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Serum biochemical profile of neonatal buffalo calves

[*Perfil bioquímico sérico de bezerros bubalinos no período neonatal*]

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ABSTRACT

Serum blood samples from 50 Murrah buffalo calves were examined in this study. The animals were allocated into three groups according to the number of parturitions of their mothers: G1 (n= 15) calves from primiparous buffaloes, G2 (n= 19) calves from buffaloes with two to four parturitions, and G3 (n= 16) calves from buffaloes with five or more parturitions. Blood samples were taken at birth, before colostrum ingestion, at 24h, 48h, and 72h after birth, and at 7, 14, 21, and 30 days after birth for determination of levels of gammaglutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase, total protein, albumin, globulins (including immunoglobulin G), iron, total calcium, ionized calcium, phosphorus, sodium, and potassium. The age of the calves was found to influence all of the biochemical parameters, with the exception of ionized calcium and potassium in the calves in groups G1 and G3. The calving order was found to influence AST, GGT, total protein, albumin, and globulins, including IgG. The high serum ALP activity in the first two days after birth indicates that measurement of the levels of this enzyme may be used as an indirect method of assessing passive immunity transfer.

Keywords: *Bubalus bubalis*, newborn, number of parturitions, murrah, passive immunity transfer (PIT)

RESUMO

Amostras de sangue de 50 bezerros de búfalo Murrah foram examinados nesse estudo. Os animais foram distribuídos em três grupos de acordo com a paridade de suas genitoras: G1 (n=15) bezerros de búfalas primíparas, G2 (n=19) bezerros de búfalas com 2 a 4 gestações, e G3 (n=16) bezerros de búfalas com cinco ou mais gestações. Amostras de sangue foram colhidas ao nascimento, antes da ingestão de colostro e 24h, 48h, e 72h após o nascimento e 7, 14, 21 e 30 dias após nascimento para determinar níveis de gammaglutamil transferase (GGT), fosfatase alcalina (ALP), aspartato aminotrasferase (AST), creatina quinase, proteínas totais, albumina, globulina (inclusive imunoglobulina G), ferro, cálcio total, cálcio ionizado, fósforo, sódio e potássio. A idade dos bezerros influenciou todos os parâmetros bioquímicos, exceto cálcio ionizado e potássio nos bezerros dos grupos G1 e G3. A ordem de nascimento influenciou AST, GGT, proteínas totais, albumina e globulinas, inclusive IgG. Intensa atividade ALP no soro nos primeiros dois dias após nascimento indica que medidas dos níveis dessa enzima podem ser utilizados como método indireto de avaliar transferência passiva de imunidade.

Palavras chave: Bubalus bubalis, *neonato, número de gestações, murrah, transferência passiva de imunidade*

INTRODUCTION

The buffalo (*Bubalus bubalis*) is an animal of great economic importance; the global buffalo population has been estimated to exceed 194 million animals (Food…, 2017). One of the critical points in buffalo production is the neonatal period, wherein high mortality rates occur, reaching up to 17% in Murrah animals (Shivahre *et al*., 2014). This is relevant because a 20% neonatal mortality rate results in a 38% reduction in farm profit (Radostits *et al*., 2007). In all species, the neonatal period represents a critical moment in which organs must adapt to extrauterine life. This is a difficult transition from intrauterine protection to the many challenges presented by the environment (Piccione *et al*., 2009).

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Knowledge of normal values of serum biochemical parameters is important when evaluating injuries to organs and tissues caused by various diseases and in the determination of animal welfare. It also allows for monitoring the metabolic condition, functional abnormalities of the organs, and the adaptation of the organism to nutritional and physiological changes. Factors such as species, breed, age, rearing systems, feeding, and number of parturitions influence serum biochemical values; thus, the identification of these factors and their interactions is crucial for the correct interpretation of the blood parameters (Klinkon and Jezek, 2012).

The available data on buffaloes are scarce, and this leads to the use of parameters obtained for cattle, which may lead to misinterpretations of the data, particularly those obtained in the first week of life that are affected by changes related to birth and by colostrum intake (Pérez-Santos *et al*., 2015). Therefore, it is necessary to determine the serum biochemical values of healthy buffaloes to evaluate whether any alterations are due to physiological changes or pathological processes. The aim of this study was to determine the serum biochemical profile of neonatal Murrah buffalo calves and to observe the influence of the age of the calves and the number of parturitions of their dams on the values of the biochemical parameters.

MATERIAL AND METHODS

Fifty healthy Murrah buffalo calves, of both sexes, born from eutocic births, from clinically healthy buffalo cows, reared on property located in the State of São Paulo, were included in this study. The animals were raised in a semi-extensive system, with annual milk production of 300,000kg, an average of 79 lactating animals, and an average individual production of 2,765kg of milk in 300 days of lactation. After birth and natural colostrum intake, the umbilical cords of the calves were treated with 2% iodine solution. The calves remained with their mothers during the first five days of age. The calves then entered a collective pen, and were fed milk from a mammary quarter, not milked, twice a day during the milking of their dams, until weaning at 90 days old. In addition to the dairy diet, calves had access to *Brachiaria* spp. pastures and were fed a diet composed of soybeans and maize.

The calves used in the experiment were examined and considered to be clinically healthy when they did not present any alterations upon physical examination (Dirksen *et al*., 1993). The calves were allocated to three experimental groups according to the number of parturitions of their mothers: G1 (n= 15) calves (10 females and 5 males) from primiparous buffaloes, G2 (n= 19) calves (7 females and 12 males) from buffaloes with two to four parturitions, and G3 (n= 16) calves (6 females and 10 males) from buffaloes with five or more parturitions.

