Scientific Paper

Influence of the complementation with sugarcane and/or citrus fruit pulp on the in vitro fermentation of diets based on Megathyrsus maximus *and* Leucaena leucocephala

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ABSTRACT: A study was conducted in order to evaluate the effect of the substitution of sugarcane by citrus fruit pulp (CFP) on the *in vitro* gas production kinetics and the energy utilization of diets based on *M. maximus* and *L. leucocephala*. For such purpose, a completely randomized design was used with three levels: 0 (control), 50 % (CFP10) and 100 % (CFP20) and three repetitions. The diets were incubated *in vitro* (96 h) with rumen inoculant from cows. The GP was measured at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation. Parameters of ruminal fermentation were estimated such as: organic matter digestibility (OMD_{24}), *in vitro* dry matter degradability ($IVDMD_{96}$), metabolizable energy (ME) and short-chain fatty acids (SCFAs). The substitution of sugarcane by CFP linearly increased ($p < 0{,}001$) the GP accumulated at 24, 48 and 96 h of incubation, as well as the asymptotic gas production (*b*), fractional gas production rate (*c*), OMD_{24}, ME and SCFAs. It also increased linearly ($p < 0{,}01$) the $IVDMD_{96}$; while the lag phase (*L*) linearly decreased ($p < 0{,}001$). The diets CFP10 and CFP20 showed a similar performance in most parameters, except in OMD_{24} and production of SCFAs. The results suggest that the partial as well as total substitution of sugarcane by CFP has a positive impact on the gas production and on the parameters of *in vitro* ruminal fermentation.

Keywords: ruminal degradability, digestibility, energy value

INTRODUCTION

In silvopastoral systems (SPS), due to the increase in the quality of the pasture in association with the legume and to the foliage contribution made by the latter to the ration, an excess of crude protein (CP) has been shown in the diet of cows; while there is an energy deficit (López, 2003). For such reason, it is important to use complements such as sugarcane which improve the energy balance of the diet under grazing conditions.

Sugarcane (*Saccharum officinarum*), crop that is produced in more than 100 countries of the world, has a high dry matter yield per hectare which exceeds that of any other forage used for animal feeding (Abdel-Aziz *et al*., 2015); besides, it is harvested mainly in the dry season (Rodríguez *et al*., 2013), coinciding with the decrease of pasture yield (Fundora *et al*., 2013), for which it is an important feed source for ruminants in the tropic (Menezes *et al*. 2011; Álvarez, 2012). Nevertheless, it shows nutritional insufficiencies, such as the low protein and mineral content, slow fiber degradation and prolonged time of NDF retention of the cell wall in the rumen (Martín, 2004).

On the other hand, food byproducts, which have low economic value as foodstuffs for human consumption, have currently become one of the main sources of nutrients and energy to support milk production (Mirzaei-Aghsaghali and Maheri-Sis, 2008).

In the world a large amount of byproducts is obtained from the citrus fruit production industry (Bampidis and Robinson, 2006), just like in America (IBGE, 2011) and in Cuba (Fernández, 2008); which are mainly used in the diet of ruminants, fresh (Arthington and Pate, 2001), or preserved through dehydration (Volanis *et al*., 2006) and/or silage (Lashkari *et al*., 2014).

The use of agroindustrial byproducts for ruminant feeding contributes in a healthy way, from the economic as well as from the environmental point of view, to reduce the deposition of garbage in the ecosystems and to decrease the costs in waste management (Salvador *et al*., 2014).

Citrus fruit pulp (CFP) represents 60 % of the fresh weight of the fruit, with 19,7 % DM as average (Mirzaei-Aghsaghali and Maheri-Sis, 2008); 6,6 % CP and 62,9 % DM digestibility (Bampidis and Robinson, 2006). Likewise, CFP constitutes an adequate energy supplement for lactating dairy cows (Bampidis and Robinson, 2006); has high ruminal degradability potential and high apparent digestibility, and it is considered a pectin-rich feedstuff (Lashkari and Taghizadeh, 2013). Hence it is classified as a fibrous concentrate feed (Bampidis and Robinson, 2006), which can even partially replace cereal grains in the rations for animals, without adverse effects on milk production and quality (Salvador *et al*., 2014).

