

# ASSOCIATION BETWEEN COLLAGEN CONTENT AND CT-DETERMINED CONNECTIVE TISSUE PROPORTION OF BEEF FROM HUNGARIAN SIMMENTAL YOUNG BULLS

G. Holló<sup>1\*</sup>, B. Húth<sup>1</sup>, E. Egri<sup>1</sup>, I. Holló<sup>1</sup> and I. Anton<sup>2</sup>

<sup>1</sup>Kaposvár University, Faculty of Agricultural and Environmental sciences Guba S. street 40., 7400 Kaposvár, Hungary, <sup>2</sup>NARIC-Research Institute for Animal Breeding, Nutrition and Meat Science, Gesztenyés str 1., 2053 Herceghalom, Hungary

\*Corresponding author email: hollo.gabriella@sic.ke.hu

**Abstract** –Our objective was to determine the relationship between collagen/hydroxyprolin content and X-ray computed tomographic (CT) connective tissue proportion of longissimus thoracis (LT) muscle in Hungarian Simmental bulls (n=34). CT examinations of muscle samples were carried out with the usage of a 16-slice CT system (Siemens Somatom Sensation Cardiac). CT value of volumetric connective tissue content at LT muscle area of each mixed scan (80+140kV) was determined. Following CT, the hydroxyproline/collagen content and intramuscular fat content of LT was determined. Close positive correlation ( $r=0.749$ ;  $P<0.001$ ) was detected between the CT connective tissue proportion of LT and collagen content. In conclusion, mixed CT scans can be used for the analysis of intramuscular connective tissue content of beef.

**Key Words** – beef quality, tenderness, non-invasive measurement

## I. INTRODUCTION

There are several factors including genetics, time on feed, diet, age, stress, chilling rate, and aging of the product, which are influencing tenderness of beef. The main methods currently employed to predict beef tenderness are consumer taste panel assessment and the Warner–Bratzler shear force (WBSF) methods. Nowadays no rapid and non-destructive measurement of tenderness is available. Therefore, it is necessary to develop a non-destructive, efficient and rapid assessment method for beef quality. The major benefit of imaging based approaches is that they provide non-destructive and non-invasive measurements. Research progress in this area suggests that the prospect of being able to estimate eating and nutritional qualities of meat may not be too far away [5]. X-ray computed tomography (CT) has also been adopted for use in animal science as it can non-invasively measure fat, muscle and bone in vivo in farm animals (up to 150 kg) [7]. In Hungary, CT has been applied - mainly for research purposes - in cattle breeding, too. CT examination of rib samples was used for the meat yield estimation of different breeds, and with the incorporation of CT data into EUROP classification, the slaughter value qualification can be achieved more objectively [3]. CT imaging is based on the attenuation of photons from an x-ray beam transmitted through the examined object. Differentiation of various materials using conventional single-energy CT is based on their x-ray attenuation expressed as CT numbers in Hounsfield units. Developments of new protocols or dual-energy (mixed) scans aim to collect better data on soft tissue composition and meat quality [4]. Our objective was to determine the collagen/hydroxyprolin content and connective tissue proportion measured by computed tomography (CT) in the longissimus thoracis (LT) muscle of Hungarian Simmental (HS) bulls in order to analyze usefulness of mixed CT-scans for the determination of intramuscular connective tissue level.

## II. MATERIALS AND METHODS

Altogether, carcasses from 34 Hungarian Simmental bulls were randomly collected at a commercial slaughterhouse. Recorded slaughter weight and average age of bulls were 510.32+101.02 kg and 535.76+151.47 days, respectively. Carcasses were assessed by a trained operator for conformation (an 18 point scale: scale 1 (poorest) to 18 (best)) and fatness (a 15 point scale: scale 1 (leanest) to 15 (fattest)) according to EU beef carcass classification scheme with the use of subclasses. Carcasses were chilled conventionally for 24 hours at 4°C. After 24 hrs chilling, rib samples were taken from the right half carcass *longissimus thoracis* (LT) cut at the 12<sup>th</sup> rib. All rib cuts from each carcass were vacuum packed in polyethylene bags and transported to Kaposvár University for X-ray computed tomography (CT) analysis. CT-examination of rib cuts was performed 3 days *post mortem* at Institute of Diagnostic Imaging and Radiation Oncology of Kaposvár University. Two consecutive helical scans at low- and high-energy settings (80 kV and 140 kV) were executed by using a 16-slice CT system (Siemens Somatom Sensation Cardiac, slice thickness: 5mm). Images from samples scanned at two different energy levels were analysed by image analysis. Images from the two energy levels were added (80kV+140kV). CT value at LT muscle area of each mixed scan (80 kV +140kV) was determined. Volumetric connective tissue content was determined (above 200 CT value) with the usage of MANGO (3.8, 2016) software. After CT examination, samples were transported to the Analytical Laboratory of the University. After removing surface fat, intramuscular fat (IMF) was determined gravimetrically by Soxhlet method, using petroleum ether as solvent. Hydroxyproline measurement was used to determine connective tissue (collagen) content in meat according to

