DOCTORATE (Ph.D) DISSERTATION THESES

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NON INVASIVE EXAMINATION OF THE CARDIOVASCULAR SYSTEM OF DIFFERENT PIG GENOTYPES

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1. INTRODUCTION

To the selection for meat production increased the skeletal muscle weight and the digestive tract volume which caused serious problems in breathing and regard of the cardiovascular system. The pig species have unfavourable cardiovascular system traits. The weak cardiovascular capacity of pigs may be due to the low relative heart weight, unfavourable blood supply of heart muscle (high systole to diastole ratio and big heart muscle fibre diameter) and small blood volume. These unfavourable characteristics are made worse by the modern races and hybrids are active, temperament, nervous, demanding to the enviroment, their adaptation ability is weak and they give strong reactions on minimal enviromental irritations. These characteristics caused changes in the cardiovascular system of the pig, consequently a growing number of cardiovascular disorders and deaths can be observed.

In our study - at first in the world - two *in vivo* cross-sectional imaging methods were applied; the functional parameters of the heart and the body composition of two extremely different swine genotypes were measured by MRI and CT examinations.

The aims of the experiments discussed in the dissertation were as follows:

- To develop narcotic and MRI examination methodology to ECG-gated dynamic MRI of the pig, which ensure an undisturbed, long and safe examination of animals in all age and live weight categories.
- Functional heart examination of meat-type pig and mangalitza by MRI to determine basic functional data of the heart.
- To compare to meat-type pig and mangalitza based on heart characteristics in the same weight (30, 60 and 90 kg) and at 170 days age, too.
- To compare the tissue composition of the two examined genotypes by computer tomography, to measure the fat and skeletal muscle volume and to find relationships between these parameters and the circulation.
- Heart anatomical study of meat- and fat-type pigs.

2. MATERIAL AND METHODS

From the start of 1998 until 2001 spring, 21 meat-type pigs and 12 mangalitzas were examined by MRI and CT imaging at the University of Kaposvár, Diagnostic and Radiation Therapy Institute.

Before the MRI and CT examination, the heart rate was measured, and an ECG was recorded. After these, the animals were examined under anaesthesia.

To work out the MRI methodology of heart examination, 15, 22-106 kg live weight meat-type pigs were investigated. 6 Hungarian large white meat-type pigs (12.5 %) x Belgian landrace (12.5 %) x Pietrain (25 %) x Norwegian landrace (50 %) genotype (3 castrates and 3 sows) and 6-6 white mangalitza castrates were examined in our research. For the determination of the examination-time the start of the average fattening time (30 kg, average 90 days old) was taken into consideration in the case of meat-type pigs. The studies were repeated in 60 and 90 kg. Mangalitzas were examined at similar live weight, but considering the different growth capacity of the two genotypes, at different age categories. The two genotypes of prominently different live weight were compared at the same age, at 170 days, as well.

The MRI examinations were carried out with SIEMENS MAGNETOM VISION PLUS 1.5 Tesla field strenght equipment by means of ECG-gated sequences. After the two-plane localisational images in the short axis of the heart, dynamic images were taken from the apex to the basis, covering all heart cavities. The complete examination protocol is found in the chapter of Results. After the MRI study, the CT examination of pigs was implemented with a spiral unit of SIEMENS SOMATOM PLUS in anaesthetized animals. Cross-sectional images were taken from the whole body (from the blow-hole till the joint of the hock). The evaluation of the swine heart images was based on the MASS 4.0 program that has been developed at the Medical University of Leiden.

After the last examination the animals were slaughtered and slaughter traits' values were determined. In the heart-morphology (anatomical) study each 20 hearts of meat and fat-type pigs were compared. From the CT data, X-ray density frequency curves were developed

in the fat and meat pixel-density range, meat percentage, meat volume, meat to fat ratio and meat average density were calculated. In the case of the results of MRI, the effects of the sex and weight were involved as factors in the multivariate variance analysis. The measured and calculated MRI and CT data were arranged based on weight and age groups. After this, in each group, the effect of genotype was analysed by independent-samples T-test. Discriminant analysis was applied to evaluate the anatomical study. For the statistical analyses we applied SPSS 9.0 program.

