University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Nebraska Beef Cattle Reports

Animal Science Department

2021

Proteomic Analysis of Oxidized Proteins in Beef

Nicolas A. Bland

Nicolas J. Herrera

Felipe A. Ribeiro

Morgan L. Henriott

Kellen B. Hart

See next page for additional authors

Authors
Nicolas A. Bland, Nicolas J. Herrera, Felipe A. Ribeiro, Morgan L. Henriott, Kellen B. Hart, and Chris Calkins

Proteomic Analysis of Oxidized Proteins in Beef

Nicolas A. Bland
Nicolas J. Herrera
Felipe A. Ribeiro
Morgan L. Henriott
Kellen B. Hart
Chris R. Calkins

Summary with Implications

To evaluate the effects of diet and quality grade on tenderness and oxidative damage to proteins, strip loins from USDA Upper 2/3rd Choice and Select- grade carcasses were obtained. Steers were fed either a diet containing dry rolled corn, steam flaked corn, dry rolled corn with 30% dried distillers grains with solubles, or steam flaked corn with 30% dried distillers grain with solubles. Results suggest that steaks from steers fed dry rolled corn are more objectively tender than steam flaked corn; in addition, steaks grading USDA Upper 2/3rd Choice steaks were more tender when compared to USDA Select quality grade. In contrast to previous research, no tenderness differences were detected between steaks from steers with or without dried distillers with solubles. Proteomic analysis revealed increased oxidative damage of myofibrillar proteins. Steaks graded as USDA Upper 2/3rd Choice steaks were determined to generally have increased oxidative damage to glycolytic, structural, and heat shock proteins, compared to USDA Select quality grade. While samples from steers fed dry rolled corn were more tender and had increased myofibrillar oxidative damage from steers fed DRC with distillers grains, steam flaked corn- related treatment displayed the inverse response. Overall, results support the relationship between marbling and tenderness, and suggest oxidative stress may be a factor involved in this difference.

Introduction

Recent proteomic research has implicated oxidative stress as a factor that damages myofibrillar antioxidant enzymes, structural, and heat shock proteins. Oxidative stress occurs as a result of increased reactive oxygen species that overwhelms antioxidant defenses in the body, causing cellular damage. While oxidative stress seems to impact tenderness in beef, research in human nutrition has determined high-fat diets can induce oxidative stress. Therefore, high-fat diets like distillers grains may induce oxidative stress in the cattle. It is commonly recognized that feeding high-fat diets such as those containing distillers grains promotes increased rate of marbling deposition. Research has long recognized the evident relationship between marbling and tenderness. Perhaps oxidative damage to proteins occurs in highly marbled beef and thereby potentially enhances tenderness. Therefore, this study was conducted to evaluate the effects of oxidative damage to myofibrillar proteins on beef tenderness, as influenced by diet and marbling.

Table 1. Warner Bratzler Shear Force of strip loin steaks (*L. lumborum*) from steers fed either with dry rolled corn with dried distillers grains with solubles, dry rolled corn without dried distillers grains with solubles, steam flaked corn with dried distillers grains with solubles, or steam flaked corn without dried distillers grains with solubles at USDA Upper 2/3rd Choice or Select quality grade (n=36).

Category					
Grain	DDGS	Quality Grade	WBSF (kgf)	*SEM*	P-value
DRC			3.46[b]	0.2	0.02
SFC			4.04[a]		
	DDGS		3.68	0.18	0.43
	No DDGS		3.84		
		Choice	3.39[b]	0.23	<0.01
		Select	4.11[a]		

[a,b] Means in the same column within a category without common superscripts differ ($P < 0.05$).
WBSF: Warner Bratzler shear force.
DRC: Dry rolled corn.
SFC: Steam flaked corn.
DDGS: Dried distillers grains with solubles.
No DDGS: Without dried distillers grains with solubles.

Procedure

A total of 240 steers were randomly block assigned by weight among 24 pens (10 head/pen) and fed for 202 d on diets containing dry rolled corn (DRC), DRC with 30% dried distillers grains with solubles (DDGS), steam flaked corn (SFC), or SFC with 30% DDGS. Thirty-six USDA Upper 2/3rd Choice and Select carcasses (21 Upper 2/3rd Choice and 15 Select) were selected and strip loins were collected. Beef strip loins were aged for 2 d and then fabricated into steaks for objective tenderness and samples were diced, frozen in liquid nitrogen, and blended into a powdered sample for proteomic analysis.

