



## Methodological study

# Automatic method for determining the number of lumbar and thoracic vertebrae in rabbits using Computer Tomography images

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**ABSTRACT** - There are several studies dealing with the phenotypic variance of the vertebral number in the spinal column of rabbits. According to the literature the number of thoracic and lumbar vertebrae varies between 11-13 and 6-8, respectively. The length of the m. longissimus dorsi (MLD) - a valuable meat part of rabbits - is determined by the length of the vertebral column therefore the number of vertebrae may have economic importance in breeding. The aim of this study was to create an automatic counter using computed tomography (CT) images. In the first step, a skeleton binary mask was created using the radiodensity range between 120 and 3071 HU, then the lumbar and thoracic regions were processed by two different methods. The lumbar part was evaluated based on the frequency of the bone voxels along the axial plane. The number of thoracic vertebrae was determined from the number of ribs. The left and right ribs were processed separately. The developed method was tested on CT examination of 40 Hycole rabbits compared to manual evaluation. The results of the automatic algorithm had few errors: in one case in the lumbar region (2.5%) and in 3 cases in the thoracic region (5%). The automated evaluation process takes a few seconds per individual and then the program visualizes the results on a graph. The incorrectly evaluated rabbits are recognizable on graphs and they can be easily corrected with a minimal time investment.

**Keywords:** computed tomography, rabbit, vertebra number, automated evaluation

## INTRODUCTION

Several studies in several animal species have been shown that the number of vertebrae in different vertebral regions has got a variance (Chilson *et al.*, 2018, Cunyuan *et al.*, 2019, Donaldson *et al.*, 2013, 2014). This has a significant effect on the length of the spine and meat production (Cunyuan *et al.* 2019, Donaldson *et al.*, 2013, Donaldson *et al.*, 2014). The carcass length is a well-inherited trait in pigs and sheeps (Berge, 1948; Cunyuan *et al.*, 2019). Early methods to measure variation in thoracic and lumbar vertebrae number required slaughtering of the animal. As technologies evolved, non-invasive techniques became available for breeding purposes. Computed tomography (CT) besides X-ray gives

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more reliable measuring with 3 dimensional representation in spine traits (*Donaldson et al.*, 2013). Selection research has been going on at the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences for years using CT (*Matics et al.*, 2014). It may be interesting to examine the dorsal skeletal system to provide additional information. The information extracted from this area has never been used in a rabbit selection program. The proposed method is able to automatically count the number of thoracic and lumbar vertebrae using images from CT examinations.

## **MATERIAL AND METHOD**

### *Animals*

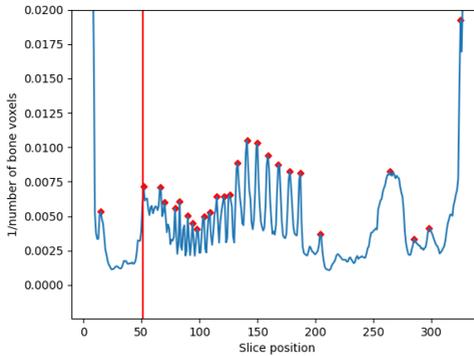
All animals were handled according to the principles stated in the *EC Directive 86/609/EEC* regarding the protection of animals used for experimental and other scientific purposes. The study was performed on 40 Hycole rabbits at the age of 11 weeks.

### *Image acquisition and pre-processing*

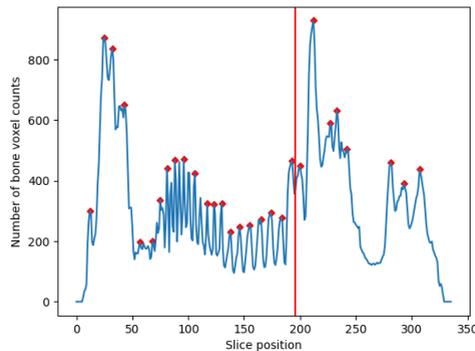
The image acquisition was carried out with a Siemens SOMATOM Definition AS+ CT scanner in 2021. Animals were immobilized as described by *Matics et al.* (2020). The rabbits were examined with the following parameters: 120 kV tube voltage, 240 mAs X-ray radiation dose, spiral data collection mode with 0.6 pitch, field of view 500 mm according to ISO 9001:2015 quality management system and ISO 14001:2015 environmental management system. Standard DICOM (Digital Imaging and Communications in Medicine) (*The DICOM Standard*) images were reconstructed by Siemens Syngo CT VA48A program with convolution kernel I30f. The resolution of the images was 0.977mm × 0.977mm × 2mm. Each exam series were converted from DICOM to MINC (Medical Image NetCDF) format. This file type allows the series images to be stored in a single file. The images were pre-processed using the OpenIP software package. The individuals were separated, and the plastic container holders were segmented out from the images using an automated pipeline (*Kovács et al.*, 2013). The individual images were converted to NIFTI (*Neuroimaging Informatics Technology Initiative*, 2005) files and the coordinate systems were set with the same starting point located at the bottom left of the first axial slice. The post-processing method was developed in python environment using the following main packages: nibabel, numpy, pandas, scipy, skimage.

