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MÁSTER EN PRODUCCIÓN ANIMAL

"Effect of carbohydrate source in diet of CH_4 and CO_2 production from dairy goats"

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Jesús Pérez Plumed

Director académico
Fernando Estellés Barber
Director experimental
Pablo Ferrer Riera

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Plumed, J.

Summary

Human activity generates several environmental impacts such as climate change as a result of the emission of greenhouse gases (GHG). Methane is a key GHG, and enteric fermentation in ruminant animals is the main source of this gas. There is a need to reduce these emissions and one option is to modify the diet of the animals, which has a direct effect on them.

The general objective of this study is to evaluate the production of methane and carbon dioxide in dairy Murciano-granadina goats fed with three mixed (forage: concentrate) rations, varying in their fiber and starch contents. The study was performed in two steps: in-vivo and in-vitro. The same diets were used in both trials, including also in the in-vitro test a control diet and the three pure diets (without forage). The technique used to measure in-vivo emissions was a dynamic camera, which is studied using a mass balance gas emissions in a volume of air enclosed and ventilated with air flow known. In-vitro emissions were determined by culture of ruminal liquor with all studied diets during 24 hours.

We concluded that diets did not statistically affect methane production from goats, although it seems that high starched diets might lead to a reduction on methane emissions than fibered diets. The in vitro study shown a direct relationship between fiber content of the diet and biogas and ammonia production.

Keywords

Ruminants, methane, dynamic chamber, enteric fermentation, gas emissions, in vitro, biogas

Resumen

La actividad humana genera diversos impactos sobre el medio ambiente entre los que destaca el cambio climático como resultado de la emisión de gases con efecto invernadero. El metano es un importante gas con efecto invernadero emitido, entre otras fuentes, en la fermentación entérica de animales rumiantes. Existe una necesidad de reducir estas emisiones y una opción es modificar la dieta de los animales, ya que tiene un efecto directo sobre éstas.

Así se planteó como objetivo general de este estudio la evaluación de la producción de metano y dióxido de carbono en cabras lecheras de la raza Murciano-granadina alimentadas con tres dietas que diferían en su fuente principal de carbohidratos (maíz, cascarilla de soja y pulpa de cítricos). El estudio se realizó en dos pasos: *in-vivo* e *in-vitro*. Las mismas dietas fueron utilizados en ambos ensayos, incluyendo también en la prueba in vitro una dieta de control y las tres dietas puros (sin forraje). La técnica utilizada para medir las emisiones *in-vivo* era una cámara dinámica, en la cual se estudia un balance de masas de gases en un volumen de aire encerrado y ventilado con flujo de aire conocido. Las emisiones *in-vitro* se determinaron por el cultivo del líquido ruminal con todas las dietas estudiadas durante 24 horas.

Llegamos a la conclusión de que las dietas no afectaron estadísticamente a la producción de metano de las cabras, aunque parece que las dietas con alto contenido en almidón pueden conducir a una reducción en las emisiones de metano mayor que las dietas fibrosas. El estudio *in-vitro* muestra una relación directa entre el contenido de fibra de la dieta, el biogás y la producción de amoníaco.

Palabras clave

Rumiantes, metano, cámara dinámica, fermentación entérica, emisión de gases, in vitro, biogás.

1.Introduction

Ruminants are commonly used for meat and milk production in most countries of the world. Sheep production is the major ruminant production in Spain, reaching in 2012 up to 20% of total EU-27 sheep heads, following the United Kingdom, which represented up to 27 % of total (MAGRAMA 2012). Spain is also the second EU-27 goat producer, with 22% of total European heads (MAGRAMA, 2012). One of the main concerns of ruminant production is related to greenhouse gases (GHG) emissions (Steinfeld *et al.*, 2006). These GHG emissions directly assigned to animal productions can be classified according to their origin in two main groups: on the one hand there is a large amount of CH_4 being released from enteric fermentation processes and, on the other hand, CH_4 and N_2O is emitted due to decomposition of organic matter and nitrogen during manure management.

