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INCLUSION DE HENO DE CHICHARO (*Pisum sativum* L.) Y PRODUCCION DE GAS IN VITRO EN DIETAS PARA CORDEROS EN CRECIMIENTO

[INCLUSION OF FIELD PEA HAY (*Pisum sativum* L.) AND IN VITRO GAS PRODUCTION IN DIETS FOR GROWING LAMBS]

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RESUMEN

La utilización de subproductos agroindustriales como el heno de chícharo (*Pisum sativum*) es una alternativa para la alimentación de ovinos, el objetivo del presente estudio fue evaluar la ingestión y digestibilidad en ovinos alimentados con diferentes niveles de inclusión de heno de chícharo (HC). Para ello se utilizaron 20 ovinos con un PV inicial de 26.0 ± 0.43 kg, los cuales fueron alimentados con uno de cuatro tratamientos (0%; 25%; 50% y 75% de inclusión de HC en base seca). El contenido de PC del HC fue de 8%. No se observaron diferencias significativas (P>0.1) entre tratamientos para la ingestión de MS (947.57 ± 32.25 g/d^{-1}), MO (856.74 ± 24.76) y FND (583.46 ± 30.6), así como para la digestibilidad (g/100g) de la MS (50.32 ± 1.0), MO (49.4 ± 2.38) y FND (57.04 ± 2.23). El consumo de N fue menor (P<0.05) para el tratamiento con 25 % HC con respecto a 0%, no se observaron diferencias (P>0.1) para la excreción de N en heces, orina y el balance de N entre tratamientos. En la producción de gas *in vitro* la fracción A (ml gas/g MS incubado) fue menor para HC con respecto a rastrojo de maíz (RM) y maíz grano (MG). La fracción b fue mayor (P<0.05) para HC respecto a los demás ingredientes, sin embargo no se encontraron diferencias (P>0.1) entre RM y MG para la fracción c. La producción de gas relativa (ml gas/ g MS desaparecida) fue menor para HC con respecto a RM y MG, La MS desaparecida *in vitro* fue menor para HC con respecto a MG, sin embargo no se encontraron diferencias (P>0.1) entre RM y HC. El heno de chícharo puede ser utilizado en dietas para ovinos hasta en un 75 % de inclusión, sin afectar la ingestión y digestibilidad. La técnica de producción de gas *in vitro* permite predecir la fermentación y degradación ruminal, mostrando una menor fermentación con un 25 % de inclusión de heno de chícharo.

Palabras claves: Corderos; Comportamiento productivo; Heno de chícharo; Producción de gas *in vitro*.

SUMMARY

The use of byproducts such as field pea (*Pisum sativum*) is an alternative to feed sheep, the objective of this study was to evaluate the intake and digestibility in sheep fed different levels of field pea hay (FPH). Twenty Rambouillet lambs (with an initial BW of 26.0 ± 0.43 kg) were fed to one of four treatments of 5 animals of each (0%, 25%, 50% and 75% inclusion of PH as dry matter basis). The content of CP for the FPH was 8%. There were no significant differences (P>0.1) between treatments for DM intake (947.6 ± 32.3 g/d), OM (856.7 ± 24.8) and NDF (583.5 ± 30.6), as well as digestibility (g/100g) of DM (50.3 ± 1.0), OM (49.4 ± 2.38) and NDF (57.0 ± 2.23). N intake was lower (P<0.05) for PH 25% compared to 0%, no significant differences (P>0.1) for N excretion in feces, urine and N balance between treatments. *In vitro* gas production in the fraction A (ml gas / g DM incubated) was lower in FPH compared to corn stover (CS) and corn grain (CG). Fraction b was higher (P <0.05) for FPH compared to the other ingredients, however there were no differences (P>0.1) between CS and CG for fraction c. Gas production (ml gas / g DM disappeared) was lower in FPH compared to CS and CG. *In vitro* DM disappearance was lower for FPH with respect to CG, however there were no differences (P>0.1) between CS and FPH. FPH can be used in diets for sheep up to 75% of inclusion, without affecting intake and digestibility. The gas production technique allows the prediction of *in vitro* fermentation and rumen degradation, showing a lower fermentation with 25% inclusion of FPH.

