Theses of Doctoral (PhD) Dissertation

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Development of Imaging Data Processing Methods for Animal Science Studies Using Elastic Registration

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Contents

1. BACKGROUND OF RESEARCH, OBJECTIVES

At the predecessor institution of the Hungarian University of Agriculture and Life Sciences (MATE), Kaposvár Campus – the Faculty of Animal Science of Pannon University of Agricultural Sciences (PATE) – livestock research based on computed tomography (CT) was launched as early as the beginning of the 1990s. Over the past three decades, various imaging techniques for animal science purposes have been applied at the institution, among which CT has played a prominent role. The first CT-based livestock research results from Kaposvár were published in 1992 on rabbits and in 1993 on pigs., followed shortly thereafter by a study estimating whole-body fat content in broiler chickens (Szendrő et al., 1992; Kövér et al., 1993; Romvári et al., 1994).

Intensive CT experiments on pigs began in the late 1990s and confirmed that carcass traits, especially lean meat content, could be reliably estimated in vivo (Romvári, 2005). At the same time, the objective of these studies was the non-destructive, in vivo determination of body composition — the ratio of muscle and fat tissues — which represented a fundamental qualitative advancement in selection practice (Romvári, 2005).

In 1991, Berényi and Kövér developed the program CTPC (Computer Tomography Picture Control), which represented a major advancement: it enabled the visualization of CT slices, the segmentation of regions of interest, the differentiation of tissues based on muscle, fat, bone, and water content, as well as the generation of histograms and the calculation of area parameters. (Berényi, and Kövér, 1991; Romvári et al., 1996). The need for such self-developed software well illustrates the difficulties faced at the beginning.

The world's first rabbit CT study was conducted in Kaposvár (Romvári, 2005). Initially, for different animal species, body composition and economically important traits were determined by manual segmentation of images taken at specific anatomical positions (Vangen, and Allen, 1984; Sehested, 1986; Bentsen et al., 1986; Szendrő et al., 1992). One such trait was the so-called L-value in rabbits, calculated as the average area of the longissimus dorsi (MLD) muscle from cross-sectional images taken between the 2nd–3rd and 3rd–4th lumbar vertebrae (Szendrő et al., 1992).

Continuous development at the Kaposvár research site — such as the introduction of spiral CT technology in the late 1990s — further improved the efficiency and accuracy of imaging. The advancement of the technique made it possible to capture the entire target volume, first with limited, then with increasingly higher resolution. To accelerate data acquisition, several simplified approaches were developed for serial imaging, such as performing scans in multiple regions (Thompson, and Kinghorn, 1992) or acquiring serial images at slice intervals (Jopson et al., 1995; Romvári et al., 1996). One advantage of serial imaging was that anatomical landmarks could be identified more rapidly afterward, compared with the precise positioning of the animal during the examination. This approach was successfully applied in poultry breeding and fish studies as well (Andrássy, 2003; Romvári, 2005).

Ongoing technological progress naturally called for advances in image processing, aiming to replace manual techniques with automated segmentation. By 2012, studies on rabbits had already reported results obtained using automatic segmentation, based on thresholding of whole-thigh CT scans performed at relatively large slice thicknesses (10 mm) (Szendrő et al., 2012). Increasing resolution allowed for more accurate distinction of small structures within the imaged object; however, in our experience, the applicability of simple thresholding techniques became limited due to increased image noise.

To address this issue, we developed a new method that applied thresholding, automatically detected anatomical landmarks, and iteratively used morphological operators for segmentation (Matics et al., 2020a). In some cases, however, its applicability was limited by the partial volume effect (PVE).

Further methodological improvements are justified by the technical and biological challenges that emerge in parallel with the continuous development of imaging technologies.

1.1. Objectives

The robust estimation method based on animal CT scans, related to my doctoral thesis, using automatic segmentation, was developed in 2019 and its first published in 2022 (Csóka et al., 2022). The method can be applied to various animal species and offers the possibility of automatically processing CT scans by estimating the objective body composition.

In connection with this research, the main objectives of my dissertation were as follows:

- to develop an open-access software package capable of estimating the weights of valuable meat cuts of different animal species by means of automatic CT image processing, applying elastic registration, stochastic feature selection, and various regression methods;
- to provide a detailed description of the developed estimation method;
- to carry out the practical testing of the method on three animal species:
  - In breeding rabbits: estimation of the weights of the longissimus dorsi, the muscles of the left hind limb, and the head;
  - In broiler chickens: estimation of the weights of breast and thigh muscles;
  - In pigs: estimation of the weights of different carcass cuts and the lean meat percentage.