Methane has 24 times the global warming potential of CO_2 (over a 100 year period) and, it is considered to contribute from 4 to 9% of the global GHG effect (Forster et al., 2007, UNFCCC, 2012).

Most of the methane produced by ruminants arises from enteric fermentation processes. That is due to the fact that some fermentation products from the rumen, like CO_2, formate and small methyl-compounds, cannot be metabolized by the animal and are transformed into CH_4. CH_4 formation is completed by a specialized group of microbes called methanogens, and their action prevents accumulation of these end-products in the rumen to keep digestive process under optimal conditions (Attwood *et al*., 2011).

In Spain, CH_4 enteric emissions from sheep are estimated to achieve up to 148.41 Gg/year, while these estimations for goats achieve 23.47 Gg/year (FAOSTAT, 2010). Both categories represent about 28% of total enteric CH_4 emissions in the country. There is scarce knowledge on practical approaches for the mitigation of enteric methane emissions from small ruminants in typical Mediterranean conditions. Attending to the contribution of goats to total methane emissions in Spain, there is a need to develop practical mitigation technologies to achieve a global reduction of GHG emissions in Spain.

The main aim of this work was to evaluate CH_4 and CO_2 emissions from lactating goats fed with three different rations, using corn, soybean hull and citrus pulp as main carbohydrate sources of concentrate in each one, and barley straw as forage. This test was performed at two different levels: I) at animal level by measuring direct emissions from animals using a flux chamber, and II) at microbial level by using ruminal in-vitro cultures.

2. Materials and methods

All experiments were developed in the small ruminant livestock unit of the Institute of Animal Science and Technology (ICTA) of the Universitat Politècnica de València (Valencia, Spain). All management and experimental procedures were developed in strict accordance with the Spanish guidelines for experimental animal protection (RD 1201/2005, October 10th, 2005).

2.1. Dynamic chamber

Twenty-four adult, non-pregnant, Murciano-granadina goats (44.36 kg ± 8.20) at mid lactation (from 3 to 5 months from birth) were used for this trial. They were randomly separated in eight groups of three animals each and fed with three experimental diets. These diets were formulated following nutritional recommendations from FEDNA (2009) for dairy goats. One of the diets used as main energy source corn (CD), while, in the other two diets, it was replaced by fibrous by products, resulting in: one based on soybean hulls (SBD) and the other one based on citrus pulp (CPD). The daily ration included (in fresh matter basis) 1 kg per animal of pelleted concentrate and 0.33 kg per animal of straw, resulting in ration forage: concentrate of 1:3. The physicho-chemical composition of the diets is shown in Table 1.

Table 1. Ingredients (g/kg dry matter, DM) and chemical composition of diets (% of DM)

	Diets[1]		
Ingredients, g/kg DM	CD	SBH	CPD
Straw	150.00	150.00	150.00
Corn	605.00	-	-
Soybean hulls	-	610.00	-
Citrus pulp	-	-	605.00
Soy meal 44% CP	202.00	174.00	222.00
Calcium carbonate	22.00	23.00	4.00
Salt	11.00	26.00	9.00
By-pass fat (Palm soap)	5.00	13.00	5.00
Corrector	5.00	5.00	5.00
Chemical composition, DM %			
Dry matter (DM)	88.36	90.15	88.41
Organic matter (OM)	92.80	89.28	91.96
Crude Protein (CP)	16.66	14.79	15.80
Ether extract (EE)	2.66	2.03	2.10
Neutral detergent fibre (NDF)	30.11	58.97	30.08
Starch (STA)	41.63	1.45	6.36
Gross energy (GE), MJ/kg DM	18.00	17.21	17.56

[1]CD = corn; SBH = soybean hulls; CPD = citrus pulp

The dynamic chamber (Martí *et al*, 2011) was located inside the farm building and was 1.80 m wide, 2.80 m long and 2.50 m high. The size of the chamber was determined by RD 1201/2005, which establishes a minimum area of 0,8 m^2/animal needed for goats housing. Materials used in the construction of the walls and roof of the chamber were polymethylmethacrylate (Plexiglas®) and multicelular polycarbonate panels, assembled on an aluminum structure. A

steel cage, to separate animals from direct contact with the chamber walls, was also placed inside. Slat panels were installed on the floor, as to it could easily be dissembled for daily cleaning and thus, prevent emissions from manure. Inside the chamber, the following equipment was also installed: a feeder, a drinker, an extraction fan (S&P TD160/100 N Silent, Barcelona, Spain), two air inlets and a small fan to homogenize the air. Above the feeder, a programmable feed automatic dispenser was placed. Figure 1 shows a scheme of the camber and the installed components.