3. RESULTS

Methodology of anaesthesia and ECG-gated dynamic heart MRI

Combined narcosis, which contained ketamin and xylazin mixture, was applied as premedication in the study. Afterwards, 2.5-3.0 vol % isoflurane gas mask inhalation was applied to the relaxation. After the deep narcosis the animals were intubated (2^{nd} human complete respiratory-system), then they were connected to the narcotic unit (Penlon evaporator, Ohmeda O_2-N_2O flow-meter). The durable narcosis necessary for the MRI examination was achieved with the mixture of 1.5-2.0 vol % isoflurane gas and oxygen as carrier gas (0.14 ml/min - 0.28 ml/min, for piglets and fattening pigs, respectively). Before awakening, the animals were given a tranquilliser (1 % Acepromazin) injection into muscle. While mangalitzas awakened among their mates, meat-type pigs did it in separate boxes.

The transmission of the ECG-sign was carried out with a so-called active electrode supplemented with an amplifier. The sensors of the electrode were located to form a regular triangle. The electrode opposite the signal cable was fixed 10 cm far from the axis of the sternum-body between the 5^{th} and 6^{th} ribs on the left, the other two electrodes were fixed between the 3^{rd} and the 6^{th} ribs in the direction of the left elbow. By using an active electrode, an adequately clear ECG-sign could be gained, even in the case of fat and heavy animals. The animals were examined in an antimagnetic container, in prone position. After the localisation images and the *angulatio*, in the short axis of the heart, multi-slice, multi-phase images were taken from the apex to the basis, covering the heart cavities; a prospective data acqusition (8-10 min) was carried out. Depending on the heart rate and the size of the heart, in 8-10 plains, 8-14 phases were taken one by one per heart cycle. The number of the applied phases was determined by the heart rate and the repetition time that could be set optionally.

Examination of the heart capacity and the tissue composition of different pig genotypes by means of ECG-gated dynamic heart MRI and spiral CT

According to the results of the dynamic MRI examinations, at the starting period (30 kg), the left ventricular stroke volume of mangalitzas showed a 30 % higher value (38.1 ml) than that of meat-type pigs (29.1 ml) by the same left ventricular mass weight (85 and 84g). However, the measured heart rate (63 %) and the calculated cardiac output (82 %) were significantly lower in the proportion of the relative data of intensive meat-type pigs. The ejection fraction was about 55 % in both genotypes. In this weight, the meat percentage of meat-type pigs was 62.9 %, while that of mangalitzas was 35.5 % and the meat to fat ratios were 3.2 and 1.

With the increase of live weight, in 60 kg, in the case of the left ventricular stroke volume and mass weight the same tendency could be seen. It increased to 54.0 ml in the case of left ventricular stroke volume in mangalitzas and to 42.1 ml in meat-type pigs. The left ventricular mass weight (127 g) measured in meat-type pigs slightly exceeded the 120 g value of the mangalitzas. The ejection fractions that had been measured in 30 kg did not change either. The heart rate fell in both genotypes in relation to the first examination occasion. Meanwhile, a lower value (72 beats/min) was measured in mangalitzas than in meat-type pigs (106 beats/min). The calculated cardiac output (82 %) increased by 1.2 - 1.3 times (3.9 and 4.4 l/min) of the previous value. According to CT examinations, the development of skeletal muscle was more expressive in meat-type pigs, while the fat deposition was more significant in mangalitzas. In the first case 58.6 % meat percentage and 3.0 meat to fat ratio was measured, and in the second the relating values were 30.9 % and 0.6, respectively.

Though significant meat building was not experienced in 90 kg live weight, a volumetric growth of fat was observed in relation to the previous examination. This time the meat percentage decreased to 28.5 % and the meat to fat ratio to 0.5. The stroke volume (55.8 ml) and the cardiac output (3.8 l/min) did not change either. The heart rate fell slightly (68 beats/min), while the left ventricular mass weight increased slightly (140.5 g). A further intensive growth could be observed in meat-type pigs in 60 kg live weight. In parallel with the building of skeletal muscle, the stroke volume increased to 63.5 ml, the cardiac output increased to 5.8 ml/min and the left ventricular mass weight increased to nearly 180 g. The

heart rate (91 beats/min), the meat percentage (53.4 %) and the meat to fat ratio decreased to 1.9.

Examining both sexes in the same weight, it can be stated that in 30 kg live weight, that higher left ventricular mass weight and cardiac output were typical of the castrates. In the animals of 90 kg, the difference in the left ventricular mass decreased, however, the 15 % difference in cardiac volume remained the same. The differences measured in ejection fraction and in heart rate were not significant. The final results proved that the castrates with more favourable fattening ability, because of the faster growth capacity, bigger muscular tissue, which became measurable in heart function as well.

