SHELF LIFE OF MEAT FROM BEEF FED ANTIOXIDANT AND WET DISTILLERS GRAINS

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***Abstract*—This study was conducted to evaluate the effects of feeding the synthetic antioxidant ethoxyquin and tertiary butyl hydroquinone (AG; AGRADO®PLUS) on shelf life of beef fed wet distillers grains (WDGS). Cross-bred steers were fed dry-rolled corn based finishing diets containing 0 or 30% WDGS with AG (0 or 150 ppm/steer/day for last 145 -160 d). Eighty USDA Choice beef short loins were collected and aged for 8 and 29 d at 2°C. The *m. longissimus lumborum* muscle was fabricated and cut into six 2.54 cm-thick steaks. Two steaks (for 0 d lipid oxidation and shear force) were vacuumed packaged and frozen. Two steaks were split into halves, packaged aerobically (OW) or under high oxygen (HiO_2-MAP) and displayed for 4 or 7 d under simulated retail display conditions for lipid oxidation. Two additional steaks were packaged OW or HiO_2-MAP and displayed for 7 d for shear force analysis. Percentage surface discoloration of steaks was visually evaluated daily by a trained 6-person panel. Lipid oxidation of steaks displayed for 0, 4, and 7 d was tested by thiobarbituric acid reactive substances (TBARS) analysis. Shear force was tested using Warner-Bratzler shear force (WBSF). Discoloration and TBARS increased during retail display time in both packaging systems and aging periods. The OW steaks were significantly more discolored than HiO_2-MAP. Steaks from corn plus AG supplemented cattle had ($P < 0.05$) less discoloration and lower TBARS at the end of retail display. The anti-discoloration and antioxidant effects of AG could not be seen when feeding WDGS. The WBSF values of HiO_2-MAP steaks significantly ($P < 0.0001$) increased during retail display. Feeding AG slightly ($P = 0.04$) decreased tenderness. Dietary supplementation of beef fed corn with ethoxyquin and tertiary butyl hydroquinone appears to be a viable means to increase lipid and color stability during retail display.**

***Index Terms*— Antioxidant, Beef, Wet distillers grains**

I. INTRODUCTION

Feeding trials with wet distillers grains plus solubles (**WDGS**), as a high protein source, for finishing cattle have shown that WDGS can be added to corn-based rations at levels ranging from 10 to 40% (DM basis) to increase feeding efficiency and average daily gain of cattle (Larson et al., 1993). However, de Mello Jr., Jenschke & Calkins (2008) and Senaratne, Calkins, de Mello Jr., Carr & Erickson (2009) have shown that WDGS linearly increase polyunsaturated fatty acids (**PUFA**) in beef. Elevated levels of PUFA in beef cause detrimental effects on beef quality including high oxidation, which can thereby severely affect color and flavor of beef, eventually affecting consumer appeal at retail. Feeding vitamin E, an antioxidant, with WDGS has shown to be a promising strategy to mitigate detrimental effects on beef due to feeding WDGS (Senaratne, Calkins, de Mello Jr. & Erickson, 2009), but it may increase the feeding cost.

Feedlot studies with a mixture of synthetic antioxidant, ethoxyquin, and tertiary butyl hydroquinone (**AG**; AGRADO®PLUS) have shown improvement in average daily gain and a decrease in morbidity and mortality of cattle by improving the antioxidant capacity at the ruminal and postruminal stages of digestion (Han, Hussein, Glimp, Saylor & Greene, 2002). Others (Moore, Vasconcelos, Erickson, Furman, Andersen & Macken, 2010) have reported AGRADO®PLUS supplementation affects neither performance nor carcass characteristics of feedlot cattle. Feeding AGRADO®PLUS may increase the antioxidant level of muscles. This study was conducted to evaluate the antioxidant effects of feeding AGRADO®PLUS with WDGS diets on color and lipid stability, and tenderness of beef during its shelf life.

II. MATERIALS AND METHODS

A. Feeding cattle

Cross-bred (British × Continental) yearling steers (n = 483; initial BW = 427 kg ± 37 kg) were randomly assigned to one of four dry rolled corn based diets, contained 0, or 30% (DM basis) wet distillers grains plus solubles (**WDGS**) with or without AGRADO®PLUS (**AG**; 150 ppm/steer/day) supplementation. Steers were fed a total of 145 (at first trial) or 160 d (at second trial) and slaughtered. Carcasses were chilled for 48 h before grading. After grading, both

sides of the beef loin, short loins (IMPS # 174; NAMP, 2007) from total of 80 (40 from each trial) USDA Choice carcasses (10 from each dietary treatment) were vacuumed-packaged and transported under refrigeration. Short loins were aged for either 8 or 29 d at 2°C.

