INFLUENCE OF ACUTE STRESS ON THE PROTEIN PROFILE OF AGED BEEF FROM CONTRASTING FEEDING SYSTEMS

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Abstract – The objective of the present study was to evaluate the effect of acute pre-slaughter stress on the quality of aged beef from animals raised and finished in contrasting production systems. The electrophoretic protein patterns (SDS-PAGE in gradient) indicated that muscle proteolysis was significantly altered in the meat of those animals submitted to the higher stress level for both feeding systems. Considering the absence of dark-cutting and normal pH 24 h of these muscles, it could be assume that the altered proteolysis is related to pH independent mechanisms.

Key Words – animal welfare, beef quality, ageing.

I. INTRODUCTION

It is known that *peri mortem* phenomena may affect the quality of meat. On this regard, muscle pH and temperature (T) have an important role in *post mortem* events taken in the muscle, like physical shortening and proteolytic enzymes activity [1, 2, 3].

Post mortem muscle metabolism may vary significantly with some factors; such as animal nutrition and pre-slaughter stress [4]. The rate and extend of glycolysis and proteolysis may be affected by these factors, being able to affect final quality of meat, either by means of pH dependant or pH independent ways [5]. Mechanisms involved remain not completely elucidated, especially when considering the pH independent way and its consequences to the ageing process.

The aim of the present study was to evaluate the effect of two different pre-slaughter stress managements in Angus steers, raised in two contrasting feeding systems, on the proteolysis ability during ageing of beef.

II. MATERIALS AND METHODS

Forty steers from the Angus breed were used. Animals raised under two contrasting feeding strategies were chosen in order to obtain the same endpoint time determined by fat cover: a) grain-based system (GS): 20 animals fed with a grain diet of 39 % corn silage (37 % grain), 59 % whole corn and 2 % mineral premix with monensin; reaching a mean weight of 461.9 kg; b) pasture-based system (PS): 20 animals fed with triticale, triticosecale Wittmack in vegetative growth stage with a daily forage allowance equivalent to 2.5 % of live weight; with a mean weight value of 509.7 kg.

Once reached the endpoint, animals were transported simultaneously to a slaughterhouse at 300 km distance. Once at the slaughterhouse installations, both groups were randomly divided into two sub-groups and placed in separate lairage pens: a) reduced-stress handling (RH): animals were slaughtered first to reduce their exposure to odors and noises. Animals waited twenty minutes in the alley next to the slaughterhouse before entering the race to stun box. All dark zones of the race were strategically illuminated to avoid shadows. The slaughter process lasted roughly 20 % less than conventional one; b) conventional handling (CH): this group was slaughtered after the previous one, without waiting in the alley -no resting or adaptation- and following usual procedures including yelling and eventual use of electric prods. Shadows and slaughter odors and noises were not minimized. All animals were stunned and immediately exsanguinated.

Blood samples were collected during exsanguinations. Hematocrit (PCV), concentrations of total proteins (plasma), glucose, lactic acid, insulin and cortisol, and creatin-kinase (CK) activity were evaluated and published recently [6].

Longissimus Ddorsi (*LD*) muscles from the animals from the different treatments were removed and submitted to two different ageing periods: a) vacuum-packaged ageing during 2 d, and b) vacuum-packaged ageing during 21 d, at 2±1 °C. After that, muscle samples were kept at -20 °C until analysis.

The texture profile analysis (TPA) and instrumental color parameters of *LD* muscles from treatment a) were also recently evaluated and published [6]. Results obtained are mentioned below.

Sarcoplasmic and myofibrillar proteins extraction from the *LD* muscles from 5 animals per treatment (GS-RH, GS-CH, PS-RH and PS-CH) was conducted as previously described [7]. Protein concentration of each extract was measured according to the Lowry method [8] using bovine serum albumin as standard.

Electrophoretic profile of protein extracts (80 µg) was monitored by discontinuous SDS-PAGE [9] using a 3-12 % acrylamide gradient gels, as previously detailed [7]. HMW-SDS (53,000-220,000) was used as molecular weight marker. Gels were stained with Coomassie Brilliant Blue dye solution. Densitometric analysis was carried out using Quantity One software (Bio Rad). T-test was used for means comparison between bands in each treatment in order to evaluate the ageing effect. The level of significance was set at 0.05.

