Influence of a microbial additive on the voluntary intake of dry matter, neutral detergent fiber and indicators of the ruminal fermentation of goats fed *Brachiaria brizantha* hay

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In order to assess the voluntary intake of dry matter (DM), neutral detergent fiber (NDF) and indicators of the ruminal fermentation in goats fed *Brachiaria brizantha* hay of low nutritive quality and with the inclusion of a microbial additive in the diet called VITAFERT (a biologically active product, reach in yeasts, lactobacilla and their metabolites), four goats of the Saanen breed, with three years of age and 43 kg (17.0±0.82 kg LWPV 0.75) of average live weight were used. A 4 x 4 Latin square design was applied for 60 d of observation. The goats were cannulated in the rumen and distributed into four treatments: T1) basic diet, with *B. brizantha* hay + goat commercial supplement, at a rate of 6 g kg LW^{-1}; T2, T3, T4) *B. brizantha* hay + goat commercial supplement and three levels of VITAFERT (4.5; 6.0 and 8.5 mL kg LW^{-1}). These levels were added to the supplement according to the treatments at the offering time (9:00 h), different of the hay that was offered twice (8:30 and 16:30 h), at a rate of 1.2 kg $animal^{-1}$ in dry basis (DB). The addition of the microbial additive in the ration, in the levels of 4.5 and 6.0 mL kg LW^{-1}, increased ($P < 0.01$) the voluntary intake of DM and NDF. However, in the indicators of the ruminal fermentation studied: short chain fatty acids (CSFAt) and bacterial biomass (BB), the level 6.0 mL kg LW^{-1} of VITAFERT differed from the rest ($P < 0.001$). It is concluded that the stimulating effect of VITAFERT in the increase of DM and NDF intake depends on the dosage. The level 6.0 mL kg LW^{-1}, together with a energy-protein supplement at a rate of 6 g kg LW^{-1}, optimizes the system in respect to the increase of the fermentative capacity of the rumen, corresponding with the increase of the of the SCFAt and BB concentrations in goats fed *Brachiaria brizantha* hay of low nutritive value.

Key words: *Saanen, VITAFERT, yeasts and lactobacilli*

Goat feeding is mainly based on free grazing of herbal species. It generally is not enough in respect to biomass and nutrients, especially during the dry season, when the pastures quality and availability decrease. This situation delays the body development of the animals, provokes weight loss, low fertility, mortality and decrease of milk and meat production (Sánchez 2001).

The good-quality forages are successfully digested, without the need of supplementation. However, generally, pastures and tropical forages have frequently nutritional deficiencies (Vera and Seré 1985), particularly low DM digestibility and reduced protein content, limiting the microbial activity in the rumen and production. In spite of this, grasses, characterized by their wide range of genera and species and plasticity, provide the basic feeding for cattle in the tropics (Peters *et al*. 2010 and Tittonell *et al*. 2010).

Pastures and tropical forages based on grasses may be used more efficiently if the rumen bacterial populations cover each energy requirement, protein and mineral constituents and other nutrients (Elías 1983), and if a level of non-structural carbohydrates is guaranteed to optimize the synthesis of microbial protein in the ration (Calsamiglia and Endres 1994).

In Cuba, the Institute of Animal Science has developed a biologically active product named VITAFERT, rich in lactobacilli, yeasts, short carbonated chain organic acids and low pH (Elías and Herrera 2008). This product, used as additive in the rations, stabilizes the microbial flora of the ruminal ecosystem, and increases, at the same time, the DM digestibility and that of the cell wall (Elías *et al*. 2010).

The objective of this study was to assess the effect of three levels of inclusion of the microbial additive VITAFERT on the voluntary intake of DM, NDF and on the ruminal fermentation of goats fed *Brachiaria brizantha* hay of low nutritive quality.

Materials and Methods

Location. The experiment was conducted during February and March 2010, in the goat metabolism area from the Department of Ruminants Management and Feeding, belonging to the Institute of Animal Science, in San Jose de las Lajas municipality, Mayabeque province, Cuba. This facility is between 22º 58 NL and 82º 02 WL, at 80 a.s.l.