The venous blood samples were collected at the following moments: at birth, before colostrum intake (M0), at 24h (M1), 48h (M2) and 72h (M3) after birth and at 7 (M4), at 14 (M5), 21 (M6) and 30 (M7) days after birth. Blood samples (10mL) were collected by jugular venipuncture after local antisepsis. A vacuum collection system in siliconized tubes without anticoagulant (Vacutainer, Bencton Dickinson, Franklin Lakes, USA) was used. Blood samples were centrifuged at 2,000g for 10min, and 2.0mL aliquots of blood serum were separated and stored in plastic tubes, labeled and maintained at -20°C until the time of the laboratory tests.

Serum activities of gammaglutamyl transferase (GGT) (modified Szasz method), aspartate aminotransferase (AST) (UV-IFCC kinetics method), creatine kinase (CK) (UV method), and alkaline phosphatase (ALP) (modified Bowers and McComb method) were determined, as well as serum concentrations of total protein (biuret method), albumin (bromocresol green method), total calcium (CPC method), phosphorus (modified Daly and Ertinghausen method), and iron (modified Goodwin method), using a set of commercial reagents (Labtest Diagnóstica, Lagoa Santa, Minas Gerais, Brazil). The samples were analyzed in a semiautomatic spectrophotometer (Labquest, Labtest Diagnóstica, Lagoa Santa, Minas Gerais, Brazil), using light of appropriate wavelength for each test.

Globulins were calculated from the arithmetic difference between the total protein and albumin concentrations. Immunoglobulin G (IgG) concentrations were determined by protein fractionation using polyacrylamide gel electrophoresis containing sodium dodecyl sulfate (SDS-PAGE), according to the technique

proposed by Laemmli (1970). After fractionation, the gel was stained for 10min in 0.25% Coomassie blue solution and then bleached in 7% acetic acid solution to remove excess dye until the protein fractions were clear. IgG concentrations were determined by computerized densitometer (Shimadzu CS-9301PC, Tokyo, Japan). For reference, a marker solution (Sigma, St Louis, MO, USA) with different molecular weights was used in addition to the purified bovine IgG protein.

The serum concentrations of ionized calcium, sodium, and potassium were determined in an ion analyzer by the ion-selective electrode method (9180 Electrolyte Analyzer, Roche Diagnostics, Mannheim, Germany).

The results were evaluated by analysis of variance (ANOVA), using the Tukey test to compare the means, after verification of the homogeneity of the samples (Zar, 1999). Significance was verified at 5% probability. Statistical analyses were performed using the SAS (Statistical Analysis System) statistical program. This study was evaluated by the Ethical Committee on the Use of Animals (CEUA) of FCAV / UNESP / Jaboticabal campus and approved under protocol number 010028/2014.

RESULTS AND DISCUSSION

The serum activities of the enzymes in G1, G2, and G3 calves, from birth to 30 days old, are presented in Table 1 and Figure 1.

Table 1. Mean ± standard deviation of aspartate aminotransferase (AST), creatine kinase (CK), gammaglutamyl transferase (GGT), and alkaline phosphatase (ALP), serum activities of neonatal buffalo calves born from primiparous buffaloes (G1), multiparous with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth, before colostrum intake (M0), at 24h (M1), 48h (M2), and 72h (M3) after birth, and at 7 (M4), 14 (M5), 21 (M6) and 30 (M7) days after birth

Group	Moments							
	M0 (0h)	M1 (24h)	M2 (48h)	M3 (72h)	M4 (7 days)	M5 (14 days)	M6 (21 days)	M7 (30 days)
	Aspartate aminotransferase (U/L)							
G1	41.0±3.94Ad	108±16.3Aa	103±11.84Aab	92.5±16.4Abc	90.1±11.7Abc	87.0±14.1Ac	88.7±28.5Ac	82.8±10.7Ac
G2	41.9±15.2Ae	105±18.3Aa	97.3±16.4Aab	87.3±14.9Abc	75.3±7.85Bcd	73.9±40.1Bd	64.5±11.0Bd	67.5±13.5Bd
G3	44.9±17.3Ad	108±16.7Aa	101±17.2Aab	91.7±12.7Abc	79.9±9.66ABc	74.3±17.0ABc	76.0±28.0ABc	75.0±11.6ABc
	Creatine kinase (U/L)							
G1	107±51.0Ad	285±106Aa	179± 45.0Aab	124±28.4Acd	170±79.8Abc	213±55.6Aab	251±92.9Aab	215±92.8Aab
G2	129±66.9Ab	231±95Aa	158±102Abc	147±113Ac	128±42.6Ac	253±143Aab	207±48.0Aa	261±128Aa
G3	115±45.8Ae	324±116Aa	170±52.6Acde	123±29.4Ae	150±51.9Ade	228±108Abcd	293±162Aabc	247±45.2Aab
	Gammaglutamyltransferase (U/L)							
G1	15.3±4.84Af	1,253±797Ba	464±297Bb	319±210Ab	139±87.4Bc	58.1±32.4ABd	32.6±17.0ABe	18.9±7.58Aaf
G2	22.9±7.65Af	1,714±814Aa	714±376Ab	403±173Ac	200±106Ad	78.9±51.8Ae	43.2±34.6Af	20.5±9.21Ag
G3	16.4±2.89Af	1,074±655Ba	341±181Bb	205±110Bc	97.1±52.3Bd	44.0±20.6Be	24.4±8.48Bf	15.3±3.95Afg
	Alkaline phosphatase (U/L)							
G1	205±102Ade	1,579±901Aa	600±283Ab	338±143Ac	191±40.8Ae	130±24.6Adef	119±28.7Adf	101±32.8Af
G2	124±38.5Ad	1,250±510Aa	530±301Ab	322±174Ac	217±136Ad	143±59.5Ae	117±41.5Ae	107±46.8Ae
G3	158±45.8Ab	1,297±780Aa	465±233Aa	275±81.2Ab	194±57.9Abc	137±44.3Acde	112±32.6Ade	97.9±37.5Ae

Mean values followed by the same upper case letters in the same column and lower case letters on the same line do not differ significantly according to Tukey's test (P> 0.05).

