Complex B Vitamin Improves Performance of Mediterranean Buffalo Calves in Artificial Suckling from Birth to Weaning

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Abstract: The aim of this study was to evaluate the ponderal development, blood parameters and hematological from buffalo calves in artificial suckling supplemented with vitamin-mineral additive from birth to weaning. 18 buffalo calves were used and divided into two random groups: 1) control group; 2) group supplemented with vitamin B complex (Metacell ®). To evaluation of the ponderal development, the calves were weighed weekly and measured the thoracic perimeter, height and body length. Blood samples were collected weekly throughout the experimental period by jugular vein puncture. The group of calves supplemented with additives showed the greatest ponderal development in relation to the control group. Similarly the group of calves supplemented with additive has higher concentration of total cholesterol, urea and total protein. However there was no additive effect on the levels of glucose, albumin and β-hydroxybutyrate. The values of leukocytes, neutrophils, eosinophils, lymphocytes increased with the oral use of the additive in relation to the control group. This study supports the definition of reference values for the evaluation of buffalo calves during suckling period. The additive use to buffalo calves improves ponderal performance without significantly alter the metabolic profile.

Keyword: Buffalo, metabolic profile, ponderal development, suckling.

INTRODUCTION

Despite nutritional and genetic improvements in the calves breeding [1], the feeding management of lactating calves is defined by breeding purposes and in this context the milk price must be considered to maximize the commercial availability of milk and minimize the amount of provided milk to the calf [2]. With increasing commercial valorization of buffalo milk is important viable ways to feed the calves minimizing the consumption of breast milk to increase the availability of milk to be marketed.

The use of supplements as growth promoters brought new perspective to the meat production in buffaloes due to its proven action on muscle development [3]. Claramella *et al*. [4] emphasized that the evaluation of the performance of calves during suckling of cattle or buffalo should be performed with metabolism trials to be monitored all the metabolic routes related to nutrients absorbed at this phase of life.

It is believed that the buffalo are subject to the same diseases that affect the cattle, but to elucidate the evolution of the diagnosis and differentiation of diseases it is necessary the knowledge of the clinical and laboratory changes. However, so that these objectives can be achieved and used in its fullness, it is fundamental the knowledge of hematology reference values of healthy animals hemogram, as well as factors causing variations [5]. In this context the evaluation of the metabolic profile may reflect the nutritional status of the animal, and has been studied in order to monitor the energy balance of animals in certain physiological stages as the birth and growth of buffalo.

Thus the objective of this study was evaluate the ponderal development and blood parameters and hematological of buffalo calves in artificial suckling supplemented with vitamin B complex.

MATERIALS AND METHODS

The study was conducted in the area of Buffaloes Production in the Department of Nutrition and Production, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, in the campus of Pirassununga.

Eighteen buffalo calves (Mediterranean breed) were used and selected with similar characteristics, according to date of birth, breed and weight. The

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experimental period included the date of birth to weaning period between the months of March to August 2009 being evaluated animals until 120 days of age. At birth the animals remained with their mother until the third day of life. Then the calves were separated from their mothers and remained on picket with salt and water ad libitum receiving corn silage and concentrate throughout the experimental period (Table **1**).

The calves were divided into two groups, a control group (only milk) composed of nine animals, and a group received the experimental treatment (complex B vitamin) composed of nine animals. 4 liters/animal/day being two liters in the morning and two liters of milk in the afternoon were offered in nursing bottles at the animals. From the supply of milk in the morning the animals had access to corn silage and concentrate, providing about 3.0 kg/animal/day of silage and 1.0 kg/animal/day of ration.

The rations were formulated to meet the nutritional requirements of lactating calves with approximately 60 kg body weight, with 4 weeks of life, to provide weight gain of 0.4 kg/day. The proportion of the ingredients in total dry matter and in concentrate is shown in Table **1**.

The other experimental group of nine animals received beyond the diet described above, the compound vitamin B complex; 16.0 mg Vitamin B12; 7.15 mg Vitamin B2 and 11.0 mg Vitamin B6. Vitamin supplement daily (10ml) was administered orally by syringe.

