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INVESTIGATION OF THE BODY COMPSITION OF DIVERGENT TYPES AND GENOTYPES OF POULTRY BY MEANS OF COMPUTER TOMOGRAPH

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1. BACKGROUND OF RESEARCH, OBJECTIVES

In meat type poultry, the breeding activity of the last decades induced basic changes with regard of body composition. From the viewpoint of animal breeding, the possible effects of this strong improvement are highly interesting. Experiments (including also those of the current dissertation) with the aim to characterize the chemical and tissue composition of broilers and turkeys may present data with regard to the effects of the selection towards meat production. The experimental work was carried out between 1996 and 2001 at the Institute of Diagnostic Imaging and Radiation Oncolgy. The practical background for animal experiments was established by the Department of Swine and Small Livestock Breeding.

From the viewpoint of the antecedent methodological work those, concerning the application of CT for the measurement of body composition and characterization of growth were of the highest importance.
At the University of Kaposvár, Faculty of Animal Science broiler and turkey performance tests have been carried out since 1973, with the co-operation of the National Institute for Agricultural Quality Control and of foreign breeding partners. In the research work data of individuals from the tested stocks were analyzed.

Our main goal was to track the growth characteristics and differences and the changes of the body composition during raising; the work was focused on divergent meat type chicken and laying hen genotypes and on merely different turkey genotypes. The aims of the study were the following:

- Gender-dependent investigation of the fat and muscle development in modern broiler genotypes, based on CT images at defined anatomical locations.
- Estimation of the broiler body fat content, on the basis of serial CT scans.
- Characterization of the relationship between the sternum length and breast muscle volume in different meat type broilers.
- Evolvement of a 3D reconstruction method from the cross-sectional CT images for the morphologic investigation of the breast muscles of broilers and laying hens
- In vivo comparison of the growth and tissue composition of bronze and giant turkeys, for the demonstration of the effects of the selection progress.

2. MATERIALS AND METHODS

2. 1. Animals

Birds were raised in a closed, air-conditioned building on deep litter at the Test Station of the Kaposvár University, Faculty of Animal Science. The CT scanning was carried out at the Institute of Diagnostic Imaging and Radiation Oncolgy, on a Siemens Somatom S40 apparatus. After weighing the chickens, three at the same time were fixed in stretched position with belts, in a specially designed holder, without using anesthetics in broilers. In case of turkeys anesthesia was applied with 10 mg/body weight kg Ketamine.

2. CT imaging

The CT images were taken by means of a Siemens Somatom DRG scanner of the Institute of Diagnostic Imaging and Radiation Oncology, after 8 hours of feed withdrawal. The examinations started with an overall topogram, which is similar to a conventional two dimensional X-ray picture. In the topogram the anatomical locations of the cross-sectional scans or body intervals were marked.

2. 3. Investigation of the body composition of a standard broiler genotype (Arbor Acres Regular)

A total of 144 Arbor Acress Regular meat type chickens were used. Six birds by sex were taken for scanning at the age 6, 12, and 18 weeks. Depending on the size of the birds 15 to 30 totally overlapping scans of 8 mm scan thickness were taken, covering the whole body.

From the serial scans, for the sake of comparison of birds of different size, 16 scans at defined anatomic locations were taken, ranging from the 9^{th} cervical vertebra to the knee joint.

CT scans were evaluated by the CTPC postprocessing software (Kövér and Berényi, 1995). Either from the total or from only a region of interest the pixel density distribution was computed. From these data, by means of the Histucut 1.0 software in the fat and muscle density range (from –200 to +200 Hounsfield unit) so-called Hounsfield variables (HUv) were produced, by summarizing all 10 neighboring density frequency values.

First the total body muscle and fat content was determined, then the distribution of the different tissue types in the body was investigated. The muscle and fat

development during growth in both sexes at different ages was demonstrated by 3D histograms, created with the negative exponential interpolation method by means of the SYSTAT 5.01. (1990) software.

Thereafter, using principle component analysis, estimation equations were developed for the crude fat content, first taking the total scan number into account, later focusing only on the abdominal region.