Key words: Lambs; Productive behavior: Field pea hay; *in vitro* gas production.

INTRODUCCION

The apparent digestibility of foods is essential to establish its nutritional value and, therefore, for the formulation of rations in the ruminants. However, the determination of *in vivo* digestibility is a laborious and expensive process, requiring the use of large amounts of food, so different methods have been proposed for estimating *in vitro* (Bochi-Brum et al., 1999). *In vitro* studies can be carried out with a large number of samples in a relatively short time, providing information on rates of digestion (Aimone et al., 1977). The interest in using agricultural waste in feed for ruminants has been increasing worldwide in recent years, as the availability of grains is reduced (Fuentes et al., 2001). There is a large amount of agricultural residues and agro-products (straw, stubble, stalks, etc.) that could be used as an alternative source of energy and protein in feed for ruminants. An example of these products is field pea hay (FPH) (*Pisum sativum*), which is left in the plot after harvesting pea pods without a rational as it would feed (Bastida Garcia et al., 2011). In Mexico's annual pea production is approximately 48031.76 ton met/ year, and the State of Mexico produces 8.13% of total production (http://www.siap.gob.mx), however, does not have a record of the remaining residue obtained after the last harvest, because not all waste is used for animal feed.

Some authors indicate a dry matter intake an efficient feed conversion and daily weight gain is similar when pea hay replaces cereals in growing and finishing diets of cattle (Reed et al., 2004), but there are few studies in sheep (Bastida Garcia et al., 2011). It was reported that the nutritional characteristics of these feed resources depress feed intake, digestibility, rate of fermentation and microbial nitrogen supply (Mekasha et al., 2003) and because nitrogen is a limiting nutrient in low-quality forages, supplementation with protein nitrogen sources or non-protein nitrogen have been used for the supply of ruminal ammonia level to meet the requirements of microbial protein (Fondevila and Barrios, 2001; Manyuchi et al., 1997).

The aim of this study was to evaluate the productive performance of growing lambs fed different levels of inclusion of FPH and estimate rumen fermentation from *in vitro* gas production.

MATERIALS AND METHODS

Animals and diets

The study was conducted in the Faculty of Veterinary Medicine at the Autonomous University of Mexico State, were used twenty Rambouillet lambs, with an average age of 5 months and 26.0 ± 0.43 kg BWi distributed homogeneously according to their weight in 4 groups of 5 animals each one. The animals were housed at random to each of the 4 treatments (Table 1) to be evaluated by considering treatment as a witness and three inclusion levels of FPH (0%, 25%, 50% and 75%). Diets were iso-protein and iso-energy (13.26% CP and 8.8 MJ ME kg DM) based on forage (pea hay: FPH; corn stover: CS) and concentrate (Corn grain: CG, soybean meal, SBM and fishmeal: FM) supplemented with vitamins and minerals (Multitec of Malta ®), the chemical composition of the ingredients is presented in Table 2, diets were formulated according to the recommendations proposed by the AFRC (1996), meeting the needs of animals for their level of growth. The FPH used for this study was obtained from the field after the last harvest (3rd crop) seed pea. The FPH was milled using a hammer mill (mill Azteca, 5 mm Ø). The concentrate and forage ratios were mixed homogeneously to prevent the selection of ingredients, using a vertical mixing mill (mill Azteca).

Table 1. Proportion of ingredients used and chemical composition of experimental diets (g / kg DM) using different inclusion levels of pea hay.

