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SHORT COMMUNICATION



## Methane emission factors for beef cows in Argentina: effect of diet quality

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### ABSTRACT

To determine the methane (CH<sub>4</sub>) emission factors for beef cows in Argentina, we carried out two experiments to test the effect of different diets on methane emission from grazing conditions. In experiment 1, diet consisted of native grass-based pasture vs. sorghum-based pasture (63.1% and 63.5% of dry matter digestibility (DMD) respectively), and in experiment 2, it consisted of native grass-based pasture vs. alfalfa hay (45.2% and 43.6% of DMD respectively). In both experiments, CH<sub>4</sub> production showed statistically significant differences (202.7 ± 11.5 and 157.5 ± 10.6 g/d for native grass-based pasture and sorghum respectively; 157.4 ± 8.9 and 190.6 ± 9.4 g/d for native grass-based pasture and lucerne hay respectively). The energy lost through eructation of CH<sub>4</sub> was less for cows grazing sorghum than for cows grazing native grass-based pasture (4.3% ± 0.3% vs 5.6% ± 0.4%), and it was similar between the latter and cows grazing alfalfa hay (7.1% ± 0.6% vs 8.2% ± 0.5%). The results support that low quality diets increase methane yield (Y<sub>m</sub>).

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## Introduction

Methane (CH<sub>4</sub>) emitted by cows and other domestic ruminants contributes significantly to the atmospheric greenhouse gas burden (Houweling 2000; Houghton et al. 2001; Lassey 2007a, 2007b).

Although numerous experiments monitor enteric methane emissions, the effects of grazing management (DeRamus et al. 2003), nutritional supply (Beauchemin and McGinn 2006; Newbold and Rode 2006; Beauchemin et al. 2007) or genetic selection (Crews 2005; Hegarty et al. 2007) are potentially relevant for emission abatement strategies, thus constituting an active area of experimental research.

Methane produced by enteric fermentation in ruminants represents 13% of total greenhouse gas emissions in Argentina (World Bank 2016). The relative weight of CH<sub>4</sub> emissions in relation to the total greenhouse gases is particularly high, mainly because of emissions from livestock production in rural areas. In order to develop and implement mitigation strategies in pasture systems, it is necessary to consider reliable emission

factors, so that determination uncertainties are minimised. The stock of cattle in Argentina represents around 5.5% of the world stock, and approximately 90% is used for meat production (SENASA 2016).

Bárbaro et al. (2008) studied methane emissions from grazing beef cattle in Argentina, where large variations in energy lost through eructation were observed (3.5%–6.5%). However, measurements of methane emission factors in Argentina are scarce.

To further investigate this issue, we conducted two experiments that measured emission factors for lactating and pregnant beef cows exposed to different diets.

## Material and methods

This work was completed at the INTA Castelar Experimental Station (Hurlingham, Argentina, GPS Coordinates: 34°36' S Latitude, 58°40' W Longitude). It was approved by the Animal Ethics Committee of INTA and animals were accordingly cared for (CICUAE 47/2016 and 49/2018).

### Experimental design, animals, pastures and management

Twenty beef cows (Aberdeen Angus x Hereford) were assigned to the two treatment diets based on live weight, 15 days prior to beginning the experiment, for the animals to acclimatise to the established diets. The CH<sub>4</sub> emissions were estimated by the sulfur hexafluoride (SF<sub>6</sub>) tracer technique (Johnson et al. 1994) and the dry matter intake (DMI) measurements were determined using chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) (Corbett and Freer 1995). The animals were weighed before and after the beginning of the CH<sub>4</sub> emission and DMI measurement. The grazing system used was continuous set stocking. The forage availability permitted *ad libitum* pasture intake (forage availability > 5% of LW).

### Experiment 1

The study was conducted for 20 days beginning in late March 2014 (autumn). It involved twenty pregnant beef cows (4–6 years old and 378 ± 57 Kg LW on average).

Treatments consisted in animals grazing pastures (8-ha. paddock) with two diets of different composition. Treatment 1 was native grass-based pasture composed of 60% grass (*Festuca arundinacea*, *Lolium multiflorum*, *Bromus unioloides*, *Paspallum dilatatum*) and 40% legume (*Trifolium repens*, *Trifolium pratensis*). Treatment 2 consisted of sorghum-based pasture, approximately 75 days after emergence (soft dough). The available forage was 3200 and 4300 kg dry matter (DM)/ha. for native grass-based pasture and sorghum-based pasture, respectively.

