Effect of protein-energy supplementation on *in situ* rumen degradability of NDF and OM of star grass (*Cynodon nlemfuensis*) in buffalo calves (*Bubalus bubalis*)

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Four male buffalo calves (*Bubalus bubalis*) of the Bufalipso breed were used to evaluate the effect of the supplementation with different amounts of protein-energy concentrate on the *in situ* rumen microbial degradation of NDF and OM of star grass forage. The animals had 175 ± 5 kg of liveweight and were fitted with rumen cannula, according to 4 x 4 Latin square design. Four treatments were used with different amounts of energy-protein supplementation in the diet: 0 (control), 3, 6, and 9 g kg LW^{-1}, given once a day. The rumen degradation of the NDF and the OM of the forage showed the highest values ($P < 0.05$) with the rise of the incubation time for the treatments with protein-energy supplementation compared with the control, since 24 h. The supplementation had effect on the increment of the degradation rate of the NDF (0.018, 0.027, 0.029 and 0.030 fraction h^{-1}) and on the decline of the colonization time or lag phase (2.8, 2.2, 2.1, 2.0 h) for the treatments with 0, 3, 6, and 9 g kg LW^{-1}, respectively, whereas the degradation rate of the OM was higher in the treatments with supplementation (2.8, 2.6, 2.2) for 9, 6, 3 g kg LW^{-1} compared with the treatment without supplementation (1.9). The effective degradability of the forage for the NDF and the OM was higher in the treatments with supplementation compared with the control. Equations of multiple regression were established permitting to describe the process of degradation of DM, NDF, and OM with the NDF N^{-1} consumed ratio in the diet and the time (t) in rumen. The results prove the positive effect of the supplementation with increasing amounts of concentrate up to 9 g kg LW^{-1} in the diet of buffalo calves fed star grass. This could contribute to the rise in the productive activity of the buffalo calves in current rearing conditions.

Key words: *buffalo calves, rumen degradability, supplementation*.

Traditionally, the good performance of the buffalo species has been generalized in conditions that are adverse to other herbivore species, for instance, in areas of native grasses and poor quality feeds (Sonia *et al*. 1998). According to these considerations, current production systems have been developed, with predominance of natural pastures and unbalanced diets (López 2009).

The buffalo calf feeding is a limitation in exploitation systems with poor nutritive value systems as those predominating in the tropical region. The low pre and post-weaning growth rates due to nutrition and management are noteworthy in this area (Berroterán *et al*. 1998).

Several scientific results support the importance of the protein-energy supplementation in order to supply the animal with the necessary nutrients for its growth and production. This option improves the availability of those nutrients limiting the fermentative processes in the rumen, with the consequent increase of the animal productivity (Elías 1983, Martínez and García 1983, and Hoover and Stokes 1991). The objective of this study was to assess the effect of the strategic supplementation with different amounts of protein-energy concentrate on the *in situ* rumen microbial degradation of the NDF and the OM of star grass forage in buffalo calves (*Bubalus bubalis*).

Materials and Methods

Four buffalo calves (*Bubalus bubalis*) of the Bufalipso breed of 175 ± 5 kg of liveweight were used and fitted with cannula in rumen. The animals were allocated in individual pens, with free access to drinking water and feeds. They were fed fresh star grass forage (*Cynodon nlemfuensis*) *ad libitum* and different levels of concentrate. It was given once a day and it was formulated in dry basis, with 67.4 % corn, 30 % soybean, 1.0 % salt, 1.0 % mineral premixture, and 0.6 % dicalcium phosphate. Table 1 shows the chemical composition of the feeds.

Treatment and design. A control (0 g kg PV^{-1}) and different amounts of protein-energy supplementation (3, 6, and 9 g of concentrate kg LW^{-1}) were used. The concentrate diet was fitted for each experimental period, according to treatment and animal weight. A 4 x 4 Latin square design was used.

Experimental procedure. The determination of the rumen degradability of the OM and the NDF of the plant material was performed according to the procedure of the nylon bags or *in situ*, as decribed by Mehrez and Orskov (1977).

The experimental periods consisted of 14 d of diet

Table 1. Chemical composition (% DM) of the feeds (n = 4).

Indicators	Forage	Concentrate
CP	7.2 ± 0.1	20.2 ± 0.2
OM	90.7 ± 0.1	92.9 ± 0.3
NDF	77.6 ± 0.1	23.2 ± 0.1
ADF	39.5 ± 0.2	13.1 ± 0.1
Ashes	9.3 ± 0.1	7.1 ± 0.1
ME (MJ kg DM^{-1})	7.8	12.2

adaptation and four of sampling. Five grams of sample of dry forage of star grass were weighed by duplicate in nylon bags (14.0 x 8.5cm), with porosity of 48 µm, in each incubation time and animal (table 2). The bags were incubated in the rumen for 8, 12, 24, 48, and 72 h post-feeding. Once extracted, they were washed manually until the water was clear and transparent. Later, they were dried in oven, with air circulation at 60 ºC for 72 h. The difference between the initial weight of the sample and that of the residue after the rumen incubation was used to determine the DM degraded in the rumen. The residues from the bags, corresponding to the two repetitions of each incubation time in the same animal, were ground up to 1 mm particle size and a homogeneous sample was formed. All the residues were determined the OM and NDF content.