In addition, CFP modifies ruminal fermentation, improves fiber digestion (Gado *et al*., 2011), and increases microbial protein synthesis in dairy cows (Gado *et al*., 2009).

It has been proven that the *in vitro* gas production technique has potential to study the associative effect between different feedstuffs (Bakhashwain *et al*., 2009); for which the objective of this work was to evaluate the effect of the substitution of sugarcane by CFP on the *in vitro* gas production kinetics and the energy utilization of diets based on *Megathyrsus maximus* and *Leucaena leucocephala*.

MATERIALS AND METHODS

Area for sample collection. The samples were taken in January and February from the grazing area of dairy unit 66, belonging to the Livestock Production Enterprise of Matanzas –Cuba–, which is geographically located at 22º 58’ 39’’ North latitude and 81º 29’ 55,66’’ West longitude, at 100 masl (Academia de Ciencias de Cuba, 1989). The soil is Brown with carbonates (Hernández *et al*., 2015). The climate is tropical warm (Centro del Clima, 2013), with annual average temperature and rainfall of 24,4 ºC and 1 300 mm, respectively.

Sample collection method. For collecting the pasture samples (~ 4 kg), the selection made by the animals when grazing was simulated. In the trees the leaves and edible fresh stems (~ 3 kg) were manually collected, simulating the browsing made by the animals, in 10 of the trees established in the paddock up to a height of 2 m, according to the methodology proposed by Herrera (2006).

The citrus fruit pulp and chopped sugarcane samples were homogeneously taken from the feeding trough of the animals, after the supply, in the morning hours.

Sample preparation. After the collection, the samples were transported to the laboratory of chemical analysis of the Pastures and Forages Research Station Indio Hatuey; and they were dried in forced-air stove at a temperature of 65 ºC during 72 h, to estimate the DM content. Afterwards, they were ground in a stationary blade mill –with sieve of 1 mm diameter in its holes–; 50 (± 1) g of sample were deposited in nylon bags, and these bags were eventually sealed.

Analytical procedures. The residual DM content of the feed was determined by drying in stove at 60 ºC during 48 h, and the OM, after incineration in a muffle furnace at 550 ºC during 4 h. The crude protein (CP, N x 6,25) was determined following the Kjeldahl method AOAC, 1990). The content of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Lig) was analyzed according to the procedure recommended by Van Soest *et al*. (1991), without ash correction, using filter bags ANKOM F-57 in a fiber analyzer ANKOM^{200} (ANKOM Technology Corporation, Fairport, NY, USA). Table 1 shows the chemical composition of the feedstuffs used in the diets.

Table 1. Chemical composition (g/kg DM) of the feedstuffs used in the diets

Chemical composition♦	Feedstuff			
	M. maximus	*L. leucocephala*	Citrus fruit pulp	*S. officinarum*
Dry matter□	309,1	311,8	188,3	254,5
Organic matter	834,0	909,6	955,5	949,4
Crude protein	122,0	233,0	64,3	23,1
Neutral detergent fiber	659,1	529,6	306,9	607,2
Acid detergent fiber	343,2	270,0	209,6	334,3
Lignin in acid detergent	55,9	151,4	43,8	67,8

♦All the values, except DM, are expressed based on DM.
□The dry matter is expressed in g DM/kg of fresh matter.

Experimental diets. Three diets were formulated to substitute the complementation with sugarcane by citrus fruit pulp: 0, 50 and 100 %. They were adjusted to satisfy the nutritional requirements of third-lactation dairy cows (480 kg LW), with a production (corrected at 4 % fat) of 12 kg/cow/day and a feed intake of 14 kg DM/cow/day (NRC, 2001). The data corresponding to the ingredients and composition of the studied diets are shown in table 2.

