EFFECTS OF CALCIUM AND ZINC INOS INJECTION ON CASPASE-3 AND TENDERIZATION IN POSTMORTEM BEEF SKELETAL MUSCLES

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Abstract –This study was designed to investigate the effects of Ca^{2+} and Zn^{2+} ions injection on postmortem tenderness and caspase activation by examining the expression of caspase-3 and cytochrome c in postmortem beef skeletal muscles treated with Ca^{2+} and Zn^{2+}. Results showed that Ca^{2+} ions injection accelerated postmortem tenderization of beef skeletal muscles, whereas Zn^{2+} retarded the process. In the Ca^{2+}-injected samples, the level of caspase-3 precursor decreased significantly ($P < 0.05$) without the production of activated caspase-3, whereas the level of cytochrome c was increased significantly ($P < 0.05$). So, Ca^{2+} possibly promoted caspases activation upstream of cytochrome c release, but inactivated caspase activity by calpain and/or fast depletion of ATP; Whereas Zn^{2+} blocked the activation of procaspase-3 with no visible change in the level of cytochrome c ($P > 0.05$), and the block possibly resulted from its direct inhibition on caspase-3 enzyme..

Key Words – conditioning, cytochrome c, tenderness

I. INTRODUCTION

The addition of calcium to muscles is known to result in accelerated PM tenderization and improved ultimate meat tenderness. Moreover, the effect was considered as a result of activation of endogenous calpain enzymes. Recent reports showed that postmortem (PM) muscle cells die through apoptosis and caspases are involved in the early tenderization process. Moreover, calcium overload promote apoptosis in physiological or pathologic apoptotic cells. Based on these observations, we want to investigate whether the effects of the calcium and zinc ions on tenderness also could be related to caspase system besides calpain by examining the level of caspase and apoptotic factors in PM beef skeletal muscles treated with calcium and zinc ions.

II. MATERIALS AND METHODS

Longissimus thoracis muscles were removed from five crossbred cattle and were cut into approximately 2.54 cm thick steaks. The steaks from the same cattle were randomly divided into three equal groups and injected (10% vol/wt) with one of the following three buffers, respectively: (1) 100 mM NaCl and 2 mM NaN_3 (control); (2) control + 200 mM $CaCl_2$; (3) control + 200 mM $ZnCl_2$. Subsequently, the samples were vacuum packaged individually and kept for 0.25, 1, 3, or 7 d at 4 °C. At the end of each storage period, Warner-Bratzler Shear Force, desmin degradation, and the expression of caspase-3 and cytochrome c were determined.

III. RESULTS AND DISCUSSION

PM tenderness is generally evaluated through cytoskeletal proteins degradation and WBSF values. Results from WBSF (Figure 1) and desmin degradation (Figure 2) showed that Ca^{2+} accelerated PM tenderization of skeletal muscles, whereas Zn^{2+} retarded the process, which demonstrated that Ca^{2+} and Zn^{2+}concentrations used in our experiment were effective and could affect the PM tenderization of meat, providing prerequisites for further studying the effect on the expression of caspase-3 and cytochrome c.

In the Ca^{2+}-injected samples, the level of caspase-3 precursor decreased significantly ($P < 0.05$) without the production of activated caspase-3(Figure 3), whereas the level of cytochrome c was increased significantly (Figure 4). So, the inactivity of caspase possibly resulted from the cleavage by calpain

and/or the depletion of ATP in PM muscle. The activation of caspas-3 in the Zn^{2+}-injected samples was effectively inhibited and meanwhile, Zn^{2+} had no visible influence on the level of cytochrome c in the PM skeletal muscle. Therefore, inhibition of Zn^{2+} on caspase-3 was possibly induced by its resistance to the cleavage of procaspase-3 in PM beef skeletal muscles.

