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Gas production in bovine feces adding tannins directly or on the diet

Producción de gas en heces de bovinos adicionando taninos directos o sobre la dieta

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RESUMEN

Se realizaron dos experimentos para conocer el efecto de los extractos de taninos (ET) en la producción de gas. En el experimento 1 se colectaron heces de cinco toretes (420 ± 10 kg) de un mismo corral durante cuatro días consecutivos para tener una muestra por día, 100 g de las heces se colocaron en frascos de 600 mL para cada muestra y se adicionaron los siguientes tratamientos: TE) heces sin tratar y HT) 7.4 % de ET condensados. En el experimento 2 se aplicaron los siguientes tratamientos: TES) dieta sin ET, TC) 0.6% de taninos condensados y TH) 0.6 % de taninos hidrolizables a la dieta de 18 becerros (220±9 kg) durante 28 días. En los dos experimentos las heces obtenidas se incubaron durante 24 h para medir su producción de gas por desplazamiento. Los datos obtenidos de gas se compararon con Diseño experimental de bloques al azar para eliminar el efecto día de muestreo. En el primer experimento la producción de gas disminuyó 44 % (*P*<0.001) con los extractos de taninos adicionados sobre las heces, pero adicionados a través de la dieta no hubo diferencias entre los tratamientos.

Palabras clave: extracto de taninos, heces, producción de gas, bovinos en engorda.

ABSTRACT

Two experiments were carried out to know the effect of tannin extracts (ET) in gas production. In experiment 1, feces were collected from five bulls (420 ± 10 kg) from the same pen for four consecutive days to have one sample per day, 100 g of the feces were placed in flasks of 600 mL for each sample and the samples were added. following treatments: TE) untreated stool and HT) 7.4% condensed ET. In experiment 2 the following treatments were applied: TES) diet without ET, TC) 0.6% of condensed tannins and TH) 0.6% of hydrolysable tannins to the diet of 18 calves (220 ± 9 kg) for 28 days. In both experiments, the stools obtained were incubated for 24 h to measure their gas production by displacement. The data obtained from gas were compared with Experimental design of random blocks to eliminate the day-sampling effect. In the first experiment, gas production decreased 44% (P <0.001) with tannin extracts added to the feces, but added through the diet, there were no differences between the treatments.

Keywords: tannins extract, feces, gas production, beef-cattle.

INTRODUCTION

The management of excreta and the increase in the size of confined cattle facilities is increasingly related to the impact on the environment (Adeola, 1999, Kebreab *et al*., 2009, Cardona-Iglesias, 2016), as a consequence Reducing the emission of solids, liquids and gases are factors that put pressure on intensive livestock (Archibeque *et al*., 2006, Grainger and Beauchemin, 2011). The gases produced by the volatilization of organic substances in cattle feces contribute to the dissemination of undesirable odors in the environment (Varel and Miller, 2000, Shabtay *et al*., 2009). An alternative to reduce the amount of gases produced by cattle feces in fattening is through the use of tannins (Cardona-Iglesias, 2016, Barros-Rodríguez *et al*., 2017). Tannins are polyphenolic compounds, a product of the secondary metabolism of a wide variety of plants (Frutos et al., 2004, Vélez-terranova *et al.,* 2014) that is related to protection against infection, insects or herbivorous animals (Duval and Averous , 2016). Tannins are classified into two groups: hydrolysable tannins and condensed tannins (Frutos *et al*., 2004, Vázquez *et al*., 2012, Kardel *et al*., 2013). The extracts of tannins (ET) modify fermentation in the rumen and reduce gas production (Bernal *et al*., 2008, Lascano and Cárdenas, 2010, Cárdenas, 2012). ETs have the ability to bind to proteins in the cell membrane of bacteria, change their structure, function, and inhibit their ability to bind to food particles and the fermentation of their nutrients (Cárdenas, 2012; Barros-Rodríguez *et al.*, 2017). Based on the above, it was hypothesized that the addition of tannin extract both condensed and hydrolyzable decreases the amount of gas produced from the feces of bovine animals. Therefore, the objective of the research was to determine the influence of gas production on cattle feces in fattening by adding direct tannins or on diet.

