

Bone Radiographic Changes in Slaughter Buffalos with Low Body Condition Index

Antonio Carlos Cunha Lacreta Junior¹, Washington Luiz Assunção Pereira²,
José Augusto Pereira Carneiro Muniz³, Mariana Avelino de Souza Santos⁴,
Thâmira Mota¹ & Luthesco Haddad Lima Chalfun¹

ABSTRACT

Background: The largest buffalo herd in Brazil is located on the Island of Marajó, in the State of Pará, northern Brazil. The pastures of the Island of Marajó consist of low quality graminaceous plants, which are generally poor in protein and mineral content. Unbalanced diets associated with low quality pastures are responsible for latent, sub-clinical diseases and metabolic disorders in bovines which affect bone health, especially in periods such as pregnancy and lactation. The purpose of this study was to point out and to describe the radiographic bone changes of buffalos with low body index bred in extensive system and intended for slaughter on the Island of Marajó, Brazil.

Materials, Methods & Results: Radiographic examinations of anatomical pieces were obtained from 34 animals of buffalo species, with no distinction of gender, age, or breed. The animals were selected among those that were in the stockyard waiting for slaughtering for the obtainment of the anatomical pieces. For this selection, low physical condition was considered, which mainly included individuals with body condition indexes (ICC) of 1 and 2, on a scale of 1 to 5. From this selection, 98 anatomical pieces were obtained, which included: 28 sets of ribs, 20 femurs, 26 metacarpus, 7 mandibles, 3 radius and ulnas, 4 sets of vertebrae, 4 sets of metacarpus and phalanges, 1 tarsus and 1 set of tarsus and metatarsus. All the pieces were separated, identified, packed in plastic bag and forwarded to the radiographic study. At least one radiographic projection was obtained of each anatomical piece. These were identified, manually processed and stored for subsequent assessment. A single observer, in order to identify and to describe the bone radiographic changes, subjectively performed the radiographic assessment.

Discussion: Bone changes were remarkable and in animals of this study, reinforcing the nutritional aspect as being of great importance for the perfect mineral homeostasis and for the osteoarticular system maintenance. Consistent radiographic findings with osteopenia are most often related to nutritional disorders that affect bone metabolism, mainly involving the homeostasis of calcium (Ca) and phosphorus (P). The nutritional hyperparathyroidism, more commonly reported in dogs, cats and exotic animals is a common example of these affections, in which the bone radiopacity reduction is the most evident radiographic aspect. Copper (Cu) deficiency has been correlated with osteochondrosis, epiphyseal fracture of the femoral head and degenerative arthropathy of the hip joint, and erosion of the articular cartilage in a deer (*Cervu selaphus*). Degenerative arthropathy through radiographs was also found in this study. From the bone radiographic analysis, it is concluded that the osteodystrophic diseases of buffalos raised in pasture system on the Island of Marajó, Pará, Brazil, present a variety of pathological conditions and the most commonly found were: osteoporosis characterized at the radiographic examination for the bone decreased radiopacity, change in bone trabeculae (medullary expansion) and cortical thinning, followed by pathological fractures with high incidence in the ribs. The low body condition, the underdevelopment and cachexia states of the animals in this study indicate the lack of an appropriate prophylactic conduct and a proper feed management. Therefore, the low reserves of P and Cu in the organism may have contributed to the osteoporotic process and, possibly, also to the protein-energy deficit, leading to secondary bone changes and causing a lack of productivity in the herd.

Keywords: buffalo, rib, fracture, mineral, osteoporosis, radiology.

INTRODUCTION

The largest buffalo herd in Brazil is located on the Island of Marajó, in the State of Pará, Brazil. According to the Ministry of Agriculture (2013) data, this region possesses approximately 320,335 thousand heads of buffalo bred in extensive livestock farming system, in which the animals are kept in large pasture areas, mostly consisting of native vegetation covering the dry fields [31]. In addition to the low nutritional quality pastures, arranged on dystrophic soils, the mineral supplementation for these animals is scarce [26].

Unbalanced diets associated with low quality pastures are responsible for latent and sub-clinical diseases, along with metabolic disorders in the bovine bones, especially in critical periods such as pregnancy and lactation [22].

