# STRESS PROTEINS IN CULL COWS: RELATIONSHIP WITH TRANSPORT AND LAIRAGE DURATIONS BUT NOT WITH MEAT TENDERNESS

M. Gagaoua[1*], E.M.C. Terlouw, V. Monteils[1], S. Couvreur[2], and B. Picard[1],

[1] UMR1213 Herbivores, INRA, VetAgro Sup, Clermont université, Université de Lyon, 63122 Saint-Genès-Champanelle, France
[2] URSE, Université Bretagne Loire, Ecole Supérieure d'Agricultures (ESA), 55 rue Rabelais, BP 30748, 49007 Angers Cedex, France
*Corresponding author email: mohammed.gagaoua@inra.fr

**Abstract – This work used *K*-means (*k*=3) after PCA analysis on 109 PDO Maine-Anjou cows according to the abundances of small and large heat shock proteins (HSPs) in the *Longissimus thoracis* muscle. Classes LP (n=24) and SP (n=33) were characterized by greater abundances of large and small HSPs, respectively, while class IP (n=52) was intermediate. The classes were compared for the abundance of other proteins in the same muscle and for meat tenderness. The effects of pre-slaughter factors (transport and lairage durations) were also investigated. Meat tenderness did not differ for any comparison. Certain proteins were influenced by pre-slaughter conditions. Among them, H2AFX and µ-calpain were impacted by both factors. The possible role of H2AFX (H2A histone family, member) in the balance of various processes involved in meat tenderization is discussed.**

**Key Words – Muscle proteome, HSPs proteins, PDO Maine-Anjou cows, Meat quality, Pre-slaughter handling.**

## I. INTRODUCTION

HSPs are chaperones expressed constitutively or inductively, which play an important role in regulating cellular homeostasis and promoting cell survival [1]. The abundances of HSPs are believed to play an important role in muscle to meat conversion due to their protective function on structural proteins and their anti-apoptotic properties [2]. However, there are few studies exploring the relationship between HSPs and meat tenderness and the relationships found vary across studies. This study compared three classes of animals varying in their abundances of HSPs and for their abundances of other muscle proteins and tenderness. The effects of pre-slaughter handling (transport and lairage times) on muscle proteome were also investigated.

## II. MATERIALS AND METHODS

109 French PDO Maine-Anjou cull cows of ~67 months old were slaughtered in a commercial abattoir in compliance with the French welfare regulations. Samples from the *Longissimus thoracis* (LT, oxido-glycolytic) muscle were excised from the right side of the carcass of each animal 24h after slaughter. They served for the quantification by Dot-Blot [3] of the levels of 20 protein biomarkers of tenderness representative of various biological pathways: ***heat shock proteins*** (αB-crystallin, HSP20, 27, 70-8, 70-1A, 70-1B, 70-Grp75), ***oxidative stress*** (DJ-1, Prdx6, SOD1), ***energy metabolism*** (ENO3, PGM1), ***structure*** (α-actin, MyBP-H, MyHC-IIx, MyLC-1F), ***proteolysis*** (µ-calpain, m-calpain), ***apoptosis*** (TP53) and ***transcription*** (H2AFX) and for electrophoresis of the proportions of myosin heavy chains I, IIa and IIx [4]. Tenderness was assessed by a trained sensory panel and Warner-Bratzler measurements [4]. Statistical analyses comprised first, principal component analysis (PCA) using the small (αB-crystallin, HSP20, 27) and large (HSP70-8, 70-1A, 70-1B) HSPs. Subsequently, a *K*-means cluster analysis ($k = 3$) using the variability explained by the axes with eigenvalues > 1.0, allowed the creation of three classes of animals. ANOVA was used to compare muscle proteins other than HSPs and tenderness between classes. ANCOVA was used to study the effect of transport and lairage times on all the proteins. Correlation analyses were performed between proteins to construct a robust correlation network (*i.e.* correlations present within and across the three classes *cf.* [3]).

## III. RESULTS AND DISCUSSION

The results show that class LP was characterized by an over-abundance of large HSPs and low abundance of small HSPs, while class SP was characterized by the opposite. Class IP had relatively low abundances of both small and large HSPs (Figure 1a and Table 1). These differences may be related to differences in muscle characteristics, as class LP had higher glycolytic properties levels than class SP and IP. In rabbits and pigs, glycolytic muscles were reported to contain greater levels of large Hsp70 than oxidative which contain mainly small Hsp [5, 6]. In cattle, no studies have reported this before. No differences were observed between sensory tenderness scores or WBSF values between the three classes. This contrasts with earlier reports and may be

Table 1. Abundances of muscle proteins (in arbitrary units) in the 3 classes and the effects of transport (TT) and lairage times (LT) (in min) on the abundances.

