

INVOLVEMENT OF APOPTOSIS IN MEAT QUALITY ATTRIBUTES USING THE CALLIPYGE LAMB MODEL: 2. HEAT SHOCK PROTEIN 27 AND MEAT TOUGHNESS

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Abstract – The objective of this study was to determine an involvement of heat shock protein (HSPs) in tenderness development of loins from callipyge lambs. Loin samples (*M. longissimus thoracis*) from sixteen lambs across four genotypes were collected throughout postmortem aging. More intact HSP27 in callipyge was coincided with higher levels of intact desmin and troponin T, less calpain-1 autolysis, and higher shears force values compared to other non-callipyge phenotype lambs (P<0.05). Positive correlations of anti-apoptotic activities (shown by elevated HSP27 and procaspase 3, and less cytochrome c) with increased toughness in callipyge lamb loins were also found (P<0.05). The results from the current study suggest that anti-apoptotic function of HSP would be likely involved in postmortem proteolytic systems and subsequent meat tenderization.

Key Words – apoptosis, postmortem proteolysis, tenderness

I. INTRODUCTION

Programmed cell death, or apoptosis, has been suggested as the first step in the process of conversion of muscle into meat. Apoptosis is initiated by release of cytochrome c to the cytoplasm, in turn activating caspase 3. As caspase 3 is an enzyme that cleaves calpastatin, a known inhibitor of calpain (primary proteolytic enzyme), a potential involvement of apoptosis in meat tenderness development has been proposed. Heat shock proteins (HSPs) are chaperone proteins that have a protective role in cell death, namely anti-apoptotic activity [1]. HSP inhibits the onset of apoptosis by binding to cytochrome c and preventing the activation of caspase 3 under stressful conditions. While several studies found the high levels of HSP in tough meat, the underlying mechanism by which HSP would impact meat tenderization process has not been fully understood. Therefore, the objective of this study was to determine an involvement of HSP and apoptosis in tenderness development of loins from callipyge lambs during postmortem aging. This investigation utilized the callipyge lamb model, as it consistently produces tough meat due to increased levels of calpastatin and subsequent decrease in myofibrillar protein degradation.

II. MATERIALS AND METHODS

A total of sixteen lambs of four different genotypes (callipyge (+/C) and non-callipyge phenotype lambs (C/C, C/+, +/+)) was slaughtered. Loin samples (*M. longissimus thoracis*) were collected at 15 min, 3, 6, and 9 days postmortem for Western-blot analyses, including desmin, troponin T, calpain-1 autolysis, calpastatin, HSP27, HSP20, α B-crystallin, caspase 3 and cytochrome c. Warner-Bratzler shear force (WBSF) data for the same loins from our recently published study [2] were used for the correlation analysis with the quantified Western-blot data. The experimental design was a randomized complete block design, and data were analyzed using mixed procedure of SAS to compare the traits across genotypes and aging times. Means were separated (F-test, P<0.05) by least significant differences and a Pearson correlation was conducted for all traits.

III. RESULTS AND DISCUSSION

A higher level of calpastatin and less extent of calpain-1 autolysis were found in the loins from the callipyge (+/C) compared to the loins from other genotypes (P <0.05; Fig.1). Furthermore, the loins from the callipyge had less degradation products of desmin and troponin T compared to the loins from non-callipyge phenotype lambs throughout the entire aging period (P <0.05; Fig. 1). These observations are in agreement with the previously reported meat toughness of the callipyge lamb loins [2]. The up-regulated calpastatin in callipyge lambs adversely affects the meat tenderization progress during aging by inhibiting calpain-mediated proteolysis of myofibrillar proteins [3]. More intact

