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Evaluation of phosphorus levels in bones and serum of buffaloes (*Bubalus bubalis*) before and after supplementation with a selective mineral mixture[1]

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ABSTRACT.- Oliveira C.M.C., Bomjardim H.A., Silveira N.S.S., Silveira J.A.S., Faial K.C.F., Silva Filho E., Salvarani F.M. & Barbosa J.D. 2023. **Evaluation of phosphorus levels in bones and serum of buffaloes (*Bubalus bubalis*) before and after supplementation with a selective mineral mixture.** *Pesquisa Veterinária Brasileira 43:e07130, 2023.* Instituto de Medicina Veterinária, Universidade Federal do Pará, BR-316 Km 61, Saudade II, Cristo Redentor, Castanhal, PA 68740-970, Brazil. E-mail: felipems@ufpa.br

This study aimed to evaluate phosphorus (P) concentration in serum and bone, the percentage of ash and the specific bone density of buffaloes on Ilha de Marajó before and after mineral supplementation. For this study, 14 crossbred buffaloes of Murrah and Mediterranean descent aged between 18 and 36 months were used. The average values of P before supplementation in serum and bone, the percentage of bone ash and the specific bone density were 5.68mg/dL±1.18, 16.53%±0.53, 59.95%±1.96 and 1.52g/cm^3±0.32, respectively, which demonstrated P deficiency in animals raised on Ilha de Marajó. After supplementation with P for a period of seven months, the values were 6.61mg/dL±0.87, 16.90%±0.56 and 60.30%±0.95 and 1.71g/cm^3±0.21, respectively. These results showed a significant increase in P concentration in blood serum, specific bone density and percentage of P in ash (P<0.05), but there was no significant increase in the percentage of ash. The average increase in P in the serum and ash did not reach normal levels in all animals; however, 28.6% of the animals had normal values of P in serum and 50% in the ash, and 64.3% had normal specific bone density values. The nonre-establishment, in some of the animals, of the variables of P serum and bone after supplementation for seven months may have occurred as a result of the low intake of the mineral mixture and by the low concentration of P in the *Brachiaria brizantha* cv. Marandu used for feeding animals during the experiment.

INDEX TERMS: Mineral deficiencies, bone biopsy, buffaloes, *Bubalus bubalis*, Ilha de Marajó.

RESUMO.- [**Avaliação dos níveis de fósforo sérico e ósseo em búfalas (*Bubalus bubalis*) antes e após suplementação com mistura mineral seletiva.**] Objetivou-se avaliar as concentrações de fósforo (P) no soro e no osso, o percentual de cinzas e a densidade óssea específica em búfalas da Ilha de Marajó antes e após suplementação mineral seletiva. Foram utilizadas 14 búfalas mestiças de Murrah com Mediterrânea, com idades entre 18 e 36 meses. Os valores médios de P, antes da suplementação, no soro, no osso, o percentual de cinzas e a densidade óssea específica foram de 5,68mg/dL±1,18, 16,53%±0,53, 59,95%±1,96 e 1,52g/cm^3±0,32, respectivamente, o que demonstra deficiência de P nos animais criados na Ilha de Marajó. Após a suplementação com P por um período de sete meses os valores foram 6,61mg/dl±0,87, 16,90%±0,56 e 60,30%±0,95 e 1,71g/cm^3±0,21 respectivamente. Esses resultados caracterizam um aumento significativo nas concentrações de P no soro sanguíneo, na densidade óssea específica e no percentual de P nas cinzas (P<0,05), porém

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não houve um aumento significativo no percentual de cinzas. O aumento médio nos valores de P no soro e nas cinzas não alcançou patamares de normalidade em todas as búfalas, entretanto 28,6% delas tinham valores normais de P no soro e 50% nas cinzas, 64,3% tinham valores normais da densidade óssea específica. O não restabelecimento, em parte dos animais, das variáveis ósseas e sanguíneas após suplementação com P durante sete meses pode ter ocorrido em virtude da baixa ingestão da mistura mineral e da baixa concentração de P em *Brachiaria brizantha* cv. Marandu utilizada para alimentação dos animais durante o experimento.