The osteodystrophies can be divided into osteopenia and osteomegalia. Among the osteopenia cases, the osteoporosis, a disease in which the nutritional deficit causes low production of osteoid tissue with consequent decrease in bone tissue and skeletal system weakening [15], is highlighted. The osteomegalias in turn is a result of multifactorial processes, with genetic, nutritional and environmental etiology. Nutritional osteodystrophies are classified as osteoporosis, rickets, osteomalacia and fibrous osteodystrophy [11]. The latter preferably affects equine and porcine, also being reported in chameleons with dietary restriction to calcium (Ca), vitamin A, and cholecalciferol [13].

The aim of this study was to identify and to describe the bone radiographic changes observed in buffalos with low physical condition, raised in the fields of the Island of Marajó, Pará, Brazil.

MATERIALS AND METHODS

Animal selection

For this study, the radiographic examinations of anatomical pieces were obtained from 34 animals of buffalo species, with no distinction of gender, age, or breed. They were raised on the Island of Marajó in an extensive production system and were intended for slaughter in the city of Belém, State of Pará, Brazil.

The animals were selected among those that were in the stockyard waiting for slaughtering for the obtainment of the anatomical pieces. For this selection, the low physical condition was considered, which included individuals with body condition indexes (ICC)

of 1 and 2, on a scale from 1 to 5 [28]. The ICC is a subjective method generally adopted in cows, which considers the relative accumulation of body fat and the thinness state, on which the 1 and 2 index represents the too skinny and thin animal, respectively [1]. This selection also considered the animals with ICC over 2, under the condition of showing at least one clinical sign of osteodystrophy, such as: underdevelopment, body bone conformation changes, step abnormalities, claudication, spine deformations, limbs and pelvic girdle.

Bone selection

After the selected animals were followed on the slaughter line, their carcasses were inspected in order to identify the skeletal changes not previously noticed on the physical examination, and to separate the anatomical pieces for the radiographic evaluation. In this selection 98 anatomical pieces from a total of 34 animals were obtained, which included: 28 sets of ribs, 20 femurs, 26 metacarpus, 7 mandibles, 3 radius and ulnas, 4 sets of vertebrae, 4 sets of metacarpus and phalanges, 1 tarsus and 1 set of tarsus and metatarsus. Some of the pieces were chosen by a noticed visual change regarding the bone anatomy and others were randomized, as long as it was from an animal that presented 1 and 2 ICC. All the pieces were separated, identified, packed in plastic bag and forwarded to the radiographic study.

Radiographic examination

For the radiographic examination of the anatomical pieces, the x-ray equipment Intecal® model CR-7¹ was used with a power of 100 Ma and 100 Kv. The radiographic technique was established according to the anatomic specimen thickness, at a focus-film distance of one meter. At least one radiographic projection was obtained of each anatomical piece. When possible, a second projection was also taken in orthogonal plan. AGFA®² films of different sizes were used, depending on the piece dimension. These were identified, manually processed and stored for subsequent assessment.

A single observer, in order to identify and to describe the bone radiographic changes, subjectively performed the radiographic assessment.

RESULTS

Radiographies presenting bone cortex thinning with medullary expansion were observed, especially in long bones (metacarpus); widespread

reduction of bone radiopacity (osteopenia) in several bones (Figure 1); focal areas of osteolysis (Figure 2); increased size of bone trabeculae (coarse trabecular pattern or trabecular rarefaction) [Figure 3], primarily reaching the ribs, vertebrae and mandibles.