The two genotypes of prominently different weight were compared at the same, 170 days old age as well. The left ventricular stroke volume was 55.8 ml in meat-type pigs and 29 ml in mangalitzas, the cardiac output was 5.8 l/min in meat-type pigs and 4.2 l/min in mangalitzas. As regards the left ventricular mass weight, a 2.6 times bigger excess can be measured in favour of meat-type pigs. The meat values were 54.3 and 46.2 %, the meat to fat ratio values were 1.9 and 1.5, and the relative cardiac output values 6.0 and 1.8 dm^3/l/min respectively. Relying upon our results, it can be sated that the ventricular output values of the animals of the two genotypes, at the same age, were primarily determined by live weight, not by the age.

When evaluating the MRI and the spiral CT results in connection, it was proven that the older an animal is, the bigger mass of skeletal muscle it has per 1 litre of cardiac volume. The relating data are 2.8, 3.9 and 4.7 dm^3/l/min in mangalitzas and 4.0, 5.6 and 6.0 dm^3/l/min in meat-type pigs. In tissue composition, the index values of the mangalitzas of less skeletal muscle weight were more favourable at all 3 times examination, which supports the more favourable heart capacity of the genotype.

Examination of slaughter value of different pig genotypes

Animals of both genotypes were slaughtered in the same live weight and the slaughter value was defined. The average meat and fat content was 48.9 and 23.2 % in case of meat-type pigs and 27.1 and 43.3 % in mangalitzas, respectively. Concerning absolute heart weight, a 30 % less value was measured in mangalitzas. The values of the relative heart weight and

the relative heart weight corrected to the skeletal muscle were 0.3 and 0.21 % and 0.77 and 0.94 % in the case of meat-type pigs and mangalitzas. The absolute heart weight values relating to the whole skeletal muscle weight were proved to be higher in mangalitzas as a demonstration of the favourable cardiovascular characteristics of the breed.

For more detailed investigations we measured the surface of the *m. longissimus dorsi* and the *m. semitendinosus* and the fat thickness of the back and the rump in the cross-sectional images in 90 kg. Concerning the lean and thigh we measured a cross-sectional surface of 45 and 31 cm^2 in the case of meat-types, 23 and 24 cm^2 in fat-type pigs. The measured fat thickness values around the kidneys and the rump of mangalitzas were 53 and 38 mm and 18 and 16 mm in meat-type pigs, respectively. In the same images, in 30 and 90 kg live weight, frequency curves were made on certain areas on the two muscles. It was proven by the measured data that mangalitzas contain a significant higher amount of intramuscular fat the meat-type pigs.

Anatomical examination of the heart of different pig genotypes

In the examination of the pig's heart anatomy 28 parameters were investigated. The 100 % classifying result gained with the help of all the measured anatomical features of the discriminant analysis, made after the anatomical examination of the pig hearts, proved that the hearts of the examined genotypes can be morphologically separated from each other very well. If only left ventricular or right ventricular parameters are analysed, the estimation in the left ventricle is of 90 %, while in the right one it is of 95 % accuracy. The accuracy of the estimation on the basis of heart lenght, heart circle and wall thickness is 87.5 %.

4. CONCLUSION

- With the help of the applied isoflurane gas we could performe examinations also on fat and heavy animals and could assure easy and balanced breathing, stable and long anaesthesia duration and immediate awakening without side-effects. With the application of endotracheal intubation the strong respiratory movements were minimalized, the animals were quiet, their heart rate and breathing showed normal values. According to our opinion to the later study the use of volumen-gated

anaesthesia could be a further adventage. Mangalitza's anaesthesia was more difficult that of meat-type pigs, another mate's presence was needed, both in the preparation and in the awakening period.

- The applied active electrode proved to be suitable, altough the sign electrodes are needed to elongated, which further allows another signs to be collected at the "electric axis" of the heart as well.

- The examination time of the dynamic MRI of one pig took between 30-40 minutes depending on the weight of the animal and on the size of the heart. During the MRI at first we applied human sequences, which were later modified to the position and function of the pig's heart. In routin studies it is not necessary to examine the whole heart-cycle, it is enough to follow the end-diastole and end-systole section, to decrease the time and cost of examination and risk of anaesthesia. To the data aquisition we suggest the application of 40 ms repetiton time, which increases the measurement time, but results in better images quality.

