Inclusion of the microbial additive Vitafert in the *in vitro* ruminal fermentation of a goat diet

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For determining the effect of Vitafert on the kinetics and fermentation indicators of a goat diet (80:20 forage: concentrate ratio), the *in vitro* gas production technique was applied. One gram of the diet was incubated alone (0 level) or with three Vitafert levels (110 µL, 150 µL and 210 µL), equivalent to 4.5, 6.0 and 8.5 mL Vitafert kg^{-1}. For each treatment specific blanks were established. Gas production was measured until 120 h and its kinetics was estimated by the Gompertz model. At 120 h, DM and NFD degradability were determined. Production and efficiency of the microbial biomass synthesis were calculated. A completely randomized design was used. MANOVA did not show treatment x hours of incubation interaction regarding gas production. On applying a linear model, there were no differences in accumulated gas production, although the specific blanks showed increase in gas production, as the Vitafert level increased. Differences between timings were only at two hours of incubation as regard the remaining hours ($P < 0.001$), Vitafert inclusion did not influence on the kinetic parameters. The time at which the maximum speed was attained ranged between 20 and 21 h. The 4.5 level increased DM and NFD degradability ($P < 0.05$), and the 8.5 level increased the production and efficiency of microbial biomass synthesis ($P < 0.01$). It is concluded that Vitafert did not affect gas production or the kinetic parameters of the diet fermentation. The 4.5 level increased DM and NFD degradability and the 8.5 level, the production and efficiency of the microbial biomass synthesis.

Key words: *Vitafert, goats, gas production, kinetic parameters, microbial biomass*

Pastures and forages are the natural feeds for ruminants. They represent the most abundant and economic feeding source in the tropics not competing with non-ruminant animal feeding (Cáceres *et al*. 2006). However, tropical pastures have a low nutritive value, due to their low nitrogen (N) content and high fiber levels, elements that limit the voluntary feed intake and nutrient contribution to the animals (Ku Vera 2010).

Concentrate supplementation allow to improve the quality of the diet and animals' productivity, but these products are expensive and generally imported. Thus, the development of technologies is required to improve the efficiency of nutrient utilization in ruminant diets (Elías 1983 and Galina *et al*. 2008). In this sense, numerous studies have been carried out to manipulate the ruminal ecosystem for obtaining improvements in the efficiency of feed use through the utilization of microbial additives (Marrero 2005, Elías and Herrera 2009, Castillo 2009, Galina *et al*. 2010 a, b and Sosa *et al*. 2010).

The addition of products containing microorganisms to the ruminant diet can positively influence on the voluntary feed consumption and on ruminal indicators, such as the number of total and celullolytic bacteria, the concentrations of short chain fatty acids and pH (Elías and Herrera 2009). In the last years the interest in the additive named Vitafert has increased as a product with biological activity (Elías and Herrera 2009). Recent studies have demonstrated its value as additive in goat feeding (Gutiérrez *et al*. 2012 a b).

The *in vitro* gas production technique allows studying the ruminal fermentation dynamics. Also, it has been used to determine the effect of microbial additives on ruminal populations (Marrero 2005, Rodríguez *et al*. 2007 and Sosa *et al*. 2010). This paper was aimed to determine the effect of the microbial additive Vitafert on the *in vitro* ruminal fermentation of a goat diet.

Materials and Methods

Preparation of the plant material. As substrate *P. purpureum* forage and a commercial concentrate as supplement was used in an 80:20 forage: concentrate ratio. The forage was collected from experimental areas of the Institute of Animal Science of the Republic of Cuba. Plants were established in a typical red ferrallitic soil, without irrigation or fertilization. Approximately 2 kg of plant stems and leaves were randomly collected. The cut was made at 20 cm of the soil. The material collected was dried in a forced air oven, with controlled temperature (60º C) for 72 h. Later, it was ground in hammer mill at 1 mm particle size.

The Vitafert additive was obtained through the fermentation of a sugar cane final molasses mixture, soybean, maize, urea, magnesium sulphate, mineral formulas and yogurt as microbial inoculum (Elías and Herrera 2011). For its production a stainless steel fermenter of 250 L capacity was used ,fitted with a central spatula for homogenizing the mixture and an automatic regulator for controlling agitation and rest times (120 and 20 min., respectively).