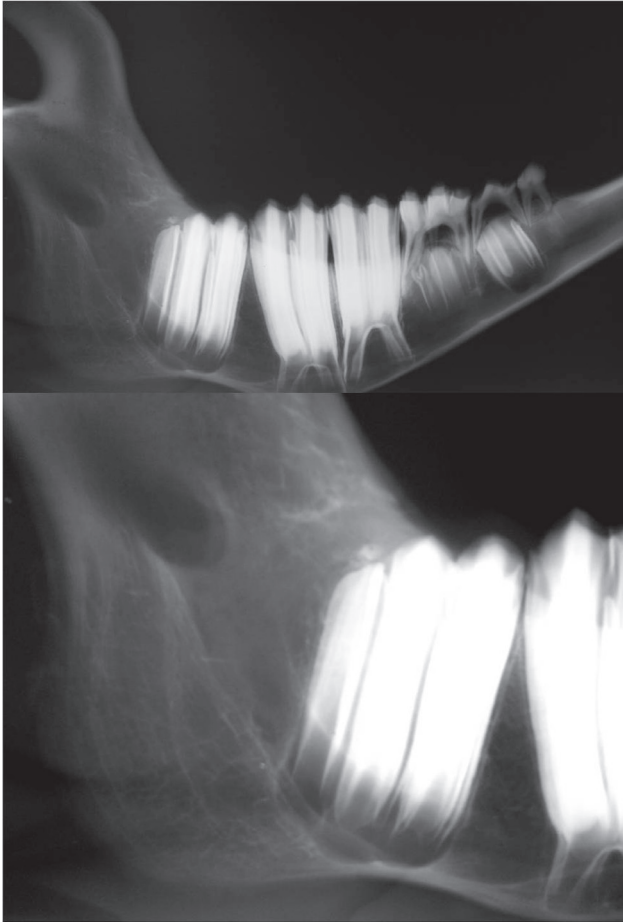


Figure 1. Laterolateral projection radiographic image in the jaw. Noted decreased bone radiopacity and coarse bone trabeculation.

Recent and old fractures were identified. The recent ones were characterized by diverse configuration fracture line (transverse, oblique, and spiral), with little or no periosteal reaction (Figure 4). Meanwhile, the old fractures showed exuberant and irregular periosteal reaction (bone callus) with bone sclerosis on the fragmented area edges, when the fracture line was still present (Figure 5). In most cases, the bone callus was poorly mineralized (Figure 5). The major bones that showed fractures were the ribs, tibia, mandible, metacarpus, phalanges. The ribs were the most affected.

In the radiographies of younger animals, it was possible to identify the growth line and bone proliferations were observed (osteophytes) at the distal physis margin of the femurs and the radiuses (Figure 6), as



Figure 2. Radiographic image in mediolateral projection of the femur, which are noted irregular areas of osteolysis.

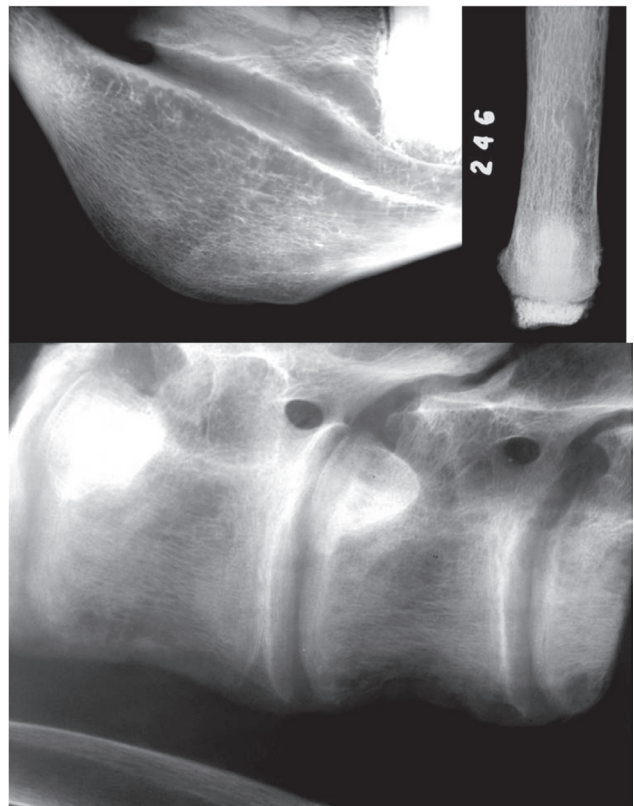


Figure 3. Radiographic images of the jaw, ribs and vertebrae showing severe changes in trabecular pattern of cancellous bone. Note the coarse trabecular pattern with areas of osteolysis and cortical thinning.

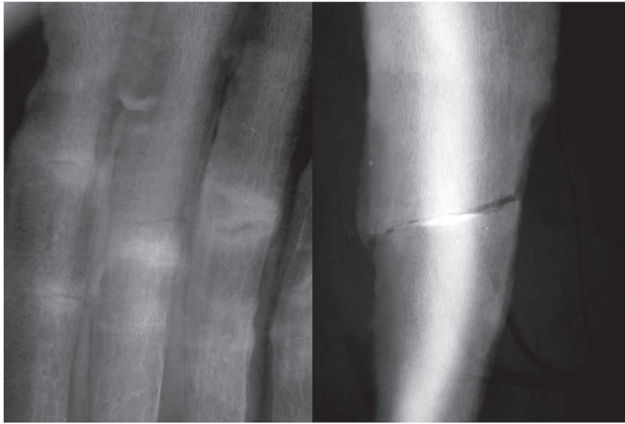


Figure 4. Radiographic images of the ribs, where it is possible to identify various pathological fractures, in addition to generalized osteopenia.