The maximum value of AST and CK was observed 24h (M1) after birth, with significant difference between the three groups (Table 1). The abrupt elevation of the values of these two enzymes between birth (M0) and 24h (M1) after birth can be attributed to injuries to the skeletal muscle during birth and by the increase in calf muscular activity, since the animal moves to a stationary position and begins to move immediately after birth (Boyd, 1989; Kaneko *et al*., 2008). The serum activity of AST was influenced by the number of parturitions from 7 (M4) to 30 (M7) days after birth, when calves in group G1 exhibited higher serum activity of the enzyme. The same did not occur with the serum activity of creatine kinase (Table 1), which originates primarily from skeletal and cardiac muscles and is used as marker of muscle injury; its activity may be increased after exercise, long recumbence, trauma, or damage to the musculature (Kaneko *et al*., 2008). Serum activities of CK decreased until 72h (M3) after birth and increased from 7 (M4) days after birth, maintaining this value until 30 (M7) days after birth (Figure 1). This increase is expected, since the growth of the calf is accelerated in the first month of life and the animal begins to exhibit greater social interaction with the other calves of the herd, such as running, jumping, and nodding (Klinkon and Jezek, 2012).

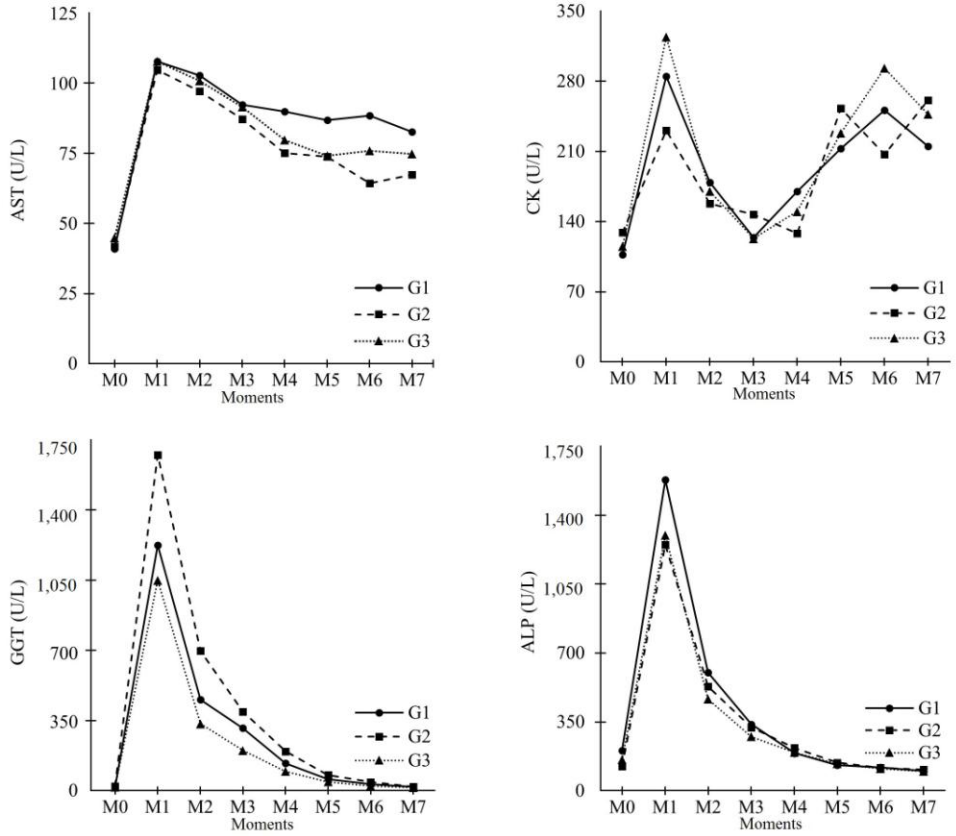

Figure 1. Serum activities of aspartate aminotransferase (AST), creatine kinase (CK), gammaglutamyl transferase (GGT), and alkaline phosphatase (ALP) of buffalo calves born from primiparous buffaloes (G1), multiparous buffaloes with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth, before colostrum intake (M0), at 24h (M1), 48h (M2), and 72h (M3) after birth, and at 7 (M4), 14 (M5), 21 (M6) and 30 (M7) days after birth.

The serum activities of GGT and ALP were influenced by age, throughout the experimental period, in all groups (Figure 1). There was an influence of the number of parturitions throughout the study, aside from the moment before the colostrum intake (M0) and at 30 (M7) days after birth (Table 1). This result differs from that reported by Feitosa *et al.* (2010), who did not find a difference in GGT values in the first 48h of life of calves of multiparous and primiparous cows, but is similar to that reported by Rocha *et al.* (2012), who observed a difference in the mean values of GGT and ALP between bovine calves born from primiparous and multiparous cows in the first 24h after birth. The buffalo calves in this study presented higher serum activity of GGT and ALP at 24 h (M1) after birth, which significantly differed from the serum activity at other moments; this result is in agreement with the results of previous studies (Knowles *et al*., 2000; Rocha *et al*., 2012; Pérez-Santos *et al*., 2015). The significant increase in the values of these enzymes between M0 and M1 is due to the absorption of GGT and ALP present in colostrum, since both are produced in large quantities by the mammary gland cells of ruminants (Kaneko *et al*., 2008). The serum GGT activity in calves after colostrum feeding is 60 to 160 times greater than the activity observed in cows; a significant correlation was previously described between the activity of this enzyme and the serum concentration of IgG (Radostits *et al*., 2007; Feitosa *et al*., 2010). The measurement of serum ALP activity is not recommended as an indirect form of evaluation of passive immunity transfer (PIT) in cattle due to the low correlation with serum IgG levels (Rocha *et al*., 2012). However, the elevation of the enzyme values at 24h (M1) and 48h (M2) after birth in buffalo calves is markedly higher than those reported in

