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CHANGES INDUCED BY SOME NUTRITIONAL FACTORS ON TIBIAL BONE STRUCTURE IN PIG

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The problems approached by our team are represented by the involvement of different sources and levels of calcium, namely calcium carbonate, fructoborate and alfalfa, in the mineralization of bone tissue. So, we made fix histological preparations of tissue fragments taken from tibia bone, from 9 pigs belonging to three batches: the control batch, where calcium was provided in a proportion of 1% through calcium carbonate, experimental batch 1, where calcium was provided in a proportion of 1.04% through fructoborate, on a calcium carbonate support, and experimental batch 2, where calcium was provided in a proportion of 1.13% through fructoborate + alfalfa, on a calcium carbonate support. The histomorphometric parameters assessed were represented by the volume of bone trabeculae (BV/TV, %) or the percentage of bone tissue in a given volume and the mean width of bone trabeculae. At the same time, in order to establish fructoborate and alfalfa implication in bone mineralization, we supervised the presence and activity of osteoblasts respectively osteoclasts. In the case of the experimental batch 1, the histomorphometric study shows an increase of bone trabeculae dimension, with a mean width of about 142,5μ μ, and also an increase of their mean volume, which is about 37,11%. The trabecular system is dense and present mineralised and ossified territory where are formed by osteoclasts with osteocytes. Peritrabeculary are presented active osteoblasts which are involved in plurilamellar stratification by deposition of young collagen. In the case of individuals from experimental batch 2, trabeculae mean width is about 140,7μ, while their volume is 35,62%.
Keywords: pig, bone, fructoborate, calcium, histomorphometry

Introduction

The process of mineralization occurs in two inseparable steps. The first step is represented by the release of the osteoid matrix, by osteoblasts, as stripes. The second step consists in the proper osteoid mineralization. Bone strength and stiffness are given by the presence of mineral salts in the osteoid matrix and, particularly, of calcium salts and phosphate hydroxide that precipitates as hydroxyapatite crystals (HAP), thermodynamically stabile. These crystals fix between and on the collagen fibres, providing in this way the osteoid mineralization. Calcium plays an important role in bone physiology and homeostasis. It is stored up in bone during its formation and it is released during bone resorption. Calcium absorption at intestinal level represents a complicated process that involves the presence of sexual hormones (Nielsen, F.H. et al., 1987), especially estrogens. But the researches carried out so far reveal boron involvement in the synthesis of estrogens, vitamin D and other steroid hormones, this one being essential in the process of –OH addition to hormone molecules, respectively vitamin D. The presence of –OH bounds determines a big difference of the hormonal characteristics, the differences between testosterone and oestrogen being especially determined by a single hydroxyl bound (-OH) (Armstrong, T.A. at al., 2000, Bentwich, Z. et al., 1994, Nielsen, F.H. et al., 1990, Samman, S. et al., 1998, Shen, V. et al., 1995).

Material and Methods

The histological study was performed on tissue fragments taken from tibia bone, from 9 pigs distributed into three batches: the control batch (M), where calcium was provided in a proportion of 1% through calcium carbonate, the experimental batch 1 (E_1), where calcium was assured in a proportion of 1.04% through Fructoborate on calcium carbonate support, and the experimental batch 2 (E_2), where calcium was provided in a proportion of 1.13% through Fructoborate + alfalfa on calcium carbonate support.

The tissue fragments were fixed in alcohol 80^0 and/or neuter formalin10%, decalcified in solution 15% of trichloroacetic acid and embedded in paraffin blocks, after a previous dehydration and clearing. The histological sections, stained with the hematoxylin-eosin, trichromic Mallory and trichromic Masson methods, were examined with the research microscope Olympux CX41 endowed with digital photo camera and software for image histomorphometric analysis (QuickPhoto Micro 2.2). Among the histomorphometric parameters recommended by the American Society for Bone and Mineral Research (Jaworski, ZFG., 1983, Eclou-Kalonji, E. et al., 1997, Mocetti, P. et al., 2000, Parfitt, A.M. et al., 1987), we evaluated the volume of bone trabeculae (BV/TV, %) and their mean width. At the same time, in order to establish fructoborate and alfalfa involvement in bone mineralization, we supervised the presence and activity of osteoblasts, respectively osteoclasts.