Figure 5. Radiographic images of metacarpal and ribs old fractures.

well as intense bone sclerosis adjacent to the radiuses and ulna bones physis (Figure 7).

In the joints, especially the distal ones (phalangeal metacarpus and interphalangeal), it could be observed: peri-articular proliferation, decreased joint space, subcondral bone irregularity and sclerosis, bone deformities, increased volume and dystrophy calcifications in peri-articular soft tissue (Figure 8).

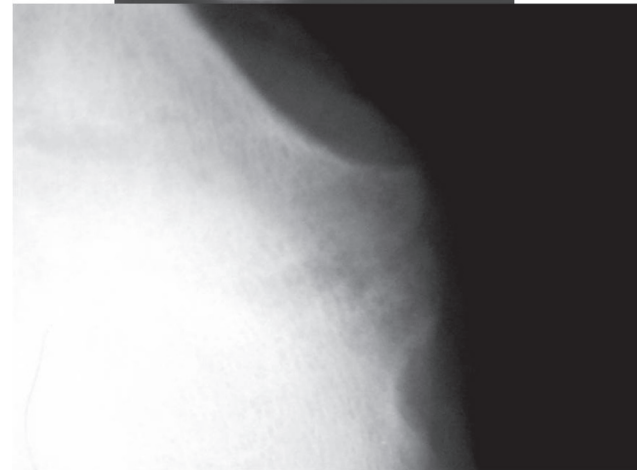
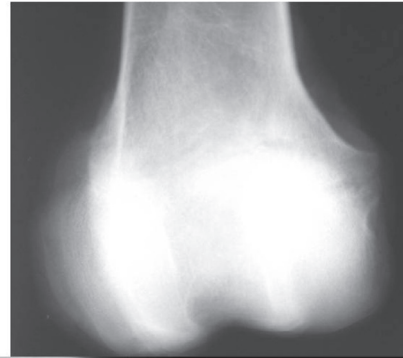


Figure 6. Radiographic image of the distal metaphyseal region of the femur. In which we note the margins of bone proliferation metaphyseal region.

DISCUSSION

The bone is a tissue that provides support to the mechanical, biological and chemical functions in the animal organism and its development can be affected by age, nutrition, hormonal phase, diseases and by intense exercise [15]. Therefore, it cannot be considered a static tissue, on the contrary: it is a target tissue that undergoes modifications due to the adverse systemic conditions that affect animals, mainly those intended for production.

Bone changes secondary to nutritional factors have already been described in several species. Studies in mice have shown the effects of food restriction on bone growth, with the reduction in bone formation, in addition to the decreased cellularity, cell size and cellular composition changes in bone [19]. The animals of this study were notably malnourished (ICC 1 and 2), and known to be from regions where the lack of minerals in soils and pastures is common [10,24]. In fact, as it had been hypothesized, the bone changes were remarkable and in animals of this study, reinforcing the nutritional aspect as being of great importance for the perfect mineral homeostasis and for the osteoarticular system maintenance.