cattle (Rocha *et al*., 2012; Pérez-Santos *et al*., 2015). Thus, the measurement of ALP in this species should be studied as an alternative to the evaluation of PIT, provided that the origin of the enzyme has been identified, since there is an increase in activity of bone isoenzyme ALP in animals with high osteoblastic activity, as in neonates (Kaneko *et al.*, 2008). The mean values of GGT and ALP enzymes decreased from 24h (M1) until 30 (M7) days after birth, at which point the enzymatic activity resembled that at the moment prior to colostrum consumption (Table 1). This reduction is a result of the degradation of the enzymes in the calf intestine over time and the process of calcification of bone epiphyses, with lower serum activity of bone isoenzyme ALP (Kaneko *et al*., 2008).

The serum concentrations of proteins in G1, G2, and G3 calves, from birth to 30 days old, are presented in Table 2 and Figure 2.

Total protein and globulin mean serum concentrations varied throughout the study period (Table 2); both parameters exhibited an abrupt increase between the time before the colostrum intake (M0) and 24h (M1) after birth. The determination of the serum total protein concentration of calves is useful as an indirect indicator of PIT, since the increase in values after the first day is primarily due to the absorption of globulins, particularly IgG, demonstrating a significant correlation with the values of total protein and globulins (Feitosa *et al*., 2010; Rocha *et al*., 2012). The serum concentrations of total protein and globulins decreased from 24h (M1) to 30 (M7) days after birth (Figure 2); this result was in agreement with previous reports (Fagliari *et al.*, 1998; Rocha *et al.*, 2012; Pérez-Santos *et al*., 2015). Such reduction is due to the degradation of the immunoglobulins acquired by colostrum ingestion. The number of parturitions influenced total protein concentrations at 24h (M1), 48h (M2) and 72h (M3) after birth, with the highest total protein levels occurring in G1 calves (Table 2). The number of parturitions also influenced the results of globulins, since the G1 calves presented higher serum globulin levels from 24h (M1) after birth until 14 (M5) days after birth. This differs from the results in bovine calves, where calves born from multiparous cows exhibited greater levels of globulins during the first week of life (Rocha *et al.*, 2012).

Table 2. Mean ± standard deviation of the total protein, albumin, globulins, and immunoglobulin G (IgG) concentrations of buffalo calves born from primiparous buffaloes (G1), multiparous with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth, before colostrum intake (M0), at 24h (M1), 48h (M2) and 72h (M3) after birth, and at 7 (M4), 14 (M5), 21 (M6), and 30 (M7) days after birth

Group	Moments							
	M0 (0h)	M1 (24h)	M2 (48h)	M3 (72h)	M4 (7 days)	M5 (14 days)	M6 (21 days)	M7 (30 days)
	Total Protein (g/dL)							
G1	4.68±0.17A	9.21±2.00Aa	8.94±1.49Aa	8.83±1.67Aa	8.34±1.30Aab	8.06±0.93Abc	7.55±0.93Acd	7.21±0.79Ad
G2	4.70±0.13A	8.36±1.03Ab	8.43±1.01Ab	8.37±1.03Ab	8.10±1.04Aab	7.68±0.90Abc	7.31±0.79Acd	6.77±0.94Ad
G3	4.88±0.32A	7.78±1.56Ba	7.97±1.65Ba	7.87±1.72Ba	7.60±1.35Aab	7.41±1.03Aabc	7.08±0.79Abc	6.77±0.59Ac
	Albumin (g/dL)							
G1	2.49±0.19A	1.95±0.24Bc	2.06±0.28Ac	2.09±0,21Bc	2.28±0.24Bbc	2.44±0.28Aab	2.46±0.20Bab	2.51±0.26Aa
G2	2.52±0.21A	2.14±0.20Ac	2.22±0.22Ac	2.34±0.25Ab	2.51±0.23Aab	2.55±0.17Aa	2.65±0.24ABa	2.58±0.37Aa
G3	2.44±0.26A	2.06±0.26Ab	2.17±0.26Ac	2.29±0.24Ac	2.46±0.17ABb	2.57±0.22Aab	2.66±0.24Aa	2.55±0.18Aab
	Globulins (g/dL)							
G1	2.19±0.08A	7.26±2.19Aa	6.88±1.71Aa	6.73±1.82Aa	6.09±1.43Abc	5.62±1.12Acd	5.09±1.05Ade	4.70±0.82Ae
G2	2.26±0.18A	6.22±1.12Ba	6.16±1.07Ab	6.04±1.05Ab	5.60±1.05ABab	5.13±0.98ABbc	4.66±0.71Acd	4.19±0.83Ad
G3	2.42±0.42A	5.72±1.65Ba	5.80±1.70Ba	5.58±1.79Ba	5.15±1.31Babc	4.84±1.06Bbcd	4.42±0.85Acd	4.22±0.59Ad
	Immunoglobulin G (mg/dL)							
G1	296±205Ac	4,093±1,558	3,682±1,207	3,642±1,289	2,913±972Ab	2,441±732Abc	1,957±647Acd	1,575±521Ad
G2	341±184Ac	3,532±858Aa	3,399±770Aa	3,107±793Aa	2,730±699Abc	2,168±577Acd	1,703±456Ade	1,387±478Aef
G3	274±203Ac	3,125±1,484	3,009±1,441	2,822±1,375	2,424±1,076Ab	1,964±859Acd	1,556±660Ade	1,356±389Ade

Mean values followed by the same upper case letters in the same column and lower case letters on the same line do not differ significantly according to Tukey's test (P> 0.05).

