CITRATE SYNTHASE ACTIVITY AS A MARKER FOR MITOCHONDRIAL CONTENT IN BOVINE *LONGISSIMUS LUMBORUM* POSTMORTEM

L. Bell[1*], B. Hawryluk[1], M. McKinney[1], P. Ramos[1,2], and T. Scheffler[1],

[1]*Department of Animal Sciences, University of Florida, Gainesville, FL, USA*,

[2]*Department of Animal Sciences, University of Sao Paulo, Piracicaba, Brazil*,

*tscheffler@ufl.edu

I. OBJECTIVES

Citrate synthase (CS) is a key enzyme in the Krebs cycle, and CS activity is a commonly used marker for mitochondrial content. However, it is unclear whether the decrease in pH of postmortem muscle affects CS activity. Our first objective was to determine whether CS from bovine *longissimus lumborum* (LL) retains activity at pH values observed in postmortem muscle and verify pH for maximal CS activity. Our second objective was to determine CS activity of bovine LL collected during the first 24 h postmortem.

II. MATERIALS AND METHODS

For the first study, LL from Angus (*Bos taurus*) ($n = 4$) and Brahman (*Bos indicus*) ($n = 2$) steer carcasses were collected at 1 h postmortem. Samples were immediately frozen using liquid nitrogen and stored at −80°C until further analysis. Pulverized muscle was diluted in homogenization buffer, sonicated, and centrifuged. Supernatants were pipetted into 96-well plates and reaction media adjusted to pH 5.5, 5.9, 6.3, 6.7 (MES as buffer); 7.4 and 8.3 (Tris as buffer) was added. Background readings were taken every 20 s for 2 min. Reaction was initiated by adding acetyl coenzyme A, and the rate was monitored by following reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) at 412 nm. CS activity was calculated as nmol/min/mg tissue. Data were analyzed using SAS JMP-Pro 11 (SAS Institute Inc., Cary, NC) with the fixed effect of pH. For the second study, LL was collected from Angus and Brahman steer carcasses ($n = 14$ per breed) at 1, 3, 6, and 24 h postmortem. Muscle processing and CS activity assay procedures were conducted as in the first study, except CS activity of muscle supernatants was determined using reaction media at pH 7.4. Data were analyzed using SAS mixed procedure with fixed effects of breed, time, and the interaction, with time as a repeated measure.

III. RESULTS

Assay pH influenced CS activity ($P < 0.0001$), with the highest amount of CS activity at pH 7.4. Activity of CS declined rapidly as pH decreased from 7.4; at pH 6.3, activity was <15% of activity at pH 7.4. For the second study, breed did not influence CS activity ($P = 0.15$); however, time postmortem affected CS activity ($P = 0.012$). CS activity was lower in 1-h LL compared to subsequent time points ($P < 0.05$), with maximal change of 10%; this was primarily associated with greater CS activity in Brahman LL at 3, 6, and 24 h postmortem.

IV. CONCLUSION

Reaction media of pH 7.4 produced maximal CS activity, but CS activity is expected to rapidly decrease in muscle postmortem, in accordance with pH decline. Somewhat unexpectedly, in postmortem samples assayed at pH 7.4, LL collected at later times (3, 6,

and 24 h) exhibited greater CS activity than 1-h LL. Therefore, the pH decline in postmortem muscle does not irreversibly impair CS activity.

Keywords: beef, citrate synthase, mitochondria, pH