# DIFFERENCES IN THE LEVELS OF Bov-SERPINA3 IN *RECTUS ABDOMINIS* MUSCLE EXPLAIN THE VARIABILITY OF BEEF TENDERNESS

M. Gagaoua[1*], N. Dunoyer[1], and B. Picard[1]

[1] UMR1213 Herbivores, INRA, VetAgro Sup, Clermont université, Université de Lyon, 63122 Saint-Genès-Champanelle, France
*Corresponding author email: mohammed.gagaoua@inra.fr

**Abstract – The aim of this study was to investigate the relationship between the abundances of Bov-SERPINA3 inhibitors (able to form pseudo-irreversible complexes with their target enzymes) and meat tenderness in *Rectus abdominis* muscle from PDO Maine-Anjou cows. The experiment included 4 tender (TE) and 4 tough (TO) samples from 20 animals, based on Warner-Bratzler shear force measurements (WBSF) and sensory tenderness scores at 14 days post-mortem (d *p-m*), taken at 24 h *p-m*. Western blotting revealed three Bov-SERPINA3 forms with MW of 114 (non yet identified), 75 and 45 kDa. The intensities of 75 and 45 kDa Bov-SERPINA3 bands were significantly lower in TE than TO group whereas no difference was observed for the 114 kDa band. The 45 and 75 kDa Bov-SERPINA3 were strongly correlated with both WB shear force (r = 0.81 and r = 0.75, respectively) and the 45 kDa was further significantly correlated with tenderness scores (r = –0.74).**

**Key Words – Serpins, Bovine muscle, Meat tenderizing, Proteolysis, Caspases, Biomarkers.**

## I. INTRODUCTION

At the beginning of 2000, the group of Ouali and co-workers found that the level of serine protease inhibitors in bovine muscle extracts sampled immediately after slaughter is the best predictor of meat tenderness amongst numerous other variables [1]. Few years later, the proteins were identified as SERPINS, acronym of **ser**ine **p**rotease **in**hibitors, and belong to the Bov-SERPINA3 like family, a group of serpins inhibiting strongly caspases (for review see [2]). While levels of Bov-SERPINA3 were previously estimated by titration using trypsin, they were quantified in this study by densitometry of the western blotting of anti-SERPINA3 bands after SDS-PAGE of muscle extracts. Relationship of their respective abundances with beef tenderness of two divergent cow groups (tough *vs.* tender) assessed by sensory panel and WBSF, was then analyzed.

## II. MATERIALS AND METHODS

The study was conducted using a dataset of 20 PDO Maine-Anjou cull cows collected from a cooperative of livestock farmers (Angers, France). The animals were slaughtered at an average age of 68 months old in a commercial abattoir and dressed according to standard commercial practice. Muscle samples from *Rectus abdominis* (RA (flank steak) were excised from the right side of the carcass of each animal. Tenderness on steaks aged for 14 days was assessed using sensory panel (at a cooking temperature of 55°C, 1 – 10 scale) and Warner-Bratzler shear force (WBSF) measurements [3]. Afterwards, based on tenderness scores and WBSF values from the total animals, two groups (4 animals each) of different grades of meat tenderness were created: animals with tender meat (TE) and tough meat (TO), respectively. The corresponding muscle samples, previously frozen at –80°C, were then used for protein extraction [3]. The soluble muscle fractions were analyzed by 12% SDS-PAGE and the Bov-SERPINA3 proteins were labeled with a rabbit polyclonal antibody (produced in our laboratory) raised against Bov-SERPINA3-1 [4]. The films were then scanned and protein bands quantified by densitometry using UN-SCAN-IT Gel 6.1. ANOVA was used to compare TO and TE groups. Correlation analysis was performed between the dependent variables (tenderness scores and WBSF values) and the levels of Bov-SERPINA3 bands.

