# Effect of Supplementing AGRADO<sup>®</sup> Antioxidant (AOX) on Feedlot Performance and Carcass Characteristics of Beef Steers and Shelf-Life of Beef

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#### **Story in Brief**

One hundred fifty mixed crossbred steers in three weight blocks and 30 pens (10 pens/block; 5 hd/pen) and consuming a high-concentrate diet were supplemented with either 0 or 150 ppm Agrado<sup>®</sup> for 25 d prior to harvest. Adding Agrado for this period of time had no affect on gain, feed intake, or feed efficiency. Supplemental Agrado did not affect lean maturity, an indicator of darkness of ribeye color. As for other quantitative carcass traits, no differences were observed. However, supplementation with Agrado did improve retail display characteristics of top loin steaks compared with controls. While no treatment differences were observed for thiobarbituric acid (TBARS) levels, the improvement in retail display characteristics did extend the shelf-life of top loin steaks by one d, determined by the d on which the subjective evaluation of overall acceptability reached a score of 3.0. Blood serum analyses were made to correlate certain systemic antioxidant levels with Agrado supplementation to better understand the mode of action by which Agrado may prevent rancidity in fat and muscle tissue. However, Agrado supplementation had no effect on serum levels of vitamins A and E, and beta-carotene compared with controls when included in the diet during the last 25 d of the feeding period.

Key Words: AGRADO<sup>®</sup>, Antioxidant, Case-life

### Introduction

Feeding higher than normal levels of vitamin E, an antioxidant, at concentrations 10 to 100 times the amount required (NRC, 1996) has been shown to improve feedlot performance slightly and has improved health of shipping-stressed cattle (Secrist et al., 1997). Agrado, a highly effective antioxidant, has received attention as a possible compliment or alternative to higher than normal levels of vitamin E and other antioxidant vitamins when they are fed for antioxidant effect. In previous experiments, Agrado supplementation has resulted in decreased morbidity (Stovall et al., 1999; Kegley et al., 2000), greater daily gains and feed intakes (Greene et al., 1999), and improved returns \$6.00/hd above controls when fed in combination with vitamin E (Texas Research Feedlot, 1999). In addition to these benefits, Agrado supplementation has resulted in extended case-life of beef, another response associated with supplementing vitamin E at levels of 500 IU or above. Krumsiek and Owens (1998) discovered that supplementing steers with 150 ppm Agrado 28 d prior to harvest increased the time period before discoloration of steaks and ground beef displayed in a commercial meat case. However, in their experiments (Krumsiek and Owens, 1998; Nelson et al., 1999; Walenciak et al., 1999), no measurements of systemic antioxidant levels were taken to quantify the potential mode of action by which Agrado reduced rancidity. Therefore, this experiment was conducted to determine the effects of feeding Agrado on performance and carcass characteristics of beef cattle, case-life of beef, and antioxidant concentration in serum.

#### **Materials and Methods**

*Feeding Period.* A 180-d feeding study was conducted at the Willard Sparks Beef Research Center near Stillwater, OK. Agrado treatments were imposed only during the final 25 d of this trial. Cattle in three weight blocks and 30 pens (10 pens/block; 5 hd/pen) which had been fed a high-concentrate diet for 137 d were divided with five pens from each block randomly allotted to receive, via a total mixed ration, either 150 or 0 ppm of Agrado for 25 d prior to harvest. Other dietary ingredients included 82.5% rolled corn grain, 8% cottonseed hulls, 3% yellow grease, and 9.5% of a protein-mineral-vitamin supplement that provided 30 g of rumensin and 10 g of tylan per ton of TMR. Cattle were fed once daily at 0800; slick bunk management was used and bunks were checked once daily at 0700 to determine the amount of feed to be delivered daily. Steers were weighed and feed bunks from all pens were weighed and cleaned prior to supplementation with Agrado. Serum blood samples were collected from a subset of two animals/pen at the initiation and conclusion of the experiment. Serum samples were analyzed for vitamins A and E, and beta carotene.