Table 2. Chemical composition of the star grass forage assessed in the *in situ* rumen degradability (% DM)

Indicators	Values
CP	6.4
OM	91.7
NDF	70.1
ADF	38.2
Ashes	8.3
ME (MJ kg DM^{-1})	7.8

Estimate of the degradation. The estimate of the *in situ* rumen degradation of the OM and the NDF was performed through the NEWAY Excel software, according to Chen (1997).

The exponential model of Orskov and McDonald (1979) was used for determining the characteristics of the degradation. It was assumed that the OM degradation curve (OMD) in time follows a kinetic process of first order, described as follows:

P = A for t0 = 0

$P=a+b(1-e^{-ct})$ t>t0

The degradation of the NDF was described according to Dhanoa (1988) by the formula:

P =A for t=t0

$P=a+b(1-e^{-c(t-L)})$ $t>t0_0$

Where,

P: rumen degradation of the indicator evaluated in the time "t" of rumen permanence.

- a: Intercept
- b: Fraction degraded in the time t
- c: Degradation rate of the fraction "b"
- t: Incubation time
- L: latency time or "lag" (hours)
- A: Easily soluble fraction. It was obtained through the incubation of the sample in water bath at 39 ºC for 30 min.

The model of McDonald (1981) was used for the determination of the rumen effective degradability (ED).

$$\mathrm{ED} = A + \left(\frac{B\ c}{c+k}\right)$$

Where,

- k: fractional rate of rumen passage. It was ass EDd k = 0.044 (NRC 1989).
- B: insoluble fraction, but potentially degradable (B = (a+b) – A) (Orskov 2002).

The comparison of the rumen degradation between treatments was performed through the RUMENAL procedure (Correa 2004).

Chemical analysis. The analyses of the OM were conducted according to AOAC (1995), whereas the NDF determinations were performed according to Goering and van Soest (1970).

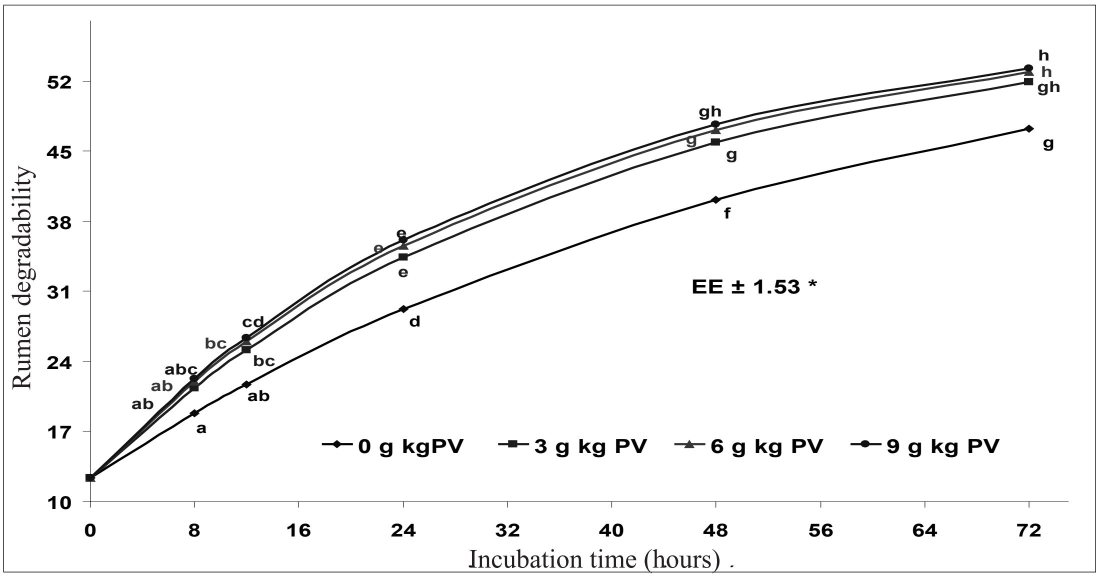
Statistical analysis. For the comparison between means, corresponding to each treatment and to the treatments of each incubation time, the statistical processor InfoStat (Balzarini *et al*. 2001) was applied. When necessary, the test of Duncan (1955) was used to compare the means. Multiple regression equations were fitted for the rumen degradability of DM, NDF, and OM in respect to the NDF N^{-1} and the time.