Measurements were made weekly of the body weight (BW), withers height (WH), body length (BL) and thoracic perimeter (TP) from birth until the 16th week of life.Weekly blood samples were taken by puncture of the jugular vein, a total of 16 samples. The blood (10 ml) was collected in two vacuntainer tubes and centrifuged at 2000 rpm for 15 minutes, being a tube with anticoagulant (EDTA) to obtain the plasma and a dried tube to obtain the serum. Serum samples were sent to the Veterinary Hospital of the Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Campus of Pirassununga, for realization of complete blood count (red blood cells count, hemoglobin concentration, packed cell volume or hematocrit, leukocytes, monocytes, lymphocytes, eosinophils, basophils, neutrophils). The mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the red blood cells, hematocrit and hemoglobin.

A count of the number of red blood cells (RBC) was performed manually by hemocytometer (modified Neubauer chamber), and the results presented in millions (10^6) cells/mL. The determination of the hematocrit (Ht) was made by microtechnique, using glass capillary tubes, with the results expressed in percentage (%). To determine the concentration of hemoglobin (Hb), used the cyanmethemoglobin method, with the results expressed in grams per deciliter (g/dL).

The hematimetric indices were calculated from values obtained for red blood cells count (RBC), measurement of hematocrit (Ht) and hemoglobin (Hb), and obtained the mean corpuscular volume (MCV) in fentoliters (fL); mean corpuscular hemoglobin (MCH), expressed in picograms (pg), and mean corpuscular hemoglobin concentration (MCHC), in percentage (%). The erytrogram constituents presented parametric distribution, according to the normality test of Kolmogorov-Smirnov.

Table 1: Composition of Concentrate and Diet of the Buffalo Calves

Ingredients (% dry matter)	Concentrate	Diet
Corn silage	-	75,63
Ground corn	54,20	13,15
Soybean meal	33,80	8,21
Urea	4,10	1,00
Dicalcium phosphate	2,70	0,65
Mineral[1]	1,30	0,31
Limestone	2,70	0,65
Salt	1,30	0,31

[1]Composition per kg of mineral mix: Ca - 180g; P - 90g; Mg - 20g; S - 20g; Na - 100g; Zn - 3.000mg; Mn - 1.250mg; Fe - 2.000mg; Co - 0,0225 mg; Cu - 0,625 mg.

Serum samples were sent to the Laboratory of Biochemistry and Animal Physiology, Department of Nutrition and Animal Production, and were determined glucose values (GL), β-hydroxybutyrate (BHB), total cholesterol (TC), total protein (TP), albumin and urea. Analyses were performed using commercial kits (Laborlab®, CELM®, Randox®), by enzymatic colorimetric (endpoint or kinetic) method. The reading was performed in automatic biochemistry analyzer (System of Automatic Biochemistry SAB-200-CELM®).

Statistical analyzes were performed by analysis of variance, verifying the normality of residuals and homogeneity of variances by PROC UNIVARIATE. The degrees of freedom were defined according to the Satterthwaite (ddfm = satterth) method. The data were analyzed as repeated measures in time, and alls variables measured weekly using SAS PROC MIXED version 9.1.3 [6], and considered as fixed effect in the model, the treatment, time and interaction time * treatment according to the following model:

$$Yi = \mu + di + tj + (di*tj) + eij$$

Where: Yi = dependent variable; μ = overall mean; di = fixed effect of treatment; tj = effect of time (weeks); di*tj = treatment time interaction; ei = random error. The random effect utilized in this model was animal.

To verify the effect of interaction between treatment and weeks evaluated pdiff option was used. The averages were obtained by the LSMEANS, adopting a significance level of 5%.

