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Methane emissions from sheep grazing pearl millet (*Penissetum americanum* (L.) Leeke) swards fertilized with increasing nitrogen levels

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ABSTRACT

This study aimed at quantifying methane emissions from sheep grazing pearl millet swards (*Penissetum americanum* (L.) Leeke) as affected by nitrogen (N) fertilization doses (50, 100, 200 and 400 kg N ha^{-1}). The experimental period was 70 days of pasture use, from February to April 2011. The grazing method was continuous stocking with variable stocking rate, so pasture structure was intended to be similar (30 cm sward height) among treatments. Thirty six tester animals aging 5 months and initially weighing 20 ± 1.6 kg were used for evaluations. Intake (OMI) was estimated by their relationship with N content on faeces. The sulfur hexafluoride (SF_6) technique was used for two sampling periods of five consecutive days each to quantify daily methane (CH_4) production. Parameters related to sward structure was not affected by N fertilization, but herbage accumulation increased linearly ($P < 0.05$). Greater ($P < 0.05$) stocking rate, weight gain per area and CP content were observed with higher fertilization levels. N doses did not affect ($P > 0.05$) OMI, and methane emissions expressed as a ratio of OMI. Results indicated that increasing nitrogen doses decrease methane emissions per animal. When expressed as grams of methane per kilogram of organic matter ingested or energy converted into methane, no difference was observed. However, emissions per unit area increase as a consequence of higher stocking rates allowed by the increase in forage production. Sheep emitted 16.3 g of methane per kg of ingested organic matter and converted 5.1% of ingested gross energy into methane.

1. Introduction

Pastoral farming systems will inevitably be faced with intensification because of the increasing world food demand. As intensification processes increase livestock pressure on natural resources, side effects encompass the emissions of greenhouse gases (GHG) from animal production (Gerber et al., 2013), and consequently their production is one of the main current concerns at a global scale (Ramírez-Restrepo et al., 2010). Bellarby et al. (2008) estimate that global GHG emissions from agriculture are (including emissions from change in land use) between 8.5 and 16.5 Gt of CO_2eq (carbon dioxide equivalent) $year^{-1}$, representing between 17% and 32% of total human emissions.

Brazil has an important role in this future scenario since it has 200 million bovine heads that produce 52% of enteric methane of total emissions from agriculture and livestock (Brazil, 2014), besides other ruminants that contribute to the emissions of GHG. Improvement in ruminant nutrition by the use of high quality forages can result in better animal performance and CH_4 (methane) reduction per unit of dry matter consumed and per unit of product (Lascano and Cárdenas, 2010).

The improvement of forage quality may be attained by the increase on N fertilization rates, among other ways. In a simulation, Bannink et al. (2010) observed that the enhancement in N fertilization in composed pastures result in significant decrease in ruminal CH_4 emissions. Lower CH_4 emissions where attributed

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to increase in nitrogen: organic matter ratio. The same concept was also used by Lovett et al. (2004) to explain the reduction of *in vitro* methane production when incubating forages fertilized with increasing nitrogen doses.

Even though nitrogen fertilization results on considerable changes in forage composition, most of the studies revised by Peyraud and Astigarraga (1998) did not find significant differences in CH_4 production related variables (intake, digestibility and microbe protein synthesis) for either grazing or feedlot animals.

The use of N fertilizers can increase pastoral systems total GHG emissions by direct and indirect emissions of N_2O (Soussana and Lemaire, 2014). Also, the use of N often results in an increase in stocking rate, causing total ruminal CH_4 to increase.

Measurements of CH_4 emissions from livestock have traditionally been obtained using indirect calorimetry techniques with housed animals. Enclosed chambers has been questioned because behaviour and environment are different in grazing systems. About 98% of the total tract CH4 production is excreted through the mouth and nostrils of ruminants. Therefore, Johnson et al. (1994) developed a technique employing sulfur hexafluoride marker (SF6) for animals in grazing systems.

