Consumption and rate of passage of digesta in buffalo calves (*Bubalus bubalis*) fed star grass (*Cynodon nlemfuensis*) and protein-energy supplementation

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Four buffalo calves (*Bubalus bubalis*) of the Buffalypso breed of 10 months of age and 214 ± 5 kg live weight were used. Animals were rumen cannulated and fed poor quality star grass for determining the effect of protein-energy concentrate supplementation on consumption and rate of passage of the liquid and solid fractions through the rumen. Four treatments were assessed with different amounts of concentrate in the diet: 0, 3, 6 and 9 g/kg LW offered once daily (8:00 a.m.), according to a 4 x 4 Latin square experimental design. Nutrient consumption was measured by supply and rejection. The determination of the passage of the liquid fraction was made at 2, 4, 6, 8, 12, 16 and 24 h after supplying the Co-EDTA marker. For the solid fraction chromium impregnated forage was used, with direct feces collection from the rectum and until 144 h after marker dosage. Supplementation, up to 9 g/kg LW with concentrate, allowed the increase ($P < 0.001$) of total dry matter consumption (DMC) and there were no differences in DMC of forage (kg animal^{-1} day^{-1}) between treatments. Total consumption of NDF was higher ($P < 0.05$) for the treatment with 6 g/kg LW regarding the control. The supplementation did not affect the passage indicators of the liquid and solid fractions through the rumen in buffalo calves. The rate of passage of the liquids was between 5.63 and 6.48 %h and rechangings between 1.35 and 1.55 times/d. The rate of passage of solids through the rumen ranged between 3.01 and 4.48 %/h. The rate of passage of solids by the rumen ranged between 3.01 and 4.48 %/h. Similar performance showed the passage of the solid fraction by the cecum-proximal colon with values between 5.88 and 7.16%/h. Supplementation with concentrates in buffalo calves consuming poor quality forage propitiated the increase of total nutrient consumption, without altering DMC of forage and the passage indicators. These results constitute elements that must be considered for developing nutritional strategies for this animal category.

Key words: *Buffalypso, buffalo calves, consumption, passage*

The production systems of buffalo calves (*Bubalus bubalis*) represent a viable alternative for developing countries and play an important role in farm economy, since they are the integrating axis of the family agricultural system (Ferdous *et al*. 2010). Natural pastures of poor nutritive value (López *et al*. 2012) are the feeding basis of these animals in the tropics. That is why the performance of the species manifests, in many cases, low growth rates pre and post-weaning, according to reports of Khare and Baghel (2010), Tatsapong *et al*. (2010) and Hassan *et al*. (2011).

Supplementation with concentrates in poor nutritive quality diets could be an alternative for providing the nutrient deficit of buffalo calves. However, there are few studies and basic understanding of the digestive physiology of this buffalo category allowing the establishment of nutritional strategies. The objective of this investigation was to study the effect of supplementation with different amounts of protein-energy concentrates on nutrient consumption and rate of passage of the ruminal digesta in buffalo calves fed star grass (*Cynodon nlemfuensis*).

Material and Methods

Localization. The experiment was carried out in productive areas of the Institute of Animal Science, located in the municipality of San José de las Lajas, Mayabeque province, Cuba between 22º 53' NL and 82º 02' WL at 80 m.a.s.l.

Animals and experimental procedure. Four male buffalo calves (*Bubalus bubalis*) were used of the Buffalypso breed, of 10 months of age and 214 ± 5 kg of average live weight, rumen cannulated. Animals were maintained stabulated all the time, with water availability. They were fed *ad libitum* fresh forage of star grass (*Cynodon nlemfuensis*) and different concentrate levels supplied once daily (8:00 a.m.). The concentrate was formulated in dry basis with 67.4 % maize, 30 % soybean, 1.0 % salt, 1.0 % mineral premix and 0.6 % of dicalcium phosphate. The chemical composition of feeds is shown in table 1. The metabolizable energy (ME) of the pasture and the concentrate was estimated from the tables of the nutritive value of feeds for ruminants of García Trujillo and Pedroso (1989).

Treatments and design. Treatments consisted of a control (0 g/kg LW) and different amounts of protein-energy supplementation: 3, 6 and 9 g of concentrates/kg LW. A 4 x 4 Latin square design with four treatments and four experimental periods was used. The amount of concentrate was adjusted for each experimental stage according to the treatment and live weight of the animal.