B. Fabrication and assignment of steaks

After aging for 8 and 29 d, *m. longissimus lumborum* (strip loin; IMPS # 1180A; NAMP, 2007) muscles were removed from the beef loins. Each strip loin was cut into six 2.54 cm-thick steaks from the anterior to the posterior. The first (for oxidation; 0 d retail displayed), and fourth (for shear force; 0 d retail displayed) steaks were immediately vacuum-packaged and stored at -20°C until they were tested. The second and third steaks were split into halves and assigned for 4 and 7 d oxidation analysis either under overwrapped (**OW**) or high-oxygen modified atmospheric packaging (**HiO_2-MAP**) systems. Fifth and sixth steaks were alloted for 7 d retail display shear force analysis under OW and HiO_2-MAP packaging systems, respectively. Surface discoloration ratings were determined on steaks assigned for 7 d retail display shear force analysis. All steaks, assigned for OW retail display were packaged (oxidation as four pieces per tray, shear force as two steaks per tray) on Styrofoam trays and overwrapped with oxygen permeable polyvinyl chloride film. All steaks, assigned for HiO_2-MAP retail display were packaged (oxidation samples as four pieces per tray, shear force samples as two steaks per tray) on high foam-barrier polypropylene trays with a gas mixture (80% O_2: 20% CO_2) and mechanically sealed with oxygen impermeable film.

C. Retail display

Overwrapped and HiO_2-MAP packaged 8 and 29 d aged steaks, placed on a table in a cooler (at 0 ± 2°C) were exposed to continuous 1000-1800 lux warm white fluorescence lighting to provide simulated retail display conditions. Steaks (8 and 29 d aged) assigned for 4 and 7 d of retail displayed were removed from tables accordingly for oxidation, and shear force analysis, immediately vacuum-packaged and stored at -20°C until they were analyzed.

D. Subjective discoloration

A six-person trained panel from the Department of Animal Science at the University of Nebraska-Lincoln subjectively evaluated the percentage surface discoloration. Discoloration was evaluated as percentage surface discoloration raging from 0 to 100%. The discoloration ratings were made on each steak from 0 d to 7 d of retail display at 24-h intervals.

E. Lipid oxidation

The 2-thiobarbuteric acid reactive substance assay (**TBARS**) described by Ahn et al. (1998), which was a modification of the TBARS assay developed by Beuge and Aust (1978), was used to measure the oxidation status of 8 d and 29 d aged steaks in both packaging systems displayed for 0, 4 and 7 d in simulated retail display.

F. Shear force

Shear force evaluation were performed by Warner-Bratzler shear force testing (**WBSF**). Steaks were thawed at 4°C for 24 h and grilled at 71°C on a Hamilton Beach Indoor-Outdoor grill, turning over once at 35°C. All steaks from same animal (including 8 and 29 d aging, 0 and 7 d retail display, and OW and HiO_2-MAP) were grilled in a single batch to avoid variation due to cooking session. After grilling, steaks were cooled at 4°C for 24 h. Six cores with 1.27 cm diameter were removed from a steak parallel to the muscle fiber arrangement using a drill press. Cores were sheared on a tabletop WBSF analyzer (3000, G-R Manufacturing Co., Manhattan, KS) with a triangular Warner-Bratzler shear attachment. An average of the peak shear force (kg) of six cores for each steak was used for statistical analysis.

G. Statistical analysis

Data were analyzed by ANOVA in the GLIMMIX procedure of SAS (version 9.2, Cary, NC., 2009) as a split-split-split-plot design with dietary treatments as the whole-plot treatment, aging period as the first split-plot treatment, packaging systems as the second split-plot treatment and retail display time (repeated measures) as the third split-plot treatment. Separation of means was conducted using LSMEANS procedure with PDIFF and SLICEDIFF options at $P \leq 0.05$. In addition, the CONTRAST statements in SAS were used to compare the effects of feeding Corn *vs.* WDGS, Corn *vs.* Corn+AG, WDGS *vs.* WDGS+AG, and No AG *vs.* AG.

