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ANIMAL PRODUCTION

Ensiling on chemical composition and *in vitro* fermentation in rabbits of different forages

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ABSTRACT: *The effect of chemical composition of silages on* in vitro *gas fermentation profiles in rabbits was examined. The study was performed using 7 silages: beans (Phaseolus vulgaris), faba bean (FB, Vicia faba), common vetch (CV, Vicia sativa), ryegrass (Lolium perenne), barley (B, Hordeum vulgare), barley with common vetch (BCV) and barley with faba bean (BFB). We used 3 New Zealand White (NZW) rabbits as donors of caecal content in each incubation run (n=3). Data were analyzed in a completely randomized experimental design. The CV silage showed higher values of crude protein (CP), followed by FB and bean silages (P<0.001). Barley silage had the lowest CP content (96g kg^{-1} DM) (P=0.001). The NDF and ADF content were lower (P<0.001) for beans and CV compared with the rest of the forage silages. Ryegrass silage had higher values of dry matter degradation, organic matter degradation, relative GP and SCFA (P<0.001). The highest values of digestible energy were for CV and bean silages (P<0.001). Ryegrass and CV silages showed higher levels in GP parameters, which could be associated with their better chemical composition characteristics, mainly protein and fiber content.*
Key words: in vitro *gas production, caecal fermentation, forage silage.*

Diferentes silagens sobre a fermentação *in vitro* em coelhos

RESUMO: *O objetivo do presente estudo foi avaliar a composição química e os padrões* in vitro *de fermentação cecal em coelhos em crescimento. Foram realizados 7 silagens: feijão (Phaseolus vulgaris), fava (FB, Vicia faba), ervilhaca (CV, Vicia sativa), azevém (Lolium perenne), cevada (B, Hordeum vulgare), cevada com ervilhaca (BCV) e da cevada com fava (BFB). oram usados 3 coelhos New Zealand White (NZW) como doadores de conteúdo cecal em cada série de incubação (n=3). Os dados foram analisados em um delineamento experimental inteiramente casualizado. A silagem de CV apresentaram maiores valores de proteína bruta (PB), seguido de silagens de FB e de feijão (P<0,001). Silagem de cevada apresentou o menor teor de PB (96g kg^{-1} de MS) (P=0,001). O conteúdo FDN e FDA foram menores (P<0,001) para Feijão e CV em comparação com o resto das silagens de forrageiras. Silagens de azevém apresentaram valores mais elevados de degradação de matéria seca, degradação da matéria orgânica, GP relativa e AGCC (P<0,001). Os maiores valores de energia digestível foram de CV e silagens de feijão (P<0,001). Azevém e CV apresentaram melhores níveis de parâmetros GP, que poderiam ser relacionadas com as suas melhores características de composição química, principalmente proteína e teor de fibra.*
Palavras-chave: *produção de gás* in vitro*, fermentação cecal, desempenho de crescimento, silagem de forragem.*

INTRODUCTION

The rabbit (*Oryctotagus cuniculus*) caecum represents 40% of the total digestive tract by weight and accounts for about two-thirds of fecal fiber digestion (MERINO & CARABAÑO, 1992); it is characterized by the presence of an abundant microbial community with wide diversity and complex interactions (RODRIGUEZ-ROMERO et al. 2011). Microbial community plays a role in feed digestion through fermentation and recycling of microbial protein through caecotrophy. The *in vitro* gas production technique has been used to evaluate the nutritive value of feed in caecal fermenters; it also provides information about kinetics and fermentation characteristics of feedstuff and diets, and is a useful indicator of caecal microbial activity (STANCO et al., 2003). The aim of the present study was to evaluate the chemical composition, the *in vitro* fermentation profiles and their correlations in silages for rabbits.