Experimental procedure. The *in vitro* gas production technique in glass bottles, described by Theodorou *et al*. (1994) was applied. One gram of the samples was incubated in 100 mL bottles in a culture medium (Menke and Steingass 1988) and with ruminal microorganism inoculum, in a 0.20 proportion of the total incubation volume (80 mL).

As inoculum was used the ruminal contents of three adult stabulated goats (*Capra hircus*), of the Nubia breed, fed with a similar diet to that evaluated *in vitro* and with free access to water and mineral salts. The ruminal contents of each goat was collected through the esophagus, before supplying the morning feed and preserved in closed thermos until arriving to the laboratory. There, it was filtered through various gauze layers and the three inocula were mixed in equal proportions. During the process inocula temperature was maintained from 39 to 40º C and anaerobiosis conditions through continuous CO_2 flow. Bottles were sealed and incubated in a water bath, with controlled temperature (39º C). That moment was taken as zero hour of incubation.

The experimental diet was incubated alone (0 level) or with three levels of the microbial additive Vitafert: 110 µL (4.5 level), 150 µL (6.0 and 210 µL (8.5), equivalent under *in vivo* conditions to 4.5, 6.0 and 8.5 mL Vitafert kg^{-1}. Also specific blanks of each treatment were incubated to know the gas contribution of the microbial inoculum and of the amounts of Vitafert added. Treatments and blanks were incubated in quadruplicate and triplicate, respectively. Gas production was measured at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, 48, 72, 96 and 120 h through a HD8804 manometer, coupled to a TP804 (DELTA OHM, Italy) pressure gauge. After each measurement, gas was released until equalizing the external and internal pressures of the bottles. The gas volume was estimated from the pressure data through a pre-established linear regression equation: gas, mL = (pressure [10^3 Pa]+4.95)/2.5858), n=132; r=0.991). The gas volume was expressed by gram of OM incubated.

At the end of the incubation, the bottles were opened and the contents filtered through nylon bags. The bags with the fermentation residues were dried in a forced air oven with controlled temperature (60º C) for 72 h.

Kinetic model for in vitro fermentation. For estimating the kinetics of gas production, the single-phase model of Gompertz was used:

$$Y = A*Exp(-B*Exp(-C*t))$$

Where:

Y is the gas production in the time (mL g^{-1} of incubated OM)

A is the potential of gas production (asymptote when $t = \infty$ [mL g^{-1} of incubated OM]).

B is the relative rate of gas production

C is a constant factor of the microbial efficiency (h^{-1})

T is the incubation time (h)

Additionaly, it was estimated the incubation time at which the maximum velocity (Tv_{max}) of gas production was attained through the second derivative of the Gompertz model, evaluated in zero (inflection point of this type of sigmoid model). Moreover the maximum gas production velocity (V_{max}; mL g^{-1} OMinc h^{-1}), on substituting Tv_{max} in the first derivative of the mathematical model.

Chemical analysis. To the forage and concentrates included in the experimental diet, dry matter (DM), organic matter (OM) and crude protein (CP) were determined (AOAC 1995). The neutral detergent fiber (NDF) was obtained as indicated by Goering and van Soest (1970). Also, the DM and NDF contents in the solid residues of the fermentation were established.

True degradability of DM (TDMD) and NDF (NDFD). By gravimetry were determined as the difference between the incubated DM and NDF and the NDF contents in the solid residue of fermentation, respectively on dividing incubated DM or NDF in each bottle as appropriate (Blümmel *et al*. 1997).

Synthesis of microbial biomass (SMB, g). It was estimated by gravimetry as the difference between DM and NDF contents in the solid residue of fermentation (Blümmel *et al*. 1997).

Efficiency of microbial synthesis (EMBS. g g^{-1} fermented DM). It was estimated as the ratio between SMB and the fermented DM. The fermented DM was assumed at the difference between the DM incubated and the NDF contents of the solid residue of fermentation.