In vitro gas production

Characteristics, management and feeding of the donor animals. For the *in vitro* incubations, two clinically healthy Limousine cows, cannulated in the dorsal sac of the rumen, were used as donors of the ruminal inoculant. The animals were confined, fed *ad libitum* with a diet constituted by 35,8 % of alfalfa (*Medicago sativa*) hay, 47,2 % of oat straw (*Avena sativa*), 14,0 % of ground corn (*Zea mays*) and 3,0 % of soybean (*Glycine max*) meal. Feed was supplied two times per day (08:00 and 16:00 h) and the animals had free access to drinking water.

Obtainment of the ruminal inoculant. The ruminal fluid was extracted from multiple sites of the rumen of the two cows in equal quantity, at 6:30 h, before supplying the morning feed. For such purpose, the rumen content was manually taken and it was squeezed in thermal containers previously heated with water at 40 ºC, for its transfer to the animal nutrition laboratory. The ruminal fluid was filtered in triple layer of gauze and fiberglass; afterwards, it was maintained for 30 min at 39 ºC and it was continuously saturated with CO_2.

***In vitro* incubations**. The *in vitro* gas production essay was performed according to the recommendation made by Theodorou *et al*. (1994). For that purpose 125-mL vial flasks were used, in which 700 (± 0,002) mg of DM of *M. maximus*, 100 (± 0,001) mg of DM of *L. leucocephala* and 200 (± 0,002) mg of *S. officinarum* and/or citrus fruit pulp (according to the proportions described in table 2), were introduced.

The incubation solution was elaborated from a mixture of macro- and microminerals, a buffer solution of bicarbonate, distilled water, reducer solution and resazurin as reduction indicator. Gassing was made with CO_2, according to the technique recommended by Menke and Steingass (1988), and 90 mL of dissolution were added to each flask, as well as 10 mL of ruminal fluid.

Three incubation series were carried out, in which three flasks per sample and three negative controls

Table 2. Chemical composition (g/kg DM) and ingredients (%) of the diets.

Indicator	Diet		
	Control	CFP10	CFP20
Chemical composition			
Dry matter[a]	939,6	940,7	941,8
Organic matter	874,1	874,8	875,4
Ash	125,9	125,2	124,6
Crude protein	104,6	108,7	112,9
Neutral detergent fiber	666,0	635,9	605,9
Acid detergent fiber	333,3	320,8	308,3
Cellulose	260,5	250,5	240,4
Hemicellulose	332,7	315,0	297,5
Lignin in acid detergent	72,7	70,3	68,0
Ingredients (%, as basis feed)			
Megathyrsus maximus	70	70	70
Leucaena leucocephala	10	10	10
Ingredients (%, as complement)			
Sugarcane	20	10	0
Citrus fruit pulp	0	10	20

[a]The dry matter is expressed in g DM/kg of fresh matter.

(blank) with only the inoculant, were included. For the incubation series oat straw was used as standard.

The content of the flasks was homogenized, and they were incubated to start the recording of accumulated gas production at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation. When incubation was finished, the content of each bottle was filtered using glass porcelain crucibles of porosity 1 (100 to 160 µm of pore size, Pyrex, Stone, UK) under pressure. The fermentation residues were dried at 60 ºC during 48 h to estimate the $IVDMD_{96}$.

Calculations. The pressure generated by the gas that is accumulated in the top part of the flasks was measured through a pressure transducer (trademark Delta OHM, model HD 8804) connected to a digital reader. The transformation of the pressure values to gas volume was done using an equation obtained through regression analysis:

$$Y = (2{,}7384X)-0{,}0243$$

Where:

Y: gas volume (mL), X: pressure (psi); $R^2 = 0{,}99$

The gas production data (mL/g DM were analyzed through the NLIN option of the statistical pack SAS (2002), using the model proposed by France *et al*. (2000):

$$A = b \times [1-e^{-c(t-L)}]$$

Where:

A: gas production volume (GP) in time t, b: asymptote of gas production (mL/g DM), c: gas production rate (/h), L (h): Lag time.