RESULTS E DISCUSSION

There was an interaction (treatment x time) for the variables, average weight, average daily weight gain ($P<0.05$; Table **2**). These results can be attributed to major exposure of animals to the treatment, in other words, as higher the supply time of the additive better the development of the animals with cumulative effect. Possibly the most nutrient concentration present in the additive provided a better utilization of nutrients by calves during growth, which could be deficient in certain vitamins and minerals only with diet of milk and concentrate, although not occurred composition evaluation of body tissues in animals evaluated in this study.

The animals supplemented with mineral additive showed higher values for the variables height, length and thoracic perimeter from the first week of measurement until the 16th of life of the calves (Table **2**), with values increasing along the time. The animals of the control group and those treated had satisfactory results for these variables.

The calves group supplemented showed in general higher values for all evaluated variables such that the average daily weight gain increased at 22% when compared to the control group (Table **2**).

Table 2: Ponderal Development of Buffalo Calves According to the Experimental Treatments Until the 16th Week of Life

Item[1]	Treatments		Mean	CV (%)	Value of P		
	Control	Additive			Treat	Time	Int
Kg							
BW	42.4	45.9	44.2	8.14	-	-	-
AW	81.1	92.8	87.0	33.2	0.002	<0.001	0.002
Cm							
HB	73.7	71.0	72.3	7.88	-	-	-
AH	85.7	87.5	86.6	8.63	0.022	<0.001	0.437
BLB	57.0	58.9	58.0	8.68	-	-	-
MBL	73.0	74.7	73.9	11.57	0.027	<0.001	0.098
TPB	75.7	80.8	78.3	8.19	-	-	-
ATP	98.3	102.1	100.2	12.94	0.022	<0.001	0.800
g/day							
ADWG	0.72	0.90	0.81	23.91	0.002	0.034	0.050

[1]Birth weight (BW), average weight (AW), height at birth (HB), average height (AH), body length at birth (BLB), mean body length (MBL), thoracic perimeter at birth (TPB), average thoracic perimeter (ATP), average daily weight gain (ADWG); coefficient of variation (CV), treatment (Treat).

In a study conducted by Schammass [7], the average daily weight gain of calves was 100g/day, a value lower than those reported by [8], who observed weight gains above 350 g/day in buffalo calves fed artificially with buffalo milk. These results differing of this study where there was an average daily weight gain of 713 grams/day for the animals that received only artificial suckling and treated group showed ADWG of 906 grams/day.

In animals supplemented serum the serum concentrations of total cholesterol, urea and total protein (P<0.05; Table **3**) were higher (with or without significant difference compared to the control). However there was no effect of the additive on the values of glucose, albumin and β-hydroxybutyrate.

The average values for concentrations of total cholesterol, urea and total protein are within the reference values for bovine calves [9]. According Pogliani [10], the values of cholesterol in Holstein calves up to 2 months of age and 2-4 months were 117.1 ± 2.1 and 108.6 ± 2.0, respectively similar to those obtained in the study.

Commonly, the values of urea are used for the assessment of renal function of the domestic animals, providing support for diagnosis and/or prognosis of several nephropathies. Considering that most of the urea is synthesized in the liver from ammonia derived of the protein catabolism and intestinal absorption [9], the urea concentration in blood is used to monitor protein content of the diet of animals.

During the development of calves occurs an intense growth of body tissues thereby increasing the demand of nutrients related to the metabolism of fat, protein and minerals. The blood biochemical components most commonly determined to evaluate the metabolic profile, represent the main metabolic routes of the organism which glucose, total cholesterol and β-hydroxybutyrate represent the energy metabolism, and the concentrations of albumin, urea and total protein, represent the protein metabolism [9]. Thus, it is possible to explain the effect of time on all blood parameters analyzed: glucose, cholesterol, urea, total protein, albumin and β-hydroxybutyrate (P<0.05; Table **3**, P <0.001).

Jenkins *et al*. [11] demonstrated the influence of age on serum levels of urea in calves Holsteins. Between 4-8 weeks of age the mean value of urea of 25.7 mg/dl was higher than that obtained in this study show that the requirements of buffaloes are lower than bovine calves.