Experimental and statistical design. A completely randomized experimental design was applied in which the inclusion levels of Vitafert (0, 4.5, 6.0 and 8.5) were considered as treatment and each bottle as an experimental unit. Results from gas production on being repeated measurements in the same experimental unit, were analyzed by MANOVA, and the gravimetric variables by ANOVA. In both cases the statistical package InfoStat (Di Rienzo *et al*. 2010) was employed. For data analysis of gas production of specific blanks for each Vitafert level, a mixed linear model (repeated option) was used through the PROC MIXED of SAS (SAS 2007). The estimation model applied was the REML (restricted maximum likelihood). For this analysis, treatments, timings and treatments*timings were taken as fixed effects and as random the repetition. The expression of the mixed linear model was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + r_k + e_{ijkl}$$

Where:

μ: common mean to all treatments

α_i: fixed effect of the i-th treatment (I = 1,….,4)

β_j: fixed effect of the j-th hours (j =0 1,….,4)

$\alpha\beta_{ij}$: fixed effect of i-th treatment in interaction with the j-th timing (ij = I,….,16)

r_k: random effect of the k-th repetition (k = 1,….4)

e_{ijkl}: common error to all observations

The linear regression of the gas production data of

the blanks was carried out regarding the Vitafert level evaluated.

When there were differences ($P < 0.05$), means of treatments were compared through Duncan's (1955) multiple range test.

Results

In table 1 is shown the chemical composition of the experimental diet and of the additive Vitafert. The concentrate used contributed to 44 % of the CP of the diet, while the forage showed high NDF contents. It is important to highlight that the Vitafert additive has low pH and contains considerable amounts of yeasts and lactobacilli (10^7-10^8 cfu and 10^9-10^{10} cfu, respectively) and concentrations of lactic (450-600 mmol.L^{-1}) and acetic (225-230 mmol L^{-1}) acids (Elías *et al*. 2010).

The profiles of *in vitro* accumulated gas production (mL g^{-1} OMinc) of the evaluated treatments as shown in figure 1. The treatment x timing interaction in the multivariate analysis was not significant, though a general linear model was used with the effects of treatments and timings (table 2). There was no effect of the inclusion level of the Vitafert additive on the accumulated gas production, although there was influence of the sampling hours ($P < 0.001$).

In table 3 is set out the gas contribution that the specific blanks realize for each treatment.

The treatment x timing interaction was not significant, but there were individual effects of the treatment and timing factors. In the case of the treatments, the gas production of the specific blanks was increased as the level of Vitafert augmented ($P < 0.001$), with linear performance according to the regression equation:

$y=0.00251x+14.613$ ($r=0.9141$, $n=4$).

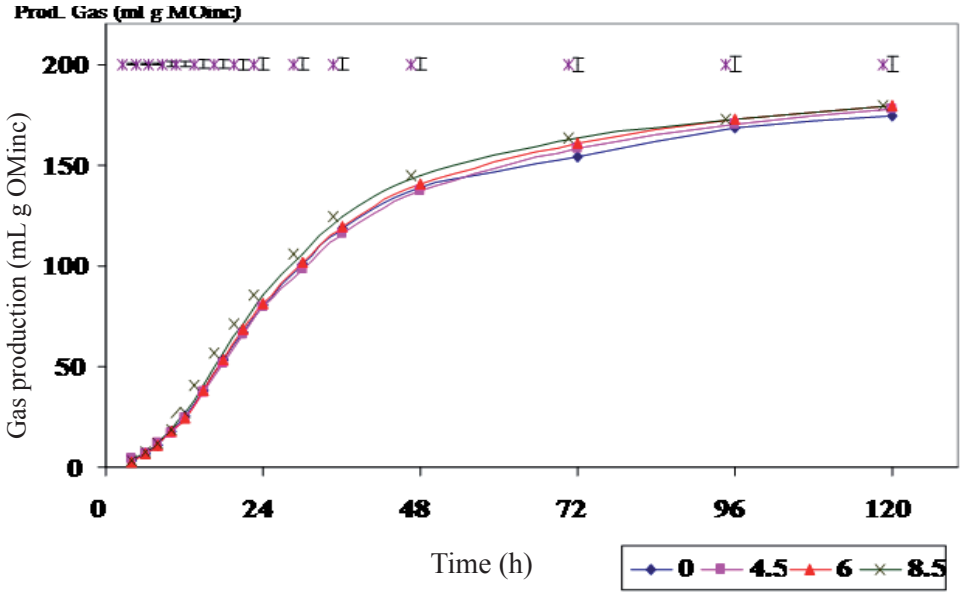
The gas production of the specific blanks only showed differences between the 2 h of incubation and the rest of the hours evaluated ($P < 0.001$).