*Carcass Traits*. At the conclusion of the feeding period, cattle were shipped to a commercial beef processor (Excel, Dodge City, KS) and harvested under humane conditions. At harvest, each animal was identified individually. After chilling the carcasses for approximately 36 h at minus 2 to 0°C, USDA yield and quality grade factors (USDA, 1997) were determined by trained personnel from Oklahoma State University.

*Steaks.* Once carcass data were collected, the *longissimus lumborum* (Strip loin; IMPS # 180, NAMP, 1997) and *triceps brachii* (Shoulder clod; IMPS # 114, NAMP) muscles were identified on 20 carcasses from each dietary treatment. The muscles were collected, vacuum packaged and transported to the Food and Agricultural Products Research and Technology Center at Oklahoma State University. The *longissimus lumborum* samples were stored for 21 d at 2°C before 2.54-cm steaks were fabricated for retail case-life evaluation. Upon arrival, the *triceps brachii* were processed into coarse-ground beef and vacuum packaged in moisture and oxygen impermeable packaging. After 21 d of storage at 2°C, the *triceps brachii* was fine-ground and formed into patties for retail case-life evaluation.

**Retail Display Panels.** Longissimus lumborum steaks and triceps brachii patties were placed on Styrofoam<sup>®</sup> trays and wrapped with oxygen permeable Saran<sup>®</sup> film to simulate industry practice. Trays were placed in coffin-style retail display cases for 8 d at 2 to 3°C under fluorescent light. Objective CIE color values were obtained daily on steaks and patties using a reflectance colorimeter (Minolta, Osaki, Japan). Subjective color scores were also obtained daily by trained personnel at Oklahoma State University. Steaks and patties were evaluated for lean color (1 = extremely dark brown or green; 8 = bright cherry color), fat color (8 = creamy white; 1 = dark brown or green), percent discoloration (7 = none; 1 = complete), and overall appearance (7 = extremely desirable; 1 = extremely undesirable).

*Rancidity*. At d-1 and d-8 of retail display, steak and patty samples were removed and analyzed for lipid oxidation by measuring thiobarbituric acid (mg malonaldehyde / kg). The analysis was conducted according to the procedures outlined by Buege and Aust (1978), with slight modifications. Briefly, meat samples were ground and homogenized in a Waring<sup>®</sup> blender that

contained 3X volume of distilled water. The homogenized sample was then centrifuged at 2500 x g for 10 min under refrigeration (4°C). A portion of the homogenate (2 mL) was mixed with 4 mL of the trichloroacetic acid (15% w/v)/thiobarbituric acid (20mM) stock solution and 100  $\mu$ l of butylated hydroxyanisole (BHA), and heated for 15 min in a boiling water bath. The sample was cooled for 10 min in a cold water bath before centrifugation at 2500 x g for 10 min under refrigeration. The absorbance of the supernate at 531 nm was read using a Beckman<sup>®</sup> DU-7500 spectrophotometer, and compared against a standard curve that was constructed using 1,1,3,3-tetra-ethoxypropane.

*Statistics*. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model included Agrado treatment as a main effect with d of display as a block effect. Least squares means were separated using the least significant difference.

### **Results and Discussion**

Supplementation with Agrado had no affect on daily gain  $(1.00 \text{ vs } 1.04 \pm .10 \text{ kg})$ , feed intake  $(9.67 \text{ vs } 10.00 \pm .17 \text{ kg/d})$  or feed efficiency  $(.104 \text{ vs } .103 \pm .01)$  compared with controls (Table 1). In addition, carcass traits for Agrado supplemented steers were not different from controls. The results from laboratory analysis of serum antioxidants are presented in Table 2. Supplementation with Agrado had no affect on serum concentrations of vitamins E and A, or beta carotene compared with controls; however, the difference between initial and final concentrations of vitamin E and total AOX did differ from 0 (P < 0.01) which shows an increase in AOX levels over time. The results of serum vitamin concentrations emphasize a need to better understand antioxidant status prior to supplementation.