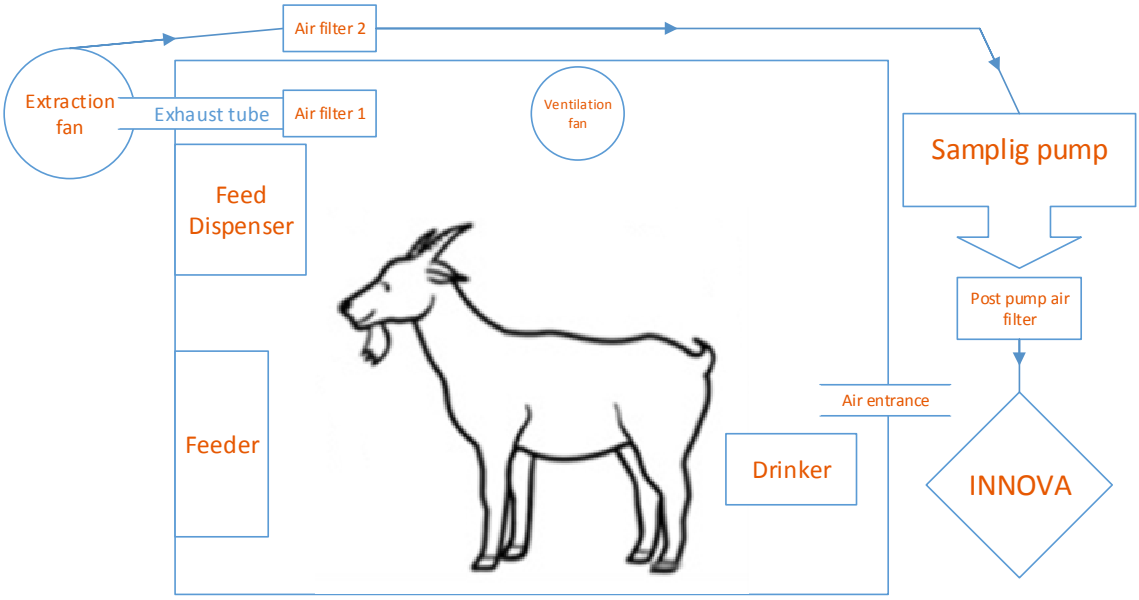

Figure 1. Scheme of the dynamic chamber and components used for the *in-vivo* trial.

All groups entered in the chamber three times, once with each diet. Fourteen days before the entrance to the chamber, each group was previously adapted to the experimental diet. Animals were introduced in the chamber at about 9 a.m. where they received their daily ration (1 kg of concentrate and 0,33 kg of straw per animal). The next day, the automatic feed dispenser was opened at 9.00 a.m. (releasing a complete new daily ration) and animals left the chamber at about 11 a.m. Food consumption in the chamber was measured daily by weighing the food refuse, straw and concentrate, separately.

Animals were milked diary at 8.30 a.m., and their production was controlled individually. When housed in the chambers, milking was performed just before entering in the chamber and after the goats were taken back to their pen. Milk production was then homogenized at 24 hours.

Gas emissions were determined by performing a mass balance in the space enclosed by the chamber, following Equation 1:

$$E_k = F \times \left(C_{o_k} - C_{i_k}\right) \times 10^{-6} \qquad \text{Eq. (1)}$$

Where E_k is the emission rate (l/h) of each gas *k*, F is the airflow rate through the chamber (m^3/h) and $C_{o,k}$ and $C_{i,k}$ are outlet and inlet gas (k) concentrations (in ppm) respectively.

