Effect of the energy-protein supplementation on the ruminal fermentation indicators of buffalo calves (*Bubalus bubalis*) fed star grass (*Cynodon nlemfuensis*)

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Four male buffalo calves (*Bubalus bubalis*) of the Bufalipso breed with 175 ± 5 kg of liveweight cannulated in the rumen were used to assess the effect of the supplementation with different amounts of protein-energy concentrate on ruminal fermentation indicators of buffalo calves fed star grass forage, according to a 4 x 4 Latin square design. A control treatment (0 g kg LW^{-1}) was established and different energy-protein supplementation levels were offered daily in the diet: 3, 6 and 9 g kg LW^{-1}. The NH_3 concentration was higher ($P < 0.001$) for the treatments with 9, 6 and 3 g kg LW^{-1} (9.37, 8.54 and 8.53 mmol L^{-1}, respectively) compared to the control (7.05 mmol L^{-1}). There was a maximum concentration of this indicator after two hours (11.89 mmol L^{-1}) and lower in the rest. The supplementation had effect ($P < 0.001$) on the increase of the SCFAt concentrations of the ruminal liquid of buffalo calves, on the bacterial biomass production, and on the organic matter fermented in the rumen. The results show that the strategic supplementation with protein-energy concentrate, up to 9 g kg LW^{-1}, in the diet of buffalo calves consuming low-quality star grass improved the ruminal environmental conditions, with favorable concentrations of ammonium and SCFAt for the increase of the bacterial biomass.

Key words: *strategic supplementation, bacterial biomass, organic matter, ruminal fermentation, forage.*

Among the ruminants useful for mankind, river buffalo (*Bubalus bubalis*) is considered the most promising species worldwide, in respect to milk and meat production, leather; apart from being a labor instrument (Cockrill 1994 and Amarjit and Toshihiko 2003). The good behavior of the river buffalo in environments unfavorable for other ruminant species has propitiated at present the development of feeding systems based on natural grasslands with low nutritional value (Sonia *et al*. 1998 and López 2009). This option is not favorable as it affects the productive potential of the bubaline species.

It has been proved that the protein-energy supplementation in animals consuming low-quality pastures improves the ruminal environmental conditions and, therefore, the efficiency of the fermentative activity in the rumen as well as the synthesis of microbial protein, with direct effect on the degradation of the pastures' nutrients and on the basal feed consumption (Martínez and García 1983, Hoover and Stokes 1991, Ramos 2005 and Suárez *et al*. 2007). The literature available on this subject, specifically referee to buffalo calves supplementation, is not enough, so widening the studies on this respect is necessary.

The objective of this study was to assess the effect of the strategic supplementation with different amounts of protein-energy concentrate on the ruminal fermentation indicators of buffalo calves fed star grass forage.

Materials and Methods

Animals, diet and experimental design. Four male buffalo calves of the Bufalipso breed, with eight months of age and 175 ± 5 kg average liveweight and fitted permanently with cannula in the rumen were used. They were allocated in individual pens, with free access to water and mineral salts, according to a 4 x 4Latin square design.

A control treatment (0 g kg LW^{-1}) and three supplementation levels with protein-energy concentrate were used: 3, 6 and 9 g kg LW-1. The animals consumed fresh star grass (*Cynodon nlemfuensis*) forage *ad libitum* and concentrate once a day (8:00 a.m.). The concentrate level was adjusted for each experimental period, according to treatment and live weight (LW) of the animal. It was formulated over the basis of 67.4 % corn, 30 % soybean, 1.0 % salt, 1.0 % mineral pre-mixture and 0.6 % dicalcic phosphate. The chemical composition of the feeds is shown in table 1. The metabolizable energy (ME) of the grassland and concentrate were from the nutritive value list for ruminants, according to García Trujillo and Pedroso (1989).

Experimental procedure and chemical analysis. The experimental periods were of 14 d of adaptation to the diet and three sampling days. The ruminal liquid was collected through the cannula of the ventral sack, from 2, 4, 6, 8, 12 and 16 h post-feeding. A sampling was conducted before feeding (zero hour). A vacuum bomb was used and the sample was stored in thermos. The liquid was filtered through muslin. The ammonium (NH_3) concentration was determined according to Conway (1957), and that of the total short chain fatty acids (SCFAt), according to Pennington (1952). The bacterial biomass production (BB) and organic matter fermented in the rumen (OMFr) were estimated according to the description of Smith (1975), who indicated that 1.3 mol of SCFAt produces 36 g BB and 100 g OMFr.