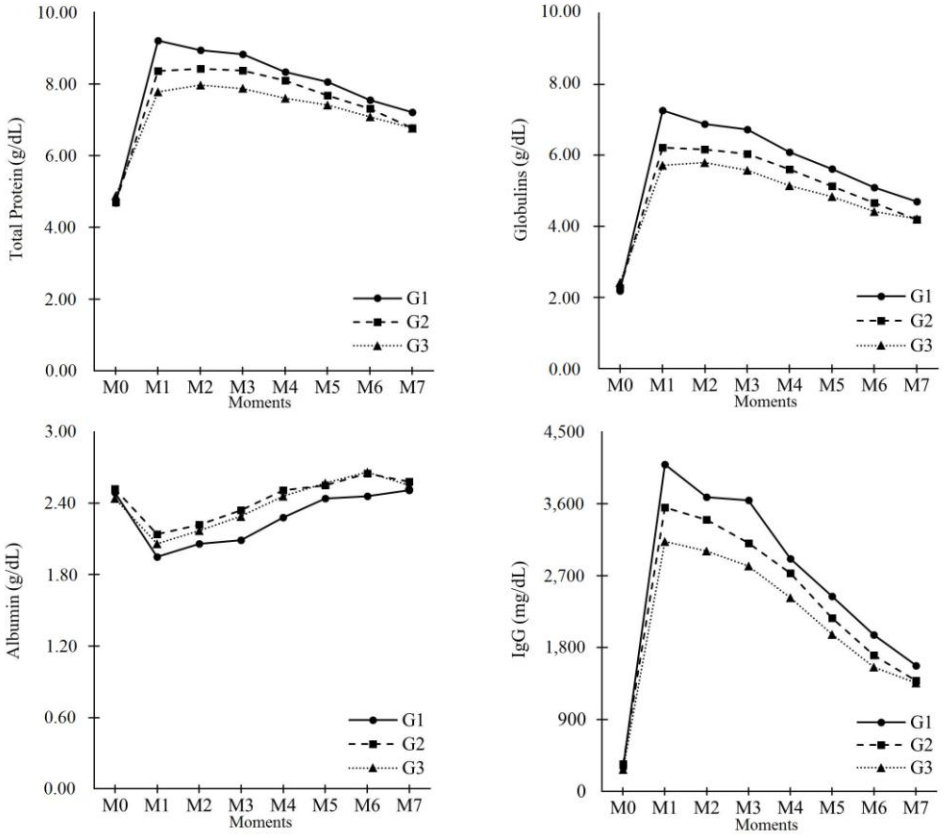

Figure 2. Serum concentrations of total protein, albumin, globulins, and immunoglobulin G (IgG) of buffalo calves born from primiparous buffaloes (G1), multiparous with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth, before colostrum intake (M0), at 24h (M1), 48h (M2), and 72h (M3) after birth, and at 7 (M4), 14 (M5), 21 (M6) and 30 (M7) days after birth.

Serum albumin concentration was influenced by the order of calving at 24h (M1) and 72h (M3) after birth, and at 7 (M4) and 21 (M6) days after birth, and the mean concentration was found to be higher in G2 and G3 calves than in G1 calves (Table 2). The buffalo calves presented variation of serum albumin concentration throughout the neonatal period, with a significant reduction in albumin concentration in the first hours after calving, most likely due to rapid plasma expansion after colostrum intake and by the absorption of immunoglobulins present in the colostrum. The serum albumin values obtained were similar to those reported in bovine calves from 24h after birth (Knowles *et al*., 2000; Piccione *et al*., 2009), when there was a gradual increase in albumin concentrations until 21 days after birth, with a subsequent decrease at the end of the first month (Figure 2). Variations in serum albumin concentrations observed over time are most likely related to the maturation of hepatic tissue and to the intake of solid food (Knowles *et al*., 2000; Birgele and Ilgaza, 2003; Kaneko *et al*., 2008).

Serum IgG concentrations peaked at 24h (M1) after birth (Table 2), with values considered optimal for PIT (Feitosa *et al.*, 2010). In contrast to previous reports in cattle (Feitosa *et al*., 2010; Rocha *et al.*, 2012), the highest serum concentrations of IgG were observed in G1 calves (Figure 2). G3 calves exhibited the lowest serum concentrations of IgG, most likely due to the lower immune capacity of the older animals, which results in a lower amount of immunoglobulin in the colostrum.

The serum concentrations of total calcium, iron, phosphorus, sodium, potassium, and ionized calcium in G1, G2, and G3 calves, from birth to 30 days old, are presented in Table 3 and Figure 3.