Animals and design. Four goats (*Capra hircus*) of the Saanen breed; clinically healthy, cannulated in the dorsal part of the rumen were used. Thay were three years old and had an average live weight of 43 kg (17.0 ± 0.82 kg LW0.75). Thay were allocated in individual metabolism cages (60 x 120 cm). The experiment lasted 60 d, distributed into four experimental periods, according to a 4 x 4 Latin square design, with nine days of adaptation to the diet and five of recollecting data.

Feeding and distribution. During the experimental development, the animals had free access to water and mineral salts. The basal diet was of *B. brizantha* hay, of low nutritive value (Pérez-Infante 2010), offered in a dry basis ration of at about 80:20 in respect to the supplement. This last was used for supplying the VITAFERT. The treatments were: T1) grass hay + goat

commercial supplement; T2, T3 and T4) grass hay + goat commercial supplement and three levels of VITAFERT (4.5, 6.0, 8.5 mL kg LW^{-1}). The levels were added to the supplement at the time of the offer (9:30 h). Differently, the hay was offered at 8:30 and 16:30 h, at a rate of 1.2 kg $animal^{-1}$ (DB). The intake was determined from the weight of the feed offered and that left in the feeder. The weighing was carried out during four continuous days and in each experimental period.

Procedure for elaborating the feeds. The VITAFERT was obtained from a mixture of the fermentation of the sugar cane final molasses, soybean, maize, urea, magnesium phosphate and mineral formulas. Yogurt was used as microbial inoculator (Elías and Herrera 2008). The *Brachiaria brizantha* hay is a fibrous material, of 85 d of age, elaborated according to the methodology proposed by Michelena and Delgado (2008). Its microbiological and chemical composition includes concentrations of yeasts and lactobacilli, oscilating between $107^{-1}08$ cfu and $109^{-1}010$ cfu, respectively. In concentrations of 450-600 mmol L^{-1} for the lactic acid and 225-230 mmol L^{-1} for the acetic acid (Elías *et al*. 2010).

Indicators assessed. The analyses of the chemical composition of feeds were conducted in the Laboratory of Analytical Chemistry of the Institute of Animal Science. The DM, CP, TP and ash were determined according to AOAC (1995). The fiber fractioning was carried out according to Goering and van Soest (1970). The prediction equations proposed by García-Trujillo and Pedroso (1989) were used to determine the ME of hay and supplement, respectively.

Samplings of the ruminal liquid dynamics were conducted in cannulated animals during the 0, 2, 4, 12 and 24 h, after the first feed supply. The samples were determined the pH with a portable pH meter, Orion 250-A. The SCFAt were determined according to Pennington (1952). The bacterial biomass (BB) production was estimated agreeing with Smith (1975) (table 1).

Statistical analysis. Duncan's test (1955) was used to establish differences between means. A linear model was used to determine the fermentation indicators showing the main effects. The data were processed with the statistical software INFOSTAT (Balzarini *et al*. 2001).

Concentrations of yeasts and lactobacilli, ranging between $107^{-1}08$ cfu and $109^{-1}010$ cfu; 450-600 mmol.L^{-1} for lactic acid and 225-230 mmol L^{-1} for the acetic, respectively, were included in the microbiological and chemical composition of VITAFERT.

Results and Discussion

In respect to the intake of DM and total NDF and their relation with the live and metabolic weight (table 2), the results favored ($P < 0.01$) the treatments of 4.5 and 6.0 mL kg LW^{-1} of VITAFERT in the ration. However, the level of 8.5 mL kg LW^{-1} and the control had inferior values. A similar performance ($P < 0.01$) had the total intake of ME and CP and the contribution of the same nutriments from the fiber source (*Brachiaria brizantha* hay). However, the ME-CP ratio, derived from the supplement, compared to that of the hay consumed (EMc: EMf vs PBc:Pbf) in the treatment with 6 mL kg LW^{-1} of VITAFERT differed only from the control ($P < 0.05$) and had the lowest and highest value in the ratio of forage- consumed concentrate (table 2).

There were no differences between treatments in the values of ruminal pH. The contrary occurred with the SCFAt and BB concentrations in the rumen, where the treatment with 6.0 mL kg LW^{-1} of VITAFERT in the ration differed significantly ($P < 0.01$) from the rest, with higher production of these indicators (table 3).