Table 1. Least squares means of feedlot performance and carcass traits for Agrado <sup>®</sup> versus control steers.									
	Treatments <sup>a</sup>								
Item	Control	Agrado	SEM <sup>b</sup>						
Feedlot Performance <sup>c</sup>									
Daily gain, kg	1.04	1.00	.10						
DM intake, kg	10.00	9.67	.17						
Gain: DMI	.103	.104	.01						
Carcass Composition									
HCW, kg	352.0	350.3	2.76						
КРН, %	2.61	2.53	.07						
PYG	3.30	3.44	.07						
12 <sup>th</sup> rib fat, cm	1.31	1.41	.06						
Adj PYG	3.53	3.60	.07						
Adj. 12 <sup>th</sup> rib fat, cm	1.48	1.58	.05						
Rib Eye Area, cm <sup>2</sup>	88.2	86.1	1.41						
Skeletal Mat.	151.4	151.2	1.80						
Lean Mat.	152.1	152.0	1.65						
Marbling score <sup>d</sup>	391.1	387.4	8.59						
U. S. Quality Grade <sup>e</sup>	337.5	338.5	6.80						

U. S. Yield Grade	2.46	2.61	.08
Liver Score	.37	.26	.09
Tenderness, kg force	4.98	4.68	.22
<sup>a</sup> No significant differences $(P > 05)$			

No significant differences (P > .05)

<sup>b</sup> Standard error of the least squares means

<sup>c</sup> Pen means of performance from d 140 – harvest. Blocks 2 & 3 were fed Agrado from d 141 – 165, whereas block 1 received Agrado from d 156 – 180

<sup>d</sup>Marbling score: 300 = Slight, 400 = Small degrees of marbling

<sup>e</sup>USDA Choice=400, USDA Select=300

<sup>f</sup>T-test (Mu = 0) P = .0001

Table 2.	Least square	s means for	r serum a	ntioxidant	levels in a	steers supp	lemented	with Agrad	.o <sup>®</sup> 25 d	prior to	D
				1							

	nui vest			
	Treati	ments <sup>a</sup>		
Item	Control	Agrado	SEM <sup>b</sup>	
Serum vitamin E, ug/mL				
Pre Agrado	2.35	2.19	.23	
Post Agrado	4.13	3.55	.31	
Difference <sup>c</sup>	1.77	1.35	.28	
Serum vitamin A, ug/mL				
Pre Agrado	.50	.48	.02	
Post Agrado	.47	.50	.02	
Difference <sup>d</sup>	.004	007	.025	
Serum beta-carotene, ug/mL				
Pre Agrado	.15	.18	.02	
Post Agrado	.15	.15	.01	
Difference <sup>d</sup>	002	026	.017	
Total AOX, ug/ mL				
Pre Agrado	2.99	2.83	.24	
Post Agrado	4.77	4.04	.34	
Difference <sup>c</sup>	1.78	1.20	.31	

<sup>a</sup>No significant differences (P > .05)

<sup>b</sup>SEM= standard error of least squares means

<sup>c</sup>Difference= Post – Pre; T-test (Mu = 0; P = .001)

<sup>d</sup>Difference= Post – Pre; T-test (Mu = 0; P > .05)

The subjective colors of top loin steaks evaluated each d of the display period are presented in Table 3. Steaks from animals supplemented with Agrado had higher ratings for lean color, fat color, percent discoloration, and overall acceptability than control steaks. These data suggest that supplementing cattle with Agrado during the feeding process improves the retail display characteristics of top loin steaks. This agrees with the findings of Krumsiek and Owens (1998) who showed an advantage in visual estimates of case life of Agrado supplemented beef. However, Walenchiak et al. (1999) showed no difference in subjective color scores of top loin steaks or beef patties, which contradicts current findings.