Results and Discussion

The microscopic examination of the transversal seriated tibia sections, stained with the trichromic Mallory and Masson methods, show the presence, at the periphery of fragments, of the periost territory, consisted of two microanatomic structures: an external one – conjunctival and internal one – osteo-collagenic; there were numerous osteoblasts at the limit between the two structures. In the periost area, the microscopic images reveal the continuation of the morphogenesis process, in which an important role is played by the periost, by forming unlamellar trabecular structures in the subperiosteal region. The superficial lamellar bone tissue is not completely differentiated, as suggested by numerous chondroclasic territories and by intense angiogenesic processes, and also by numerous osteons in formation. The angiogenesis association with the process of ossification, respectively with the formation of the bone trabecular structures, may be explained with the help of the vascular endothelial growing factor (VEGF), which couples the remodelling of the hypertrophic cartilage with the processes of ossification and angiogenesis. The inactivation of this factor blocks the sanguine vessels invasion, leading to the reduction of bone length growing (Ferrara et al., 1997, quoted by Dragoi G.S., 2004).

The histomorphometric analysis of the seriated sections, stained with the trichromic Mallory method led, to the conclusion that, in the case of the control variant, the mean bone trabecula dimension was approximately 128.5 μ and their mean volume was about 30.5%. The osteoblastic areas are reduced (fig. 1), the osteoblasts are peritrabeculary disposed, having a cubic aspect; in some territories, they are plat and inactive (fig. 2). The osteoclastic activity is reduced (fig.3). The marrow parenchyma is loaded with adipose cells (fig. 1) and manifests intense granulocytopoiesis processes.

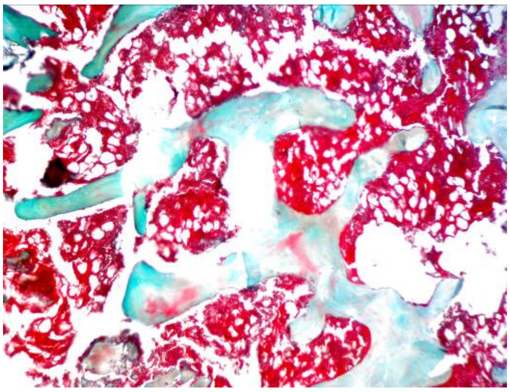


Fig. 1. Superior tibia extremity LM assembly (trichromic Mallory staining; 100x)

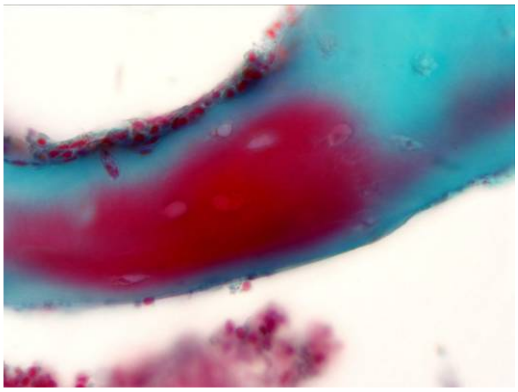
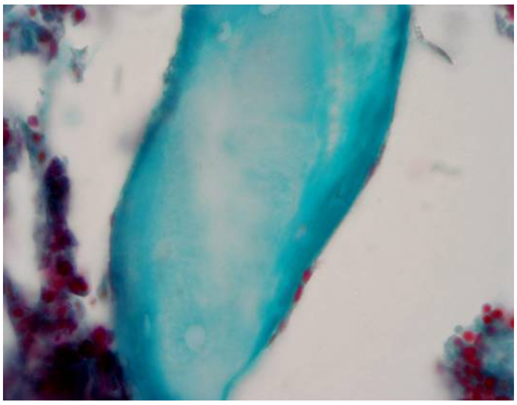


Fig. 2. Tibia LM – active and inactive osteoblasts peritrabeculary disposed (trichromic Mallory staining; 1000x)



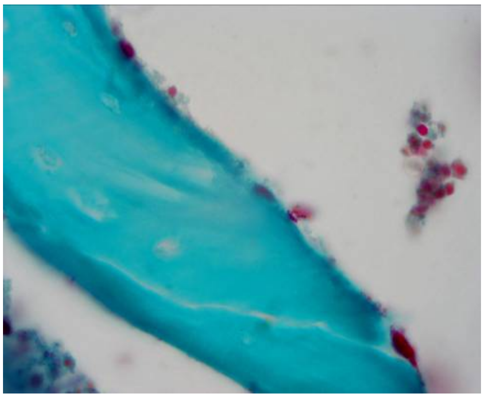
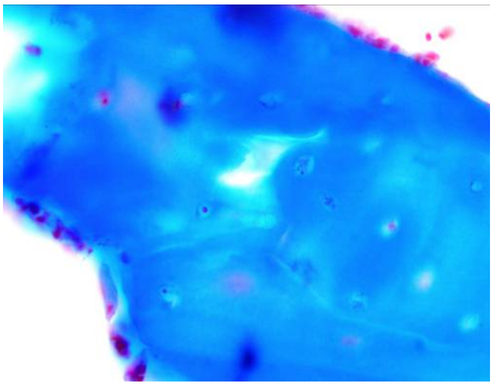