Table 4 presents the values of the kinetic parameters of the accumulated gas production, estimated from the Gompertz model. The inclusion level of the Vitafert

Table 1. Chemical composition of the substrates (g kg^{-1}DM)

Substrate	DM residual	OM	CP	NDF
P. purpureum	919.0	852.5	64.0	829.7
Concentrate	875.5	761.6	198.4	245.6
Activator Vitafert*	97.0	45.1	48.0	-

*Taken from Gutiérrez (2012)



Punctual values in the upper part of the graph indicate SE of means at each sampling timing

Figure 1. Profile of the *in vitro* accumulated gas production of the treatments evaluated

Table 2. Effects of the treatment and sampling hours on the *in vitro* accumulated gas production of the evaluated treatments

Vitafert level	Level 0	Level 1	Level 2	Level 3	SE ± and Sign.
	70.75	71.25	72.20	73.74	1.172
Sampling hours	4	12	24	120	1.172
	3.13[a]	25.32[b]	81.70[c]	177.79[d]	$P < 0.001$

additive was found to have no effect on the estimated kinetic parameters, which fitted the similarity in the profiles of accumulated gas production (table 2). It is important to indicate that the model showed high coefficient of determination ($R^2 > 0.9865$), which allows to state that it was capable of explaining the high percentage of the variability of the experimental data obtained.

In the same way, there were no differences in the maximum gas production speed of the treatments, although there was a decreasing tendency with the 4.5 level, and to increase with higher levels, regarding the treatment without Vitafert (table 4). The time at which this value of maximum speed (inflection point of the sigmoid curve of Gompert type) was attained varied a little between treatments, ranging between 20 and 21 h of incubation. Additionally, important changes were not observed either in the values of gas production speed of the treatments, regardless the Vitafert levels used during the incubation period (figure 2).

Regarding the effect of the inclusion of the Vitafert additive on the DM and NDF degradability (figure 3), the 4.5 level increased the TDMD and NDFD, with respect to the treatment without Vitafert. To this the 8.5 level of this additive ($P < 0.05$) was included. The treatment with the 6.0 level did not differ from the rest.

In figure 4 are shown the effects of the inclusion level of Vitafert on the MBS and on the EMBS. Both indicators

Table 3. Effects of the treatment and sampling hours on the accumulated *in vitro* gas production of the specific blanks of each Vitafert level evaluated in the experiment

Variable	Level 0	Level 1	Level 2	Level 3	SE± and Sign
Gas production (mL g^{-1} OMinc)	15.11[a]	15.92[b]	19.07[c]	20.16[d]	0.16 P < 0.001
Hour / Variable	2	4	6	8	0.15
Gas production (mL g^{-1} OMinc)	16.77[a]	17.68[b]	17.69[b]	17.81[b]	P < 0.00

Table 4. Kinetic parameters of the accumulated gas production of the treatments evaluated according to the mathematical model of Gompertz

Level	A Parameter (± SE)[1]	B Parameter (±SE)	C Parameter (±SE)	SE^2	R^2	V_{max}	T_{Vmax}
0	166.90 (±1.317)	4.43 (±0.154)	0.072 (±0.0018)	4.419	0.995	4.42	20.67
4.5	169.59 (±1.654)	4.27 (±0.173)	0.069 (±0.0021)	5.397	0.993	4.30	21.04
6.0	171.46 (±1.776)	4.38 (±0.195)	0.071 (±0.0023)	5.878	0.992	4.48	20.80
8.5	171.63 (±2.208)	4.45 (±0.258)	0.074 (±0.0031)	7.466	0.987	4.67	20.17

[1]Standard error of the parameter, all parameters were significant ($P < 0.0001$)
[2]Standard error of the curve