Statistical analysis. Analysis of variance according to linear model was conducted for comparing the means

Table 1. Diet chemical composition of the animals under experiment (% DM) n = 4.

Indicators	Forage	Concentrate
CP	7.17 ± 0.01	20.17 ± 0.23
OM	90.73 ± 0.03	92.86 ± 0.28
NDF	77.58 ± 0.14	23.15 ± 0.08
ADF	39.47 ± 0.15	13.01 ± 0.01
Ash	9.27 ± 0.06	7.14 ± 0.02
ME (MJ kg DM^{-1})	7.82	12.18

of treatments, time and interaction of the treatments x time. The statistical software InfoStat (Balzarini *et al*. 2001) was used for the data processing. The Duncan's (1955) test was used for comparing between means when necessary.

Results and Discussion

There were no interaction between treatments and the sampling times for the final indicators of the fermentation studied. However, when analyzing the results, it was proved that the strategic supplementation with protein-energy concentrates, up to 9 g kg LW^{-1}, in the diet of buffalo calves had effect on the NH_3 concentrations. They were superior ($P < 0.001$) for the treatments with 3, 6 and 9 g kg LW^{-1} (8.53, 8.54 and 9.37 mmol L^{-1}, respectively) compared to the control (table 2). This result could be related with the reports of Hardy and Cruz (1979) and Elías (1983), who indicated that the supplementation with optimum levels of protein-energy concentrates in the diet of ruminants consuming low-quality pastures improves the ruminal environmental conditions and, with that, the microbial growth and degradation of nutrients.

The highest NH_3 concentration (11.93 mmol L^{-1}) in the ruminal liquid after two hours (figure 1) could be associated with a higher content of nitrogen degradable in rumen during the first hours, when it passes at higher degradation rate compared with its use by the rumen microorganisms (Ramos 2005). This could be related with the results of Paliwal and Sagar (1990), Souza *et al*. (2000), Wanapat (2001), Maeda *et al*. (2007) and Wanapat y Chanthakhoun (2009), who found higher NH_3 concentration in the rumen content after two hours of offering the feed. This concentration decreased in time. The diminishing of NH_3 concentration from the four hours could be determined due to its use by the ruminal microorganisms to incorporate them to their cells as silliars of amino acids and for the synthesis of microbial protein (Ruiz and Ayala 1987 and Oba and Allen 2003). It could also be attributed to its exit from the rumen to the abomasum, together with the liquid fraction or through the absorption of NH_3 by the ruminal epithelium (Rotger *et al*. 2006).

The results of López *et al*. (2009) inform ruminal pH values between 6.83 and 6.99 in similar experiments. In this study, the ammonium concentrations could influence the ruminal pH values over six, because the ammonium constant basic dissociation allows the absorption of hydrogen ions from the environment and be part of controlling the ruminal pH, although Sauvant *et al*. (1999) stated that the pool of ammonium available in the rumen is too small to contribute significantly with the pH control.