display in the retail case <sup>a</sup>									
Display, d	Treatment	Lean color	Fat color	Discoloration	Acceptance				
1	Control	6.34	7.13	6.92	6.63				
	Agrado	7.04	7.35	6.99	6.89				
2	Control	4.99	5.96	5.90	4.87				
	Agrado	5.79	6.32	6.35	5.91				
3	Control	4.08	5.14	4.43	3.39				
	Agrado	4.81	5.67	5.35	4.36				
4	Control	3.00	4.41	3.24	2.17				
	Agrado	3.91	5.09	4.30	3.14				
5	Control	1.76	3.62	1.40	1.02				
	Agrado	2.93	4.64	2.76	1.76				
6	Control	1.55	3.97	1.13	1.04				
	Agrado	2.12	4.20	1.59	1.28				
7	Control	2.01	2.99	1.67	1.40				
	Agrado	2.53	3.37	2.25	1.69				
8	Control	1.55	2.34	1.35	1.06				
	Agrado	1.89	2.68	1.73	1.17				
<sup>a</sup> Within each displ	lay time means for	each subjective colo	or score were sign	ificantly different (P <	(0.05) between				

Table 3.	The effect of Agrado <sup>®</sup>	supplementation on subjective color scores of top loin steaks at each d of								
display in the retail case <sup>a</sup>										

treatment groups

The TBARS values for top loin steaks and beef patties are presented in Table 4. Supplementation with Agrado had no affect on the TBARS values of steaks and patties. TBARS values increased over the display period. These results are inconsistent with those reported by Krumsiek and Owens (1998), Nelson et al. (1999) and Walenciak et al. (1999), who showed decreased TBARS values for Agrado supplemented cattle. Marbling was similar between treatments (Table 1) and explained little of the variation ( $r^2 = .04$ ; TBARS = .006 [marbling score] + .599) in TBARS concentrations.

Table 4. The effect of Agrado<sup>®</sup> supplementation on the thiobarbituric acid (TBARS, mg/kg) values of ground beef and top loin steaks at d-1 and d-8 of retail display

	Ground b	eef TBA	Top loin stea	ık TBA				
Display, d	Control	Agrado	Control	Agrado				
1	$1.04 \pm 1.1^{a}$	$2.66 \pm 1.1^{a}$	$1.42 \pm .34^{a}$	$1.28\pm.34^{\rm a}$				
8	$7.25 \pm .34^{a}$	$6.03 \pm .34^{a}$	$3.94 \pm .35^{a}$	$3.47 \pm .35^{a}$				
<sup>a</sup> Within each row and cut type, means lacking a common superscript letter differ ( $P < .05$ )								

Objective color values, as measured by colorimetric reflectance, are presented in Tables 5 and 6. Occasionally, there were differences in  $L^*$ ,  $a^*$ , and  $b^*$  values within d of display. However, distinguishable trends in the data could not be identified. This is consistent with the results of Walenciak et al. (1999) who showed no difference in  $L^*$ ,  $a^*$ , and  $b^*$  values between Agrado treated animals.

Table 5.	The effect of Agra	ndo <sup>®</sup> supplementation or	n the Minolta color	value (CIE L*,	a*, b*) of beef top	loin
		steaks on eac	h d of retail display	y		

			F		Display, d		2	
CIE	Treatment	1	2	3	4	5	6	7
L*	Control	40.8 <sup>a</sup>	40.6 <sup>a</sup>	40.2 <sup>a</sup>	40.4 <sup>a</sup>	41.0 <sup>a</sup>	41.7 <sup>a</sup>	41.7 <sup>a</sup>
	Agrado	42.3 <sup>b</sup>	41.9 <sup>b</sup>	41.0 <sup>a</sup>	41.7 <sup>b</sup>	42.0 <sup>a</sup>	42.6 <sup>a</sup>	43.0 <sup>a</sup>
a*	Control	16.9 <sup>a</sup>	15.3 <sup>a</sup>	13.0 <sup>a</sup>	10.5 <sup>a</sup>	9.0 <sup>a</sup>	7.8 <sup>a</sup>	6.9 <sup>a</sup>
	Agrado	17.8 <sup>b</sup>	16.3 <sup>a</sup>	14.5 <sup>b</sup>	12.0 <sup>a</sup>	10.6 <sup>a</sup>	9.1 <sup>a</sup>	8.3 <sup>a</sup>
b*	Control	7.6 <sup>a</sup>	7.1 <sup>ª</sup>	6.6 <sup>a</sup>	6.2 <sup>a</sup>	6.1 <sup>a</sup>	6.4 <sup>a</sup>	5.9 <sup>a</sup>
	Agrado	8.4 <sup>b</sup>	7.9 <sup>b</sup>	7.5 <sup>b</sup>	8.1 <sup>a</sup>	6.8 <sup>a</sup>	6.8 <sup>a</sup>	6.5 <sup>b</sup>

