Antioxidant Capacity of Calcium Lactate on m-Calpain Activity In Vitro

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Yuan Kim, postdoctoral research associate; Ed Steadham, research associate; Steven Lonergan, professor; Elisabeth Huff-Lonergan, professor, Department of Animal Science

Summary and Implications
To investigate the antioxidant capacity of calcium lactate on m-calpain activity, porcine m-calpain was pre-incubated with various combinations of hydrogen peroxide (H₂O₂), calcium chloride (CaCl₂), and/or different concentrations of calcium lactate (CaLac). The m-calpain was activated by CaLac, and the extent of m-calpain oxidation by H₂O₂ was decreased with increasing CaLac concentrations. These results suggest that CaLac addition to early postmortem muscle may lead a significant improvement of meat tenderness by providing an activation of endogenous calpain enzymes and protection against protein oxidation.

Introduction
Calpains play significant role in postmortem proteolysis of cytoskeletal proteins and thus affect tenderization of meat. Oxidation of postmortem muscle negatively affects proteolytic activity of calpains, and subsequently decreases tenderness of meat. CaLac has free radical scavenging and antioxidant effects. Hence, it can be hypothesized that CaLac may both activate calpain and retard oxidative inhibition of calpain under oxidative conditions. The objective of this study was to determine the effects of CaLac on calpain activity under oxidizing conditions in vitro.

Materials and Methods
Porcine skeletal muscle m-calpain (66 U/mg) was incubated with 40mM Tris-HCl, at 23°C for 5 min with various combination of 100 μM H₂O₂, 5 mM CaCl₂, and/or 5 mM CaLac generating 8 treatments; 1) control, 2) H₂O₂, 3) CaCl₂, 4) CaLac, 5) H₂O₂ + CaCl₂, 6) H₂O₂ + CaLac, 7) CaCl₂ + H₂O₂, and 8) CaLac + H₂O₂. The m-calpain activity was measured in a standard casein assay under both reducing and non-reducing conditions (with and without 0.2% mercaptoethanol) in triplicate. In order to determine the effects of lactate concentration on calpain activity, m-calpain (66 U/mg) was incubated at the same conditions as above but with 50 μM H₂O₂ and with 5 mM CaCl₂, or CaLac (5, 10, 15, and 20 mM).

Results and Discussion
Under both reducing and non-reducing conditions, CaLac activated m-calpain. Pre-exposure of calpain with H₂O₂ + CaLac treatment significantly lowered calpain activity compared to the H₂O₂ + CaCl₂ under the reducing condition. These results suggest that CaLac scavenges oxidants, which allows more calpain activity and thus more autolysis during pre-incubation, and consequently it results in lower calpain activity (P < 0.05). Increasing CaLac concentration decreased (P < 0.05) the activity of m-calpain pre-incubated with H₂O₂ under the reducing condition (Fig. 1). Taken together these data suggest that the antioxidant capacity of CaLac may be concentration dependent, because the extent of m-calpain oxidation by H₂O₂ was decreased with increasing lactate levels. Thus, it can be concluded that CaLac is able to activate m-calpain, and may provide antioxidant capacity against m-calpain oxidation.

![Figure 1. Effect of calcium lactate on m-calpain activity under oxidizing condition. Porcine skeletal muscle m-calpain was pre-incubated with different concentrations of CaLac. Activity was measured in a reducing buffer. The lower residual calpain activity indicates the more activity during pre-incubation. Means with a different letters (a-e) are different (p < 0.05). The standard error of the mean was 0.33.](image-url)