	Inclusion of pea hay			
Ingredient	0%	25%	50%	75%
Pea hay	0.0	261.4	516.1	760
Corn stover	620	585.8	387.7	194
Corn grain	170	39.1	24.4	11.7
Fish meal	0.0	62.4	27.1	0
SoyBean Meal	170	40.4	21.7	0
Minerals[1]	40	34.3	34.3	34.3
Total	1000	1000	1000	1000
Chemical composition				
DM	935	920	920	930
OM	907	890	900	910
CP	110	107	113	119
NDF	562	513	529	510
ME MJ/kg DM[2]	8.80	8.08	8.05	8.03

[1]*Multitec de Malta* (1.0 kg DM) containing antioxidant 25 mg, calcium carbonate 4.5 g, salt 6 g, ionophore 30 g, zinc oxide 50 g, sodium bicarbonate 6 g, copper sulphate 6 g, ferrous sulphate 20 g, sodium sulphate 125 g, vitamins E 18 000 IU, A 3 000 000 UI, D 3 750 000 IU, potassium chloride 140 g, *E.D.D.* I ethylene-dynamine 0.500 g, cobalt carbonate 0.090 g, magnesium oxide 500 mg, manganese oxide 36 g, selenium 0.090 g

[2]Estimates from Ewing (1997), Young (2002).

Intake and apparent digestibility

Sheep were housed in metabolic cages (1.20 x 0.80 m) with an individual fed at 0800 and 1600 h and having free access to drinking water, food intake and the residues were weighed daily. The experimental period lasted 28 days, 21 days for diet adaptation and 7 days for sample collection. Samples of feces and urine were collected daily in its entirety and they took 10%. The feces were placed in plastic bags and urine containers where there was a mixture of samples taken per animal and frozen at -20 °C for further analysis. After the experimental period proceeded to estimate voluntary food intake, food conversion, feed efficiency and digestibility.

Table 2. Chemical composition (g/kg DM) of the ingredients used in the present study.

	PH	CS	CG	FM	SBM
DM	910	930	890	930	920
OM	920	920	900	915	910
CP	80	36	89	592	460
NDF	636	670	141	-	120
ME, MJ/kg DM[1]	8.7	6.5	14.5	14.5	13.6

PH: Pea hay, CS: Corn stover, CG: Corn grain, FM: Fish meal, SBM: Soybean Meal

[1]Estimates from Ewing (1997), Young (2002).

In vitro gas production

To determine the kinetics of ruminal degradation, it was used technique of *in vitro* gas production according to the method proposed by Menke et al. (1979) and modified by Theodorou et al. (1994). Approximately 0800 g DM of each ingredient and each diet mixtures were incubated in glass flasks with 90 ml of buffer solution and 10 ml of rumen fluid having a replica of three bottles per sample. Three Rambouillet lambs (BW 20 ± 0.5 kg) cannulated in rumen as a fluid donor, which were fed *ad libitum* (0900 and 1600 h) with a diet based on alfalfa hay and straw oats in a 50:50 ratio, to which was added 2% of a vitamin and mineral supplement. About 0.5 L of rumen fluid and 100 g of rumen solid (0830 h) were collected from each lamb, extracted and filtered in triple layer of gauze and cheese cloth, and was homogenized with CO_2 for five minutes, then were mixed and used as inoculum. The flasks were incubated in a water bath at 39 °C. The volume of gas was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation using a Delta brand pressure transducer (Model 8804 HD). After incubation the samples were filtered and dried (48 h, 65 °C) to measure the proportion of dry matter disappeared (DMD). Gas production at 96 h was correlated with dry matter disappeared for relative gas production (RGP: ml gas g DMD) (González Ronquillo et al., 1998).

Gas production was adjusted according to the model proposed by France et al. (1993) $y = A [1 - \exp(-b (t - T) - c (\sqrt{t} - \sqrt{T}))]$. Where: "y" is the cumulative gas production (ml) "t" is the incubation time (hours), A is the curve asymptote (total gas produced, ml), b (h-1) c (h - ½) are the constants of gas produced, T is the delay time (hours) that colonize the microorganisms to begin the fermentation. The values shown in Figures 1 and 2 were obtained from gas production split between the hours reading for each of the ingredients and diets.

