Comparative evaluation of the integrative parameters of the bone tissue of lower limbs of broiler chickens when using osteotropic additives in the diet

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Abstract. The article presents the results of studies of the comparative assessment of the bone tissue integrative parameters of the lower limbs of broiler chickens of the Arbor Acres cross when using in their diet the drug siliostin and feed additives for correcting mineral metabolism – acidic calcium and bentonite clays of the Kantemirovskoye deposit of the Voronezh region. Based on the measurements of the width of the sum of the cortical zone of the diaphysis of the X-ray images of the bones, the assessment of its average brightness, as well as the assessment of the average brightness of the middle part of the bone, it was found that in hens and cocks of meat poultry during the period of intensive growth and development, the use of various feed mineral additives leads to unequal changes in the structure and degree of mineralization of the femur and tibia bones of the extremities. Moreover, the most stable positive picture of changes was noted in chickens treated with siliostin + acidic calcium. The absence of a stable picture from other radiological parameters should be considered as a part of complex biochemical processes in the body that accompany the growth and development of young poultry.

1 Introduction

Violations of mineral metabolism significantly affect the growth and development of young animals, as well as the productivity and health of adult animals and poultry. Quite often the pathology of mineral metabolism is diagnosed to a greater extent at those stages when structural changes occur in the bones of the skeleton associated with softening, osteoporosis and osteofibrosis of the bone tissue and leading to its deformations and fractures that occur even with minor injuries [1, 2].

Biochemical parameters of blood serum characterizing the state of mineral metabolism may indicate the development of dystrophic processes in the bones of the skeleton only at the second stage of the pathology (beginning of clinical manifestations) and later [3, 4].

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Whereas in the early stages of mineral metabolism disorders, other diagnostic methods are required to detect dystrophic changes in the bones of the skeleton with the subsequent possibility of timely therapeutic measures [5, 6].

The main characteristics of bone strength are bone mass or its equivalent - bone mineral density (BMD) and bone quality [7, 8].

Changes in the bone mineral density during radiography are visualized only when the loss of bone mass is more than 30-40%. Therefore, the standard methods of radiographic assessment are mainly focused on an obvious destructive pathology with deformation of the bones of the skeleton and the presence of pronounced elements of its demineralization [9, 10].

In human medicine, the diagnosis of osteopathology is based on the WHO criteria that determine the threshold level of bone mass destruction using densitometers (radiographic and ultrasound) - devices that allow to assess the level of calcium, density, structure and thickness of the surface layer of the bone for the subsequent appointment of specific therapy to the patient [11, 12].

In veterinary medicine, studies using a densitometric assessment of the state of the bones of the skeleton were carried out in our country in the 50-70s of the twentieth century. In animal husbandry practice, the method of X-ray densitometry was used using a wedge standard from the femur bone of cattle, used by I. G. Sharabrin (1953). In the 90s of the same century, A. A. Samotaev (2002) proposed a technique for ultrasonic densitometry of bones from various parts of the skeleton, according to which the mineralization of bone tissue was indirectly assessed by the speed of an ultrasonic wave passing through the bone in the longitudinal direction.

Currently, because of the lack of appropriate materials and equipment for veterinary purposes, manufactured in an industrial way, these methods for assessing the state of bone tissue are not used in diagnostic practice.

However, the development of modern computer technologies and software makes it possible to develop and apply new methods of X-ray densitometry with high visualization of a computer image and accurate digital measurements of images of animal skeleton bones [13-15].

2 Materials and Methods

An experiment on the comparative assessment of the integrative parameters of the bone tissue of the lower limbs of meat-producing poultry when using the drug siliostin and feed additives in their diet to correct mineral metabolism was carried out on broiler chickens of the Arbor Acres cross, formed into 5 groups (n=30). In the starting period, the poultry of all groups received only complete compound feed, but already from the age of two weeks and up to 42 days of life (growth and finish periods), the design of the experiment provided the introduction of mineral metabolism correction agents into the feed rations in the following doses (Table 1).

Table 1. Scheme of the experiment (n=30)

Group	Experiment conditions / doses
1 experimental	basic diet feed + siliostin 1%
2 experimental	basic diet feed + acidic calcium 2.5%
3 experimental	basic diet feed + siliostin 1% + acidic calcium 2.5%
4 experimental	basic diet feed + bentonite 1% + acidic calcium 2.5%
Control	basic diet feed

Siliostin is a complex osteotropic drug with the high pharmacological activity in metabolic osteopathology. The component composition of the drug includes various forms of silicon with high bioavailability, organic acids, vitamins, polyphenols and a number of macro- and microelements.