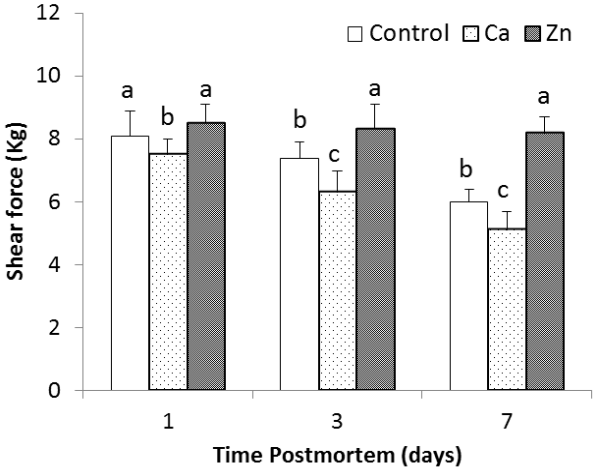

Figure 1. Change of WBSF during PM aging

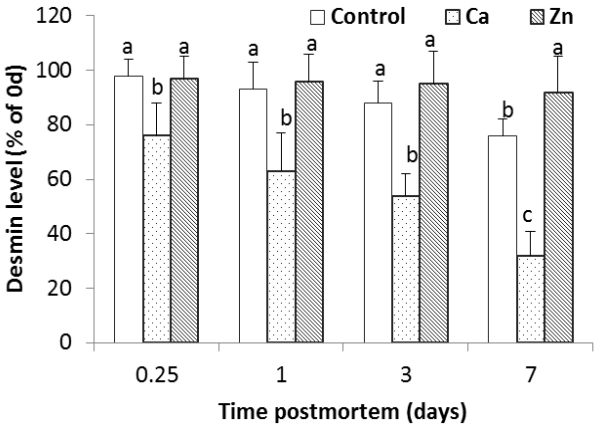

Figure 2. Densitometric analysis of desmin expression

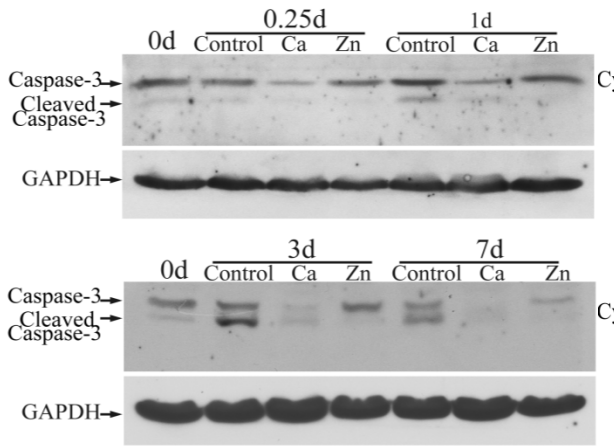

Figure 3. Expression of caspase-3 during PM aging

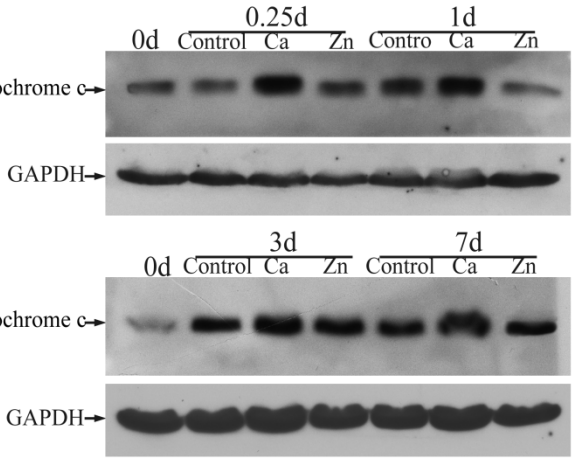

Figure 4. Expression of cytochrome c during PM aging

IV. CONCLUSION

Ca^{2+} possibly promoted caspases activation upstream of cytochrome c release, but inactivated caspase activity by calpain and/or fast depletion of ATP; Whereas Zn^{2+} blocked the activation of procaspase-3 with no visible change in the level of cytochrome c ($P > 0.05$), and the block possibly resulted from its direct inhibition on caspase-3 enzyme.

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