The Impact of Oxidative Stress on Postmortem Meat Quality

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Summary with Implications

This study was conducted to evaluate the relationship between animal oxidative status, using lipopolysaccharide (LPS) as a promoter for oxidation. This was used as a model to evaluate tenderization and meat quality factors early postmortem. Lambs were administered an intravenous injection of either saline, 50 ng/kg bodyweight (LPS50), or 100 ng/kg bodyweight (LPS100) every 72 hours for a 9-day period to stimulate physiological oxidative stress. After a day of rest, lambs were harvested, and pre-rigor Longissimus dorsi muscles were obtained for transcriptomic analysis. Loins, aged for 1 and 14 days, were analyzed for attributes relating to oxidative potential, meat tenderness, color, and lipid stability. Results show lambs administered lipopolysaccharide treatments exhibited greater oxidative potential, as indicated by increased rectal temperatures, and upregulated expression of mRNA protein pathways essential for cellular differentiation, proliferation, and apoptotic events. Lambs administered LPS50 tended to be more tender early postmortem, with significantly increased proteolysis (Troponin T). Interestingly, LPS treatment was not detrimental to meat quality, as indicated by more ideal color values and no significant changes in lipid oxidation. These data indicate that oxidative potential via oxidative stress can potentially increase tenderization early postmortem, which may provide more tender meat with no detriment to other meat quality factors.

Introduction

Tenderness is repeatedly cited as the primary element associated with both eating quality and consumer purchasing decisions. Inconsistent meat tenderness and its impact on consumer satisfaction is an obstacle to optimizing demand for U.S. meat products. Thus, investigations into the process of postmortem tenderization and the role of cellular organelles and mechanisms involved have strong, practical application.

Recent research using beef muscles with little aging has identified different oxidized proteins across different tenderness group. The tender (~3.9 kg) samples, compared to the intermediate (~5.3 kg) and tough (~7.6 kg) groups, had highly oxidized structural, contractile, and regulatory proteins, all directly associated with muscle contraction and tenderization mechanisms. Predisposition to oxidative stress may promote an increase in oxidized proteins.

It is hypothesized that states of oxidative stress may activate proteolytic mechanisms responsible for the structural degradation of muscle proteins during postmortem tenderization. The influence of oxidative stress is also being investigated for its impact on other factors of meat quality, such as lipid oxidation and color stability.

We hypothesized that controlled levels of oxidative stress modify mechanisms responsible for meat quality. The objectives of the research were to understand the mechanism related to meat quality in lamb from wethers administered defined levels of an oxidative stress promoter (lipopolysaccharides).

Procedure

A total of 29 lambs were individually housed and fed a standard finishing ration. Lambs were blocked by weight and randomly assigned to one of three intravenous injection treatments: Saline Control (n =10), 50 ng lipopolysaccharide O111:B4/kg bodyweight [LPS50] (n = 9), or 100 ng LPS O111:B4/kg bodyweight [LPS100] (n = 10). Each lamb was injected with 2 mL every 72 hours, totaling 3 injections across a 9-day challenge. Rectal temperatures were taken at 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-injection times. After the immune challenge, lambs were given 48 hours lairage and then harvested. Pre-rigor loin muscle (80 mg) from Control and LPS100 lambs was obtained for transcriptomic analysis, evaluation of mRNA pathways as they relate to muscle development and function. After 1 or 14 d of aging, 1-inch thick chops were cut from the Longissimus dorsi for measuring tenderness (shear force), objective color, subjective discoloration, and lipid oxidation (TBARs). Samples were obtained to evaluate calcium concentration, fatty acids, sarcomere length, pH, proximate composition, proteolysis (Troponin-T; Desmin), and isoprostane content. Chops used for color analysis and TBARs were overwrapped with oxygen permeable film and placed under retail display (RD) for 7 d at 37°F. Chops for sarcomere length, proximate composition, and isoprostane content were analyzed at 1 d postmortem. Transcriptomics was measured using log fold change (total gene expression) and z-score (upregulated pathway, positive – LPS100, negative – Control). Tenderness was measured using the Warner-Bratzler shear force (WBSF) method and proteolysis was determined using protein electrophoresis and immunoblotting. Sarcomere length was measured via laser diffraction, free Ca^{2+} concentration was analyzed via inductively coupled plasma spectroscopy following high-speed centrifugation, and pH was measured via pH meter. Fatty acid profile was measured via gas chromatography. Isoprostane content was evaluated using an ELISA test kit, with final values calculated as picograms/mL. Proximate composition (%) included: fat content via ether extraction, moisture and ash via Thermogravimetric Analyzer, and protein content was calculated by difference. Lipid oxidation, Thiobarbituric acid reactive substances (TBARs), was measured

Table 1. Transcriptomics expressed by Conical Pathways in Contol vs 100ng LPS treated lambs. P-values for negative logarithmic (-log) expression set for (P_{raw} < 0.05).