Experimental procedure. The experimental periods were of 14 d for adaptation to the diet and seven sampling days for the determination of the rate of passage of the liquid and solid fractions from the rumen. The forage was supplied *ad libitum* and DM

Table 1. Chemical composition of the feeds supplied, values expressed as percentage of the dry matter (DM) n = 4

Indicators[1]	Forage	Concentrates
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)	7.82	12.18

[1]CP – crude protein, OM – organic matter, NDF neutral detergent fiber, ADF – acid detergent fiber, ME – metabolizable energy

consumption (DMC) was determined by the supply-rejection method.

Co-EDTA was used as marker for determining the liquid phase, according to Uden *et al*. (1980). To the animals, via cannula, 25 g of Co-EDTA were supplied dissolved in 250 mL of distilled water in only one dosage which were mixed with the digesta. The ruminal liquid was collected from different parts of the rumen, with the help of a vaccum pump, starting 2, 4, 6, 8, 12, 16 and 24 h after supplying the marker.

Cobalt concentration (Co) of the ruminal liquid was determined in a spectrophotometer of atomic absorption (PAE UNICAM SP9), according to the technique proposed by Williams *et al*. (1962).

The parameters of the dynamics of the liquid phase were calculated as indicated by Colucci *et al*. (1990) who indicated that:

Ruminal volume. $V(L) = \dfrac{\textit{Initial Co concentration}}{\textit{Anti}\ \log.\ A}$

Liquid flow F (L/h) = $k * V$

Liquid passage rate kCo (%/h) = $k * 100$

Ruminal rechanging R (day) = $\dfrac{k * 24}{V}$

Mean retention time MRT (h) = $k * F$

Where:

A = Concentration of the marker at zero hour

k: Fractional rate of the ruminal passage

As marker for estimating the kinetics of the solid particles was used star grass forage impregnated with chromium (Cr), as the procedure described by Uden *et al*. (1980). Via cannula it was supplied to the animals 150 g of marked fiber equivalent to 5 g of Cr^{3+}. This was mixed with the content of the ruminal digesta.

Feces samples were directly collected from the animal's rectum from 4, 8, 12, 16, 24, 32, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 h after the marker dosage. These were dried in an oven at 105º C, ground to 1 mm particle size and stored until further analysis. Chromium concentration in feces was determined in a spectrophotometer of atomic absorption (PAE UNICAM SP9) as stated by the technique proposed by Williams *et al*. (1962).

For the kinetic estimation of the solid fraction, the bi-compartmental mathematical model proposed by Grovum and Williams (1973) was used.

The parameters of the dynamics of the solid phase were calculated according to Colucci *et al*. (1990).

MRT k_1 and MRT k_2 (h) = Mean retention times in rumen and cecum proximal colon, respectively

$$\left[\frac{1}{K_1} \text{ and } \frac{1}{K_2}\right]$$

TT = Transit time $\dfrac{\operatorname{Ln} A_2 - \operatorname{Ln} A_1}{k_2 - k_1}$

MRT = Mean retention time:

Where: $\dfrac{1}{k_1} + \dfrac{1}{k_2} + T$

k_1 = rate of passage in the reticulo-rumen

k_2 = rate of passage cecum-proximal colon

A_1 = marker concentration in the rumen

A_2 = marker concentration in the cecum proximal colon

Statistical analysis. An analysis of variance was made through the InfoStat statistical system, Version 1 (Balzarini *et al*. 2001). In the necessary cases, Duncan's (1955) multiple range test was used for mean comparison.

Results and Discussion

The increase of concentrate till 9 g/LW in the diet did not affect DM consumption (DMC) of the base feed (kg/d), even though buffalo calves consumed forage with high NDF and ADF and low CP contents, values that could have influenced negatively on voluntary intake of the animals (table 2). Tatsapong *et al*. (2010) reported lower values (3.84 – 3.89 kg DM/d) to those obtained in this study, when buffalo calves of 12-18 months of age were fed different protein levels in the diet and they indicated that the increase of the protein level did not affect DMC.

