

Differential microRNA expression in skeletal muscle from dark cutting beef carcasses

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Introduction: Despite efforts to reduce handling and transport stress, and improve management practices, dark-cutting beef remains an industry problem. While it has been known that dark-cutting can result from a number of factors including long-term stress and depleted glycogen stores, some aspects of the physiological mechanisms that cause dark-cutting phenotypes remain poorly understood. Recently it has been shown how profoundly microRNAs can regulate the response to stress factors in fully developed tissues. We hypothesize that certain microRNA signatures expressed in skeletal muscle in response to environmental factors and stresses may regulate physiological networks that result in dark cutting beef (also known as dry, firm, dry or DFD) in some animals. Therefore, the objective of this project was to investigate microRNA expression in Longissimus lumborum samples from a contemporary group of steers that resulted in a high incidence of dark cutting carcasses.

Materials and Methods: From a contemporary group of 78 F2 and F3 steers that were part of a designed genetic mapping herd of *Bos indicus* - *Bos taurus* cattle, we observed a 32% incidence of dark cutting carcasses. At harvest, biopsies obtained from L. lumborum samples from each of the steers was frozen in liquid nitrogen. Total RNA was extracted from the frozen samples, followed by small RNA sequencing from prepared libraries via Illumina NextSeq. Following a quality check and deletion of the adapter sequences, the RNA sequence data were aligned to the *B. taurus* genome sequence, as well as to known microRNAs in the miRbase database. Further analysis was conducted to identify miRNAs differentially expressed between samples from DFD carcasses and normal counterparts.

Results: A total of 296 unique microRNAs were identified that were expressed at the rate of at least 1 count per million sequencing reads (CPM). When DFD samples were compared to non-DFD (normal) samples, 39 differentially expressed microRNAs were identified in which expression was at least 20% greater in the DFD samples. In addition, 10 differentially expressed microRNAs were expressed at least 20% less in the DFD samples compared to (normal) samples. These microRNAs may play a role in stress response.

Conclusions: Our long-term goal is to improve meat quality by reducing the costly incidence of dark-cutting meat. We aim to capitalize on technological advances to understand the variation in genetic and physiological mechanisms that result in dark-cutting meat from some, but not all, animals exposed to certain conditions of stress. In this study we identified microRNAs that are differentially expressed in muscle samples obtained from DFD carcasses compared to samples from normal carcasses. These findings are a first step to understanding other physiological mechanisms that may play a role in stress responses that can affect meat quality. Better understanding of these phenotypes may enable actionable strategies - including post-harvest opportunities - to reduce the economic impact of dark cutting.

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