CHANGES INDUCED BY SOME NUTRITIONAL FACTORS ON FEMUR HISTOLOGICAL STRUCTURE IN PIG

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Abstract

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 femure, 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 115.4µ µ, and also an increase of their mean volume, which is about 36.46%. 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 111,5µ, while their volume is 33.60%.

Key words: 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 hydroxyde 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 estrogen 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 METHOD

The histological study was performed on tissue fragments taken from femur, 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 introduced 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 DISCUSSIONS

In the case of the control batch, the histological study shows the area of reserve cartilage consisted of hyaline cartilage, idling, that binds the rest of the growth cartilage to epiphyses. Between this area and epiphysis, there are sanguine capillaries involved in the trophicization of bone tissue and diaphysis-epiphysis cartilage (fig. 1). In the peripheral epiphysis area, we may observe the ossification process, marked through fibroblast metaplasia in osteoblasts, cells with hyperchromatic nucleus and basophile cytoplasm that synthesizes young conjunctival tissue (fig. 6), and also by disposing osteoblasts on the areolar bone trabecula face (fig. 2). They lay down a thin young collagen layer, on the bone lamella surface (fig 2). Below this layer, we may notice a territory where bone trabeculae are submitted to a more advanced differentiation process (fig. 3), and in the central epiphysis area, we may observe differentiated trabeculae that, by association, generate large cavities, occupied with marrow. The histomorphometric study performed on transversal and longitudinal sections reveals a mean bone trabecula dimension of about 109µ. The myeloid parenchyma is submitted to intense changes, expressed through stromal fibroblasts metaplasia in adipocytes (fig. 4). This aspect suggests the fact that, in this area, osteogenesis is very slow, a big number of fibroblasts turning into fat cells and not in osteoblasts.

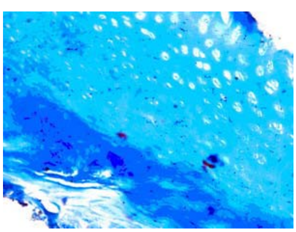

Fig. 1. Femur CB –reserve cartilage (Trichromic Mallory st.; 200x)

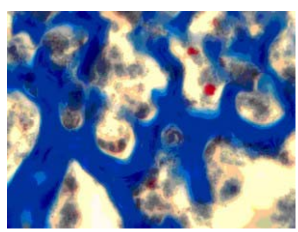

Fig. 2. Femur CB – active osteoblasts disposed on the areolar face *of bone trabeculae* (Trichromic Mallory st.; 200x)

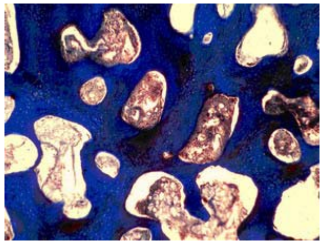

Fig. 3. Femur CB –differentiated bone trabeculae (Trichromic Mallory st.; 200x)

The longitudinal sections performed through femur reveal the presence of the periost (fig. 6) and its involvement in the process of osteogenesis through apposition.

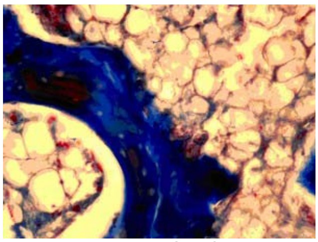

Fig. 4. Femur CB: fibroblasts metaplasia in adipose cells (Trichromic Mallory st.; 400x)

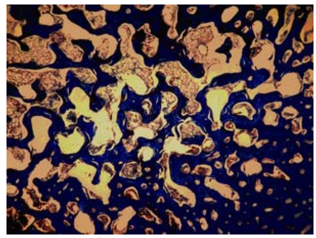

Fig. 5. Femur CB –transversal section assembly (Trichromic Mallory st.; 40x)

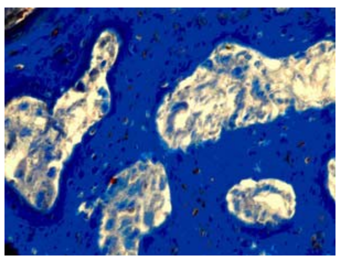

Fig. 6. Femur CB – processes of osteogenesis through apposition (Trichromic Mallory st.; 400x)

The histomorphometric study of the transversal sections performed through the superior femur extremity, in the individuals from the experimental batch 1, leads to the conclusion that the mean trabecula dimension is bigger compared with the control batch, this one being about 115,4µ, and their mean volume is about 36.46%. Trabeculae have a compact aspect and present territories of bone deposits and mineralization, leading to the formation of osteoplasts with osteocytes (fig.7, 8). Peritrabeculary (fig. 9), we may observe active osteoblasts, involved in plurilamellar stratification through deposition of young collagen. The myeloid parenchyma of the hematogenous marrow has a rarefied aspect and, as a general aspect, is represented by the tendency of fibroblasts metaplasia in adipose cells (fig. 8). We may also notice a slight hypertrophic hyperplasia of blood vessels, with lymphocytes being concentrated around them.