III. RESULTS AND DISCUSSION

Four-way interaction effects of treatment × packaging × aging × day on steaks surface discoloration were significant (Table 1; $P < 0.0001$). Discoloration increased during retail display time in both packaging systems and both aging periods. However, steaks in the OW packaging system were significantly more discolored compared to steaks in HiO_2-

MAP system (less than 20% surface discoloration). Similar results have been reported by de Mello Jr., Watanabe, Calkins, Senaratne, Carr and Erickson (2010). A possible reason for less discoloration when beef exposed to higher levels of oxygen is due to conversion of myoglobin into stable oxymyoglobin (cherry red color). Steaks (29 d aged in both packaging systems) from cattle fed corn plus AG had significantly ($P < 0.05$) lower discoloration at the end of the retail display period ($P < 0.05$). The effectiveness of AG supplementation in reducing discoloration was prominent when meat was aged longer. The anti-discoloration effect of AG supplementation could not be seen when cattle were fed WDGS. The reason for high discoloration even after adding AG into the diet is likely due to the increase of easily oxidizable, polyunsaturated fatty acids in beef from feeding WDGS. de Mello Jr. et al (2008) and Senaratne et al. (2009) reported that feeding WDGS increased PUFA level in beef compared to corn control diets.

There were significant (Table 2; $P = 0.04$) three-way interaction effects of treatment × aging × day on lipid oxidation. As aging and retail display time increased lipid oxidation also increased. However, there were no significant differences among 8 d aged steaks from cattle fed different diets. The only significant ($P < 0.05$) difference in dietary treatments could be seen in 29 d aged steaks at the end of retail display. Similar to discoloration results, steaks from corn plus AG diets had the lowest oxidation. Steaks from AG supplemented cattle had significantly ($P < 0.05$) lower lipid oxidation compared to steaks from cattle not on AG supplementation. Feeding AG helped to reduce oxidation of increased PUFA content when cattle were fed WDGS. As expected, TBARS analysis did not show significantly higher lipid oxidation on steaks from HiO_2-MAP systems compared to steaks from OW packaging systems (although, numerically higher). This might be due to the dilution effect of oxidized lipid on the surface of the thick steaks when preparation for TBARS analysis.

The WBSF values of steaks significantly ($P = 0.03$) decreased when aging and retail display time increased. Steaks from HiO_2-MAP systems after 7 d retail display had significantly ($P < 0.0001$) higher WBSF value (in 0.46 kg) compared to steaks from OW packaging systems. Only the main effect of dietary treatments was significant (Table3; $P = 0.02$). Steaks from corn plus AG fed cattle significantly less tender compared to steaks from other diets. When comparing steaks from AG fed cattle and steaks from AG non supplemented diets, steaks from AG supplemented cattle were significantly ($P = 0.04$) tougher. Complete understanding for decreasing tenderness of steaks from AG supplemented cattle is still lacking, although protein oxidation and polymerization are suspected.

IV. CONCLUSION

Feeding feedlot cattle with a mixture of antioxidants (ethoxyquin and tertiary butyl hydroquinone) contained within AGRADO®PLUS shows positive antioxidant effects against myoglobin and lipid oxidations of beef strip loins during retail display. However, the antioxidant effect of AGRADO®PLUS in reducing lipid and color oxidations of strip loin steaks is reduced with feeding wet distillers grains due to increase of polyunsaturated fatty acids in beef. The AGRADO®PLUS feed supplementation appears as a viable means to increase lipid and color stability of beef during retail display.

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Table 1. Means of percentage discoloration of overwrapped (OW) and high oxygen (HiO_2-MAP) packaged strip loins (m. *longissimus lumborum*) aged for 8 and 29 d, during 7d of simulated retail display conditions. (treatment×packaging×aging×day - <0.0001)