2. 4. Comparative investigation of the fat content of Arbor Acres Regular and Arbor Acres Yield Pack broilers

In both sexes 30 Arbor Acres and Arbor Yield Pack birds were scanned in a serial manner with total overlapping (8 mm slice thickness) at the age of 6, 12 and 20 weeks. Following the experimental slaughtering, the total body fat content was determined. The fat content was estimated by the fat index (Σ HU (-)10 - (-)200) / Σ (-)200 – 200) also used in former studies (Romvári, 1996), which can be handled as a measure independent of the bodyweight. It can accurately (R^2 = 0.8-0.9) be used for the estimation of the total body fat content.

2. 5. Growth of the breast muscle and the sternum in broilers and laying hens

Altogether 10-10 Arbor Acres Regular and Foxy Chick broilers, between the age 2 and 9 weeks were scanned weekly, while 5 - 5 Bábolna Tetra SL and Shaver Starcross 288 birds were exposed to imaging at the ages of 8, 12 and 20 weeks. Depending on the weight, 25-70 cross-sectional images, covering the breast region, were taken, using the Siemens Somatom Plus 40 spiral CT equipment. The slice thickness was 3 mm, and total overlapping was applied. First the topogram, later, after the marking of the breast region the cross-sectional tomograms were taken. The zoom factor was set to the birds' size, ranging form 3 to 5.3.

The determination of the muscle volume in a defined HU interval was performed by means of the CTPC software; the morphologic study of the sternum and breast muscle was based on the Able 3D Doctor 3.0 software (Able Inc., 2000).

The sternum volume could not be measured by the method based on the pixel densities, since the bones matter is not homogenous and changes also during growth. The volumetric estimation on this basis would, therefore, be hardly biased. To avoid this, the Able 3D software was used for the measurement of the sternum surface area and volume. The different tissue types were marked in the consecutive images by manual demarcation. Segmentation methodology was used for the morphologic and volumetric characterization of the sternum and breast muscle. The

muscle was marked in the consecutive tomograms and the covering surface model of the pectoralis musculature was developed by 3D rendering. By the volumetric measurement of these models the zoom factor was set as a calibrator.

The breast muscle growth was expressed as percentage related to the value determined at the 2nd week, which was set as 100%. Thereafter the sternum area to breast muscle volume index was developed, for the determination of the muscle mass falling onto one sternum area unit. The length of both breast muscle and sternum was given from the slice thickness and the number of slices covering this region. The largest breadth value of the breast muscle was measured with the Able 3D Doctor, at the anatomic location crossing the 2nd costa.

Proportional changes of the breast muscle during growth were characterized by means of the index values calculated form the breadth and length data. The relationship between the sternum length and breast muscle volume was characterized by exponential curves.

2. 6. Changes of the body composition of the BUT Big 6 and bronze turkeys

Two, from a genetic viewpoint highly divergent turkey genotypes, namely BUT Big 6 and bronze birds were scanned for total body, at the ages of 5, 12, 16 and 21 weeks by a Siemens Somatom Plus S 40 CT equipment. Six birds form each genotype and sex were investigated in a serial manner. Depending on the body size 30-50 cross-sectional, 10 mm thick images were taken, with total overlapping, under anesthesia, using body size dependent zoom factors (3.7, 2.2, 1.7, 1.5).

The measurement of the total body fat and muscle volume was based on pixel frequency distributions. After this, to estimate the total body fat content HU indices were calculated. Applying the negative exponential interpolation method 3D histograms were constructed, concerning 15 images at identical anatomic locations.

Morphological properties of the breast musculature of the different genotypes were described by the above-mentioned 3D reconstruction method. This was conducted on the individuals reaching the highest bodyweight (BUT Big 6: 21 kg, Bronze: 6.7 kg) at 21 weeks of age, which were scanned using 3 mm slices.