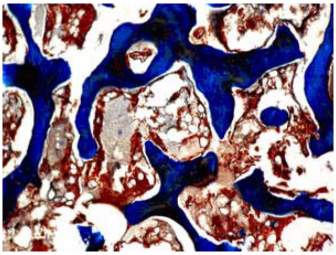

Fig. 7. Femur superior extremity EB1 assembly (trichromic Mallory st.; 100x)

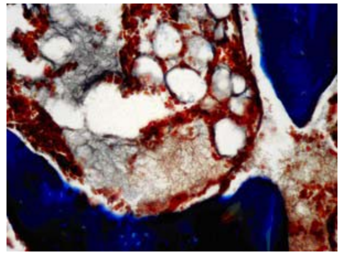

Fig. 8. Femur superior extremity EB1 - fibroblasts metaplasia in adipose cells (trichromic Mallory st.; 400x)

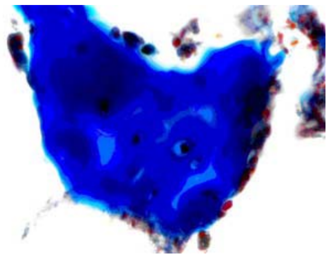

Fig. 9. Femur superior extremity EB1 active osteoblasts involved in plurilamellar stratification. We may also notice osteocytes in the trabecular territories (trichromic Mallory st.; 1000x)

In the case of the experimental batch 2, the transversal sections performed through the superior femur extremity reveal intense processes of osteogenesis, with the involvement of the periost, respectively of the osteoprogenitor cells from its cellular layer structure, cells that enter pre-formed cavities and, by turning into osteoblasts, initiate the synthesis of young conjunctival tissue (fig.10). The osteoblasts within cavities present an intensely basophil cytoplasm, justifying their active implication in the process of protein synthesis.

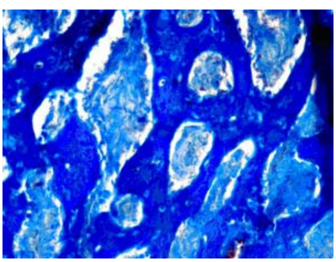

Fig. 10. Femur – EB2 –processes of osteogenesis (trichromic Mallory st.; 400x)

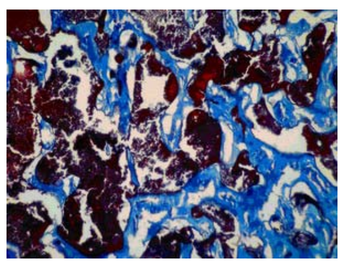

Fig. 11. Femur – EB2 – assembly (trichromic Mallory st.; 100x)

Bone trabeculae (fig. 11) are thinner compared with the control batch and with the experimental batch 1, having a mean width of about 111,5µ and have, on the whole, a "rarefied" aspect (fig. 12). The histomorphometric analysis of sections reveals the fact that the volume of bone trabeculae, respectively the proportion "full/empty" is 33.60%. The areas occupied by osteoblasts are more reduced (fig. 13); on the contrary, we may remark an intenser activity of osteoclasts (fig. 14), large cells involved in bone resorbtion. Bone areolas are wide, loaded with hematoforming tissue, where intense processes of erythropoiesis are made evident.

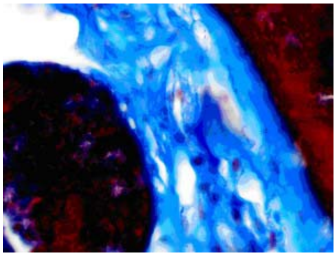

Fig. 12. Femur – EB2 – Bone trabecula with aspect (trichromic Mallory st.; 1000x)

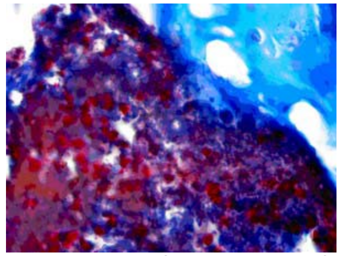

Fig. 13. Femur – EB2 – Osteoblasts rarefied – matrix deposition (trichromic Mallory st.; 1000x)

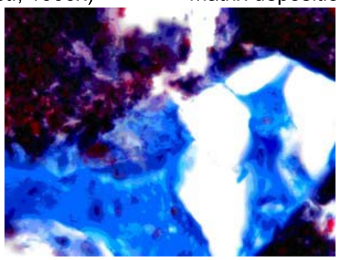

Fig. 14. Femur – EB2 – Osteoclastic and osteoblastic activity (trichromic Mallory st.; 1000x)

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 resorbtion (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 hypocalcemia 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 hematogenous marrow we observed the tendency of fibroblast metaplasia in adipose cells.

CONCLUSIONS

1. The histomorphometric study reveals, in the control batch, a mean bone trabecula dimension of about 109µ, and a mean volume of about 31.20%. In the myeloid parenchyma, fibroblasts are metaplasied into adypocytes, suggesting a very slow process of osteogenesis.

2. The histomorphometric analysis of the transversal sections performed through the superior femur extremity in the experimental batch 1 leads to the conclusion that the mean trabecula dimension is bigger compared with the control batch, being about 115.4µ, and the mean volume is about 36.46%. Trabeculae have a compact aspect and present territories of bone deposition and mineralization, leading to the formation of osteoplasts with osteocytes. Peritrabecularly, we may observe active

osteoblasts, involved in the plurilamellar stratification through deposition of young collagen.

3. In the case of the experimental batch 2, the transversal sections performed through the superior femur extremity reveal intense processes of osteogenesis, with the involvement of the periost, respectively of the osteoprogenitor cells from its cellular layer structure.

4. Bone trabeculae, in the experimental batch 2, are thinner compared with the control batch and with the experimental batch 1, having a mean width of about 111,5μ and have, on the whole, a "rarefied" aspect, their volume being about 33.60%. The areas occupied by osteoblasts are more reduced; on the contrary, we may remark an intenser activity of osteoclasts. Bone areolas are wide, loaded with hematoforming tissue, where intense processes of erythropoiesis and leucopoiesis are made evident.

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