MATERIALS AND METHODS

The present study was performed in the Faculty of Veterinary Medicine and Livestock, of the Universidad Autonoma del Estado de Mexico

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(19º14'20" and 19º33'01" north latitude and 99º42'07" and 99º56'13" west longitude at 2600m altitude), annual rainfall of 788mm^3, humid temperate climate and an annual temperature range of 13.5 to 30.5ºC.

We evaluated traditional forages that small farmers use for feeding rabbits, such as gramineae: Ryegrass (*Lolium perenne*), Barley (*Hordeum vulgare*); legumes: Beans (*Phaseolus vulgaris*), faba bean (FB, *Vicia faba*), common vetch (CV, *Vicia sativa*); and their mixtures B with FB (BFB) and B with CV (BCV) as an intercrop. The experimental design was completely randomized. The study included the seven forages with three replicates per treatment; hence, each forage was cultivated in three experimental plots (10x10m^2) with a total of 21 experimental plots; crops were harvested after 120 days (1 August 2014 in Toluca, Mexico), having been fertilized previously with 50kg N ha^{-1}, 90kg $Ca(H_2PO_4)_2$ ha^{-1} and KCl 70kg ha^{-1}, except ryegrass which was fertilized with 44kg N ha^{-1} and KCl 60kg ha^{-1}, 35 days before harvest.

One sample from each replicate (plot) was taken from the seven forages and this was considered the experimental unit. One silage was made from each sample. For the *in vitro* analysis, three bottles were assigned to each one of the silages in three runs (three different weeks), plus two bottles as blanks (i.e., caecal fluid and buffer only), so that a total of 189 bottles were analyzed and each bottle was the observational unit.

The crop was cut manually, chopped with a mill (3cm screen size) and compacted in nylon bags (25kg capacity as fresh matter); the excess air was removed and the silos were tightly sealed and stored for 90 days. They were then transported to the lab for sampling and analysis. Forage samples for chemical analysis post-conservation were collected from silages at opening. A minimum of 6 cores were taken from each bag and pooled to produce one sample per bag. Samples for chemical analysis were frozen at -18ºC before chemical composition analysis. Pooled samples of feeds were ground to a 1mm maximum size with a Wiley mill and analyzed in duplicate following the procedures of AOAC (1997). The pH was measured immediately after opening a silo; dry matter (DM) was determined by oven drying at 105°C to constant weight (ref. 934.01), organic matter and total ash by muffle furnace (ref. 942.05), and crude protein (CP) by the Kjeldahl method (ref. 976.05). The content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were determined according to Van SOEST et al. (1991) using an Ankom 200 Fiber Analyzer (Ankom Technol. Co. Macedon, NY, USA). NDF was assayed without α-amylase, with sodium sulfite. Both NDF and ADF were expressed without residual ash. Moisture content of the silages was determined through distillation with toluene (HAIGH & HOPKINS, 1977).

For the *in vitro* caecal microbial fermentation, forages were incubated 48h in a water bath at 38°C. In each incubation run (n=3) we used three White New Zealand (WNZ) rabbits (50 days of age and average weight of 1.5kg) slaughtered by cervical dislocation between 07:00 and 08:00h. The rabbit donors of inoculums were fed with fresh alfalfa 15 days prior to the experiment. Once slaughtered, the rabbit caecum was excised, and total caecal contents (around 300g) were removed and diluted under a CO_2 stream in 300ml of incubation solution, without resazurin and micromineral solution, using HCl-cysteine as the reducing agent (MOULD et al., 2005) and filtrated in three gauzes of cheesecloth, after which caecal content (10ml) was taken, supplemented with 90ml of buffer solution and inoculated into triplicate bottles (100ml total volume) with 0.8g of forage silage as substrate, according to the method proposed by THEODOROU et al. (1994). Internal pressure of the bottles was recorded at 1, 2, 3, 4, 8, 10, 12, 24, 36 and 48h with an HD 8804 manometer (Delta OMS, Caselle di Selvazzano, Italy). Pressure readings (pres) were converted into volume (vol) by using a pre-established linear regression between pressure recorded in the same type of bottles and known inoculated air volumes (ml gas=2.7384x–0.0243, where x = psi h^{-1}). The gas volume for each incubation time was corrected for the inoculum (ml gas g^{-1} DM).