Nonetheless, studies evaluating the effect of N fertilization in CH_4 emissions in grazing conditions are rarely available, particularly in tropical and subtropical regions. Thus, the data presented in this paper will be useful in constructing a national inventory of methane emission from sheep grazing. Therefore, the hypothesis tested in this study was that increasing N fertilization rates result in ruminal CH_4 emissions decrease. Our objective was to elucidate the relationships among forage quality, animal production and methane emissions from sheep.

2. Material and methods

2.1. Experimental site, treatments and experimental design

The experiment was conducted at the Federal University of Rio Grande do Sul Agronomic Experimental Station (EEA- UFRGS), 46 m above sea level ([a.s.l.]), at latitude 30°05′22″S and longitude 51°39′08″W, South Brazil. The soil is classified as Typic Paleudul (US taxonomy) and sandy clay loam Acrisol (FAO classification). The climate is subtropical humid (Cfa classification, Köppen), with annual precipitation of 1440 mm, well distributed throughout the year; June is the wettest month (168.2 mm), and December is the driest (97.7 mm).

The experimental design used was completely randomized block, with four treatments, replicated three times, with three lambs grazing on each. Treatments consisted of increasing nitrogen levels nitrogen doses (50; 100; 200 and 400 kg N ha^{-1}).

Experimental units were constituted of 12 paddocks with area varying between 800 m^2 and 1200 m^2 for the highest and lowest doses, respectively. Three animals were used in each experimental unit, totaling 36 non castrated male Texel lambs. Animals were grouped according to weight, being the blocking criteria. Average weight was 20 ± 1.6 kg and average age was 5 months.

2.2. Sward management

Pearl millet (*Penissetum americanum* (L.) Leeke) was sown with 25 kg ha^{-1} of seed in December 2010, in a no-tillage system over black oat residue. Fertilization at sowing was 300 kg ha^{-1} of N-P-K (5-30-15 formula). Approximately 40 days after sowing, there was intense grazing for one week, later the sward was clipped to 15 cm of ground level. Nitrogen fertilization (50, 100, 200 and 400 kg N ha^{-1}) was applied in a single urea application one week before starting the grazing. Experimental period was from February to April 2011, totaling 70 days of pasture use.

Continuous stocking with variable stocking rates was applied (Mott and Lucas, 1952) to impose similar sward structures in all treatments. An additional 0.7 ha pearl millet area was used to keep put-and-take animals during periods on which they were not used to regulate sward. Sward height was the management variable to impose similar sward structure among treatments. The targeted sward height was 30 cm as determined by Castro (2002) to optimize live weight gain per area and per animal.

Sward height was taken using a sward stick, in 140 spots per experimental unit. A marker runs through the stick and canopy height is considered as the height on which the marker touches the first leaf in the canopy (Barthram, 1985).

Herbage mass (HM) was estimated every 20 days, using the relationship between sward height (SH) and HM (Santillan et al., 1979). Two sites per experimental unit representing average HM were sampled. At these sites, five SH measurements were taken and the area within the quadrat (0.5 × 0.5 m) was clipped to the ground level. These values were used to develop linear regression equations ($y = a + bx$) relating HM (y) and SH (x) for each level of fertilization and general equation (including 4 levels of fertilization) was developed. The selection of the equations to be used was based on the highest coefficient of determination (R^2) and the P value ($P < 0.05$). SH was measured weekly in 140 spots per experimental unit, and regression equations were used to estimate HM and stocking rate (SR) was adjusted.

Herbage accumulation rate (HAR) was determined every 20 days using three grazing exclusion cages per experimental unit (Klingman et al., 1943), placed in spots that represented average herbage mass in each paddock.

Samples were taken to a forced circulation drying oven at 60 °C for 72 h, after which they were weighed for determining HM as kg dry matter (DM) ha^{-1}

2.3. Chemical analysis

The hand-plucking technique (De Vries, 1995) was proceeded during the CH_4 sampling period (see below) to collect forage samples aiming to mimic diet (Table 1). To determine forage composition the following methodologies were used: dry matter content (DM) in oven at 105 °C for 12 h (Easley et al., 1965); ashes (MM) through incineration at 550 °C (AOAC, 1975, method no. 22.010, and no. 7.010); crude protein (CP) through Kjeldahl method (AOAC, 1975, methods 2036, 1960 and no. 2049), obtained by total nitrogen (TN) x 6.25. Acid detergent fiber content (ADF) was analyzed excluding the ashes content and neutral detergent fiber (NDF) without amylase usage. Fiber analysis was made according to the methodology described by Van Soest and Robertson (1985). The gross energy (GE) was determined by bomb calorimetry.