The parameters REL_1 and REL_2 were calculated from the proportion between GP_{48} and GP_{96} (REL_1) and the proportion between GP_{96} and b (REL_2) (Bueno *et al*., 2005).

The organic matter digestibility (OMD_{24}) was estimated according to Menke *et al.* (1979), as follows:

$$OMD_{24}\ (g/kg\ DM) = 148{,}8 + 8{,}89\ GP_{24} + 4{,}5\ CP + 0{,}651\ A$$

Where:

GP_{24}: net GP (mL/200 mg DM) at 24 h, CP: crude protein (% DM), A: ash (% DM).

The metabolizable energy (ME, MJ/kg DM) was estimated according to the procedure proposed by Menke and Steingass (1988), through the following equation:

$$ME\ (MJ/kg\ DM) = 2{,}20 + 0{,}136\ GP_{24}\ (mL/200mg\ DM) + 0{,}057\ CP$$

Where:

GP_{24}: gas volume at 24 h, CP (% DM): crude protein content of the feed.

The short chain fatty acids (SCFAs) were calculated according to the equation suggested by Getachew *et al*. (2002):

$$SCFA\ (mmol/200\ mg\ DM) = 0{,}0222\ GP_{24} - 0{,}00425$$

Where:

GP_{24}: net gas production at 24 h (mL/200 mg DM).

Experimental design and statistical analysis. The *in vitro* gas production, gas production parameters (b, c, L), *in vitro* degradability (OMD_{24} and $IVDMD_{96}$), ME and SCFAs were compared (previous testing of the normal distribution and variance homogeneity assumptions) through a variance analysis (SAS Institute, 2002), in a completely randomized design with three treatments (experimental diets) and three repetitions. The differences among the means were determined through Duncan's multiple range comparison test (Steel and Torrie, 1980). In addition, a linear and quadratic contrast analysis of the citrus fruit pulp level was made.

RESULTS AND DISCUSSION

SPSs, characterized by associations of cultivated grasses with tree legumes such as *L. leucocephala*, with moderate and high plant density per hectare, contribute a diet of high nutritional quality for dairy cows, mainly regarding digestibility and CP content; nevertheless, this diet is insufficient with regards to the energy requirements, for an adequate milk production. For such reason, the partial substitution of an abundant feedstuff in the tropic but with a relatively low nutritional value, such as sugarcane forage, by a potential environment pollutant agroindustrial by_product, but with a higher nutritional value, like CFP, would allow to increase fiber degradability and, thus DM degradability, as well as to improve the energy balance of the ration.

The CP content of the diets (105-113 g/kg DM) exceeded the minimum (80 g/kg DM) that is considered as the limit from which the growth of bacteria in the rumen can be affected (Ramírez, 1998).

Figure 1 shows the accumulated gas production profile (mL/g DM) for the three diets during 96 h of incubation. The GP increased rapidly in the first 12 h of fermentation, with higher values for the diets CFP10 and CFP20. Between the 12 and 24 h of incubation there was a slight decrease of GP in all the diets, but the same trend regarding the difference was maintained, for which the contrast in accumulated GP of the diets CFP10 and CFP20 increased with regards to the control. The divergence increased even more between 24 and 48