Kinetic parameters of gas production (GP) were estimated through an iterative procedure of nonlinear regression analysis (PROC NLIN, SAS Institute 2002) according to KRISHNAMOORTHY et al. (1991), calculated as: $GP = B\ (1\text{-}e\ ^{-C(t-l)})$ (1)

Where GP is the volume of gas at time t; B is the asymptotic GP (ml/g DM); C is the fractional rate of GP (g h^{-1}), and *l* (h) is the discrete lag time prior to gas production.

At the end of the incubation DM degradability (DMD, mg g^{-1}) and OM degradability (OMD, mg g^{-1}) were determined by filtering the residue and drying 60ºC, 48h for DM and OM by muffle furnace; gas yield (GY_{24}) was calculated as the volume of gas (ml gas g^{-1} DM) produced after 24h of incubation divided by the amount of DMD (g) as:

Gas yield (GY_{24}) = ml gas × g DM_{24h} g^{-1} DMD (2)

Digestible energy (DE, Mj kg^{-1} DM) was estimated according to FERNANDEZ-CARMONA et al. (2004): DE = 15.3 - ADF (3)

Relative gas production (RGP) was calculated as milliliter of gas per gram of DMD after 48h incubation (DMD_{48h}). RPG = ml gas g^{-1} DMD_{48h} (4)

Short chain fatty acid concentrations (SCFA) were calculated according to GETACHEW et al. (2002): SCFA (mmol $200mg^{-1}$ DM) = 0.0222 GP - 0.00425 (5)
where GP is the 24h net gas production (ml $200mg^{-1}$ DM).

Microbial CP biomass production was calculated according to BLUMMEL et al. (1997): MCP (mg g^{-1} DM) = mg DMD - (ml gas × 2.2 mg ml^{-1}) (6)
where 2.2mg ml^{-1} is a stoichiometric factor that expresses mg of C, H and O required for the production of SCFA gas associated with production of 1ml of gas.

The data were subjected to analysis of variance (ANOVA) using the General Linear Model (PROC GLM, SAS Institute 2002); the following model was used:

$$Y_{ij} = \mu + S_i + P_j + \varepsilon_{ijk} \quad (7)$$

where Y_{ij} = is every observation of the i_{th} silage species (*Si*); μ is the general mean; S_i (i = 1–7) is the fixed effect of silage, P_j is the plot (j=3) of each forage and ε_{ijk} is experimental error.

Tukey's Multiple Range Test was used to determine the difference among groups (P<0.05). Also the correlation coefficient (r) between *in vitro* parameters and chemical composition of the forages was determined (PROC CORR, SAS Institute 2002).

RESULTS

Chemical composition of the silages is presented in table 1. pH values in ryegrass, FB and bean silages were lower than in the other silages analyzed (P<0.001). The DM was higher in beans, followed by ryegrass, in comparison with the rest of the silages. The CV silage showed higher values of CP, followed by FB and bean silages (P<0.001);

Table 1 - Chemical composition (g/kg DM) and effect of forage silage on *in vitro* caecal gas kinetics of rabbits.