TERMOS DE INDEXAÇÃO: Deficiências minerais, biópsia óssea, bubalinos, búfalas, *Bubalus bubalis*, Ilha de Marajó.

INTRODUCTION

Phosphorus (P) performs several functions in animals that are related to the transfer of energy, composition of bones, teeth, cell membranes and nucleic acids. It is also considered a growth factor for the ruminal microbiota, participating in the control of ruminal pH and acid-base balance (Breves & Schroder 1991, Underwood & Suttle 1999, Satter et al. 2005).

In Brazil, phosphorus deficiency affects cattle and buffaloes in different regions, and there is no doubt that this deficiency is the most common and economically important mineral disorder (Tokarnia et al. 2010). The occurrence of this deficiency is very common on Ilha de Marajó (Cardoso 1997, Barbosa et al. 2005, Pereira & Cardoso 2009, Oliveira et al. 2009, Pinheiro et al. 2011), which is characterized by low-fertility soils (Falesi 1972, Kendall et al. 1974) and low-nutritional-value pastures (Teixeira Neto et al. 1991).

Even with these adverse characteristics, Ilha de Marajó has a herd of approximately 670,000 bovids, and of this total, approximately 310,000 are buffaloes (IBGE 2020). In general, these animals are reared in systems characterized by a lack of technical information by producers, low technology diffusion, low zootechnical indices and high rates of infectious and contagious diseases (Arima & Uhl 1996). According to Barbosa et al. (2005), among the diseases that affect buffaloes and cattle in the state of Pará, mineral deficiencies stand out because they cause severe losses in productivity, being a limiting factor for raising these animals in the region if there is no adequate mineral supplementation. Given the above information, this work aimed to determine the levels of P in blood serum and bone, as well as the percentage of ash and specific density in the bone of buffaloes from the Ilha de Marajó before and after selective mineral supplementation.

MATERIALS AND METHODS

Animals and place of experiment. The experiment was conducted in accordance with the National Council for Animal Experimentation (CONCEA), and it was submitted to the Animal Use Ethics Committee of the "Universidade Federal do Pará" (CEUA-UFPA) and approved under license registration number 8117280421.

The study was carried out on a property located in the northeast region of the state of Pará. Fourteen crossbred Murrah and Mediterranean buffaloes, aged between 18 and 36 months, were maintained on two properties located in the municipality of Cachoeira do Arari on Ilha de Marajó, state of Pará. The animals were raised in an extensive production system on native pastures and without mineral supplementation.

During the entire experimental period, the animals were kept on pastures of *Brachiaria brizantha* cv. Marandu, with fertilization of 100kg/hectare/year triple superphosphate and 100kg/hectare/year urea, and received mineral supplementation and water *ad libitum*. The experiment lasted 210 days.

Animal weight control. To adjust for weight gain, the animals were weighed at the beginning and end of the experimental period. Weighing was performed in the morning without fasting the animals.

Fecal production determination and dry matter intake. To estimate P intake by grazing, purified lignin was used as an external marker (LIPE®) (Saliba 2005), and indigestible neutral detergent fiber (iNDF) were used as internal markers. LIPE® was also used to estimate fecal output. Five hundred milligrams capsules containing LIPE® were orally administered to the animals in three periods for five days in each period, two days for adaptation and three days for feces collection.

Administration periods were from 11/11 to 11/15 and 12/12 to 12/17 in 2011 and from 10/01 to 14/01 in 2012. Feces were collected from the rectal ampulla, placed in plastic bags and frozen. The three fecal samples from each animal, obtained at the end of each collection period, were mixed and homogenized to form a composite sample, forming three composite samples per animal at the end of the experiment. These feces were used to estimate fecal production (FP) in kg of dry matter (DM)/day and iNDF.

