



## Early Postmortem Metabolism and Protease Activation in Bovine Muscles

P. Ramos<sup>1</sup>, L. Bell<sup>1</sup>, M. Pedrao<sup>1,2</sup>, and T. Scheffler<sup>1\*</sup>

<sup>1</sup>Department of Animal Sciences, University of Florida, Gainesville, FL, USA

<sup>2</sup>Department of Science and Technology, Federal Technological University of Parana, Londrina, Londrina, Brazil

\*Corresponding author. Email: tscheffler@ufl.edu (T. Scheffler)

**Keywords:** calpain-1, caspase-3, muscle fiber type, pH  
Meat and Muscle Biology 3(2):171

### Objectives

The conversion of muscle to meat is largely controlled by postmortem energy metabolism and pH decline. These biochemical changes influence activity of enzymes implicated in proteolysis and meat tenderization. Therefore, our objective was to investigate pH decline, muscle energy metabolism, and protease activation in functionally distinct bovine muscles.

### Materials and Methods

Steers ( $n = 6$ ) were harvested at approximately 18.5 mo and 630 kg live weight. Samples from the *longissimus lumborum* (LL) and *diaphragm* (Dia) were taken at 1, 3, and 24h postmortem, immediately frozen using liquid nitrogen, and stored in ultra-freezer until analysis. Muscle pH was obtained using a pH meter at the same time points. Myosin heavy chain composition (I, IIa, and IIx) was determined using gel electrophoresis. Substrate (residual glycogen), as well as glycolytic metabolites, glucose, glucose-6-phosphate, and lactate, were quantified by enzymatic methods; muscle ATP at 1 and 3h was also determined. Western blotting was used to evaluate protease activation (calpain-1 and caspase-3). Data were analyzed using a randomized block design, with slaughter date as block. Animal within slaughter date was considered as random effect and fixed effects of muscle, time, and the interaction tested. Time was considered a repeated measure.

### Results

*Diaphragm* contained a greater percentage of slow myosin heavy chain compared to LL (80% vs. 12%, respectively). Consistent with fiber type, LL contained

greater glycogen than Dia at 1h ( $P < 0.05$ ), but not at subsequent times postmortem. Overall, a greater decline in glycogen occurred in LL. Accordingly, lactate concentration increased markedly in LL postmortem and to a lesser extent in Dia (interaction effect;  $P < 0.01$ ). Although muscles exhibited similar lactate content at 1h, at 24h the LL showed elevated lactate relative to Dia (88 vs. 53  $\mu\text{mol/g}$  tissue, respectively). Accumulation of glucose and glucose-6-phosphate were affected by muscle ( $P < 0.01$ ) and time ( $P < 0.01$ ), with greater final content in LL compared to Dia. Muscles exhibited different patterns of postmortem pH decline (muscle  $\times$  time,  $P < 0.0001$ ). Initially, pH of LL was higher than Dia ( $P < 0.01$ ) and remained different at 3h ( $P < 0.05$ ); but by 24h, pH values were similar. Content of ATP was influenced by muscle ( $P < 0.01$ ) and time ( $P < 0.01$ ). Initial ATP was greater ( $P < 0.01$ ) in LL than in Dia and remained greater ( $P = 0.002$ ) at 3h postmortem. From 1 to 24h, the pattern of calpain autolysis differed between muscles (interaction effect;  $P = 0.01$ ). Calpain-1 autolysis was similar at all times in Dia, whereas autolysis increased in LL from 3h to 24h postmortem. Caspase-3 was identified by one band (32 kDa) that represents the zymogen (procaspase-3). Procaspase-3 content is affected by muscle ( $P < 0.01$ ), with Dia containing greater content than LL.

### Conclusion

Although the Dia is considered a slow muscle, it exhibited a more rapid pH decline and lower ATP levels than LL early postmortem. These parameters were expected to coincide with more rapid calpain-1 autolysis in Dia, but this was not the case. Further work is necessary to understand the interaction between pH decline, muscle type, and postmortem proteolysis.