BIOMARKERS OF BEEF TENDERNESS IN YOUNG BULLS OF THREE BREEDS

B. Picard[1*], M. Gagaoua[1,2], M. Kammoun[1], C. Terlouw[1], J.F. Hocquette[1] and D. Micol[1]

[1] INRA, VetAgro Sup, UMR1213 Herbivores, F-63122 Saint-Genès-Champanelle, France

[2] INATAA, Constantine 1 University, Route de Ain El-Bey, 25000, Algeria

Abstract –The aim of this study was to validate the relationships between tenderness and the abundance of 21 proteins previously identified as potential biomarkers of beef tenderness in several genomic experiments. Seventy 17-month-old young bulls of three breeds, Angus (AA), Blond d'Aquitaine (BA) and Limousin (LIM) were used. Proteins were quantified by dot-blot with specific antibodies. The results confirm a negative relationship between tenderness and proteins associated with fast glycolytic fibres in *Longissimus thoracis* muscle. They confirm that proteins protecting against oxidative stress such as PRDX6 and Heat shock proteins (Hsp) are associated with tenderness but the direction of the correlation is breed dependent. Results are coherent with the hypothesis that tenderness is related to the ratio "small Hsp's/Hsp70s". Regression equations predicting tenderness were specific for breed and cooking temperature.

Key Words – Meat, Sensory quality, Cattle, Proteins

I. INTRODUCTION

For the beef sector, it is of major interest to be able to predict for consumers tenderness of the meat of live animals, carcass or cuts, as variability in beef tenderness causes dissatisfaction of consumers. For several years, various scientific programs aimed to identify genomic biomarkers for tenderness [1]. Transcriptomic and proteomic analysis on bovine muscles with low or high tenderness assessed by sensory analysis and/or mechanical measurements allowed us to produce a list of several protein tenderness biomarkers [2]. The present study investigated in more detail the relationships between the abundance of these proteins and tenderness in young bulls of three breeds differing in their earliness, as part of the EU ProSafeBeef project.

II. MATERIALS AND METHODS

The study used 70 young bulls: Aberdeen Angus (AA; n =20), Blond d'Aquitaine (BA; n =25) and Limousin (LIM; n =25). AA are known as fatty, BA for their high muscle and low intramuscular fat content and LIM have intermediate properties. The young bulls (12-month-old) were assigned to a 100 day finishing period. They were housed in straw pens. Diets consisted of concentrate (75%) and straw (25%). Animals were slaughtered at about 17 months of age at a live weight around 665 kg. They were slaughtered at the experimental abattoir of the INRA Research centre in compliance with the current ethical guidelines for animal welfare. The experience was carried out in two replicates (2 consecutive years, during the spring/summer seasons) using a balanced experimental design. *Longissimus thoracis* (LT, mixed fast oxido-glycolytic muscle) samples were excised from the 6th rib 15 minutes after slaughter, frozen in liquid nitrogen and stored at -80°C until use. Total protein extractions were performed according to Bouley *et al.* [3]. The abundance of the 21 proteins was quantified by dot-blot analysis according to the protocol described by Guillemin *et al* [4] using specific antibodies previously validated by western-blot. Protein abundance for each sample, given in arbitrary units, was normalised using a mix of different samples being the reference. The proteins studied represented 5 different biological functions: (i) muscle fibre structure with CapZ-β, α-actin, Myosin Light Chain (MyLC-1F), Myosin Binding Protein (MyBP-H), Myosin Heavy Chain (MyHC) IIx and II (IIa+IIx); (ii) muscle metabolism with Enolase (Eno3), Lactate dehydrogenase (LDH), and Malate Dehydrogenase (MDH1); (iii) proteolysis with μ-calpain; (iv) oxidative resistance with DJ-1, Peroxiredoxin (PRDX6), and (v) Heat shock proteins (Hsp) with Hsp20, 27, αβ-crystallin, Hsp40, Hsp70-1B, Hsp70-8, Hsp70/GRP75 [4]. Tenderness of LT steaks aged for 14 days was assessed by sensory analysis with a trained panel as described by Dransfield *et al*., [5]. Two grilled cooking temperatures were used: 55 and 74 °C.

Variance analysis (ANOVA) was used to compare breeds. The relationship between protein abundance and tenderness was evaluated by principal components analysis (PCA) and across breeds and per breed by multiple regression selecting parameters amongst protein abundance, breed (where applicable) and replicate to produce best models in terms of variability explained. XlSTAT 2009 and SAS 9.2 software's were used.