In respect to the differences found in the DM intake and its relation with the LW, the VITAFERT levels of 4.5 and 6.0 mL kg LW^{-1} were higher compared to the rest but were below the reports of Sauvant *et al*. (1991) for goats (4.0 % of LW), according to studies developed in the tropics with diversity of management and feeding systems, but included in the range of 2.0 to

Table 1. Bromatological composition of the feeds used

	Feed		
	Supplement	Hay	Act. 1VITAFERT 1
DM, %	88.10	86.26	9.70
CP,%	16.45	5.99	4.80
TP,%	-	-	2.88
ME,MJ	11.80	7.70	-
NDF,%	-	85.18	-
Lignin , %	-	7.16	-
Cellulose, %	-	44.82	-
Hemicellulose,%	-	33.21	-
Ash, %	6.25	6.15	5.19
Calcium,%	2.10	0.45	1.20
Phosphorous,%	0.30	0.24	0.17

Table 2. Effect of VITAFERT on the voluntary intake of total dry matter, basic nutrients and their relation

Indicators	Treatments				± SE Sig.
	Control	4,5 mL kgLW^{-1}	6.0 mL kgLW^{-1}	8.5 mL kgLW^{-1}	
VITDM, kg anim^{-1}	0.69^{a}	0.94^{b}	1.00^{b}	0.70^{a}	0.06**
VITDM,g LW0,75	37.86^{a}	56.25^{b}	63.02^{b}	37.36^{a}	4.66**
VITDM, %LW	2.06^{a}	2.85^{b}	3.16^{b}	2.20^{a}	0.17**
VITDM PV, g kg LW	20.65^{a}	28.48^{b}	31.58^{b}	21.99^{a}	1.87**
CFND, % LW	1.36^{a}	2.03^{b}	2.29^{b}	1.48^{a}	0.15**
CFND, g kg LW,75	32.74^{a}	48.40^{b}	54.19^{b}	32.28^{a}	3.98**
CFNDf, % LW	1.36^{a}	2.03^{b}	2.29^{b}	1.47^{a}	0.15**
IME total, MJ anim.$^{-1}$	5.93^{a}	7.89^{b}	8.38^{b}	6.03^{a}	0.48**
ICP total, g anim^{-1}	57.16^{a}	72.37^{b}	76.22^{b}	57.79^{a}	3.77**
VITDMf , kg anim.$^{-1}$	0.52^{a}	0.78^{b}	0.84^{b}	0.54^{a}	0.06**
IMEf, MJ anim.$^{-1}$	4.03^{a}	5.99^{b}	6.49^{b}	4.13^{a}	0.48**
ICPf, g anim.$^{-1}$	31.39^{a}	46.61^{b}	50.46^{b}	32.16^{a}	3.77**
Ratio ME intake: EMf $^{-1}$ MJ anim^{-1}	0.60b	0.45^{ab}	0.35a	0.52^{ab}	0.07*
CP conc: CPf^{-1} g anim^{-1}	1.05^{b}	0.78^{ab}	0.61^{a}	0.91^{ab}	0.13*
Ratio F: C (DB), %	73:27^{a}	79:21^{ab}	82:18^{b}	75:25^{ab}	2.33*

Different letters on the same row differ at P < 0.05. **P < 0.01, *P < 0.05

Voluntary intake of total dry matter (VITDM), versus metabolic weight, of the forrage dry matter (VITDMf), neutral detergent fiber intake (NDFI), intake of metabolizable energy (IME) and intake of crude protein(ICP)

Table 3. Performance of the ruminal pH, concentrations of short-chain fatty acids (SCFA) and estimation of the bacterial biomass (BB)

Indicator	Treatments				± SE Sig.
	Control	4.5 mL kg PV^{-1}	6.0 mL kg PV^{-1}	8.5 mL kg PV^{-1}	
pH	6.96	6.99	6.98	6.89	0.04
AGCCt, mmol L^{-1}	68.91^{a}	70.70^{b}	74.08^{c}	68.95^{a}	0.44***
BB, g mol AGCCt^{-1}	1.91^{a}	1.96^{b}	2.05^{c}	1.92^{a}	0.01***

Different letters on the same row differ at P < 0.05. ***P < 0.001

2.8 % informed by Meneses (2010), and even higher, as the VITAFERT level of 6 mL in the ration. This shows that the values obtianed are in correspondance with maintainance intakes for goats out of production, as those used in this experiment (NRC 2005), and corroborates the previous analysis in respect to age and maturity of the plant material used for elaborating the hay and its influence on the expected intake of DM and NDF in goats.