Concomitant with the collection of feces, pasture was collected through simulated grazing to determine the NDF of the pasture. Samples were collected on the same day as the first fecal collection of each experimental period. After collection, pasture samples were placed in plastic bags and frozen until analysis. Subsequently, the feces and pasture samples were thawed, weighed and placed in an oven with forced ventilation, set at a temperature of 55°C for 72 hours and ground in Willey-type mills with a 1mm sieve. Then, LIPE® from the fecal samples was analyzed at the Animal Nutrition Laboratory of the "Escola de Veterinária" (Veterinary School) of the "Universidade Federal de Minas Gerais" using a Varian 099-2243 spectrophotometer with an infrared light detector (FTIR). The FP was calculated as the logarithmic ratio of the spectral bands between wavelengths 1050nm and 1650nm (Saliba et al. 2003). The formula according to Saliba (2005) was used to calculate the PF.

The quantification of iNDF in feces and pasture was obtained after incubation in the rumen of fistulated buffaloes, where 0.8g was placed in TNT bags (4cm x 4cm). The bags were then placed in plastic nets and incubated for 288 hours. After ruminal incubation, the bags were washed in running water and boiled for one hour in a neutral detergent solution (Van Soest & Robertson 1985) and then washed with hot water and acetone, and the residue was considered NDF.

Dry matter intake (DMI) based on iNDF was estimated using the equation DMI=FPx(CIF%/CIP%), where FP is the fecal production and CIF is the concentration of the indicator in the feces and CIP is the concentration of the indicator in the pasture.

Phosphorus determination in the pasture. To determine P, part of the pasture samples collected were used to determine the iNDF. P was determined according to the methodology developed by Carmo et al. (2000) for dry leaf analysis. For P reading, vanadate yellow spectrometry was used (Malavolta et al. 1989).

Mineral supplementation. The animals were supplemented for 210 days with a selective mineral mixture (SMM) containing the following composition per kilogram: sodium (Na), 196.5g; phosphorus (P), 97.5g; copper (Cu), 875mg; cobalt (Co), 126mg; selenium (Se),

36mg; and zinc (Zn), 1010mg (adapted from Tokarnia et al. 2010). The mineral sources used were sodium chloride, dicalcium phosphate, copper sulfate, sodium selenite and zinc sulfate. The control of the average consumption of minerals was carried out by measuring the amount of mineral mixture offered and the leftovers in the trough at the end of the experimental period. SMM was offered to the animals in a covered trough with lateral protection against rain.

Blood collection and measurement of P in the blood serum. Blood samples were collected upon arrival of the animals from Ilha de Marajó and at the end of the experimental period. Collection was performed using sterile 5ml vacuum tubes. The serum was obtained by centrifugation (800 × g for 10 minutes), stored in polyethylene bottles (Eppendorf) and frozen at -20°C until analysis.

The analyses of inorganic P levels in blood serum were carried out at the Veterinary Clinical Pathology Laboratory of the "Instituto de Medicina Veterinária" (Faculty of Veterinary Medicine) of the UFPA using commercial kits (Cepa®) and the readings obtained in a semiautomated biochemical analyzer (BIOPLUS – BIO 2000, SP, Brazil).

Bone collection and measurement of P in bone. Bone samples were collected through biopsies performed in the upper third of the 12th intercostal space on the right side using a professional automated impact drill, model GSR 14.4 VE-2, coupled to a Starret 25mm hole saw with guide support A01 – 3/8 chuck. To perform the biopsies, the animals were initially sedated intramuscularly with 2% xylazine hydrochloride at a dose of 1mL for each 100kg of body live weight. After sedation, animals were properly restrained with ropes and kept in left lateral decubitus.

The region of the upper right third of the 12th rib was washed with clean water, and a neutral detergent was applied to carry out a wide trichotomy. Then, local anesthesia of the intramuscular and subcutaneous infiltration type was performed using 40mL of 2% lidocaine hydrochloride. Subsequently, a second wash of the clipped area was performed with water and neutral detergent, followed by proper disinfection using 10% iodized alcohol.