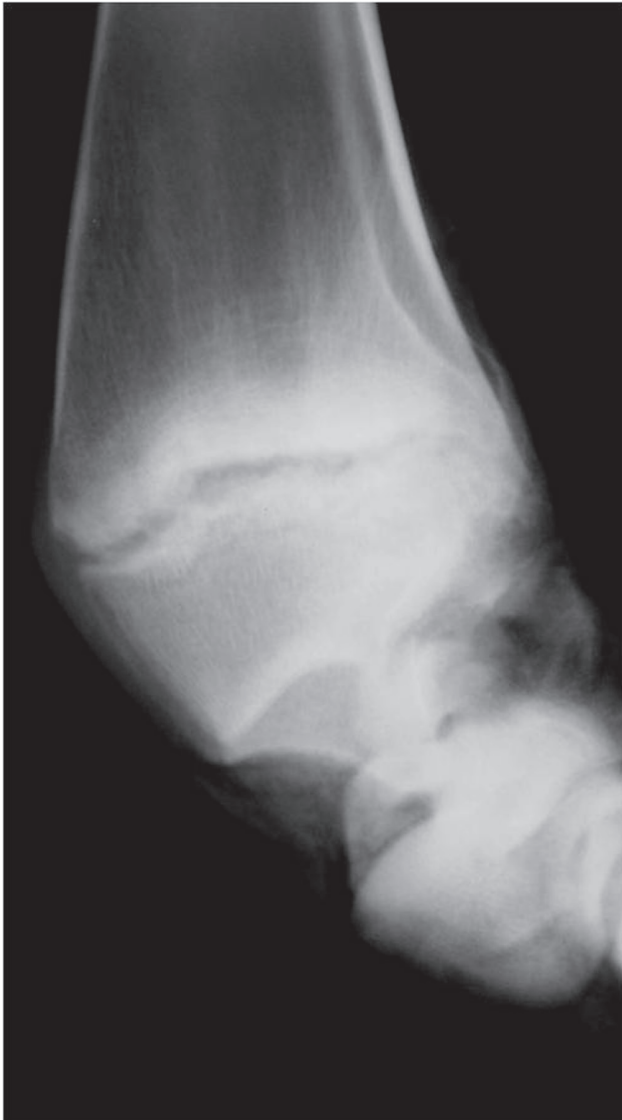


Figure 7. Radiographic image of the distal metaphyseal region of the radio. Note the intense bone sclerosis adjacent physal line.

Consistent radiographic findings with osteopenia are most often related to nutritional disorders that affect bone metabolism, mainly involving the homeostasis of calcium (Ca) and phosphorus (P). The nutritional hyperparathyroidism, more commonly reported in dogs, cats and exotic animals is a common example of these affections, in which the bone radiopacity reduction is the most evident radiographic aspect [12]. The radiographic findings of bone changes encountered in this experiment could be related and were consisted with nutritional disorders, occurring more frequently bone rarefaction lesions (osteopenia).

Mineral deficiencies are among the main occurrences in ruminants bred in the pasture system in Brazil [3]. The P deficiency is prevalent in tropical countries, including Brazil [24], and it has already

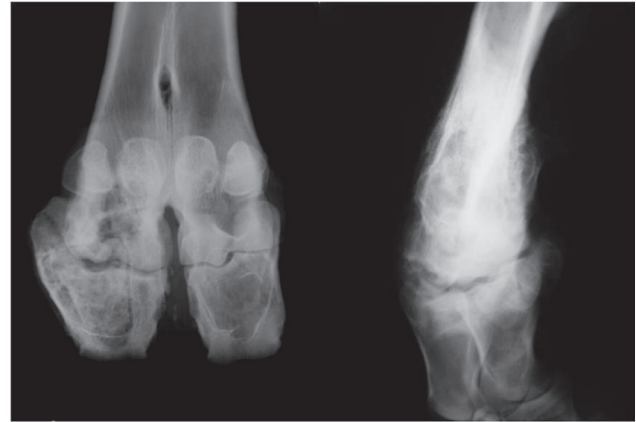


Figure 8. Radiographic images of the metacarpal-phalangeal, the note is severe degenerative arthropathy.

been identified in buffalos from the Island of Marajó and from some other cities in the State of Pará [3,10].

The nutritional problems on the Island of Marajó, regarding the breeding in pasture regime, were reported by several authors. When analyzing the soil, gramineous and tissues (blood and bone), it had been demonstrated the lack of Ca and P in the soil and in the native gramineous, in addition to high levels of acidity and concentrations of iron (Fe) and aluminum (Al) in the soil; in the bones, the Ca and P are faulty, mainly in the drought season [8].

The deficiency of these minerals (Ca and P) influences in the formation, development and maintenance of the skeletal system, predisposing the animals to osteodystrophies. Low concentration diets of P in cattle (5.13 to 6.62 g/P/day) result in demineralization, decreased bone mass and osteomalacia [29]. At histopathological bone examination of Ilha do Marajó's animals presenting low body index, it is possible to notice, osteoporotic patterns of various intensities [23]. Bone phosphorous and hepatic copper (Cu) deficiency in buffalos was also observed in animals at the same region [24,26].

Young bovines with Cu deficiency are subjected to osteoporosis changes in the entire skeleton, with a decrease in bone intensity by rarefaction of trabeculae and thickness reduction of cortical bone. In the radiographic findings of buffalos in this condition, the possible findings are bone radiopacity reduction, bone lysis, cortical thinning, and coarse or rarefied trabecular pattern, running with osteopenia, possibly secondary to osteoporosis and osteomalacia, which are conditions directly related to the mineral deficiency, particularly P and Cu [17].