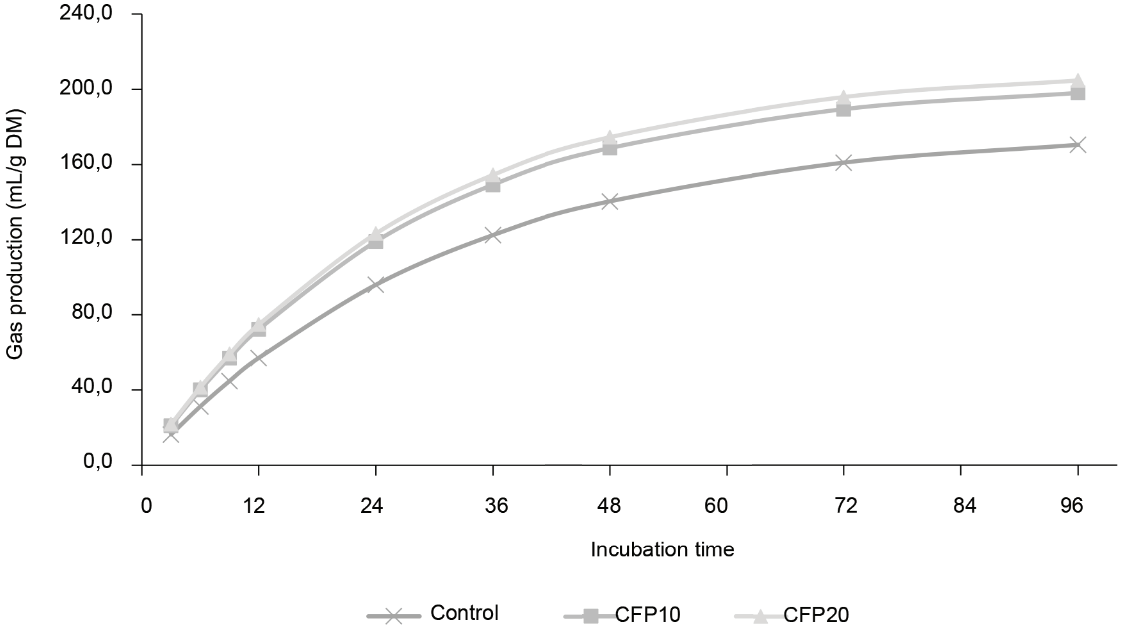

Figure 1. In vitro gas production profiles of the diets with different levels of substitution of sugarcane by citrus fruit pulp.

h of incubation; while since that moment the same trend was maintained until the 96 h of fermentation.

The increase of the GP of the experimental diets (CFP10 and CFP20) throughout the fermentation process, indicates that they had a higher concentration of energy and higher digestibility, because the quantity of gas produced through the *in vitro* incubation of a substratum is intrinsically related to its digestibility and, thus, to its energy value (Getachew *et al*., 2004; Van Dung *et al*., 2014).

On the other, by increasing the CFP in the diet the NDF content was reduced, which could have also led to the increase of gas production (Soltan *et al*., 2012).

The increase of gas production at 24 h in the diets CFP10 and CFP20 indicates that degradable nitrogen did not limit the microbial activity either, which allowed the carbohydrate fraction to be degraded according to its potential (Hamid *et al*., 2007). Thus, the synchronization between the energy contribution, from the fermentation of carbohydrates, and the contribution in nitrogen, through protein degradation, is essential to achieve adequate efficiency of ruminal fermentation (López *et al*., 2014).

The substitution of sugarcane by citrus fruit pulp linearly increased ($p < 0{,}001$) the GP accumulated at 24, 48, 72 and 96 h of incubation. It also increased linearly ($p < 0{,}001$) the asymptotic gas production (b) and the fractional gas production rate (c); while the starting time for the fermentation of the insoluble but potentially fermentable fraction (lag phase, L) linearly decreased ($p < 0{,}001$).

The linear increase of the asymptotic production and the fractional rate of gas production with the increase of CFP in the diet could have occurred due to a higher availability of nutrients for the rumen microbiota, especially nitrogen (Calabro *et al*., 2012), soluble carbohydrates and soluble fiber present in this feedstuff (Lashkari and Taghizadeh, 2015).

When there is a higher available nitrogen content in the presence of sufficient carbohydrates, the bacterial cell takes up more amino acids, which leads to accelerated microbial growth and, consequently, to an increase of fermentation with higher gas production (Berumen *et al*., 2015).

When calculating the relation between GP_{48} and GP_{96} (REL_1) and the correspondence of GP_{96} with b (REL_2) it was observed that the diets CFP10 and CFP20 increased these two indicators in 0,03 and 0,02 units, respectively, compared with the control diet.