Fig. 3. Tibia LM – inactive osteoblasts (trichromic Mallory staining; 1000x)

Fig. 4. Tibia LM - osteoclast (trichromic Mallory staining; 1000x)

In the case of the experimental batch 1, the histomorphometric study reveals the increase of the mean bone trabecula width, of approximately 142.5 μ, and also of the trabecula volume, to 37.11%. In batch 2, the values of these two parameters are about 140.7 μ, respectively 35.62%. In both cases, we could observe the increase of the osteoblastic areas (fig. 5), manifestation of the processes of collagen deposition and of the apparition of osteocytes in the trabecular territories, these processes being slightly more intense in the experimental batch 2 (fig. 6).



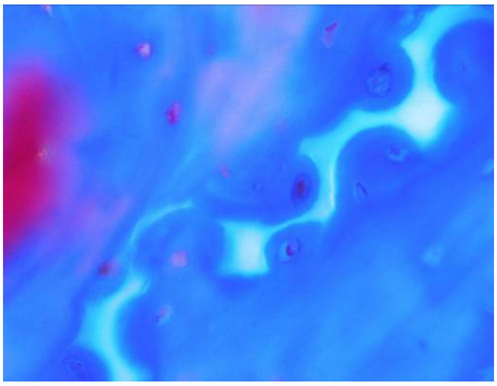


Fig. 5. Tibia LE1 – osteoblastic areas and collagen deposition (trichromic Mallory staining; 1000x)

Fig. 6. Tibia LE2 – collagen deposition and apparition of osteocytes (trichromic Mallory staining; 1000x)

Among the changes induced by the utilization of calcium-deficient diets, the literature mentions: the decrease of bone forming level, bone loss or diffuse osteoporosis (Ohya, K., 1994; Shen, V. et al., 1995); the increase of the number of osteoclasts and, consequently, bone resorption (Liu and Baylink, 1984; Ohya, K., 1994); the increase of the number of endosteal cells (Stauffer, M. et al., 1973) and, in young, osteomalacia (Pettifor, J.M. et al., 1984, quoted by Mocetti, P. et al., 2000). Although the literature presents little data where the effects exerted by hypocalcaemia are corroborated with histomorphometric measurements, on the whole, they show a reduction of bone trabecula volume, especially in adult animals (Thomas, M.L. et al., 1991; Weinreb, M. et al., 1991; Shen, V. et al., 1995). In t his study, we attempted to analyze from a histomorphometric view point the effect exerted by calcium supplementation from various sources, like Fructoborate + alfalfa on calcium carbonate support, compared with the control batch where calcium was provided only through calcium carbonate. The histomorphometric data achieved show that, in both experimental batches, trabeculae volume increases and their width as well, the biggest values being available in the experimental batch 1. In both cases, we noticed the presence of osteoblasts, disposed on one row, peritrabeculary, involved in plurilamellar stratification through deposition of young collagen, the presence of a reduced number of osteoclasts, and in the myeloid parenchyma of the haematogenous marrow we observed the tendency of fibroblast metaplasia in adipose cells.

Conclusions

1. The histomorphometric analysis of the seriated sections coloured with the trichromic Mallory method led to a mean bone trabecula dimension, in the control variant (LM), of about 128.5 μ and a mean trabecula volume of about 30.5%. The osteoblastic areas are reduced, the osteoblasts are peritrabeculary disposed, with cubic aspect; in some territories, they are plat and inactive. The marrow parenchyma is loaded with adipose cells and manifests intense granulocytopoiesis processes.

2. In the case of the experimental batches 1 and 2, the histomorphometric study reveals, on one hand, the increase of the mean bone trabecula width, of about 142.5 μ in the experimental batch 1, respectively of about 140.7 μ, in the experimental batch 2, and also of the mean trabecula volume to 37.11, respectively 35.62%, and on the other hand, the increase of the osteoblastic areas.

3. In both experimental batches, we may remark processes of collagen deposition and of apparition of osteocytes in the trabecular territories, these processes being slightly more intense in the experimental batch 2.

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