2.4. Animal performance

Animals were weighted on the beginning and at the end of the experimental period. Before weighing the animals had a 12 h solid and liquid fasting period. Average daily weight gain (ADG) was obtained by difference of initial and final weight of tester animals, divided by the number of days in the experimental period. Stocking rate was obtained by summing the weight of all animals in each paddock divided by the area of each pasture and the values were expressed as kg of live weight per hectare (kg LW ha^{-1}). Live weight gain ha^{-1} (LWG) was obtained by multiplying the stocking rate by tester animals ADG in the experimental period.

Table 1
Sward characteristics and chemical composition of pearl millet (*Penissetum americanum* (L.) Leeke) swards fertilized with increasing nitrogen levels.

Variables	N fertilization levels ($kg\,ha^{-1}$)				P Value	R^2	SEM[d]
	50	100	200	400			
Sward characteristics							
SH (cm)	19.9	26.5	20.8	24.3	0.4839	–	2.15
HM ($kg\,ha^{-1}$)	1684	2286	1761	2082	0.4823	–	105.3
HAR ($kg\,day\,ha^{-1}$)[e]	146	192	216	270	0.0155	0.69	11.67
Measures from chemical composition							
DM ($g\,kg^{-1}$)	953.0	932.9	944.1	955.5	0.0870	–	0.35
OM ($g\,kgDM^{-1}$)	885.9	887.3	891.6	899.5	0.2678	–	0.32
CP ($g\,kgDM^{-1}$)	234.5[b]	261.8[ab]	280.1[a]	294.3[a]	0.0046	–	0.77
NDFc ($g\,kgDM^{-1}$)	592.1	555.9	549.8	552.6	0.2883	–	1.03
ADFc ($g\,kgDM^{-1}$)	296.1	262.6	262.3	246.6	0.2791	–	0.86

[a,b,c] Means followed by lowercase letters on line differ by Tukey test ($P < 0.05$).
[d] SEM: Standard error mean.
[e] $y = 156.2 + 0.27x$; SH, sward height; HM, herbage mass; HAR, herbage accumulation rate; DM, dry matter; OM, organic matter; CP, crude protein; NDFc, neutral detergent fibre corrected to ash and crude protein; ADFc, acid detergent fibre corrected to ash and crude protein.

2.5. *Organic matter intake*

Organic matter intake was estimated by their relationship with nitrogen content on faeces. The faecal index technique is based on the assumption that the amount of crude protein (CP) excreted in faeces per unit of ingested organic matter (OM) is constant (Lancaster 1949).

David et al. (2014), developed equations to estimate the intake and digestibility of pearl millet (*Pennisetum americanum* (L) Leeke.), through digestibility trials with sheep kept in metabolism cages. Subsequently, the equations generated in these metabolic studies were applied to test grazing with sheep consuming pearl millet.

According to David et al. (2014), the following equation was used:

$$\mathrm{OMI} = 16.52 + 29.15 \times \mathrm{CPf} - 5.38 \times \mathrm{NDFf};\ P < 0.0001;$$

$$R^2 = 0.94;\ \text{relative predictionerror(RPE)} = 9.25$$

Where: OMI: organic matter intake ($g\,d^{-1}$); CPf: faecal crude protein ($g\,d^{-1}$); NDFf: fecal neutral detergent fiber.

To make these analyses, total faeces collections were made using bags. The collections were made once a day, for five consecutive days. After the field collections, faeces were weighed and a subsample 20% of the total was dried in a forced-air oven at 60 °C for 72 h for pre-dry matter determination. After dried, subsamples were grouped in a per animal basis, grinded and referred to a laboratory for analysis. Dry matter, organic matter, nitrogen, NDF and ADF were determined according to previously described methodologies.

