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POTENTIAL OF METHANE AND CARBON DIOXIDE *IN VITRO* PRODUCTION OF INGREDIENTS USED IN DIETS FOR SHEEP

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ABSTRACT

This study aimed to evaluate the potential of CH_4 and CO_2 *in vitro* production of soybean hulls, sunflower meal, corn, citrus pulp and corn silage. Four rumen-cannulated sheep were fed diets containing the evaluated ingredients at 40:60 forage:concentrate ratio. The gases produced by samples incubation were measured by injection into a gas chromatograph equipped with flame ionization detector. The experimental design was completely randomized with repeated measures, with three replicates for each evaluated food at four different periods. Under the experimental conditions, we verified different potential gas production among the ingredients. The citrus pulp meal was the ingredient with the greatest potential for CO_2 production. Corn silage and soybean hulls showed the greatest potential while citrus pulp and sunflower meal showed the least potential for CH_4 production, when expressed in mL/g of degraded dry matter; therefore, they can be considered, among the evaluated ingredients, those with the lowest environmental impact.

KEYWORDS: CH_4; CO_2; degradation; feed; ruminant.

POTENCIAL DE PRODUÇÃO DE GÁS METANO E DIÓXIDO DE CARBONO *IN VITRO* DOS INGREDIENTES UTILIZADOS EM DIETAS PARA OVINOS

RESUMO

Objetivou-se avaliar o potencial de produção dos gases CH_4 e CO_2 *in vitro* dos ingredientes casca de soja, farelo de girassol, milho, polpa cítrica e silagem de milho. Quatro ovinos canulados no rúmen foram alimentados com dietas contendo os ingredientes avaliados na proporção volumoso:concentrado de 40:60. Os gases produzidos na incubação das amostras em líquido ruminal foram mensurados pela injeção em cromatógrafo gasoso equipado com detector de ionização de chama. O delineamento utilizado foi o inteiramente ao acaso em medidas repetidas no tempo, com três repetições para cada alimento avaliado em quatro diferentes períodos. Nas condições experimentais deste trabalho, foram verificados diferentes potenciais para produção de gases entre os ingredientes. A polpa cítrica foi o ingrediente de maior potencial de produção de CO_2. A silagem de milho e casca de soja foram os ingredientes de maior potencial e a polpa cítrica e o farelo de girassol os de menor potencial para produção de CH_4 em relação à matéria seca degradada, podendo ser considerados, entre os ingredientes avaliados, os de menores impactos ao ambiente.

PALAVRAS-CHAVE: Alimentos; CH_4; CO_2; degradação; ruminantes.

INTRODUCTION

Food fermentation in the rumen is a great source of CH_4 production, because it is a byproduct of anaerobic fermentation (MOSS et al., 2000; BEAUCHEMIN et al., 2008), and it represents 2 to 12% loss of the energy from the food consumed by the ruminants (JOHNSON & JOHNSON, 2005), besides contributing to global warming. The emission of CH4 by ruminants depends on different factors such as animal species, composition and amount of concentrate in the diet (SEJIAN et al., 2011), intake level, type of carbohydrate in the diet, rations processing, addition of lipids or ionophores to the diet and changes in the ruminal microflora (JOHNSON & JOHNSON, 2005).

The differences in the nutritional composition of the food may cause diverse fermentative patterns, resulting in different proportions of the final fermentation products such as acetic, propionic and butyric fatty acids, which represent an important energy source for ruminants' metabolism. Besides these fermentative processes, there is CO_2 and CH_4 production, in higher or lower amounts, depending on the individual proportion of produced acids that is affected by the organic matter composition of the diets, due mainly to the fermentation nature and rate of the carbohydrates (MOSS et al., 2000).

Due to nutritional differences among ingredients and hence the differences in fermentation rates, this study aimed at evaluating the potential of in vitro CO_2 and CH_4 production of the ingredients soybean hull, sunflower meal, corn, citrus pulp and corn silage used in the formulation of ovine diets.

MATERIAL AND METHODS

The experiment was carried out at the Animal Unity of Digestive and Metabolic Studies belonging to the Animal Science Departament of the Agrarian and Veterinary Sciences, Unesp, Jaboticabal Campus.

