Glycogen supplementation in-vitro promotes pH decline in dark-cutting beef by reverting the muscle's metabolome towards normal postmortem muscle state

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I. INTRODUCTION

The rate and extent of postmortem pH decline plays a significant role in determining meat color, with consumer-preferred characteristics such as a cherry bright-red color typically achieved at a postmortem muscle pH of approximately 5.6 (1). Deviations from this norm, particularly exceeding 5.8, are associated with muscle darkening, a well-documented phenomenon in dark-cutting beef (2). Previous research has demonstrated that dysregulated muscle glycogen metabolism pre-slaughter in dark-cutting beef phenotypes significantly affects substrate metabolism compromising glycolytic flux leading to less accumulation of lactic acid postmortem, thereby contributing to abnormal muscle pH (> 5.8). However, the underlying mechanisms have remained elusive. In this study, we aimed to examine the metabolic role of glycogen in regulating postmortem muscle darkening and pH decline in beef. We hypothesized that increasing glycogen levels in dark-cutting muscles could induce a metabolic shift capable of restoring normal postmortem metabolic programs. Our objective was to assess the impact of glycogen supplementation on muscle pH decline, as well as the activities of glycogen phosphorylase and lactate dehydrogenase enzymes, and the metabolite profiles of dark-cutting beef muscles.

II. MATERIALS AND METHODS

Longismuss lumborum muscles from six bright red normal-pH and six dark-cutting beef loins (grain-finished, spray chilled) sourced from A maturity carcasses were procured from a commercial beef processing facility. Upon procurement, samples were transported on ice to Oklahoma State University where they were fabricated into steaks of 2.54 cm thick, powdered in liquid nitrogen, and stored at -80 ˚C until further analysis. Muscle glycolysis was stimulated as described (3) by homogenizing 100 mg of powdered *longissimus lumborum* muscles from twelve samples (n = 6 dark-cutting and n = 6 normal-pH beef) and incubating them in 1mL of an anaerobic buffer (10 mM Na_2HPO_4, 5 mM $MgCl_2$, 60 mM KCl, 5 mM ATP, 0.5 mM ADP, 0.5 mM NAD+, 25 mM carnosine, 30 mM creatine and 10 mM sodium acetate; pH 7.4), with or without glycogen at 0 and 10 mM. Normal-pH beef samples without glycogen served as a negative control. The pH of all treatments was adjusted to the same point (pH = 7.0) using pH adjusting solutions. Subsequently, the reaction was monitored for 24 hours at room temperature (25 ºC). Post-incubation, pH, enzyme activities (glycogen phosphorylase and lactate dehydrogenase), and metabolite profiles were assessed. pH decline was measured using an Acumet 50 pH meter, while enzyme activities were determined using standard enzyme assay kits from Abcam, and metabolomics profiling was conducted via a non-targeted gas chromatography mass spectrometry approach. Statistical analyses, including two-way ANOVA for pH decline and enzyme activities, and pairwise t-tests for metabolomics data, were performed using GraphPad Prism V.10 and Metabolome Analyst V.6.0, respectively, with significance at $\alpha = 0.05$.

III. RESULTS AND DISCUSION

Results showed that in vitro glycogen supplementation at 10 mM in dark-cutting beef led to a significant pH decline (pH = 5.87; P < 0.05) after 24 hours of incubation compared to both normal beef and untreated dark-cutting beef. This decline in pH following glycogen supplementation suggests substrate-mediated activation of enzymes involved with glycogen mobilization and utilization. Additionally, glycogen supplementation stimulated approximately a two-fold increase in glycogen phosphorylase (7.06 mUnit/mg tissue) and lactate dehydrogenase enzyme (61.87 pmol of NADH/min/µL) activities in dark-cutting samples. While lactate dehydrogenase activity was significantly lower in untreated dark-cutting compared to normal beef control (P> 0.05), glycogen phosphorylase activity exhibited a numerical increase in untreated dark-cutting beef. Metabolite profiling identified 132 metabolites, with 122 showing differential abundant across sample groups after 24 hours of incubation. Principle component analysis (PCA) revealed distinct clustering by treatments, with glycogen-supplemented dark-cutting treatments exhibiting separation from both untreated dark-cutting and normal-pH beef treatment groups. This indicates that glycogen is a key discriminative factor in the metabolite profiles. Pairwise comparison of metabolic profiles demonstrated differential abundance of 25 up-regulated and 31 down-regulated metabolites with > 2-fold change (FDR > 0.05) in glycogen-supplemented dark-cutting samples compared to untreated dark-cutting control while 22 up-regulated and 55 down-regulated metabolites were observed in comparison with normal-pH beef control samples (FDR > 0.05). Furthermore, examination of the specific differentially abundant metabolites between glycogen-supplemented dark-cutting samples and un-treated controls demonstrated a greater abundance of glycolytic metabolites and reduced levels of tri carboxylic acid (TCA) cycle, amino acids, and nucleotide metabolites (FDR > 0.05). This metabolic reprograming resemblence normal beef metabolite profiles. Thus, our findings suggest that glycogen levels rather than enzyme abundances, may be the primary limiting factor in dark-cutting beef muscle pH decline.

IV. CONCLUSIONS

In this study we provide insights into the metabolic dynamics underlying dark-cutting beef, highlighting the crucial role of glycogen in modulating postmortem muscle pH decline and metabolic processes. The substantial decrease in pH observed upon glycogen-supplementation in dark-cutting beef illustrates the influence of substrate-mediated activation of key enzymes involved in glycogen utilization and mobilization. Moreover, the pronounced metabolic reprograming towards a profile resembling normal postmortem muscle, characterized by increased abundance of glycolytic metabolites and decreased levels of TCA cycle intermediates and amino acids, suggests that that pre-slaughter developmental events may contribute to substrate inherent inhibition mechanisms in dark-cutting beef. These findings imply that optimizing glycogen levels could represent a promising strategy for mitigating dark-cutting beef phenotypes and improving meat quality.

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