2.6. *Methane emissions determination*

The sulphur hexafluoride (SF_6) tracer technique was used to quantify daily methane (CH_4) production (Johnson et al., 1994). This technique is performed by dosing each animal with a SF_6 controlled release tube, which is calibrated to release a constant flux of tracer. It is assumed that the SF_6 emission patter simulates the CH_4 emission.

The permeation tubes (PT) used were the small version described in Lassey et al. (2001). The PT were provided by the NIWA (National Institute of Water and Atmospheric Research, New Zealand) and were introduced *per os* into the rumen. The permeation rates were 0.793 ± 0.157 mg/d (mean and standard deviation, respectively), as given by two months calibration assay in a 39 °C water bath.

Gas collection was performed five days after the collection faeces. The sample gases were taken in twice to get greater number of repetitions. The collection periods were in March 2011. The sample system consisted in 0.5 L stainless steel collecting canisters (provided by Solydes, Argentina) with air inflow regulator adjusted to the animal's nostrils. Prior to the sample period, canisters were cleaning with pure nitrogen and then were evacuated (<0.5 mb).

The inflow regulator was an adjustable geometric leaks based on cheap commercially available components. Basically, we assemble these by pressing a steel bearing-ball (diameter 8 mm) against the border of the cylindrical central hole of a standard brass tube union by means of a threaded brass plug. The closure between the ball and the hole border leaves open few micro-channels, whose overall conductance depends on the torque applied to the hexagonal plug head (Gere and Gratton, 2010).

The air inflow regulators were calibrated to reach 0.5 bar (one half of the atmospheric pressure) for a five-day long collection period. Hence, one air sample represented the air expired by the animal's mouth and nostrils for five days. Two additional canisters were placed in the experimental area to collect background air samples during each five-day sub-period.

After the collection period the pressure of the tubes was measured (to check the operation of the sampling system) and was pressured with pure nitrogen and the final pressure was taken (useful to calculate the dilution factor).

Analyses were performed in the Environmental Biogeochemistry Laboratory of the Soils Department/UFRGS in a Shimadzu 2014 gas chromatographer to determine CH_4 and SF_6 concentrations. The gas chromatographer was of greenhouse model, equipped with injectors connected to two automatic valves, flame ionization detection (for methane readings) and electrons capturing (for SF_6 readings). The pattern curve was calibrated using the following concentrations: 30, 100 and 1000 ppt of SF_6 and 5, 10 and 20 ppm of CH_4, provided by the White Martins, Brazil.

After the chromatography readings the CH_4 and SF_6 were corrected for dilution. With the known ruminal SF_6 release rate and concentrations of CH_4 and SF_6 in the collected samples, animal methane release flux was calculated using the CH_4/SF_6 ratio of concentrations in breath samples, after correction for background gas concentrations Thus, methane emissions would be given by:

$$QCH_4 = QSF_6 \times ((CH_4 - CH_4B)/(SF_6 - SF_6B))(\text{Johnsonet al., 2007}).$$

Where:

QCH_4 is the methane emission rate in $g\,day^{-1}$

QSF_6 is the SF_6 release rate from the permeation tube

CH_4 and SF_6 are the concentrations measured in the collecting tube

CH_4B e SF_6B are the concentrations measured in the ambient collecting tube

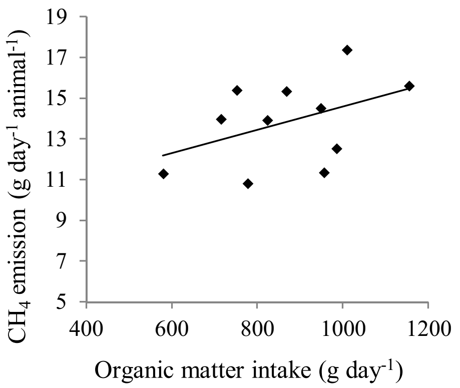

Fig. 1. Relationship between organic matter intake (g day animal^{-1}) and methane emission (g day animal^{-1}) per sheep in pearl millet pastures. $y = 8.86 + 0.0057x$; $P = 0.0390$; $R^2 = 0.44$; SEM = 1.76.

