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COMPARATIVE STUDY OF THE CARDIOVASCULAR SYSTEM OF DIFFERENT GENOTYPE TURKEYS WITH IN VIVO AND IN VITRO METHODS

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Abbreviations

B.U.T. = ***B***ritish ***U***nited ***T***urkey *(it was a turkey breeder company, now it is a part of the Aviagen Turkeys Corp.)* • CT = ***c***omputed ***t***omography • ECG = ***e***lectro***c***ardio***g***raphy • LVEF% = ***l***eft ***v***entricular ***e***jection ***f***raction% • LVEDV = ***l***eft ***v***entricular ***e***nd ***d***iastolic ***v***olume • LVESV = ***l***eft ***v***entricular ***e***nd ***s***ystolic ***v***olume • LVSV = ***l***eft ***v***entricular ***s***troke ***v***olume • ME = metabolisable energy • MRI = ***m***agnetic ***r***esonance ***i***maging • WL = ***w***indow ***l***evel • WW = ***w***indow ***w***idth

1. INTRODUCTION

In recent decades, such turkey types were bred, proper to the consumers' needs, which with high growth rate constitute significant more lean muscle mass at the end of the fattening period. However the life support systems, including particularly the heart growth and the cardiac capacity keep up with difficulty with the large body mass and skeletal muscle growth, in this way the circulatory system reserves have become constrained. Meat quality problems, skeletal diseases, respiratory and circulatory system disorders are experienced increasingly. Despite that these difficulties play a more and more increasing role during the breeding and fattening period only few publications are dealing with the cardiovascular system and its problems of hybrid turkey.

Since there was no selection impact on the bronze turkey in the last fifty years, this genotype can be considered as native because the autochtonous breeds were placed under protection in the 1960's in Hungary. The bronze type turkeys can achieve a much smaller body size and can yield much smaller valuable cuts compare to the hybrids, however cardiovascular diseases are not common among them.

The liveweight, the slaughter properties of the two genotypes were examined in our faculty under different conditions many times. The question was how the intensive selection and the feeding affect the performance of the turkey.

Body composition and conformation were compared during the breeding of the two genotypes with computed tomography (CT). Cardiac output relative to the skeletal muscle of hybrid turkeys, were determined on the 12^{th}, 16^{th} and 20^{th} week with cardiac magnetic resonance imaging (MRI).

The comparative analysis of the cardiac output relative to the skeletal muscle of the two genotypes was not performed. In this way we do not know

how the one-sided selection - intent to the increase of valuable body parts - affects the circulatory system and the cardiac output.

2. OBJECTIVES

1. To present the cross-sectional and CT anatomy of the turkey.

2. To compare the cardiac output during breeding of the two genotypes of male turkeys with ECG controlled MR imaging.

3. To determine the quantity of skeletal muscle and the body surface of the hybrid and bronze turkey on the 12^{th}, 16^{th} and 20^{th} week with CT scanning.

4. To determine the relative cardiac output related to the body surface and skeletal muscle volume of the hybrid and bronze turkey on the 12^{th}, 16^{th} and 20^{th} week.

5. The analysis of the arteries of hybrid turkey heart with micro- and macrocorrosion anatomic methods on the 20^{th} week.

3. MATERIAL AND METHODS

3.1. First experiment, introducing of CT and cross-sectional anatomy

Two, 20 weeks old B.U.T. Big 6 meat-type male turkey were used for the CT- and cross-sectional anatomic examinations. The turkeys were healthy on the day of CT scanning according to the physical examination. Inhalation anesthesia was applied without premedication and intubation and T61 was used for euthanasia. The bodies were taken in PVC tubes in sternal recumbence for the CT examination. 620 transverse records were taken, with the following parameters: exposure time 6 sec, 120 kV, 80 mAs, collimation 0.75; pitch 1.25;

spiral scanning mode, 1mm slice thickness. After that the bodies were cooled to -70 °C, and 1cm thick slices were cut with a brandsaw, then we took pictures of the slices. The visible anatomy sctructures on the photos were identified and compared with the CT pictures.