Bentonite (Kantemirovskoye deposit, Voronezh region) is a natural layered silicate mineral, a distinctive feature of which is a large amount of amorphous silicon and a number of nutrient elements that are easily absorbed in the body.

Acidic calcium is a compound obtained during the interaction of feed sources of calcium (chalk, shell rock and others) with the corn extract. Corn extract is a by-product of deep processing of corn grain. During the production of cornstarch, the corn kernel is soaked in an aqueous environment under controlled conditions, producing an extract containing the soluble substances of the kernel. Due to the low storage capacity, native corn extract is concentrated using vacuum evaporators to a solids content of 35–50%, representing a thick or pasty liquid. Deoxidation of corn extract with calcium compounds increases its ability to activate digestive enzymes at the molecular level and provides the ability to maintain calcium-phosphorus balance in the animal organism.

The chickens were kept in single-tier cage batteries with a mesh floor, grooved (outer) feeders, vacuum and nipple drinkers. The conditions of keeping such as light and temperature conditions, humidity, planting density, corresponded to the recommendations of All-Russian Research and Technological Institute of Poultry (2005). There were no intergroup differences in the content of normalized nutrients and bio-logically active substances.

On the 42nd day of the experiment from each group by random sampling (6 chickens from each group: 3 hens and 3 cocks) a control slaughter was performed with anatomical cutting, separation of the pelvic extremities from broiler carcasses and their subsequent mechanical deboning to separate the tibial and femoral bones.

The cleaned bones were subjected to X-ray diagnostics (in direct projection) using an Orange 1040HF Collimator (S/N: 1908-5CL01) with subsequent assessment of radiographs in the skiological aspect (measurements of the cortical zone of the diaphyses and detection of the intensity of light-optical zones). X-ray digitizer was FireCR Spark.

Measurements of the width of the sum of the cortical zone of the diaphysis of the X-ray images of the bones, the assessment of its average brightness, as well as the assessment of the average brightness of the median part of the bone were made in the middle part of the bone using digital tools of the VideoTest - Size 5.0 computer program for analytical work with X-ray images (Fig. 1-2)

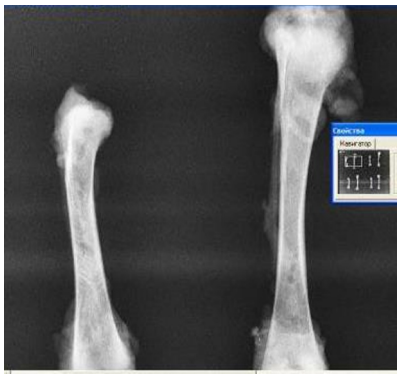

Fig.1. X-ray image of the femur and tibia bones. VideoTest program interface –Size 5.0.

Fig.2. Median zone of the bone to determine the width of the cortex zone, average brightness of the cortex zone and the middle part of the bone. VideoTest program interface – Size 5.0.

3 Results and Discussion

The results of the X-ray studies of the bones of broiler chickens, taking into account gender, are presented in tables 2–4.

Gender determinant.

As a result of comparing the tibia bones of chickens of the control group by gen-der, the following changes were determined (Table 2).

Table 2. Comparison of X-ray parameters of the bones of poultry of the control group in the gender aspect

Indicator	The sum of the width of the cortex zone, mm	Average brightness of the cortex zone, pixels	Average brightness of the middle zone of the bone, pixels
cocks ♂			
Tibia bone	4.27±0.066*	219.4±3.13	194.5±6.04*
Femur bone	2.95±0.047	227.7±4.87*	214.7±5.72
hens ♀			
Tibia bone	3.59±0.233	213.36±4.419	176.92±4.725
Femur bone	2.80±0.088	214.82±2.428	200.74±4.926

Note: *p≤0.05 in relation to the control values

Young cocks had a wider bone cortex in its middle part, which in the total value of the left and right sides was 18.8% (p≤0.05) higher than in hens.

At the same time, the average brightness of the bone cortex zone in cocks was 2.8% higher than in hens. The average brightness of the middle zone of the bones in cocks was also higher than in hens by 9.9% (p≤0.05).

A comparative analysis of the femur bones of chickens of this group revealed the following differences: in cocks, the sum of the femoral cortex in the middle part exceeded the similar parameter in hens by 5.5%, the average brightness of the bone cortex was 6.0% higher than in hens (p ≤0.05), and the average brightness of the middle zone of the femur bone was 7.0% higher.