2.7. Statistical analysis

Data referring to pasture characteristics and animal production refer to the periods on which gas collections were taken. Only the LW gain ha^{-1} variable was the result of summing pasture utilization periods. One paddock (400 kg N ha^{-1}) was excluded at the start of sampling due to inadequate establishment of the sward.

The collected data was subjected to analysis of variance and adjusted to regression models. Treatment and block effects were considered in the models. For variables that were measured in each period, this effect was included in the model as a repeated measure over time. The procedure used for stats was Mixed. The means were compared with the Tukey test at 5% level. Linear and non-linear regressions were tested, being the best model choice defined by the higher R^2 and significance level ($P < 0.05$). The statistical package SAS version 9.3 (SAS Institute Inc., Cary, NC, USA, 2011) was used.

3. Results

Parameters related to sward structure were not affected by N fertilization, with SH average of 22.8 ± 2.15 cm and HM averege of 1946 ± 105.3 kg DM ha^{-1}, SH was below to the target proposed (30 cm) for treatments (Table 1). However, herbage accumulation rate increased linearly ($P < 0.05$) with the increase on N rates. DM values, OM, NDF and ADF of pearl millet were similar between treatments, while the crude protein content increase ($P < 0.05$) with N fertilization (Table 1).

OMI (% LW and g day^{-1}) and GEI (Mj day^{-1}) was similar for all treatments ($P > 0.05$), with an average of 3.82 ± 0.15%LW and 15.04 ± 0.98 Mj day^{-}. As well as individual animal performance (gLW day^{-1}) was similar ($P > 0.05$) (Table 2). SR increased linearly ($P < 0.05$) with N fertilization, enhancing animal performance per area (LWG) (Table 2).

Emission of CH_4 differ ($P < 0.05$) between treatments, CH_4 emission per sheep (g day animal^{-1}) decreased ($P < 0.05$) as greater levels on nitrogen were applied in the experimental area, however, methane emission per unit area increases ($P < 0.05$) due to the greater stocking rate on pearl millet pasture (Table 3). CH4 emission per kg of organic matter intake (CH_4OMI) and CH_4 emission per kg of GEI (Ym) by sheep were similar ($P > 0.05$) between N fertilization. On average, animals emitted 16.3 g of methane per kg of OMI and loss 5% of GEI as methane (Table 3).

Relationship between OMI (g day animal^{-1}) and CH_4 emission (g day animal^{-1}) per sheep on pearl millet pastures was positive. CH_4 emission (g day^{-1}) increased linearly ($P < 0.05$) with the increase of OMI (Fig. 1).

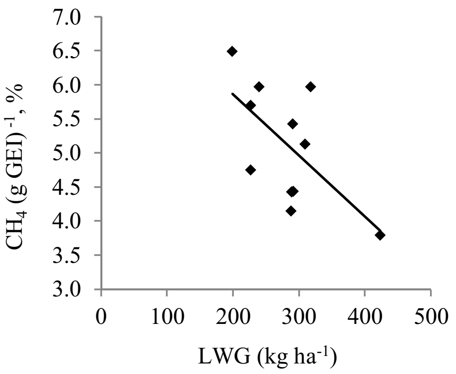

Fig. 2. Relationship between methane emission per kg of gross energy intake (CH_4 kg GEI^{-1}) and live weight gain per area (LWG ha^{-1}); $y = 7.64 - 0.009x$; $P = 0.0387$; $R^2 = 0.39$; SEM = 0.71.

Methane emission per kg of GEI decreased when LWG per area was greater ($P < 0.05$; Fig. 2).

4. Discussion

Our objective this study was to elucidate the relationships among forage quality, animal production and methane emissions from sheep grazing pearl millet swards fertilized with N.

According Poppi et al. (1987), DMI is maximized by lambs on pasture with HM of approximately 1800 kg DM ha^{-1}. HM in this study had an average value of 1946 ± 105.3 kgDM ha^{-1} (Table 1) which leads to the conclusion that performance of lambs was not limited by allowance herbage.