An incision of approximately 10cm was made in the skin of the 12th rib, followed by blunt dissection of the subcutaneous and muscle tissue to expose the rib. Then, two fragments (2.5cm in diameter) of bone tissue from the cranial region of the rib were removed. The fragments were placed in plastic bags and frozen at -20°C until analysis.

After removing the bone fragments, the peritoneum was sutured with a simple #0 catgut. Then, the subcutaneous tissue was sutured with a simple continuous pattern using #0.50mm nylon thread. Finally, approximation of the skin was performed with nylon thread #0.80mm with a Wolf-type suture pattern. The animals were treated with a single dose of 20mg/kg oxytetracycline and flunixin meglumine at a dose of 2.2mg/kg body weight. The surgical wound was treated daily using a topical ointment (based on zinc oxide, pine oil, kaolin and xylene) until healing. The biopsies were repeated at the end of the experimental period in the same region as the previous one, and fragments of size, thickness and vitality similar to those of the first biopsy were removed to avoid influencing the final result.

After thawing at room temperature, all soft tissue and bone marrow material were removed. Then, the samples were weighed on an analytical balance to obtain the fresh weight and placed in a test tube with 10mL of water to determine the volume of displaced water. After this procedure, the samples were dried in an oven at 105°C for 12 hours and degreased with ethyl ether in the Goldfish extraction for 48 hours. After drying and free of fat, the samples were weighed, calcined in a muffle furnace at 600°C for 12 hours and crushed with an agate mortar and pestle to obtain ash (adapted from Fick et al. 1980).

For the determination of P in the bone, the ash of the samples were weighed between 0.25 and 0.26g and placed in a Teflon digestion tube (Xpress model) by digestion with 3mL of 65% nitric acid P.A., 1 mL of 30% hydrochloric acid and 1mL of 30% hydrogen peroxide P.A. and then diluted with deionized water to form solutions for P analysis (adapted from the techniques of Nomura et al. 2005).

The analysis was carried out at the Toxicology Laboratory in the Environment Sector of the "Instituto Evandro Chagas" using ion chromatography in the ICS 2000 DUAL system (THERMO SCINTIFIC-DIONEX, USA).

Determination of specific density and percentage of ash in bone. The calculation of bone density was performed considering the formula d=m/v, expressed in g/cm^3, according to the description by Fick et al. (1980). The percentage of ash in the bone was determined according to the recommendations of Mendes (1977). All results are expressed as a percentage based on fat-free DM.

Statistical analysis. The data were initially submitted to the Kolmogorov–Smirnov normality test and presented a Gaussian distribution. Means and standard deviations were determined descriptively. The *t*-test was used to compare moments (before vs. after SMM supplementation). The significance level considered was 5%. All analyses were performed using the BioEstat 5.0 computer program (Ayres et al. 2007).

RESULTS

The average results of iNDF in the feces and in the pasture, of the FP and DMI in the pasture, with the average values of the initial, final and average weights, the percentage of DMI in relation to the average live weight and the average daily weight gain are described in Table 1 and 2.

The individual values of the initial, final and average weight, as well as the DMI and the DMI in relation to the percentage of live weight (%LW), the weight gain in the period (WG) and the average weight gain (AWG), are related in Table 2.

The average percentage of P in the pasture ingested by the animals, the average consumption of SMM, the consumption of P through the pasture and the SMM, and the average total consumption of P by the animals are shown in Table 3.

The reference values for the bovine species of bone and blood serum variables and the mean values obtained from buffaloes before and after supplementation with SMM are described in Table 4.

The percentages of deficient and subdeficient animals and animals with normal levels of blood and bone variables evaluated before and after supplementation with SMM are described in Table 5 and 6.

DISCUSSION

The iNDF of feces was below the values found by Soares et al. (2009) (36%) in buffalo fed *Pennisetum purpureum* Schumach.