We used four ½ Santa Inês ½ Dorper sheep with mean body weight of 47.3 kg, cannulated in the rumen, housed in stalls with cement floor and individual feeders and drinkers. The animals were fed twice a day with diet with 40% roughage and 60% concentrate.

The samples of the ingredients soybean hull, sunflower meal, corn, citrus pulp and corn silage were pre-dried in an oven at 55 °C for 72 hours, ground in knife mill with 1 mm sieve for determining the levels of dry matter (DM), ashes and ether extract (EE), according to the AOAC (1995), neutral detergent fiber (NDF), according to VAN SOEST et al. (1991), acid detergent fiber (ADF), according to VAN SOEST & ROBERTSON (1985), right after the NDF, neutral detergent-soluble fiber (NDSF), quantified as methodology described by HALL (2000), and starch, estimated according to the method described by HENDRIX (1993). For extraction and colorimetric reading we used dinitrosalicylic acid (DNS), according to the methodology described by MILLER (1959). Chemical composition of the evaluated ingredients is displayed in Table 1.

Table 1. Chemical composition of the ingredients

Ingredient	Nutrient							
	DM	CP	MM	EE	FDN	FDA	NDSF	Starch
Corn silage	40.00	8.30	3.93	3.10	46.00	27.79	4.9	37.36
Corn	89.43	9.72	1.29	4.36	10.51	2.83	6.07	68.17
Citrus pulp	88.27	7.40	7.18	2.20	26.36	22.08	49.81	7.56
Soybean hull	89.45	11.29	4.13	1.42	73.11	55.57	7.17	6.05
Sunflower meal	89.67	29.19	4.87	1.19	52.97	44.87	3.48	4.08

DM = dry matter, CP = crude protein; MM = mineral matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; NDSF = neutral detergent-soluble fiber

For the evaluation of the ruminal disappearance rate of the ingredients, we made 100% polyamide (nylon) not resined bags, with 50 µm porosity, 14 cm length per 7 cm width, sealed by an

automatic sealer. The bags received approximately 4 g of the sample, maintaining the relation 20 mg of dry matter of the feed per square centimeter of the tissue, as proposed by NOCEK (1988) as ideal for evaluating in situ degradability. The bags were incubated in the rumen of the sheep for 12 hours in order to compare with gases production. After this period, the bags were withdrawn from the rumen and immersed in cold water with ice for 30 minutes to cease microbial activity, and then they were washed in running water until the water was clean and without solids. The bags with residues were dried in a forced-air circulation oven at 55 °C for 72 hours and then weighed. The dry matter disappearance rate was calculated by the formula:

$$DMD(\%) = \left(1 - \frac{WBI - WGB}{WBB - WGB}\right) 100,$$

where DMD% is the percentage of DM degradability; WBI is the weight of the bag after ruminal incubation; WEB is the weight of the empty bag before ruminal incubation and WBB is the weight of the bag before ruminal incubation. The calculation of degradability percentage of each nutrient was carried out by means of the formula above and the differences (WBI - WEB) and (WBB - WEB) were multiplied by the respective percentage of each nutrient. The nutrient content of the residues was obtained by using the same techniques for analysis of the chemical composition of the ingredients.

The assessments of CH_4 and CO_2 production were repeated during four periods (days) for each ingredient, by using the methodology by GASTALDI (2003) adapted to sheep and consisting of three stages. The first stage consisted of preparing the sample: before feeding, approximately 500 mL of the ruminal content of each animal were collected, filtered through nylon tissue (100 µm) and mixed, forming, thus, an homogenous environment. In an Erlenmeyer flask with 250 mL capacity, we placed 150 mL of ruminal liquid containing 2.1 g of sample of each ingredient previously added to serve as substrate for microbial fermentation, maintaining the relation 1.25 g DM/100 mL of ruminal liquid. We also incubated three samples containing only ruminal liquid, without ingredients. The second stage comprised the gases production and storage: the Erlenmeyer flasks containing the samples and the ruminal liquid were maintained in a double boiler for 12 hours at 39 ºC, in a dark environment, and the gases produced were stored in a PET container adapted to the system, with intern volume of 600 mL. The third stage included the qualitative and quantitative analysis of the gas: we collected an aliquot directly from the plastic containers with the aid of a plastic syringe with valve, with 1.0 mL capacity, and immediately injected it in a gas chromatograph Trace GC Ultra by Thermo Scientific, equipped with a flame ionization detector, using argon as carrier gas with flow of 25 mL per minute and oven temperature of 70 ºC. The calibration was carried out with a standard mixture of CH_4 and CO_2 gases. Peak areas were integrated using the software Chromquest 5.0.