<sup>ab</sup>Within each CIE color value and display time, means lacking a common superscript letter differ (P < .05).

 $L^*$  = measure of lightness; 0 = black and 100 = white

 $a^*$  = measure of redness; +60 = red and -60 = green

 $b^*$  = measure of yellow; +60 = yellow and -60 = blue

Table 6.	The effect of Agrado <sup>®</sup> supplementation on the Minolta color value	(CIE	L*, a*,	b*) of	ground	beef
	patties on each d of retail display					

					Display, d			
CIE	Treatment	1	2	3	4	5	6	7
L*	Control	49.9 <sup>a</sup>	50.1 <sup>a</sup>	51.6 <sup>a</sup>	51.6 <sup>a</sup>	52.6 <sup>a</sup>	53.1 <sup>a</sup>	51.5 <sup>a</sup>
	Agrado	50.0 <sup>b</sup>	49.5 <sup>a</sup>	50.5 <sup>b</sup>	51.2 <sup>a</sup>	50.5 <sup>b</sup>	51.0 <sup>a</sup>	51.2 <sup>a</sup>
a*	Control	18.4 <sup>a</sup>	11.2 <sup>a</sup>	6.8 <sup>a</sup>	5.2 <sup>a</sup>	4.8 <sup>a</sup>	4.5 <sup>a</sup>	4.5 <sup>a</sup>
	Agrado	19.8 <sup>b</sup>	13.7 <sup>b</sup>	8.2 <sup>a</sup>	5.4 <sup>a</sup>	5.3 <sup>b</sup>	5.1 <sup>b</sup>	5.1 <sup>b</sup>
b*	Control	10.6 <sup>a</sup>	9.6 <sup>a</sup>	10.0 <sup>a</sup>	10.4 <sup>a</sup>	10.6 <sup>a</sup>	10.6 <sup>a</sup>	10.7 <sup>a</sup>
	Agrado	11.1 <sup>b</sup>	9.9 <sup>b</sup>	9.6 <sup>b</sup>	10.1 <sup>a</sup>	10.4 <sup>a</sup>	10.1 <sup>a</sup>	10.5 <sup>a</sup>

<sup>ab</sup>Within each CIE color value and display time, means lacking a common superscript letter differ (P < .05).

Minolta CIE color scale:

 $L^*$  = measure of lightness; 0 = black and 100 = white

 $a^*$  = measure of redness; +60 = red and -60 = green

 $b^*$  = measure of yellow; +60 = yellow and -60 = blue

### Implications

Agrado<sup>®</sup> supplementation during the last 25 d of the finishing period had no effect on feedlot performance, carcass characteristics, or serum levels of vitamins A and E, and beta-carotene. Furthermore Agrado did not decrease thiobarbituric acid values compared with controls; however, it did improve retail display characteristics and overall case-life of top loin steaks by one d compared with controls. When fed for less than 30 d, the ability of Agrado to consistently improve feedlot performance and case-life of beef is still unknown. Therefore, more experiments are needed to answer this question as well as better understand the mode of action by which Agrado functions as a dietary antioxidant. Also, more research is needed to determine the antioxidant status of feedlot cattle and feedstuffs to help understand the role of antioxidants in beef production and to better utilize them in the beef industry.

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