## III. RESULTS AND DISCUSSION

Average WBSF values and tenderness scores were 41±1.2 N/cm$^2$ and 4.8±0.14 for the TE group and 75±6.5 N/cm$^2$ and 4.1±0.21 for the TO group (Fig. 1a). The Bov-SERPINA3 antibody revealed three bands with MW of 114 (unidentified band or Enzyme-inhibitor (EI) complex), 75 (Bov-SERPINA3-1 or A3-3 like) and 45 kDa (unidentified low MW Bov-SERPINA3 like). According to the large diversity of the Bov-SERPINA3 family [2,4,5], we cannot exclude that each band may contain several isoforms. Fig. 1b clearly shows that intensity of the 75 and 45 kDa bands are higher in the TO than TE group. This was confirmed by ANOVA showing significant differences between groups for the 75 ($P$<0.01) and 45 ($P$<0.001) kDa bands whereas

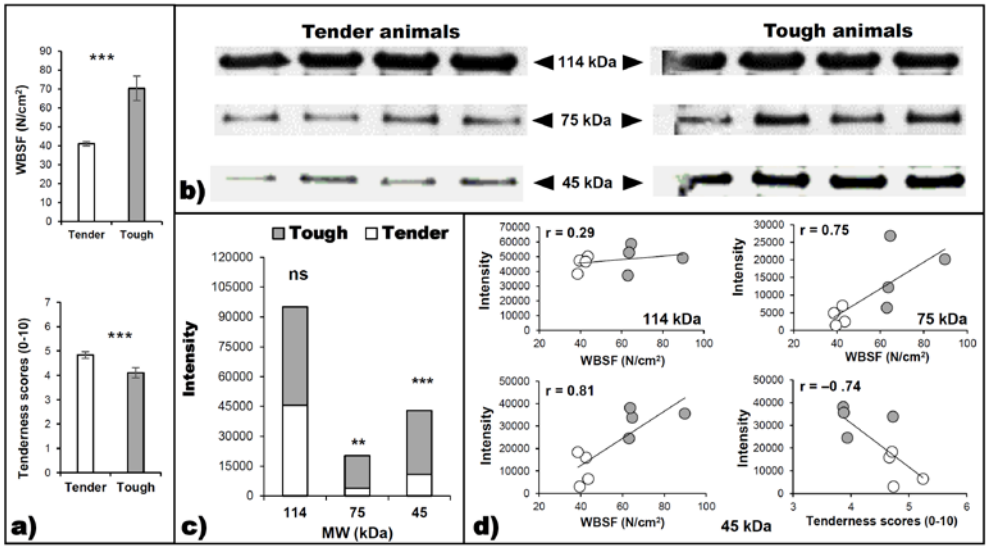


Figure 1. Characteristics of TE and TO animal groups. **a**) Comparison of WBSF values and tenderness scores between TE and TO; **b**) Representative Western blot of the 3 revealed Bov-SERPINA3 bands (114, 75 and 45 kDa, respectively) after 12% SDS-PAGE gel; **c**) Comparison of the relative band density of the 3 bands between TE and TO; **d**) Correlation analyses between Bov-SERPINA3 band intensities and WBSF. ns, not significant; ** $P < 0.01$; *** $P < 0.001$. MW:

very similar amounts of the 114 kDa were present in both groups (Fig. 1c). Both 75 (r=0.71) and 45 (r=0.81) kDa bands were positively correlated with WBSF and the 45 kDa band was further negatively correlated with sensory tenderness (Fig. 1d). The correlation between 75 kDa and tenderness score was not significant (r = –0.32, *P*=0.43). Such relationships between Bov-SERPINA3 members and meat tenderness reflect very likely their ability to inhibit caspases and hence apoptosis, an essential process in meat texture development [6].

## IV. CONCLUSION

The role of muscle protein inhibitors, namely those of Bov-SERPINA3, have been neglected in the meat science field. These preliminary results confirm once again and in line to our previous studies [3] that this position would have to be reconsidered for a better understanding of meat tenderizing involving myriad proteolytic systems. This study is the first to correlate Bov-SERPINA3 members to meat tenderness. These suggest that meat would be easily classified using Bov-SERPINA3 members as a prime candidate biomarker. Finally, such caspase inhibiting serpins are not well known and much remains to be done by exploring their presence in different muscles, breeds and animal types in order to better understand their biological functions and their exact role in the onset and progress of apoptosis, and hence in muscle to meat conversion.

## ACKNOWLEDGEMENTS

This study was conducted in the French PSDR project “Région Grand-Ouest” (PA-PEss-597 AOCMA_2009). We gratefully thank Dr S. Couveur (ESA Angers) for his help in muscle sampling and tenderness evaluation.

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