III. RESULTS AND DISCUSSION

The PCA on the abundance of 19 most relevant proteins and tenderness scores for the three breeds is illustrated in Figure 1.

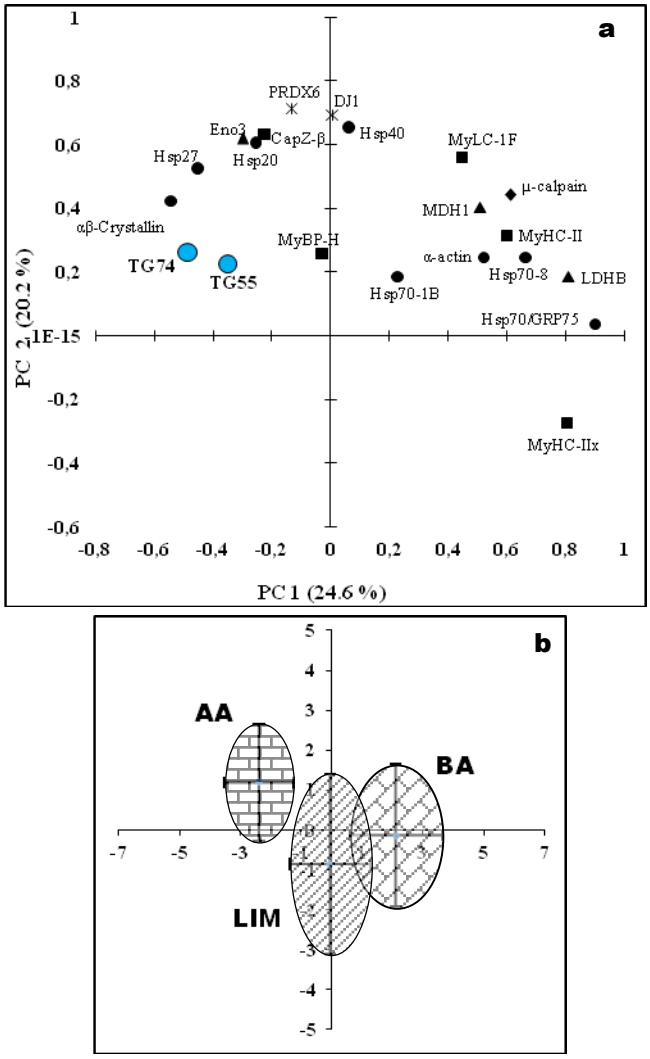

Figure 1. Principal component analysis. a) Distribution of protein abundance and tenderness scores; b) Distribution of the three breeds on the first two principal axes (mean ± standard errors).

The PCA shows that when considered across breeds, high tenderness scores whether obtained at 55 or 74°C were associated with small Hsp proteins (Hsp27, Hsp20, αβ-crystallin), Enolase 3, structural protein CapZ-β and antioxidants PRDX6 and DJ1. Low tenderness was associated with proteins of the Hsp70 family (Hsp70-8, Hsp70-1B and Hsp70/GRP75), or related to fast glycolytic muscle fibres (e.g. LDHB, fast MyHC). These data are coherent with the results of previous experiments on the LT muscle of young bulls, which found positive relationships between tenderness and slow oxidative; and negative relationships with fast glycolytic properties [6]. Our results are also in agreement with Guillemin *et al.* [7] who suggested that small Hsp are tenderness, and Hsp70s toughness markers. The authors hypothesized that tenderness depends on the Hsp20s/Hsp70s ratio which is coherent with our results. Proteins involved in oxidative stress such as DJ-1 and PRDX6 were also earlier found to be associated with tenderness (for review see Picard *et al.* [1]).

Multiple regression analyses across breeds (Table 1) found models with relatively weak predictive power for tenderness obtained at 55°C and 74°C. The three most explicative proteins were MyHC IIx, Hsp 20 and Hsp70-1B at 55°C and PRDX6, Hsp70/GRP75, and Hsp70-1B at 74°C.

The equations of prediction of tenderness established for each given breed were tested on the two other breeds. In none of the cases the models were significant, indicating that each model was specific for each breed (data not shown). This is in accordance with the observation that the direction of the correlations between tenderness and certain proteins depended on the breed. For example, MyHC-IIx was positively correlated with tenderness in Angus but negatively in the across-breed model (Table 1). Negative correlations between MyHC-IIx and tenderness were earlier observed in young bulls of French beef breeds including BA, LIM or Charolais [6, 8], but not in the Angus breed. The relationships found may depend on the contractile and metabolic properties of the muscle [9].

