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Potential of *Samanea saman* pod meal for enteric methane mitigation in crossbred heifers fed low-quality tropical grass

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ABSTRACT

Pods of *Samanea saman* have been reported to be effective in mitigating methane (CH_4) emissions in ruminants. The aim of this study was to evaluate the effect of inclusion of *S. saman* pod meal in the ration on feed intake, apparent digestibility, rumen fermentation, rumen degradation, protozoa population, and enteric CH_4 emissions in crossbred heifers fed low-quality tropical grass. Four crossbred heifers (*Bos taurus* × *B. indicus*) with an average live weight (LW) of 261.5 ± 1.29 kg were used in a 4 × 4 Latin square design with four periods of 23 days (17 days for adaptation and 6 days for measurements of response variables). Levels of inclusion of *S. saman* ground pods in the ration were 0, 10, 20 and 30% of dry matter (DM). Enteric CH_4 emissions were measured using open-circuit respiration chambers. Additionally, rumen degradation of the different components of *S. saman* pods was assessed. Incorporation of *S. saman* levels had no effect on dry matter intake (DMI) ($P > .05$), apparent digestibility of dry matter (DM) and organic matter (OM) ($P > .05$) nor protozoal count ($P > .05$). It was found that molar proportion of propionic acid in rumen liquid was increased ($P < .05$), while that of acetic acid and acetic:propionic ratio was linearly decreased ($P < .01$) when heifers were supplemented with *S. saman*. sasAdditionally, emissions of enteric CH_4 showed a linear reduction as the inclusion of *S. saman* in the ration was increased ($P = .007$). A reduction of 50.9% in L CH_4/day and 56.9% in L CH_4/kg DM intake was observed at the highest inclusion level of *S. saman* compared to the control diet ($P < .05$). Potential degradation of DM of pods of *S. saman* was 79.6%. It is concluded that, the inclusion of *S. saman* pods in the ration has the capacity to reduce energy losses in the form of methane emissions in heifers fed low-quality tropical grass.

1. Introduction

At present, there is a worldwide concern regarding global warming as a result of the accumulation of greenhouse gases (GHG) in the atmosphere arising from anthropogenic activities. Livestock production is one of the activities that contributes the most to GHG emissions and has been severely criticized for its effects on soil, biodiversity, deforestation and water depletion (Herrero et al., 2013). Livestock represents about 14.5% of total GHG emissions (IPCC, 2007) with 80 million tons of methane annually (Patra, 2012). Concomitantly, there is a growing demand for animal products (meat and milk) originating mainly from developing countries due to population growth and the increase in income (FAO, 2011, 2016). The increase in demand is a challenge for agricultural systems around the world, as it means producing more food to support a growing population more efficiently within the constraints of available natural resources (FAO, 2016).

In Latin American countries most of the daily dry matter consumed by cattle arises from grasses of medium to low quality which are grazed in the vast grasslands of the "llanos" present in several countries or in the extensive "cerrados" of Brazil. Under traditional grazing conditions of low quality grass, cattle raising has been associated to high GHG emissions (Archimède et al., 2011). Traditional grazing results in the degradation of natural resources and low production parameters, making production systems less profitable and unsustainable in the long term. Methane is the main GHG by-product of rumen fermentation and constitutes a loss of energy (up to 12% of gross energy intake) for the animal (Johnson and Johnson, 1995). Considering this, enteric methane contributes to 39.1% of GHG from livestock (Gerber et al., 2013).

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Therefore, it is important to establish alternatives that reduce these emissions, or increase the efficiency of animal production.

Inclusion of supplements in the ration with high levels of protein and fermentable energy renders ruminal fermentation more efficient when basal rations are low-quality grass (Poppi and McLennan, 1995). This improves animal performance and reduces CH_4 emissions per unit of synthetized product (L CH_4/kg of meat produced). In addition, the implementation of diets containing secondary metabolites which have the capacity to alter the rumen microbial population and rumen fermentation processes constitutes an alternative to reduce CH_4 emissions in the tropics. Several authors have reported that the use of compounds such as saponins, condensed tannins (CT) and essential oils reduce the synthesis of CH_4 in the rumen (Knapp et al., 2014; Patra et al., 2017). In recent years, research has been carried out on the use of tropical forages, such as trees and shrubs, which, in addition to their potential for ruminant feeding, contain a wide variety of secondary metabolites (Patra et al., 2017; Albores-Moreno et al., 2017). *S. saman* is an alternative as a dietary supplement due to the high production of pods during the dry season (Delgado et al., 2014; Anantasook et al., 2015) and its high nutritional quality. *S. saman* contains secondary metabolites (CT and saponins) with anti-methanogenic potential (Anantasook et al., 2015) and high content of starch which can increase the proportion of propionic acid and consequently decrease the availability of H_2 for microorganisms (*archaea*) that synthesize CH_4.

The objective of this study was to evaluate the effect of different levels of inclusion of ground pods of *S. saman* in the ration on feed intake, apparent digestibility, protozoa population and enteric CH_4 emissions in heifers fed a basal ration of *Pennisetum purpureum* grass.