Table 1. Mean values and standard deviation (SD) of indigestible neutral detergent fiber (iNDF) in feces, pastures and fecal production (FP) in dry matter (DM) of buffaloes during the experimental period

Variables	Mean ± SD
iNDF (feces) em %	24.75 ± 2.29
iNDF (pasture) em %	15.95 ± 0.82
FP (kg/DM)	3.19 ± 0.04

Table 2. Initial, final and average weight, dry matter intake (DMI) in kg/day and percentage of live weight (% BW), weight gain in the period (WG) and average daily gain (ADG) during the experiment

Animal	Initial weight (kg)	Final weight (kg)	Average weight (kg)	DMI (kg)	DMI (%BW)	WG (kg)	ADG (g)
312	200	365	282.5	5.07	1.79	165	785.7
313	227	370	298.5	5.43	1.82	143	681.0
314	153	305	229.0	4.30	1.88	152	723.8
315	158	330	244.0	5.23	2.14	172	819.0
316	285	405	345.0	4.86	1.41	120	571.4
317	400	455	427.5	4.19	0.98	55	261.9
318	385	400	392.5	5.15	1.31	15	71.4
319	385	445	415.0	5.07	1.22	60	285.7
320	405	435	420.0	5.13	1.22	30	142.9
321	325	380	352.5	4.05	1.15	55	261.9
322	305	350	327.5	5.47	1.67	45	214.3
323	370	435	402.5	4.73	1.18	65	309.5
324	400	425	412.5	4.99	1.21	25	119.0
325	340	380	360.0	5.63	1.56	40	190.5
Mean ± SD	309.9 ± 91.40	391.40 ± 45.12	350.64 ± 66.40	4.95 ± 0.48	1.47 ± 0.34	81.60 ± 56.06	388.40 ± 266.96

Table 3. Phosphorus (P) percentage in the pasture (%PP), P intake through pasture (PIP) and mineral mixture (MM), mineral mixture intake (MMI) and total P intake (TPI) in buffaloes during the trial period

Animal	%PP	PIP (g)	MM (g)	MMI (g)	TPI (g)
312	0,17	8.68	34.6	3.37	12.05
313	0.17	9.14	34.6	3.37	12.52
314	0.17	7.25	34.6	3.37	10.62
315	0.17	8.92	34.6	3.37	12.30
316	0.17	8.20	34.6	3.37	11.57
317	0.17	8.81	34.6	3.37	12.19
318	0.17	8.65	34.6	3.37	12.02
319	0.17	8.66	34.6	3.37	12.03
320	0.17	8.75	34.6	3.37	12.12
321	0.17	8.24	34.6	3.37	11.61
322	0.17	9.33	34.6	3.37	12.70
323	0.17	8.09	34.6	3.37	11.46
324	0.17	8.57	34.6	3.37	11.94
325	0.17	9.48	34.6	3.37	12.85
Mean ± SD	0.17 ± 0.0	8.63 ± 0.57	34.6 ± 0.0	3.37 ± 0.0	12 ± 0.57

Table 4. Reference values for bovine species and mean values of phosphorus (P) in blood serum and ash, specific density and percentage of bone ash of buffaloes from Ilha de Marajó before and after seven months of supplementation with selective mineral mixture (SMM)

Variables	Reference			Mean ± SD	
	Deficient	Subdeficient	Normal	Before SMM	After SMM
P in blood serum (mg/dL)[A]*	< 4.0	4.0-7.0	> 7.0	5.68 ± 1.18b	6.61 ± 0.87a
P in ash (%)[A]*	< 17		> 17	16.53 ± 0.53b	16.90 ± 0.56a
Bone specific density (g/cm)[B]*	< 1.69		> 1.69	1.52 ± 0.32b	1.71 ± 0.21a
Ash (%)[C]	< 66.8		> 66.8	59.95 ± 1.96b	60.30 ± 0.95b

[A] Riet-Correa & Timm (2007), [B] Valdes et al. (1988), [C] Little (1972); * Reference values for cattle described by these authors; a,b = values in the same line with different letters are statistically different (P<0.05).

