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# Effect of energy sources inclusion in diet on methane production of cattle determined by sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique

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## Introduction

Ruminant animals have a great advantage over simple-stomached animals, as their digestive process is able to release the energy contained in cellulosic material through carbohydrate fermentation by microorganisms enzymes from the rumen environment. However, carbohydrate fermentation results not only in short chain fatty acids (SCFA) but also in less desirable products such as heat, as well as methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) gases which represents energy loss for the animal estimated in 2 to 12% of gross energy from feed (Johnson and Johnson, 1995). As an adult ruminant can produce up to 17 liters of methane per hour and this gas cannot be metabolized even by rumen microorganisms, most of it is removed from rumen by expiration or eructation (Moss, 1993), and released in the environment.

There are different techniques for methane emissions measurements by ruminants in production conditions; among them there is one that uses an inert gas tracer, sulphur hexafluoride (SF<sub>6</sub>). This technique results in a precise estimative of methane production by the animal,

besides enabling the evaluation of animals in normal pasture conditions, it consists in placing a permeation tube, which releases SF<sub>6</sub> at a previously known rate in the rumen, where by the contractions of this organ, CH<sub>4</sub> and SF<sub>6</sub> gases are released by eructation and samples are collected close to mouth and nostrils. This method assumes that the standard of SF<sub>6</sub> emission simulates the standard of CH<sub>4</sub> emission. The flow of CH<sub>4</sub> released by the animal is calculated in relation to SF<sub>6</sub> flow (Westberg et al., 1998).

The objective with the present study was to evaluate energy sources inclusion in cattle diets on methane production determined by sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique.

## Material and Methods

The trial was conducted at the College of Veterinary Medicine and Animal Science, University of São Paulo (USP), Brazil. Six Holstein nonpregnant and nonlactating cows (mean  $730 \pm 70$  kg of BW) fitted with ruminal cannulas were randomly assigned to a replicated 3 x 3 Latin square (n = 18) in three isoenergetic (1.55 Mcal NE/kg of DM) and isoproteic (12% CP) experimental diets: control (CON): diet with low ether extract (3.5% of EE); soybean (SOY): diet with high ether extract (5.30% of EE) with 15% inclusion of whole soybean grain; citrus pulp (CiPu): diet with low ether extract (3.00% of EE) and high participation of pectin with 15% inclusion of citrus pulp. Diets were fed as total mixed rations with a ratio of concentrate to forage of 50:50 (DM basis). Diets were offered twice daily at 0800 and 1600 h throughout the experiment for ad libitum consumption (minimum of 5 - 10% feed refusal). In all diets, the forage source was corn silage and the concentrate consisted of dry-ground corn grain, soybean meal, white salt, dicalcium phosphate, limestone and vitamin and mineral premix. Cows were housed in a barn equipped with individual feed bunks, rubbermatted floors, and automatic water fountains shared by 2 animals. Body weight was measured at the beginning of period 1 (d 1) and at the end of each of the 3 periods (d 21) at the same time each day. Each experimental period had 21 days, where 15

days were destined for diet adaptation and the last 6 days for sampling collection. Dry matter intake (DMI) was daily evaluated at the last six days of each experimental period. Sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique for methane measurement was described by Johnson and Johnson (1995) and adapted in Brazil by Primavesi et al. (2004). After the adaptation of animals to the sampling apparatus (PVC canister), the measurement of methane production was performed over six days at 24 h intervals, after morning meal (08:00h). The concentrations of CH<sub>4</sub> and SF<sub>6</sub> were determined by gas chromatograph in EMBRAPA Environment Laboratory Jaguariúna/SP. The quantification of methane released by the animal in the sample was calculated in function of SF<sub>6</sub> concentrations, associating the results to the known rate of the tracer gas in the rumen (Westberg et al., 1998). From the primary data was calculated potential emission of methane in g per day (CH<sub>4</sub>, g/d); kg of methane per year (CH<sub>4</sub>, kg/yr); g of methane per kg of DM ingested (CH<sub>4</sub>, g/kgDMI); % of Gross energy lost as methane (CH<sub>4</sub>, %GE) considering the % of gross energy of the diet; Megacalories of methane produced per animal per day (CH<sub>4</sub>, Mcal/An.d), considering 13.16 kcal/g of CH<sub>4</sub> and gross energy ingested per animal per day (GEI, Mcal/Ani.d). The data were submitted to variance analysis and the effects of treatment were separated by Tukey test ( $P < 0.05$  or  $P < 0.10$ ), using the Statistical Analysis System (Version 9.1, 2002-2003).