the method described by Reddy and Enwemeka [6]. Total collagen was calculated as hydroxyproline  $\times$  7.25 and expressed as g of collagen per 100 g of muscle. Statistical analysis was performed by using IBM SPSS 20.0 software package. Bivariate Correlations procedure was used for the measurement of linear association among CT data and laboratory results, and Pearson's correlation coefficient was determined as well.

### III. RESULTS AND DISCUSSION

The average hot carcass weight, EU muscle and fat score as well as CT and laboratory results of bulls was shown in Table 1. For bulls the majority of carcasses were placed to conformation score O+ and to fat score 2+. Average IMF content was  $2.81 \pm 1.1\%$ , slightly lower than the minimum amount (3%) of IMF to achieve acceptable consumer satisfaction [2]. Intramuscular connective tissue (IMCT) plays a significant role in determining meat tenderness. The main component of IMCT is collagen and it determines the basal toughness of meat. Mean collagen content was  $0.49 \pm 0.15$  mg/100g, similar to literature data [1]. CT-number of collagen is not well documented in the literature, though a few human CT studies determine the collagen density to be higher than both in muscle and fat tissue. The ability to differentiate soft tissue structures within muscle is limited, because the former mainly contain small atomic numbers and showed similar X-ray attenuation values. Based on our hypothesis, material differentiation is with the usage of dual energy more feasible. In our study the examined average area of LT muscle on CT scans was  $59.51 \pm 16.22$  cm<sup>2</sup> and average scans ( $10.51 \pm 3.39$ ) were evaluated per samples depending on size of rib joint. The CT connective tissue proportion of LT correlated positively with the collagen content ( $r=0.749$ ). A weak relationship was found between the IMF and connective tissue content ( $r=-0.20$ ) as well as CT connective tissue proportion and carcass gain ( $r=-0.243$ ).

Table 1. Summary statistics of slaughter and carcass data

Variable	Mean	Standard deviation
Hot carcass weight, kg	272.79	63.83
EU muscle score, point	5.31	1.42
EU fat score, point	5.69	1.56
Carcass gain, g/d	531.48	124.74
Weight gain, g/d	998.81	224.20
Hydroxyproline content, %	0.07	0.02
Connective tissue (CT), %	0.51	0.18
Collagen content, %	0.49	0.15
Intramuscular fat, %	2.81	1.07

### IV. CONCLUSION

Mixed CT scans can be used for the analysis of IMCT content. Further research is needed on this topic.

### ACKNOWLEDGEMENTS

We gratefully acknowledge support from the Hungarian Scientific Research Fund (Project 111645).

### REFERENCES

- Christensen M., Ertbjerg P., Failla S., Sañudo C., Richardson R.I., Nute G.R., Olleta J.L., Panea B., Albertí P., Juárez M., Hocquette J.F. & Williams J.L. (2011). Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. *Meat Science* 87: 61–65.
- Hocquette J.F., Gondret F., Baéza E., Médale F., Jurie C., & Pethick D.W. (2010). Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. *Animal* 4: 303-309.
- Holló G., Szűcs E., Tózsér J., Holló I. & Repa I. (2007). Application of X-ray Computer Tomography (CT) in Cattle Production. *Asian Australasian Journal of Animal Sciences* 20: 1901-1909.
- Kongsro J. (2014). Genetic gain on body composition in pigs by Computed Tomography (CT): return on investment. In: Maltin C. Craigie C. & Bünger L. (ed): *Farm Animal Imaging Copenhagen 2014*, 28-30.
- Maltin C.A. & Craigie C.R. 2012. Overview to the FAIM I. Meeting; *Farm Animal Imaging Opportunities and Challenges*. In: Maltin C. Craigie C. & Bünger L. (ed): *Farm Animal Imaging Dublin 2012*, 6-8.
- Reddy G.K. & Enwemeka C.S. (1996). A simplified method for the analysis of hydroxyproline in biological tissues. *Clinical Biochemistry* 29: 225-229.
- Scholz A.M., Bünger L., Kongsro J., Baulain U., & Mitchell A.D. (2015). Non-invasive methods for the determination of body and carcass composition in livestock: dual-energy X-ray absorptiometry, computed tomography, magnetic resonance imaging and ultrasound: invited review. *Animal* 9: 1250-1264.