Function	Pathways	Fold change -log	Z-score
Cell Biosynthesis and Turnover	IGF-1	2.9	0.707
	EGF	2.6	0.816
	ErbB2-ErbB3	2.24	-0.447
	ILK	2.05	1
	cAMP	1.88	-0.302
	PI3K	1.63	1.414
	ERK5	1.46	1.342
	Ceramide	0.848	1.89
Nucleic Modification	Unfolded Protein	5.93	0.378
	Telomerase	3.43	0.707
	HMGB1	2.5	1.414
	EIF2	1.94	0.333
	Neurotrophin/TRK	1.91	0.816
	JAK/Stat	1.81	0.816
Oxidative Response/ Autophagy	NRF2 Oxidative Stress Resp.	6.48	1.265
	IL-6	3.49	-0.905
	p38 MAPK	3.09	1
	Sumoylation	2.35	1.633
	TNFR-2	2.19	2
	CXCR4	2.01	0.707
	IL-8	1.52	0.632
	NO/ROS prod. In Macrophages	1.31	0.333
	IL-3	1.31	1.342
Muscle Function Oxidative-Stress	eNOS Signaling	2.63	-2.121
	Agrin	1.84	1.342
	Calcium Signaling	1.81	-1.633
	PPARα/RXRα	1.65	-1.265
	D-myo-inositol-tetrakiphosphate	1.59	0.707

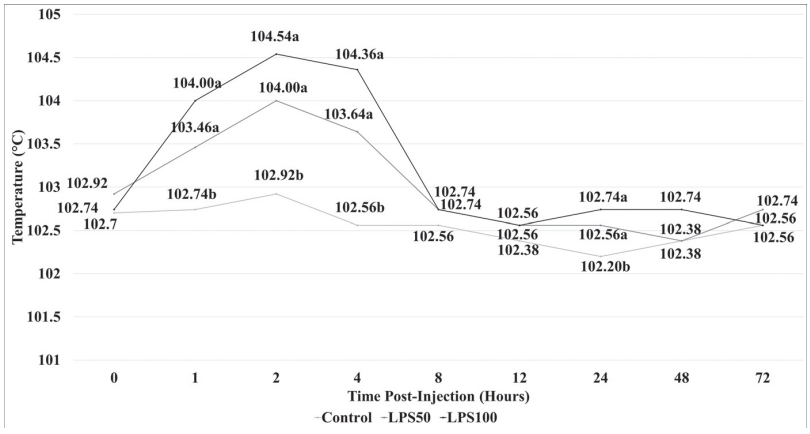

Figure 1. Rectal temperatures of lambs administered intravenous injections of Control, LPS50, or LPS100. Superscripts denote statistical differences (P < 0.05) within hours.

by the amount of mg of malonaldehye per kg of muscle tissue following retail display periods of 0 or 7 d. Instrumental color was measured using a colorimeter to detect lightness (L*), redness (a*), and yellowness (b*). Subjective discoloration was also evaluated daily during retail display by a panel of five trained panelists using a percentage scale where 0% meant no discoloration and 100% meant total surface discoloration.

Statistical analysis was conducted with SAS (version 9.4, Cary, NC). Transcriptomic data were quality trimmed using Trim Galore!, and aligned to the Oar_rambouillet_v1.0 reference genome STAR (Dobin et al., 2016). Differential expression (control vs LPS 100) was evaluated using transcript counts in DESeq2 (Love et al., 2014). Loci with P_{adj}<0.05 were considered to be differentially expressed; those with P_{raw}<0.05 were utilized for pathway exploration in Ingenuity Pathway Analysis (Qiagen). Objective and subjective color data were analyzed as a split-plot repeated measures design with treatment as the whole-plot, aging period as the split-plot and retail display as the repeated measures. Tenderness, troponin-T, desmin, calcium, and pH were analyzed as a spilt-plot design with treatment as the whole-plot and aging period as the split-plot. Lipid was evaluated using free thiols and carbonyls were a split-split-plot design with treatment as the whole plot, aging period as the split-plot and retail display time as the split-split-plot. Sarcomere length, fatty acids, and isoprostanes were analyzed as a completely randomized design. Lamb was the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS and animal was the experimental unit. Correlations were evaluated using the PROC CORR procedure of SAS across all postmortem analyses. All means comparing within aging periods were separated using SLICE function in SAS. All means were separated using the LS MEANS statement with an α level of 0.05 and tendencies were considered at an α level of 0.15.

