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# Enteric methane emissions in crossbred heifers fed a basal ration of low-quality tropical grass supplemented with different nitrogen sources

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**Abstract:** The aim of the present study was to assess enteric methane ($CH_4$) emissions by crossbred heifers fed a basal ration of low-quality tropical grass supplemented with different nitrogen sources. Four crossbred heifers (*Bos taurus* × *Bos indicus*) with an average live weight (LW) of 355 ± 6.01 kg were used in a 4 × 4 crossover Latin square design with four periods of fifteen days each. Basal ration was chopped low-quality tropical grass *Pennisetum purpureum* fed to cover ~70% of metabolizable energy requirements for maintenance of heifers and it was supplemented with either poultry litter (control ration, T1), urea (T2), canola meal (T3) or soybean meal (T4). Enteric $CH_4$ emissions of heifers were measured in open-circuit respiration chambers for 23 hours. Dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) intakes decreased when feeding urea (1.6% of ration) as a source of nitrogen (7.64, 3.78, and 1.83 kg/d, respectively). Rations including urea (T2) or canola meal (T3) given to heifers fed a basal ration of low-quality *Pennisetum purpureum* grass significantly reduced acetic acid concentration and increased propionic acid concentration in the rumen and decreased the loss of gross energy as methane ($P = 0.004$). Incorporation of urea or canola meal in the ration of cattle fed low-quality tropical grass can decrease methane emissions and improve rumen fermentation patterns.

**Keywords:** greenhouse gas; crude protein; heifers; rumen fermentation; digestibility

Methane ($CH_4$) is the second most important greenhouse gas (GHG) contributing to global warming and climate change. Methane affects the energy balance on the Earth by its radiative forcing properties along with carbon dioxide ($CO_2$), ozone, hydroxyl radicals, carbon monoxide and stratospheric chlorine (Harper et al. 1999). Ruminant species represent one of the largest sources of methane emissions with 81–92 million tons produced per year globally, which is equivalent to 23–27% of total anthropogenic methane (Opio et al. 2013). From all livestock species, cattle are responsible for most (77%) of enteric methane emissions. Methane emission is not only related

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to environmental concerns, but also it is associated with feed efficiency, losses arising from methane production range from 2–3% of gross energy intake (GEI) when ruminants are fed high grain rations (Moumen et al. 2016) to 11.3% when consuming low-quality forages (Johnson and Johnson 1995; Beauchemin et al. 2020).

In tropical conditions, during the long dry season, nutritional quality of grasses decreases, particularly its nitrogen content, so farmers depend on locally available feed resources to formulate rations and increase nitrogen intake. Under those conditions, protein supplements are usually recommended for the improvement of feed intake, digestibility and productive performance (Poppi and McLennan 1995).

In Southern Mexico, seasonal forages are commonly supplemented with dried poultry litter as a nitrogen source, and this waste is considered to be one of the basic feedstuffs for cattle in this region. Poultry litter contains around 25% of crude protein, about half of which derives from uric acid that can be efficiently used by rumen microbes for protein synthesis (Lanyasunya et al. 2006). Nitrogen sources are important for maintaining an adequate level of $NH_3$–N suitable for growth, metabolism and microbial activity in the rumen (5–25 mg/100 ml rumen liquor) (Orskov 1977). Supplementation with nitrogen sources such as urea, soybean meal, canola meal or poultry litter can prevent weight loss and maintain milk production in cows during the dry season when the crude protein content in pastures is low (2–4%). However, nitrogen sources under these conditions are worth being evaluated as regards methane production in the tropics.

Many trials have been carried out for the measurement of enteric $CH_4$ production using tropical forages and it has been found that feeding foliage and pods of tropical legumes (and other plant species) to ruminants represents a promising enteric $CH_4$ mitigation strategy at a low cost (Ku-Vera et al. 2018; Valencia-Salazar et al. 2018; Molina-Botero et al. 2019). Some of those forages contain a wide variety of condensed tannins, saponins and starch which can increase the proportion of propionic acid in rumen liquor and consequently decrease the availability of metabolic hydrogen ($H_2$) to reduce $CO_2$ for $CH_4$ synthesis by archaea (Valencia-Salazar et al. 2018). Some nitrogen compounds may work as hydrogen sinks thus decreasing methane synthesis, or they could also upgrade low-quality forage rations improving animal performance and reducing methane intensity per kg of milk or meat produced.