With the development and application of this method, my aim was to produce reliable and reproducible data based on imaging techniques that can effectively support selection decisions in animal breeding.

2. MATERIAL AND METHOD

2.1. Description of the Animal Species Used in the Studies

During the experiments, CT examinations were carried out on three different livestock species — rabbit, broiler chicken, and pig. The estimation method was based on CT image data and reference data obtained from the carcass dissections of the same animals.

In the case of rabbits, two groups with different genotypes were examined. The first group consisted of 170 ten-week-old rabbits of the Pannon White genotype (genotype A). The smaller group included 30 rabbits of a different large genotype (genotype B), also at 10 weeks of age. Animals in both groups were randomly selected from larger populations.

For the CT examinations, chickens were selected from healthy individuals of a pre-established trial at 10 weeks of age. Selection was carried out in a mixed-sex design, with an equal proportion of males and females. The body weights of slaughtered animals could differ by no more than ±3–5% from the sex-specific average weight, in accordance with the recommendations of *Hen and Turkey Performance Testing Code IV.*. In total, 60 animals were examined within approximately one hour using CT, and the same individuals were slaughtered the following day. All animals belonged to the slower-growing broiler genotype TETRA HB Colour, originated from the Bábolna Ltd. hatchery, and were reared at the test farm of Kaposvár University.

During the examination of chickens and rabbits, three individuals were scanned simultaneously without anaesthesia, using a specially designed triple trough, with the animals fixed by straps. In the smaller rabbit group, the head was also immobilized, while in the larger group (Pannon White) the head was left free to move.

In the case of pigs, the right half-carcass of 48 animals of the Swiss Large White genotype was examined by CT. The animals were reared at the Agroscope research institute within the framework of the PIGWEB project. Slaughtering took place at 22–24 weeks of age. Half-carcasses were transported to our institute in frozen condition (–20 °C) and examined after thawing.

2.2. CT Examinations

CT examinations of Pannon White rabbits were performed one day prior to slaughter, on 9 January 2018, at the Institute of Diagnostic Imaging and Radiation Oncology of Kaposvár University, using a Siemens Somatom Sensation Cardiac 16 MDCT scanner. The applied settings were as follows: tube voltage 140 kV, total radiation dose 90 mAs, spiral data acquisition with a pitch factor of 1 (table feed/collimation). Overlapping images were reconstructed using the Siemens Syngo CT VA48A software with an I30f convolution kernel, within a 500 mm field of view and an image matrix of 512×512 per slice.

On 2 December 2020, CT examinations of 60 broiler chickens originating from the experimental poultry farm of MATE Kaposvár Campus were performed by the staff of Medicopus Nonprofit Ltd. at the SM KMOK Dr. József Baka Diagnostic, Oncoradiological, Research and Educational Center, using a Siemens SOMATOM Definition AS+ scanner. The scanning parameters were as follows: tube voltage 120 kV, total radiation dose 120 mAs, spiral data acquisition with a pitch factor of 0.5, and a 500 mm field of view. Image reconstruction was carried out with Siemens Syngo CT VA48A software using an I30f convolution kernel.

The examinations of the smaller rabbit group were carried out on 23 March 2022, also by the staff of Medicopus Nonprofit Ltd. at the SM KMOK Dr. József Baka Diagnostic, Oncoradiological, Research and Educational Center, using a Siemens SOMATOM Definition AS+ scanner. The applied parameters were: tube voltage 120 kV, total radiation dose 200 mAs, spiral data acquisition with a pitch factor of 0.6, field of view 500 mm, and an image matrix of 512×512 per slice. Compared to the earlier rabbit CT examinations, in these cases we also attempted to immobilize the head of the rabbits using fixation straps.

Pig examinations were likewise performed with a Siemens SOMATOM Definition AS+ scanner by the staff of Medicopus Nonprofit Ltd. at the SM KMOK Dr. József Baka Diagnostic, Oncoradiological, Research and Educational Center. The parameters were: tube voltage 120 kV, total radiation dose 200 mAs, spiral data acquisition with a pitch factor of 1, field of view 500 mm, and an image matrix of 512 × 512 per slice. Images were reconstructed using the Siemens Syngo CT VA48A software with an I30f convolution kernel.

All examinations were performed in compliance with the ISO 9001:2015 quality management system and the ISO 14001:2015 environmental management system requirements (*ISO 9001:2015 Quality management systems*; *ISO 14001:2015 environmental management system*).