Concerning the beneficial effect the levels 4.5 and 6.0 mL kg LW^{-1} of VITAFERT could have caused in the ration, the associative effect of VITAFERT should be considered first, together with that of synchronization and synergy with the nutriments of the supplement, although the levels of fresh supplement did not surpass 5.57 ± 0.93g kg LW^{-1}. According to Ruiz and Vázquez (1983), these levels are within the optimum range to increase the rations used in basically fibrous diets, like that used in this experiment. Besides, a low concentration of nitrogen in the rumen, diminishes the digestion of the cellulose, confirming that a minimum nitrogen source is always needed to achieve a higher microbial ruminal activity, even more knowing that the microorganisms with cellulolytic activity have low growth rate and, also, low maintenance requirements. Therefore, the response of the animal to the intake increase and emptying of the fermentative organ is low (Stritzler *et al*. 1983).

In spite of the influence of the concentrate intake on all treatments as the level supplied was equal, those of 4.5 and 6 mL kg LW^{-1}of VITAFERT, different from the rest, used more the hay nutrients, at about 76 % for ME and 65 % for CP, for the development of the microbial activity. Its maintenance and the synthesis and degradation of the material are also included. A prove of all this is the relation between ME and the CP of the concentrate consumed compared to that of the hay, which was low. This shows that the ingestion of the hay and concentrate protein was not enough to influence the growth and development of the ruminal cellulolytic microorganisms, and with that, achieve changes on the

magnitude of its activity.

The contribution of VITAFERT CP and LW amino acids, as well as certain amount of natural or soluble protein, like those included in the supplement offered and the contribution of the ramified SCFAt (valeric, isovaleric and isobutyric), of VITAFERT (Elías and Herrera 2008), could stimulate the ruminal cellulosis (Elías 1983), because the content of these last in the rumen is null or almost insignificant, mainly when thay are used in the diet with low-quality forage (Elías 2000), as the case of this study. Chalupa (1977), among other authors, refer that these ramified chain fatty acids are needed as bacterial growth factors.

The use of VITAFERT at dosages of 4,5 and 6 mL kg LW^{-1}, could have beneficial effects, fibrolitic and functional, similar to those referred by Galina *et al*. (2010), when using mixed cultures of lactic bacteria and yeasts. These cellulolytic microorganisms, on the concentration present, being stimulated with the activator product, produced higher degradation of the cell walls, diminishing of the mean time of appearance of the fibrous material and, therefore, higher emptying of the fermentative organ. According to reports of Galina *et al*. (2007), this mixture of microorganisms, in goats, has a substitutive effect of the ruminal lactic bacteria by those of the product. In this study, although the mixtures were different, the results are similar to those found with VITAFERT, produced a favorable environment for the ruminal fermentation. This corroborates that a nitrogen donor, enough energy and microbial growth co-factors are needed for a high initial microbial population, in a complex system as rumen is (Elías 1983).

When analyzing the ruminal environment, the pH values obtained (±6.9), even when there is not a unique agreement of the optimum pH value, were within the physiological limits, in a range between 6.2 and 7.2. These values have been defined as optimum to guarantee the cellulose digestion (Cardozo *et al*. 2001 and Calsamiglia *et al*. 2002 and Krause and Oetzel 2006) and increase the growth rhythm of cellulolytic and hemicellulolytic microorganisms, their activity and, with it, the products of their metabolism (Marrero 2005). This is today, establishing a pH value over 6.0 is necessary for the prolificacy of cellulolytic bacteria more than for a higher enzymatic activity. Likewise, it is possible that the pH of the ruminal environment allows keeping a dense population of diverse genera and protozoa species, due to the associative effect for the presence of cotton micelles of the concentrated supplement, which will stimulate such microorganisms (Elías 1983).