Following 15 days of adaption to pastures, the CH<sub>4</sub> emission and DMI measurements were completed over a 5-day period (days 16–21).

During the experimental period, the daily mean of air temperatures and relative humidity were 18.1°C and 76.5%, respectively and the rainfall averaged c. 146 mm/month.

### Experiment 2

The study was conducted over 25 days beginning in late October 2016 (spring). It involved twenty lactating beef cows (6–8 years old and 388 ± 66 Kg LW on average). The calves age ranged from 60 to 86 days.

Treatments consisted of two groups of animals: for treatment 1, animals grazed native grass-based pasture (same paddock with herbage covering offered in experiment 1 with 2500 kg of DM/ha. of available forage) and for treatment 2, animals were fed with lucerne hay in a bare paddock (*ad libitum* access).

Following 15 days adaption to pastures, the CH<sub>4</sub> emission and DMI measurements were completed over 10 days (two 5-day period: days 16–21 and 21–26).

The daily mean air temperature and relative humidity at the farm were 18.5°C and 72.7%, respectively. The rainfall averaged c. 91 mm/month (however, during the months before the experiment, the rainfall was very scarce: c. 45 mm/month).

### **Pasture sampling**

At the end of the acclimatisation period, six random samples at ground level were obtained in each paddock within a 1000 cm<sup>2</sup> frame. The samples were dried together at 60°C until reaching a constant weight. Once ground and mixed, the composite sample of each treatment was analysed to determine its chemical composition and digestibility. For experiment 2, we repeated this procedure five days later.

### **Feed intake measurements**

The DMI (kg/day) of the animals was determined using chromic oxide (Cr<sub>2</sub>O<sub>3</sub>). From the start of the acclimatisation period, cows were dosed daily with a gelatin capsule containing 10 g of Cr<sub>2</sub>O<sub>3</sub> (99% purity). Faeces were rectal-sampled daily in the morning (10 am) during the measurement of enteric CH<sub>4</sub>, and oven-dried at 60°C for 96 h in order to measure dry matter and chromium concentration.

Using the daily Cr<sub>2</sub>O<sub>3</sub> dose and the chromium concentration in the faeces of each animal, it is possible to estimate faecal organic matter output, according to Equation 1 below.

$$\text{Faecal DM output (kg/d)} = \frac{\text{Cr}_2\text{O}_3\text{dose(mg/d)}}{\text{Cr}_2\text{O}_3\text{in faeces (mg/kg DM)}} \quad (1)$$

The daily mean DMI (kg/d) per animal was calculated using the faecal DM output (kg/d) and the dry matter digestibility (DMD) according to Corbett and Freer (1995) (Equation 2):

$$\text{DMI (kg/d)} = \frac{\text{Faecal DM output (kg/d)} \times 100}{100 - \text{DMD} \times 100} \quad (2)$$

### **Methane measurements**

On the third day of the acclimatisation period, the cows were orally dosed with SF<sub>6</sub> permeation tubes, which were chosen based on their high linearity of mass loss ( $R^2 > 0.99$ ) and proximities of precalibrated permeation rates (PR) ( $4.90 \pm 0.52$  and  $4.71 \pm 0.12$  mg/day for experiment 1 and 2, respectively). The sample system consisted of a polyvinyl chloride (PVC) yoke-shape collection device (2.5 L volume) with a sample flow regulated by a capillary system (Johnson et al. 1994).

During experiment 1, five consecutive samples were taken per animal using a sampling collection period of 24 h (traditional use of SF<sub>6</sub> tracer technique) (Johnson et al. 1994). For

experiment 2, two consecutive samples were collected per animal and the sampling collection period was 5 days long (adapted technique extending the collection duration of breath samples) (Pinares-Patiño et al. 2012).