Osteodystrophic diseases in buffalos raised in pasture system on the Island of Marajó do not present exclusively a single pathological condition. In most animals, osteoporosis and osteoidosis changes coexist [24]. The consequences of osteoporosis are related to the widespread weakening of trabecular and cortical bones and bone fractures and deformities may exist [25]. Osteoporosis resulting of P deficiency in the diet is due to the mobilization of these mineral reserves in the bones, being this deficiency tolerated for a short period of time without showing clinical signs [16]. If the deficiency is prolonged or severe, it will cause abnormalities in bones and teeth, with a high probability of development of claudication and locomotion difficulty.

Therefore, as reported by other authors [3, 25], several configurations of pathological fractures and changes in trabecular bone pattern were identified through radiological examination of the anatomic pieces of the animals in this study, mainly in the ribs. Necropsy findings in buffalos with phosphorus deficiency detected the presence of bone callus in different bones [3], which was also observed in the radiographies previously mentioned, especially in the ribs, resulted from old fractures.

The utilization of rib bone fragments as phosphorus levels indicators in cattle have been used successfully [27] suggesting the sensitivity of this specific bone to the adequacy of homeostasis of minerals in cattle. We believe that the ribs may be more susceptible to the demineralization of bone mineral deficiencies, which is the main reason why there was a greater incidence of fractures in these bones.

Cu deficiency has been correlated with osteochondrosis, epiphyseal fracture of the femoral head and degenerative arthropathy of the hip joint, and erosion of the articular cartilage in a deer (*Cervu selaphus*) [2]. Degenerative arthropathy through radiographs was also found in this study.

Radiographic changes observed in physis (growth lines) of the femur and metacarpus of animals in development in this study were very similar to those observed in dogs with nutritional osteopathy and in foals and cattle with physeal dysplasia. The nutrition osteopathy in dogs is characterized by sclerosis of the metaphyseal regions that also feature flat to the radiographic examinations [20]. Thus, in the physeal dysplasia of foals, the physis are sclerotic to the radio-

graphic examination, both bone proliferation (marginal osteophytes) and, sometimes, with subchondral defects similar to osteochondrosis [5]. These affections tend to occur in opposition to the excessive supplementation of minerals and not to shortages, as noticed in the animals of study.

Physeal dysplasia in foals appears to be sclerotic at radiographic examination, also presenting bone proliferation (marginal osteophytes) and sometimes osteochondrosis-like subchondral defects [5]. These conditions often occur due to the excessive mineral supplementation and not to its deficiencies, as observed in the animals. The physeal dysplasia in cattle has also been correlated with the age of the animals, being observed in 52.9% of a group of up to 36 months of age confined animals [14]. Copper deficiency was identified as a determinant factor of epiphysitis in young cattle⁴ and such deficiency may also be involved in physisitis (physeal dysplasia) observed in the radiographs of this experiment. In Ca deficiency the most significant injuries occur in the long bones, in the epiphysis of the proximal humerus and distal radius, ribs and femur, and the ends of the tibia and ulna in ruminants and especially in the growth plate of the distal metacarpal bone growth and metatarsus [21], regions of which we have also identified radiographic changes involving the physis.

Studies considered that the clinical evaluation by itself is not able to determine, accurately, the focal lesion extent and nature, diagnosed in the cattle locomotor system [14]. It is assumed that the safest and the most correct procedure for the bone diseases diagnosis secondary to mineral deficiencies is the clinic-pathological inspection of the herd, in addition to the chemical analysis of animal tissues [24] and to the imaging examinations, particularly the radiographic examination. Therefore, is it crucial to create important tools for treatment protocols and for mineral supplementation propositions, thus avoiding the premature mutilation or discarding of buffalos raised in extensive pasture system under poor nutrients soils and graminaceous.

From the bone radiographic analysis, it is concluded that the osteodystrophic diseases of buffalos raised in pasture system on the Island of Marajó, Pará, Brazil, present a variety of pathological conditions and the most commonly found were: osteoporosis characterized at the radiographic examination for the bone

decreased radiopacity, change in bone trabeculae (medullary expansion) and cortical thinning, followed by pathological fractures with high incidence in the ribs.