Thus, in accordance with the measurements, the significant gender differences were determined, indicating a more intensive structural development and mineralization of the estimated skeletal bones in cocks in relation to hens of the same age.

When evaluating the radiological parameters of the poultry of the experimental groups, a number of changes were determined (Tables 3, 4). Thus, the inclusion of the drug siliostin in the diet of poultry of the meat direction had an impact on the mineral density and bone quality of the experimental chickens.

In the tibia bones of broilers of this group in relation to the control analogues the following aspects are revealed:

– in hens - the total width of the cortex in the middle part was higher by 4.5%, the average brightness of the cortex - by 3.2%, the average brightness of the middle zone of the bone - by 2.6%;

– in cocks, on the contrary, there was a decrease in the sum of the cortex width in the middle part of the bone by 2.7% and the average brightness in the cortex zone by 3.8%, while in the middle zone of the bone there were no changes in brightness be-tween the evaluated groups. Taking into account the conjugation of the average brightness in the cortex zone and in the middle part of the bone, the noted changes can be regarded as insignificant due to the natural variability of the trait.

Table 3. X-ray indicators of the bones of cocks (M±m; n=3)

Indicator	The sum of the width of the cortex zone, mm	Average brightness of the cortex zone, pixels	Average brightness of the middle zone of the bone, pixels
1 experimental group			
Tibia bone	4.15±0.050	211.1±0.300	194.45±1.350
Femur bone	3.15±0.118	218.3±1.508	192.8±0.471*
2 experimental group			
Tibia bone	4.15±0.15	232.25±8.05	209.65±15.35
Femur bone	3.505±0.186*	224.2±5.11	208.5±6.51
3 experimental group			
Tibia bone	3.65±0.450	195.9±13.95	170.0±12.45
Femur bone	3.50±0.141*	202.8±6.91*	174.7±5.40**
4 experimental group			
Tibia bone	4.00±0.100*	228.4±6.80	209.1±8.10
Femur bone	3.40±0.28	220.4±4.36	214.2±4.64
Control group			
Tibia bone	4.27±0.066	219.4±3.13	194.5±6.04
Femur bone	2.95±0.047	227.7±4.87	214.7±5.72

Note: *p≤0.05; **p≤0.01 in relation to the control values

Table 4. X-ray indicators of the bones of hens (M±m; n=3)

Indicator	The sum of the width of the cortex zone, mm	Average brightness of the cortex zone, pixels	Average brightness of the middle zone of the bone, pixels
1 experimental group			
Tibia bone	3.75±0.35	220.25±1.35	181.6±4.3
Femur bone	2.355±0.068*	223.85±2.475*	182.25±0.071*
2 experimental group			
Tibia bone	4.305±0.605	203.55±5.55	168.1±6.8
Femur bone	3.35±0.212*	212.05±4.031	180.25±5.72*
3 experimental group			
Tibia bone	4.05±0.05	237.15±0.15**	202.65±11.55
Femur bone	2.9±0.094	217.3±3.064	194.4±2.734
4 experimental group			
Tibia bone	3.59±0.233	213.36±4.419	176.92±4.725
Femur bone	3.15±0.165	201.5±5.28*	175.3±0.61**
Control group			
Tibia bone	3.59±0.233	213.36±4.419	176.92±4.725
Femur bone	2.80±0.088	214.82±2.428	200.74±4.926

Note: *p≤0.05; **p≤0.01 in relation to the control values

When evaluating the X-ray picture of the femur bones in hens, a decrease in all assessed indicators was determined. The sum of the cortex width decreased by 15.9% (p≤0.05), the average brightness of the cortex zone - by 2.6% (p≤0.05), the median bone zone - by 9.2% (p≤0.05). Whereas the X-ray parameters of the cocks' femur bones revealed an increase in the total width of the cortex by 6.6% with a simultaneous decrease in the average brightness

in the cortex zone by 4.1% and the average brightness in the middle part of the bone by 10.2% (p≤0, 05).

Thus, the introduction of 1% siliostin into the poultry diet has an effect on a certain increase in the studied parameters in the tibia bones of hens on the background of a pronounced decrease in the same parameters in the femur bones. In the tibia bones of cocks, there was a slight decrease in the width of the cortex with insignificant fluctuations in the light saturation of the bone. In the femur bones there was an increase in the width of the bone matrix on the background of a decrease in mineralization indices.

When evaluating the radiological parameters of the poultry of the 2nd experimental group, it was found that in hens in the tibia bones there was an increase in the total cortex by 19.9% in relation to the control analogues, while there was a decrease in the intensity of the average brightness in the cortex area by 4.6% and by 5.0% in the area of the median part of the bone of the substance.