Prior to each sample collection period, the PVC canisters were cleaned with high purity nitrogen (N<sub>2</sub>) and pre-evacuated (until 1 mbar). The flow regulators were calibrated to allow a residual vacuum of about 500 mbar at the end of the sample collection period. Sample flow collection was set to ~1.2 and 0.25 mL/min for daily and five-day sample collection systems, respectively. For the setting of the sample flow rate, a small segment (5 cm) of the metal capillary was pressed (using a vice) until the desired flow was achieved (Pinares-Patiño et al. 2007). Simultaneously to the breath sample collections, background air samples were also collected in duplicate, facing the incoming wind flow direction at the edge of the paddock (1.2 m). At the end of the collection, the pressure in sample containers was checked, and those with residual pressure in the range of 400–600 mbar were retained for analysis (Gere and Gratton 2010). The sampling procedure achieved 88% efficiency, and this percentage was similar for both experiments and treatments.

Concentrations of CH<sub>4</sub> and SF<sub>6</sub> were analysed using gas chromatography (Perkin Elmer 600, INTA Castelar, Hurlingham, Argentina). For CH<sub>4</sub> measurements, the gas chromatograph was equipped with a flame ionisation detector and a 30 m Elite Plot Q column (0.53 mm I.D.). The temperatures of the oven, the injector, and the detector were 40°C, 120°C, and 380°C, respectively. The carrier gas (N<sub>2</sub>) flux was 21 psi. The flammable gases (hydrogen and air) fluxes were 45 and 450 mL/min, respectively. For SF<sub>6</sub>, the gas chromatograph was equipped with an electron capture detector and a 30 m Elite Molesieve column (0.53 mm ID). The temperatures of the oven, the injector, and the detector were 40°C, 120°C, and 350°C, respectively. The carrier gas (N<sub>2</sub>) flux was 30 mL/min.

The CH<sub>4</sub> emission per animal was calculated using the PR of each SF<sub>6</sub> capsule and concentration of CH<sub>4</sub> and SF<sub>6</sub> (Equation 3).

$$\text{CH}_4(\text{g/d}) = \frac{\text{PR SF}_6(\text{g/d}) * [\text{CH}_4 - \text{BG}_{\text{CH}_4}]}{[\text{SF}_6 - \text{BG}_{\text{SF}_6}]} \quad (3)$$

where: [CH<sub>4</sub>] and [SF<sub>6</sub>] are the concentrations of these gases in the samplers and BG are the background atmospheric concentrations. The background values were 1.9 ± 0.2 and 2.2 ± 0.3 ppm for CH<sub>4</sub> and 8.1 ± 3.2 and 9.7 ± 2.5 ppt (for experiment 1 and 2, respectively).

### Chemical analysis

Crude protein (CP) was measured by means of the Kjeldhal technique; neutral detergent fibre (NDF) and acid detergent fibre (ADF), using the Van Soest technique (Goering and Van Soest 1970); and *in vitro* dry matter digestibility (DMD) with the Tilley and Terry technique (Tilley and Terry 1963). Gross energy (GE) (MJ per kg of DM) was also calculated, using the equation of Schiemann et al. (1971).

### Data analysis and statistics

Infostat Statistical Software (Balzarini et al. 2008) was used to analyse differences in the mean values of CH<sub>4</sub> emission, DMI, gross energy intake (GEI), intensity of emission

(CH<sub>4</sub>/kg LW) and energy lost through eructation of CH<sub>4</sub> or methane yield (Ym) (ANOVA, Fisher's LSD Test).

Origin Lab 6.0 software (OriginLab Corporation 2016) was used to calculate the slopes of the linear regressions for Ym versus DMD and NDF.

## Results

Table 1 shows the chemical composition of pasture for both experiments. DMD was similar in the forages treatments offered in both experiments: 63.1% and 63.5% for native grass-based pasture and sorghum-based pasture, respectively (experiment 1), and 45.2% and 43.6% for native grass-based pasture and alfalfa hay, respectively (experiment 2).

DMI and CH<sub>4</sub> emission parameters are presented in Table 2. In experiment 1, a statistically significant difference in CH<sub>4</sub> emissions was observed ( $p < 0.05$ , 23% in absolute terms). As no differences in intake (DMI) were observed ( $p = 0.7369$ ), such difference was maintained when calculating Ym. Sorghum-fed animals presented a CH<sub>4</sub> energy loss through eructation of  $4.3\% \pm 0.3\%$ , while pasture-fed animals showed  $5.6\% \pm 0.4\%$ .