Tenderness

Internal temperature and initial weight of raw steak (1 inch thick) were recorded. Steaks were cooked to a target temperature of 160°F on a belt grill. The cooked steaks were measured and recorded for internal temperature and weight. The steaks were individually bagged and stored overnight at 36°F for WBSF analysis. On the following day, six (0.5-inch diameter) cores were removed using a drill press going parallel to the muscle fibers and were sheared

Table 2. Characteristics of carbonylated protein spots derived from USDA Upper 2/3rd Choice or Select beef from steers fed dry rolled corn with or without distillers grains.

Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical	Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical
DRC without DDGS													
Increased oxidative damage in USDA Upper 2/3rd Choice Quality Grade							Increased oxidative damage in USDA Select Quality Grade						
289	P00570	AK1	Adenylate kinase	1873	9.52/ 24,579	8.40/ 21,664							
DRC with DDGS													
Increased oxidative damage USDA Upper 2/3rd Choice Quality Grade							Increased oxidative damage in USDA Select Quality Grade						
110	P19120	HSPA8	Heat shock cognate 71 kDa protein	139	4.82/ 64,857	5.37/ 71,241	293	P02510	CRYAB	Alpha-crystallin B chain	2675	8.05/ 24,026	6.76/ 20,037
	Q27975	HSPA1A	Heat shock 70 kDa protein 1A	100		5.67/ 70,259	133	P19483	ATP5F1A	ATP synthase subunit alpha, mitochondrial	668	9.29/ 59,143	9.21/ 59,720

Comparisons are made across the table.

Table 3. Characteristic of carbonylated protein spots derived from beef from steers fed dry rolled corn with or without distillers grains at the same quality grade.

Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical	Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical
DRC–USDA Upper 2/3rd Choice Quality Grade													
Increased oxidative damage without DDGS							Increased oxidative damage with DDGS						
293	P02510	CRYAB	Alpha-crystallin B chain	2675	8.05/ 24,026	6.76/ 20,037	200	Q8MKH6	TNNT1	Troponin T, slow skeletal muscle	903	6.67/ 38,640	5.71/ 31,284
								Q8MKI3	Tnnt3	Troponin T, fast skeletal muscle	280		5.99/ 32,126
								Q3T145	MDH1	Malate dehydrogenase, cytoplasmic	468		6.16/ 36,438
							156	Q3ZC09	ENO3	Beta-enolase	506	6.93/ 52,000	7.60 /47,096
								Q9XSJ4	ENO1	Alpha-enolase	473		6.37 /47,326
							71	P79334	PYGM	Glycogen phosphorylase, muscle form	248	9.05/ 102,222	6.65 / 97,293
DRC–USDA Select Quality Grade													
Increased oxidative damage without DDGS							Increased oxidative damage in DRC with DDGS Select						
360	O97680	TXN	Thioredoxin	358	5.02/ 16,030	4.97/ 11,813	306	Q148F1	CFL2	Cofilin-2	571	6.69/ 22,842	7.66/ 18,737
358	P11116	LGALS1	Galectin-1	802	5.41/ 16,446	5.32/ 14,744	126	P31081	HSPD1	60 kDa heat shock protein, mitochondrial	686	5.28/ 61,048	5.60/ 61,108
	P00426	COX5A	Cytochrome c oxidase subunit 5A	564		6.42/ 16,735	125	O62654	DES	Desmin	3187	5.14/ 61,048	5.21/ 53,532
104	Q08DP0	PGM1	Phosphoglucomutase-1	1362	8.82/ 68,190	6.36/ 61,589							
	Q3ZBD7	GPI	Glucose-6-phosphate isomerase	730		7.33/ 62,855							

Comparisons are made across the table.

using a texture analyzer using a Warner-Bratzler shear blade. The WBSF values were averaged from each steak for statistical purposes.

Proteomics

About 50 mg of powdered beef samples were utilized to extract, separate, and identify proteins for proteomic analysis. Sample comparisons include evaluating differences due to dietary treatment of DRC with or without DDGS that were Choice or Select quality grade or SFC with or without DDGS that were Choice or Select quality grade.

Statistical analysis

The processing method of corn, addition or absence of DDGS, and quality grade served as the main plot factors. For proteomic analysis, if the protein oxidative damage score was greater than 31, then the comparison was significant. Tenderness determination was analyzed as a randomized complete block design in a 2×2×2 factorial. Data were analyzed using PROC GLIMMIX program of SAS with LSMEANS statement. Specific to the proteomic assay, if the protein score exceeded 31, the difference of protein oxidation, to treatment comparison, was significant. Statistical significance was determined at $P< 0.05$.