At the similar assessment of the femur bones in hens, there was an increase in the total width of the cortex by 19.6% (p≤0.05) with a decrease in the average brightness of the cortex by 1.3% and the middle part of the bone by 10.2% (p≤0.05).

In cocks of this group, when examining radiographs of the tibia bones, a decrease in the total width of the cortex by 2.7% was noted with a simultaneous increase in the average brightness of the cortex by 5.9% and the middle part of the bone by 7.8%.

The study of X-ray images of the femur bones of cocks showed an increase in the total width of the cortex relative to the parameters of the poultry of the control group by 18.6% (p≤0.05). However, at the same time, in cocks of the 2nd experimental group, a decrease in the average brightness in the area of the cortex by 1.6% and in the area of the middle part of the bone by 2.9% was revealed.

So, the use of acidic calcium in the diet of poultry contributes to an increase in the width of the cortical layer of the femur bones on the background of a decrease in their mineralization. In the tibia bones, an increase in mineral components occurs due to minor changes in the cortex.

With the combined use of siliostin and acidic calcium in the diet in the tibia bones of hens of the 3rd experimental group relative to the control analogues, an increase in the total cortex of the middle part of the bones by 12.8%, the average brightness of the cortex - by 11.2% (p≤0, 01) and average brightness in the middle part of the bones - by 14.5% were noted.

When evaluating the femur bones of hens of this group, the following differences were determined: the sum of the cortex width exceeded the control values by 3.5%, the average brightness of the cortex - by 1.2%, while the average brightness of the middle part of the bones was lower by 3.2%. Considering that the values of the aver-age brightness of the cortex and the middle part of the bone are conjugated indicators, the identified changes should be attributed to the variability of the trait, while the absence of true structural deviations should be recognized.

The study of radiographic parameters of the tibia bones of cocks of the experimental group revealed a decrease in all assessed indicators. Thus, the sum of the cortex width in cocks of the 3rd group was lower by 14.5%, the average brightness of the cortex was lower by 10.7%, the average brightness of the middle zone was lower by 12.6% in comparison with the same indicators of control cocks.

Whereas the study of the parameters of their femur bones, on the contrary, revealed an increase in the total cortex by 18.5% (p≤0.05). At the same time, in the experimental group the average brightness of the cortex zone decreased by 11.0% (p≤0.05) and the middle part of the bone decreased by 18.6% (p≤0.01).

The introduction of siliostin and acidic calcium into the diets revealed an unequal picture of changes in the density and quality of bone tissue based on the gender differences in poultry and the type of bone. In hens, on the background of the use of drugs in the tibia bones an

increase in all indicators was noted, and in the femur bones an increase in the width of the peripheral bone matrix with slight fluctuations in mineralization was revealed. In cocks, a decrease in all the studied bone parameters was revealed in the tibia bones, while in the femur bones there was an increase in the width of the peripheral matrix on the background of a significant decrease in mineralization.

These differences in bone formation can be explained by the more intensive growth rates of cocks, at which bone formation and their mineralization do not follow the development rates. In hens, growth processes occur more evenly, therefore, on the background of the inclusion of additives in the diet, a positive trend was recorded.

When adding bentonite and acidic calcium to poultry feed, in the X-ray picture of broilers of the 4th experimental group in comparison with the indicators of control analogues in the tibia bones of hens a decrease in the total cortex width in the middle part of the bone by 6.3% and brightness in the cortex zone by 6.6% were recorded, while the average brightness in the middle part of the bone increased by 1.3%. Considering that different projections of the bone are evaluated in determining the radio-graphic brightness, in this case we can talk about its insignificant changes as a result of the natural variability of the sign.

When assessing the femur bones of hens, an increase in the width of the total cortex by 12.4% was revealed on the background of a decrease in the average brightness in the cortex zone by 6.2% ($p \leq 0.05$) and in the middle zone of the bone by 12.7% ($p \leq 0.05$).

The X-ray picture of the tibia bones of cocks showed a decrease in the total width of the cortex by 2.7%, the average brightness in the cortex zone - by 3.8%. No changes were found in the area of the middle part of the bones.

In terms of the density of the femur bones of cocks of this group, an increase in the total width of the cortex by 15.1% was noted with a decrease in the average brightness in the cortex zone by 3.2%, with no changes in the median zone of the bones.