According Peyraud and Astigarraga (1998) and Canto et al. (2009) animal performance is not affected by nitrogen fertilization rates. The sward structure (HM and HS) offered to sheep was similar between treatments, resulting in OMI average of 3.82 ± 0.15% LW (Table 2). Therefore individual performance animal was not influenced by fertilization rates, resulting in average value of 60 g day animal^{-1} (Table 2), corroborating with results found in the literature.

Nitrogen improves leaf elongation and leaf appearance rates and the length of the leaf blade (Paciullo et al., 2011). Therefore expected that N fertilization increases the growth of herbage, enabling greater SR. In this study the SR increased linearly ($P < 0.05$), thus increased performance per area (LWG) with increasing N fertilization (Table 2).

N fertilization rate influenced enteric CH4 emission depending on the unit in which CH4 emission is expressed. The methane emission, expressed in absolute values (g day animal^{-1}) decreased linearly with increasing N fertilization (Table 3). Study of Warner et al. (2015), evaluating N fertilization rate on methane emission by lactating dairy cows, the daily CH4 production decreased by 4% with increasing N fertilization rate (65 vs. 150 kg ha^{-1}).

Some authors report that pastures from tropical climates (C4 metabolism, with more fiber and lignin), when compared with temperate pastures (C3 metabolism, with less fiber and less lignin), produce a greater amount of CH_4 (Eckard et al., 2010; Beauchemin et al., 2008), while legumes produce less CH_4, when compared to grasses (Archimède et al., 2011). Methane production tends to decrease when feed protein content increases, and to increase when fiber content in the diet is enhanced (Shibata et al., 1992; Johnson and Johnson, 1995). In present study the protein content of pasture was 23.4, 26.2, 28.0 and 29.4% in the 50; 100; 200 and 400 kg N ha^{-1}, respectively. This indicates that the herbage quality variation influences CH_4 emissions.

When CH_4 emissions are expressed per unit area (Table 3), a linear increase is verified according to fertilization levels. This is jus-

Table 2
Organic matter intake and performance variables for sheep grazing in pearl millet (*Penissetum americanum* (L.) Leeke) swards fertilized with increasing nitrogen levels.

Variables	Nitrogen fertilization rates (kg ha^{-1})				P > F	R^2	SEM[a]
	50	100	200	400			
OMI, % PV	3.84	3.94	3.82	3.64	0.8396	–	0.15
OMI, g day^{-1}	924.33	889.67	860.67	752.06	0.2809	–	48.67
GEI, Mj day^{-1}	15.88	15.89	15.10	13.30	0.3910	–	0.78
ADG, g day $^{-1}$	65	60	63	52	0.9497	–	6.86
SR[b], kg LW ha^{-1}	620[b]	997[ab]	795[ab]	1266[a]	0.0064	0.82	87.54
LWG[c], kg ha^{-1}	237[b]	252[ab]	296[ab]	364[a]	0.0004	0.84	18.41

OMI, organic matter intake, GEI, gross energy intake; ADG, average daily gain; SR, stocking rate; LWG, live weight gain.

[a] SEM: Standard error mean.

[b] $y = 594.1 + 1.2x$.

[c] $y = 216.3 + 0.39x$.

Table 3
Methane emission by sheep grazing in pearl millet (*Penissetum americanum* (L.) Leeke) swards fertilized with increasing nitrogen levels.

Variables	Nitrogen fertilization rates (kg ha^{-1})				P Value	R^2	SEM[1]
	50	100	200	400			
CH_4 [3], g day animal^{-1}	15.47[a]	15.31[a]	12.45[ab]	10.93[b]	0.0050	0.65	1.4
CH_4 [4], g day ha^{-1}	404.97	585.07	599.57	622.48	0.0124	0.68	73.80
CH_4OMI, g kg^{-1}	17.3	17.4	14.6	15.4	0.2287	–	0.89
Y_m, % GEI	5.55	5.39	4.58	4.79	0.2194	–	0.26

[1] SEM, Standard error mean.