The total amount of gas produced was measured by the determination of the volume occupied by the produced gas inside the plastic containers after 12 hours of fermentation. We considered this time because of the feeding frequency of two daily meals, with a 12-hour interval between each other, to which the animals were submitted.

Regarding the evaluation and comparison of CH_4 and CO_2 production among the ingredients, we used a completely randomised design with measures repeated in time, with three replications for each food evaluated (treatments), in four different periods (times), using the mixed models of SAS (2008) (Statistical Analysis System). Means comparison was made by Tukey test, considering a 5% significance level.

RESULTS AND DISCUSSION

The mean values of the disappearance rate of DM, CP, NDF and starch after 12 hours of in situ incubation may be verified in Table 2.

The high degradation rates of CP, FDN and starch of citrus pulp in addition to the high level of neutral detergent-soluble fiber (49.81%), which is rapidly and extensively degraded by ruminal microorganisms (HALL, 2000), may explain the higher rate of DM degradation of this ingredient after 12 hours of fermentation. According to HALL (2000), 85% NDF of citrus pulp is fermented within 24 hours, similar to the fermentation rate of the neutral detergent-soluble fiber (NDSF), constituted by fructan, β-glucan and pectic substances.

Table 2. Disappearance (%) of dry matter, crude protein, neutral detergent fiber and starch of the ingredients after twelve hours of in situ incubation

Nutrient	Ingredient				
	Citrus pulp	Corn	Soybean hull	Sunflower meal	Corn silage
Dry matter	70.36	40.84	35.60	58.02	45.28
Crude protein	71.07	46.97	51.26	86.99	69.42
Neutral detergent fiber	50.49	62.67	28.52	35.17	20.16
Starch	78.96	44.18	69.62	77.71	90.71

Starch degradation varied among the evaluated ingredients, being almost complete for corn silage 12 hours after incubation and presenting the lowest rate for corn. According to OFFER et al. (2003), corn silage degradability is affected by the plant genotype and maturity, and largely by the dry matter content of the silage. ROONEY & PFLUGFELDER (1986) stated that the digestibility of food starch may vary, depending on factors such as amylose/amylopectin relation in starch structure, type of endosperm (cornea or flour-like), type of cereal grain, processing method.

Nutrients content of sunflower meal varies according to the oil extraction process and quantity of hull present, which gives high fiber content. The high crude protein content is the main reference for the use of this meal in animal feeding and, as we verified, CP is rapidly degraded in the rumen (86.99% within 12 hours of incubation), which may explain the high DM degradation rate of this ingredient.

The high NDF (73.11%) of the soybean hull and the smallest degradation of this component compared to the citrus pulp, corn and sunflower meal during the 12-hour incubation period may have caused the soybean hull to present the smallest DM degradation in the established incubation time. According to BACH et al. (1999), soybean hull is an ingredient with high content of highly digestible NDF, high cellulose content and low lignin content, making it largely degradable in the rumen with high short-chain fatty acids production, due to the high fiber fermentation in the rumen. The low NDF degradation of corn silage may be attributed to the reduced incubation time (12 hours), once the fibrous fraction of the food has low ruminal fermentation rate.

The mean values of the production potential of CH_4, CO_2 and gas total volume of the ingredients may be observed in Table 3.

Table 3. In vitro gas production of the evaluated ingredients

Variable	Ingredient					Pr>F
	Citrus pulp	Corn	Soybean hull	Sonflower meal	Corn Silage	
CH_4	10.47	8.38	8.69	9.66	11.03	0.05
CO_2 (mL/g incubated DM)	43.31a	35.90b	29.79c	37.14b	31.88bc	<0.01
Total (mL/g incubated DM)	50.07a	45.30bc	38.27c	49.50ab	45.72bc	<0.01
CH_4 (mL/g degraded DM)	14.88c	20.53b	24.41a	16.66c	24.36a	<0.01

Pr>F = effect of the treatments. Means followed by the same letter in the row do not differ between each other by Tukey test (p>0.05).