III. RESULTS AND DISCUSSION

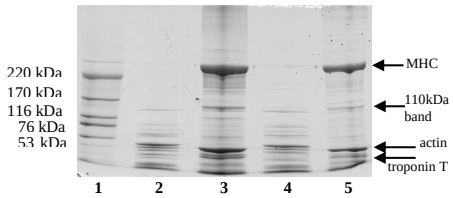
The results obtained in this study and previously published [6], showed significant differences in plasma levels of glucose and lactate between CH and RH of both production systems, indicating different levels of pre-slaughter stress in both feeding systems.

It has been also recently published that all *LD* muscles presented pH 24 h below 5.8, what means that no dark-cutting was found [6]. In the same study, color and texture analysis of *LD* muscles of these animals only displayed significant difference for the hardness parameter. Thus, animals raised and finished in GS presented less hardness of *LD* muscles when submitted to RH prior to slaughter when compared to their counter mates [6]. No such difference was found with animals raised and finished on pasture system.

SDS-PAGE analysis of sarcoplasmic and myofibrillar proteins extracted from *LD* muscles submitted at to two different ageing times are shown below (Fig. 1-4).

As can be seenillustrated in Figure 1, the electroforetic electrophoretic pattern of myofibrillar proteins from *LD* muscles of GS-animals submitted to reduced pre-slaughter stress levels changed as a consequence of ageing during 21 d. This is particularly evident in the decreased ($p<0.05$) optical density of several bands, especially: 220 kDa (myosin heavy chain -MHC-) (0.42 vs 0.38), 110 kDa band (0.27 vs 0.15), 43 kDa band (actin) (0.69 vs 0.39), 37 kDa band -troponin T- (0.50 vs 0.17), in aged samples. These results provide evidence of an important proteolysis that has taken place during ageing. Nevertheless, *LD* muscles from animals from the same production system submitted to CH prior to slaughter did not display the same proteolysis behavior (Fig. 2). As can be clearly seen in Figure 2, the protein pattern of these samples did not differ significantly as a consequence of ageing, demonstrating that increased levels of pre-slaughter stress can be associated to a decreased proteolysis ability of these muscles. This finding agrees with previous published results and discussions. [5].

Figure 1. Representative SDS-PAGE figure of sarcoplasmic and myofibrillar proteins of *LD* muscle from GS-animals submitted to RH, and aged 0 (t0) or 21 days (t21).



1. HMW-SDS standard; 2. Sarcoplasmic proteins t0; 3. Myofibrillar proteins t0; 4. Sarcoplasmic proteins T21; 5. Myofibrillar proteins t21. Arrows indicate bands mentioned in the text.

Commenté [rw1]: Indicate the entire name of the muscle

Answer: Ok, agree. Done

Mis en forme : Police :Italique

Commenté [rw6]: Statistically different ?

Answer: Yes, p<0.05. Done

Commenté [rw2]: Indicate the amount of proteins that was analyse by SDS-PAGE

Answer: Ok, agree. Done

Commenté [rw3]: Was a statistical analysis of data done ?

Answer: Yes, it was recently finished. Done.

Commenté [rw4]: How many replicates were performed per animal ?

Answer: It was done one gel per animal.

Commenté [rw7]: Indicate in the text that the figure representative of all the gels ran in the experiment

Answer: Ok. Done

Commenté [rw8]: 1 animal/gel ?

Answer : Yes, one animal per gel, at two ageing times: 0 and 21 days.

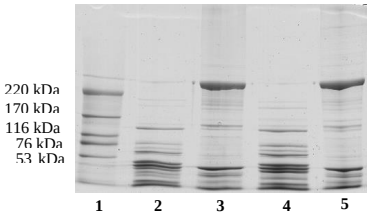
Commenté [rw5]: Please discuss the results according to the littérature.

Answer: Ok, agree. Done.

Commenté [rw9]: Indicate name or abbreviation of proteins

Answer: Ok, Done.