The airflow rate through the chamber was fixed during the whole experiment. The fan was calibrated twice (at the beginning and at the end of the experiment) following AMCA (1999) recommendations resulting in an average value of 90 $m^3/h \pm 6.1$. A thermal anemometer (Testo425, Testo, Germany) was used to this aim.

A photoacoustic monitor (INNOVA 1412, Lumasense, Denmark) was used to determine CH_4 and CO_2 concentrations. Measurements of the outlet air were taken each 5 minutes while animals were housed in. In order to characterize inlet concentrations, gas concentrations were measured out of the chamber, during the days in which the chamber remained empty (3 days per week).

Outlet gas samples were taken from the ventilation exhaust tube, thus being representative of the exhaust air of the chamber. Inlet samples were taken from the surrounding environment of the chamber, close to the air inlets. In both cases, the transport of the sample to the gas-measuring device was performed using plastic pipes (PVC Ø 6mm) and a sampling pump (Bravo plus M, Tecora, Italy). Gas concentration measurements from the first 30 minutes after the entrance of animals were discarded, in order to avoid unrepresentative values caused by the stress of the animals.

Methane conversion rate (Ym) was calculated in order to see how gross energy (GE) intake affects to enteric methane production, and relationship between them and digestibility of the diets.

2.2 In vitro analysis

An *in-vitro* analysis was also conducted in order to determine methane and biogas production with the studied diets. To this aim, seven substratum were used in the test: three of them were the mixed rations (concentrate + straw, at the same mixing ratio) tested in the *in-vivo* trial, other three consisted in the three concentrates without forage (pure corn diet, PCD, pure soy-hulls diet, PSD and pure citrus pulp diet, PCP) and finally, a commercial concentrate used as a control (C). Each analysis was performed by duplicate and two batches (B1 and B2) were conducted. For each one, 0,5 g of diets was introduced into the vials. 50 ml of rumen fluid and Goering and Van Soest (1970) medium was added into vials. Rumen fluid was obtained by esophageal way from 16 dry, non-pregnant Murciano-Granadina goats (not included in the *in-vivo* experiment and fed with the control diet). Goering and Van Soest medium was composed by a mineral solution, a trace element solution, a buffer solution, a reducer solution and a part of resazurine. Rumen fluid was transported to the laboratory in a thermos and once there it was filtered through 2 layers of gauze with continuous bubbling of CO_2, and added to the culture medium at ratio 1/4.

Samples were incubated at 39ºC for 24 hours. Biogas (mixture of CH_4 and CO_2) produced was extracted every 2 hours. Biogas production was determined by extracting gas vials until reducing the flask pressure to approximately 15 psi. Difference between atmospheric pressure and vial pressure was written down in a register for subsequent calculates.

After 24 hours of incubation, vials were removed from heat and cooled with ice, to stop all microbial activity. Then, 0.2 ml of biogas from each vial was placed into a Vacutainer for CH_4

concentrations analysis. Later, 0.5 ml of rumen fluid was taken into new vials and used to determine ammonia concentration though alkaline digestion (Foss Tecator, ENGLAND). All analyses were performed by duplicate.

The remaining rumen fluid was weighed and dried at 110 ºC to obtain the dry matter content. Neutral detergent fiber (NDF) content was also determined following Van Soest methodology (Van Soest *et al.* 1991). To this aim, 0,5 g of sample were placed in Tedlar® bags and washed for 75 minutes with a neutral detergent solution in a fiber digestor (Ankom, USA). Once washed, 20 gr of anhydrous sodium sulfite and 1 ml of α-amylase were added to the solution. Three consecutive washings were conducted with distilled water and, after draining, samples were placed in an acetone bath for 3 minutes. After a second drainage, samples were dried at 100ºC during one day and weighted.

To determine volatile fatty acid content (VFA) 0,9 ml of rumen solution after 24 hours of digestion was added into an Eppendorf flask with 0,1 ml of conserving solution with internal standard. Samples were filtered using 0.5 µm filters (Thermo Scientific, USA). These samples were centrifuged at 24000 rpm during 10 min, and then the supernatant was collected and placed in chromatographic vials. Samples were frozen at -32ª until chromatographic analysis.