Table 3. Mean ± standard deviation of the total calcium, iron, phosphorus, sodium, potassium, and ionized calcium, of buffalo calves born from primiparous buffaloes (G1), multiparous with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth, before colostrum intake (M0), at 24h (M1), 48 h (M2), and 72h (M3) after birth, and at 7 (M4), 14 (M5), 21 (M6) and 30 (M7) days after birth

Group	Moments							
	M0 (0h)	M1 (24h)	M2 (48h)	M3 (72h)	M4 (7 days)	M5 (14 days)	M6 (21 days)	M7 (30 days)
					Total calcium (mg/dL)			
G1	9.44±0.72Aab	9.83±0.44Aa	10.1±0.90Aa	10.7±0.85Aa	10.5±0.85Aa	10.1±0.80Aab	10.2±0.96Aab	9.50±0.65Ab
G2	9.69±1.47Aabc	9.80±1.53Ac	10.6±1.07Aa	10.8±1.37Aa	10.9±1.28Aa	10.3±1.24Aabc	9.96±1.47Abc	9.70±1.64Ac
G3	9.98±0.82Aabc	9.48±1.06Ab	9.16±1.02Aa	10.4±0.88Aa	10.4±0.86Aa	9.76±0.96Aabc	9.87±1.16Aabc	9.19±0.85Ac
					Iron (µg/dL)			
G1	81.5±24.3Aab	98.9±32.3Ab	119±41.5Aal	112±49.9Ab	102±51.1Ab	147±94.0Aab	156±70.2Aab	214±89.2Aa
G2	89.3±41.6Aabc	74.6±39.0Ab	106±45Abcd	101±76Ab	137±133Abd	131±92.0Abcd	172±104Aac	194±75.9Aa
G3	81.3±20.7Ac	88.0±47.2Ab	135±81.9Aal	133±65.4Aal	160±141Aabc	146±70.5Aabc	213±97.0Aa	178±85.8Aac
					Phosphorus (mg/dL)			
G1	5.34±0.32Ab	7.70±1.88Aa	8.40±1.60Aa	8.94±1.44Aa	8.99±1.29Aa	8.70±0.77Aa	8.33±1.05Aa	8.13±0.60Aa
G2	5.31±0.64Ac	6.47±1.13Ac	7.77±1.84Ab	8.98±1.59Aa	9.44±0.98Aa	8.65±0.74Aab	8.53±1.02Aab	8.44±1.04Aab
G3	5.46±0.83Ad	6.70±1.83Ac	7.36±1.96Ab	9.10±1.96Aa	9.30±1.30Aa	8.61±1.04Aab	8.38±0.75Aab	8.09±1.16Aab
					Sodium (mMol/L)			
G1	136±3.78Aab	138±2.37Aa	135±1.41Aal	135±2.44Ab	136±5.53Ab	136±1.68Aab	134±2.16Ab	133±3.37Ab
G2	133±6.00Aab	141±12.8Aa	138±4.00Aa	138±8.25Aal	136±2.97Aa	133±2.59Abc	133±3.27Abc	132±4.96Ac
G3	137±2.19Aabc	137±1.24Aa	136±1.74Aal	135±2.25Aal	136±3.17Aa	134±3.21Aabc	134±3.01Abc	132±4.98Ac
					Potassium (mMol/L)			
G1	4.70±0.28Aa	4.59±0.40Aa	4.73±0.33Aa	4.81±0.39Aa	5.03±0.38Aa	4.83±0.53Aa	4.76±0.62Aa	4.79±0.33Aa
G2	4.38±0.31Aab	4.69±0.64Ab	4.81±0.50Aa	4.99±0.55Aa	5.17±0.40Aa	4.92±0.47Aab	4.99±0.46Aab	4.96±0.37Aab
G3	4.67±0.43Aa	4.72±0.35Aa	4.73±0.25Aa	4.89±0.36Aa	5.15±0.33Aa	5.00±0.49Aa	4.98±0.54Aa	4.89±0.46Aa
					Ionized calcium (mMol/L)			
G1	0.90±0.10Aa	1.02±0.09Aa	0.97±0.07Aa	0.90±0.11Aa	0.95±0.09Aa	0.93±0.07Aa	0.94±0.09Aa	0.94±0.08Aa
G2	1.01±0.09Aa	1.03±0.13Aa	0.98±0.13Aa	0.94±0.12Aa	0.93±0.09Aa	0.94±0.11Aa	0.93±0.10Aa	0.94±0.07Aa
G3	0.97±0.08Aa	1.00±0.14Aa	0.99±0.12Aa	0.90±0.10Aa	0.94±0.09Aa	0.97±0.07Aa	0.93±0.09Aa	0.97±0.07Aa

Mean values followed by the same upper case letters in the same column and lower case letters on the same line do not differ significantly according to Tukey's test (P> 0.05).

Serum concentration of total calcium was not influenced by the number of parturitions (Table 3), as previously reported in bovine calves (Rocha *et al*., 2012). The mineral concentrations varied over time (Figure 3). An increase in calcium concentration was observed from birth (M0) to 7 (M4) days old in all groups, which can be attributed to the absorption of calcium from the colostrum (Jezek *et al*., 2006). From 14 (M5) days after birth, the results changed slightly, with a reduction observed at 30 (M7) days after birth. Evaluation of serum total calcium concentration is important for the adjustment of this element in the diet and in detecting possible metabolic and kidney problems (Kaneko *et al.*, 2008).

Serum iron levels increased throughout the study (Table 3), mainly after 7 (M4) days after birth (Figure 3). The buffalo calves exhibited an increase in serum iron levels during the first month of life, in contrast to observations in cattle, wherein a decrease in iron levels can be observed until 30 days after birth with lower mean values than those of buffalo calves (Knowles *et al*., 2000). This difference is due to the higher concentration of iron in buffalo milk than in cow milk (61 ppm vs. 37ppm) (Verruma and Salgado, 1994). The serum iron concentration was not influenced by the number of parturitions (Table 3). The evaluation of serum iron levels is of great importance because this mineral participates in several metabolic processes, such as hematopoiesis, hemoglobin synthesis, activation of the cellular immune response, and pathogen-host interactions (Kaneko *et al*., 2008). Iron levels are also relevant to type 1 insulin-like growth factor (IGF-1), which is directly related to animal weight gain and growth (Prodanovic *et al*., 2014). While iron supplementation is required in bovine calves (Atyabi *et al*., 2006), it is not recommended in buffaloes during the neonatal period due to the risk of intoxication.