The low body condition, the underdevelopment and cachexia states of the animals in this study indicate the lack of an appropriate prophylactic conduct and a proper feed management. Therefore, the low reserves of P and Cu in the organism may have contributed to the osteoporotic process and, possibly, also to the protein-

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MANUFACTURERS

¹Casa do Radiologista - Centro Brasileiro Eletro-Médicos Ltda. Rio de Janeiro, RJ, Brazil.

²Agfa-Gevaert N.V. Mortsel, Belgium.

Declaration of interest. The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

- 1 Alapati A., Kapa R.S., Jeepalyam S., Rangappa S.M.P. & Yemireddy K.R. 2010. Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. *Journal Veterinary Science*. 11(1): 1-8.
- 2 Audigé P., Wilson P.R., Morris R.S. & Davidson G.W. 1995. Osteochondrosis, skeletal abnormalities and enzootic ataxia associated with copper deficiency in a farmed red deer (*Cervus elaphus*) herd. *New Zealand Veterinary Journal*. 43(1): 70-76.
- 3 Barbosa Neto J.D., Oliveira C.M.C., Duarte M.D., Albernaz T.T., Oliveira Júnior C.A., Riet-Correa G. & Riet-Correa F. 2007. Phosphorus Deficiency in Buffaloes in the State of Pará, Northern Brazil. *Italian Journal Animal Science*. 6(Supl 2): 971-973.
- 4 Blood D. & Radostits O.M. 1992. *Clínica Veterinária*. 8.ed. Rio de Janeiro: Guanabara Koogan, 412p.
- 5 Butler J., Colles C., Dyson S., Kold S. & Poulos P. 2008. *Clinical radiology of the horse*. 3rd edn. Sterling Heights: Wiley-Blackwell Publishing, 748p.
- 6 Carciofi A.C. & Oliveira L.D. 2011. *Doenças Nutricionais*. Disponível em: <<http://veterinariosnativa.com.br/books/Doencas-Nutricionais-Silvestres.pdf>>. [Accessed online in April 2013].
- 7 Cardoso E.C., Teixeira Neto J.F., Silva A.W.C., Veiga J.B., Vale W.G., Souza Filho A.P.S. & Alencar N.X. 1992. *Deficiência mineral em bubalinos no Município de Santa Maria, Estado do Pará*. Comunicado Técnico 71. Belém: Embrapa-Cpatu, pp.1-4.
- 8 Cardoso E.C., Teixeira Neto J.F., Veiga J.B., Oliveira R.P. & Vale W.G. 1997. Calcium and phosphorus deficiency in water buffaloes raised on the Island of Marajó, State of Pará, Brazil. *Buffalo Journal*. 13(1): 15-22.
- 9 Cardoso E.C., Teixeira Neto J.F., Silva A.W.C., Veiga J.B., Vale W.G. & Alencar N.X. 1993. *Embrapa. Deficiência de cálcio e fósforo em bubalinos no município de Portel, Estado do Pará*. Disponível em: <<http://www.infoteca.cnptia.embrapa.br/handle/doc/380486>>. [Accessed online in March 2013].
- 10 Cardoso E.C., Vale W.G., Veiga J.B. & Simão Neto M. 1999. Condição mineral de bubalinos e bovinos na Ilha de Marajó, Estado do Pará. *Revista Brasileira de Medicina Veterinária*. 21(5): 197-202.
- 11 Doige C.E. & Weisbrode S.E. 1998. *Patologia Veterinária Especial de Thomson*. 2.ed. Porto Alegre: Artes Médicas Sul, 672p.
- 12 Farrow C.F. 2006. *Veterinária Diagnóstico por imagem do Cão e Gato*. São Paulo: Editora Roca, 768p.
- 13 Hoby S., Wenker C., Robert N., Jermann T., Hartnack S., Sener H., Aebischer C.P. & Liesegang A. 2010. Nutritional Metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptratus*) and its prevention. *Journal Nutrition*. 140(11): 923-931.
- 14 Lima I. R. 2009. Estudo Clínico e Radiográfico das Extremidades Distais dos Membros Locomotores de Bovinos Machos de Corte. 70f. Goiânia, GO. Dissertação (Mestrado em Ciência Animal) - Programa de Pós-graduação em Ciência Animal, Universidade Federal de Goiás.
- 15 Martig S., Chen W., Lee P.V. & Whitton R.C. 2014. Bone fatigue and its implications for injuries in race horses. *Equine Veterinary Journal*. 46(4): 408-415.
- 16 Masters H. & McCormick B. 1998. Phosphorus for cattle and sheep. Disponível em <<http://www.agric.wa.gov.au/agency/pubns/farmnote>>. [Accessed online in April 2012].