| Variables[1] | C1-LP (n=24) | C2-SP (n=33) | C3-IP (n=52) | SEM | Treatments (p-values)[2] | | |
|---|---|---|---|---|---|---|---|
| | | | | | **Class** | **TT** | **LT** |
| **αB-crystallin** | 211[b] | **297[a]** | 185[b] | 7.97 | *** | | |
| **HSP20** | 160[b] | **208[a]** | 138[c] | 4.32 | *** | | |
| **HSP27** | 68[b] | **95[a]** | 76[b] | 1.92 | *** | | |
| HSP40 | 123[b] | 125[b] | 139[a] | 1.48 | *** | | |
| **HSP70-8** | **133[a]** | 115[b] | 99[c] | 2.40 | *** | | * |
| **HSP70-1A** | **143[a]** | 126[b] | 105[c] | 2.50 | *** | | |
| **HSP70-1B** | **223[a]** | 199[b] | 153[c] | 4.19 | *** | * | |
| HSP70-Grp75 | 151[a] | 157[a] | 133[b] | 2.97 | *** | | |
| Prdx6 | 109[a,b] | 113[a] | 100[b] | 1.67 | ** | * | |
| DJ-1 | 97[a] | 91[a,b] | 86[b] | 1.24 | ** | * | |
| ENO3 | 147[a] | 155[a] | 137[b] | 3.48 | * | | |
| PGM1 | 119[a] | 98[b] | 96[b] | 2.62 | ** | | |
| α-actin | 162[a] | 112[b] | 111[b] | 3.89 | *** | | |
| MyBP-H | 135[a] | 128[a] | 119[b] | 1.79 | *** | | |
| MyHC-IIx | 101[a] | 91[a,b] | 85[b] | 2.65 | * | | |
| μ-calpain (*CAPN1*) | 167[a] | 156[a,b] | 142[b] | 3.70 | * | * | * |
| H2AFX | 116[b] | 127[a] | 114[b] | 2.14 | * | * | * |
| TP53 | 97[b] | 107[a] | 95[b] | 1.80 | * | | |
| ***Variables related to animal handling (pre-slaughter conditions)*** | | | | | | | |
| Transport time (**TT**) | 700[a] | 512[b] | 510[b] | 56 | * | | |
| Lairage time (**LT**) | 328[b] | 631[a,b] | 921[a] | 105 | * | | |

[1]Only variables that were different are shown; [2] *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

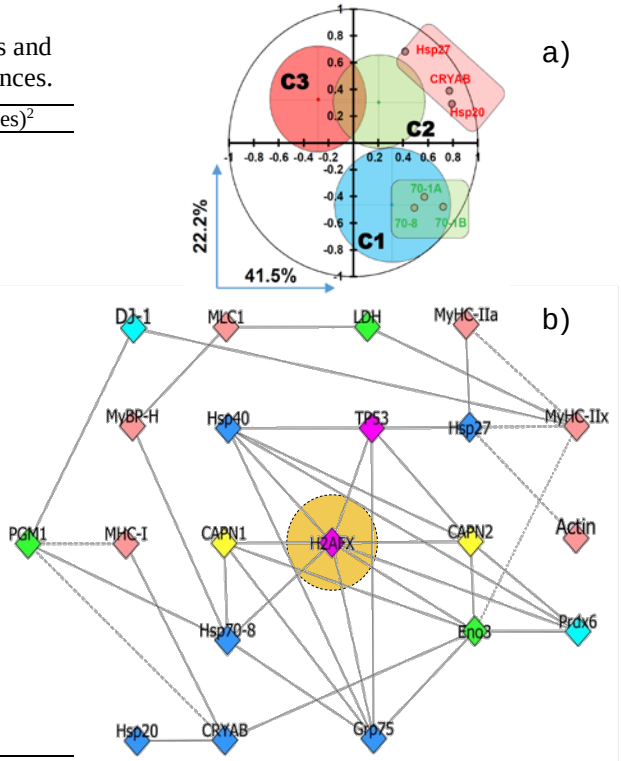


Figure 1. Loading and score plots (**a**) of the variables used to discriminate between the animals. The classes LP, SP and IP (1-3) are illustrated by blue, green and red circles, respectively. Robust correlation network (**b**) constructed between the proteins studied highlighting H2AFX at the crossroad of the network.

related to the multifactorial character of the tenderizing process, which involves not only apoptosis, but also oxidation and proteolysis. Specifically, oxidative (Grp75, Prdx6 and DJ-1) and proteolytic enzymes (μ-calpain) showed different abundances between classes which may have disturbed a straightforward relationship between HSPs and tenderness. Possibly, H2AFX has played a role in the balance between the different processes. This histone binds to DNA and to various enzymes, modulating many cellular pathways [2], and is at a crossroad of the correlation network with 8 connectors (Figure 1b). In addition, H2AFX and μ-calpain were both affected by transport and lairage times. Three other proteins (HSP70-1B, Prdx6, DJ-1) were affected by TT and one (Hsp70-8) by TL (low abundance for long lairage duration).

## IV. CONCLUSION

This study shows that various proteins are influenced by pre-slaughter stress conditions and suggests that some of these may orient *post-mortem* biochemical processes involved in the tenderizing process.

## ACKNOWLEDGEMENTS

The authors would thank “Pays de Loire Region”, the defense Trade Union of PDO Maine-Anjou, Adema and Elivia.

## REFERENCES

1. Schmitt, E., Gehrmann, M., Brunet, M., Multhoff, G., & Garrido, C. (2007). Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. *Journal of leukocyte biology* 81: 15-27.
2. Picard, B. & Gagaoua, M. (2017). Chapter 11: Proteomic investigations of beef tenderness. In *Proteomics in Food Science: From Farm to Fork,* Colgrave, M., Ed. Elsevier Science: Netherlands; p 538.
3. Gagaoua, M.; Terlouw, E. M.; Boudjellal, A. & Picard, B. (2015). Coherent correlation networks among protein biomarkers of beef tenderness: What they reveal. *J Proteomics* 128: 365-74.
4. Picard, B.; Gagaoua, M.; *et al.* (2014). Inverse relationships between biomarkers and beef tenderness according to contractile and metabolic properties of the muscle. *J Agric Food Chem*, 62: 9808-18.
5. Xu, Y. J., Jin, M. L., Wang, L. *et al.* (2009). Differential proteome analysis of porcine skeletal muscles between Meishan and Large White. *J. Anim. Sci.* 87: 2519-2527.
6. Neufer, D. & Benjamin I.J. (1996). Differential expression of B-crystallin and Hsp27 in skeletal muscle during continuous contractile activity. Relationship to myogenic regulatory factors. *J. Biol. Chem.* 271: 24089–24095.