Therefore, this study was carried out to better understand the effect of dried poultry litter compared to other different nitrogen sources (urea, canola meal, soybean meal) on enteric methane emissions, feed digestibility and ruminal fermentation patterns in heifers fed a low-quality tropical grass as a basal ration.

## MATERIAL AND METHODS

Heifers were treated in accordance with guidelines and regulations for animal experimentation of the Faculty of Veterinary Medicine and Animal Science (FMVZ), University of Yucatan (UADY), Merida, Mexico.

### Location

The experiment was conducted at the Laboratory of Climate Change and Livestock Production of FMVZ-UADY located in the central region of Yucatan, Mexico, from November 2017 to January 2018. The region has a warm weather with rainfall (984 mm) in summer (May–September), average annual temperature is 26 °C and relative humidity ranges from 66% to 89% (Garcia 1981).

### Experimental design and animals

Four crossbred heifers (*Bos taurus* × *Bos indicus*) with an average live weight (LW) of 355 ± 6.01 kg were used in a 4 × 4 crossover Latin square design. Each period lasted 15 days (9 days for adaptation to rations and management followed by 6 days for measurements). Heifers were housed individually in metabolic crates located in a roofed building with concrete floor, without walls. Before the experiment, cattle were accustomed to the respiration chamber environment by entering them inside for a period of ~3 weeks to reduce the effect of stress on voluntary feed intake and behaviour in and out of the chambers. Then, heifers were randomly assigned to each treatment in every period.

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Table 1. Experimental diets fed to crossbred heifers receiving low-quality grass

| Ingredients (%DM) | Treatments | | | |
|---|---|---|---|---|
| | control (T1) | urea (T2) | canola (T3) | soybean (T4) |
| *Pennisetum purpureum* | 70 | 72 | 71 | 71.6 |
| Dried poultry litter | 14 | 0 | 0 | 0 |
| Urea | 0 | 1.6 | 0 | 0 |
| Ground canola meal | 0 | 0 | 17 | 0 |
| Soybean meal | 7 | 0 | 0 | 12.5 |
| Cane molasses | 7 | 24.4 | 10 | 13.9 |
| Minerals | 1 | 1 | 1 | 1 |
| Ca-carbonate | 1 | 1 | 1 | 1 |

DM = dry matter

## Experimental treatments

The ration supplied an estimated dry matter (DM) intake of 2.5% of LW of heifers. Formulations of the experimental rations are given in Table 1.

Basal ration was composed of chopped grass (*Pennisetum purpureum*) aimed to cover ~70% of metabolizable energy requirements for maintenance of dried cows according to NRC (2001) and it was supplemented with dried poultry litter (control diet, T1), urea (T2), canola meal (T3) or soybean meal (T4). Daily feed offered and refusals were weighed for each heifer to estimate actual feed intake. Rations were adjusted every two weeks according to changes in the live weight of heifers. Chemical composition of each ingredient and of the experimental rations is shown in Table 2.

## Measurement of $CH_4$ emissions

Methane production (g/d) was measured when cattle were housed in two open-circuit respiration chambers (Canul-Solis et al. 2017). Chamber dimensions were 2.1 m in height, 1.6 m in width and 3.10 m in length, with an internal volume of 9.38 $m^3$. Air was drawn out from the respiration chambers by the action of mass flowmeters (Sable Systems International, Las Vegas, NV, USA) with a capacity of 500 l/min, generating a slight negative pressure of around –475 Pa inside the chambers. For the measurement of the $CH_4$ concentration, an infrared analyzer (MA-10; Sable Systems International, Las Vegas, NV, USA) was used. The methane analyzer was tested for linearity before each run with $N_2$ for zeroing the apparatus,

Table 2. Chemical composition of feed ingredients and experimental diets

| Component | Chemical composition (g/kg/DM) | | | | GE (MJ/kg/DM) |
|---|---|---|---|---|---|
| | DM | CP | ADF | NDF | |
| *Pennisetum purpureum* | 918 | 84 | 327 | 677 | 17.17 |
| Dried poultry litter | 870 | 199 | 192 | 320 | 13.44 |
| Ground canola meal | 952 | 320 | 175 | 272 | 18.06 |
| Soybean meal | 916 | 402 | 117 | 130 | 17.53 |
| Cane molasses | 858 | 42 | 0 | 0 | 14.7 |
| **Experimental diets** | | | | | |
| Control (T1) | 887 | 115 | 264 | 527 | 16.13 |
| Urea (T2) | 879 | 116 | 235 | 487 | 15.88 |
| Canola meal (T3) | 899 | 118 | 262 | 527 | 16.68 |
| Soybean meal (T4) | 81 | 116 | 247 | 497 | 16.34 |

