PROJECT SUMMARY
PRODUCT QUALITY

BEEF
RESEARCH

Effect of Implant Strategy and Supplementation of Zilpaterol Hydrochloride on the Skeletal Muscle Proteome from Beef Steaks Aged up to 14 Days

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Study Completed

June 2016

This project was funded in part by the Beef Checkoff.

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Background

Growth promoting technologies, such as anabolic implants and β-adrenergic agonists, increase beef cattle performance during the finishing period. Cattle administered anabolic implants increase final body weight (BW) but maintain a similar body composition to non-implanted cattle (Samber et al., 1996; Guiroy et al., 2002; Bryant et al., 2010), while β-adrenergic agonists fed for the last 20 to 40 days of finishing improve weight gain and increase muscle leanness (Avendano-Reyes et al., 2006; Boler et al., 2012). Zilpaterol hydrochloride (ZH) is a β1-adrenergic agonist that works as a repartitioning agent to redirect nutrients toward lean muscle accretion.

Cooked meat tenderness is heavily influenced by muscle ultrastructure. The myofibrillar proteins consist of the contractile and cytoskeletal proteins that are responsible for muscle contraction and structure (Aberle et al., 2003). The focus of much of the literature on meat tenderness is in the ability of proteolytic enzymes to degrade cytoskeletal proteins during post-mortem aging and increase tenderness. Also, connective tissue or collagen is a major part of muscle structure than can affect cooked meat tenderness. Connective tissue serves to provide structure to the muscle and transmit the force of muscle contraction. As collagen becomes more mature, the number of intra- and intermolecular crosslinks increase, decreasing solubility of collagen and meat tenderness.

Performance and meat quality data from this study presented previously (Ebarb et al., 2016) indicated that the implant treatment (IMP)-produced steaks were tougher than control steaks at three days post-mortem, however, by day 14, shear values were similar. Additionally, steaks from the implant/zilpaterol-hydrochloride (COMBO) treated heifers had tougher steaks through 14 days of aging. While protein degradation and collagen content and solubility were evaluated for this project, different analytical approaches are currently being utilized that allow researchers to look beyond these focus areas of tenderness research. The use of 2-D DIGE evaluates the muscle proteome and finds proteins that are differentially expressed and may contribute to differences in growth or quality attributes. This technique allows a more global evaluation of the protein in the muscle samples, potentially identifying pathways or processes that have been overlooked in the literature that may impact beef quality.

Objectives

The objectives of the study were to 1) Determine differentially expressed proteins from the sarcoplasmic and myofibrillar protein fractions of beef strips due to use of growth promotant technologies (implant and zilpaterol hydrochloride) by utilizing a novel proteomic approach; and 2) Establish if different finishing or aging strategies may be warranted when utilizing different growth promotant technologies to maximize beef tenderness or other quality attributes.

Methods

In a previous experiment (Ebarb et al., 2016) where samples for the current study were utilized, cattle were separated into three treatment groups: no implant or beta-agonist (CON); implant, no beta-agonist (IMP); implant and beta-agonist (COMBO). On day zero of the study, animals designated to receive anabolic implants were administered Component TE-200 (Elanco Animal Health, Greenfield, IN). Cattle that were designated to receive beta-agonists received 8.3 ppm of ZH for 21 day with a three-day withdrawal. After completion of the designated feeding period, cattle were harvested in commercial abattoirs and frozen strip steaks that had been aged 14 days were shipped to North Dakota State University. Steaks were cut into lateral, lateral/medial, and medial sections. Sarcoplasmic and myofibrillar protein was extracted from the lateral/medial section only. Fractionated samples were labeled with cyanine 3 red minimal dye or cyanine 5 blue minimal dye. Protein in samples were separated utilizing a 2-dimensional approach with the first dimension being isoelectric focusing and the second dimension being SDS-PAGE. Each gel was imaged on a biomolecular imager. All images were compared on DeCyder software (GE Healthcare) and analyzed to identify differences by relative abundance of individual protein spots due to treatment group. Spots were hand picked from gels and transported to the University of North Dakota proteomic facility for identification by HPLC and mass spectrometry.

Findings

The use of zilpaterol hydrochloride in combination with a steroidal implant had the greatest impact on expression of proteins from aged Strip Steaks. Proteins with differentially abundant expression are related to functions of carbohydrate metabolism, muscle structure and remodeling, antioxidant properties, and chaperone properties. Interestingly, eight spots were identified as actin, alpha skeletal muscle. In the largest molecular weight spot, actin was more abundant in the COMBO treatment, however, in the lower MW spots identified as actin, there was less abundant COMBO. This change in abundance due to treatment may indicate less post-mortem proteolysis in the COMBO treatment, which correlates to the tenderness data presented in Ebarb et al. (2016). However, most literature indicates that actin is not a protein used as a marker of post-mortem proteolysis as traditional methods of evaluation fail to find these changes.

Industry Impact

Changes in abundance of proteins that regulate energy usage, muscle protein turnover, and mitochondrial capacity can have underlying impacts on meat quality as muscle converts to meat in early stages post-mortem as well as continued protein changes that occur during typical aging protocols. Identification of proteins will help to target management and processing protocols for the most advantageous outcomes in meat quality when utilizing growth promotant technologies. This study identified proteins of interest that have not previously been determined to be related to beef quality traits and will help direct future studies to consider the role these proteins may have in providing the highest quality beef to consumers.

Photos

Figure 1. Dr. Christina Hayes picking spots for identification from pick gels.

Figure. 2 Ms. Wanda Keller scanning 2-D DIGE gels for detection of differences in protein spots.

Graphs/Tables

Table 1. Myofibrillar protein spots of 2D DIGE identified as actin by LC-MS/MS that were differentially abundant in *Longissimus lumborum* aged 14 days from crossbred heifers subjected to three exogenous growth promoting programs.

Spot[1]	pI[2] from DeCyder	MW[2] from DeCyder	Comparison	Average ratio[3]	*P*-value
60	5.1	58	COMBO/IMP		
				1.13	0.0031
			COMBO/CON		
				1.12	0.0034
			IMP/CON	-1.01	0.74
74	5.0	52	COMBO/IMP		
				1.13	0.0031
			COMBO/CON		
				1.12	0.0034
			IMP/CON	-1.01	0.74
75	5.0	49	COMBO/IMP		
				-1.23	≤ 0.001
			COMBO/CON		
				-1.27	≤ 0.001
			IMP/CON	-1.04	0.79
110	5.6	42	COMBO/IMP		
				-1.16	≤ 0.001
			COMBO/CON		
				-1.14	0.0018
			IMP/CON	1.02	0.58
120	5.4	41	COMBO/IMP		
				-1.22	≤ 0.001
			COMBO/CON		
				-1.12	0.044
			IMP/CON	1.09	0.041
126	5.2	42	COMBO/IMP		
				-1.27	≤ 0.001
			COMBO/CON		
				-1.12	0.015
			IMP/CON	1.14	0.001
133	5.5	40	COMBO/IMP		
				-1.31	≤ 0.001
			COMBO/CON		
				-1.31	≤ 0.001
			IMP/CON	-1.00	0.77
160	5.3	36	COMBO/IMP		
				-1.32	≤ 0.001
			COMBO/CON		
				-1.22	≤ 0.001
			IMP/CON	1.08	0.073

[1]Spot number refers to the numbered spots in the Cy2 Master gel image.
[2]pI = isoelectric point, MW = molecular weight.
[3]Average ratio of the Cy2 pooled standard is set to a value of 1. Positive values indicate an increase over that of the comparative treatment; negative values indicate a decrease over that of the comparative treatment.

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