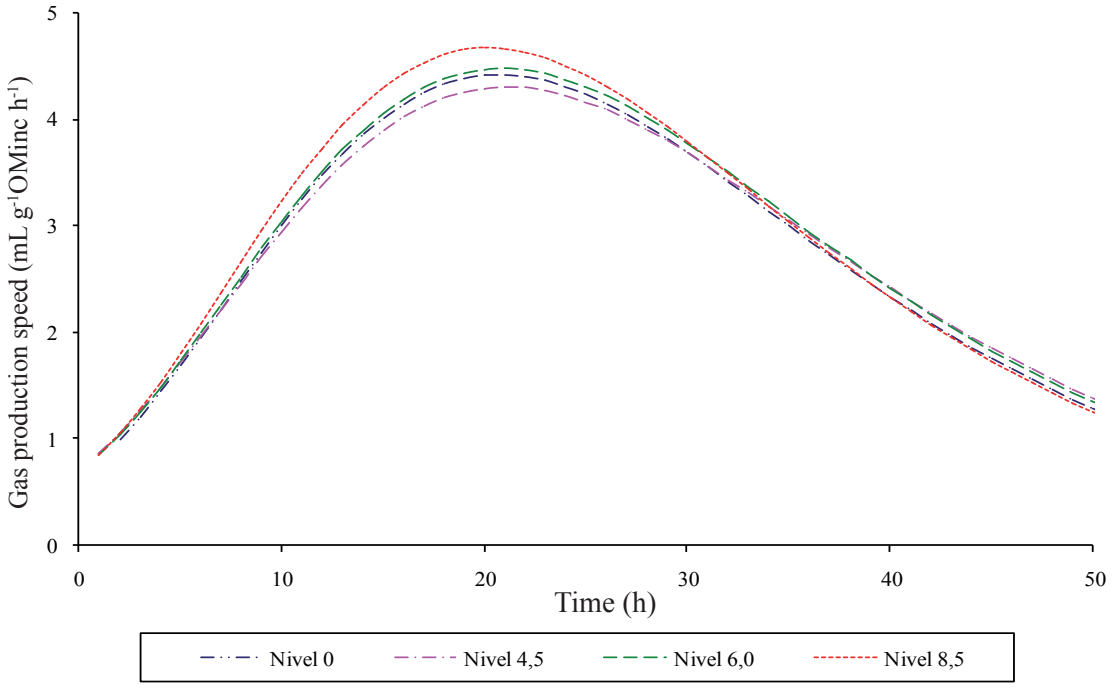


Figure 2. Performance of the gas production speed (mL g^{-1} OMinc h^{-1}) in time

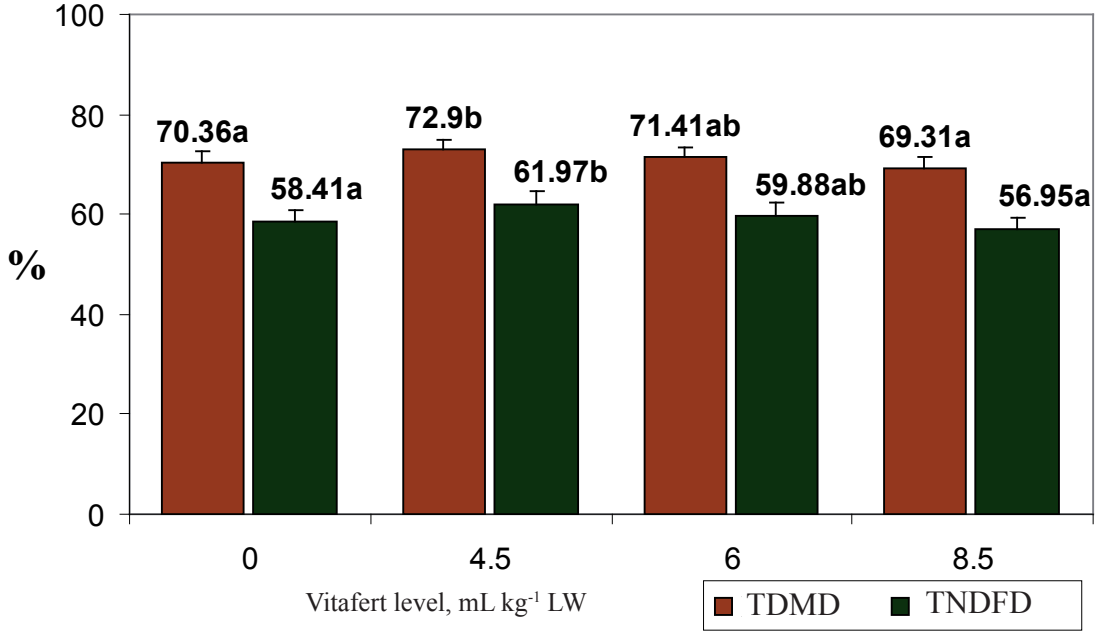


Figure 3. Effect of the Vitafert level on the DM and NDF degradability of the goat diet

