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**Summary**

*Crossbred steers (n = 483) were fed dry-rolled corn based finishing diets containing 0 or 30% wet distillers grains with the synthetic antioxidants, ethoxyquin and tertiary butyl hydroquinone (AGRADO®PLUS). Synthetic antioxidants reduced lipid and color deterioration of strip steaks at the end of the retail display period under high or atmospheric oxygen packaging conditions.*

## Introduction

de Mello Jr. et al. (*2008 Nebraska Beef Cattle Report*, pp. 116-117) and Senaratne et al. (*2009 Nebraska Beef Cattle Report*, pp. 110-111) have shown that WDGS increase polyunsaturated fatty acids (PUFA) in beef. Elevated levels of PUFA in beef may 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 et al. *2009 Nebraska Beef Cattle Report*, pp. 113-115), 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 et al. *2002 Journal of Animal Science*, *80,* pp. 1117-1123). Others (Moore et al. 2010 *Proceedings 43rd Midwestern Sectional Animal Science Meetings*, pp. 86) have reported AGRADO®PLUS supplementation affects neither performance nor carcass characteristics of feedlot cattle. Feeding AGRADO®PLUS may increase the antioxidant level of muscles. Our study evaluated the antioxidant effects of feeding AGRADO®PLUS with WDGS diets on color and lipid stability, and tenderness of beef m. *longissimus lumborum* during its shelf life.

## Procedure

Crossbred (British × Continental) yearling steers (n = 483; initial BW = 942 kg ± 2.2 lb) 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 days (at second trial) and slaughtered. Carcasses were chilled for 48 hours before grading. After grading, both sides of the beef loin, short loins (IMPS # 174; NAMP, 2007) from a total of 80 (40 from each trial) USDA Choice carcasses (10 from each dietary treatment) were vacuum-packaged and transported under refrigeration. After aging for either 8 or 29 days at 36ºF, m. *longissimus lumborum* muscles were removed from the beef loins. Each strip loin was cut into six 1-inch-thick steaks from the anterior to the posterior. The first (for oxidation; 0 day retail displayed) and fourth (for shear force; 0 day retail displayed) steaks were immediately vacuum-packaged and stored at -4ºF. The second and third steaks were split into halves and assigned for four and seven days oxidation analysis either under overwrapped (OW) or high-oxygen modified atmospheric packaging ($HiO_2$-MAP) systems. Fifth and sixth steaks were allotted for seven days retail display shear force analysis under OW and $HiO_2$-MAP packaging systems, respectively. Surface discoloration ratings were determined on steaks assigned for seven days 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 (steaks arrangement was similar to OW packaging system) on high foam-barrier polypropylene trays with a gas mixture (80% $O_2$: 20% $CO_2$) and mechanically sealed with oxygen impermeable film. Overwrapped and $HiO_2$-MAP packaged 8 and 29 days aged steaks, placed on a table in a cooler (at 0 ± 36ºF) were exposed to continuous 1,000-1,800 lux warm white fluorescence lighting to provide simulated retail display conditions. Steaks (8 and 29 days aged) assigned for four and seven days of retail display were removed from tables accordingly for oxidation and shear force analysis, and immediately vacuum-packaged and stored at -4ºF.

A six-person trained panel subjectively evaluated the percentage surface discoloration. Discoloration ratings were made on each steak from 0 to 7 days of retail display at 24-hour intervals.

The 2-thiobarbuteric acid reactive substance (TBARS) assay was used to measure the oxidation status of 8- and 29-day aged steaks in both packaging systems displayed for 0, 4 and 7 days in simulated retail display.

Tenderness evaluations were performed by Warner-Bratzler shear force testing (WBSF).

Data were analyzed by ANOVA in the GLIMMIX procedure of SAS (version 9.2, Cary, N.C., 2009) as a split-split-split-plot design with dietary treatments as the whole-plot treatment, aging period as the first split-


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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 days, during 7 days of simulated retail display conditions.**