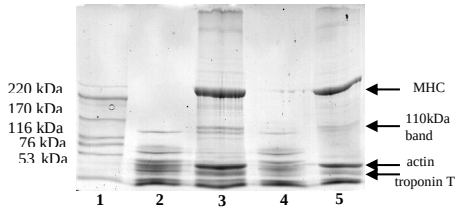
Figure 2. Representative SDS-PAGE figure of sarcoplasmic (x µg) and myofibrillar (x µg) proteins of *LD* muscle from GS-animals submitted to CH, and aged 0 (t0) or 21 days (t21).



1. HMW-SDS standard; 2. Sarcoplasmic proteins t0; 3. Myofibrillar proteins t0; 4. Sarcoplasmic proteins T21; 5. Myofibrillar proteins t21.

Fig~~-~~ure 3 and Fig~~-~~ure 4 show the electrophoretic protein pattern of both sarcoplasmic and myofibrillar proteins from *LD* muscles of PS-animals submitted to reduced and conventional pre-slaughter stress levels, respectively. The ~~behavior~~ evolution of these protein patterns with respect to ageing is similar to that of the grain system ~~one~~. Thus, it can be seen in Fig~~-~~ure 3 that ageing led to reduced bands from myofibrillar proteins, specially 220 kDa band -MHC- (0.63 vs 0.51, $p<0.05$), 110 kDa band (0.40 vs 0.35), 43 kDa band -actin- (0.63 vs 0.52, $p<0.05$) and 37 kDa band -troponin T- (0.49 vs 0.41).

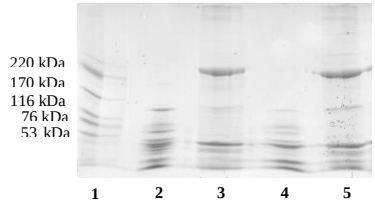
Figure 3. Representative SDS-PAGE figure of sarcoplasmic and myofibrillar proteins of *LD* muscle from PS-animals submitted to RH, and aged 0 (t0) or 21 days (t21).



1. HMW-SDS standard; 2. Sarcoplasmic proteins t0; 3. Myofibrillar proteins t0; 4. Sarcoplasmic proteins T21; 5. Myofibrillar proteins t21. Arrows indicate bands mentioned in the text.

On the other hand, *LD* muscles from animals from the same production system submitted to CH prior to slaughter did not display the same proteolysis behavior (Fig. 4). As can be seen in Fig~~-~~ure 4, the protein patterns of *LD* muscles from the two ageing periods did not displayed differences when assayed by SDS-PAGE, demonstrating an~~d~~ altered capacity of endogenous proteolysis ~~of~~ in these muscles.

Figure 4. Representative SDS-PAGE figure of sarcoplasmic and myofibrillar proteins of *LD* muscle from PS-animals submitted to CH, and aged 0 (t0) or 21 days (t21).



1. HMW-SDS standard; 2. Sarcoplasmic proteins t0; 3. Myofibrillar proteins t0; 4. Sarcoplasmic proteins T21; 5. Myofibrillar proteins t21.

These findings agree with previous published results and discussions regarding pH independent mechanisms involved in the meat quality alteration due to acute animal stress [5, 10, 11]. On this regard, Gruber *el al.*, [10] had recently demonstrated a delayed response to ageing -until 14 d- in beef from stressed cattle, in absence of dark-cutting or high pH values. Warner *et al.* [11] had previously published that 15 min of pre slaughter stress with electric prods also altered the ageing capacity of beef, leading to conserved sarcomere length and Warner-Bratzler shear force of *longissimuss lumborum* muscle at 2, 6 and 21 d. Similarly, in the present study, ageing capacity of *LD* muscle was affected until 21 h as a result of pre slaughter stress at the slaughterhouse installations. Moreover, ~~Finally,~~ it is important to remark that since the *peri mortem* stress only affected the texture profile (hardness) of *LD* muscles from animals raised and finished in grain system and aged during 2 days, it also affected the proteolysis ability of beef from both feeding systems when aged 21 days.

Commenté [rw10]: Statistics ?

Answer : Done.

IV. CONCLUSION

Results shown that acute pre-slaughter stress modified the muscle protein pattern during 21-days of ageing, due to different responses to maturation.

Acute stress before slaughter plays an important role in the quality development of aged beef for both feeding system, GS and PS, throughout pH-independent mechanisms. ~~Further research is guarantee in order to deep present results.~~

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Commenté [rw11]: Not necessary

Answer : Ok, agree. Done