Chemical composition

The DM content of feed, refusals and feces was determined in a forced air oven (60 °C, 48 h), and then milled (mill Willey, 3 mm Ø Arthur H. Thomas Philadelphia, PA) to determine organic matter (OM) AOAC (1991), and total nitrogen (N) by the kjeldahl procedure (AOAC, 1991), neutral detergent fiber (NDF) was determined according to Van Soest (1991) adding sodium sulfite and alpha amylase, using a digester fiber ANKOM. Urine samples were subjected to the determination of nitrogen (N) (AOAC, 1991). Residues from the *in vitro* incubation were dried (60 °C, 48 h) to determine the DM disappeared at 96 h.

Calculations and Statistical Analysis

Feed intake was the difference in the amount of feed offered and refused each day. The daily weight gain was calculated based animal weight every seven days until the completion of the experiment; the animals were weighed the morning before fasting the day before. Feed conversion was calculated by dividing the dry matter intake between daily gain and feed efficiency by dividing the daily weight gain between the daily intake. The relationship forage: concentrate ratio (F:C) was obtained considering the forage intake multiplied by 10 between the total intake (g /d) (Bastida Garcia et al., 2011).

To test *in vivo*, we performed an analysis of variance using a completely randomized design. $Y_{ijk} = \mu + T_j + \varepsilon_{ijk}$. Where: μ is the overall mean, T is the effect due to treatment, ε is the experimental error.

For the *in vitro* study conducted an analysis of variance, which included treatment (n = 4) and repetition (3 rounds of incubation). The corresponding variance analysis was done using the GLM procedure of SAS program (1999). Comparisons between means were performed using Tukey's test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Intake and apparent digestibility

Table 1 shows the chemical composition of experimental diets. The content of CP for the FPH was similar to that found by Mekasha et al. (2003) who evaluated several products, including field pea, however, was less than that found by Garcia Bastida et al. (2011) using diets containing FPH, containing 16% CP at second cut, this last quotation that the court used the second FPH. The NDF content was lower than found by Rotger et al. (2006) who in assessing digestibility FPH, mentioned a value of 82%, possibly due to containing a few pods and leaves because they came from three cuts of the product, however was higher than that found by Gilbery et al. (2007) using FPH diets for cattle. The content of CP and NDF in the CS was lower than found by Fuentes et al. (2001), who report a CP content of 490 g / kg DM and 724 g / kg DM of NDF, this may be due to lignification of hay to be packed, with respect to CG, CP content was less than reporting Oropeza et al. (1989) who found 12% in different hybrids of corn, however, about the NDF content is no different to that found in the present study with 1.4%.

The chemical compositions of experimental diets are presented in Table 1. The CP content was higher for 75% inclusion of FPH, being less than that found by Bastida et al. (2011) and Abdel-Magid et al. (2008) who found a concentration of 13.7% of CP using the same inclusion of FPH in diets for sheep. The NDF was higher for the control treatment, because it contained CS, however, the concentration of NDF in all treatments was higher than that found by Pol et al. (2009), Loe et al. (2004) and Bastida Garcia et al. (2011) who reported a concentration of NDF in the range of 381.6 to 553.7 g / kg DM.

The daily weight gain, feed efficiency and feed conversion (Table 3) were not affected by the inclusion of pea hay in the diet (P>0.1), no clutch, although no significant difference, the animals were fed the diet containing 75% inclusion of FPH have a better efficiency compared to other treatments.

Table 3 shows the DM intake, which was lower than found by Loe et al. (2004) of 115 $g/kg^{0.75}$, and higher than that reported by Salawu et al. (2002), who reported 53.7 $g/kg^{0.75}$ of intake. OM intake did not differ (P>0.1) among treatments, while OM intake was increased with 25% inclusion of FPH, however, it was higher (P <0.05) than that found by Salawu et al. (2002) using pea hay field third cut in diets for lambs with 52 $g/kg^{0.75}$ of intake. The NDF intake showed no significant differences (P> 0.1) in treatments, however, differ from that found by Mekasha et al. (2002) using field peas in diets for lambs (49.24$g/kg^{0.75}$). With regard to the average values of ingestion (g/d) and balance and digestibility (g/100g) there were no significant differences (P>0.1) among treatments.