In experiment 2, even though there is a statistically significant difference in total CH<sub>4</sub> emissions ( $p < 0.05$ ), this difference is no longer observed in DMI and energy lost through eructation of CH<sub>4</sub>. The resulting values were  $7.1\% \pm 0.6\%$  and  $8.2\% \pm 0.5\%$ , for native grass-based pasture and lucerne hay, respectively ( $p = 0.2875$ ).

## Discussion

The two experiments described were planned to provide data on enteric CH<sub>4</sub> emission from lactating and pregnant grazing beef cows in Argentina.

**Table 1.** Chemical composition and in vitro digestibility of the pastures used in the study.

	Experiment 1		Experiment 2	
	Native grass-based pasture	Sorghum-based pasture	Native grass-based pasture	Alfalfa hay
CP (%)	17	7.1	14	8
NDF (%)	60	44.7	69.7	75.2
ADF (%)	37.5	24.7	36.5	42.1
DMD	63.1	63.5	45.2	43.6
GE (MJ/Kg DM)	17.4	16.8	12.4	11.3

Note: CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; DMD = dry matter digestibility; GE = gross energy (MJ/Kg dry matter).

**Table 2.** Methane emission, dry mater intake and methane yield for beef cows in the two experiments.

	Experiment 1			Experiment 2		
	Native grass-based pasture	Sorghum-based pasture	<i>p</i> value	Native grass-based pasture	Alfalfa hay	<i>p</i> value
CH <sub>4</sub> Emission (g/d)	202.7 ± 11.5	157.5 ± 10.6	0.0243	157.4 ± 8.9	190.6 ± 9.4	0.0169
DMI (kg/d)	11.75 ± 1.83	12.05 ± 1.87	0.7300	10.3 ± 1.7	11.86 ± 1.86	0.0716
GEI (MJ/d)	204.6 ± 10.1	207.5 ± 11.3	0.8312	126.8 ± 6.7	135.56 ± 7.53	0.3569
CH <sub>4</sub> /kg LW	0.55 ± 0.04	0.42 ± 0.03	0.0491	0.45 ± 0.03	0.49 ± 0.03	0.4702
Ym (%)	5.6 ± 0.4	4.3 ± 0.3	0.0441	7.1 ± 0.6	8.2 ± 0.5	0.2875

Note: Emissions = daily CH<sub>4</sub> emissions (g/day); DMI = dry matter intake (kg/day); GEI = gross energy intake (MJ/day); CH<sub>4</sub>/kg LW = intensity of emission; Ym = methane yield (%). Values are means and standard deviations.

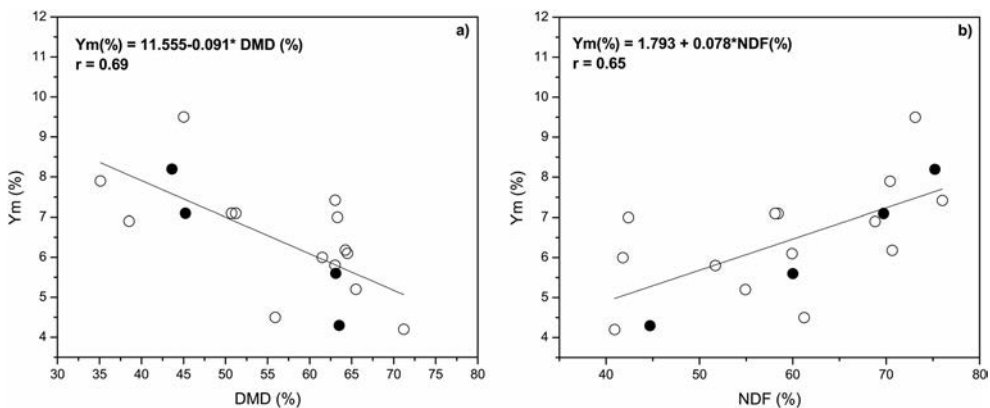
The total CH<sub>4</sub> emission was similar to that reported by Bárbaro et al. (2008). The differences between experiments regarding total CH<sub>4</sub> emissions can be explained by feed intake and digestibility (Blaxter and Clapperton 1965). In experiment 1 although the DMD for both treatments was similar, a lower Y<sub>m</sub> was observed in sorghum-based pasture, where the content of NDF (fibrous carbohydrates) was lower than that of native grassland (44.7% vs 60% respectively). Ulyatt et al. (2005) reported that higher contents of NDF would increase CH<sub>4</sub> emitted per unit digested. At the same time, the presence of tannins in sorghum, as reported by Cummins (1971), could contribute to reducing CH<sub>4</sub> emissions. Feeding sorghum silages with variable tannin levels could improve protein digestibility and reduce CH<sub>4</sub> emissions (Oliveira et al. 2007).