3.2. Second expeiment, cardiac output examination of the two different genotypes

Fifteen B.U.T. Big 6 meat-type turkeys and fifteen bronze turkeys were used for the measurements. Measurements were made on five-five male turkey from both genotypes on the 12^{th}, 16^{th} an 20^{th} week. The B.U.T. Big 6 meat-type turkeys were bred under the conditions 14 hours per day in light, post-breeding suitable composition of feed (12-15th week: 12.5MJ/kg ME and 21.4% crude protein; 16-19th weeks: 12.9MJ/kg ME and 19.0% crude protein; 20th week: 13.1MJ/kg ME and 17.2% crude protein) and intensive conditions. The bronze turkeys were placed outdoors, and fed in various stages (12-14th weeks: 12.9MJ/kg ME and 18.6% crude protein; 14-20th weeks: 13.1MJ/kg ME and 17.0% crude protein) with mixed feed. Feed and water were available ad libitum in both conditions. Siemens Magneto Avanto type 1.5T field strength equipment was used for the MR examinations. From the apex of the heart to the basis multi-slice, multiphase ECG-triggered turbo gradient echo (TEG) records were done in sagittal and transversal planes. The left ventricular end systolic volume (LVESV [ml]) and the left ventricular end dyastolic volume (LVEDV [ml]) were calculated separately from the transversal records. The left ventricular stroke volume (LVSV [ml]) was calculated by extracting the left venticular systolic end volume from the left ventricular end dyastolic volume. The quotient of the LVSV and the LVEDV is the left ventricular ejection fraction (LVEF%). After the MR examination CT scans were made form the whole body using a Siemens Somatom Plus Expert (voltage: 120kV, dose: 90mAs; slice thickness: 10mm). Considering the muscle (20-200 HU)

and fat ((-)20-(-)200 HU) density values, the slice thickness and the magnification, muscle volume (MV [dm^3]) was calculated. The body surface area (BSA [m^2]) of the animals was determined from the CT images for every turkey.

SPSS for Windows 10.0 (1999) programme was used to statistical analysis (two-sample T-test and univarite analysis).

3.3. Third experiment, examination of the coronaries and blood vessels of myocardium

In a slaughterhouse two separate occasions, a total of forty, 20 weeks old B.U.T. Big 6 meat-type turkey hearts were collected. Immediately after removal the ascending aorta was cannulated and the heart was perfused with heparinized saline solution untill the liquid from the right heart ran out clearly. The own blood vessels of the heart were filled with two different viscosity resin throw the aorta, depending on that the aim was the visualization of the main blood vessels or the capillary system. The organic substrate was macerated with potassium hydroxide (KOH) in both cases.

4. RESULTS AND DISCUSSION

4.1. Results and discussion of the first experiment

Our aim was to represent more organ systems in details (respiratory, gastrointestinal, circulatorry and locomotor system) in one image, therefore the used window level (WL) and window width (WW) paremeters was (WL 860, WW 3200) different from the parameters used in general diagnostic. These settings enabled to illustrate in details the parts of the skeletal system (free vertebrae, notarium, synsacrum, sternal and vertebral ribs, sternum), the parts of the respiratory system (lungs, bronchia, air bags, septas between the air

sacs), the parts of the circulatory system (heart, great vessels) and the parts of the gastrointestinal system (liver, pancreas, stomach, intestines) on the CT images. The muscles, muscle groups can be isolated from each other on the CT images with these settings, but there were areas where the fascias were too thin between them to do so. On the hind limbs fat between the muscles facilitated the separation.

In human medicine a large number of CT and cross-sectional atlases are available; livestock and veterinary medicine miss this variety. From birds, especially domestic birds such work have not been made.