In regard to digestibility of different treatments, DM digestibility was similar to that found by Mekasha et al. (2002) using FPH, digestibility of OM in the present study was lower than found by Gilbery et al. (2006) of 65% using field peas in diets for cattle. The digestibility of NDF was higher than found by Meshaka et al. (2002) who found a digestibility of 45.8% using pea hay in diets for sheep, but differs from Reed et al. (2002) with a 75.5% digestibility of NDF when replacing 100% of the corn with field peas in diets for growing calves.

Nitrogen balance

The results obtained of nitrogen balance are shown in Table 4. N intake (g/d) was higher (P <0.05) with the inclusion of 25% of FPH compared to 75%, without differences (P>0.1) among other treatments, being higher than the found by Mekasha et al. (2002) and Salawu et al. (2002), who recorded a intake of 9.1 and 11.3 g N/d with field pea diets for lambs, this is because diets that included forage used only for their studies, which makes the N intake was less, however, the results obtained in this study were lower than found by Abdel-Magid et al. (2008) who reported a intake of 24.8 g N/d using diets for growing lambs. Fecal excretion shows no difference (P>0.1) among treatments, being lower than found by Mekasha et al. (2002) and Abdel-Magid et al. (2008), with an excretion of 6.85 g N/d which assessed various agro-products including field pea, and 7.82 g N/d using FPH in diets for growing lambs, this may be that in this study used FM and SBM, which are used as protein source on passage to be better used in the intestine (Guada, 1993). The loss of N in urine did not differ (P>0.1) in treatments being higher than that found by Mekasha et al. (2002) who show a loss of N of 1.13 g N/d, however, are less than Abdel-Magid et al. (2008) with an excretion of 11.97 g N/d. Regarding the N retained no differences (P>0.1) among treatments. The diets used in this study were made isoenergetic and isoproteic for which there were no differences between intake and digestibility, coupled with a low concentration of CP and NDF in forages used. The minimum N excretion in urine and feces found in this study indicates a greater utilization of nutrients, which reduces environmental pollution (soil) excess N excreted into the environment, avoiding being made nitrous oxide (N_2O), which contributes to the greenhouse effect (Elizondo, 2006).

Table 3. Intake (g/d) and digestibility (g/100g) in growing lambs fed different levels of supplementation of pea hay.

	Pea hay inclusion					
Item	0%	25%	50%	75%	SEM	*P-value*
No. of animals	5	5	5	5		
Live weigth (kg)	26.4	26.5	25.8	26.0	0.37	NS
DGW (g/d)	132.6	143.4	140.0	143.5	13.0	NS
Feed conversion	7.0	7.2	6.8	6.2	0.6	NS
Feed eficiency	0.144	0.138	0.146	0.162	0.01	NS
Intake (g/d)						
Field pea intake	0.0[d]	270.7[c]	494.1[b]	671.3[a]	18.9	0.001
Total Roughage intake (R)	571.6[b]	877.4[a]	865.3[a]	842.7[a]	28.1	0.001
Total Concentrate intake (C)	350.3[a]	182.5[b]	102.9[c]	40.6[d]	13.0	0.001
R:C ratio	6.2:3.8[b]	8.5 :1.5[a]	9.0:1.0[a]	9.5:0.5[a]	0.03	0.001
DM	922.0	1035.6	957.4	883.3	13.6	NS
OM	839.0	921.7	861. 7	803.8	11.1	NS
NDF	562.4	621.4	593.6	556.5	7.6	NS
Digestibity (g/100 g)						
DM	47.8	52.6	51.6	49.4	1.08	NS
OM	51.8	54.3	54.9	47.7	1.64	NS
NDF	54.6	56.6	60.0	56.9	1.11	NS

DWG, Daily Weight Gain (g/d); SEM, standard error of the mean. NS, not significant (P>0.1)

Table 4. Nitrogen balance (g / d) in growing lambs fed different levels of supplementation of pea hay.