### Image post-processing

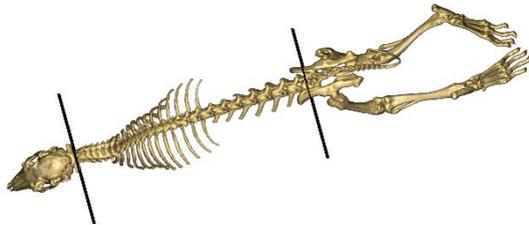
The first image processing approach was the segmentation of the rabbit skeleton by thresholding with 120 HU down threshold value. The skeletal system with associated bones was segmented by connected-component analysis (CCA), where the small bones causing disturbance and first limbs were omitted. The interesting thoracic and lumbar part of the remaining skeleton was cut. The first cutting point was found after the head by signal processing using *slice position* and  $1/\text{number of bone voxel counts}$  data (Figure 1.1). The second cutting point was found after the last lumbar vertebra by signal processing of the changes in the *number of bone voxel* by *slice position* (Figure 1.2). The results are showed on the Figure 2.1 and 2.2 where the red lines represent the cutting points.



**Figure 1.1:** Determining of the cutting point after the head



**Figure 1.2:** Determining of the cutting point after the last lumbar vertebra

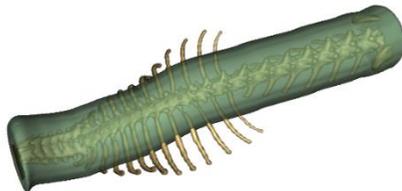


**Figure 2.1:** Two cutting plane on the truncated skeletal system



**Figure 2.2:** 3D rendered image of the cut skeletal system

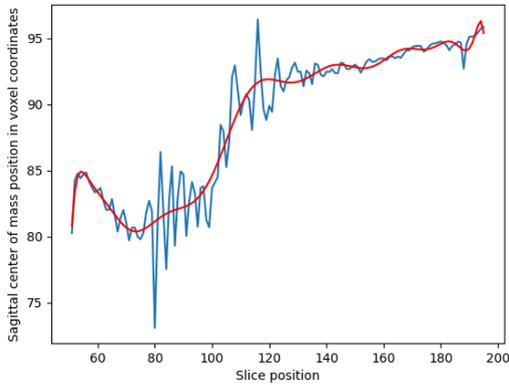
The curved ribs complicate the image evaluation, therefore we cut it close to the vertebrae by elastic cylinder (*Figure 3.1 and 3.2*). The cylinder was placed on a curve defined by two functions with 10 degrees of freedom (*Figure 4.1 and 4.2*). These functions represented the axial centerline of the skeleton.



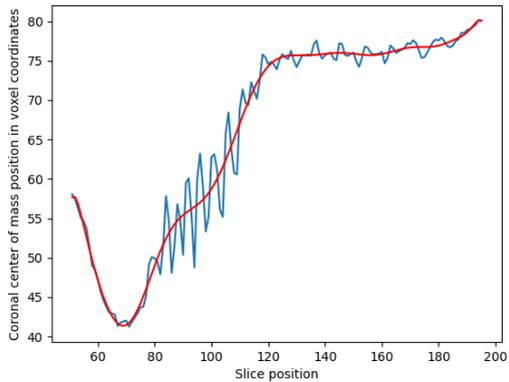
**Figure 3.1:** Fitted elastic cylinder on the vertebrae