CP = crude protein; DF = acid detergent fibre; DM = dry matter; GE = gross energy; NDF = neutral detergent fibre

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and then with a mixture of methane (1 000 ppm) diluted in $N_2$ for calibratation (Arceo-Castillo et al. 2019). Cattle were housed inside the chambers and kept at a temperature of 23 °C and a relative humidity of 55%, a small fan mixed the air inside the chambers. Methane measurements were carried out during three consecutive days on each heifer for 23 h in each run (Pinares-Patino et al. 2007; Kennedy and Charmley 2012); the data obtained were extrapolated to 24 h with ExpeData software (Sable Systems International, Las Vegas, NV, USA). Energy loss as $CH_4$ was determined on the basis of the heat of combustion of $CH_4$ (55.22 MJ/kg $CH_4$; Brouwer 1965).

## Pattern of VFAs in the rumen

Samples of rumen liquor were taken by an oesophageal tube and were subjected to visual and tactile examination to ensure that it was not contaminated with saliva (Ramos-Morales et al. 2014). Animals were sampled for 6 days, during the period the heifers spent outside the respiration chambers, and 6 h after feeding as suggested by Bhatta et al. (2013), with the aim of determining pH and molar proportions of volatile fatty acids (VFAs). Rumen pH was measured immediately after obtaining the sample of rumen liquor, filtered through two layers of cheesecloth and with a portable potentiometer (HANNA® Instruments, Woonsocket, USA), which was calibrated with reference buffer at pH's 4, 7, and 10. For VFA analysis, 4 ml of rumen liquor were taken and preserved in 1 ml of deproteinizing solution consisting of metaphosphoric acid and 3-methylvaleric acid. For VFA determination, the technique described by Ryan (1980) was employed using a gas chromatograph (Hewlett-Packard, 5890 series III), equipped with a flame ionization detector (FID); the column was HP-FFAP 30 m × 0.53 mm.

## Feed intake

Heifers were fed *ad libitum* once per day and were offered the complete ration at 9:00 h, allowing a refusal of 15% of the amount offered the previous day. Voluntary intake of dry matter (DM), organic matter (OM), and neutral detergent fibre (NDF) was determined as the difference between the amount offered and that refused. Fresh water was available to the heifers at all times.

## Apparent digestibility

Apparent digestibility of DM, OM, NDF, and crude protein (CP) were determined using the total collection of faeces technique (Schneider and Flatt 1975). A sample of 10% of the total faecal production every day during the last 6 days of each experimental period was collected. Samples were stored in freezers at −20 °C. Faecal samples were pooled for each treatment, and a 10% aliquot was taken for DM quantification and chemical analysis.

## Chemical analysis

Dry matter determination was carried out in a forced air oven at 55 °C for 48 h (constant weight). Nitrogen (CP = N × 6.25) determinations were carried out with LECO CN-2000 series 3740 instruments [LECO Corp., St. Joseph, MI, USA (#2.057); AOAC (1990)]. Organic matter content of the sample was determined by combustion in a muffle furnace at 600 °C for 6 h and the concentration of NDF was determined as suggested by Van Soest et al. (1991).

## Statistical analysis

Data on feed intake, apparent digestibility, and molar proportions of VFAs and enteric $CH_4$ were subjected to analysis of variance for a 4 × 4 crossover Latin square design; the model was:

$$Y_{ijk} = \mu + H_i + C_j + T(_k) + E_{ijk} \quad (1)$$

where:

$Y_{ijk}$ – response variable in row $i$, column $j$, $k$ treatment;
$\mu$ – general mean;
$H_i$ – effect of the row;
$C_j$ – effect of the column;
$T_k$ – treatment effect;
$E_{ijk}$ – random error (Cody and Smith 1991; SAS 2006).

Means were compared by Tukey's test using the SAS software for Windows® (SAS 2006). Results were considered significant at < 5%.