MATERIAL AND METHODS

The present investigation was constituted by two experiments, both were carried out in the Experimental Unit for Bovines of Fattening in Trópico Seco, located in the commercial fattening cattle Los Migueles, S.A. of C.V. (24º 51 'N, and 107º 26' W) and in the Research Laboratory in Nutrition and Animal Production of the FMVZ of the Autonomous University of Sinaloa, both located in Culiacán, Sinaloa. All the animals used in the research were managed following international recommendations for the care of animals used in research (FASS, 2010).

Experiment 1. In this experiment, the influence of the addition of tannin extract directly to the feces of cattle in fattening on *in vitro* gas production was evaluated. Five Brahman bulls (420 ± 10 kg) housed in a corral with a dirt floor (6 x 12 m) with 24 m2 of metallic roof were used, providing 4.8 m2 of shade for each bull, 2.4 m of linear concrete trough and 0.6 m drinking fountain with permanent access to clean and fresh water for animals.

For 60 days before taking the stool samples, the bulls were fed free access with a finishing diet that had a 10:90 ratio of forage: concentrate (13.6% CP, 2.1 Mcal EN_m), formulated with corn stubble , soybean paste and ground corn grain (Table 1). Once adapted perfectly to the diet, for four continuous days stool samples were taken from the rectum of each of the bulls (± 200 g), the samples were mixed in a blender for 5 min to obtain a composite sample for each one of the four days of sampling.

Table 1. Composition of the diets used in the stool gas production experiments

Ingredients	Proportion in the diet, % in BS	
	Experiment 1	Experiment 2
Corn silage	-	46.11
Corn stubble	10.24	25.33
Ground corn	68.57	-
Soybean paste	7.17	16.28
Molasses cane	6.82	8.29
Tallow	4.50	-
Ganamin Total Sinaloa[¶]	-	2.61
Ganamin Los Migueles[§]	2.70	-
Ganabuffer [Þ]	-	1.38
Total	100%	100%
Calculated analysis (on dry basis) [†]		
PC,%	13.60	15.21
ENm, Mcal kg^{-1}	2.113	1.358
ENg, Mcal kg^{-1}	1.438	0.793

Ganamin Total Sinaloa (vitamin and mineral premix, Mineral Peculiar Technique S.A. de C.V., Guadalajara, Jalisco, Mexico). They contain 68% PC as NNP and 25 g of monensin sodium (Rumensin 200®, ElancoAnimal Health).
[§] Ganamin Los Migueles (premix of vitamins and minerals; Mineral Peculiar Technique S.A. de C.V .; Guadalajara, Jalisco, Mexico). They contain 112% PC a as NNP and 25 g sodium monensin (Rumensin 200®, Elanco Animal Health, Indianapolis, IN, USA).
[Þ] Ganabuffer (premix of pH buffers; Mineral Pecuarian Technique S.A. de C.V .; Guadalajara, Jalisco, Mexico).
[†] Values calculated based on published values NASEM, 2016.

From the stool sample, 20 g aliquots were taken to determine the dry matter (MS) content in a forced air oven at 105 °C for 24 h (AOAC, 1997).

Separately, 400 g of feces were divided into 2 parts of 200 g, each one was added 200 g of distilled H_2O and randomly assigned to one of the two treatments described below: 1) stool alone without the addition of tannin extract (Control); and 2) stools added with 7.4% extract of condensed tannins in hydrated base (TC). The TCs were provided using Bypro

® (INDUNOR, Buenos Aires, Argentina) a preparation based on condensed tannin extract from the red quebracho tree with an average content of 75 % condensed tannins.