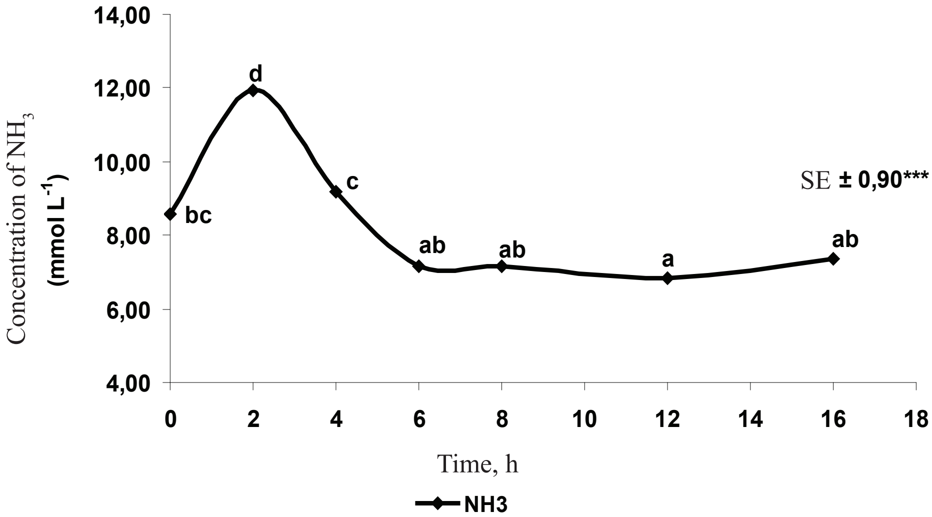
In respect to the SCFAt concentration (mmol L^{-1}) in the ruminal content, this indicator increased ($P < 0.001$) as the concentrate in the diet of buffalo calves increased. The supplementation increased the concentration in 15.05, 15.36 and 18.17 mmol L^{-1} of SCFAt for 3, 6 and 9 g kg LW^{-1} of supplementation in respect to the control (69.17 mmol L^{-1}). The values of SCFAt are in correspondence with the type of diet offered and could contribute to the stability of the pH values reported by López *et al*. (2009), in favor of the DM degradation and a better use of this metabolite by the microorganismos (Sauvant *et al*. 1999).

Souza *et al*. (2000) indicated concentrations of SCFAt of 69.94 mmo L^{-1} in buffaloes, fed 54, 60, 66 and 72 % NDF in the diet, very similar to those of this study. This lower concentration could be associated to the higher passage of this metabolite through the

Table 2. Effect of the strategic supplementation with protein-energy concentrate on some final products of the ruminal fermentation of buffalo calves fed star grass forage

Indicators	Concentrate g kg LW^{-1}				EE ± and Sig.
	0	3	6	9	
NH_3 (mmol L^{-1})	7.05[b]	8.53[a]	8.54[a]	9.37[a]	0.35 ***
SCFAt (mmol L^{-1})	69.17[b]	84.22[a]	84.53[a]	87.34[a]	1.80 ***
BB (g mol $AGCCt^{-1}$)	1.92[b]	2.33[a]	2.34[a]	2.42[a]	0.05 ***
OMFr (g)	5.32[b]	6.47[a]	6.50[a]	6.72[a]	0.13 ***

[abc]Means with different letters in the same row differ at $P < 0.05$ (Duncan 1955) *** $P < 0.001$



abcdMeans with different letters differ at P < 0.05 (Duncan 1955)
*** P< 0.001

Figure 1. Effect of the sampling time on the NH_3 concentration (mmol L^{-1}) of the ruminal liquid of buffalo calves

ruminal fluid to the abomasum or through higher absorption of the ruminal epithelium walls (Ruiz 1979 and Wanapat *et al*. 2009a).

The increase of SCFAt concentrations, from the strategic supplementation with different amounts of protein-energy concentrate, allowed estimating higher bacterial biomass production and of organic matter fermented in rumen (table 2). When the concentrate in the diet of buffalo calves fed star grass increased, the values of these indicators increased (P< 0.001) in respect to the control. The bacterial biomass ranged between 2.33 and 2.42 g mol $SCFAt^{-1}$ for the treatments with supplementation, while the organic matter fermented in rumen differed (P < 0.001) for the treatments with supplementation in respect to the control (5.32 g), expressing lower production. Similar results obtained Wanapat *et al*. (2009 a) and Wanapat *et al*. (2009 b), when comparing the rumen microorganisms of buffaloes and cattle.

If we consider that the bacterial biomass has at about 60 % of protein and that this is the most expensive resource in ruminants' production, then any improvement in the efficiency of bacterial biomass would be related with the economic efficiency of the system (Vargas *et al*. 2004). Consequently, the results of this study offer the initial data to establish studies related with the ruminal microbial efficiency in buffalo calves.

Generally, the strategic supplementation with growing amounts of protein-energy concentrate, up to 9 g kg LW^{-1} in the diet for buffalo calves consuming low-quality star grass allowed improving the conditions of the ruminal environment, with NH_3 concentrations and SCFAt favorable for the microbial growth. The strategic supplementation of buffalo calves with protein sources to maximize the ruminal fermentation and improve the animal response under similar conditions is recommended.

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