EFFECT OF ALPHA-KETOGLUTARATE (AKG)
ON MINERALISATION, MORPHOLOGY
AND MECHANICAL ENDURANCE
OF FEMUR AND TIBIA IN TURKEY

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Abstract

The aim of this study was to determine the influence of alpha-ketoglutarate (AKG) on volumetric bone density, geometrical and mechanical properties of femur and tibia in farm turkeys. All animals 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 control turkeys. The second group of animals consisted of turkeys that were administered AKG from the 22nd to 110th d of life. Using Quantitative Computed Tomography (QCT) method, volumetric bone density of femur and tibia were estimated for trabecular and cortical bone. Both the mechanical and geometrical properties of femur and tibia were assessed according to Ferretti’s method. The obtained results indicate positive influence of enteral AKG administration on skeletal system quality in turkeys.

Key words: turkeys alpha-ketoglutarate, femur, tibia.

Leg weakness and lameness are considered among the major factors that influence production results and determine profitability of poultry farming. Lame birds may weight even 25% less than the healthy ones at market age as the result of reduced feed and water consumption (15). To achieve genetic potential of final body weight, the optimal nutrition should guarantee proper growth, development, mineralisation and sufficient mechanical endurance of skeletal system. The mortality of turkeys, connected with their impaired locomotory functions may reach even few percent of population.

Current genetic selection pressure for higher body weight in turkeys places increased demands on skeletal integrity (4). Increased body weight, mainly conditioned by breast and leg muscles and imbalance in bone growth (higher meat to bone ratio) result in bone deformities or fractures as an insufficient skeletal adaptation to heavy body weight (5, 15). Created overloading or abnormal loading conditions may result in structural damages and deformities occurring especially in long bones (femur and tibia) in turkeys. Because of the most dynamic growth rate, commonly occurring weakness of bone tissue in turkeys such as bone atrophy, tibial dyschondroplasia, chondrodystrophy, and leg deformities may affect the tibia (13).

Alpha-ketoglutarate (AKG) is a central molecule in Krebs cycle determining the overall rate of the citric acid cycle in organism. It is a nitrogen scavenger and a source of glutamine that stimulates protein synthesis and inhibits protein degradation in muscles. AKG is an important metabolic fuel for cells of the gastrointestinal tract as well (8, 9). Glutamate that is released from nervous fibers penetrating into bone tissue and regulating its metabolism is synthesised by the reductive amination of AKG in perivenous hepatocytes (2, 3, 12, 17, 22, 23). Moreover, AKG, ascorbate, and Fe2+ steer hydroxylation of peptide-bound proline to hydroxyproline via prolyl hydrodase, increasing the
conversion of pro-collagen to collagen and bone matrix formation (11, 21).

To assess the influence of alpha-ketoglutarate administration on growth, development, and mineralisation of skeletal system in turkey, the volumetric bone density, geometrical and mechanical properties of femur and tibia were estimated.

**Material and Methods**

**Experimental design and sampling procedure.** The experiment was performed on 16 HB-91 turkeys. The turkeys were kept under standard rearing conditions with 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 separation of the experimental birds from the remaining farm stock was possible due to a special wire fencing construction, which assured identical feeding, visual and acoustic contact for all of the turkeys. The separated area was lined with straw, just like the rest of the building. The experiment was conducted from the beginning of the 4th week after hatching until the moment that the production cycle was completed 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 administered alpha-ketoglutarate (AKG) in the dose of 0.4 g/kg body weight 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 sacrificed, tibia and femur were X-rayed, isolated with VR 3 software were used for volumetric bone density determination. Bone density was measured both for trabecular and cortical bone using 2 mm thick, cross-sectional, metaphysial QCT scans at 7% of femur length and at 10% of tibia length along the longitudinal axis, measuring from distal extremities of these bones.