We did not observe differences for CH_4 production, expressed in mL/g incubated DM among the evaluated ingredients. However, when expressed in mL/g degraded DM, corn silage and soybean hull showed the highest potential to CH_4 production, whereas citrus and sunflower meal presented the lowest. LEE et al. (2003) evaluated CH_4 in vitro production of different ingredients in 24 hours of incubation and verified that soybean hull produced more methane among the meals and hulls of the byproducts evaluated. These authors stated that food with high crude protein content tend to produce lower quantity of methane, because the NH_4 resulting from the degradation of protein sources, such as oilseed meal, may combine with the CO_2, which is a substratum for CH_4 production, resulting in lower

production, which may explain the low methane production for sunflower meal.

Corn showed medium potential for the production of CH_4/g of fermented DM among the evaluated ingredients, because the production was higher than for citrus pulp and sunflower meal and lower than for corn silage and soybean hull. According to MOSS et al. (2000), high levels of starch in the diets favor propionate production and decrease CH_4/organic matter fermented in the rumen, because the bacteria that ferment starch may compete with the methanogenic ones for free hydrogen for propionate production.

We observed negative correlation (-0.9) between dry matter degradation and CH_4 production in mL/g of degraded DM (Table 4), which may explain the lower CH_4 production in mL/g degraded DM for citrus pulp and sunflower meal which presented higher dry matter degradation among the ingredients evaluated in 12 hours of incubation.

Table 4. Correlation coefficient (r) among the nutritional composition and nutrients degradation of the food and the in vitro gas production

	CH_4 (mL)	CO_2 (mL)	Total (mL)	CH_4 (mL/g degraded DM)
OM	-0.84**	0.21	-0.17	-0.47
CP	-0.07	0.18	0.20	-0.35
Starch	-0.28	-0.28	0.26	0.31
NDF	0.19	-0.48	-0.37	0.43
CNF	-0.17	0.31	0.21	-0.23
NDSF	0.19	0.74	0.59	-0.64
DM degradation	0.40	0.97*	0.94*	-0.90*
CP degradation	0.63	0.61	0.34	-0.55
NDF degradation	-0.82**	0.30	-0.10	-0.48

*p<0.05; ** 0.05>p<0.10

The high positive correlation between DM degradation and total gas production, and between DM degradation and CO_2 production may explain the higher potential of total gas and CO_2 production of the citrus pulp and the smaller potential of the soybean hull, which presented the highest and the lowest dry degradation, respectively.

According to SANTOSO et al. (2009), the high NDF content of the food is related to the high CH_4 production, due to the high positive correlation observed between methane production and NDF content of the food, in 24 hours of incubation. MOSS et al. (2000) verified positive correlation between CH_4/g degraded OM and the NDF content of the diet in 24 hours of incubation. In the present study we did not verify correlation between the NDF content of the ingredients and CH_4 production, which may be explained by the limited incubation time (12 hours) that generated low fermentation rate of the food NDF, since this fraction degrades slowly. The ingredients with high fiber content, such as soybean hull and corn silage, presented the lowest NDF disappearance rate; these ingredients presented also greater potential for CH_4/g degraded DM production compared to the ingredients with high NDF disappearance rate, such as citrus pulp and corn, agreeing with the results obtained by PEREIRA et al. (2006), who evaluated bovines within 12 hours of in vitro incubation. According to ALUWONG et al. (2011), the higher the digestibility of the food, the smaller the remaining time of the food in the rumen and lower the CO_2 to CH_4 conversion by the methanogenic archaeas that use H_2 to produce CO_2.

CONCLUSIONS

The variable potential for CH_4 and CO_2 production is related to the chemical composition of the food, where rapidly fermentable ingredients tend to produce lower amounts of CH_4/g fermented DM compared to ingredients with high fiber content; therefore, they can be considered for the formulation of diets aiming at a lower environment impact.

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