Results

No differences ($P= 0.43$) in tenderness were observed to be associated with the addition or absence of DDGS in the diet (Table 1). In previous research, a tenderness advantage has been reported for steaks from cattle fed DDGS, especially early postmortem. Steaks from steers fed DRC had significantly lower WBSF than steaks from steers fed SFC ($P< 0.05$) indicating that steers fed DRC were more tender than steers fed SFC. Also, there was a difference in tenderness between USDA Upper 2/3rd Choice and Select strip loins ($P< 0.05$), with USDA Upper 2/3rd Choice having lower WBSF. The lower the WBSF value, the more tender the sample. These results suggest that while the addition of DDGS may not improve tenderness, the substitution of DRC for SFC can improve tenderness along with improving USDA quality grade through fat deposition in the marbling adipocytes.

Protein oxidation in dry-rolled corn treatment

When comparing the different quality grades from steers fed DRC without DDGS diet (Table 2), there was an increase of oxidative damage in adenylate kinase for USDA Upper 2/3rd Choice, compared to Select. While adenylate kinase is a nucleotide myofibrillar protein involved in maintaining muscular homeostasis, it had increased oxidative damage of myofibrillar proteins in tender beef in previous research. In beef from steers fed DRC with DDGS, some heat shock proteins exhibited oxidative damage for USDA Upper 2/3rd Choice, compared to Select. Conversely, USDA Select carcasses had greater oxidative damage to α crystallin β chain and ATP synthase proteins. Given the tenderness advantage for the USDA Upper 2/3rd Choice carcasses, these results suggest that oxidative damage to certain proteins can be associated with increased tenderness.

For the USDA Upper 2/3rd Choice carcasses from steers fed a DRC diet, the addition of DDGS was associated with a wide array of oxidative damage of proteins (Table 3), including slow-twitch skeletal muscle and fast-twitch skeletal muscle fiber troponin T, and β-enolase, and malate dehydrogenase. While troponin T degradation has long been recognized as an indicator of improved tenderness, degradation of β-enolase is a protein only recently been reported to indicate improved tenderness. Intact malate dehydrogenase, alternatively, has been positively related to improved tenderness. For the USDA Select beef from steers fed DRC diet treatment, the addition of DDGS was associated with increased oxidative damage to the structural protein desmin and heat shock protein 60kDa. As heat shock proteins help stabilize cells and have been related to increased toughness in beef, increased oxidative damage of heat shock proteins are related to improved tenderness. Similarly, damage to desmin would support improved tenderness. For USDA Select beef from cattle fed DRC without DDGS, oxidative damage was associated with apoptotic proteins galectin and cytochrome-c oxidase. Apoptotic proteins have been hypothesized to improve tenderness, so increased oxidative damage of those proteins may negatively impact tenderness.

Protein oxidation in the steam-flaked corn treatment

When comparing quality grades beef from steers fed SFC without DDGS diet (Table 4), there was an increase of oxidative damage in a heat shock protein in USDA Select beef carcasses. Increased oxidation of heat shock proteins is often associated with more tender meat; however, the tenderness data do not support that USDA Select beef was more tender than USDA Upper 2/3rd Choice beef carcasses.

When including DDGS in the SFC diet (Table 4), USDA Upper 2/3rd Choice beef carcasses had considerably more proteins that were oxidized than USDA Select beef, including oxidation of structural proteins (the actinins) and proteins associated with glycolysis. The glycolytic enzymes are not only valuable to producing energy in low oxygen conditions but during slaughter when the lack of oxygen shunts energy production to mostly glycolysis and the lactic acid pathway. Damage to such systems could conceivably allow early postmortem release of calcium, stimulating calpain enzymes which accelerate tenderization. Alternatively, there was more sustained oxidative damage in myosin and a few glycolytic proteins in USDA Select beef when compared to USDA Upper 2/3rd Choice beef. The impacts of these changes are unknown.

In contrast to DRC, SFC without DDGS resulted in more proteins sustaining oxidative damage within USDA Upper 2/3rd Choice beef from steers fed SFC containing DDGS (Table 5). The oxidized proteins include myosin, tropomyosin, and cytochrome b-c1 complex. With myosin and tropomyosin being structural proteins, increased oxidative damage may indicate decreased structural integrity at the actomyosin cross-bridge, which may improve tenderness. Damage to cytochrome b-c1 complex can impact ATP production by negatively impacting the electron transport chain. Furthermore, it may impact cytochrome c, a protein that can influence apoptotic processes. Similar to these observations with USDA Upper 2/3rd Choice, the

Table 4. Characteristics of carbonylated protein spots derived from USDA Upper 2/3rd Choice or Select beef from steers fed steam flaked corn with or without distillers grains.

Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical	Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical
SFC without DDGS													
Increased oxidative damage in USDA Upper 2/3rd Choice Quality Grade							Increased oxidative damage in Select Quality Grade						
							150	P19120	HSPA8	Heat shock cognate 71 kDa protein	4116	3.45/ 65,684	5.37/ 71,241
SFC with DDGS													
Increased oxidative damage USDA Upper 2/3rd Choice Quality Grade							Increased oxidative damage in Select Quality Grade						
282	P19858	LDHA	L-lactate dehydrogenase A chain	2382	9.33/ 36,495	8.12/ 36,598	390	Q0P571	MYLPF	Myosin regulatory light chain 2, skeletal muscle isoform	3298	4.87/ 14,602	4.88/ 19,010
249	Q9XSC6	CKM	Creatine kinase M-type	2068	8.01/ 41,484	6.63 /42,989	245	Q5EA88	GPD1	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	1463	7.88/ 42,000	6.42/ 37,648
	Q5EA88	GPD1	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	1475		6.42/ 37,648		Q2KJJ9	FBP2	Fructose-1,6-bisphosphatase isozyme 2	449		7.52/ 36,767
218	Q3ZC09	ENOB_BOVIN	Beta-enolase OS=Bos taurus	9527	9.17/ 48,296	7.60 /47,096							
	Q9XSJ4	ENO1	Alpha-enolase	4021		6.37 /47,326							
	Q3T0P6	PGK1	Phosphoglycerate kinase 1	1453		8.48/ 44,538							
181	P10096	G3P_BOVIN	Glyceraldehyde-3-phosphate dehydrogenase	379	9.60/ 59,368	8.51/ 35,868							
88	Q3ZBT1	TERA	Transitional endoplasmic reticulum ATPase	1603	4.98/ 85,600	5.13 / 89,330							
	Q0III9	ACTN3	Alpha-actinin-3	1514		5.31/ 103,151							
	Q3ZC55	ACTN2	Alpha-actinin-2	1300		5.31/ 103,779							

Comparisons are made across the table.

Table 5. Characteristic of carbonylated protein spots derived from beef from steers fed steam flaked corn with or without distillers grains at the same quality grade.

Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical	Spot ID	Protein ID	ABV	Protein	Score	PI/Mw experimental	PI/Mw theoretical
SFC - USDA Upper 2/3rd Choice Quality Grade													
Increased oxidative damage without DDGS							Increased oxidative damage with DDGS						
455	Q0P571	MYLPF	Myosin regulatory light chain 2, skeletal muscle isoform	94	4.87/ 14,602	4.88/ 19,010	456	P02584	PFN1	Profilin-1	238	9.25/ 14,472	8.46/ 15,057
390	Q0P571	MYLPF	Myosin regulatory light chain 2, skeletal muscle isoform	3298	4.87/ 14,602	4.88/ 19,010							
335	Q3SZX4	CA3	Carbonic anhydrase 3	3126	9.46/ 28,237	7.71/ 29,370							
239	Q5KR49	TPM1	Tropomyosin alpha-1 chain	466	3.57/ 43,111	4.69/ 32,695							
	Q5KR48	TPM2	Tropomyosin beta chain	294		4.66/ 32,837							
179	P31800	UQCRC1	Cytochrome b-c1 complex subunit 1, mitochondrial	951	5.98/ 59,368	5.94/ 52.736							
SFC – USDA Select Quality Grade													
Increased oxidative damage without DDGS							Increased oxidative damage with DDGS						
100	P79334	PYGM	Glycogen phosphorylase, muscle form	1870	8.73/ 76,945	6.65/ 97,293							
109	P20004	ACO2	Aconitate hydratase, mitochondrial	518		7.87/ 85,359							

Comparisons are made across the table.

SFC without DDGS diet resulted in more oxidative damage within the USDA Select beef, as well, when compared to USDA Select beef from diets containing SFC and DDGS. In this study, the effects of DDGS in SFC diets are contrary to the effects of DDGS in DRC diets, indicating the need for further investigation.

Conclusions

USDA Upper 2/3rd Choice beef was more tender and generally had increased oxidative damage in proteins, compared to USDA Select beef. This gives credence to the hypothesis that there is a relationship between marbling and tenderness which may be mediated through oxidative damage to proteins. Conflicting results were observed on the effects of DDGS when comparing DRC-based diets to SFC-based diets.

Acknowledgment

This project was funded in part by the Beef Checkoff.

Nicolas A. Bland, graduate student

Nicolas J. Herrera, graduate student

Felipe A. Ribeiro, graduate student

Morgan L. Henriott, former graduate student

Kellen B. Hart, former graduate student

Chris R. Calkins, professor, Animal Science, University of Nebraska–Lincoln