	Barley	Ryegrass	Beans	CV	FB	BFB	BCV	SEM	P value
Item	--------------------	--------------------	Silage chemical composition characteristics	--------------------					
pH	4.6^{bc}	4.1^{d}	4.4^{cd}	5.3^{a}	4.1^{d}	4.6^{bc}	4.8^{b}	0.08	0.001
DM	220^{b}	240^{ab}	260^{a}	210^{b}	220^{b}	230^{b}	220^{b}	11.2	0.001
OM	969^{ab}	973^{a}	955^{b}	961^{ab}	965^{ab}	972^{a}	973^{a}	4.31	0.005
CP	96^{d}	139^{c}	146^{bc}	179^{a}	157^{b}	133^{c}	144^{bc}	4.92	0.001
NDF	613^{a}	651^{a}	516^{b}	476^{b}	658^{a}	612^{a}	608^{a}	24.6	0.001
ADF	314.9	294.5	303	335.9	298.5	299	287.4	25.1	0.800
ADL	34.4^{b}	49^{b}	110^{a}	77.1^{ab}	59.3^{ab}	54^{b}	52.8^{b}	9.72	0.010
Hemicellulose	298.5^{a}	357^{a}	213^{b}	140.5^{b}	360^{a}	313^{a}	321^{a}	15.1	0.001
Cellulose	277.4	245.5	193	258.9	239.2	234.6	234.6	29.31	0.600
Item	--------------------	--------------------	--------------------	Gas production parameters	--------------------				
B	586^{a}	174^{c}	354^{abc}	242^{bc}	255^{bc}	222^{bc}	454^{ab}	53.9	0.001
C	0.0036^{c}	0.0236^{a}	0.0080^{bc}	0.0113^{bc}	0.014^{abc}	0.016^{ab}	0.0060^{bc}	0.002	0.001
lag time	0.96^{b}	0.50^{b}	2.00^{b}	4.63^{a}	2.43^{a}	2.10^{b}	2.43^{a}	0.44	0.001
	--------------------	--------------------	*In vitro* gas production (ml gas g^{-1} DM)	--------------------					
GP8	9.2^{cd}	25.2^{a}	9.7^{cd}	6.5^{d}	13.4^{bc}	18.5^{b}	9.5^{cd}	1.96	0.001
GP12	21.6^{d}	49.2^{a}	25.6^{cd}	22.5^{d}	36.1^{bc}	40.4^{ab}	30.1^{bcd}	3.13	0.001
GP24	39.1^{d}	70.7^{a}	47.3^{cd}	44.9^{cd}	66.2^{ab}	61.1^{ab}	54.2^{bc}	3.90	0.001
GP48	87.1^{b}	121.2^{a}	89.7^{b}	90.4^{b}	119.3^{a}	111.1^{a}	106.8^{a}	4.81	0.001
	--------------------	--------------------	--------------------	Fermentation profile	--------------------				
DMD	668^{abc}	677^{a}	658^{c}	664^{bc}	670^{ab}	674^{a}	675^{a}	2.13	0.001
OMD	654^{abc}	662^{a}	645^{c}	650^{bc}	656^{ab}	660^{a}	661^{a}	20.8	0.001
GY24	58^{e}	104^{a}	71^{cde}	98^{ab}	68de	90^{abc}	80^{bcd}	4.14	0.001
DE	4.73^{b}	4.16^{bc}	6.00^{a}	6.73^{a}	3.50^{c}	4.06^{bc}	4.30^{bc}	0.17	0.001
RGP	130^{c}	179^{a}	136^{bc}	136^{bc}	178^{a}	165^{a}	158^{ab}	5.11	0.001
SCFA	1.70^{d}	3.13^{a}	2.06^{cd}	2.00^{cd}	2.96^{ab}	2.73^{ab}	2.40^{bc}	0.124	0.001
MCP	477^{a}	410^{c}	461^{ab}	465^{ab}	408^{c}	430^{bc}	440^{abc}	7.76	0.001

CV, common vetch; FB, faba bean; Barley Faba bean (BFB); Barley common vetch (BCV); DM, Dry matter; OM, Organic matter; CP, Crude protein; NDF, Neutral detergent fiber, ADF, Acid detergent fiber; ADL, Acid detergent lignin; *B*, asymptotic gas production (ml/g DM); *C*, rate of gas production (h-1); *lag time*, initial delay before gas production begins (h);DMD, dry matter degradability (mg/g); OMD, organic matter degradability (mg/g); DE, Digestible energy (Mj kg^{-1} DM); RGP, relative gas production (ml gas g^{-1} DMD); GY24, gas yield at 24h; SCFA, short chain fatty acids; MCP, microbial CP production.
Values within columns followed by different letters are significantly different (P<0.05).