2.2.1. Slaughter of Animal

The experimental slaughter of rabbits and subsequent weighing were carried out in accordance with the recommendations of the World Rabbit Science Association, following the slaughter procedure developed by Blasco, and Ouhayoun (1996). After dissection and cutting, the weights of the longissimus dorsi (MLD), the head, and the whole loin were measured using a balance with gram-level precision.

In the case of broiler chickens, carcass dissection was performed according to the procedure described by Jensen (1983). Following dissection, the weights of the valuable meat parts — breast and thigh muscles — were measured.

The slaughter of pigs and the cutting of the left half-carcass were carried out at the experimental slaughterhouse of Agroscope (*Agroscope, Federal Office for Agriculture - FOAG, Switzerland*), according to the EU reference method described by Walstra, and Merkus (1995). After slaughter, carcasses were split into halves and chilled for over night (more than 15 hours), after which their weights were recorded. The left half-carcasses were subsequently dissected, and the weights of the resulting cuts were measured.

2.3. Image Processing

In each case, the CT images were converted from DICOM format (*DICOM File Format*) into **MINC** format (Vincent et al., 2004) for preprocessing. During preprocessing, the three individuals were separated from each other and the troughs were removed from the images. The **MINC** image files containing the individual animals were then converted into **NIFTI** (Neuroimaging Informatics Technology Initiative) format (*Neuroimaging informatics technology initiative* 2005). The center of mass of the resulting images was aligned to the origin of the coordinate system in order to facilitate faster and more accurate image registration. In the case of pigs, only one half-carcass was included in each scanning series. Therefore, for these images only a DICOM to NIFTI conversion was performed. The examination table was automatically removed, and the center of mass of the images was likewise aligned to the origin of the coordinate system.

2.3.1. Manual Image Segmentation

For the segmentation of the longissimus dorsi and the muscles of the left hind limb of Pannon White rabbits, 16 previously acquired exams were used. For manual segmentation of the heads in rabbits, 20 image series from earlier studies were processed for each genotype. In broiler chickens, 16 CT images from previous studies were randomly selected for the segmentation of the breast and thigh muscles. In the case of pigs, no earlier database was available; therefore, five carcasses out of the existing 48 individuals were manually segmented and virtually dissected.

Manual segmentation of the images was performed using the open-source software 3D Slicer (Kikinis et al., 2014; *3D Slicer image computing platform*). For each animal species, the regions corresponding to the measured traits were delineated (segmented). In pigs, the lean meat percentage (LMP) was also determined, which was estimated using the entire half-carcass.

In the case of rabbit head and skull segmentation, the created segments did not correspond to the dissection. To ensure reproducibility, two virtual dissection procedures were developed, based on clearly identifiable anatomical landmarks in the CT images.

In the first method, the proximal edge of the atlas wing (left or right) closest to the nose in the axial plane was used as a reference point. Based on this landmark, the head was separated in the axial plane, and two segments were created by simple thresholding: head (threshold values: –200 to 3071 HU) and skull (threshold values: 200 to 3071 HU).

In the second method, five anatomical landmarks were used to define two planes, which enabled head separation and the subsequent creation of the two segments (head and skull) using thresholding with the same values as above. The anatomical landmarks for constructing the first plane were the right and left angular process and the external occipital protruberance (nape). For the second plane, the external occipital protuberance and the distal edges of the right and left atlas wings were identified and used.

The first segmentation approach (single-plane cut) could only be applied to the CT images of genotype A rabbits, which had immobilized heads. For the CT images of genotype B rabbits, this method was not feasible, as the animals' heads almost always touched the forelimbs, which prevented its use. In addition, in several cases the heads were twisted or turned sideways (to the right or left), making consistent segmentation impossible with the first method. The second segmentation procedure, however, could be applied to both sets of images prepared for segmentation, since the two-plane virtual cut based on five anatomical landmarks was able to overcome the difficulties arising from variations in head positioning.

2.3.2. Image Postprocessing (Registration)

For image registration, the Elastix software package (*Elastix*) was used with default parameter settings (Klein et al., 2010; Shamonin et al., 2014). The method applied a multi-step alignment with a B-spline transformation grid, defined similarity based on Mutual Information, and optimized the transformation field using Adaptive Stochastic Gradient Descent, with a maximum of 200 iterations. All manually segmented individuals were registered to the CT images of non-segmented individuals of the same genotype, and the corresponding masks were transformed. In this way, a multi-atlas registration-based segmentation was performed. In estimating the lean meat percentage (LMP) of half-carcasses, registration and the use of atlases were not required, as segmentation could be carried out using a simple threshold-based method.

