<sub>4</sub> production was expressed in relation to DMI. However, the authors observed a decrease in digestibility by approximately 5%, suggesting that the tannins indeed form a complex with dietary nutrients, but probably in lower concentrations than at lower intensity without impairing the digestibility of nutrients. In fact, we observed in the present study that, when total tannins from *Acacia mearnsii* were used at dose of 6 g kg<sup>-1</sup> of DM, it did not interfere with digestibility of nutrients. However, the potential to reduce the CH<sub>4</sub> production was not efficient when compared with higher doses. Although not significant, doses of tannins used in the present study suggest that this is the starting point for reduction of CH<sub>4</sub> production in cattle.

## Conclusions

Feed supplementation with monensin (18 mg kg<sup>-1</sup> of DM) or *Acacia mearnsii* extract (total tannins equivalent to 6 g kg<sup>-1</sup> of DM) in short-term use does not alter feed intake or the apparent digestibility of



nutrients by cattle. At the levels of additives used, monensin is more effective than tannins in reducing CH<sub>4</sub> emissions in the short term.

### Conflict of Interest

The authors declare no conflict of interest.

### Author Contributions

Formal analysis: D.C. Zapata Vásquez and R.T.S. Friguetto. Investigation: D.C. Zapata Vásquez and R. Gardinal. Methodology: R.T.S. Friguetto. Project administration: F. Perna Junior, D.C. Zapata Vásquez and P.H.M. Rodrigues. Supervision: A. Berndt and P.H.M. Rodrigues. Writing-original draft: F. Perna Junior and D.C. Zapata Vásquez. Writing-review & editing: P.M. Meyer, A. Berndt and J.J.A.A. Demarchi.

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