Pkg	Aging (d)	Treatments	Retail display (d)								Contrasts (*P* value)			
			0	1	2	3	4	5	6	7	Corn vs. WDGS	Corn vs. Corn +AG	WDGS vs. WDGS +AG	No AG vs. AG
HiO_2-MAP	8	Corn	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.47^{b}	3.12^{a}				
		30%WDGS	0.00	0.00	0.00	0.00	0.00	0.23	0.64	2.23				
		Corn+AG	0.00	0.00	0.00	0.00	0.00	0.04	0.21	0.76	0.68	0.20	0.18	0.06
		30%WDGS+AG	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.28				
	29	Corn	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.93^{c}	8.42^{b}	17.07^{aA}				
		30%WDGS	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.30^{c}	5.16^{b}	18.12^{aA}				
		Corn+AG	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.49^{c}	4.42^{b}	11.42^{aB}	0.73	0.43	0.89	0.64
		30%WDGS+AG	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.04^{c}	0.65^{c}	6.65^{b}	17.99^{aA}				
OW	8	Corn	0.00^{d}	0.09^{d}	0.09^{d}	0.33^{d}	1.65^{d}	5.20^{c}	12.12^{b}	24.06^{aA}				
		30%WDGS	0.00^{d}	0.00^{d}	0.00^{d}	0.32^{d}	1.53^{d}	5.46^{c}	12.61^{b}	27.71^{aA}				
		Corn+AG	0.00^{d}	0.00^{d}	0.00^{d}	0.32^{d}	1.94^{d}	6.63^{c}	13.52^{b}	26.24^{aA}	0.34	0.65	0.09	0.39
		30%WDGS+AG	0.00^{d}	0.00^{d}	0.00^{d}	0.01^{d}	1.62^{cd}	3.79^{c}	8.44^{b}	15.72^{aB}				
	29	Corn	0.00^{f}	0.00^{f}	0.24^{f}	2.53^{e}	9.73^{dA}	19.57^{cA}	28.38^{bA}	39.49^{aA}				
		30%WDGS	0.00^{e}	0.00^{e}	0.06^{e}	1.18^{e}	5.73^{dAB}	15.68^{cAB}	27.30^{bA}	42.86^{aA}				
		Corn+AG	0.00^{e}	0.00^{e}	0.03^{e}	0.74^{e}	4.80^{dB}	11.69^{cB}	19.33^{bB}	29.13^{aB}	0.56	0.14	0.98	0.29
		30%WDGS+AG	0.00^{e}	0.00^{e}	0.15^{e}	1.25^{e}	5.01^{dB}	14.65^{cB}	28.03^{bA}	43.08^{aA}				

[a-f] Comparison within a row, means lacking a common superscript were different at $P < 0.05$.
[A-B] Comparison within a column by aging, means lacking a common superscript were different at $P < 0.05$.

Table 2. Means of TBARS of (OW) and high oxygen (HiO_2-MAP) packaged strip loins (m. *longissimus lumborum*) aged for 8 and 29 d, during 7d of simulated retail display conditions. (treatment×aging×day – 0.0388)

Aging (d)	Day (d)	Treatments				Contrasts (*P* value)			
		Corn	30% WDGS	Corn +AG	30%WDGS +AG	Corn vs. WDGS	Corn vs. Corn+AG	WDGS vs. WDGS+AG	No AG vs. AG
8	0	0.09^{b}	0.02^{b}	0.09	0.05^{b}	0.18	0.83	0.66	0.64
	4	0.10^{b}	0.22^{b}	0.15	0.19^{ab}	0.32	0.64	0.80	0.87
	7	0.44^{a}	0.67^{a}	0.37	0.38^{a}	0.39	0.70	0.14	0.19
29	0	0.01^{c}	0.32^{c}	0.16^{c}	0.18^{c}	0.11	0.32	0.37	0.95
	4	1.00^{b}	1.06^{b}	0.66^{b}	0.69^{b}	0.76	0.13	0.11	0.03
	7	2.07^{Aa}	2.17^{Aa}	1.11^{Ba}	1.62^{ABa}	0.18	0.003	0.09	0.001

[A-B] Comparison within a row by treatment, means lacking a common superscript were different at $P < 0.05$.
[a-c] Comparison within a column, means lacking a common superscript were different at $P < 0.05$.

Table 3. Means of Warner-Bratzler shear force value (kg) of overwrapped (OW) and high oxygen (HiO_2-MAP) packaged strip loins (m. *longissimus lumborum*) aged for 8 and 29 d, during 7d of simulated retail display conditions. (treatment – 0.0200)

Treatments				Contrasts (*P* value)			
Corn	30% WDGS	Corn +AG	30%WDGS +AG	Corn vs. WDGS	Corn vs. Corn+AG	WDGS vs. WDGS+AG	No AG vs. AG
2.77^{B}	2.75^{B}	2.98^{A}	2.79^{B}	0.06	0.01	0.62	0.04

[A-B] Comparison within a row, means lacking a common superscript were different at $P < 0.05$.