During the four days, 100 g of the homogenized mixture of feces with water from each of the treatments were placed in bottles of 600 mL with a screw cap fitted with a gas collection tube (Tygon®, Saint-Gobain, France) placed in a water bath at 37 °C. At the opposite end of the tube was placed a 250 mL graduated glass test tube filled with distilled water placed in an inverted position in a water bath (Miller and Varel 2001; Miller *et al.,* 2006) identified with the respective treatment data. The samples were incubated for 24 h and gas production was expressed in mL as the amount of water displaced by the gas within each specimen (Miller and Varel 2001; Miller *et al*., 2006).

Experiment 2. In this experiment the influence of the consumption of diets added with extract of condensed and hydrolysable tannins in the production of gas in the feces of cattle in fattening was evaluated. 18 Brahman calves (220 ± 6 kg) recently arrived at the feedlot were used; at the time of their arrival at the feedlot, the calves were identified with numbered, individually weighted plastic earrings and received bacterins to prevent diseases caused by *Clostridia, Histophilus somni* (Ultrabac-sumnovac) and *Mannheimia haemolytica* (OneShot, Zoetis). In groups of three calves, the animals were housed in six pens with ground floor (6 x 12 m), with 24 m^2 of metallic roof that provides 8 m^2 of shade for each calf, 2.45 m of linear concrete feeder and 0.6 m of drinker with permanent access of clean and fresh water for animals. The calves were fed in conditions of free access with a diet of growth with proportion 70:30 of forage: concentrate (15% PC, 1.4 Mcal ENm), formulated with corn silage, soybean paste and ground corn grain (Table 1). The animals had an adaptation period of 21 d to the diet, social interaction and experimental management. After 21 d of the adaptation period, fecal samples were taken directly from the rectum of each calf for three consecutive days. The stool samples were handled individually, placed in plastic bags, identified with the number of the calf and day of sampling; the feces were transported immediately to the laboratory, where *in vitro* gas production was measured with a procedure similar to that described in Experiment 1.
Once the stool samples were obtained, the calves were randomly assigned to one of three treatments: 1) growth diet without ET (Control); 2) Control plus 0.6% on dry base of condensed tannin extract (TC); and 3) control plus 0.6 % on dry basis of extract of hydrolysable tannins (TH). The TC was provided in the form of an extract of condensed tannins from the quebracho tree (*Schinopsis balansae*), available on the market as Bypro® (INDUNOR, SA, Buenos Aires, Argentina); and the TH was provided from an extract of chestnut tannins (Castanea sativa), was provided as NutiP® (SilvaTeam; San Michele Mondavi, Italy); both extracts with a content of 75% tannins.

The corresponding daily dose of TC and TH according to the treatment assigned per pen, was dispersed in 1000 g of ground corn. Then it was mixed and homogenized manually for five minutes inside a transparent plastic bag (labeled with the pen number and treatment); the mixture of ground corn with the corresponding ET was added in the feeder of each pen at the time of serving the food (11:00 h), it was mixed manually with the food contained in the upper third of the feeder (Top dress procedure). In the corrals assigned to the control treatment, 1000 g of ground corn was offered daily to match the handling of the feed and the energy intake with the rest of the animals involved in the experiment. The calves were fed during 28 d with their respective treatments; once this period was completed, stool samples were taken from each calf for three consecutive days with the procedure described above. The feces were placed in plastic bags, closed, identified and transported to the Laboratory to measure *in vitro* gas production as in the procedure described above in Experiment 1; constituting each day an independent run, which in turn formed a block and the bottles fermented with the feces of each animal in each run constituted the experimental unit.