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## RESULTS AND DISCUSSION

### Dry matter intake and apparent digestibility

Results of feed intake and apparent digestibility of DM, NDF and ADF are shown in Table 3. Although heifers were fed rations formulated to be isoenergetic and isonitrogenous, intakes of DM were nearly similar for heifers fed *P. purpureum* grass supplemented with poultry litter (T1), canola meal (T3), and soybean meal (T4) except for animals fed urea (T2) (7.64 kg DM/day) and there were significant differences between treatments ($P < 0.05$). NDF and ADF intakes followed the same trend as DM intake. Apparent digestibility of DM, NDF and ADF was 45.69, 53.43 and 74.12%, respectively, and there were no differences between treatments ($P > 0.05$).

In tropical regions, low-quality pastures induce a reduction in apparent digestibility and rumen degradability of the fibrous fractions consumed by ruminants, this result in rumen conditions not being adequate (concentration of ammonia, pH and rumen osmolarity) for the growth of microbial population. Under those circumstances, feed intake, rate of passage and apparent digestibility are affected due to the limited supply of nitrogen for the rumen microbes. Supplementations with protein and energy sources during the dry season avoid these negative effects and improve weight gain and milk production. In the present study, DMI was affected by feeding urea as a source of nitrogen (T2) which resulted in the lowest intake, which agrees with Burque et al. (2008) in studies of buffalo calves that reported a decrease in DMI as the level of urea in the ration was increased. Moreover, Hulshof et al. (2012) and Klop et al. (2016) recorded lower DMI when nitrate was added to cattle rations. It seems that rations supplemented with NPN (i.e., urea, nitrate, etc.) as a source of nitrogen tended to negatively affect the intake of the ration by heifers probably due to an effect on palatability or perhaps to the increased $NH_3$-N concentration in the rumen. As a result of the lower dry matter intake with the urea ration (T2), NDF and ADF intakes followed the same trend and gave the lowest values with the T2 ration. Apparent digestibility of DM, NDF and ADF was not affected by the different nitrogen sources. This result was in contrast to Kostenbauder et al. (2007), who reported that the ration which included hay with urea and molasses increased NDF and ADF digestibility in Holstein steers. In other studies (Olijhoek et al. 2016), adding nitrate caused no significant effect on the digestion processes in various parts of the digestive tract, this conflicting result could be related to the feeding strategy used.

### Rumen fermentation characteristics

Rumen pH was not affected ($P > 0.05$) by experimental rations while total volatile fatty acids (VFA) and molar proportions of acetic and pro-

Table 3. Feed intake and apparent digestibility of crossbreed heifers fed low-quality *Pennisetum purpureum* grass supplemented with different nitrogen sources

| | Treatments | | | | SE | *P* value |
|---|---|---|---|---|---|---|
| | control (T1) | urea (T2) | canola (T3) | soybean (T4) | | |
| Live weight (kg) | 352 | 356 | 356 | 354 | 6.01 | 0.22 |
| Intake (kg/d) | | | | | | |
| DM | 8.17[a] | 7.64[b] | 8.18[a] | 8.14[a] | 0.08 | 0.03 |
| NDF | 4.29[a] | 3.78[b] | 4.22[a] | 3.98[a] | 1.33 | 0.007 |
| ADF | 2.15[a] | 1.83[b] | 2.10[a] | 1.98[a] | 6.63 | 0.009 |
| Digestibility (%) | | | | | | |
| DM | 47.40 | 41.43 | 44.70 | 49.23 | 5.34 | 0.13 |
| NDF | 56.23 | 43.16 | 55.03 | 59.30 | 7.36 | 0.22 |
| ADF | 79.21 | 66.13 | 74.63 | 76.53 | 5.36 | 0.42 |

Means in the same row with common superscripts are not different ($P < 0.05$)

ADF = acid detergent fibre; CP = crude protein; DM = dry matter; NDF = neutral detergent fibre; SE = standard error

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pionic acids in rumen liquor significantly varied among treatments (Table 4). A reduction in acetic acid concentration was observed in T2 and T3 *vs* T1 and T4 (67.5 and 66.4 *vs* 71.3 ml/100 ml rumen liquor, respectively: $P < 0.05$). The molar proportion of propionic acid in rumen liquor was similar between T1 and T4 ($P > 0.05$). No difference was found between T1, T2 and T3 ($P > 0.05$), however, T2 and T3 were higher than T4 ($P < 0.05$).