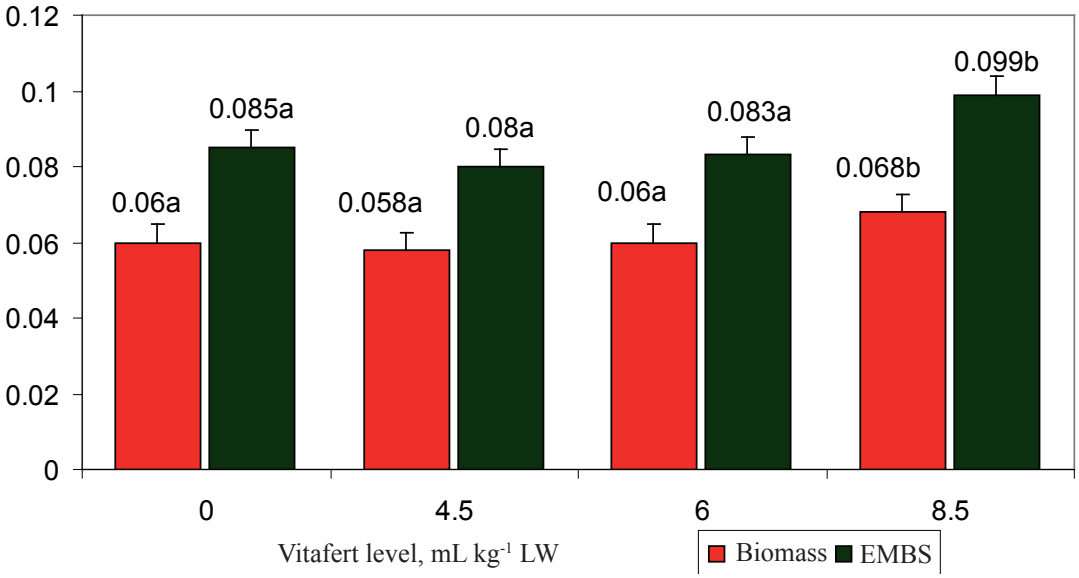


Figure 4. Effect of the Vitafert level on the MBS (g) and the EMBS (g biomass g^{-1} fermented DM)

had similar performance regarding the microbial additive level included. Both were increased with 8.5, regarding the rest of the treatments ($P < 0.01$), while 4.5 and 6.0 of the additive did not exhibit differences compared to treatment without Vitafert.

Discussion

The utilization of Vitafert as microbial additive in fattening cattle and dairy cows produce positive results on voluntary intake, fibrous feed degradability, body weight gain as well as yield and milk composition (Elías *et al*. 2010). Although there are few results on its utilization in goat feeding, some investigations show new application perspectives in this species (Gutiérrez *et al*. 2012ab).

Results achieved in this study indicated that the inclusion of different levels of Vitafert had no effect on the accumulated gas production. On the other hand, the increase found in gas production of the specific blanks, as the additive level was increased, was low regarding total gas production of the treatments. It was only limited at the beginning of fermentation (first 2 h of incubation), since from hour 4 no differences were observed between timings in gas production.

These effects of Vitafert in the first hours of incubation coincide with Newbold *et al*. (1990) and Rodríguez *et al*. (2007), who used yeast cultures as additives. These authors also determined that the metabolic activity of those microorganisms in the rumen was only maintained for few hours. This could be related to the fact that these microorganisms cannot maintain a viable population for long time under the incubation conditions (Marrero

2005). In this study these effects did not affect the gas production of the treatments or the kinetic indicators of its production (parameters of the model, speed, and inflection point of the curve). However, the tendency found in the increase of the maximum gas production speed on augmenting the Vitafert levels from 6.0 could be related to the increase in the yeast contribution made by the additive, as its inclusion level is increased, since it is known that *Saccharomyces cerevisiae* inclusion increased the fractional rate of *in vitro* gas production, on fermenting tree substrates (Rodríguez *et al*. 2007). Even though these two parameters are not mathematically equal, from the biological point of view both are used as referents of the dynamics with which the gas is produced inside the bottles.