2.3.3. Data Collection (Feature Extraction)

Using the transformed masks, features were collected from the target regions covered by the masks (number of voxels, sum of voxel HU values, mean HU value, standard deviation, skewness, kurtosis, and histograms).

2.3.4. Data Processing and Statistical Analysis

For weight estimation, different regression methods (linear, PLS, lasso, ridge, KNNR) were applied. Since the number of features was relatively large compared to the number of samples, and multicollinearity among the variables could not be excluded, feature selection was performed for each regression procedure. To reduce the number of features and to determine the parameters of the regression models, simulated annealing (SA) was applied. Among linear models, we used linear regression, ridge regression, lasso regression, and partial least squares regression (PLS), and as a non-linear method, K-nearest neighbors regression (KNNR) was employed. The regression parameters were set as follows:

- Conventional linear regression: only a single parameter was varied.
- PLS regression: the number of components was varied between 2, 3, $\ldots$, up to $\sqrt{\text{number of variables}}$.
- Lasso regression: the regularization parameter was varied between 0.01 and 10.
- Ridge regression: the regularization parameter was varied between

0.01 and 10.

The parameters of the simulated annealing (SA) were as follows:

- N = 8000 (number of iterations)
- T = 1 (initial system temperature)
- $T_0 = 10^{-7}$ (minimum target temperature)

To evaluate the performance of the models, 10-fold cross-validation repeated 20 times was applied.

The case of broiler chickens and Pannon White rabbits, the R^2 values obtained from the 20-times repeated 10-fold cross-validation did not follow a normal distribution according to the Shapiro-Wilk test, with p-values being smaller than 3×10^{-10} in all cases. Based on the normality test, the Mann-Whitney U test was applied to compare the results obtained with different atlases, while the Wilcoxon signed-rank test was used to compare the results with and without feature selection.

The steps of multi-atlas segmentation, feature extraction, and model selection were made publicly available as an open-source Python package on GitHub (*maweight*), `https://github.com/cseka7/maweight`. The test data as well as the Jupyter notebooks used for evaluation are available in the following repositories:

- Rabbits - notebooks used for rabbit data processing (*Jupyter Notebooks Used for Rabbit Data Processing*): `https://github.com/cseka7/rabbit_ct_weights`
- Chickens - notebooks used for chicken data processing (*Jupyter Notebooks Used for Broiler Chicken Data Processing*): `https://github.com/cseka7/chicken_ct_weights`
- Pig half-carcasses - notebooks used for pig half-carcass data processing (*Jupyter Notebooks Used for Processing Pig Half-Carcasses*): `https://github.com/cseka7/pork_ct_weight`

3. RESULTS

The developed method was applied to three animal species. Regression analysis of the features obtained from CT-based image processing enabled accurate estimation of slaughter traits, using live animals in rabbits and chickens, and slaughtered animals in pigs.

In rabbits, for the estimation of the weights of the left hind limb muscles and the musculus longissimus dorsi (MLD), the best performance was achieved – when combined with feature selection – by the multi-atlas models using linear, PLS, lasso, and ridge regression. For the left hind limb muscles, model performance (R^2) ranged between 0.925 and 0.975, while for the MLD it ranged between 0.86 and 0.94. Without feature selection, the models performed less effectively.

For rabbit heads, better estimation accuracy was achieved in the genotype A population compared to genotype B. The difference can most likely be attributed to the fact that the heads in the genotype B population were not immobilized during the examinations. The results confirmed that the segmentation masks created could be effectively applied across rabbit populations of different genotypes, supporting the robustness and generalizability of the method, particularly in light of the substantial size differences observed between the genotypes.

In broiler chickens, for the estimation of breast and thigh muscle weights, the multi-atlas regressions combined with feature selection again proved to be the most effective. Among these, ridge and lasso regressions performed outstandingly (breast muscle: R^2 = 0.995 and 0.993; thigh muscle: R^2 = 0.965 and 0.976).

For pigs, the estimation of the major meat cuts (ham, shoulder, loin, ribs) achieved excellent results, with R^2 values ranging between 0.96 and 0.99 and low error rates. For LMP prediction, the ridge model reached an R^2 of 0.996 with an RMSE of 0.17%, indicating very high accuracy.