3. RESULTS

3. 1. Investigation of the body composition of standard broiler genotype (Arbor Acres Regular)

In the first experiment, the tissue and chemical composition of a standard broiler genotype (AA Regular) was determined. This work was mainly focused on the growth properties and gender dimorphism of modern meat-type hybrids kept far above the usual slaughter age. After the CT imaging the gender-dependent differences of the muscle and fat tissue development were described in three time points (6, 12 and 18 weeks of age). The muscle tissue development was found to be the characteristic process in both sexes until the standard slaughter age of 6 weeks. The degree of muscle development of males was higher than that of the females. The muscle development of the thigh and back was stronger than that of the breast musculature, in the males in all the timepoints examined; the muscle building of females was more balanced in the two main body regions. After the age of 12 weeks, the abdominal fat deposition of females became progressive, showing a further increasing tendency until the 18^{th} week, when also a considerable neck-fat deposit was detected. The to main body parts, i.e. the fore and the hind part, could be accurately differentiated on the basis of the fat index calculated. According the relative fat deposition (where the fat content at the 6^{th} week was accepted as 100%) the following order was established at the age of 18 weeks: male fore part, female fore part, male hind part and female hind body part (180, 240, 245 and 375%).

Furthermore, based on the CT-dataset obtained either from the total body or from the abdominal region and on the chemical analysis, by means of principal component analysis estimation methods were developed for the prediction of the total body fat content. Testing the equation on an independent dataset, the correlation between the measured and the estimated fat content could be characterized as good. The practical applicability of the method was mainly supported by the R^2=0.84 value in case of the abdominal region. Based on this strength, this method could effectively replace the direct chemical analysis; moreover, since it is non-invasive the serial investigation of identical animals is also possible.

3. 2. Comparative investigation of the fat content of Arbor Acres Regular and Arbor Acres Yield Pack broilers

Using the fat index, the total body fat content of AA Regular and AA Yield Pack genotypes was estimated at the ages of 6, 12 and 20 weeks, partly as a continuation

of the work performed on other species (meat-type rabbit, common carp). The direct volumetric body composition measurement, based on the density values of single voxels is well applicapble over the body fat content of 10%, being true for this whole experimental period (R^2 = 0.91 – 0.92). A further advantage of the Σ HUv 6-12 / Σ HUv 1-40 index computed from the (–200) – 200 interval of the Hounsfield scale is its independence from the body weight. The body fat content on the basis of the above index at the age of 20 weeks was the following: AA Regular male, AA Yield Pack male, AA Regular female and AA Yield Pack female, 0.18, 0.19, 0.27 and 0.27, respectively.

3. 3. Growth of the breast muscle and the sternum in broilers and laying hens

In the investigation of the breast musculature and sternum growth, changes of the breast properties of two meat-type hybrids were tracked between weeks 1 and 9. An exponential relation was found between the breast muscle volume and sternum length (R^2 = 0.91 and 0.85 by AA Regular and Foxy Chick, respectively). For the numeric characterization of the relation between the sternum surface area and the breast muscle volume index value was calculated; this showed an age dependent decrease throughout the trial (AA Regular, Foxy Chick; 2. and 9. week – 0.43, 0.56, and 0.22, 0.25, respectively). This index value was always lower by the AA Regular genotype, consequently, it develops more muscle volume on an identical sternum area unit.

For the demonstration of the differences between the original double-purpose poultry genotypes and the current, specific ones our investigations were also performed on weak and a medium-weight laying hybrid genotypes (Shaver Starcross, Terta SL). Real 3D comparisons were carried out at identical body weight categories and ages, where the breast muscle and sternum-geometric differences arising from the divergent utilization types were emphasized.