The use of microbial activators can modify the ruminal fermentation so to stimulate cellulolysis (Elías 1983). In this study an increase in DM and NDF degradability was observed when the 4.5 level was included. This coincides with what was reported on the positive effects on the fiber degradability on adding *S. cerevisiae* (Newbold *et al*. 1995, Marrero 2005 and Di Francia *et al*. 2008) and *Lactobacillus sp*. (Galina *et al*. 2007). Gutiérrez *et al*. (2012b) found an increase in the voluntary intake of DM of a goat ration including 6.0 mL kg LW^{-1} of Vitafert. This was associated to the beneficial effect of Vitafert on fiber degradation. In this study, that inclusion level of the microbial additive did not show differences compared to the 4.5 level, but neither regarding the treatment without Vitafert.

The action of the microbial additive could be related to the contribution of growth factors of the celullolytic microorganisms, since Vitafert is also rich in nitrogenous compounds and organic acids (Elías and Herrera 2009). Some authors state that the highest availability of branched chains of fatty acids stimulates the celullolytic bacteria growth (Bryant 1973) and the *in vitro* fiber digestion (Stern *et al*. 1985).

However, the increase in the substrate degradability could not be associated to the increase in gas production which is the product of the energy metabolism of the ruminal microorganisms, having a directly proportional relationship with the production of short chain fatty acids (Makkar 2000). This apparent contradiction is due to the fact that part of the substrate that is degraded, is incorporated to anabolic routes for the microbial biomass synthesis. Additionally, the efficiency with which this synthesis occurs varies in function of the incubation conditions and the nature of the fermented substrate (Makkar 2005). Thus, the determination of SMB and EMBS, combined with the calculation of gas production, allow the best selection of the products or treatments evaluated, since feed must be selected on the basis of greater degradability, but low gas production per unit of substrate degraded. That is, greater MBS per unit of degraded substrate (Makkar 2000).

In this study SMB was not established at the initial fermentation time but at 120 h, when *in vitro* fermentation was practically exhausted (gas production speed approximately zero), which could induce a bias in the results obtained. Optimum would have been to determine MBS and EMBS at the time of greatest fermentation of the substrate, between 20 and 21 h of incubation, when in the four treatments evaluated the maximum gas production velocity was attained. However, at 120 h, results indicated that the treatment with 8.5 Vitafert increased SMB and EMBS, although it did not increase the substrate degradability. The effect of the additives on the microbial synthesis is controversial, since results are variable and depend on the quality, type of diets and inclusion dosage of the additive.

Results obtained for the 8.5 level of Vitafert contradict the findings of Gutiérrez *et al*. (2012b), on evaluating *in vivo* the use of this additive in goat diets. Nonetheless, the indicators measured in previous studies did not include the determination of SMB and EMBS, but both were estimated from the proportion established by Smith (1975). The utilization of this relationship is based on assuming that the microbial efficiency is constant in any condition. However, it is known that there is great variability in the SMB per unit of ATP generated during fermentation (YATP) (Blümmel *et al*. 1997 and Makkar 2005).

Moreover, the inclusion of microbial additives in the ruminal medium is known that provokes modifications in the efficiency of synthesis, due to changes in the relative proportions of specific groups of microorganisms in the total populations (Bach *et al*. 2003), mainly by increments in the ruminal concentrations of celullolytic and anaerobic bacteria (Newbold *et al*. 1995). This beneficial effect is attributed to the elimination of oxygen from the environment that stabilizes pH and stimulates the SMB, specifically the strict anaerobic bacteria growth as the celullolytic (Broderick *et al*. 1991 and Wallace 1994). There are also *in vitro* evidences of the favorable effect of the branched chain of volatile fatty acids on SMB and EMBS (Chalupa and Block 1983 and Russell and Sniffen 1984) that are rich in the Vitafert additive (Elías and Herrera 2009).

Results obtained *in vitro* indicate that the addition of the product Vitafert to a goat diet based on *P. purpureum* forage and a concentrate supplement did not affect gas production nor had influence on the kinetic parameters of fermentation. However, the inclusion of the additive at 4.5 % improved TDMD and NDFD. Its addition at 8.5 increased MBS and EMBS during the fermentation of the diet.

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