barley silage had the lowest CP content (P=0.001) and CV had the highest The NDF content was lower (P<0.001) for beans and CV compared with the rest of the forage silages. Beans silage showed the highest ADL content, followed by the other two legume silages (CV and FB). There was a difference among the silages with regard to hemicellulose content (P<0.001). Beans and CV silages had consistently lower hemicellulose content than the other silages.

The *B* parameter, which represents potential cumulative gas production, was higher (P=0.001) in barley, beans and BCV in comparison with the rest of the forage silage varieties. Ryegrass, FB and BFB showed the highest rate of gas production (parameter *C;* P=0.001) compared with the other silages. In contrast, barley silage presented the lowest value of *C* parameter, immediately followed by beans, CV and BCV silages. The lag time was higher (P<0.001) in CV and BF silages in comparison with the other analyzed forages. Ryegrass silage had higher values of DMD, OMD, RGP and SCFA (P<0.001). The GY24 was higher in ryegrass silage (P<0.001), immediately followed by CV and BFB. The highest values of DE were shown for CV and bean silages (P<0.001).

The correlation between *in vitro* parameters and chemical composition of silage are shown in table 2. The correlation of CP with the *B in vitro* parameter showed a negative relationship (r=-0.65, P<0.001). NDF and ADF showed a negative relationship with lag time (P<0.05) and MCP (P<0.05), and a positive correlation with DMD, OMD, DE (P<0.001) and RGP (P<0.01).

DISCUSSION

Dry matter of beans, CV, FB and mixed silages was within the optimum range of 240-320g kg^{-1} for legume silages (MAHANNA, 1994). The CV silage showed the highest values of pH, which is in agreement with PURSIAINEN & TUORI (2008); this result in both studies could reflect the low content of water-soluble carbohydrates of legume silage, which enhances *clostridial* fermentation and butyric acid production (BORREANI et al., 2009). The CV silage had the highest values of CP, which is a desirable nutritional component in rabbit nutrition; however, it should be taken into account that a higher protein content can be associated with higher rates of proteolysis in legume silage (CAVALLARIN et al., 2007).

The pH of bean, FB and BFB silages was within the acceptable range for good-quality legume silage (MUSTAFA & SEGUIN, 2003a, 2003b). However, BCV and CV silages showed high values of pH, possibly explained by the high content of fiber, or by the low DM content of forages, which could enhance poor compression. Excessive moisture promotes the proliferation of *clostridial* bacteria that degrade amino acids extensively, producing ammonia and amines that tend to slow or reverse the normal pH reduction (THOMAS & RAE 1988).

Mixed silages did not exhibit different levels of DM, OM and NDF in comparison with single silages. The CV had higher CP, ADL and pH values when compared with BCV silage; likewise, BFB and BCV had higher levels of CP than barley silage. This is

Table 2 - Correlation matrix between *in vitro* cecal fermentation in rabbits compared with chemical composition silages.

In vitro GP	DM	CP	NDF	ADF
B	-0.13 (0.58)	-0.65 (0.001)	-0.01 (0.95)	0.11 (0.63)
C	0.17 (0.47)	0.36 (0.10)	0.31 (0.18)	0.16 (0.49)
lag time	-0.51 (0.01)	0.32 (0.16)	-0.51 (0.01)	-0.47 (0.03)
DMD	-0.26 (0.25)	-0.12 (0.60)	0.71 (0.001)	0.69 (0.001)
OMD	-0.26 (0.25)	-0.12 (0.60)	0.71 (0.001)	0.69 (0.001)
GY24	0.15 (0.49)	0.38 (0.08)	0.51 (0.01)	0.39 (0.07)
DE	0.08 (0.73)	0.24 (0.29)	-0.95 (0.001)	-0.94 (0.001)
RGP	0.01 (0.94)	0.32 (0.15)	0.59 (0.001)	0.49 (0.02)
SCFA	0.12 (0.59)	0.38 (0.08)	0.52 (0.01)	0.41 (0.06)
MCP	-0.06 (0.77)	-0.37 (0.09)	-0.53 (0.01)	-0.42 (0.05)