Table 1. Equations of best models (parameters including the direction and level of significance) to predict tenderness across breeds and for each breed

	Parameter 1	*P*-value[1]	Parameter 2	*P*-value	Parameter 3	*P*-value	Predictive power[2]	*P*- value model
			Cooking temperature 74°C					
All breeds	+ PRDX6	**	– Hsp70/GRP75	+	– Hsp70-1B	**	27	<.0001
AA	– ENO3	***	+ MyBPH	***	+ Hsp27	**	79	<.0001
BA	+ αβ-crystallin	+	+ α-actine	*	/	/	32	0.005
LIM	+ PRDX6	+	– Hsp70-1B	*	/	/	30	0.008
			Cooking temperature 55°C					
All breeds	– MyHC-IIx	+	+ Hsp20	+	– Hsp70 1B	+	17	0.002
AA	– MyLC-1F	**	+ MyHC-IIx	+	Replicate	±	35	0.02
BA	+ DJ1	***	– MyBPH	+	Replicate	*	40	0.003
LIM	– Hsp70-1B	*	+ MyLC-F1	*	/	/	35	0.003

[1]. *P*-value: significance of differences, ***: $P<0.0001$; **: $P<0.001$; *: $P<0.01$; +: $P<0.05$; ± : $P=0.06$
[2]. % of variability between animals explained by the model.

Table 2. Breed effects on tenderness

Cooking temp.	Tenderness score (1-10)			SEM	*P-value*
	AA	BA	LIM		
55 °C	5.27 a	4.85 b	4.75 b	0.08	0.04
74 °C	4.82 a	4.05 b	4.16 b	0.08	0.0003

On a same line, different superscripts indicate significant differences ($P<0.05$).

Multiple regression analysis carried out for each breed separately revealed different prediction equations of tenderness (Table 1; Figure. 2). Moreover, ANOVA analysis found that Angus produced more tender meat (Table 2).

Our earlier results showed that the LT muscle of Angus young bulls is more slow oxidative and has a higher intramuscular fat content compared to LIM and BA [10]. Present results may indicate that within breeds with muscles presenting slow oxidative properties, animals with fewer oxidative fibres were more tender. In breeds having muscles presenting fast glycolytic properties, animals with relatively high proportions of oxidative fibres were more tender. This hypothesis was already proposed by Morzel *et al.* [11] for BA young bulls. We observed the same differences for the Hsp from the small Hsp family. Guillemin *et al.* [9] showed that the abundance of small Hsp depended on the type of muscle and of animal.

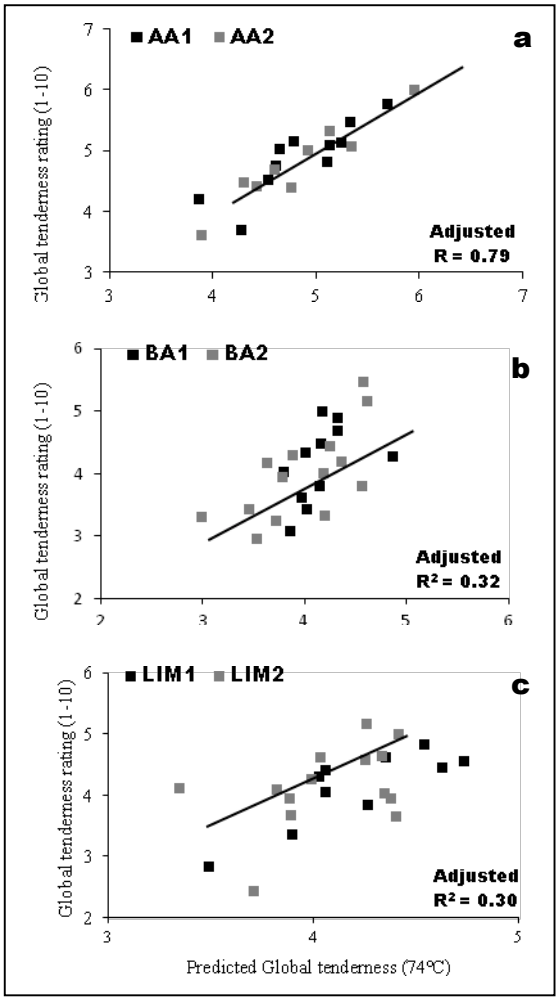

Figure 2. Correlation between predicted and measured tenderness of meat cooked at 74°C for the AA (a), BA (b) and LIM breed (c).