3. 4. Changes of the body composition of the BUT Big 6 and bronze turkey

The biggest selection progress in the production of valuable meat ratio was reached in the turkey between all poultry species. In connection with this, an ancient, basic genotype, the bronze turkey and a current giant hybrid (BUT Big 6) were compared, to describe the differences in their growth characteristics. Our results indicate a continuous age-dependent increase of the dressing percentage in both genotypes until the age of 12 weeks (BUT 62 %, bronze 47 %). Afterwards, between the 12th and 21st weeks, by the bronze turkey practically no difference could be shown, while by the BUT genotype a slight decrease was measured in the total body muscle

ratio (60%); latter might be attributed to the increasing fat deposition. The meat content of the modern genotype was 5-12% higher in all examined time points, while its bodyweight advantage continuously increased reaching a threefold value at the age of 21 weeks. Based on the 3D histograms, in the breast muscle ratio of the total body serious differences were described between the 12^{th} and 21^{st} weeks, with the predominance of the modern genotype. Though at the age of 5 weeks the fat content of both genotypes was similar, at the age of 21 weeks this order, according the total body fat index, was the following: BUT male (0.12), bronze male (0.13), bronze female (0.14), BUT female (0.20).

4. CONCLUSION

The quantitative increase and the qualitative improvement of the products of animal origin is a central question in animal breeding. This can be reached by the selection of breeder candidates, resulting in higher performance of the offsping in a defined trait, e.g meat production. Conventional methods obtain indirect information, either from the parents of from littermates. In contrast, cross-sectional imaging methods, like CT and MRI provide information on the real self performance in an *in vivo* manner.

A considerably novel possibily is the application of tomography, since the slaughter value (dressing percentage, fat content) of animals can *in vivo* be accurately estimated. This means a great quality step in the selection procedure for different traits. The method is not only more accurate than the evaluation of the offspring, but it is also much more quick in progress.

Body compositon changes during the growth of different pultry species can be accurately modelled by the method using serial imaging. The 3D histograms plotted from the pixel density distributions sensitively reflect both qualitative and quantitative changes of the tissue components. Since the CT imaging can also be repeatedly performed, it is possible to follow changes during the raising, moreover, the comparative investigation of different genotypes can also be carried out. This methodology could be an effective tool in the evolvement of crossing programs for a lower body fat content.

The very reliable measurement of the most valuable meat of chickens and turkey, the breast, is based on the possibility that in the cross-sectional images this muscle area can be demarcated, consequently, the muscle volume can be measured. Since by the fat, similarly to other farm animal species, this method is not applicable, the

crude fat content of the whole body was determined form the pixel density distribution data. According to our earlier results (rabbit, fish) the „fat index" has been used and estimation equations were created.

The fat index, a measure independent of body weight has been proven to be very suitable in the measurement of the body fat content over the 10% limit value. However, in our opinion the estimation equations arising from the principal component analysis are more accurate. An advantage of this method is that it strongly lowers the effect of multicolinearity existing between the original HU variables. The accuracy and applicability of the equations created was also proven by their very good testing results on independent datasets. On this basis this method could substitute the direct chemical analysis, in particular becasue is does not only characterize a single timepoint, but also the tracking of the changes in the body composition is possible this way. Applying the threefold plastic animal holder, evolved in rabbit investigations, the method is more cost-effective than the laboratory chemical analysis.

A novel 3D reconstruction methodology has been worked out when compared to those applied in earlier studies. A great advantage of this method is that morphological properties can only be investigated alive, e.g. the real geometry of the breast muscle cannot be attained after the slaughtering procedure. The relationship bewteen the sternum aera and the breast muscle is a further important condition, this again underlines the importance of the CT investigations. This methodolgy is not only effective in the analysis of the relationship between the different carcass traits, but it may also be applicable in the determination of the heritability of meat production related traits.

It is well-marked that 3D reconstruction of good quality requires high number of images and a relatively complicated image processing. Accordingly, the muscle surface to muscle volume index was constructed, characterizing the breast muscle morphology. In our further experimental work the evolvement of geometrical model, that, based on only a few images located at pre-defined anatomical locations, can also effectively demonstrate the breast muscle volume and surface, is planned.

A basic aspect of our investigations was to demonstrate body compositional changes both in broilers and turkeys that are highly characteristic in course of the selection for meat production. As a final conclusion of our work it can be stated that both the CT imaging method evolved and the personal and technical background at the Institute of Diagnostic Imaging and Radiation Oncology are highly suitable for the routine imaging of broilers and turkeys.