Rumen pH is an important parameter to assess the appropriate functioning of rumen fermentation. Rumen pH was not affected by experimental rations and it was 6.6 on average. This value was optimal for normal rumen fermentation, microbial activity and VFA production (Anantasook et al. 2013; Gunun et al. 2013) and it is a typical pH of a cellulose-based ration with supplementation of nitrogen or energy sources. While the experimental rations affected rumen fermentation as evidenced by changes in VFA patterns (Table 4). Total concentration of volatile fatty acids (ml/100 ml) was decreased when heifers were fed diets containing urea. This result suggests that using urea as a source of nitrogen in the diet reduced voluntary feed intake (Table 3) and rumen fermentation as well. Furthermore, urea (T2) and canola meal (T3) in the diets decreased the proportion of acetic acid and increased the proportion of propionic acid in rumen liquor and the population of cellulolytic bacteria was therefore decreased. Consequently, the apparent digestibility of fibre and $CH_4$ production were reduced while the synthesis of propionic acid was increased. This result was in agreement with Wanapat et al. (2009), who reported that volatile fatty acid concentrations particularly those of acetic acid were decreased and those of propionic acid increased in cows fed 5.5% urea-treated rice straw and 2.2% urea + 2.2% calcium hydroxide-treated rice straw, respectively, in dairy cow rations. Although a reduction in DMI was observed with urea treatment, the ration of better quality was ingested, having a lower amount of NDFI and ADFI and higher digestibility of the diet; these factors can explain a reduction in acetic acid production.

The significant effect of canola meal on acetic and propionic acids is due to the composition of canola meal which has a higher concentration of oil that contains 54% oleic acid, 22% linoleic acid, and 11% linolenic acid (NRC 2001). Unsaturated fatty acids in canola meal provided alternative hydrogen sinks which could affect biohydrogenation processes (Boadi et al. 2004) which serve as an alternative electron sink for $H_2$ produced during acetic acid synthesis. This reduction in metabolic $H_2$ provokes a stoichiometric reduction of the synthesis of enteric methane (Beauchemin and McGinn 2014).

## Enteric methane emissions

Enteric methane emissions and energy loss as methane are shown in Table 5. Mean values of total methane emissions of heifers for all experimental rations were 119 g/d (SE = 4.48). No statistical differences in methane emissions were found between control diet (T1; poultry litter) and the other experimental rations, however, differences were found in T2 and T3 compared to T4. Enteric methane emissions were not statistically different between urea (T2) and canola meal (T3). Methane emis-

Table 4. Rumen fermentation pattern of crossbred heifers fed low-quality *Pennisetum purpureum grass* supplemented with different nitrogen sources

| | Treatments | | | | SE | *P* value |
|---|---|---|---|---|---|---|
| | control (T1) | urea (T2) | canola (T3) | soybean (T4) | | |
| pH | 6.77 | 6.73 | 6.67 | 6.53 | 0.064 | 0.649 |
| VFA (ml/100 ml) | | | | | | |
| Total VFA | 88.7$^{a}$ | 79.0$^{b}$ | 87.3$^{a}$ | 87.7$^{a}$ | 1.634 | 0.108 |
| Acetic acid | 71.3$^{a}$ | 67.5$^{b}$ | 66.4$^{b}$ | 71.3$^{a}$ | 0.739 | 0.002 |
| Propionic acid | 18.5$^{ab}$ | 20.7$^{a}$ | 19.5$^{a}$ | 17.5$^{b}$ | 0.457 | 0.044 |
| Butyric acid | 7.67 | 7.1 | 7.17 | 6.6 | 0.275 | 0.662 |

Means in the same row with common superscripts are not different ($P < 0.05$)

SE = standard error; VFA = volatile fatty acids

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Table 5. Enteric methane emissions of crossbreed heifers fed low-quality *Pennisetum purpureum* grass supplemented with different nitrogen sources as measured in open-circuit respiration chambers

| Items | Treatments | | | | SE | *P* value |
|---|---|---|---|---|---|---|
| | control (T1) | urea (T2) | canola (T3) | soybean (T4) | | |
| $CH_4$ (g/d) | 125$^{ab}$ | 105$^{b}$ | 104$^{b}$ | 141$^{a}$ | 4.48 | 0.007 |
| $CH_4$ (g/kg DMI) | 15.4$^{ab}$ | 13.6$^{b}$ | 12.6$^{b}$ | 17. 4$^{a}$ | 0.61 | 0.033 |
| Energy loss as $CH_4$ (MJ/d) | 6.91$^{ab}$ | 5.64$^{b}$ | 5.76$^{b}$ | 7.92$^{a}$ | 0.26 | 0.004 |
| Energy loss as $CH_4$ (%GEI; $Y_m$) | 5.44$^{ab}$ | 4.21$^{c}$ | 4.49$^{bc}$ | 5.88$^{a}$ | 0.19 | 0.004 |