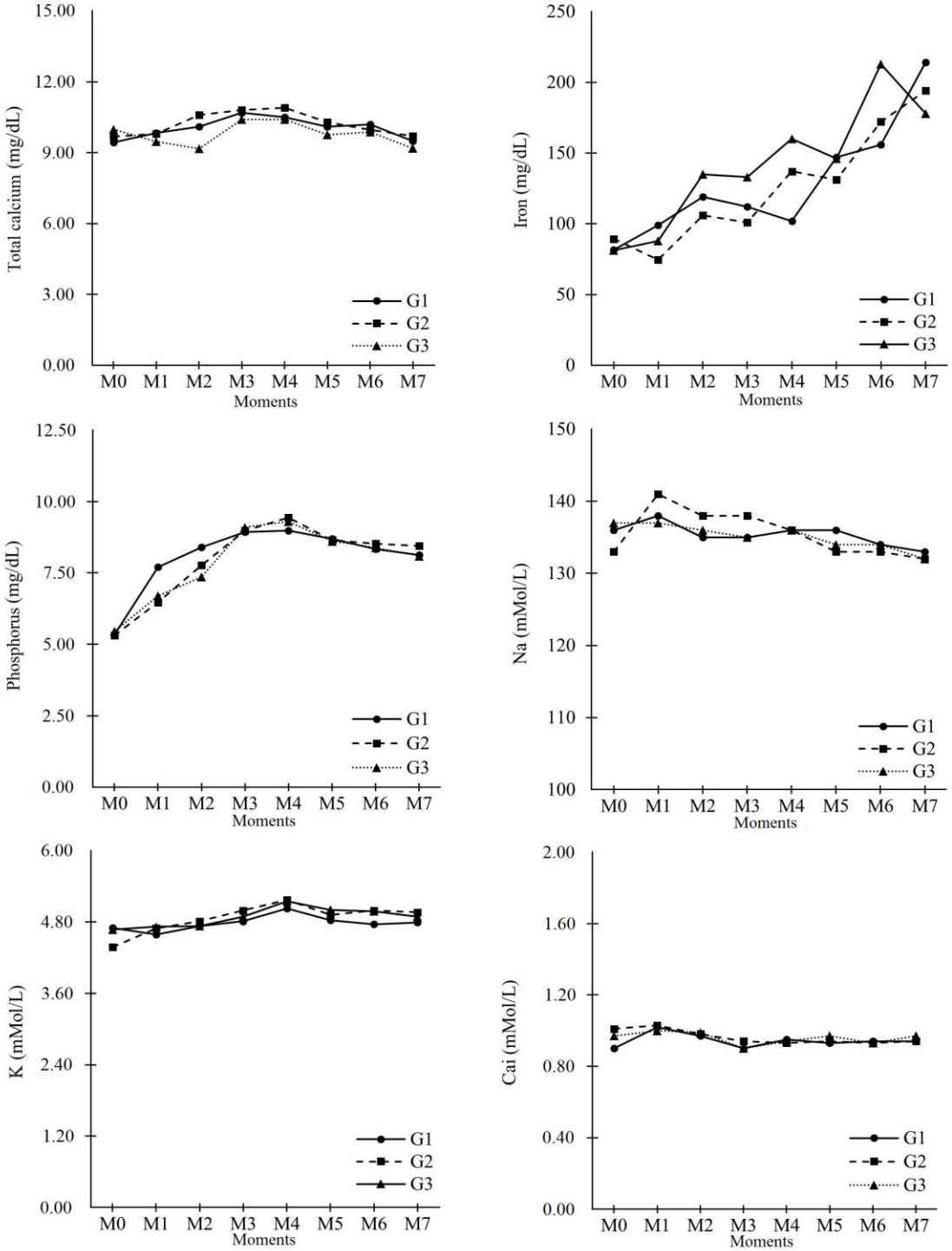

Figure 3. Serum concentrations of total calcium, iron, phosphorus, sodium (Na), potassium (K), and ionized calcium (Cai) of buffalo calves born from primiparous buffaloes (G1), multiparous with two to four parturitions (G2), and multiparous buffaloes with more than five parturitions (G3) at birth; before colostrum intake (M0); at 24h (M1), 48h (M2), and 72h (M3) after birth; and at 7 (M4), 14 (M5), 21 (M6), and 30 (M7) days after birth.

The number of parturitions did not influence the serum concentration of inorganic phosphorus (Table 3). The serum concentration of the mineral increased from birth (M0) to 7 (M4) days after birth (Figure 3), most likely due to the increased renal reabsorption of phosphate by young animals due to the action of growth hormone (Rosol and Capen, 1997). From 7 (M4) days after birth, there was a decrease in phosphorus levels until 30 (M7) days after birth. The serum concentrations of inorganic phosphorus of studied animals were higher than those previously reported in buffalo calves (Fagliari *et al*., 1998) and lower than those of bovine calves (Rocha *et al*., 2012).

The number of parturitions did not influence the serum concentrations of the measured electrolytes (Table 3). The serum concentrations of sodium, potassium, and ionized calcium exhibited slight variation over the study period (Figure 3). The ionized calcium and potassium concentrations were not affected by age in the G1 and G3 groups. Electrolyte dosing is of great importance in assessment of animal health, since electrolyte imbalances lead to changes in the pH of body fluids, blood volume, heart rate, muscle contractions, and stability of cell membranes; it is also essential for the correction of acid-base imbalances and for cation-anion balance of diets (Kaneko *et al*., 2008).