## Results

There was no difference ( $P > 0.05$ ) between treatments for dry matter intake, when expressed in kilogram per day (kg/d), in relation to body weight (%BW) or in relation to metabolic weight (g/kg BW<sup>0.75</sup>) (Table 1). This was probably, due to the fact that the animals were fed with isoenergetic and isoproteic diets in total mixed rations what may had provided better supply of nutrients along the day, favoring ruminal fermentation, especially the concentration of SCFA.

Table 1. Values of dry matter intake and methane production in cattle fed different energy sources determined by sulphur hexafluoride (SF6) tracer gas technique.

Variable <sup>1</sup>	Treatment <sup>2</sup>			SEM <sup>3</sup>	P-value
	CON	SOY	CiPu		
DMI, kg/d	16.2	14.9	15.8	0.36	0.2559
DMI, % BW	2.12	1.94	2.08	0.06	0.1309
DMI, g/kg M <sup>0.75</sup>	111.4	102.2	109.3	3.00	0.1571
CH <sub>4</sub> , g/d	286.2	284.1	344.2	17.7	0.0703
CH <sub>4</sub> , kg/yr	110.4	103.7	125.6	6.46	0.0703
CH <sub>4</sub> , g/kgDMI	17.4	19.1	22.0	1.20	0.2418
CH <sub>4</sub> , %GE	5.17	5.52	6.58	0.36	0.1885
CH <sub>4</sub> , Mcal/An.d	3.98	3.74	4.53	0.23	0.0704
GEI, Mcal/An.d	71.7	67.9	69.6	1.55	0.5106

<sup>1</sup>DMI = Dry matter intake; DMI, % BW = Dry matter intake in relation to body weight; DMI, g/kg M<sup>0.75</sup> = Dry matter intake in relation to metabolic weight; GEI = Gross energy ingested; <sup>2</sup>CON = Control; SOY = Soybean; CiPu = Citrus pulp; <sup>3</sup>SEM = Standard error of the mean.

Average values of methane production was not affected ( $P > 0.05$ ) by energy source inclusion in the diets when methane was expressed in gram of methane per day (CH<sub>4</sub>, g/d); kilogram of methane per year (CH<sub>4</sub>, kg/yr); gram of methane per kilogram of ingested dry matter (CH<sub>4</sub>, g/kg DMI); percentage of gross energy loss as methane (CH<sub>4</sub>, %GE) and megacalories per animal per day (CH<sub>4</sub>, Mcal/An.d), as well as the quantity of gross energy ingested per animal per day (GEI, Mcal/Ani.d) (Table 1). When evaluated at 10% probability, it was a significant effect of energy source for the average methane production in g/d; kg/yr and Mcal/An.d, resulting in increased methane emission for the treatment with citrus pulp compared to treatment with soybean and not differing from any of these variables for the control treatment.

In the present experiment, there was no lipid source effect, probably due to the lipid source used, as the shell of soybean does not provide oil directly to the ruminal microbiota, not presenting thus a toxic effect on microorganisms mainly on those who are involved in fiber digestion. On the other hand, the inclusion of a feed source rich in pectin, such as citrus pulp, generates higher CH<sub>4</sub> production, as pectin favors acetate production despite of propionate or lactate (Van Soest, 1994), featuring a fermentative pattern similar to forages. According to ruminal fermentation stoichiometry, changes in ruminal fermentation profile that favor acetate production results in higher

CH<sub>4</sub> (Boadi et al., 2004). However, this fact was not observed in the present study.

## Conclusions

The inclusion of a rich source of pectin such as citrus pulp or unsaturated fatty acids such as soybean results in changes in the rumen, although these changes are of small amplitude.

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