Results and Discussion

The increase in the protein-energy supplementation (up to 9 g kg LW^{-1}) in the diet of buffalo calves had effect on the degradation of the NDF and the OM of the star grass forage.

The supplementation with different amounts of protein-energy concentrate in the diet of buffalo calves had positive effect on the kinetics of the *in situ* rumen degradation of the NDF (NDFD) of star grass (figure 1). There was higher ($P < 0.05$) degradation of the NDF with the rise in the incubation time for the treatments with supplementation compared with the control, since 24 h. The rise in the NDFD of star grass forage, obtained with the protein-energy supplementation, could be related to the statements of Hardy and Cruz (1979), Elías (1983), and Souza *et al*. (2000) about the effect of the protein-energy supplementation on the increment of the utilization of the ration and the enhancement of the rumen cellulolytic activity.

Thus, the increase in the NDF degradability of star grass forage could be related to results obtained by López *et al*. (2009). These authors reported larger amount of bacterial biomass in the rumen of buffalo calves supplemented with protein-energy concentrate. This could be associated with the statements of Cheng *et al*. (1991), who noted that the increment in the bacterial biomass favors the degradative capacity of the fibrous fraction of the pasture, because the bacteria are



abcdefgh Means with different letters differ at P < 0.05 (Duncan 1955)

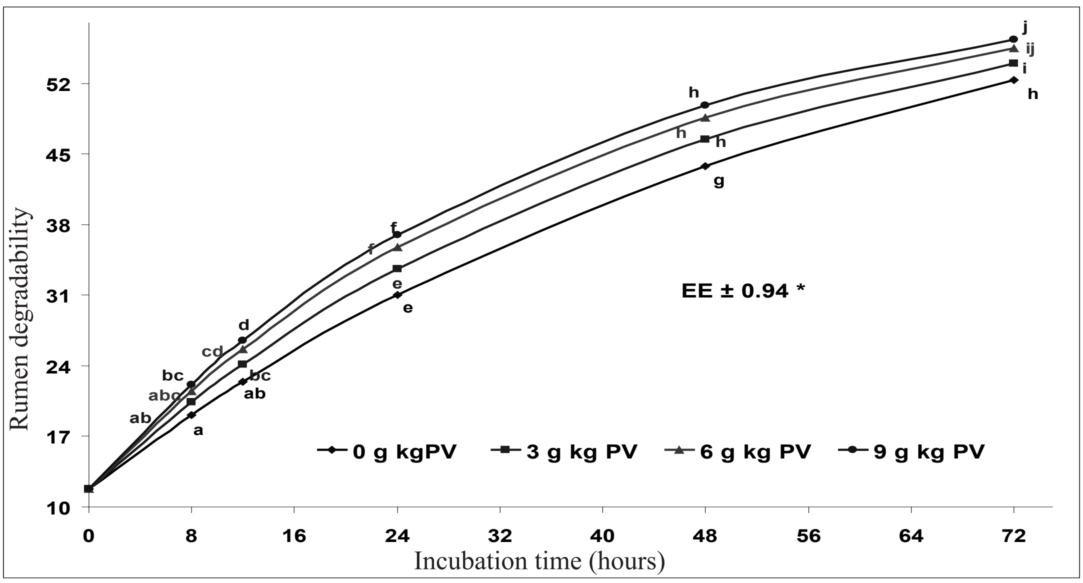
*P < 0.05

Figure 1. Effect of the protein-energy supplementation on the kinetics of NDFD of star grass in buffalo calves

the group of microorganisms representative of the microbial biomass and of greatest rumen metabolic activity.

According to García and Kalscheur (2006), when giving a diet with high NDF levels, the processes of rumination and mastication can be favored. In this regard, the rumination and the mastication reduce the particle size and thus, the action of the microorganisms with the consequent increment of the degradative capacity of the pasture nutrients. This result could be related to the stability in the pH values in the rumen of buffalo calves (López *et al*. 2009). Also, it could be associated with the balance between the production of protons (H^+) and their neutralization through the buffering capacity of the rumen environment, roughly due to the amount of segregated saliva. It provides the greatest rumen buffering capacity, primarily by phosphate and bicarbonate ions (Calsamiglia and Ferret 2002).

The kinetics of *in situ* rumen degradation of the OM (OMD) of the sample (figure 2) kept a similar performance for all the treatments. The OMD was increased (P < 0.05) with the rise in the amount of the protein-energy concentrate in the diet and the incubation time in the rumen. The treatments with supplementation (6 and 9 g kg LW^{-1}) showed the highest degradation values at 24 h, and they were superior (P < 0.05) to the



abcdefghij Means with different letters differ at P<0.05 (Duncan 1955)

* P<0.05

Figure 2. Effect of the protein-energy supplementationon the kinetics of OMD of star grass in buffalo calves

rest of the treatments.