CONCLUSIONS

The age of the calves influenced the biochemical parameters, with the exception of ionized calcium and potassium concentrations in calves of primiparous and multiparous buffaloes with more than five parturitions. The number of parturitions influenced the serum activities of AST and GGT and the serum concentrations of total protein, albumin, globulins, and IgG in neonatal buffalo calves. Such variations are important because they allow for discrimination of physiological and pathological processes. The high serum activity of ALP in the first two days after birth, after colostrum intake, indicates that measurement of this parameter may be used as an indirect method to determine PIT failure. The serum iron concentrations of neonatal buffalo calves were high, and therefore contraindicate the supplementation of iron in these animals.

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REFERENCES

ATYABI, N.; GHARAGOZLOO, F.; NASSIRI, S.M. The necessity of iron supplementation for normal development of commercially reared suckling calves. *Comp. Clin. Pathol.*, v.15, p.165-168, 2006.

BIRGELE, E.; ILGAZA, A. Age and feed effect on the dynamics of animal blood biochemical values in postnatal ontogenesis in calves. *Vet. Zoot*., v.22, p.5-10, 2003.

BOYD, J.W. Serum enzyme changes in newborn calves fed colostrum. *Vet. Clin. Pathol*., v.18, p.47-51, 1989.

DIRKSEN, G.; GRÜNDER, H.D.; STÖBER, M. *Exame clínico dos bovinos*. Rio de Janeiro: Guanabara Koogan, 1993. 419p.

FAGLIARI, J.J.; SANTANA, A.E.; LUCAS, F.A. *et al*. Constituintes sanguíneos de bovinos recém-nascidos da raça Nelore (*Bos indicus*) e Holandesa (*Bos taurus*) e de bubalinos (*Bubalis bubalus*) raça Murrah. *Arq. Bras. Med. Vet. Zootec*., v.50, p.253-262, 1998.

FEITOSA, F.L.F.; CAMARGO, D.G.; YANAKA, R. *et al*. Índices de falha de transferência de imunidade passiva (FTIP) em bezerros holandeses e nelores, às 24 e 48 horas de vidas: valores de proteína total, de gamaglobulina, de imunoglobulina G e da atividade sérica de gamaglutamiltransferase, para o diagnóstico de FTIP. *Pesqui. Vet. Bras*., v.30, p.696-704, 2010.

FOOD and Agriculture Organization of the United Nations. FAOSTAT 2017. Available in: <http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor>. Accessed in: 24 Jan. 2017.

JEZEK, J.; KLOPCIC, M.; KLINKON, M. Influence of age on biochemical parameters in calves. *Bull. Vet. Inst. Pulawy*, v.50, p.211-214, 2006.

KANEKO, J.J.; HARVEY, J.W.; BRUSS, M.L. *Clinical biochemistry of domestic animals*. 6.ed. San Diego: Academic Press, 2008. 916p.

KLINKON, M.; JEZEK, J. Values of blood variable in calves. A bird's-eye view of veterinary medicine. intech. 2012. Available in: <http://www.intechopen.com/books/a-bird-s-eye-view-of-veterinary-medicine/values-of-blood-variables-in-calves>. Accessed in: 4 Nov. 2015.

KNOWLES, T.G.; EDWARDS, J.E.; BAZELEY, K.J. *et al*. Changes in the blood biochemical and haematological profile of neonatal calves with age. *Vet. Rec*., v.147, p.593-598, 2000.

LAEMMLI, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, v.227, p.680-685, 1970.

PÉREZ-SANTOS, M.; CASTILLO, C.; HERNÁNDEZ, J. *et al*. Biochemical variables from Holstein-Friesian calves older than one week are comparable to those obtained from adult animals of stable metabolic status on the same farm. *Vet. Clin. Pathol.*, v.44, p.145-151, 2015.

PICCIONE, G.; CASELLA, S.; GIANNETTO, C. *et al*. Influences of age on profile of serum proteins in calf. *Acta Vet. Beograd*, v.59, p.413-422, 2009.

PRODANOVIC, R.; KIROVSKI, D.; VUJANAC, I. *et al*. Relationship between serum iron and insulin-like growth factor-I concentrations in 10-day-old calves. *Acta Vet. Brno*, v.83, p.133-137, 2014.

RADOSTITS, O.M.; GAY, C.C.; HINCHCLIFF, K.W. *et al*. *Veterinary medicine*: a textbook of the diseases of cattle, horses, sheep, pigs and goats. 10.ed. Londres: W.B. Saunders, 2007. 2065p.

ROCHA, T.G.; NOCITI, R.P.; SAMPAIO, A.M. *et al*. Passive immunity transfer and serum constituents of crossbred calves. *Pesqui. Vet. Bras*., v.32, p.515-522, 2012.

ROSOL, T.J.; CAPEN C.C. Calcium regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: KANEKO, J.J.; HARVEY, J.W.; BRUSS, M.L. *et al.* (Eds.). *Clinical biochemistry of domestic animals*. 5.ed. San Diego: Academic Press, 1997. p.619-702.

SHIVAHRE, P.R.; GUPTA, A.K.; PANMEI, A. *et al.* Mortality pattern of Murrah buffalo males in an organised herd. *Vet. World*, v.7, p.356-359, 2014.

VERRUMA, M.R.; SALGADO, J.M. Análise química do leite de búfala em comparação ao leite de vaca. *Sci. Agric*., v.51, p.131-137, 1994.

ZAR, J.H. *Biostatistical analysis.* 4.ed. New Jersey: Prentice Hall, 1999. 663p.