The proportion of fermentation that is developed in the first 48 h of incubation (REL_1), as well as the relation between the total gas production (GP_{96}) and the gas production potential (*b*) of the feed (REL_2) constitute a new approach to evaluate feedstuffs and/or diets (Sallam *et al*., 2007). In that sense, the increase in the values of these two parameters in CFP10 and CFP20 showed the increase in the quality of both diets with regards to the control diet.

Although the gas is a waste product of nutrition, it provides useful bases from which the OMD, ME and SCFA production can be predicted (Babayemi, 2006).

The increase of the citrus fruit pulp linearly increased ($p < 0,001$) the OMD_{24} and $IVDMD_{96}$ ($p < 0,01$), as well as the ME and SCFAs ($p < 0,001$). The organic matter digestibility value is a good indicator of the quantity of feedstuff that is accessible for the rumen microorganisms (Abiola-Olagunju *et al*., 2015).

The control diet, with 20 % of sugarcane in its composition, showed the lowest values for the OMD_{24} as well as for the $IVDMD_{96}$. Sugarcane is recognized as an energy source, mainly of highly digestible soluble carbohydrates, with low protein content and high indigestible fiber content (Romão *et al*., 2014).

Thus, the highest proportion of soluble carbohydrates present in the control diet could have caused certain depression in the digestibility of the forage NDF by the ruminal cellulolytic population. The inhibitory effect of the available carbohydrates in the cellulolytic bacteria of the rumen, can occur even when the rumen pH remains over 6 (Miron *et al*., 2002). As has been shown in previous studies (Miron *et al*., 1997), this effect is probably related to the preference of bacterial species for using the available carbohydrates instead of investing energy for the production of binding organelles and cellulolytic enzymes.

On the contrary, when sugarcane was substituted by citrus fruit pulp at 50 and 100 %, the OMD_{24} as well as the $IVDMD_{96}$ increased, which indicates that there was an increase in the digestion of the diet NDF.

In citrus fruit pulp, approximately 80 % of the DM is constituted by carbohydrates (Lashkari and Taghizadeh, 2015), from which around 35 % are soluble carbohydrates that can ferment easily and rapidly. In addition, more than 36 % is soluble fiber,

Table 3. Characteristics of the *in vitro* fermentation of diets based on *M. maximus* and *L. leucocephala* with different levels of substitution of *S. officinarum* by citrus fruit pulp.

Indicator	Diet			SEM	Effect, P	
	Control	CFP10	CFP20		Linear	Quadratic
Gas production (mL/ g DM)						
GP_{24}	96,1[b]	119,1[a]	123,3[a]	4,32	0,001	0,003
GP_{48}	140,5[b]	168,8[a]	174,6[a]	5,38	0,001	0,005
GP_{72}	161,0[b]	189,5[a]	195,9[a]	5,49	0,001	0,009
GP_{96}	170,5[b]	198,1[a]	204,7[a]	5,38	0,001	0,013
Parameters of gas production						
b	178,7[b]	204,3[a]	211,0[a]	5,09	0,001	0,022
c	0,032[b]	0,037[a]	0,037[a]	0,0008	0,001	0,003
L	2,43[a]	1,61[b]	1,67[b]	0,137	0,001	0,003
REL_1	0,82	0,85	0,85	-	-	-
REL_2	0,95	0,97	0,97	-	-	-
In vitro DM degradability and energy utilization						
OMD_{24}	376,5[c]	415,8[b]	427,0[a]	7,77	0,001	0,007
$IVDMD_{96}$	506,9[b]	549,9[ab]	608,8[a]	17,14	0,01	0,721
ME	5,41[b]	6,06[a]	6,20[a]	0,124	0,001	0,003
SCFA	0,42[c]	0,52[b]	0,55[a]	0,019	0,001	0,003

a, b, c: averages with different superscripts in the same row differ ($p < 0,05$). SEM: standard error of the means (n = 3). GP: gas production (mL/g DM at 24, 48, 72 and 96 h); *b*: asymptotic gas production (mL/g DM); *c*: gas production fractional rate (/h); *L*: lag time (h). REL_1: GP_{48}/GP_{96}, where GP_{48} and GP_{96}: accumulated gas production at 48 and 96 h of incubation, respectively; REL_2: GP_{96}/b, where *b*: potential gas production. OMD: organic matter degradability; IVDMD: *in vitro* dry matter degradability (g/kg DM); ME: metabolizable energy (MJ/kg DM); SCFA: concentration of short chain fatty acids (mmol).