- 17 Mills C.F., Dalgarno A.C. & Wenham G. 1976. Biochemical and pathological changes in tissues of Friesian cattle during experimental induction of copper deficiency. *British Journal of Nutrition*. (35): 309-331.
- 18 Monnier J.P., Tubiana J.P. & Lederman H.M. 1999. *Manual de Diagnóstico Radiográfico*. 5.ed. Rio de Janeiro: Medsi, 478p.
- 19 Nicodemo M.L.F., Moraes S.S., Thiago L.R.L.S., Caxias E.L., Macedo M.C.M., Pires P.P., Madruga C.R., Vaz E.C. & Barrocas G.E. 2005. Metabolismo Ósseo de Vacas Jovens Nelore em Pastos de *Brachiaria brizantha* Suplementadas ou não Durante a Seca, com Fósforo, Cálcio e Concentrado. *Revista Brasileira de Zootecnia*. 34(1): 316-326.
- 20 Owens J.M. & Biery D.N. 1982. *Radiographic Interpretation for the Small Animal Clinician*. Saint Louis: Ralston Purina Company, 207p.
- 21 Palmer N. 1993. *Pathology of Domestic Animals*. 4th edn. Orlando: Academic Press, 318p.
- 22 Pereira W. L. A. 2000. Ocorrência de Osteodistrofias em Bubalinos Procedentes da Ilha de Marajó, Estado do Pará. 175f. Tese (Doutorado em Ciência Animal) - Programa de Pós-graduação em Ciência Animal, Universidade Federal do Pará.
- 23 Pereira W.L.A. & Cardoso E.C. 2009. Aspectos Histológicos da Osteoporose em Bubalinos e a Condição Físico-Química Óssea e do Cobre Hepático. *Revista Ciências Agrárias*. (51): 25-36.
- 24 Pereira W.L.A. & Cardoso E.C. 2010. Osteoporose em bubalinos em relação à idade, à condição clínica animal e ao estado físico-químico ósseo e do cobre hepático. *Brazilian Journal of Veterinary Research and Animal Science*. 47(2): 159-167.
- 25 Pereira W.L.A. & Cardoso E.C. 2011. Avaliação da ocorrência de fraturas de arcos costais de bufalinos de abate, nas deficiências de fósforo e cobre. *Revista Portuguesa de Ciências Veterinárias*. 106(577-580): 53-37.
- 26 Pinheiro C.P., Bomjardim H.A., Andrade S.J.T., Faial K.C.F., Oliveira C.M.C.E. & Barbosa J.D. 2011. Níveis de fósforo, cobre, cobalto e zinco em bubalinos (*Bubalus bubalis*) na Ilha de Marajó, Estado do Pará. *Pesquisa Veterinária Brasileira*. 31(3): 193-198.
- 27 Read M.V.P., Engels E.A.N. & Smith W.A. 1986. Phosphorus and Grazing Ruminant. 3. Rib Bone Samples as na Indicator of the P Status of Cattle. *South Africa Journal of Animal Science*. 16(1): 13-17.
- 28 Ribeiro H.F.L. 1996. Puerpério na Búfala (*Bubalus bubalis*): Aspectos Clínicos e Histológicos da Involução Uterina e Atividade Ovariana. 125f. Tese (Mestrado em Ciência Animal) Programa de Pós-graduação em Ciência Animal, Universidade Federal de Minas Gerais.
- 29 Serrão E.A. 1986. *Pastagens nativas do trópico úmido brasileiro: conhecimentos atuais*. Belém: Embrapa-Cpatu, pp.109-115.
- 30 Shupe J.L., Butcher J.E., Call J.W., Olson A.E. & Blake J.T. 1988. Clinical signs and bone changes associated with phosphorus deficiency in beef cattle. *American Jornal Veterinary Research*. 49(9): 1629-1636.
- 31 Teixeira Neto J.F., Souza Filho A.P.S. & Serrão E.A.S. 1991. Elementos Limitantes à Produção de Pastagens Nativas Consorciadas com Leguminosas na Ilha do Marajó. *Boletim de Pesquisa* 119. Belém: Embrapa-Cpatu, pp.8-16.