INFLUENCE OF ALPHA-KETOGLUTARATE ON CORTICAL BONE DENSITY, GEOMETRICAL PROPERTIES AND MECHANICAL ENDURANCE OF THE HUMERUS IN TURKIES

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Abstract

The aim of this study was to estimate an influence of alpha-ketoglutarate (AKG) on volumetric bone density and geometrical and mechanical properties of the humerus in farm turkeys. All birds were kept under standard rearing conditions with constant access to water and appropriate feed, supplied in accordance with the stage of production cycle. Bone samples were collected 14 weeks after beginning of the experiment. To the first group belonged the control turkeys. The second group of animals consisted of turkeys that were treated with AKG from the 22nd to 110th d of life. Using Quantitative Computed Tomography (QCT) method, volumetric bone density of the cortical bone in the humerus was estimated. Both the mechanical and geometrical properties of the bone were assessed according to Ferretti method. The obtained results showed anabolic influence of enteral AKG administration on cortical bone density, geometrical properties and mechanical endurance of the humerus. Moreover, the humerus in turkey may be utilised as a model of cortical bone for further investigations of factors conditioning skeletal homeostasis.

Key words: turkeys, alpha-ketoglutarate, humerus.

Bone tissue in vertebrates fulfils three main functions such as supportive and locomotory, protective for the central nervous system and bone marrow, and metabolic as the main reservoir of calcium and phosphorus in the body. The locomotory and protective functions in vertebrates are generally provided by the cortical part of bone tissue, whereas the trabecular bone is characterised by relatively high rate of metabolic processes (16). As opposite to the femur, tibia or ulna that consist the cortical and the trabecular bone in turkey, the humerus is built of the cortical bone only. It enables the utilisation of this bone in turkey as an experimental model for investigations of factors determining volumetric bone density, geometrical properties, and mechanical endurance of long bones, conditioned by cortical bone only.

The advances made in the field of genetics and nutrition during last 30 years have resulted in increased growth rates and body weight in livestock (4, 5, 15). On the other hand, the incidence of skeletal system growth problems, such as rickets, avian tibial dyschondroplasia and osteochondrosis rapidly increased as well. To guarantee proper skeletal growth and development, the growth plate cartilage that regulates long bone development, have to maintain a tightly controlled balance between cartilage synthesis and degradation as well as chondrocytes proliferation and apoptosis (15). In rapidly developing bones like the tibia, femur, humerus or ulna in poultry, the growth plate cartilage determines the rate of longitudinal growth as well as the ultimate length and width of bones. The investigations of high rate skeletal development in vertebrates elucidated that the growth plate chondrocytes turnover in broiler chickens is four times faster than in rabbits (15). Due to their intensive growth and relatively short fattening time (about 16 weeks in Poland), turkeys are an attractive experimental model for studies on conditions that determine proper growth, development, and healing of the skeletal system (20, 22). Moreover, the turkey model offers a possibility of investigations of feeding, physiological, pharmacological, and toxicological factors influencing development and mineralisation of the skeletal system (21).

Alpha-ketoglutarate (AKG) is a molecule determining the overall rate of the citric acid cycle in the organism. It is a nitrogen scavenger and a source of
glutamine that stimulates protein synthesis and inhibits protein degradation in muscles. Moreover, AKG as a precursor of glutamine is a metabolic fuel for the gastrointestinal tract (9, 10). Glutamate released from nervous fibers penetrates into bone tissue and regulates its metabolism. It is synthesised by the reductive amination of AKG in perivenous hepatocytes as well (14, 17, 24, 26). Together with ascorbate, and Fe^{2+}, AKG steers hydroxylation of peptide-bound proline to hydroxyproline via prolyl hydrodase, increasing the conversion of pro-collagen to collagen and bone matrix formation (12, 23).

To investigate the influence of alpha-ketoglutarate enteral administration on growth, development, and mineralisation of cortical bone in turkey, the volumetric bone density and geometrical and mechanical properties of the humerus were estimated.