**Figure 3.2:** 3D rendered image of the cut skeletal system

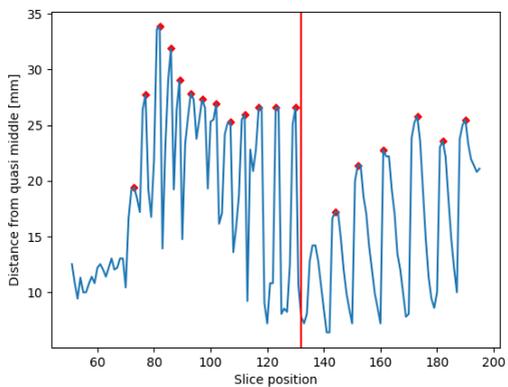


**Figure 4.1:** Fitting ten degree of freedom equation on the sagittal mass centres along the axial axis

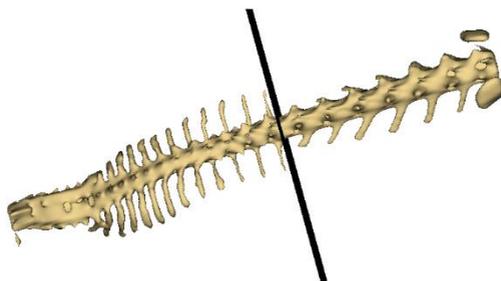


**Figure 4.2:** Fitting ten degree of freedom equation on the coronal mass centres along the axial axis

After the removal of the disturbing parts, we could easily separate the lumbar and thoracic regions by signal processing of *distance from middle* and *slice position* (Figure 5.1, 5.2). At the thoracic part, the number of vertebrae was examined by rib detection. The right and left ribs were examined separately, because the two-sides disturbed each other (Figure 6.1, 6.2). The number of vertebrae at the lumbar part was determined by signal processing of the skeletal voxel frequency with the slice position (Figure 7.1, 7.2). The above-described image post-processing method is fully automated.



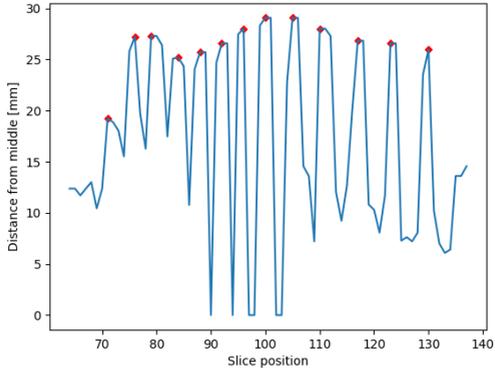
**Figure 5.1:** Detection of separating point between lumbar and thoracic part



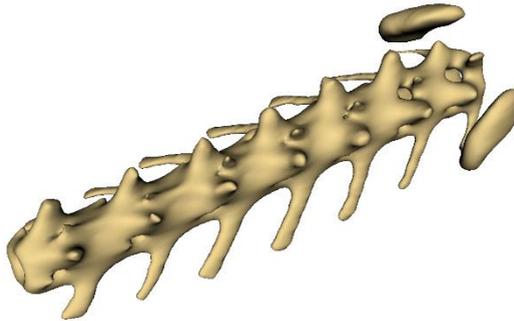
**Figure 5.2:** Separating plane between lumbar and thoracic part



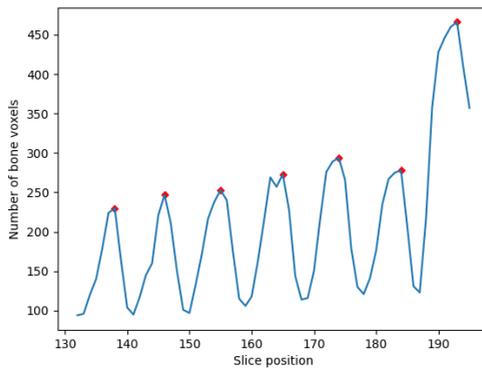
**Figure 6.1:** 3D rendered image of the left cut vertebrae.