Results

Treatment affected rectal temperatures (Figure 1). Lambs administered LPS treatments exhibited an increased ($P \leq$ 0.02) rectal temperature at 1, 2, 4, and 14 h

Table 2. Analytical measures of 1 and 14 day aged chops from lambs administered Saline Control, LPS50, or LPS100. Superscripts denote statistical differences ($P < 0.05$).

	Treatment										
	Days Aging										
	1				14				*P*-value		
	Control	LPS50	LPS100	*P*-value	Control	LPS50	LPS100	*P*-value	Trt	Age	Trt*Age
WBSF, lbs of force (kg)	17.73 (8.06)	14.50 (6.59)	15.99 (7.27)	0.10	6.09 (2.77)	5.32 (2.42)	5.19 (2.36)	0.9	0.11	< 0.0001	0.13
pH	5.71	5.68	5.73	0.54	5.84	5.86	5.91	0.27	0.13	< 0.0001	0.76
Calcium (µm)	46.72	40.71	43.63	0.33	108.02	104.51	103.12	0.41	0.50	< 0.0001	0.88
Troponin-T Degradation (%)	6.85b	10.32a	6.24b	0.02	47.73	49.19	41.68	0.78	0.27	< 0.0001	0.88
Desmin Degradation (%)	3.01	4.17	3.54	0.85	55.02	41.73	54.94	0.1	0.15	< 0.0001	0.08

LPS: Lipopolysaccharides

WBSF: Warner-Bratzler Shear Force

[a,b] Means within an aging period with different superscripts are different ($P < 0.05$).

post-injection, as the increase in LPS content increased the temperature at these time points. Rectal temperature is an indicator of acute physiological inflammation, suggesting increased oxidative stress occurred.

Transcriptomics is an analytical method that identifies molecules that have been transcribed from genes into RNA. These protein precursors can provide the cellular instructions for synthesis of specific proteins, which can be associated with particular metabolic pathways. For this experiment, transcriptomic analysis identified different gene expressions across Control and LPS100 treated lambs ($P_{raw} < 0.05$) (Table 1). In particular, pathways with positive z-scores denote increased expression of pathways for LPS100 lambs. Increased expression in LPS100 treated lambs primarily focus on RNA responsible for cellular biosynthesis and turnover, nucleic modification, oxidative stress response systems, and muscle functionality and apoptotic activation. Given the impact oxidative potential can have on cellular damage and dysfunction, it is reasonable to expect that lambs treated with a compound such as LPS that triggers an immune and oxidative response would induce pathways responsible for cellular death and turnover. Additionally, the increase in oxidative stress-related pathways link the concept between increased oxidative stress with muscle response systems, which could impact meat quality. Negative z-scores denote an increased expression in pathways as they relate to Control samples. This analysis validates the occurrence of oxidative stress.

Treatment tended to affect shear force ($P = 0.1343$) values [Table 2]. In particular, LPS50 and LPS100 chops aged 1 day postmortem tended to have a lower shear force compared to the control (14.50 lbs, 15.99 lbs, and 17.73 lbs of force, respectively).

Proteolysis of troponin-T and desmin were utilized as indicators of protein degradation. During tenderization, proteolytic enzymes break down different proteins related to structures within the sarcomere and myofibril, reducing shear force and improving tenderness. There was no treatment main effect on desmin, however, a treatment-by-days of aging trend ($P = 0.08$) was identified, as LPS50 chops tended to have a lower percent degradation compared to Control and LPS100 chops at 14 days aging (41.73%, 55.02%, 54.94%, respectively), with no impact at 1 day of aging. A treatment effect was found at 1 day aging for troponin-T analysis ($P = 0.02$), as LPS50 samples were higher in percent degradation compared to Control and LPS100 (10.32%, 6.85%, 6.24%, respectively). A days of aging effect was found, as 14 days aging had significantly ($P < 0.0001$) greater percent degradation compared to day 1 aged chops. This indicates LPS50 treated lambs exhibited greater degradation early postmortem compared to the other treatments.