**Material and Methods**

**Experimental design and sampling procedure.** The study was performed on 16 HB-91 male turkeys that were kept under standard rearing conditions with a constant access to fresh water, appropriate feed supplied in accordance with the stage of production cycle, and air temperature set at the level of 28°C. The experiment was conducted from the beginning of the 4th week after hatching until the moment that the production cycle was completed i.e. in the 17th week of life. On the 22nd d of life, all experimental turkeys were divided into two weight-matched groups. The first group (Group 1) consisted of 8 turkeys that received placebo (physiological saline – PhS). To the second group (Group 2) belonged 8 turkeys that were treated with alpha-ketoglutarate (AKG) in the dosage of 0.4 g/kg body mass per day. Both AKG and PhS were administered every day throughout 14 weeks at the same volumes, directly into the crop with the use of a tube. After 14 weeks of the experiment, the turkeys were slaughtered; left and right humeri were X-rayed, isolated and frozen at –25°C for further analyses.

**Volumetric bone density.** Using Quantitative Computed Tomography (QCT) method and SOMATOM AR. T – SIEMENS apparatus supplied with VR 3 software, the volumetric bone density of the humerus was determined. Bone density of the cortical bone was measured using 2 mm thick, cross sectional, diaphyseal QCT scans at approximately 40 and 47.5% of humerus length along the longitudinal axis, measuring from distal extremities of this bone.

**Analysis of mechanical and geometrical properties.** Mechanical properties of right and left humeri were estimated using three-point bending test according to Ferretti’s et al. (7, 8) method in INSTRON 4302 apparatus linked with a computer, registering relationship between forces perpendicular to length of bone and resulting in displacement. Results were presented graphically and maximum elastic strength (Wy) and ultimate strength (Wf) were estimated. On the basis of the measurement of horizontal and vertical diameters of cross section of the humerus, both external and internal diameters, cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT) and cortical index (CI) were estimated (2, 7, 8).

**Statistical analysis.** All data are presented as mean ± standard error (±S.E.). Statistical analyses were performed using Statistica software (version 5.1). All investigated parameters in both groups of turkeys were found to be normally distributed in accordance to Kolomogorov-Smirnov test. The Student’s t-test was used to determine statistical significance of differences in variables between the analysed groups. A significance level of P ≤ 0.05 was used for all comparisons.

**Results**

The body mass of turkeys at the beginning of the 4th week was 522.6 g (±30.6) in control group and 520.7 g (±9.25) in AKG treated group, whereas at the age of 17 weeks its value reached 8602.5 g (±304.1) and 8678 g (±395.2), respectively. No significant differences between the investigated groups were found in body mass both at the beginning and at the end of the experiment (P = 0.95 and P = 0.88). Considering the weight and length of the humerus no significant differences between experimental and control groups were observed as well (P = 0.15 and P = 0.18) (Table 1). Results of volumetric bone density measurement of the cortical bone showed its significantly higher values in AKG treated animals. 14.93% and 12.69% increase in volumetric bone density of cortical bone was obtained in AKG group, analysing measuring scans placed in the 40 and 47.5% of humerus length, respectively (Fig. 1). The administration of alpha-ketoglutarate for 14 weeks induced higher values of all analysed geometrical parameters and the obtained differences were statistically significant (Table 1). The analysis of mechanical properties of the humerus revealed significantly higher values of the maximum elastic strength of the bone in turkeys that received AKG, when compared to the control group. The ultimate strength of the humerus was significantly increased in the AKG treated birds as well (Table 1).

**Discussion**

Due to different properties and metabolic rate, the investigation on cortical bone model is complementary to the models including both the cortical and the trabecular bone. To evaluate an influence of various factors determining bone development, growth, and skeletal homeostasis in mammals the calvaria was used as a cortical bone model in numerous studies (18, 19). Because of very intensive growth and development of the skeletal system in poultry they are attractive for investigations in this field. The humerus of turkey is pneumatic and it is built-up from the cortical bone, what makes this bone suitable for investigations on cortical bone properties in birds.
Table 1
Characteristics of turkey humerus

<table>
<thead>
<tr>
<th>Investigated parameter</th>
<th>Group 1 (control group)</th>
<th>Group 2 (AKG treated group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of investigated bones</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>48.2 (±2.32)</td>
<td>52.86 (±2.24)</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>138.75 (±1.25)</td>
<td>136.37 (±1.24)</td>
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<tr>
<td>Cross-sectional area (mm²)</td>
<td>49.5 (±2.04)</td>
<td>64.43 (±2.42)</td>
</tr>
<tr>
<td>Second moment of inertia (mm⁴)</td>
<td>779.8 (±38.5)</td>
<td>987.7 (±68.7)</td>
</tr>
<tr>
<td>Mean relative wall thickness</td>
<td>0.244 (±0.014)</td>
<td>0.329 (±0.008)</td>
</tr>
<tr>
<td>Cortical index</td>
<td>19.39 (±0.97)</td>
<td>24.6 (±0.47)</td>
</tr>
<tr>
<td>Maximum elastic strength (N)</td>
<td>626 (±26.7)</td>
<td>782 (±36.9)</td>
</tr>
<tr>
<td>Ultimate strength (N)</td>
<td>1059 (±47.4)</td>
<td>1188 (±45.1)</td>
</tr>
</tbody>
</table>

* statistically significant differences (P<0.05) between the control and AKG treated group parameters.