The percentage of iNDF of *Brachiaria brizantha* was in agreement with that determined by Perreira et al. (2012), who verified values from 12.9 to 17.2%. The FP values were higher than those found by Soares et al. (2009) in buffaloes with an average weight of 300kg and fed Cameron grass (*Pennisetum purpureum* Schumach), who verified an average PF of 2.1kg of DM using LIPE® as an external marker. The greater production of feces by the buffaloes in this experiment possibly occurred due to the higher average weight of the animals (391.4kg).

The average DMI (4.95kg) and the average DMI of the animals in relation to live weight (1.47%) are below the values stipulated by Punia & Singh (2001), who described that DM requirements for buffaloes between 300 and 400kg and with a GPM of 500g per day are from 5.1 to 9.7kg, which corresponds to between 1.7 and 2.4% of the live weight. The low DMI by the animals in this experiment may have occurred due to the handling and the aloof behavior of the animals presented throughout the experimental period. During handling for placement of the external marker (LIPE), the animals were restrained in a squeeze chute, and the nose tong was used to facilitate the application of the marker orally; this procedure caused trauma in this region during each period of LIPE® application, and it was observed that the animals did not completely fill the rumen. According to Borges (2007) and Soares et al. (2009), the excessive manipulation of the animals during the processes of application of external markers of digestibility and FP can cause stress and significantly decrease DMI. When evaluating the percentage of DMI, it can be seen that in animals 312, 313, 314, 315 and 316 that had more docile behavior, the average DMI (1.81%±0.26) was higher than the average DMI (1.28%±0.20) of the other animals that had less docile behavior.

The AWG between the periods before and after supplementation was 81.60kg±56.06, with an average daily gain of 388.40g±266.96. When comparing the WG values and the daily AWG, it was verified that the most docile animals had a daily AWG (716.2g±97.0) higher than the other animals (210g±80). This is explained by the higher DMI of these animals. The AWG values observed in this study were lower than those obtained by Cardoso et al. (2008) in two experiments with mineral supplementation in buffaloes aged between eight and ten months for 14 months. In these experiments, the GP ranged from 550 to 580g per day. The greater weight gain in these experiments may have occurred due to the greater growth of these animals, as they were young animals and were in full body development.

The average percentage of P in the pasture (0.17%) verified between the three pasture collection periods is much lower than that established by Terramoccia et al. (2005) (0.32%) as minimum values of P in the pasture to meet the daily requirements of maintenance and weight gain of 500g per day in buffaloes with weights between 300 and 400kg. When adding the P intake through the pasture and the mineral mixture, an average intake of 12 g±0.57 was obtained.

According to Punia & Singh (2001), the daily P requirements for buffaloes weighing between 150 and 500kg and with a daily weight gain of 500g are 9 to 16g. It is possible that the intake of P through the pasture is underestimated since the intake of DM is below the daily requirements of the animals. Another factor that contributed to a lower P intake was the low consumption of SMM, which was approximately 34.6g per day, which contributed to an average P intake of only 3.37g per day. Tokarnia et al. (2010) reported that the consumption of 70g of a mineral mixture containing 9% P would be enough to meet the needs of this mineral for growing animals, considering the intake of P through forage. The consumption values of the mineral mixture are much lower than those found by Cardoso et al. (2008), who

Table 5. Number and percentage of deficient and subdeficient animals and mean values of phosphorus (P) in serum and bone, specific density and percentage of ash in the bone of 14 buffaloes before supplementation with selective mineral mixture (SMM)

Variables	Deficient			Subdeficient			Normal		
	Amount	%	Mean ± SD	Amount	%	Média ± DP	Amount	%	Mean ± SD
P in blood serum (mg/dL)[A]*	1	7.2	3.8 ± 0.0	10	71	5.35 ± 0.70	3	21.4	7.4 ± 0.38
P in ash (%)[A]	10	71.4	16.26 ± 0.34				4	28.6	17.2 ± 0.22
Bone specific density (g/cm^3)[B]*	9	64.3	1.33 ± 0.20				5	35.7	1.87 ± 0.14
Ash (%)[C]*	14	100	59.95 ± 1.96				-	-	-

[A] Riet-Correa & Timm (2007), [B] Valdes et al. (1988), [C] Little (1972); * Reference values for cattle described by these authors.