DM, Dry matter; CP, Crude protein; NDF, Neutral detergent fiber, ADF, Acid detergent fiber. *B*, asymptotic gas production (ml g^{-1} DM); *C*, rate of gas production (h-1); *lag time*, initial delay before gas production begins (h);DMD, Dry matter degradability; OMD, Organic matter degradability; GY24, gas yield at 24h; DE, Digestible energy (MJ kg^{-1} DM); RGP, relative gas production (ml gas g^{-1} DM disappeared); SCFA, short chain fatty acids; MCP, microbial CP production; correlations between variables are reported and their significance is indicated within parentheses.

agreement with TITTERTON & MAASDORP (1997) and LITHOURGIDIS et al. (2007) who concluded that mixed legume-graminean intercrop and silages provided higher forage quality, mainly in CP content, in comparison with cereal silages. FB silage showed similar values of CP to those reported by LOUW (2009) (158g kg^{-1}), higher levels of NDF (567g kg^{-1},) and lower ADF (511g kg^{-1}), which could be associated with differences between mature stages in both studies, being that LOUW (2009) was ensiled 166 days after planting.

In rabbits, the level of dietary fiber has a critical role in maintaining gut health, stimulating gut motility and preventing enteritis (IRLBECK, 2001). In the current study NDF and ADF showed acceptable values in all silages analyzed (600-650 and 300-350g kg^{-1} DM, respectively) (MAHANNA, 1994). GIDENNE (2000) mentioned that when the dietary fiber level is very high (>25% ADF), this dilutes the calorific content of the diet and the animal is unable to increase its intake sufficiently to meet its energetic needs. Also, fiber fermentation is time-dependent and its caecal retention time can greatly affect cell wall digestibility, as has been observed with the negative correlation of ileo-rectal retention time with dietary NDF content (GIDENNE et al., 2002) as well as the positive relationship between digestibility of non starch polysaccharides and caecal mean retention time (GARCIA et al., 1999).

The effective utilization of dietary fiber by rabbits depends on its chemical composition (e.g. lignification) and physical characteristics (particle size, moisture, etc.) High levels of lignification of the cell wall (ADL) in rabbit rations are related with a decrease in the efficiency in the use of digestible energy and feed digestibility, and are associated with a reduction of digestion retention time in the entire tract (-20%), an increase in the feed conversion ratio (GIDENNE, 2000) and a decrease in feed intake and body growth (ALVAREZ et al., 2006).

Linkages between cell wall components, especially lignin and polysaccharides, limit physical access of microbes. It has been widely reported that species with high cell wall and lignin contents showed an increase of *lag time* (HAMID et al., 2007; CHANG et al., 2007), and a decrease in the rate of gas production (*C* parameter) and *in vitro* DMD and OMD (SANDOVAL-CASTRO et al., 2005), which is in accordance with the findings of the present study, in which bean silage showed the highest values of ADL associated with the lowest values of DMD and OMD; also, ryegrass silage showed the lowest *lag time* associated with its significant lower level (P<0.001) of ADL.

CHANG et al. (2001) suggested that the current fiber of the diets based on the total NDF content can hardly predict the requirements for dietary fiber in rabbits due to the complex chemical composition of the total dietary fiber and its multiple interactions with the microbial caecal ecosystem. For this reason the use of *in vitro* gas production techniques could allow the adequate characterization of feed fermentation in the caecum of post-gastric fermenters such as rabbits (LAVRENČIČ, 2007).