The effect of increasing the number of atlases was examined in two species (chickens and rabbits) using multi-atlas ridge regression combined with feature selection. In both cases, increasing the number of atlases improved predictive performance. At the same time, the error rate and the variance of the R^2 values decreased. However, increasing the number of atlases also considerably increased the processing time, particularly in broiler chickens, while in rabbits this increase was more moderate.

Using the Mann–Whitney U test, I compared the multi-atlas technique with the single-atlas approaches. The tests revealed highly significant differences ($p < 1.6 \times 10^{-6}$). When comparing individual atlases, the differences were not significant in most cases, and none of the single atlases could be considered to have consistently outperformed the others. In contrast, the multi-atlas approach yielded statistically proven superior results across all regression techniques.

4. CONCLUSIONS AND SUGGESTIONS

The automated, multi-atlas segmentation-based approach proved to be effective for estimating the weights of dissected cuts across different animal species. The main advantage of the system is that it can handle anatomical variability with minimal manual intervention, while regression models combined with feature selection ensure both the accuracy and robustness of the predictions.

The multi-atlas approach yielded better results for all examined species compared to single-atlas methods. Ridge and lasso regressions performed consistently well, whereas linear and KNNR models were less reliable, particularly in the absence of feature selection.

In the practical application carried out in rabbit breeding, the method demonstrated better predictive performance than the previously applied procedures based on manual segmentation (Nagy et al., 2006; Matics et al., 2014), resulting in an approximate relative R^2 increase of 23% for the left hind limb muscles and about 27% for the MLD. Consequently, from 2020 onwards, the presented technique has been implemented in the selection program of Pannon White rabbits at the MATE Kaposvár Campus.

Increasing the number of atlases continuously improved predictive performance, although it also led to longer processing times. Nevertheless, the fully automated process enables large-scale application, where weight estimation can be carried out with minimal time investment.

The results of rabbit head weight estimation confirmed the robustness of the method: the segments proved to be applicable across animals of different genotypes. Measurement conditions – such as head immobilization – had a significant impact on predictive performance, highlighting the importance of applying standardized examination protocols.

The method, first applied to rabbits, was successfully adapted to other species. In broiler chickens and pigs, high-accuracy results were also achieved, supporting the generalizability of the approach. In pigs, beyond estimating LMP and the weights of major cuts, the method also offers opportunities for estimating chemical composition.

Overall, the developed method provides an accurate, reliable, and widely applicable tool for the objective and automated estimation of body composition, thereby contributing to more precise determination of breeding value and to improved selection efficiency in modern animal breeding.

5. NEW SCIENTIFIC RESULTS

1. I developed a general, automated, and easily adaptable method for estimating the weights of different body parts of livestock species using computed tomography (CT) image processing. The major advantage of the method lies in the multi-atlas approach, which allows the determination of a large number of features. Their reduction and the selection of regression parameters were performed by stochastic optimization, which made the weight estimation process reliable.

2. In rabbits, the application of the developed method resulted in a substantial improvement in predictive performance: for the musculus longissimus dorsi, the absolute R^2 value increased by 0.2, while the relative predictive performance improved by 27% compared with the previously used procedure based on morphological operators.

3. In broiler chickens, a registration-based segmentation technique was applied for the first time to process the CT images of the examined individuals. The fully automated procedure yielded reliable results for both examined muscle groups (breast and left thigh muscles). The ridge regression model provided the most accurate estimate of breast muscle weight ($R^2 = 0.995$), while the lasso model performed best for the left thigh muscle ($R^2 = 0.976$).

4. In pigs, the lean meat percentage (LMP) and the weights of dissected cuts were estimated by parametric regressions with cross-validation, achieving R^2 values ranging between 0.958 and 0.9975. These results confirm that the automated method can be applied with high reliability to determine the slaughter value of pigs.

5. In rabbit head weight estimation, the method demonstrated good generalizability, since atlases derived from animals of a given genotype and body size could also be successfully applied to populations of different genotypes.

6. According to my investigations, increasing the number of atlases significantly improved the performance of regression models, particularly for muscle groups where the initial predictive accuracy was lower.

7. The complete image processing and regression workflow was published as an open-source Python package (*maweight*), with test data, manual segmentations, and Jupyter notebooks also made available on GitHub (*Jupyter Notebooks Used for Rabbit Data Processing*; *Jupyter Notebooks Used for Broiler Chicken Data Processing*; *Jupyter Notebooks Used for Processing Pig Half-Carcasses*). The open-source toolkit and the published datasets facilitate community validation, further development, and adaptation of the method to other species.

6. Publications on the Subject of the Dissertation

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