**Volumetric bone density of femur and tibia.** Quantitative computed tomography (QCT) method and SOMATOM AR. T – SIEMENS apparatus supplied with VR 3 software were used for volumetric bone density determination. Bone density was measured both for trabecular and cortical bone using 2 mm thick, cross-sectional, metaphysial QCT scans at 7% of femur length and at 10% of tibia length along the longitudinal axis, measuring from distal extremities of these bones. Analysis of mechanical and geometrical properties. Mechanical properties of femur and tibia were estimated using three-point bending test according to Ferretti’s method in INSTRON 4302 apparatus linked with a computer, registering relationship between forces perpendicular to length of bone and resulting in displacement (6, 7). Results were presented graphically and maximum elastic strength (Wy) and ultimate strength (Wf) were estimated. On the basis of measurement of horizontal and vertical diameters of cross section of femur and tibia, both external and internal diameters, cross-sectional area (A), second moment of inertia (Ix) and mean relative wall thickness (MRWT) were estimated (6, 7).

**Statistical analysis.** All data are presented as mean ± standard error (±S.E.). Statistical analyses were performed using Statistica software version 5.0. The Student’s t-test was used to determine statistical significance of differences in variables between analysed groups. A significance level of P<0.05 was used for all comparisons.

**Results**

At the age of 22 d the body weight of turkeys belonging to group 1 and group 2 reached 524.12 g (±26.12) and 527.12 g (±16.64), respectively. No significant difference was stated between control and AKG treated turkeys at the beginning of the experiment (P=0.92). The final body weight of turkeys at the age of 17 weeks reached 8425 g (±283.8) in group 1 and was not significantly different from 8462.5 g (±217.3) in group 2 that received alpha-ketoglutarate (P=0.91).

The weight of femur in turkeys from group 1 reached 46.47 g (±0.86) and was significantly higher when compared to 43.83 g (±0.96) obtained in group 2. No significant difference was stated between tibia weight in turkeys from control and AKG treated groups, where these values reached 61.76 g (±1.07) and 60.16 g (±1.93), respectively.

Results of volumetric bone density measurement, mechanical and geometrical properties analyses are presented in Tables 1 and 2.

The measurement of trabecular bone density (Td) in femur and tibia revealed its significantly higher values in turkeys that received AKG. The cortical bone density (Cd) of tibia were higher in AKG administered birds as well.

The analysis of maximum elastic strength (Wy) elucidated its higher values both in femur and tibia of turkeys that received alpha-ketoglutarate. Moreover, the higher ultimate strength (Wf) value was noted in tibia of turkeys from group 2.

No significant differences between investigated groups of turkeys were stated in geometrical parameters of femur, like cross-sectional area (A), second moment of inertia (Ix) and mean relative wall thickness (MRWT). The analysis of geometrical properties of tibia revealed higher values of A, Ix and MRWT in turkeys that were administered AKG, when compared to control birds.

**Discussion**

Regarding their intensive growth and relatively short fattening period, turkeys serve as an attractive model for investigations on proper development and function of skeletal system (4, 5, 18-20). Genetic selection of poultry towards maximum intensive production and increased body weight has created a predisposition to disorders in skeletal system (4, 15). Disturbances of growth, development and mineralisation of skeletal system, resulting in common femur and tibia weakness are very important factors of excessive mortality in poultry. Leg deformities and bone fractures disturb animal welfare and cause serious economic losses due to following infections, decreased final body weight, and lower carcass quality. It was stated that leg weakness and impaired locomotory
functions of turkeys are the most important factors influencing final results. Higher body weight of turkeys, mainly conditioned by increased breast and leg muscles weight, achieved in accordance to their current genetic potential, causes an abnormal loading of femur and shank bones, leading to their structural damages. Common leg weakness in poultry is a result of disturbances of endochondral ossification, high growth rate and relatively very high body weight of these animals (5).