In experiment 2, even though there is a statistically significant difference in the total CH<sub>4</sub> emissions, this difference is no longer observed in terms of GEI. In this case, chemical composition and in vitro digestibility were similar for both treatments.

The resulting emission values were within the range of the ones reported by McCaughey et al. (1999) for lactating beef cows. Lassey (2007a) studied the effect that DMD has on CH<sub>4</sub> emissions, and the values reported are comparable to the results obtained in the present study.

The two experiments reported are independent of each other, so they cannot be directly compared. However, the lower quality of the feed offered in experiment 2 could contribute to a considerable increase of Y<sub>m</sub> compared to experiment 1. If the native grass-based pasture is compared in the two experiments, it can be observed that in spring Y<sub>m</sub> increased by 27% compared to autumn, while DMD and GE decreased. This feed quality decrease might be explained by lower precipitation in the months prior to the experiment, which could have accelerated the maturity of the pasture, thus increasing dead material.

The Y<sub>m</sub> values obtained in this paper are in the range of those reported in other trials for beef cows using the SF<sub>6</sub> tracer technique in Brazil (Nascimento et al. 2016), Uruguay (Dini et al. 2017) Argentina (Bárbaro et al. 2008) and Canada (McCaughey et al. 1999; Boadi and Wittenberg 2002). In all cases, we observe a negative correlation of Y<sub>m</sub> with DMD ( $r = 0.69$ ,  $p < 0.001$ ) (Figure 1A) and a positive correlation with NDF ( $r = 0.65$ ,  $p < 0.001$ ) (Figure 1B),



**Figure 1.** Energy lost through eructation of CH<sub>4</sub> (Y<sub>m</sub>) as function of DMD (%) (A) and NDF (%) (B) for our results (in full circles) and other trials (in open circles), for beef cattle using the SF<sub>6</sub> tracer technique in some experiments of Argentina, Brazil, Uruguay and Canada.

consistent with previous analyses. The strong data dispersion in [Figure 1](#) can be explained by greater intra-animal and inter-animal variability in CH<sub>4</sub> emission measurements (Pinares-Patiño and Clark 2008) combined with errors in the estimation of digestibility and intake, resulting from inter-animal variations in recovery of the external marker (Cordova et al. 1978).

Dini et al. (2017) demonstrated that using high quality pastures could reduce enteric CH<sub>4</sub> emissions. A reduction of about 14% of the CH<sub>4</sub> emitted per unit of DMI was reported. For DMD between 35.1% and 71.2%, Ym was in the range 4.20% and 7.90%.

The IPCC (2006) estimated a  $6.5 \pm 1\%$  loss of gross energy for beef cattle and suggested that, although the mean value is appropriate for most applications, lower limits may be used for high-digestibility and high-energy feeds, whereas upper limits may be more appropriate for poor quality feed. This is consistent with the results obtained in this work for native grass-based pasture.

## Conclusion

In this study, the lowest methane yield values were obtained in cows fed with sorghum-based pasture, and the highest values, in animals fed with alfalfa hay.

Cows fed with native grass-based pasture showed a higher energy lost through eructation when the quality of the offered diet was lower (spring trial presented a lower quality than autumn trial). We can conclude that low quality diets, with low DMD and high NDF, considerably increased enteric CH<sub>4</sub> emissions.

The information presented in this work may be useful to develop national inventories that calculate CH<sub>4</sub> emission factors for livestock according to forage quality.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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