Means in the same row with the same superscripts are not different ($P < 0.05$)

$CH_4$ = methane; d = day; DMI = dry matter intake; GEI = gross energy intake; SE = standard error

sions (g/d) recorded the highest value ($P < 0.05$) for heifers fed soybean meal (T4) as a nitrogen source, then they decreased in descending order for those fed poultry litter (T1), canola meal (T3) and urea (T2) ($P < 0.05$). A reduction in $CH_4$ emissions (g/d) for T3 and T4 was also observed when expressed per kilogram of dry matter intake (DMI). Energy lost as $CH_4$ (MJ/d or as % of gross energy intake; $Y_m$) was affected by different rations and recorded the highest value for heifers fed soybean meal while the lowest value ($P < 0.05$) was recorded in cattle fed the urea ration.

Several factors are known to affect enteric methane emissions such as dry matter intake, type of carbohydrate fermented in the rumen (cellulose *vs* starch), forage processing method and lipid addition (Johnson and Ward 1996; Beauchemin et al. 2020). The mode of action has two possible mechanisms which can be rationalized as:

1. The amount of carbohydrate that is fermented in the reticulorumen;
2. the amount of hydrogen available for carbon dioxide reduction and the resulting methane synthesis mediated by the proportions of VFA in the rumen.

Other studies reported that changes in $CH_4$ production in the rumen were related to the sources of carbohydrate and protein, and to the rumen environment (Johnson and Johnson 1995; Ulyatt and Lassey 2001; Singh et al. 2012). In the present experiment, methane yield ranged from 12.6 g/kg to 17.4 g/kg DMI (Table 5), these values are comparable with those reported by Lage et al. (2017) when they estimated methane production in cattle (Gyr and $F_1$ Holstein × Gyr) under tropical conditions. Niu et al. (2016), using two dietary forage levels (37.4% *vs* 53.3% DM) and two dietary CP levels (15.2% *vs* 18.5% DM) in lactating cows, estimated methane emissions of 20.3 *vs* 18.0 for 19.2 *vs* 19.1 g/kg DM intake, respectively. The values mentioned above by those authors are higher than the values obtained in the present work due to higher content of the concentrate used, higher intake and to the differences in the feeding strategy when compared with that in our study.

As expected, a significant reduction in methane emission was recorded in heifers fed urea (T2) or canola meal (T3) rations compared to those fed soybean meal (Table 5). Decreased methane emission for the urea supplemented ration (T2) could be due to increased ammonia accumulation from urea breakdown in the rumen and inhibition of methane synthesis by archaea (He et al. 2005; Sujiang et al. 2016).

In *in vitro* studies, Ramirez-Bribiesca et al. (2018) demonstrated that rations including different types of canola meal decreased methane production and enhanced propionate production as well. This result agrees with that reported by Mathison (1997), who found that daily enteric methane emissions could be reduced by 33% when canola oil was added to a high concentrate feedlot ration.

Energy loss as $CH_4$ (MJ/d or as %GEI) gave a higher value for heifers fed soybean meal (T4), then it decreased gradually for those fed poultry litter (T1), canola meal (T3) and urea (T2) in descending order. A significant reduction in gross energy loss as methane for the urea supplemented ration of 23% and of 17% for the canola meal ration (with respect to control treatment) was ob-

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served in the present trial. The mean estimated value of energy loss as $CH_4$ (%GEI) agrees with Pineiro-Vazquez et al. (2017) in heifers fed low-quality tropical grasses. Similar results were found by Valencia-Salazar et al. (2018), who reported that energy loss as methane represented 4.2% of GEI. In general, the estimates of enteric methane emissions at present have been derived by using the equations proposed by the Intergovernmental Panel on Climate Change (IPCC 2006) which are known to be inaccurate because of the several assumptions implicit in those equations. Based on the results of the work hereby described, it can be concluded that supplementation with urea or canola meal as sources of nitrogen to heifers fed a basal ration of low-quality *Pennisetum purpureum* grass reduced acetic acid concentration in the rumen and decreased enteric methane emissions.

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## Conflict of interest

The authors declare no conflict of interest.

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