[2] $y = -0.013x + 435$ 16.04.

$R^2 = 0.6527$; CH_4, methane emission; CH_4OMI, methane emission per kg of organic matter intake; Y_m, methane emission per kg of GEI.

[a,b,c] means followed by lowercase letters on line differ by Tukey test ($P < 0.05$).

tified by the higher stocking rate, since a greater number of animals per unit area were supported in nitrogen-fertilized pastures.

Animals on pearl millet pastures in this study emitted on average 16.3 ± 0.89 g CH_4 per kg of OMI (Table 3). According to Waghorn et al. (2002), sheep fed on red clover (*Trifolium pratense*) emitted 18 g CH_4 (kg DMI) $^{-1}$, while animals fed with perennial ryegrass (*Lolium perenne*) emitted 26 g CH_4 (kg DMI) $^{-1}$. Therefore the values found in this study are considerably lower, and it should be considered that grazing management imposed allowed for animals grazing only the highest quality leaf lamina.

According to literature, besides the carbohydrate type on diet (Johnson and Johnson, 1995), forage: concentrate proportion, forage species and forage quality (Shibata and Terada, 2010) can also influence methane emissions. Blaxter and Clapperton (1965) determined the quantity effect of the amount and type of diet on methane production in sheep and cattle and observed that methane production can be modified by variations in digestibility and feed level.

Hegarty (2001), while working with sheep receiving diets with different digestibility levels, observed that better quality diets resulted in greater intake and methane daily production. However, methane emissions in relation to digestible energy intake and live body weight (g of methane kg of LWG^{-1}) decreased with the increase of diet digestibility, indicating the occurrence of a greater energy retention.

CH_4 emission (g day^{-1}) increased linearly ($P < 0.05$) with the increase of OMI (Fig. 1). Similar result was found by Warner et al. (2015), evaluating N fertilization rate of grass silage on methane emission by lactating dairy cows. The greatest effect of intake level on methane production is explained by feed passage rate in the rumen (Owens and Goetsch, 1986). According to Moss et al. (2000) and Pinares-Patiño et al. (2003), less methane production is expected when feed retention time is reduced.

According to Johnson and Johnson (1995) ruminants lose between 2 and 12% of consumed energy as methane, known as methane conversion rate. Literature reports average values for methane conversion rate of 6.2% of gross energy intake (GEI) by different categories of grazing cattle, while grazing sheep is reported to lose 5.4% of GEI as methane (Lassey, 2007). The conversion factor of GEI into methane reported by IPCC for sheep younger than one year is 4.5% (IPCC, 2006). Savian et al. (2014), reported average value of 7.3% for growing lambs grazing ryegrass (*Lolium multiflorum*, Lam.). Values of methane conversion rate in this study had an average value of 5%, corroborating with results found in the literature (Table 3).

Even though emissions per unit area are enhanced, methane emissions per kg of gross energy intake (CH_4 Kg GEI^{-1}) decreased ($P < 0.05$) with higher live weight gain per area (Fig. 2). Kurihara et al. (1999) presented results that emphasized that methane emission (g CH_4 (Kg LWG) $^{-1}$) varies with live weight gain and this is related to diet quality and fattening efficiency. This study corroborates with the literature, since methane emissions decreased when average live weight gain per area was greater (Fig. 2). Therefore, the increase in animal production efficiency results in smaller methane per weight gain. This improve in feed energy use can generate a reduction in individual methane emission. However, Allard et al. (2007) conclude that the reduction in fertilization use and smaller grazing pressure decrease CH_4 and N_2O emissions per area.

5. Conclusions

Methane emissions, expressed as absolute values (g CH_4 day^{-1} animal^{-1}), decrease with higher nitrogen doses. When expressed as grams of methane per kilogram of organic matter ingested or energy converted into methane, there is no difference in relation to nitrogen doses. However, emissions per unit area increase as a consequence of higher stocking rates allowed by the increase in forage production.

Conflict of interest

The authors declare that there are no conflict of interest.

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