CH_4 and VFA concentrations were determined using a Fisons GC gas chromatograph, equipped with a FID detector, and a Supelco SP 2560 (60m x 0.25 mm ID, 0.20 µm film) column.

2.3 Statistical analysis

For the flux chamber trial, the effect of each diet and group of animals on CH_4 and CO_2 production was assessed by means a multiple factor analysis of variance (ANOVA) The model used was:

$$Gas_{i,j} = \bar{x} + diet_i + group_j + \varepsilon \quad \text{Eq.(2)}$$

Where $Gas_{i,j}$ represents gas production for each diet (*i*) and group (*j*), *x* is the overall average, and ε is the error of the model.

The effect of introducing animals in the chamber on milk yield was also determined. To this aim, a single factor ANOVA analysis was developed following the model:

$$Milk_k = \bar{x} + animal_k + chamber + \varepsilon \quad \text{Eq.(3)}$$

Where *Milk* is the daily milk production of each animal (*k*), and *chamber* is the presence (1) or absence (0) of each animal in the chamber when it was milked.

In order to analyze the effect of each diet and both batches on methane and biogas production as well as on the characteristics of digested matter, a multiple factor ANOVA was also performed following the next model:

$$y_{l,m} = \bar{x} + diets_l + batch_m + \varepsilon \quad \text{Eq.(4)}$$

Where $y_{l,m}$ depicts the value of each analyzed factor for each diet (*l*) and batch (*m*).

All analyses were performed using the software *Statgraphics Centurion XVI (V.1.16.7. Statpoint Technologies Inc. 1982-2011).*

3. Results

3.1 Dynamic Chamber

Gas emissions and productive traits observed during the flux chamber experiment are summarized in Table 2:

Table 2. Methane and carbon dioxide production, milk production and chamber results averages

	DIET			SEM	P VALUE	P-VALUE
	CD	SBH	CPD		DIET	GROUP
CO_2, l/animal/d	400.49	350.48	383.86	101.149	0.7500	0.0883
CH4 l/animal/d	21.45	25.45	26.33	7.321	0.5631	0.0184
CH4 g/d DM	13.59	14.65	16.65	1.738	0.5074	0.0430
CH4 l/kg milk	0.01	0.01	0.01	0.011	0.5407	0.0193
CH4 l/kg DM intake	25.29	22.80	25.61	3.775	0.8763	0.8715
CH4 l/kg OM intake	22.67	20.64	22.90	3.391	0.8987	0.8698
CH4 l/d MW^4	2.26	1.49	1.54	0.178	0.5631	0.0184
CH_4/CO_2 ratio	[a]0.05	[b]0.07	[b]0.07	0.004	0.0037	0.9450
Concentrate intake DM basis	0.55	0.67	0.61	0.139	0.8630	0.0641
Straw intake DM basis	0.39	0.43	0.42	0.027	0.0558	0.6968
Ym (%)	5.07	4.86	6.22	0.927	0.5099	0.9001
Milk production kg/d/animal	1.78	1.77	1.77	0.277	0.8680	0.0659

* SBH: Soybean hull diet; CPD: Citrus pulp diet; SEM: Standard error of the mean in diets; DM: dry matter; OM: Organic matter; MW: metabolic weight

Regarding methane production, no statistically significant differences were found among diets in terms of total production neither expressed as a function of intake or milk production. The group of animals presented a statistically significant effect when expressing emissions as l/animals and day, l/kg of MW and l/kg of milk, which indicates the high variability found on CH_4 emissions among animals.

For CO_2 production we can see that the statistical analysis show us that there is not significant differences between the three diets, (p=0,7500). Despite no differences were found for gas emissions, statistically differences were found in CH_4/CO_2 ratio (p=0.0037). So, it can be stated that CH_4/CO_2 ratio was affected by the diet, finding a lower value for the corn based (CD) diet than for the other two ones.

Milk production was not affected by diet, neither by the entrance of the goats to the chamber, finding an average (± standard deviation) milk yield of (1.84 ± 0.25 kg). Milk production depends only of the animal. Diets hadn't either any effect on milk production.