4.2. Results and discussion of the second experiment

The relative hearth weight was different in the two genotypes during growth. In the B.U.T. Big 6 meat-type turkey it was decreasing during ageing: from 0.45±0.05% to 0.37±0.02% ($p=.07$). In the bronze turkeys there were no significant changes. Significant difference between the two genotypes was present in all ages. Regarding the parameters of the left ventricle (LVEDV, LVESV, LVSV) in the two genotypes in all ages, in the given genotype during ageing there were significant differences ($p<.05$).

The measured cardiac output, the heart rate and the stroke volume index (SVI) values showed significant differences ($p<.05$) in the two genotypes and also in the given genotype during ageing. Considering the relative cardiac output [1iter/min/dm^3] per unite of skeletal muscle volume it is prominent that the B.U.T. Big 6 meat-type turkeys have significantly smaller values. The value measured on the 12^{th} week 0.48±0.01liter/min/dm^3 decreased to 0.19liter/min/dm^3 on the 20^{th} week. Comparing this with the values of the bronze turkey (0.72±0.03liter/min/dm^3 and 0.44±0.01liter/min/dm^3) it was very little.

In this experiment, we tried to measure the impact of the one-sided selection which focuses on bigger skeletal muscle - mainly the valuable parts (breast

muscle, thights) - on the circulatory system, especially the heart performance. We used male turkeys because females are fattened until the 16th week that wouldn't have shown the different tendencies between the two genotypes during the short time of examination.

4.3. Results and discussion of the third experiment

With the applied method we could prepare sufficient number of specimens for the examination of the blood vessels of the turkey heart. The myocardium has a high metabolic rate, the two coronary arteries the *A. coronaria sinistra, A. coronaria dextra* and its branches are supplying it. Their course is different from mammals, where they run in the subepicardium, while in birds they are located in the myocardium. In mammals the two coronaries are developed in different ways (right and left dominance). In birds, also in turkeys the right coronary artery is stronger; it supplies a larger part of the myocardium. Cardiac problems, such as round heart disease, coronary artery rupture in meat-type turkeys make it necessary to study the heart of this species extensively. It was possible to study the capillaries of the turkey heart on microcorrosion casts. The bigger arterioles and precapillar arterioles run on the capillaries generally obliquely. At the bifurcations of the precapillar arterioles we found signs refer to spincters.

5. CONCLUSIONS AND SUGGESTIONS

The anatomy of the body cavity of birds differ greatly in the two sexes in this way it is subservient to introduce the male and female individulas of the same genus, the latter in the egg-laying period.

Mostly cross-sectional images were done during the scanning. It is also necessary to take anatomical images in the two other main planes (horizontal and saggital).

During the CT scaning of the body composition of the meat-type hybrid turkeys the high ratio of the skeletal muscle to the body weight was remarkable. Birds' heat loss happens mostly through the respiratory system with panting. Combined testing is appropriate when we compare the surface area of the respiratory system with the muscle volume. Tests were performed on anesthesied animals; cardiac output was compared in rest. There was no opportunity to measure the reactions to the increased stress. We plan to do stress tests to receive information about circulatory reserves. According to expects, this kind of analysis would show better results in bronze turkeys.

The development of data recording and the invazive techniques make it possible the complex analysis of the circulatory system. There are many ideas about the reasons of aorta ruptures in meat-type turkeys. It would be practical to examine the status of the vascular system (different sections of the aorta, the main vessels supplying the breast muscle) in an invazive way paralell with the heart capacity.

We presented the qualitative analysis of the coronaries. Quantitative comparison would be useful of the capillary density of heart and the ratio of capillaries of the skeletal muscle and the muscle fibers. Selection based on the described imaging tests would be help in the optimization of physiological properties of new genotypes.