	Pea hay Inclusion					
	0%	25%	50%	75%	SEM	*P-value*
N Intake	16.2[ab]	16.8[a]	15.3[ab]	15.7[b]	0.33	0.05
N Excretion						
Feces	4.3	4.7	4.3	4.4	0.09	NS
Urine	6.1	6.5	6.4	5.8	0.16	NS
N Balance	5.8	6.4	7.9	5.8	0.49	NS

[abc] Means with different literal in the same row are statistically different (P <0.05), NS, not significant (P> 0.1). SEM, standard error of the mean.

In vitro gas production

Table 5 presents the parameters of gas production adjustment obtained *in vitro* incubation of the different ingredients used in the experimental diets, which shows that the fraction A (ml gas / g DM incubated) was lower for FPH with respect to CS and CG, the latter who showed the increased production of gas. The fraction b (h^{-1}) was higher (P <0.05) in FPH compared to the other ingredients, however there were no differences (P>0.1) between CS and FM for the fraction c ($h^{1/2}$). The lag time phase, FM and SBM had a higher start of fermentation compared to the other ingredients. Relative gas production (ml gas / g DMD) was lower in FPH compared to CS and CG. The DMD *in vitro* was lower for PH with respect to CG, but no differences (P>0.1) between CS and FPH, on the other hand, the DMD between CS and FM were not significantly different (P>0.1). The ability of rumen bacteria to degrade to a greater extent than the structural soluble carbohydrates (fodder) has been evidenced in the literature, as reflected in the production levels of gas or DMD (Bastida Garcia et al. 2011). Relative gas production was lower (P<0.001) in FPH compared to the other ingredients, with the CG that has the highest amount of gas production.

Figure 1 shows the gas production of up to 96 hours of the ingredients used in the experimental diets, SBM results in increased production of gas to the first 3 h and found no difference (P>0.1) between remaining ingredients, at 3 and 6 h the CS showed a higher production compared to the other ingredients, this is due to the maturation stage of the cut stubble as the first cut, the leaves and stems contain a number low fiber which indicates containing rapidly fermentable soluble components in these parts (leaves and stems) so it has a higher gas production in the early hours of fermentation and then decline rapidly (Tolerates et al., 1999) , from 12 h onwards, the largest gas production is presented by the CG. The low gas production of the CG in the initial time of incubation compared with the other ingredients is due to increased slowly fermentable carbohydrate (Hamid et al., 2007), but the production of the FPH although it was lower compared to CS and CG has a constant gas production, this may be related to the content of hemicellulose in FPH, which is more available than in the CS which presents unions linked to the lignin that makes it less digestible (Bach et al., 2006). The FM gas production shows a lower gas production with respect to other ingredients used, this may be due to increased temperature during preparation, in which the proteins are denatured by breaking hydrogen bonds and disulfide bonds responsible for their secondary structure, the result of the distortion, reduces the solubility of the protein as well as susceptibility to degradation and rumen fermentation (Guada, 1993).

Table 6 shows the fermentation parameters of the different levels of inclusion of FPH in the diets, showing that the total gas production for 25% FPH was lower compared to the control diet (0% FPH). The average rate of degradation (h) shows that 50% of FPH was lower (P <0.05) compared to other treatments, but no significant differences (P>0.1) between 25 and 75%. For the fraction $h^{1/2}$ and lag time there were no differences (P>0.1) between treatments. It has been shown that the production of gas is related to the disappearance of the NDF (Nsahlai et al., 1995) and about Pell et al. (1997) found that the relationship between variables is linear with a slope remarkably constant. Also found a high correlation between *in vitro* gas production and availability of starch in cereal grains (Posadas et al., 2005). Gas production was higher on the diet containing 50% of FPH compared with other treatments, while the diet containing 25% of FPH inclusion had the lower value. For the DMD no differences were found (P>0.1) between treatments.