Statistical analysis

The displacement data obtained in the two experiments were analyzed with a statistical design of random blocks, considering the sampling day as a block (Hicks, 1973). For the statistical calculations the Statistix® version 9 program (Analytical Software, 2007) was used and the mathematical model was:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \varepsilon_{ijk}$$

RESULTS AND DISCUSSION

Experiment 1. The results of the influence of the addition of tannin extract to bovine feces in *in vitro* gas production are presented in Table 2. The addition of 7.4% of TC (BS) to bovine feces was reduced in 45% (*P* <0.001) *in vitro* gas production.

According to the hypothesis, the decrease in the amount of gas produced in the feces of the cattle to which they were added TC, is an expected response, considering that the bacteria degrade the organic matter components, and as a consequence of their metabolism are gas producers (Dhanoa *et al*., 2004). When these microorganisms are in contact with the condensed tannins they bind to the cell membrane proteins of the bacteria, inhibit their ability to bind to substrates, reduce fermentation of nutrients and gas production (Bae *et al*., 1993 Jones *et al*., 1994; Cárdenas, 2012).

Table 2. Influence of the addition of tannin extract to cattle feces in *in vitro* gas production (Experiment 1).

Variables	Treatments		EEM†	*P*<
	Control	Tannins		
Tannin extract, g §				
Grams ET sample $^{-1}$	0.00	1.003		
% of the feces, BH	0.00	2.00		
% of the feces, BS	0.00	7.41		
MS of the feces §	26.90	26.90	0.532	1.00
Sample of feces §				
g (per vial BH)	50.20	50.29	0.037	0.12
g (per vial BS)	13.50	13.53	0.010	0.12
Production of gas §				
Total, in 24 h, mL	218.33	121.33	7.636	< 0.001
Per g of feces BH, mL	4.35	2.41	0.153	< 0.001
Per g of feces BS, mL	16.24	8.92	0.603	< 0.001

† Standard error of the mean

§ Average values of 12 observations.

Dhanoa *et al*. (2004) tested the utility of using bovine feces as an inoculum instead of using ruminal fluid to develop the *in vitro* fermentation test; so it is possible to infer that the tendency in the fermentation processes is similar with both types of inocula, although the magnitude of the response is lower when feces are used given the lower amount of microbial cells per gram of inoculum. Based on the above, the fermentative behavior of the bacteria present in the feces as a result of the addition of ET, is explicable in the same terms as when this phenomenon has been appreciated using ruminal fluid as an inoculum.

In a series of *in vitro* studies, a decrease in gas production by rumen microbes was attributed to the presence of tannins in several species of tropical legumes (Carmona et al., 2005, Bernal et al., 2008; Cárdenas 2012). Likewise, the addition of different types of TC from Leucaena (*Leucaena leucocephala*) decreased *in vitro* gas production in direct relation to its molecular weight when ruminal microbes were used as inoculum (Huang et al., 2011); therefore, in the present experiment, the lower production of gas in the faeces to which the CTs were added is presumably due to the inhibitory action of the tannins on the microbial activity due to the structural damage that occurs in the membrane cellular, especially in Gram-negative bacteria (Bae *et al*., 1993; Jones *et al*., 1994). Other researchers Gilroyed *et al*. (2015) appreciated a decrease in the population of bacteria and protozoa, as well as in the production of feces gas from cattle when 2.5 % of black acacia (*Acacia mearnsii*) condensed tannins were added to the diet.

Experiment 2. The results of the influence of the addition of tannin extract to the diet in the production of gas from calf feces are presented in Table 3. The addition of 0.6% of ET to the diet did not modify the production of gas in the stool. These results indicate that when the quebracho TC or chestnut TH are added to the diet in proportions lower than 1%, they do not induce any change in the amount of gas produced from the feces, unlike what was found in Experiment 1, in which the addition of 7.4% TC (BS) directly to the feces after being excreted, decreased gas production.