3.2 In vitro methane and biogas production

Results of in vitro cultivate are shown in Table 3:

Table 3. In vitro results of biogas, methane, ammonia, and NDF production for all diets

	DIET								BATCH			P- VALUE		
	CD	SBH	CPD	PCD	PSB	PCP	CT	SEM	B1	B2	SEM	DIET	BATCH	DIET x BATCH
CH_4 µm ^{2}CN/ml	7.59	7.81	6.62	7.72	8.01	6.78	6.03	0.787	8.74	7.90	0.420	0.0515	0.1705	1.112
Biogas (ml)	^{b}105.00	^{a}90.33	^{c}119.61	^{b}108.83	^{a}94.11	^{c}115.38	^{a}89.88	2.374	108.97	105.07	1.268	0.0000	0.0381	0.0193
Ammonia mg NH_3/l	^{b}107.18	^{a}85.47	^{c}119.00	^{c}120.61	^{a}86.13	^{c}127.91	^{d}165.94	13.634	98.02	116.53	7.288	0.0023	0.0833	0.8275
NDF %	^{b}5.87	^{b}6.47	^{b}5.94	^{a}3.96	^{b}5.67	^{a}4.21	^{c}8.02	0.366	6.46	5.32	0.195	0.0000	0.0003	0.1015

According to the ANOVA analysis, CH_4 production did not present significant differences among diets ($p=0.052$). Nevertheless, we observed differences in terms of biogas production, finding the highest biogas production for the CPD diet, while SBH and CT diets produced the lowest amounts of biogas. Biogas production depended also of the batch ($p<0.05$), finding slightly lower production during the second batch. Biogas production was similar between diets, (with forage content or not). Most similar diet to commercial one is SBH diet (90.33-89.88 ml).

Ammonia production was closely related to the diet used ($p<0.05$). A positive relationship between the fiber content of the diet and ammonia production was found. In this regard, diets without forage (PCD, PSB, PCP) produced more ammonia than their corresponding mixed diets (CD, SBH and CPD). This fact can be explained by the fiber content of each diet. Previous studies (Isaacson *et al.,* 1975; Agle *et al*., 2010, Animut *et al*., 2007) demonstrated that when providing an energetic substrate to the ruminal microorganisms, the efficiency of ruminal fermentation increases, enhancing then the capture of ruminal ammonia into microbial protein. Diets with limited energy produce that ruminal microorganisms degrade feed proteins to ammonia (Russell *et al*. 1983, Lana *et al*.,1998). The results of our experiment agreed with this information, since we found higher ammonia production as the digestibility of the diets energy decreased (as fiber content increased).

NDF must be in relation with diet. The highest NDF content was found for the C diet, while the lowest ones were those from diets PCD and PCP. No differences were found among mixed (concentrate and forage) diets (CD, SBH and CPD), neither between them and PSD diet. The batch ha a strongly significant effect on NDF content of samples after digestion ($p<0.005$) finding lower values for the second batch.

3.3. VFA´s analysis

Chromatographic analysis gave us VFA's results, as we can see in Table 4.

Table 4. Volatile fatty acids (VFA's)

	CD	SBH	CPD	PCD	PSD	PCP	C	SEM	PVALUE
Total (mmol/l)	^{b}57.95	^{a}50.79	^{d}90.95	^{c}63.02	^{b}57.19	^{c}64.49	^{a}49.79	5.3126	0.0000
Acetic (% of total)	^{a}58.71	^{b}67.78	^{b}64.56	^{a}58.22	^{b}68.67	^{b}65.36	^{b}63.40	3.4101	0.0000
Propionic (% of total)	^{b}26.18	^{a}21.33	^{b}24.36	^{b}27.21	^{a}21.30	^{b}23.86	^{b}24.36	1.4100	0.0000
Butiric (% of total)	^{c}11.66	^{b}7.32	^{a}0.51	^{a}0.82	^{b}7.25	^{b}7.76	^{c}8.64	0.5452	0.0000
Isobutiric (% of total)	^{b}0.87	^{b}0.72	^{c}4.09	^{a}0.63	^{c}13.09	^{a}0.68	^{b}0.92	0.0583	0.0053
Other (% of total)	^{c}2.24	^{a}1.21	^{c}2.25	^{b}2.04	^{d}2.41	^{c}2.34	^{e}2.69	0.0366	0.0007

As we can see in table 4, VFA's production presented statistically significant differences in all fatty acid analysis, except N-caproic (p=0.1014) and heptanoic (p=0.2569). Acetic (58-68%) and propionic (21-27%) were the fatty acids with the highest proportion in the analysis.