The SCFAt concentrations in the rumen and the differences found in the level of 6.0 mL kg LW^{-1} of VITAFERT in respect to the rest of the treatments seemed to be due to, the possibility of creating ideal conditions to increase the fermentative capacity, related with the improvement of the ruminal environment because, among other factors, the pH values of the environment and the increase of hay intake as consequence of the increase of the microbial activity and its degradation (Ortiz-Rubio *et al*. 2002). The SCFAt production decreases as pH increases in the rumen and vice versa (Valdez *et al*. 1997). An increase of these organic compounds gives the enough energy so the microorganisms and the host can use the available nitrogen. This favors the higher total DM intake (Herrera 2007 and Elías and Herrera 2008).

Similar results, in respect to concentrations of SCFAt during the dynamics and their relation with the pH, in basically fibrous diets, were obtained by Elías (2000) and Castañeda *et al*. (2010). The latter found increase of the SCFAt concentrations in goats and sheep from the three and six hours after the feed ingestion, informing, at the same time, negative correlation ($r2 = -0.454$, $P < 0.01$) between the pH and the concentrations of SCFAt. An analogue performance was evidenced in this study ($r2 = -0.30$ $P < 0.01$) in the treatment with 6 mL kg LW^{-1} of VITAFERT. This low correlation of this level of VITAFERT indicated that other metabolites, not determined in this study, could influence on the mixed ecosystem found in the rumen.

The stability of the pH values could be the positive effect of the microbial activators when stimulating the bacterial growth and contributing to the higher DM intake, mainly of NDF. This provokes more rumination, mastication and, possibly, more saliva secretion, as well as the possibility of buffering amounts of SCFA, acetic and lactic acids present in VITAFERT and produced in the rumen.

Due to the importance of the rumen pH, in spite there were no interaction between treatments and during the dynamics, the calculated regression between the pH values and the SCFAt concentration in the treatment with 6 mL kgPV^{-1} of VITAFERT was negative ($R^2 = -0.23$, SE±=0.20) and lower in the rest of the treatments. In them, the relations, still negative, were within the range of moderate to strong, so the pH values were not only influenced by the concentration of these organic acids but also by other factors such as the weakening capacity of the environment due to the increase of mastication and rumination, with the subsequent increase of saliva production and segregation (Sauvant *et al*. 1999). These buffer substances (carbonates and phosphates), apart from the urea of the activator, together with the pH stability, should improve, substantially, the synthesis of microbial protein (Elías 1983) and increase the animal response. This criterion coincides with that referred by Fernández (1998).

In spite of the knowledge on ruminal fermentation, it is necessary to estimate the microbial biomass when researching a digestive process and considering the nutritive value of animal feeds. This indicator is of great importance for obtaining a reliable estimation of the microbial protein contribution. According to Smith (1975), the SCFAt concentrations allow estimating the

rumen microbial mass. According to this author, the treatment with 6.0 mL kg LW^{-1} of VITAFERT in the ratio, achieved higher microbial production, showing higher fermentation and, as a consequence, higher hay degradation. This microbial mass turns into the digestive part, as a protein with excellent aminoacidic composition (80 % LW), coming out of the rumen and absorbed in the small intestine (López 2009). Concerning Henning *et al*. (1993), the efficiency of the microbial growth and the improvement of the microbial protein production would be possible with the energy balance of the ration, the nitrogen available in the rumen and its synchronization.

Regarding Aguilera (1989), the formation efficiency of the microbial mass is related with the increase of the degradation rate and the decrease of the volume or time or feed retention in the rumen. These indicators, in the treatment with 6.0 mL kg $LW^{-1}$of VITAFERT, could be higher, increasing the microbial protein relation per VFA produced. The synthesis of microbial protein is complicated and, according to Elías and Herrera (2008), the increase of the efficiency corresponds with the availability of the energy required for maintaining the microorganism.

It is concluded that in goats fed *Brachiaria brizantha* hay, of low nutritive value, the stimulating effect of VITAFERT on the increase intake of DM and NDF depends on the dosage. Level 6.0 mL kg LW^{-1} in in the ration, together with protein-energy supplement at a rate of 6 g kg LW^{-1}, optimizes the system, in respect to fermentative capacity of the rumen, which is coherent with the increase of SCFAt an BB concentrations.

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