There were no differences in forage DMC (kg animal^{-1}day^{-1}) between the different treatments (table 2). On increasing the supplementation level from 3, 6 and 9 g kg LW^{-1} of concentrates in the buffalo calves diet, it was obtained total DMC (550, 1330 and 1610 g, respectively) regarding the control. These results

Table 2. Effect of strategic supplementation with different amounts of protein-energy concentrate on nutrient consumption of buffalo calves

Consumption	Concentrate, g kg/LW				
	0	3	6	9	SE (±)
			DM/kg/d		
Forage	4.15	4.14	4.34	4.09	0.14
Concentrate	0.00	0.56[c]	1.14[b]	1.67[a]	0.02***
Total	4.15[b]	4.70[ab]	5.48[a]	5.76[a]	0.13***
% LW	2.06[c]	2.30[b]	2.63[a]	2.82[a]	0.06**
Forage % LW	2.06	2.03	2.09	2.01	0.06
			CP/kg/d		
Forage	0.30	0.30	0.31	0.30	0.01
Concentrate	0.00	0.11[c]	0.23[b]	0.34[a]	0.01***
Total	0.30[d]	0.41[c]	0.54[b]	0.64[a]	0.01***
% LW	0.15[c]	0.20[c]	0.26[b]	0.31[a]	0.01***
			ME MJ/kg/DM		
Forage	32.45	32.36	34.07	32.05	1.11
Concentrate	0.00	6.78c	13.85[b]	20.33[a]	0.30***
Total	32.45[b]	39.13[b]	47.91[a]	52.34[a]	1.02***
			NDF/kg/d		
Forage	3.22	3.21	3.38	3.18	0.11
Concentrate	0.00	0.13[c]	0.26[b]	0.39[a]	0.01***
Total	3.22[b]	3.34[ab]	3.64[a]	3.57[ab]	0.10*
% LW	1.60	1.64	1.75	1.75	0.05

[abc]Means with different letters in the same row differ at P < 0.05 (Duncan 1955)
MS – dry matter, CP – crude protein, ME – metabolizable energy,
NDF – neutral detergent fiber,
*P < 0.05 **P < 0.01 ***P < 0.001

agree with Suárez *et al*. (2007) who demonstrated that increases of protein-energy concentrates in the diet of up to 6 g kg LW, stimulate DMC and degradation of fibrous feeds.

Total dry matter consumption, expressed as % LW, was higher (P < 0.01) for treatments with 6 and 9 g kg/LW, regarding the rest. These results are similar to those reported by Yunnus *et al*. (2004) who compared the utilization of sunflower and cotton seeds in multi-nutritional blocks with a commercial supplement in buffalo calves of 11 months of age. The high DMC are in correspondence with what was reported by Zicarelli (2006), who found increase up to 2.7 % LW in growing buffalo calves. This increase in DMC in buffalo calves could be associated to the requirements of attaining the optimum body status for the age. In consequence, DM consumption of forage in % LW was similar between treatments. Therefore, the concentrate levels in the diet influenced on the difference in total DM consumption.

Likewise, Bhatia *et al*. (2001) obtained DM consumptions of 1.7 % LW in adult buffaloes fed wheat straw and rapeseed cake diets, while Montes (2006) reported dry matter consumptions from 1.43 to 1.57 % LW in river buffaloes fed integral diets based on sugar cane forage. Both results were lower to those found in the study with growing buffaloes that could be related to the type and quality of the base feed.

CP and ME consumption of the forage revealed similar tendencies. However, the increase of the different concentrate levels produced increase (P < 0.001) in total CP consumption for the treatment with 9 g/kg LW. In regard total ME consumption it was higher (P < 0.001) for treatments with 6 and 9 g/kg LW, respecting the treatment with 3 g/kg LW and the control, respectively.

Consequently, total NDF consumption was higher (P < 0.05) for the treatment with 6 g/kg LW and differed from the control. However, there were no differences between treatments for consumption in % LW. This was higher than 88 % of the total of the diet for the different treatments studied that could have favored the stability of the pH values above six, reported by López *et al*. (2011). With that favorable conditions were created for nutrient utilization of the pasture in similar experiments.

Concentrate supplementation did not produce variations in the indicators of passage of the liquid phase (table 3). Values of the liquid rate of passage (KCo) obtained were similar to those reported by Bartocci and Terramoccia (2006) who found KCo of 6.60 % h^{-1} in adult buffaloes and values of 7.02, 6.86, 6.33 and 6.02 % h^{-1} with the increase of concentrate in the diet (12, 75, 37 and 50 %, respectively). These authors found lower ruminal liquid flow (F) in diets with higher amount of concentrate.

The kCo values were between 5.63 and 6.48 % h^{-1}. These were no differences between treatments for MRT with values between 15.84 and 18.13 h. Liquid rechangings was between 1.35 and 1.55 times d^{-1}, while liquid flow ranged between 1.89 and 2.20 L/h.