Figure 2 shows the gas production at different levels including the FPH, it was noted that gas production at 3, 6 and 9 h of incubation was higher (P>0.1 in the treatment that no containing FPH (0%) compared with the other diets, the production of gas at 3 h shows no significant difference (P>0.1) between treatments containing 25, 50 and 75% inclusion of FPH, with respect to the 6 h gas production was lower (P> 0.1) for the diet containing 50% compared with 25 to 75% of FPH which show no significant differences (P>0.1), compared to 12 hours of incubation, there was no significant differences (P>0.1) among treatments, the production of gas from 24 h onwards was lower (P>0.1) for the diet containing 25% of FPH with respect to the 50 and 75%. Usually as the starch in corn is more digestible than the NDF is expected that the substitution of concentrate feed by resulting in an increase in total digestibility of the ration, thus an increase in the fermentation as in diet with 25% inclusion of FPH, without clutch, although the potential for fiber digestibility of grasses is higher, the high rate of passage through the rumen resulting in higher effective fiber digestibility in legumes in grasses (Bach et al. 2006; Guada, 1993; Hoffman et al., 2007).

Table 5. Gas production parameters obtained from the *in vitro* incubation of adjustment and digestibility of the ingredients used in the diets, using the model proposed by France et al. (1993).

Item	FPH	CS	CG	FM	SBM	SEM	*P-value*
A	94.8^{d}	118.2^{e}	229.7^{f}	210.1^{g}	206.00^{g}	3.79	0.001
b	0.187^{d}	0.072^{e}	0.088^{e}	0.004^{f}	0.001^{f}	0.008	0.001
c	-0.287^{d}	-0.122^{d}	-0.244^{d}	-0.109^{d}	-0.111^{d}	0.05	NS
T	2.31^{d}	2.46^{d}	3.91^{d}	2.66^{e}	2.41^{e}	0.78	0.001
DMD_{96h}	59.71^{e}	61.79^{de}	66.72^{d}	66.12^{d}	66.33^{d}	1.08	0.01
RGP	158.7^{f}	191.26^{e}	344.27^{d}	317.69^{g}	310.56^{g}	5.4	0.001

[defg] Means with different literal in the same row are statistically different (P <0.05), NS, not significant (P>0.1); SEM, standard error of the mean.

A total gas production (ml gas / g DM incubated); b, fermentation rate (h^{-1}), c, fermentation rate ($h^{-1/2}$) T, Lag Time (the time in which start the fermentation). DMD_{96h}, DM disappeared at 96 h (mg/100 mg), RGP (ml gas $_{96h}$ / g DMD_{96h})

PH: pea hay, CS: corn stover, CG: corn grain, FM: fish meal, SBM: soybean meal.