such as pectin, which can be equally used by rumen microorganisms as a fermentable energy source (Lashkari and Taghizadeh, 2013).

In that sense, Arthington *et al*. (2002) stated that citrus fruit pulp has in its composition a high concentration of pectins (approximately 250 g/kg DM), which are highly digestible: > 980 g/kg DM (Hall *et al*., 1998; Arthington *et al*., 2002); this conditions their fast degradation in the rumen and the release of energy for a rapid microbial growth (Gholizadeh and Naserian, 2010). Besides, as pectin is considered a soluble fiber, its fermentation produces more acetate (Liu *et al*., 2014) and less lactate than starch (Hall *et al*., 1998) and soluble carbohydrates (Dušková and Marounek, 2001), creating better conditions in the rumen for fiber fermentation (Gholizadeh and Naserian, 2010).

Zhao *et al*. (2013), with use of the rumen simulation technique, reported that when the soluble neutral detergent fiber was increased there was a lower production of ammoniac N and a higher daily flow of N in the form of solid pellets of microbial associations, as well as of total microorganisms; in addition, higher efficiency was observed in the synthesis of microbial protein.

On the other hand, in *in situ* studies it has been found that the true protein of silages (Lashkari *et al*., 2014) and of pastures (Ramírez *et al*., 2004) increases the effective DM degradability. Thus, the higher CP content of the citrus pulp with regards to sugarcane, as well as of the diets CFP10 and CFP20 compared with the control, is an effect that could also have had incidence on the increase of the OMD_{24} and $IVDMD_{96}$ in the experimental diets.

The citrus fruit pulp has a high ruminal degradation potential and high apparent digestibility (Lashkari and Taghizadeh, 2013); for such reason, when this feedstuff increases its proportion in forage-based diets it contributes to increase the OMD_{24} as well as the $IVDMD_{96}$.

The values of the OMD_{24} for CFP10 and CFP20 (41,6 and 42,7 %, respectively) were similar to the one reported by Tona (2014), of 42,4 %, in a diet constituted by 60 % of *M. maximus*, 30 % of *Gliricidia sepium* and 10 % of cassava (*Manihot esculenta*) shell. Nevertheless, in this study, the ME (6,06-6,20 MJ/kg DM) as well as SCFAs (0,52-0,55 mmol) were in a range higher than the values reported by this author (5,52 MJ/kg DM and 0,35 mmol).

Gas production, which is generally a good indicator of digestibility (Sommart *et al*., 2000), showed the same performance as the IVDMD. This coincides with the results obtained by Hernández *et al*. (2012), who evaluated whole rations constituted by sorghum grain, soybean meal, urea, molasses, *Cenchrus ciliaris*, mineral pre-mixture and different levels of CFP inclusion (0, 10, 20 and 30 % of the total DM); and found a linear increase in the GP, IVDMD, ME and SCFAs with the increase of CFP in the diet.

The above-explained facts indicate that with the increase of the CFP the nutrient profile of the diet improves (Hernández *et al*., 2012) and with it, the nutrient availability for rumen microorganisms (Paya *et al*., 2007), mainly fermentable carbohydrates and available nitrogen, which in turn stimulates the metabolism of rumen microorganisms (Harikrishna *et al*., 2012).

CONCLUSIONS

The partial as well as total substitution of sugarcane by citrus fruit pulp, as complement of a diet based on *M. maximus* and *L. leucocephala*, contributed to increase the gas production and to improve the parameters of *in vitro* ruminal fermentation and energy utilization.

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