Variations in results found in the literature may thus be explained by the existence of different mechanisms according to muscle properties.

Our results are also coherent with earlier suggestions that proteins associated with oxidative stress are related to tenderness in cattle [1] and pigs [12]. The underlying cellular mechanism may involve Hsp, which protect structural proteins against oxidative stress and proteolysis, known to be involved in the tenderisation process. However, the relationship between antioxidative proteins such as PRDX6 and tenderness deserves further investigation as its association with tenderness is positive in some experiments as in the present study, and negative in others (for review see Picard *et al* [1]). The present results demonstrate that to understand the mechanisms underlying tenderness, it is necessary to establish prediction equations of tenderness for each type of animal separately.

IV. CONCLUSION

The present results indicate that several proteins are involved in the determinism of tenderness, but that the proteins and the underlying mechanisms depend on breed characteristics and on cooking temperature. The antibodies corresponding to the proteins validated as tenderness biomarkers will be used to develop an antibody micro-array as a new practical tool for the evaluation of tenderness.

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REFERENCES

1. Picard, B., Lefèvre F. & Lebret, B. (2012). Meat and fish quality improvement with proteomic applications. Animal Frontiers 2: 18-25.
2. Guillemin, N., Bonnet, M., Jurie, C. & Picard, B., (2011a). Functional analysis of beef tenderness. Journal of Proteomics, 75(2): 352-365.
3. Bouley, J., Chambon, C. & Picard, B., (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. Proteomics 4 (6): 1811-1824.
4. Guillemin, N, Meunier, B, Jurie, C, Cassar-Malek, I, Hocquette, JF, Levéziel, H &Picard, B (2009). Validation of a Dot-Blot quantitative technique for large-scale analysis of beef tenderness biomarkers. Journal of Physiology and Pharmacology 60: 91-97.
5. Dransfield, E., Martin, J.-F., Bauchart, D., Abouelkaram, S., Lepetit, J., Culioli, J., Jurie, C., & Picard, B. (2003). Meat quality and composition of three muscles from French cull cows and young bulls. Animal Science 76 : 387-399.
6. Chaze, T., Hocquette, J.F., Meunier, B. , Renand, G., Jurie, C., Chambon, C., Journaux, L., Rousset, S., Denoyelle, C., Lepetit & J., Picard, B. (2013). Biological Markers for Meat Tenderness of the Three Main French Beef Breeds Using 2-DE and MS Approach. In: Proteomics in Foods. Edited by Toldrá F, Nollet LML, vol. 2: Springer US; 2013: 127-146.
7. Guillemin, N., Jurie, C., Renand, G., Hocquette, J. F., Micol, D., Lepetit, J., & Picard, B. (2012). Different phenotypic and proteomic markers explain variability of beef tenderness across muscles. International Journal of Biology 4: 26-38.
8. Chriki, S., Gardner, G. E., Jurie, C., Picard, B., Micol, D., Brun, J. P., Journaux, L., & Hocquette, J. F., 2012. Cluster analysis application in search of muscle biochemical determinants for beef tenderness. BMC Biochemistry: 13, 29.
9. Guillemin N., Jurie C., Cassar-Malek I., Hocquette J.-F., Renand G &, Picard B., (2011b). Variations in the abundance of 24 protein biomarkers of beef tenderness according to muscle and animal type. Animal 5, 885-894.
10. Jurie C., Picard B., Micol D., Listrat A., Kammoun M. & Hocquette J.F. (2011). Muscle characteristics from young bulls of different beef breeds. 57th International Congress of Meat Science and Technology, August 7th – 12th 2011, Ghent - Belgium.
11. Morzel, M, Terlouw, C, Chambon, C, Micol, D & Picard, B 2008. Muscle proteome and meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: A central role of HSP27 isoforms. Meat Science 78: 297-304.
12. Jia, X., Veiseth-Kent, E., Grove, H., Kuziora, P., Aass, L., Hildrum, K. I. & Hollung, K., 2009. Peroxiredoxin-6 - a potential protein marker for meat tenderness in bovine m. Longissimus thoracis muscle. Journal of Animal Sciences: 2391-2399.