- When evaluating the functional parameters of the two genotypes measured by MRI results together, the skeletal muscle volume determined the ventricular volumes in same age and weight as well. The ejection fraction values were between 48 and 68 % in both genotypes and in all examination times. However, the measured heart rate decreased with the growth in both genotypes, but the data were lower in the fat-type pigs. Our study was carried out in relaxed animals, we can only suppose the different of cardiovascular capacity of the two genotype. Furthermore measurements during a stress condition use also planned. The functional parameters are planned to measured *in vivo,* during exercise.

- In the case of meat-type pigs, animals of different sexes were examined in the research process. Examining both sexes in the same weight, the final results proved that the castrates possess a more favourable fattening ability, because of the faster growth capacity, bigger muscular tissue, which became measurable as well in heart function.

- The functional MRI examinations together with the CT imaging give important opportunity to define body composition, and for the description of heart capacity of different genotypes of pigs. The relative cardiac output value showed a bigger heart capacity of mangalitza. In our future experiments we are going to estimate the skeletal muscle volume from images of some fixed pre-defined anatomical locations, instead of CT imaging of the whole body, to decrease the anaesthesia time and the examination costs.

- During the postprocessing of MRI images, the applied programs were found to be well applicable. In the future an additional alternative is the MEDIMAGE Practice Builder 3.4 system, which can provide a new estimating method for outside researchers, through Internet connection. The postprocessing of MRI images took 2-3, in the case of CT 3-4 hours per animal. The applied MASS program was suitable for extra functions, too, which were not published in this dissertation and give new possibilities for the image evaluation.

- The anatomical examination proved that with this applied method the hearts of the two genotypes can be morphologically accurately classified. New research work is planned to find connections between the measured morphological data and functional features by MRI.

- The developed and succesfully used methodology may be applicable in the selection. The widening of the database to another pig genotypes may have important consequences in the pig breeding. It can be an aim in the future to develop new breeding lines, featuring outstanding meat production, suitable cardiovascular system and few heart diseases.

- The personal and technical possibilites of Diagnostic and Radiation Therapy Institute ensure the opportunity to the parallel CT and MRI examinations also an international level. The present study makes the co-operation with famous foreign collaborators also possible.

5. NEW EXPERIMENTAL RESULTS

- The endotracheal intubation, isoflurane gas narcotic procedure and the signal transmission of ECG measurement were developed to the heart MR imaging of meat-type and mangalitza pigs.

- Based on the application of human MRI sequences, a new methodology was developed, which proved to be well applicable for the functional heart examinations of different pig genotypes. With the help of this method in both genotypes the basic heart characterictics were measured in the weight of 30, 60 and 90 kg. The left ventricular stroke volumes (29.1, 41.2 and 63.5 ml in meat-type pigs, 38.1, 54.0 and 55.8 ml in mangalitzas) were found to be analogous to the left ventricular mass weight (84.8, 120 and 177 g in meat-type, 83.6, 127 and 141 g at mangalitza), increasing in all intervals continuously. Relying on the comparison at 170 day old pig, it can be stated that the heart parameters changed according to the change of the skeletal muscle mass.

- The heart rates of mangalitzas were sigificantly lower in all 3 time points of examination suggesting a higher capacity for the increase of the cardiac output. The average cardiac output values of meat-type pigs (3.8, 4.4 and 5.8 l/min) increased parallel with the skeletal muscle weight, while in the case of mangalitzas there were no important changes above 60 kg live weight (3.1, 3.9 and 3.8 l/min).

- It was proven by the CT data that mangalitzas have no important skeletal muscle growth, while the examined *m. longissimus dorsi* and *m. semitendinosus* contained a higher amount of intramuscular fat, than in the case of meat-type pigs.

- When evaluating the functional heart parameters and skeletal muscle volumes measured with MR equipment and the spiral CT results in connection, it was proven that the older an animal is, the higher mass of skeletal muscle it has per 1 litre of cardiac volume, at least in the examined interval. Results gained from anaesthesia can be analogical with those from relaxed state. The 4.0, 5.6 and 6.0 (meat-type pigs), 2.8, 3.9 and 4.7 dm^3/l/min (mangalitzas) cardiac output values showed that mangalitza has larger cardiovascular capacity.

- The traits involved in the anatomical examination were proven to be suitable to classify the two genotypes, by means of biometric methods.

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

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