Thus, for this group as a whole (both in hens and cocks), we can speak of insignificant changes in bone mineral density in the tibia bones. At the same time, in poultry of the both gender groups an increase in the width of the bone matrix was found on the background of a decrease in mineralization in hens and the absence of significant changes in cocks.

4 Conclusion

Based on the obtained results, it can be concluded that in hens and cocks of meat poultry during the period of intensive growth and development the use of various feed mineral additives leads to unequal changes in the structure and degree of mineralization of the femur and tibia bones of the extremities.

The most stable positive picture of changes was noted in the 3rd experimental group of chickens treated with siliostin + acidic calcium.

At the same time, in all experimental groups the only stable positive sign was determined - an increase in the width of the total cortex zone in the middle part of the femur bones. Significant changes for this trait were noted in the 2nd group of hens, as well as in all groups of cocks.

We believe that the absence of a stable picture from other radiological parameters should be considered as part of complex biochemical processes in the body that ac-company the growth and development of young poultry

References

1. S. Bertazzo, Biomineralization, Semin Cell Dev Biol., **46(1)**, (2015) doi: 10.1016/j.semcdb.2015.11.010.

2. L.R. McCabe, N. Parameswaran, Advances in Probiotic Regulation of Bone and Mineral Metabolism, Calcif Tissue Int., **102(4)**, 480-488 (2018) doi: 10.1007/s00223-018-0403-7.
3. A. During, Osteoporosis: A role for lipids, Biochimie, **178**, 49-55 (2020) doi: 10.1016/j.biochi.2020.08.004.
4. M. Semenenko, E. Kuzminova, K. Semenenko, A. Vlasenko, I. Zholobova, Biochemical markers of bone tissue metabolism in broiler chickens, E3S Web Conf., **273**, 02016 (2021) doi: https://doi.org/10.1051/e3sconf/202127302016.
5. S. Bianchi, Ultrasound and bone: a pictorial review, J Ultrasound, **3(3)**, 227-257 (2020) doi: 10.1007/s40477-020-00477-4.
6. D. Krakow, Skeletal dysplasias. Clin Perinatol., **42(2)**, 301-19 (2015) doi: 10.1016/j.clp.2015.03.003.
7. L.P.B. Elbers, H.G. Raterman, W.F. Lems, Bone Mineral Density Loss and Fracture Risk After Discontinuation of Antiosteoporotic Drug Treatment: A Narrative Review, Drugs, **81(14)**, 1645-1655 (2021) doi: 10.1007/s40265-021-01587-x.
8. B.C. Lupsa, K. Insogna, Bone Health and Osteoporosis, Endocrinol Metab Clin North Am., **44(3)**, 517-30 (2015) doi: 10.1016/j.ecl.2015.05.002.
9. L. Wang, L. Zhang, M. Gao, Metabolic Disorders and Mineral Density of the Bone Tissue in the Early Pathogenesis of Osteonecrosis: Study on Rabbits with Steroid-Induced Osteonecrosis., Bull Exp Biol Med., **170(6)**, 724-728 (2021) doi: 10.1007/s10517-021-05141-3.
10. J. Serra-Vinardell, N. Roca-Ayats, L. De-Ugarte, L. Vilageliu, S. Balcells, D. Grinberg, Bone development and remodeling in metabolic disorders, J Inherit Metab Dis., **43(1)**, 133-144 (2020) doi: 10.1002/jimd.12097.
11. F. Jakob, F. Genest, L. Seefried, E. Tsourdi, C. Lapa, L.C. Hofbauer, Diagnostik in der Osteologie, Internist (Berl), **57(7)**, 631-7 (2016) doi: 10.1007/s00108-016-0081-3.
12. Y.L. Fan, W.C. Peh, Radiology of Osteoporosis: Old and New Findings, Semin Musculoskelet Radiol., **20(3)**, 235-245 (2016) doi: 10.1055/s-0036-1592371.
13. A. Bazzocchi, F. Ponti, U. Albisinni, G. Battista, G. Guglielmi, DXA: Technical aspects and application, Eur J Radiol., **85(8)**, 1481-92 (2016) doi: 10.1016/j.ejrad.2016.04.004.
14. P. Sawicki, M. Tałałaj, K. Życińska, W.S. Zgliczyński, W. Wierzba, Current Applications and Selected Technical Details of Dual-Energy X-Ray Absorptiometry, Med Sci Monit., **27**, e930839 (2021) doi: 10.12659/MSM.930839.
15. D. Martel, A. Monga, G. Chang, Osteoporosis Imaging, Radiol Clin North Am., **60(4)**, 537-545 (2022) doi: 10.1016/j.rcl.2022.02.003.