6. NEW SCIENTIFIC RESULTS

1. To prepare the cross-sectional anatomical and comuted tomographic comparison of the turkey.
2. Comparison of cardiac output basic data of the bronze turkey and the B.U.T. Big 6 genotype turkey with MR imaging used in human diagnostics. Observation: the left ventricular ejection fraction% (LVEF%) of the bronze turkey showed increasing tendency (69.42±0.34; 70.20±0.92; 71.74±0.60) during the period of examination (12^{th}, 16^{th} and 20^{th} week), while it decreased in B.U.T. Big 6 meat-type turkeys (69.42±1.16; 68.16±0.78; 66.98±0.89).
3. The value of stroke volume ratio relative to body surface significantly decreased in B.U.T. Big 6 meat-type turkeys (27.2±0.63ml/m^2, 25.0±0.57ml/m^2, 21.5±0.6ml/m^2). In the case of bronze turkey there was no significant change (21.1±0.1ml/m^2; 21.1±0.8ml/m^2; 21.6±0.36ml/m^2).
4. The cardiac output per a volume unite of skeletal muscle of the bronze turkey 0.72±0.03liter/min/dm^3, 0.55±0.04liter/min/dm^3, 44±0.01liter/min/dm^3) was higher in every examined ages than in B.U.T. Big 6 meat-type turkeys (0.48±0.01liter/min/dm^3, 0.29±0.02liter/min/dm^3, 0.19±0liter/min/dm^3).
5. Describe the anatomy of the coronary arteries' branching pattern of the B.U.T. Big 6 meat-type turkey in referenced international literature for the first time.
6. Analysis of the myocardium vessel structure of the B.U.T. Big 6 meat-type turkey with qualitative method—as far as we know—previously has not been done.

7. PUBLICATIONS AND ORAL PRESENTATIONS FROM THE THESIS STUDY

7.1. Publication in English

PETNEHÁZY, Ö. – BENCZIK. J. – TAKÁCS, I. – PETRÁSI. ZS. – SÜTŐ, Z. – HORN, P. – REPA, I.: Computed tomographical (CT) anatomy of the thoracoabdominal cavity of the male turkey (Meleagris gallopavo) *Anat. Histol. Embryol.*, 2011. (Accepted, Article first published online: 4 Aug. 2011, DOI: 10.1111/j.1439-0264.2011.01099.x)

7.2. Publications in Hungarian

PETNEHÁZY Ö. – TAKÁCS I. – PETRÁSI ZS. – DONKÓ T. – SÜTŐ Z. – BOGNER P. – HORN P. – REPA I.: A szelekció hatása a pulyka szívének teljesítményére *Magy. Állatorv. Lapja*, 2009 (131): 543–551.

PETNEHÁZY Ö. – LELOVICS ZS. – BENCZIK J. – TAKÁCS I. – REPA I.: A hibrid pulyka (Meleagris gallopavo) szív koszorús artériáinak morfológiája. *Acta Agraria Kaposváriensis,* 2011. (Accepted)

7.3. Publication in Proceedings in Hungarian

PETNEHÁZY, Ö. – TAKÁCS, I. – PETRÁSI, ZS. – DONKÓ, T. – SÜTŐ, Z. – BOGNER, P. – HORN, P. – REPA, I.: A bronz- és a gigantpulyka kardiovaszkuláris teljesítményének összehasonlító vizsgálata. [10. Nemzetközi Baromfitenyésztési Szimpózium. Kaposvár, 2011. április 6.] In: *10. Nemzetközi Baromfitenyésztési Szimpózium Proceedings.* 63–66. o.

7.4. Abstracts in English

PETNEHÁZY, Ö. – PETRÁSI, ZS. – TAKÁCS, I. – HORN, P. – SÜTŐ, Z. – SÓTONYI, P. – BOGNER, P. – REPA, I.: Cross sectional, CT and MR anatomy of the turkey (Meleagris gallopavo). [27th Congress of European Association of Veterinary Anatomists. Budapest, 23–26th July 2008.] *Magy. Állatorv. Lapja,* 2008. 130: 129.

PETNEHÁZY, Ö. – TAKÁCS, I. – PETRÁSI, ZS. – MAGYARI, T. – HORN, P. – BOGNER, P. – REPA, I.: Coronary arteries of the meat-type turkey. A corrosion cast study. [27th Congress of European Association of Veterinary Anatomists. Budapest, 23–26th July 2008.] *Magy. Állatorv. Lapja,* 2008. 130: 49.