A probable explanation of the result is that tannins passing through the digestive system of cattle can present interactions; first with the initial components of the diet and then with the products of digestion, since tannins have pH dependence to express or not their ability to bind proteins in a stable manner (Jones *et al*., 1994). When tannins are ingested, in the rumen stable tannin-protein complexes are formed in the pH range of 3.5 to 7.0 (Makkar, 2003, Beserra *et al*., 2011), but they dissociate in the abomasum and duodenum, where the pH in cattle fattening is generally <3.0 (Frutos *et al*., 2004, Jones *et al*., 1994).

Table 3. Influence of the addition of tannin extract to the diet in the production of gas from calf feces (Experiment 2).

Variables	Treatments†			EEM¶	*P*<
	Control	TC†	TH†		
Production of gas, mL §					
Previous production	36.0	40.9	40.1	3.681	0.57
Subsequent production	48.5	56.1	49.1	4.854	0.43
Difference between periods	12.5	15.2	9.0	6.360	0.78
Production of gas, mL g of MS^{-1} §					
Previous production	4.36	5.07	4.80	0.447	0.53
Subsequent production	5.60	6.36	5.85	0.486	0.54
Difference between periods	1.25	1.29	1.05	0.672	0.97

† TC = Condensed tannin extract; TH = Extract of hydrolysable tannins.
¶ Standard error of the mean.
§ Average values of 18 observations.

As the chyme advances in the small intestine, the pH gradually rises due to several secretions (Owens *et al*., 1986; Brake *et al*., 2014), especially those from the pancreas with high concentrations of bicarbonate ions (Huntington *et al*., 2016). In media in which the pH values range from slightly alkaline to slightly acidic such as those prevailing in the ileum and large intestine (Owens *et al*., 1986; Brake *et al*., 2014), it is feasible that the tannins form again complexes with undigested components of the diet, and with endogenous proteins of the digestive secretions (Frutos *et al*., 2004, Bae *et al*., 1993). In this way, by the time the tannins in the amount used in this experiment reached the large intestine, they were probably already associated with other substrates and therefore, were not available to form new complexes with the cell membrane proteins of the bacteria present in this organ, consequently, did not modify its fermentative activity. In the same sense, when the fecal bolus is excreted, the tannins were previously bound to other substances and unable to bind with the proteins of the fecal bacteria, so under these circumstances the tannins are not able to modify the production of gas in the feces of cattles.

Another possibility is that the level of inclusion of tannins in the diet of 0.6% BS of the extract in the current experiment, has been insufficient to allow showing its activity, compared with the four times higher quantity of black acacia TC (*Acacia mearnsii*) that Gilroyed *et al*. (2015) used in a ruminal metabolism experiment in which they found a decrease in gas production in the feces when they added 2.5% acacia TC. However, the authors (Gilroyed *et al*., 2015) do not describe the impact of this level of tannins on dry matter intake and diet digestibility in the cattle they used.

In the current experiment, the maximum amount of tannins to be offered was limited to a level in which food consumption was not affected (0.6%), given that the calves were under feeding conditions similar to those used in the industry with the purpose that the results could have practical application in the production of cattle meat. In general, the level of 1.5% is considered as the maximum threshold of tannins present in the diet of ruminants and that when hydrolysable tannins are provided in higher concentrations the metabolites products of the hydrolysis of the tannins in the rumen can be absorbed at blood and adversely affect other organs (Makkar, 2003).

Henke *et al*. (2016) found that the addition of 1.5% or more of quebracho TC in the diet of dairy cows negatively affected the digestibility of the various components of the same. The consumption of tannins in amounts equivalent to 2% of the diet is sufficient to induce undesirable effects such as a decrease in the digestibility of the protein in the diet (Beauchemin *et al*., 2007). Although there are reports from other authors (Krueger *et al*., 2010, Ebert *et al*., 2017), who add between 1 and 1.5% of tannins to the diet and do not modify the voluntary consumption or the productive response in cattle.

CONCLUSION

The direct addition of tannins in cattle feces decreases their gas production; however, when they are included as part of the diet offered to cattle, they have no impact on the production of gas from the feces of bovine animals.

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