**Figure 6.2:** Counting of left thoracic ribs



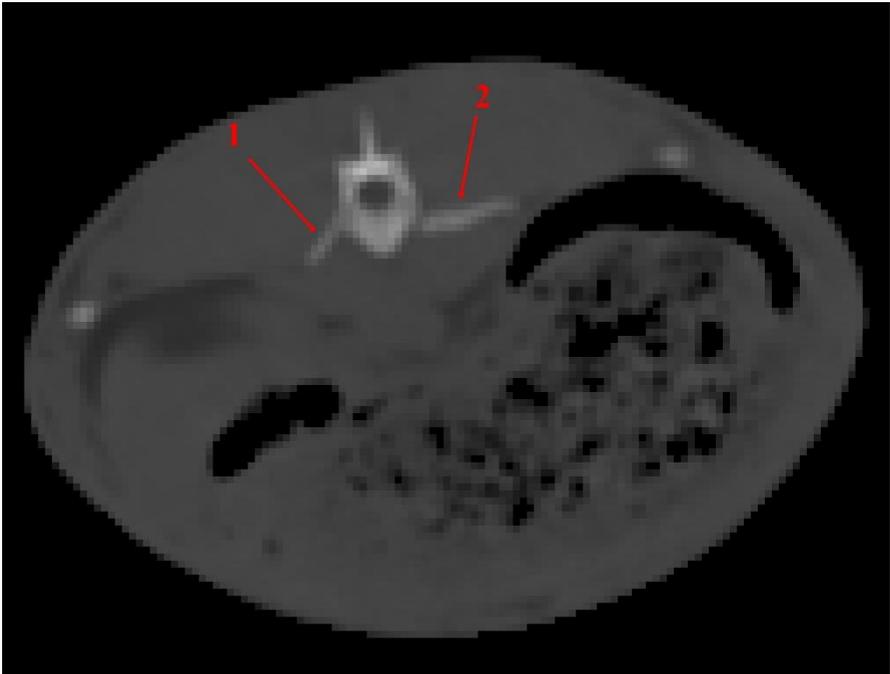
**Figure 7.1:** 3D rendered image of the lumbar vertebrae



**Figure 7.2:** Counting of the lumbar vertebrae

*Difficulties:*

1. High-density substances in the intestines originated from feed. These materials adhere to the intestinal wall and may fuse to the ribs on the CT images. The rib becomes inseparable using our proposed method.
2. A non-typical vertebra was found with characteristic of the lumbar and thoracic vertebra (*Figure 8*). This case cannot be handled by the program. The vertebra was classified as a part of lumbar region.



**Figure 8:** Typical transverse process of lumbar vertebra (left side of image, red mark 1). Rib like structure beside of the same lumbar vertebra (right side of image, red mark 2).

In addition, the length of each vertebral region (thoracic and lumbar) was calculated using the differences between the appropriate anatomical points.

*Statistical analysis*

The normality (Kolmogorov-Smirnov) and variance equality (two side F-test, conf. level 0.95) of lengths by groups were tested. All of the grouped lengths

are normally distributed and there are no significant differences in variances between examined groups, therefore analysis of variance was performed. R statistical software were used for all statistical processing.

## RESULTS

After manual and automated examination of all the rabbits, the results were compared to each other. At the lumbar region one error occurred while at the thoracic region we found three errors.

Frequencies of the dorsal vertebrae system in rabbits are described in *Table 1*. It is encouraging that the number of cases where either the number of lumbar or thoracic vertebrae increases is more frequent than when only one increases and the other decreases.

**Table 1**

Variability of the vertebral skeletal system

Number of lumbar vertebrae	Number of thoracic vertebrae	Frequency [animals]	Frequency [%]
7	12	23	57.5
8	12	8	20
7	13	8	20
6	13	1	2.5

All of the collected and comparable lengths were summarized in *Table 2*.

**Table 2**

Lengths of spine regions according to the number of vertebrae (mm)

Region	Number of vertebrae	Mean	Standard Deviation	Minimum	Maximum
Thoracic	12	108.3 <sup>a</sup>	3.1	100	116
	13	122.9 <sup>b</sup>	5.0	114	130
Lumbar	7	110.4 <sup>a</sup>	3.8	100	118
	8	130.0 <sup>b</sup>	3.2	126	136
Thoracic-lumbar	12-7	229.8 <sup>a</sup>	5.8	220	240
	12-8	251.0 <sup>b</sup>	5.2	246	262
	13-7	249.7 <sup>b</sup>	5.3	240	256

a, b: means with different superscripts within the same region differ significantly ( $P < 0.001$ )

Based on analysis of variance, length of thoracic region and length of lumbar region grouped by number of vertebrae have got significant differences. Process the two regions' lengths (thoracic and lumbar) together with analysis of variance by multiple comparisons of means (Tukey Contrast) grouped by thoracic-lumbar vertebrae there is not significant differences between 13-7

and 12-8 and significant differences are between 12-8 and 12-7, 13-7 and 12-7. Generally, the larger number of vertebrae resulted longer regions.

## CONCLUSIONS

The described technique provides a quick automated image processing method (2-3 seconds per individual) for counting the lumbar and thoracic vertebrae. The software visualizes the results on graphs. The incorrectly evaluated rabbits are recognizable on the graphs. It is possible to correct the data with minimal time investment and the perfect result can be achieved. Examination of the dorsal skeletal system can serve extra information for the rabbit selection program. The robustness of the proposed method may increase with better image resolution. Based on the virtual measurements, the larger number of vertebrae resulted longer regions.

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