Table 6. Number and percentage of deficient and subdeficient animals and mean values of phosphorus (P) in serum and bone, specific density and percentage of ash in the bone of 14 buffaloes seven months after supplementation with selective mineral mixture (MMS)

Variables	Deficient			Subdeficient			Normal		
	Amount	%	Mean ± SD	Amount	%	Média ± DP	Amount	%	Mean ± SD
P in blood serum (mg/dL)[A]*	-	-	-	10	71.4	6.19 ± 0.56	4	28.6	7.68 ± 0.51
P in ash (%)[A]	7	50.0	16.45 ± 0.42				7	50.0	17.35 ± 0.20
Bone specific density (g/cm^3)[B]*	5	35.7	1.46 ± 0.09				9	64.3	1.84 ± 0.10
Ash (%)[C]*	14	100	60.3 ± 0.95				-	-	-

[A] Riet-Correa & Timm (2007), [B] Valdes et al. (1988), [C] Little (1972); * Reference values for cattle described by these authors.

reported an average intake of 77.14g of a mineral mixture for buffaloes and 83.18g of a conventional mineral mixture used for cattle in buffaloes. Possibly the greater consumption of the mineral mixture in these experiments may have occurred due to the lower sodium concentrations (5 and 7%) when compared to the Na concentration (19.5%) of the SMM used in this experiment. The authors attributed the difference in consumption to Na concentrations of 7% in the mineral mixture with the lowest consumption and 5% in the one with the highest consumption. However, Na concentrations in the mixtures are far below the needs of buffaloes (4 to 6g for each 100kg of live weight) (Sekerden 2001). Viana (2006), in two experiments with supplementation carried out with buffaloes raised in upland areas and in floodplain areas that were influenced by the tides (brackish water), it was found that in upland animals, the consumption of the mineral mixture was higher than in animals from the floodplain area, despite the use of a mineral mixture with low sodium concentration (7.4%) and with the addition of 13.9% corn bran. It is probably necessary to use a mineral mixture without sodium and with the addition of a higher concentration of corn bran, wheat bran or sugarcane molasses to encourage consumption by animals in flooded areas.

Mean blood serum P levels before and after SMM supplementation increased significantly ($P<0.05$) from 5.68±1.18mg/dL to 6.61±0.87mg/dL. Even with a significant increase, these values are below the reference values (>7mg/dL) for cattle with normal values (Riet-Correa & Timm 2007). P values in blood serum of 5.51±1.03mg/dL (Oliveira et al. 2009) and 6.26±1.81mg/dL (Pinheiro et al. 2011), similar to this study and considered subdeficient, have also been described in buffaloes from Ilha de Marajó. A mean value of P in blood serum of 2.67mg/dL±0.79, lower than that of this study, was found by Mahmood et al. (2013) in buffaloes with parturient hemoglobinuria. Jayachandran et al. (2013) described mean values of P in blood serum of 4.22mg/dL±0.13, below those found in this study, in buffaloes in *postpartum* anestrus and of 6.15mg/dL ±0.17, similar to this work, in buffaloes that were cycling. Regarding the response of P levels in blood serum to mineral supplementation, Sharma et al. (2002) also observed a positive response (increase from 4.29mg/dL±0.42 to 5.46mg/dL±0.44) in buffaloes after ingesting 40g of a mineral mixture containing 31.25% phosphate dicalcium for 75 days in northern India.