Fig. 1. Volumetric bone density of the cortical bone (Cd) of the humerus in placebo (control) and AKG treated turkeys. The measured scans were placed at the 40% (Cd I) and 47.5% (Cd II) of total humerus length. * Statistically significant differences (P<0.001) between the investigated groups.

Quantitative Computed Tomography (QCT) method and estimation of geometrical and mechanical properties of bones are commonly accepted methods, making it possible to assess the quality of the skeletal system in vertebrates. They also enable in-depth analysis of the changes occurring during development, growth, and bone metabolism modulation (20, 22). QCT is non-invasive in determination of the volumetric bone density (g/cm³) and enables the assessment of skeletal system mineralisation. In comparisons to other methods like DEXA (Dual-energy X-ray Absorptiometry) that determines areal bone mineral density (g/cm²) - BMD, it guarantees the measurements without errors occurring in the case of DEXA due to presence of surrounding soft tissues (6, 13). Considering that even 0.1% X-ray attenuation is detectable by QCT, the application of this method in investigation of the skeletal system in vertebrates enables very precise bone density measurement (20, 25). Moreover, it enables to assess changes in the bone density that can be difficult to state by simple bone weight measurement. The mechanical properties of bones are conditioned mainly by the bone structure, their mineralisation, maturity, and geometrical properties which include such parameters as cross-sectional area, second moment of inertia, mean relative wall thickness and cortical index. The quality of the material and the structure of the trabecular bone, however, play the decisive role (2, 7, 8, 20). Considering the humerus that consists of no trabecular bone in turkey, its mechanical endurance is provided due to geometrical properties, cortical bone density and quality of bone tissue only.

The obtained results prove advantageous influence of alpha-ketoglutarate administration via digestive tract on humerus quality in turkeys slaughtered at the age of 17 weeks. Similar body mass in the
beginning of the experiment and no statistically significant difference in the final body mass value between the investigated groups confirm that geometrical properties, mechanical endurance and volumetric bone density of the humerus were not influenced by differentiated body mass of turkeys. The increased volumetric bone density in the mid-shaft of this bone indicates that the bone tissue synthesis and mineralisation was higher in the cortical bone. This anabolic effect on bone tissue can be explained by digestive processes that are influenced by enteral AKG, which acts as an energy donor and as a substrate for the synthesis of amino acids, mainly proline. Improved proline synthesis, regulated by AKG, ascorbate, and Fe²⁺ increases its following hydroxylation to hydroxyproline that is a principal amino acid of collagen, main organic constituent of bone tissue (12, 21, 23). Increase in the geometrical properties like cross-sectional area, second moment of inertia, mean relative wall thickness and cortical index may indicate that AKG may stimulate chondrocytes proliferation on the epiphyseal cartilage level that is responsible for longitudinal growth and bone width (15). Similar effect of AKG was observed in other investigations performed on the osteotomised and denervated ulna model in turkey (20). The higher values of MRWT and CI of the humerus in experimental turkeys indicate that the cortical bone thickness was increased due to 14 - week AKG administration. Due to the higher volumetric bone density of the cortical bone and risen geometrical properties, the higher mechanical endurance of the humerus was demonstrated as well. These results are in accordance with other studies performed on rat, pig, and turkey models (1, 11, 20, 21).

Next to the mechanism of increased proline synthesis, the other action that may be engaged in bone metabolism regulation by AKG is enhanced glutamate synthesis and its utilisation as neuro-signaling by the peripheral nervous system, which was previously reported (14). All obtained results indicate that enteral AKG administration in turkeys improves skeletal system quality by anabolic effect on cortical bone formation. Due to positive effect of dietary supplementation of AKG on humerus properties it may be considered as an efficient factor in prevention of skeletal disorders during fast growth and development in turkeys and perhaps other poultry species.

References