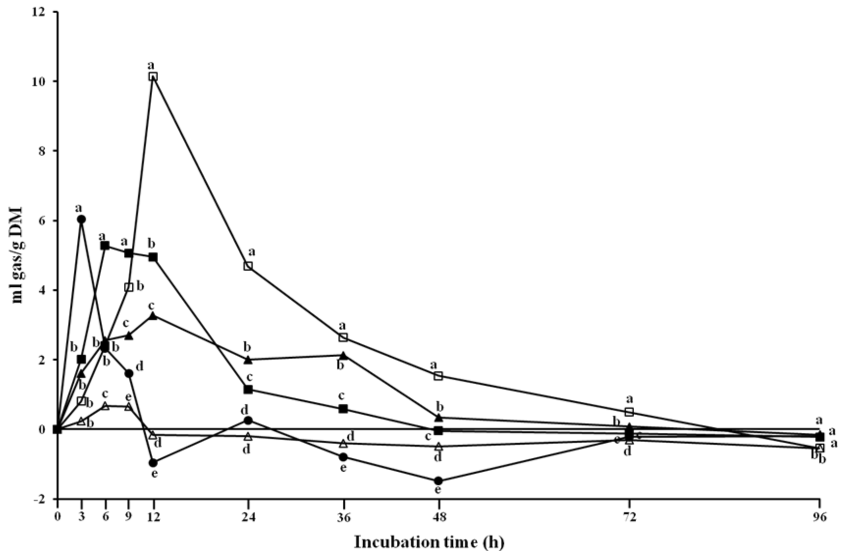

Figure 1. The curves of gas production per hour (ml gas /g DM) of ingredients used in the experimental diets (▲, pea hay, ■, corn stover, Δ, Fish meal; □, corn grain, •, Soybean Meal). [abcde] different letters at the same time (P <0.05).

Table 6. Gas production parameters obtained *in vitro* incubation of adjustment and digestibility of the different inclusion levels of pea hay in the diets, using the model proposed by France et al. (1993).

	Pea hay inclusion					
Item	0%	25%	50%	75%	SEM	*P-value*
A	216.81[d]	161.55[e]	211.75[de]	187.58[de]	12.67	0.01
b	0.0507[ef]	0.0661[d]	0.0427[f]	0.0605[de]	0.0051	0.001
c	-0.0492	-0.0525	-0.0270	-0.0490	0.0058	NS
T	1.2740	0.9434	0.7927	0.9489	0.1014	NS
DMD_{96h}	61.85	62.76	62.66	58.55	1.51	NS
RGP	350.54[d]	257.40[d]	377.93[e]	320.37[f]	7.06	0.001

A, total gas production (ml gas / g DM incubated); b, fermentation rate (h^{-1}), c, fermentation rate ($h^{-1/2}$) T, Lag Time (the time in which start the fermentation). RGP, relative gas production (ml gas 96h / g DMi); DMD_{96h}, DM disappeared 96 h (mg / 100 mg). Means with different literal in the same row are statistically different (P <0.05), NS. No significant (P> 0.1).
SEM, standard error of the mean.

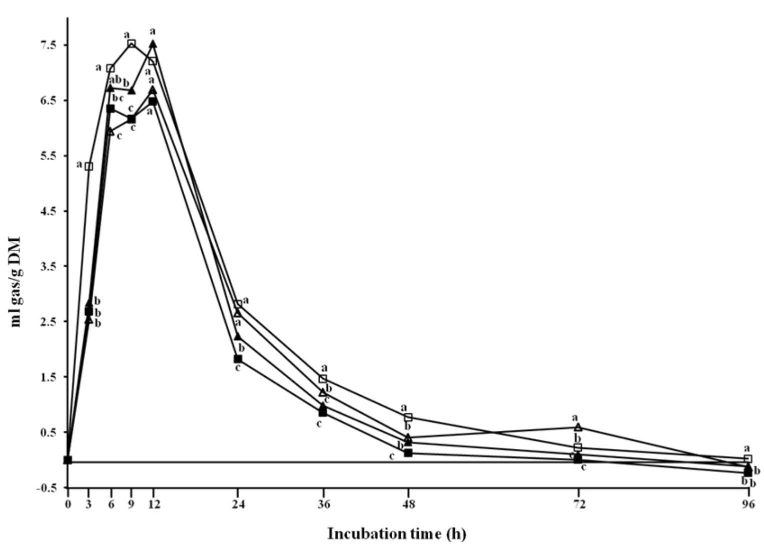

Figure 2. Production curves of gas per hour (ml gas / g DM) of diets used different inclusion levels of pea hay (□ 0% ■ 25%, 50% Δ, ▲ 75%). [abc] Different letters to the same time are statistically different (P <0.05).

CONCLUSIONS

Pea hay can be used in diets for sheep up to 75% inclusion, without affecting intake and digestibility. The method of *in vitro* gas production is a useful tool for selecting ingredients for balancing rations, and the data obtained from the digestibility provide an adequate and reliable estimate of the nutritional quality of food.

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