| | | Retail display (days) | | | | | | | | Contrasts (*P*-value) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aging (days) | Treatments | 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 | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ | $0.47^{b}$ | $3.12^{a}$ | 0.68 | 0.20 | 0.18 | 0.06 |
| | 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 | | | | |
| | 30%WDGS+AG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.28 | | | | |
| $HiO_2$-MAP 29 | Corn | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.93^{c}$ | $8.42^{b}$ | $17.07^{aA}$ | 0.73 | 0.43 | 0.89 | 0.64 |
| | 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 | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.49^{c}$ | $4.42^{b}$ | $11.42^{aB}$ | | | | |
| | 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 | $0.00^{d}$ | $0.09^{d}$ | $0.09^{d}$ | $0.33^{d}$ | $1.65^{d}$ | $5.20^{c}$ | $12.12^{b}$ | $24.06^{aA}$ | 0.34 | 0.65 | 0.09 | 0.39 |
| | 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 | $0.00^{d}$ | $0.00^{d}$ | $0.00^{d}$ | $0.32^{d}$ | $1.94^{d}$ | $6.63^{c}$ | $13.52^{b}$ | $26.24^{aA}$ | | | | |
| | 30%WDGS+AG | $0.00^{d}$ | $0.00^{d}$ | $0.00^{d}$ | $0.01^{d}$ | $1.62^{cd}$ | $3.79^{c}$ | $8.44^{b}$ | $15.72^{aB}$ | | | | |
| OW 29 | Corn | $0.00^{f}$ | $0.00^{f}$ | $0.24^{f}$ | $2.53^{e}$ | $9.73^{dA}$ | $19.57^{cA}$ | $28.38^{bA}$ | $39.49^{aA}$ | 0.56 | 0.14 | 0.98 | 0.29 |
| | 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 | $0.00^{e}$ | $0.00^{e}$ | $0.03^{e}$ | $0.74^{e}$ | $4.80^{dB}$ | $11.69^{cB}$ | $19.33^{bB}$ | $29.13^{aB}$ | | | | |
| | 30%WDGS+AG | $0.00^{e}$ | $0.00^{e}$ | $0.15^{e}$ | $1.25^{e}$ | $5.01^{dB}$ | $14.65^{cB}$ | $28.03^{bA}$ | $43.08^{aA}$ | | | | |

$^{a\text{-}f}$Comparison within a row, means lacking a common superscript were different at $P < 0.05$.
$^{A\text{-}B}$Comparison within a column by aging, means lacking a common superscript were different at $P < 0.05$.
WDGS – Wet distillers grains plus solubles, AG – AGRADO®PLUS.
*P*-value for treatment×packaging×aging×day < 0.0001.

plot treatment, packaging systems as the second split-plot treatment, and retail display time (repeated measures) as the third split-plot treatment, with the animal as the experimental unit. 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.

## Results

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. et al. (*2010 Nebraska Beef Cattle Report*, pp. 99-101). A possible reason for less discoloration when beef is exposed to higher levels of oxygen is conversion of myoglobin into stable oxymyoglobin (cherry red color). Steaks (29 days aged in both packaging systems) from cattle fed corn plus AG had significantly ($P < 0.05$) less discoloration at the end of the retail display period ($P < 0.05$) than steaks from cattle fed any other diet/treatment. The effectiveness of AG supplementation in reducing discoloration was prominent when meat was aged longer. Although the trends were the same, anti-discoloration effect of AG supplementation was not statistically significant 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 Nebraska Beef Cattle Report*, pp. 116-117) and Senaratne et al. (*2009 Nebraska Beef Cattle Report*, pp. 110-111) 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 eight day aged steaks from cattle fed different diets. The only significant ($P < 0.05$) difference in dietary treatments could be seen in 29-day 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 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 seven days retail display had significantly ($P < 0.0001$) higher WBSF values (by 1.01 lbs) compared to steaks from OW packaging systems (data not shown). The main effect

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of dietary treatments significantly influenced WBSF, with no confound effects from aging time (Table3; $P = 0.02$). Steaks from corn plus AG fed cattle were significantly less tender compared to steaks from other diets. When comparing steaks from AG fed cattle and steaks from AG nonsupplemented 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.

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 toward the end of the retail display period. However, the antioxidant effect of AGRADO®PLUS in reducing lipid and color oxidations of strip loin steaks is reduced with feeding wet distillers grains. The AGRADO®PLUS feed supplementation appears as a viable means to increase lipid and color stability of beef during retail display.

**Table 2. Means of thiobabituric acid reactive substances values of overwrapped (OW) and high oxygen ($HiO_2$-MAP) packaged strip loins (m. *longissimus lumborum*) aged for 8 and 29 days, during 7 days of simulated retail display conditions.**

| | | Treatments | | | | Contrasts (*P* value) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Aging (days) | Day (days) | 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 $P < 0.05$.
WDGS – Wet distillers grains plus solubles, AG – AGRADO®PLUS
*P*-value for treatment×aging×day = 0.0388.

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

| 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 |
| $6.11^{B}$ | $6.06^{B}$ | $6.57^{A}$ | $6.15^{B}$ | 0.06 | 0.01 | 0.62 | 0.04 |

$^{A-B}$Comparison within a row, means lacking a common superscript were different at $P < 0.05$.
WDGS – Wet distillers grains plus solubles, AG – AGRADO®PLUS.
*P*-value for treatment = 0.0200.

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