2. Materials and methods

Heifers were treated in accordance with guidelines and regulations for animal experimentation and welfare of the Faculty of Veterinary Medicine and Animal Science (FMVZ), University of Yucatan, Merida, Mexico. Experimental methodology was approved by the Ethics Committee of FMVZ.

2.1. Description of the location

Collection of pods of *Samanea saman* was carried out from April to May 2016 in the central region of Merida, Yucatan, Mexico. This region has a sub humid warm weather with regular rainfall in the summer (May–July), an annual average temperature of 26 °C, rainfall of 984.4 mm and relative humidity fluctuates from 66 to 89% (García, 1981). Soils are calcareous, shallow and with high percentages of rocks, called latosol and "tzek'el" in Maya language (Duch, 1988). The vegetation is low dense and the shrubs reach between 4 and 7 meters height. The jungle area of the region is characterized by low deciduous forest (Gutierrez-Báez and Zamora-Crescencio, 2012). The experiment was carried out from August to November 2016 at the Laboratory of Climate Change and Livestock Production of FMVZ, University of Yucatan located at 21°15´N 83° 32´W in Merida, Mexico.

2.2. Experimental animals

Four commercial crossbred heifers (*Bos taurus x B. indicus*) with an average LW of 261.5 ± 1.29 (μ ± SD) kg were used. Heifers were housed individually in metabolic crates located in a roofed building with concrete floor and without walls. Before the experiment began, animals were dewormed with Dectomax® (1 mL/50 kg LW) and ADE (Vigantol ADE®) vitamins were applied intramuscularly (1 mL/10 kg LW). For one month before the experiment (June 2016), heifers were habituated to the respiration chambers by housing them inside for increasingly longer periods of time as shown in Fig. 1. Data on DMI was taken the last two weeks of the month to ensure same level of intake both inside as outside the chambers, and heifers were considered to be accustomed when no variation was observed in DM intake.

To assess ruminal degradation of the different components of the pods, three ruminally cannulated cows (*Bos taurus* × *B. indicus*) were used. Cows had an average weight of 504.5 ± 58.4 (μ ± SD) kg and were housed in individual metabolic crates located in a roofed building with concrete floor and without walls with free access to water. This facility is located 20 meters away from the chambers. These measurements took place in June 2016.

2.3. Experimental design

A 4 × 4 crossover Latin square design was used (four levels of *S. saman*, four heifers, four periods) (Cochran and Cox, 1991), each period lasted 25 days. In order to minimize the residual effect of the previous treatment, heifers were given a 17-day buffer period to adapt to the new ration and management (starting on day 0), and 7 days for the measurement of response variables (day 17–23), on day 24 rumen fluid was collected, and on day 25, heifers were weighed. Heifers were randomly assigned to each level of inclusion of *S. saman* in every period, assuring that treatments were not repeated for heifers as shown on Fig. 2. The experiment lasted one hundred days.

2.4. Experimental diets

After collection, pods of *S. saman* were dried in a forced-air oven at 55 °C for 48 h, ground in a hammer mill (Azteca, Monterrey, Nuevo León, México) to a ~3 mm particle size and stored in plastic containers. The basal diet was composed of chopped green grass fodder *P. purpureum*. Percentages of inclusion of the dried and ground pods of *S. saman* were 0, 10, 20 and 30% of the offered DM. Chemical composition of each ingredient and of the diets fed in the experiment is shown in Table 1. All rations were supplemented with soybean meal, wheat bran, and sugarcane molasses in different proportions as shown in Table 2. Additionally, minerals were offered at 65 g/day/animal and heifers had free access to water at all times. Rations were offered at 0900 h considering a dry matter intake of 2.5% of LW (NRC, 2001). In order to ensure that heifers consumed the total amount of *S. saman* pod meal, they were first offered a mixture of the pods and the other ingredients, and then chopped grass of *P. purpureum*.

2.5. Intake and apparent digestibility

Dry matter, organic matter (OM), and neutral detergent fiber (NDF) intakes were determined as the difference between the amount of nutrients offered and what was refused (24 h period). Feed refusals were collected, weighed, and sampled each day for chemical analysis. Apparent digestibility was determined by the method described by Schneider and Flatt (1975). Total production of feces was collected and weighed every day, and an aliquot of 10% was taken. Fecal samples were pooled for each period and treatment, and a subsample was used for chemical analysis.

2.6. Methane production

Enteric CH_4 (L/d) production by heifers was measured using two open-circuit respiration chambers (Pinares-Patiño and Garry, 2012; Canul-Solis et al., 2017) with an internal volume of 9.38 m^3 (Fig. 3). The air inside the chambers was extracted by mass flow generators (Flowkit¸ Sable Systems International®, USA) at a rate of 300 L per minute creating a pressure of 275 Pa below external pressure. A subsample of the air extracted from the chamber was sent into an infrared CH_4 analyser (MA-10 Sable Systems International, USA) using a multiplexer. Heifers entered the respiration chambers in pairs (one in each chamber) for 23 h per day for three consecutive days of every 6-day measurement period (Fig. 4). Since each of the heifers were housed inside the chambers for three days, there were three days for methane