**Table 1**
Characteristics of femur in investigated turkeys

<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>No. of investigated bones</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>46.47 (±0.86)</td>
<td>*</td>
</tr>
<tr>
<td>Trabecular bone density (g/cm³)</td>
<td>1.046 (±0.015)</td>
<td>*</td>
</tr>
<tr>
<td>Cortical bone density (g/cm³)</td>
<td>1.436 (±0.029)</td>
<td>1.481 (±0.023)</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>57.78 (±2.46)</td>
<td>59.38 (±1.91)</td>
</tr>
<tr>
<td>Second moment of inertia (mm⁴)</td>
<td>1118 (±70.9)</td>
<td>1166.7 (±72.1)</td>
</tr>
<tr>
<td>Mean relative wall thickness</td>
<td>0.251 (±0.009)</td>
<td>0.260 (±0.008)</td>
</tr>
<tr>
<td>Maximum elastic strength (N)</td>
<td>533 (±18.8)</td>
<td>*</td>
</tr>
<tr>
<td>Ultimate strength (N)</td>
<td>703.1 (±24.5)</td>
<td>711.4 (±19.93)</td>
</tr>
</tbody>
</table>

* Significance of differences between groups.

**Table 2**
Characteristics of tibia in investigated turkeys

<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>No. of investigated bones</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>61.76 (±1.07)</td>
<td>60.16 (±1.93)</td>
</tr>
<tr>
<td>Trabecular bone density (g/cm³)</td>
<td>1.186 (±0.015)</td>
<td>*</td>
</tr>
<tr>
<td>Cortical bone density (g/cm³)</td>
<td>1.598 (±0.018)</td>
<td>*</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>51.91 (±1.5)</td>
<td>*</td>
</tr>
<tr>
<td>Second moment of inertia (mm⁴)</td>
<td>650.7 (±24)</td>
<td>*</td>
</tr>
<tr>
<td>Mean relative wall thickness</td>
<td>0.286 (±0.014)</td>
<td>*</td>
</tr>
<tr>
<td>Maximum elastic strength (N)</td>
<td>447 (±21.7)</td>
<td>*</td>
</tr>
<tr>
<td>Ultimate strength (N)</td>
<td>578.4 (±16.6)</td>
<td>*</td>
</tr>
</tbody>
</table>

* Significance of differences between groups.
Our study revealed positive effect of alpha-ketoglutarate administration on skeletal system quality in fast growing turkeys. Almost identical mean values of body weight at the beginning of experiments and similar final body weight in both groups indicate that investigated parameters of femur and tibia were not influenced by differentiated body weight of turkeys. Increased trabecular bone density values of femur and tibia, and higher cortical bone density of tibia after AKG administration proves its influence on osteoblastic activity. Higher mechanical endurance of tibia in turkeys receiving AKG is a result of increased geometrical parameters of this bone and its higher trabecular and cortical bone density. The three-point bending test of femur showed that even in case of significantly lower femur weight of turkeys in AKG group, the mechanical properties of this bone were improved as well. The maximum elastic strength of femur in AKG treated turkeys was significantly increased, whereas the ultimate strength has not shown any difference between the investigated groups.

The comparison of the result obtained on femur and tibia with studies performed on turkey’s ulna, elucidated similar effect of AKG on bone tissue (18, 19, 20). Previous investigation on turkey’s ulna showed that AKG administration inhibits osteopenial changes, occurring as a result of bone denervation. The beneficent influence of AKG on callus formation and ulna fracture healing in turkeys was stated as well (18, 20). Moreover, the similar influence of AKG administration on bone tissue was observed in studies performed on rats, pigs and humans (1, 10, 14, 16, 21). Considering current knowledge of AKG, its metabolites and peripheral nervous system functions, it can be concluded that improved bone quality may be induced by higher glutamate synthesis and its utilisation as neuro-signalling molecule in bone metabolism regulation (2, 3, 17, 22). The other mechanism that may be involved in bone metabolism regulation by AKG is increased collagen formation as the result of higher proline synthesis and its following conversion to hydroxyproline, what was previously reported (11).

All these results indicate that enteral administration of AKG in vertebrates improves skeletal system quality and guarantees bone tissue homeostasis maintenance. Better mechanical endurance of skeletal system provided by dietary AKG supplementation may be considered as an effective way in the reduction of bone-related disorders during the production cycle in turkeys.

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**References**