The mean percentage of P in ash increased significantly ($P<0.05$) from 16.53%±0.53 to 16.90%±0.56 after SMM supplementation. However, when compared with the reference values for cattle, it appears that, on average, the animals remained with P concentrations in the bone below normal values. Possibly, these findings are a consequence of the low intake of the mineral mixture verified in this study. Bone P concentrations below reference values were also described by Cardoso (1997), Pereira & Cardoso (2009) and Pinheiro et al. (2011) in different studies carried out in buffaloes in Ilha de Marajó, which is a region with soils and pastures known to be deficient in P.

The specific density values increased from 1.52g/cm^3±0.32 to 1.71g/cm^3±0.21 after mineral supplementation ($P<0.05$). This reveals that, on average, the bone density values reached normal levels when compared with the reference values (Table 4). Low bone density values of 1.59g/cm^3±0.18 were also described by Pinheiro et al. (2011) and 1.46g/cm^3 by Pereira & Cardoso (2009) in buffaloes from Ilha de Marajó without mineral supplementation. The mean percentage of bone ash before and after supplementation was 59.95%±1.96 and 60.30%±0.95, respectively. When comparing these values with the reference ones, it is verified that the mineral supplementation was not enough to increase the ash concentrations in the animals' bones. Bone ash concentrations of 60.24 and 60.87%, similar to the values of this study, were verified by Pereira & Cardoso (2009) and Pinheiro et al. (2011) in buffaloes from Ilha de Marajó without mineral supplementation.

When comparing the P values found in the serum before supplementation with SMM with the reference values, it was verified that one animal (7.2%) presented P values below 4 mg/dL, which indicates deficiency; 10 animals (71.4%) had values between 4 and 7mg/dl, which characterizes subdeficiency, and three (21.4%) had normal values above 7mg/dL. After supplementation, 10 buffaloes (71.4%) still had P values between 4 and 7mg/dL (6.19±0.56 mg/dL), and four (28.6%) had normal P levels (Table 6). A high percentage of animals with P subdeficiency was also described by Oliveira et al. (2009) and Pinheiro et al. (2011) in buffaloes from Ilha de Marajó, where 94.05% and 48.08% of the animals presented values between 4 and 7mg/dl, respectively.

Evaluating P concentrations in the bone before SMM supplementation, we found that 10 (71.4%) animals adopted mean P values of 16.26%±0.34 and that four (28.6%) adopted values of 17.2%±0.22, which is considered normal when compared to cattle. Similar findings were reported by Pereira & Cardoso (2009) and by Pinheiro et al. (2011), who, when evaluating the P concentrations in the bone of buffaloes from Ilha de Marajó, verified that 81.8% and 57.3%, respectively, presented P values in the bone of buffaloes below the recommendations for bovines. Cardoso (1997) reported a similar finding in buffaloes on Ilha de Marajó. Before the experiment, 64.3% of the animals had bone density values below the reference values used, and 100% of the animals had a percentage of ash below the reference values. After supplementation, 35.7% of the animals had bone density below the reference values, but the ash percentage remained below the reference values in 100% of the animals (Table 6). The percentage of animals with low bone density before supplementation is similar to that found by Pinheiro et al. (2011), who found that 70.79% of the 104 buffaloes evaluated on Ilha de Marajó had bone density values below 1.69g/cm^3. These authors also found that 100% of the evaluated animals had bone ash concentrations below the value used as a reference. However, the bone density values of 1.84g/cm^3±0.10 presented by 64.3% of the animals after the experiment are higher than those found by Prabowo et al. (1991), who found values of 1.75g/cm^3 in buffaloes in Indonesia. It is possible that the increase in phosphorus concentrations in bone, blood and bone density occurred due to mineral supplementation; however, the variation in the individual response of the animals may have occurred due to the variation in the intake of the mineral mixture.

CONCLUSION

According to the bone and blood parameters evaluated, it can be concluded that most buffaloes from Ilha de Marajó in this experiment were deficient in phosphorus (P). The low recovery of bone and blood variables after the experimental

period may have occurred due to the low intake of the mineral mixture offered to the animals and the low concentration of P in *Brachiaria brizantha* cv. Marandu used for animal feed.

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