Days of aging had an effect on free Ca^{2+} concentration ($P < 0.0001$). Chops aged for 14 days exhibited higher amounts of free calcium concentration than chops aged for 1 day postmortem. However, no LPS treatment effect was observed for free Ca^{2+} concentration ($P = 0.33$). Calcium plays a critical role in the tenderization of meat postmortem. Calcium acts as a regulator for muscle contraction in live tissue, but functions to activate proteolytic enzymes in meat postmortem. Free Ca^{2+} concentration was measured as an indicator of proteolytic enzyme activity, since the increase in Ca^{2+} would activate proteolytic enzymes used to breakdown muscle proteins (Troponin-T, Desmin). However, the lack of statistical differences in Ca^{2+} concentration does not explain observed differences in tenderness.

There was no LPS treatment effect on pH ($P = 0.27$). A higher pH would allow greater water retention and stearic hindrance between muscle structures, facilitating an increase in tenderness. Days of aging had an effect on pH ($P < 0.0001$), as chops aged 14 days increased their pH compared to 1 day aged chops. However, the increase in pH was not within the range recognized for dark cutting meat (≥ 6.0), meaning that the increase to pH was not seen as a detrimental aspect to meat quality.

Sarcomere length and proximate composition were measured as potential indicators of meat tenderness (Table 3). Typically, a longer sarcomere length and greater moisture and fat content are associated

Table 3. Analytical measures of 1 day aged chops from lambs administered Saline Control, LPS50, or LPS100.

	Treatment			
	Control	LPS50	LPS100	*P*-value
Sarcomere Length (μm)	1.7	1.73	1.71	0.7
Moisture (%)	75.1	75.51	75.45	0.31
Protein (%)	15.13	14.43	13.91	0.68
Fat (%)	8.18	8.29	8.99	0.82
Ash (%)	1.59	1.77	1.66	0.44
SFA (mg/100g tissue)	3,289	3,301	3,560	0.86
MUFA (mg/100g tissue)	4,100	4,139	4,542	0.77
PUFA (mg/100g tissue)	738	810	839	0.82
Trans Fatty Acid (mg/100g tissue)	365	346	419	0.55
Total (mg/100g tissue)	8127	8250	8941	0.82
Isoprostane Content (pg/mL)	165.51	239.51	219.95	0.2

LPS: Lipopolysaccharides

SFA: Saturated Fatty Acids

MUFA: Monounsaturated Fatty Acids

PUFA: Polyunsaturated Fatty Acids

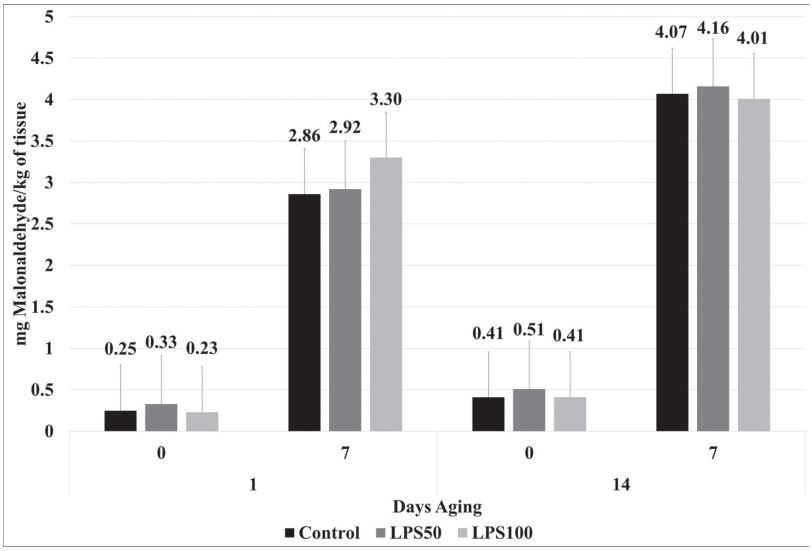

Figure 2. Lipid Oxidation (Thiobarbituric acid reactive substances) for 1 and 14 days aged chops after 0 and 7 days of retail display from lambs administered Saline control, LPS50, or LPS100.

with greater tenderness. LPS treatments, however, had no effect on sarcomere length and proximate composition.

Fatty acid profiles were measured as a potential confounding variable on meat quality. Composition of fatty acids found within muscle are critical when examining tenderness, as recent literature has associated an increase in unsaturated fatty acids (UFAs) with increased tenderness early postmortem. Additionally, fatty acids impact color and lipid oxidation, with increased UFA content associated with increased discoloration and lipid oxidation in muscle tissue, negatively impacting meat quality. Fortunately, there were no differences among any fatty acid attributes across treatments, suggesting that fatty acid composition was not the source of differences in tenderness across treatments.