Although it has been reported that *in vitro* gas production techniques estimate the digestibility of compound diets with only moderate precision (60% to 70% of explained variability) due to the fact that the majority of digestible nutrients are not involved in caecal fermentation (STANCO et al*.,* 2003), the *in vitro* technique has simple, practical, animal welfare and timesaving advantages, which allow it to be used to differentiate the microbial fermentation of fibrous feed provided to rabbits (CHANG et al., 2007), depending on the NDF and ADF content.

The *in vitro* digestibility parameters (GP, DMD, OMD) showed variability within botanical species according to their chemical composition, in agreement with STANCO et al. (2003), who evaluated different feeds in New Zealand rabbits utilizing *in vitro* gas production technique with caecal inoculum. Also, these last reported a negative correlation between DE and NDF (r=-0.76, P<0.01) and ADF (r=-0.79, P<0.01), which agrees with the present values of the correlation coefficient between DE with NDF and ADF (r=-0.91and r=-0.94, P<0.001, respectively).

CV silage also had the lowest levels of NDF, which determined its lowest performance of *in vitro* gas production at initial incubation times (GP8 and GP12). These finding are associated with its higher values of ADL, which confirm the observations of LOPEZ (2005) that feeds with low levels of NDF and consequently high proportions of rapidly degradable fractions, including the soluble fraction, have high propionic acid production, which determines a low yield of gas production at the start of incubation. The CV showed high levels of ADL and lower hemicellulose that can affect the degradation rate due to the fact that the lignin is covalently liked to hemicelluloses (GIDENNE et al., 2010).

RODRIGUEZ-ROMERO et al. (2011) analyzed the effect of fiber in growing rabbits in caecal *in vitro* fermentation and reported a decrease in DMD and OMD by 14-15% as NDF increases. In this sense, according to the data shown in the present study, the type and level of dietary fiber was the main factor that affected the levels of almost all *in vitro* parameters, except the *B* and *C* parameters, with a strong negative correlation between NDF and ADF with DE (P<0.001).

BOVERA et al. (2008) reported a similar value for ryegrass hay to OMD to that reported in the present study, mentioning that it is not only associated with the highest potential gas production (parameter *B*)*;* these authors considered the higher content of CP of ryegrass as a factor that contributed to its elevated OMD values. These authors also reported that ryegrass hay had significantly higher levels (P<0.01) of gas production and VFA production in comparison with legume hays, which is in agreement with the findings of the present research, in which ryegrass silage showed the highest values of GY24, RGP and SCFA, which can be associated with the easy fermentability of structural carbohydrates.

The importance of individual feeds cannot be estimated considering only the values of total potential gas production, as the retention time of substrates in the caecum is limited to about 10h (GIDENNE et al., 2002). Moreover, it is important to consider that caecal parameters, such as MCP and SCFA concentration, represent an equilibrium between two processes – production and absorption – so a direct relationship between microbial activity and caecum parameters is not always necessarily true. According to LARBI et al. (1998) there is a positive correlation between CP and GP (r=0.63), which coincides with the observations in the present study.

CONCLUSION

The digestibility parameters of the *in vitro* technique provided a useful approximation of the fermentative digestion process for analyzing the nutritive value of several feeds offered to rabbits, such as the silages tested in the present research. Common vetch and bean silages showed the highest values of digestible energy. Ryegrass and Common vetch silages showed better levels of GP parameters, which could be associated with their better chemical composition characteristics, mainly protein and fiber content; however, *in vivo* performance is needed to complement the findings of the current research, in order to achieve better practical recommendations about the inclusion of silage in rabbit rations.

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Protocols and animal handling throughout this experiment were approved by the Committee of Ethics of the Autonomous University of Mexico State.

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