In respect to the parameters of rumen degradation of NDF and OM in the star grass for buffalo calves, it should be noted that in all the indicators the soluble fraction (A) did not vary, because the incubated substrate was the same (table 3). However, Martínez (2006) reported that the insoluble, but potentially degradable (B) fraction depends, largely, on the time of permanence of the feed in the rumen. In this study, it was found that this indicator diminished with the inclusion of concentrate in the diet, which could be related to the improvement of the rumen medium conditions, due to the supply of CP and ME from the concentrate. These outcomes were in correspondence with those of Rotger *et al*. (2006).

It was proved that the rise in the concentrate of the diet in buffalo calves increased the degradation rate (c), from 1.8 to 3.0 % h^{-1} and from 1.9 to 2.8 % h^{-1}, for the NDFD and the OMD of the star grass forage in buffalo calves, respectively (table 3). Maeda *et al*. (2007) reported similar results when assessing different levels of concentrate in the diet of buffalos and cattle.

In this work, the following multiple regression equations were fitted for the degradation of DM, NDF and OM, according to the NDF N^{-1} ratio as consumed in the diet and the time (t) in the rumen.

$$DMD = 26.61 - 0.18\ (\pm 0.03)\ NDF\ N^{-1} + 0.53\ (\pm 0.01)\ t$$

(R^2 0. 94; Sign: ***; SE of estimation = ± 3.21; MSE= 10.33)

$$NDFD = 29.30 - 0.19\ (\pm 0.04)\ DNF\ N^{-1} + 0.53\ (\pm 0.02)\ t$$

(R^2 0.89; Sign: ***; EE de estimación = ± 4.53; MSE = 20.53)

$$OMD = 25.62 – 0.17\ (\pm 0.03)\ NDF\ N^{-1} + 0.54\ (\pm 0.02)\ t$$

(R^2 0.94; Sign: ***; SE of estimation = ± 3.34; MSE = 11.17)

The equations were characterized by having high coefficients of determination (R^2), high significance, low standard errors (SE) of estimation and mean squares of the error (MSE) and adequate distribution of the residues. These equations permitted describing how as the strategic protein-energy supplementation was increased in the diet (up to 9 g kg LW^{-1}) the NDF N^{-1} ratio of the diet was narrowed. This improved the rumen conditions in the buffalo calves and thus, the processes of degradation of nutrients from the pasture.

The responses could be the result from the biochemical reactions established in the rumen of buffalo calves, due to the energy and protein supply of the concentrate as complement of diets deficient in nutrients. Thus, the energy-protein supply is enhanced, as well as the microbial growth and the degradation of the nutrients (McAllister *et al*. 1994 and Suárez *et al*. 2007). Similar responses were reported by Wanapat and Chanthakhoun (2009) and Wanapat *et al*. (2009) in studies on buffaloes and cattle.

The equations of regression could be used as theoretical elements to establish adequate strategies of supplementation, when using poor nutritive value pastures in buffalo calf feeding. They could also serve as reference for the application of agroindustrial byproducts, harvest residues, among other alternatives that could be used as nutritional supplements.

In general, unlike the control, the supplementation with protein-energy concentrate, up to 9 g kg LW^{-1}, favored the degradation of star grass nutrients. The protein-energy supplementation to buffalo calves is recommended for Cuban exploitation conditions. Further

Table 3. Characteristics of the parameters of the NDFD and the OMD of star grass in buffalo calves supplemented with different amounts of protein-energy concentrate

Parameters		Concentrate g kg LW^{-1}			
		0	3	6	9
NDF	A %	12.40	12.40	12.40	12.40
	B %	48.0	46.1	46.20	46.20
	c Fraction h^{-1}	0.018	0.027	0.029	0.030
	L h	2.80	2.20	2.10	2.00
	SE ± and Sign.	4.57*	2.54***	3.39***	2.68***
	R^2	91.03	97.04	95.54	96.69
OM	A %	11.80	11.80	11.80	11.80
	B %	57.40	53.10	51.60	51.40
	c Fraction h^{-1}	0.019	0.022	0.026	0.028
	SE ± and Sign.	2.87**	2.21***	1.23***	1.85***
	R^2	95.66	97.73	99.31	98.43

SE: Standard error, Sign: Significance, R^2: Coefficient of determination of the exponential model of Orskov and McDonald (1979) and Dhanoa (1988) in each treatment, for the NDFD and the OMD, respectively research is encouraged recommending more adequate inclusion levels from the biological and economic standpoint.

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