In this experiment no variations in KCo and F were encountered. This could be related to the high NDF content of the forage supplied, allowing ND consumption higher than 88 % in total for all diets. This provoked greater saliva production interrelating with the stability in the pH values. In this way are demonstrated the results reported by López *et al*. (2011). Similar performance referred Bartocci *et al*. (2005).

In turn, these indicators are very much related to the performance of the ruminal liquid volume obtained (table 3) which were between 31.27 and 36.78 L, representing approximately 16 % of the live weight of buffalo calves. There were not evidenced with the concentrate increase in the diet. The similarity in the values obtained are a response to that, as well as the physical and chemical characteristics of the diet and the consumption capacity of buffalo calves until ruminal filling up.

Bartocci *et al*. (2005) estimated ruminal volume of 15 % of LW in adult buffaloes with 450 kg LW fed different proportions of concentrates in the diet. These authors found decrease of the ruminal volume with the increase of concentrate in the diet. Other articles reported ruminal volumes between 16 and 17 % LW in buffaloes with LW between 300 and 577 kg, fed with different diets (Ichinoche et al. 2004).

The indicators of the solid fraction of rumen did not show differences with the concentrate increase in the diet of buffalo calves consuming poor quality forage (table 4).

Values of k_1 and k_2 of the particles in the respective compartments showed with the increase of the concentrate in the diet, higher figures in the rumen and lower in the cecum-proximal colon regarding to what was obtained by Bartocci *et al*. (2005) and Bartocci and Terramoccia (2006). This could be due to the high NDF and low CP content of the feed supplied, to the DMC of similar forage between treatments, to the weight of the animals and to the physiological status, as well as to having supplied the diet in only one frequency, unlike the procedure the above mentioned authors followed.

When a forage-concentrate proportion (80:20) was utilized, Di Francia *et al*. (1997) found lower value of k_1 in adult buffalo (2.28 % h^{-1}) than those obtained (3.57 % h^{-1}) with similar forage concentrate proportion. This could have been influenced by the type of diet supplied, the age and physiological status of the animal,

Table 3. Effect of strategic supplementation with protein-energy concentrate on the kinetics of the liquid fraction of the rumen of buffalo calves

Indicators	Concentrate, g/kg LW				
	0	3	6	9	SE (±)
K Co (% h)	6.48	6.01	6.09	5.63	0.53
MRT (h)	15.84	16.76	16.48	18.13	1.47
F (Lh)	2.20	2.17	1.89	1.92	0.12
R (times/d)	1.55	1.44	1.46	1.35	0.13
V (L)	34.33	36.78	31.27	34.47	4.26
V (L/kg LW)	16.04	17.18	14.61	16.11	1.99

Table 4. Effect of the strategic supplementation with protein-energy concentrate on the kinetics of the solid fraction in the GIT of buffalo calves

Indicators	Concentrate, g kg LW^{-1}				
	0	3	6	9	SE (±)
K_1 (% h)	4.48	3.01	3.57	3.07	0.44
$MRTk_1$ (h)	26.89	47.54	31.46	37.68	7.25
K_2 (% h^{-1})	6.43	7.16	6.67	5.88	0.46
$MRTk_2$ (h)	15.96	14.25	15.81	17.03	1.11
TT (h)	12.44	12.13	16.55	17.51	2.27
MRT (h)	55.29	73.91	63.83	72.23	7.18

the management of the feeding and environmental conditions. These aspects determine, to a great extent, the time of stay of the feed in the rumen and its better use by the animal.

The k_l values were in correspondence with the $MRTk_l$ found. They allow assuming higher stay time of the particles in the rumen that could favor more efficient nutrient degradation with the concentrate increase in the diet, even though there are no differences between treatments in these indicators. Such values are similar to those obtained by Masucci *et al*. (1997) who found MRT of 65 h in adult buffalo fed different forage concentrate proportions in the diet.

For the transit time of particles (TT) there were no differences between treatments. Mean values of TT were in the range of 14.8 h. On the other hand, the MRT estimation of the solid fraction in the digestive tract of buffalo calves was between 55.29 and 73.91 h.

The similarity of the indicators of the rate of passage of the liquid and solid fractions in the different treatments could be related with the similar DMC of forage between treatments, what in turn is interrelated with the additive effect of the concentrate in the diet and the supply of feed once daily.

Supplementation with protein-energy concentrate to buffalo calves consuming poor quality forage propitiated the increase of total nutrient consumption, without altering DMC of the base feed. There was no effect of supplementation on the indicators of the passage of the liquid and solid fraction in the rumen. These results are elements to be taken into account for the development of nutritional strategies for this animal category when intended diet supplementation.

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