Summed up, the in vivo CT imaging was found to be a suitable method for the accurate characterization of the changes of tissue and chemical composition of different poultry species during growth. The volume of both muscle and fat tissue, and the total body fat content can be estimated by means of the density frequencies ordered to the image-forming pixels. Using 3D reconstruction methods, the morphological properties of the breast muscle and sternum can be characterized and also exactly measured. The comparative investigation of strongly divergent utilization types and genotypes is informative with regard to the consequences of the selection progress, moreover, its possible directions can also be forecasted.

5. NEW EXPERIMENTAL RESULTS

Gender-dependent changes in the muscle development and fat tissue deposition were characterized between the age of 6 and 18 weeks, with the application of 3D histograms adapted to broiler investigations. The muscle development was more pronounced in males, in particular at the thighs and back during the whole period investigated. The difference existing and showing and age-dependent increase between the sexes in the body fat content of the fore and hind part showed a further increase.

A principal component analysis based estimation method was developed for the pixel density distribution datasets. Using this, the accuracy of the estimation between the chemically measured and CT computed body fat content was $R^2 = 0.86$ and 0.89, as calculated either from the whole body of from the abdominal region. Testing the estimation method on an independent dataset resulted in $R^2 = 0.81$ and 0.84 values, proving its high practical applicability.

In AA Regular and Foxy Chick genotypes an exponential relationship was described between the breast muscle volume and sternum length ($R^2 = 0.91$ and 0.85). It was stated that by the AA Regular genotype the muscle volume increment related to sternum length unit is higher between the age of 1 and 9 weeks.

A 3D reconstruction method was developed for the morphological investigation of the breast musculature during growth both in broilers (AA Regular, Foxy Chick) and laying hens (Shaver Starcross, Tetra SL). Index values were calculated form the muscle surface and volume, as measured from the models. The lower indices by the growth indicate a morphologic change towards a more compact form. Calculated values for AA Regular and Foxy Chick on the 2^{nd} and 9^{th} weeks were: : 2.2 and 2.6, and 0.95 and 1.05.

6. SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

6. 1. Articles in foreign languages

Andrássy-Baka, G., Romvári, R., Sütő, Z., Szabó, A., Horn, P. (2003): Comparative study of the body composition of different turkey genotypes by means of CT. Archiv für Tierzucht, 46:1-3, 285-293 pp.

Andrássy-Baka, G., Romvári, R., Milisits, G., Sütő, Z., Szabó, A., Locsmándi, L., Horn, P. (2003): Non-invasive body composition measurement of broiler chickens between 4 – 18 weeks of age by computer tomography. Archiv für Tierzucht. *(megjelenés alatt)*

Andrássy-Baka, G., Romvári, R., Sütő, Z., Csapó, J., Szabó, A., Locsmándi, L. (2003): The study of the broiler chickens' growth by X-ray computerized tomography. Acta Agraria Kaposváriensis, 2, 19-29 pp.

Andrássy-Baka, G., Romvári, R., Takács, I., Sütő, Z. (2002): Comparative investigation of sternum surface area and breast muscle volume of different broiler genotypes by means of computer tomography. Krmiva, 2, 81-85 pp.

6. 2. Full conference papers in proceedings

Andrassy-Baka, G., Romvári, R., Petrási, Zs. (1999): In vivo measurement of breast muscle volume of broiler chicken by CT. 7th Int. Symp. "Animal Science Days", Balatonföldvár, 203-211 pp.

Andrássy, G., Romvári, R., Takács, I., (2000): Broilercsirkék izom- és zsírbeépülésének in vivo CT vizsgálata. XXVIII. Óvári Tudományos Napok, 13-18 pp.

Romvári, R., Andrássy- Baka, G., Repa, I., Závoda, F., Sütő, Z., Horn, P. (2000): In vivo 3D evaluation of breast muscle of broiler chickens by means computer tomograph XXI World's Poultry congress, Montreal, 2000, august 20-24 Procceding CD.