bands and less extent of degraded HSP27 products were found in callipyge (+/C) compared to other genotypes during aging ($P = 0.02$; Fig.2), which might indicate greater anti-apoptotic activity in callipyge [4]. Cytochrome c was present in lesser abundance in callipyge compared to the normal genotype counterparts throughout aging ($P = 0.009$), suggesting that cytochrome c was less released into cytosol in callipyge. Furthermore, the loin samples from the callipyge showed the most procaspase 3 at the 32kDa band across aging among other genotypes ($P = 0.04$), indicating that less caspase 3 could be activated in callipyge. No significant differences between genotypes were found in HSP20 or aB-crystallin. HSP27 degradation product was negatively correlated with intact desmin ($r=-0.43$, $P=0.0003$), troponin T ($r=-0.50$, $P<0.0001$), and calpain-1 autolysis ($r=-0.69$, $P<0.0001$). Further, procaspase 3 was positively correlated with intact troponin T ($r=0.32$, $P=0.009$) and WBSF ($r=0.53$, $P=0.0001$).

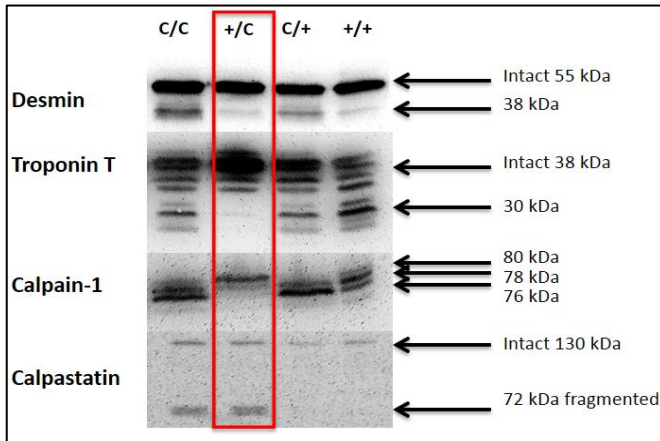


Figure 1. Representative Western-blots for desmin, troponin T, calpain-1 and calpastatin from loins from callipyge lamb (+/C) and other non-callipyge phenotype lambs (C/C, C/+, +/+) after 9 days postmortem aging.

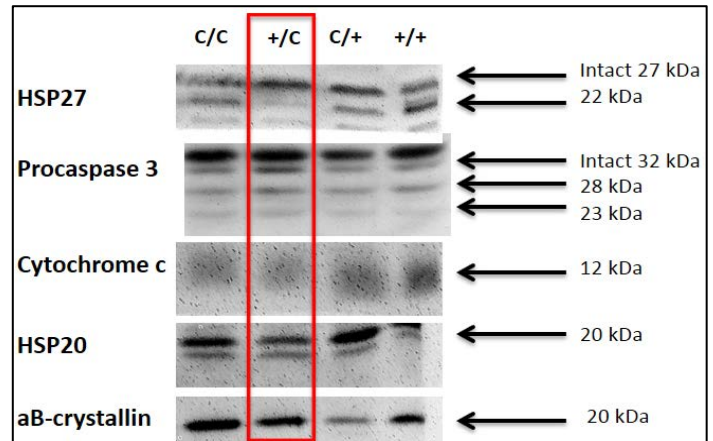


Figure 2. Representative Western-blots for small heat shock proteins (HSP27, HSP20, and aB-crystallin) and apoptotic factors (procaspase 3 and cytochrome c) loins callipyge lamb (+/C) and other non-callipyge phenotype lambs (C/C, C/+, +/+) after 9 days postmortem aging.

IV. CONCLUSION

The results from the present study found that the up-regulation of HSP27 in callipyge was coincided with higher levels of intact desmin and troponin T, less calpain-1 autolysis, and higher shears force values compared to other non-callipyge phenotype lambs. Positive correlations of anti-apoptotic activities (shown by elevated HSP27 and procaspase 3, and less cytochrome c) with increased toughness in callipyge lamb loins indicate that apoptosis would be likely involved in postmortem proteolytic systems and subsequent meat tenderization. We believe that the findings of the current study advance our understanding and provide novel insight into the toughness of callipyge lamb loins by brining apoptosis perspective.

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