There was a time effect for concentrations of β-hydroxybutyrate (Table **3**). The calves went from pre-ruminant to ruminant, with the addition of solid diet, and as result was the development of rumen papillae, responsible for important physiological functions such as absorption, transport, protection and metabolism of short chain fatty acids, consequently the higher carbohydrate fermentation, producing volatile fatty acids, especially butyrate.

The butyric acid produced in the rumen is transported in the epithelium of the pre-stomach, *via* acetoacetate in β-hydroxybutyrate, which is the main blood ketone body [12]. Thus the values of β-hydroxybutyrate were increased, reflecting the increase in concentrate intake by animals with advancing age. The data are consistent with results of Suarez *et al*. [13] that observed increasing variations in plasma

Table 3: Blood Parameters in Function of the Experimental Treatments

Variables	Treatments		Mean	CV%	Value of P		
	Control	Additive			Treat	Time	Int
mg/dL							
Glucose	110.98	113.20	112.32	6.96	0.612	<0.001	0.083
Cholesterol	106.91	119.83	114.00	7.98	0.012	<0.001	0.249
Urea	16.90	13.92	15.40	12.57	0.010	0.006	0.129
g/L							
Total protein	4.43	4.77	4.60	5.37	0.012	0.046	0.610
Albumin	1.95	1.90	1.93	4.27	0.210	<0.001	0.722
mmo/l							
β-hydroxybutyrate	0.238	0.258	0.250	11.54	0.263	<0.001	0.622

concentrations of acetate and β-hydroxybutyrate with rumen development of calves.

Quigley *et al*. [14] evaluated changes in plasma concentrations of β-hydroxybutyrate in calves weaned, and observed increasing values of β-hydroxybutyrate during weeks, compatible with the development of the function of the ruminal epithelium. Therefore, the study shows that with the development of age the metabolic profile of animals in growth phase improves physiologically the development of animals demonstrating greater evolution of pre-stomachs.Considering the critical phase in which the calves are due to their susceptibility to infectious diseases, the hematological study allows demonstrate the conditions of tissue physiology.

The values of leukocytes, neutrophils, eosinophils, lymphocytes increased with the use of the additive oral compared to the control group (Table **4**). Due to scarcity of studies reporting the hematologic profile, mainly reference values in studies with blood cells of buffalo calves, does not allow conclusive considerations about the parameters mentioned above.

There was a time effect (P<0.05; Table **4**) for the values of red blood cells, hematocrit, hemoglobin, mean corpuscular volume, mean hemoglobin concentration, neutrophils, segmented, typical lymphocytes, neutrophils/lymphocytes relation, platelet and fibrinogen for both groups. However, the concentration values of mean corpuscular hemoglobin, leucocytes, eosinophils, basophils, monocytes and atypical lymphocytes were not affected by time (P>0.05; Table **4**).

Akhtar *et al.* [15] and Ciaramella *et al*. [4] evaluated the influence of age on the constituents of erythrogram of Murrah buffaloes. These authors verified a decreased number of red blood cells, hemoglobin concentration and hematocrit with development of age, disagreeing with the behavior found in this study, where the values of red blood cells, hematocrit and hemoglobin increased from birth until the 16^{th} week of life of the calves. Gomes *et al*. [16] studied the number of red blood cells and hemoglobin in 12 buffalo calves (Mediterranean breed) and observed average values of 8.29×10^6/ml and 13.24 g/dl, respectively, numbers very similar to those found in this study (Table **4**).

Gomes *et al.* [16] evaluated the erythrogram of buffalo calves (Murrah breed) with up to 3 months of age and found mean values for red blood cells of 8.2 ±