4. Discussion

The average methane emission recorded was 24.41±0.12 l/animal and day, which is in the range of the values reported by other authors (Lopez et al., 2010) for similar diets, about 20±0.2 l/animal and day. Despite the corn-based diet (CD) presented a higher proportion oh highly degradable carbohydrate, and lower methane emissions should be expected than when using more fibrous diets such as SBH and CPD (Lopez *et al*., 2010), no differences were found. This might be caused by the high variability found between animals.

Regarding CO_2 production, we can see that SBH diet seems to present a lower CO_2 production in comparison with corn diet and citrus pulp diet despite observed differences were not statistically significant. Average CO_2 emission recorded in this experiment (378.27±101.15 l/animal and day) was lower to the theoretical gas production calculated following CIGR recommendations (522.98±99.75) for animals with the same live weight and milk yield (CIGR, 2002).

The CH_4/CO_2 ratio, which was the only factor affected by the diet, explains that, in diets with a higher degradability of carbohydrates (as CD in this experiment), a higher proportion of carbon in diet is oxidized to CO_2, so CH_4/CO_2 ratio is found to be lower than in fiber-based diets, in which ruminal degradability is lower (Hellwing *et al.,* 2012, Chandramoni *et al*.,2000., Nhu Thuc *et al*.,2012, Madsen *et al*., 2008). This ratio could be therefore used as an indicator of enteric methane production, helping to identify diets with may lead to mitigate the emissions.

Despite no statistically differences were found, CH_4 production was slightly lower in both diets based on citrus pulp (CP and PCP) as well as in the control diet.

Biogas production was much lower for both diets based in soybean hulls (SBH and PSD). This fact could be explained by their high level of fiber content, which is hardly digested by rumen microorganisms leading to lower emissions than diets with a higher content of easy degradable carbohydrates (Moss *et al., 1995,* Dabiao *et al*,. 2008, Makoto *et al*., 2007). The control diet also presented a low biogas production. Moreover, diets presented similar biogas production rates when tested purely or after mixing with the forage source (probably due to the low proportion of forage in the mixed rations). This finding might help to reduce experimental load for future experimental layouts with similar forage: concentrate ratios.

The acetic/propionic ratio is an indicator of ruminal metabolic fermentation processes and can be related to methane emissions (Sauvant *et al.,* 2011). The more fermentation in the rumen, leads to the more propionic acid production, resulting in a lower acetic/propionic ratio (Chandramoni *et al.*, 2000, Moss *et al*. 1995, Eknasa *et al*., 2009). This might be directly related with the NDF content of the diet, finding more propionic acid in these diets rich in easily fermentable carbohydrates such as starch (Jaroslav *et al*., 2009). The results of this work are in accordance with this knowledge, so the acetic/propionic ratio decreased as decreasing the NDF content of the diets.

5. Conclusions

Methane and carbon dioxide emissions from animals measured in a dynamic chamber were not affected by the diet used. Nevertheless, the CH_4/CO_2 ratio, which is an indicator of enteric methane production, was lower for the diet with higher starch content (CD). The group of animals had a strongly significant effect on methane emissions.

When cultured in-vitro, fiber content of the diet had a positive effect on ammonia and biogas production.

The acetic/propionic ratio was found to be lower for the corn based diets (CD and PCD), which indicates higher degradability of the carbohydrates for these diets than for the rest.

No differences were found for all studied parameters in the in-vitro trial between pure diets and their mixed ration equivalents (CD vs PCD, SBH vs PSD and CPD vs PCP).

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