There was no treatment effect ($P = 0.20$) found for isoprostane content. Isoprostanes are regarded as one of the best biomarkers available to detect sustained oxidative stress in a tissue. Isoprostanes are generated during oxidation of arachiodonic acid (20:4) via reactive oxygen species, constituents of oxidative stress. The generation of isoprostane content can be used as an indicator of oxidative stress damage produced in a living system, and used to telegraph the degree of oxidative damage which has occurred in a sample. While not significant, it was interesting to see that both LPS50 and LPS100 treatments had a numerically greater isoprostane content compared to the Control (239.51 pg/mL, 219.95 pg/mL, and 169.51 pg/mL, respectively), suggesting an increase in LPS-induced oxidative stress in those samples.

Lipid oxidation was determined as an indicator of oxidation or rancidity of meat. LPS had no effect on lipid oxidation ($P = 0.9687$). The TBARs values displayed in Figure 2 show a day effect ($P < 0.0001$), as 7 days of RD significantly increased oxidation compared to 0 days of RD. A potential trend ($P = 0.06$) occurred during aging, as 14 day aged chops tend to increase in lipid oxidation compared to 1 day aged chops. While there was no days of aging-by-days of RD interaction ($P = 0.1786$), all samples aged 14 days exhibited the greatest numerical amount of oxidation at 7 days of RD compared to all other measures. From these comparisons, it is noteworthy that LPS treatments did not induce greater oxidation of muscle tissue, which relate to extreme off-flavors or detrimental effects on quality.

Color is the primary factor associated with consumer purchasing decisions, as consumers use visual evaluation of meat quality when product is sold. Consumers desire a bright cherry-red color in meat. Objective color measures include lightness score, L* (0 = black, 100 = white), redness score, a* (-60 = green, 60 = red), and yellowness score, b* (-60 = blue, 60 = yellow). As seen in Table 4, the L* values decreased as days of RD increased for both aging periods, however, the L* values were significantly lighter in LPS100 samples compared to Control ($P = 0.0017$). The a* values had an LPS treatment-by-days of aging interaction ($P = 0.0008$). In total, 14 day aged chops had greater a* values

Table 4. Instrumental color (L*, a*, b*) and discoloration (%) of 1 and 14 days aged chops from lambs administered Saline Control, LPS50, LPS100. Different superscripts denote differences ($P < 0.05$) within row.

		Treatment			*P*-value		
Measure	Days of Aging	Control	LPS50	LPS100	*Trt*	*Age*	*Trt x Age*
L*	1	44.37	45.47	45.92	0.0017	0.68	0.92
	14	44.36	45.73	46.06			
Mean		44.37^{b}	45.6^{ab}	45.97^{a}			
a*	1	13.7^{a}	13.67^{a}	13.31^{a}	0.01	<.0001	0.0008
	14	15^{b}	16.01^{a}	15.7^{a}			
b*	1	6.93^{c}	8.2^{a}	7.39^{bc}	0.12	0.12	0.02
	14	7.88^{a}	7.55^{a}	8.04^{a}			
Discoloration	1	7.81^{a}	3.34^{a}	9.27^{a}	0.35	0.22	0.02
	14	16.43^{a}	3.32^{b}	5.58^{b}			

^{a,b} Superscripts denote differences (P < 0.05) within a trait.

LPS: Lipopolysaccharides

compared to 1 day aged chops. Within 14 day aged chops, chops from both LPS treatments had significantly greater a* scores, denoting greater redness stability compared to the Control. The b* values had an LPS treatment-by-days of aging interaction ($P = 0.02$), as 14 day aged chops had greater b* values compared to 1 day aged chops. Within 1 day aging, LPS50 chops had the highest b* score compared to the other treatments, denoting a greater degree of yellowness within the samples. As a result, chops from the LPS treated lambs exhibited had greater color stability compared to control samples.

Conclusions

The results suggest that LPS-induced oxidative stress *in vivo* could explain the trend of increased tenderness and the significant increases in proteolysis early postmortem for LPS-treated lambs, in particular LPS50 treated lambs. Additionally, the increased oxidative stress was not detrimental to meat color or lipid oxidation, suggesting that low levels of oxidative stress alter meat tenderization early postmortem, without negatively impacting other meat quality attributes.

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