Table 4: Hematologic Parameters in Function of the Experimental Treatments

Variables	Treatments		Mean	CV%	Value of P		
	Control	Additive			Treat	Time	Int
(10^3/mm^3)							
Hemaceas	8.89	8.71	8.81	2.80	0.688	<0.001	0.048
Hematocrit (%)	41.52	40.42	41.05	2.40	0.388	<0.001	0.100
(*g/dl*)							
Hemoglobin	13.22	12.86	13.06	2.52	0.268	<0.001	0.260
MCV[1](fl)	46.83	46.57	46.72	1.76	0.703	0.003	0.923
MCH[1](pg)	14.90	14.82	14.87	1.66	0.599	0.005	0.593
MCHC[1] (*g/dl*)	31.83	31.83	31.83	0.64	0.815	0.234	0.123
Absolute values/mm^3							
Leukocytes	11193	15237	12943	6.71	0.022	0.123	0.244
Neutrophils	3707	4176	3910	8.07	0.018	0.009	0.139
Eosinophils	86.00	144.00	111.00	45.28	0.026	0.096	0.013
Basophils	74.00	79.00	76.00	40.17	0.962	0.354	0.774
Lymphocytes	6484	6915	6702	10.44	0.050	<0.001	0.101
Monocytes	352	326	341	15.33	0.341	0.184	0.221
Platelets	430000	366000	399800	-	0.035	<0.001	0.304
Neutr/lymph	0.64	0.70	0.68	12.12	0.426	<0.001	0.084
(g/L)							
Fibrinogen	0.73	0.69	0.71	5.80	0.238	0.002	0.397

[1]Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

1.0, 13.5 ± 0.9 for hemoglobin, 40.6 ± 3.2 for hematocrit, 50.1 ± 5.7 for MCV, 33.8 ± 3.7 for MCHC, 16.4 ± 1.6 for HCM and all the values cited similar to those observed in this study [4, 16].

Neutrophils are cells of first line of defense against infections and in inflammatory reactions. The neutrophilia may occur due to variations in stress, exercise, diet and others factors [9]. The values of neutrophils observed for the animals treated with the additive was higher (P<0.05) (4176 verses 3707 Absolute values/mm^3) compared to untreated animals, respectively (Table **4**).

Birgel Júnior *et al.* [5] observed changes in total number of neutrophils. These authors demonstrated, initially, a decrease of 3109±2067 neutrophils/mm^3, in animals with up to 3 months of age, to 1995±1211 neutrophils/mm^3 in calves of 3-6 months. They also demonstrated that the absolute number of neutrophils with rod-shaped nucleus in the blood of calves with up to three months of age (171±206 neutrophils/mm^3) and with age ranging from of 3 to 6 months (101±97 neutrophils/mm^3) differed, significantly, from those observed in adult animals, with ages ranging between 24 and 48 months (40 ± 80 neutrophils/mm^3). In relation to the absolute number of neutrophils with segmented nucleus, it was observed maximum and minimum value, respectively, in calves with up to three months of age (2938±1957 segmented neutrophils/mm^3) and with age ranging between 3 and 6 months (1894±1179 segmented neutrophils/mm^3). The differences between the average value obtained from segmented neutrophils in these animals and in those animals with age ranging between 12 and 24 months and between 24 and 48 months were, respectively, 2645±976 cells/mm^3 and 2833±1351 cells/mm^3, demonstrating the influence of age factors in bovine calves. These results indicate that the values found in this study are presented within the normal values observed for cattle in suckling phasis. However, it is necessary emphasize that there are few studies that reported values of erythrocyte and hemogram in buffalo calves becoming somewhat difficult comparisons with reference values.

The comparison of results obtained by Birgel Júnior *et al*. [5] with those obtained in this study, demonstrated significant differences, and the relationship between lymphocytes/neutrophils obtained by Birgel Júnior *et al*. [5] equal to 3.00 for the Jersey calves, values higher than found this study (Table **4**).

Peixoto *et al*. [17] found values for leucogram of Holstein calves with up to 30 days of age for segmented 4991±2201, 5376±2368 for lymphocytes, and to calves of 30 to 90 days of age; observed results of 3700±1677, 7690±1985 for values segmented and lymphocytes, respectively.

CONCLUSION

The use of Complex B vitamin for buffalo calves in suckling phases improves the ponderal performance, increases the concentrations of biochemical compounds related to energy and protein metabolism as well as influence the hematological parameters along the weeks of growth.

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