AGRADO™ FOR FINISHING CATTLE: EFFECTS ON PERFORMANCE, CARCASS MEASUREMENTS, & MEAT QUALITY

By

CHARLES LUTHER KRUMSIEK

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The greatest things on earth are not by chance, there is a reason and purpose for everything we encounter. We, as individuals, have a purpose for being who we are and dealing with everyday situations in the manner our personalities dictate. My greatest acknowledgment is to the one who gave me the gift of intelligence to accomplish this goal and the gift of life. May the gifts and abilities that God has given me brighten the lives of those I meet and possibly put a little laughter in their hearts.

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This thesis is dedicated to my wife Melissa A. Krumsiek for her support and encouragement for the past 18 months. By putting her career on hold and working to support us was an unselfish act of love that has been an asset to the completion of this project. Now she can advance in her career of Radiography and Ultrasound.

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Format of Thesis

This Thesis is presented in the Journal of Animal Science style format, as outlined by the Oklahoma State University graduate college style manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

Chapter I

INTRODUCTION

By definition, color is the visual perception that enables one to differentiate between objects with an identical shape. People equate shades of color to materialistic or idealistic thoughts in their everyday activities. Green often is equated to youthfulness and vigor. Safety is symbolized by green; when operating a motorized vehicle green means "Go." On the other hand, green can depict envy. The color red symbolizes strength, power and boldness attributes to warriors and athletes. Red also is the sign of danger. We stop at a "Red" light. We have been conditioned to perceive the color green as "good" and the color red as "bad." The consumer perceives these colors exactly opposite when making purchasing decisions. Red and yellow, being warm colors, are attractive to customers whereas the cooler colors, blue and green, are less attractive. When buying fresh beef, consumers have been conditioned to believe that bright cherry equates to freshness; any deviation from this color is perceived as undesirable (Faustman and Cassens, 1990; Kropf, 1980). Preservation of the bright cherry red color of beef in the retail case is imperative for selling beef; the amount of time meat is displayed to sell before it discolors is called "case life."

Case life can be extended by including certain supplemental antioxidants in the diet or by applying antioxidants directly to fresh beef.

A mixture of ascorbic acid and propyl gallate or butylated hydroxyanisole (BHA) extends case life of ground beef (Greene et al., 1971). Although adding an antioxidant to the meat after fabrication is effective, the addition of an antioxidant to the animals diet antemortem rather than to the meat postmortem reduces the equipment and labor needed for product application. Supplementation of cattle diets with 500 to 1000 IU of vitamin E daily, at a cost of $3 to $4 per head for the total feeding period, markedly extends case life of beef retail cuts (Sherbeck et al., 1995; Liu et al. 1996). This increase in case life has been attributed to an increased concentration of alpha tocopherol in the meat. Because they cannot recover the expense of supplementing vitamin E, this cost of supplementation concerns feedlot operators . An increased case life inevitably benefits the retail merchant in the short term or the consumer in the long term. In a quest to find a less expensive antioxidant to preserve the bright cherry color while at the same time benefiting the feedlot producer, we investigated Agrado, a mixture that contains 6-ethoxy-1,2-dihyrdo-2,2,4-trimethylquinoline and other chemicals produced by Solutia, Inc., St. Louis MO, that is used widely as an antioxidant by the feed industry.

The purpose of this research was to evaluate the efficacy of Agrado supplemented at 150 ppm on performance, carcass measurements and meat quality of finishing cattle.

Chapter II

LITERATURE REVIEW

6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

Nomenclature

Several names have been used in the chemical and food industry to identify 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline. Names and abbreviations found in the literature to describe this compound include Antioxidant EC, Kurasan (e), Niflex, Stop Scald, EQ, and EMQ. To reduce confusion, only the terms ethoxyquin and the trade names Agrado (Solutia, Inc.) and Santoquin (Monsanto) will be used throughout this report for identification.

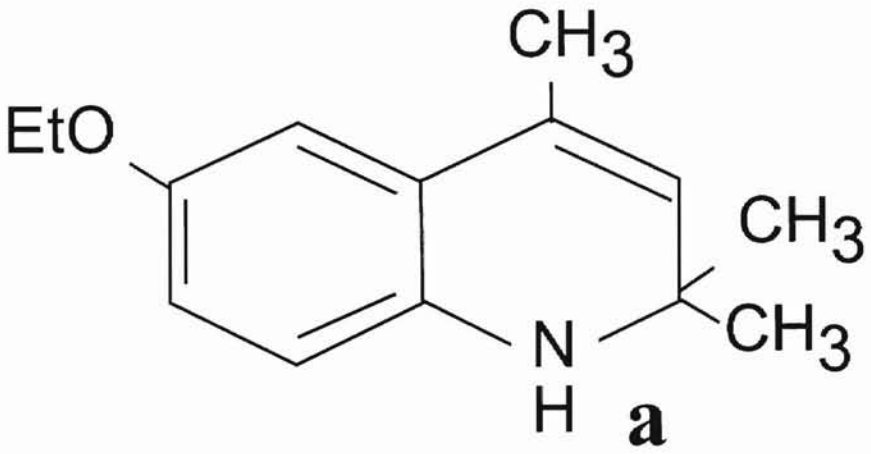

Figure 1. Presumed chemical structure of ethoxyquin. From Burka et al., 1996.

Overview

Discussion below was derived primarily from personal communication (W. Samuels). Ethoxyquin, an aromatic amine shown in Figure 1, was first synthesized in 1921. The product exists in a partially nitroxide free radical form which is presumed to be formed by the dissociation of the hydrogen atom (point **a** of Figure 1) from the secondary amine moiety. This unique feature may be responsible for the antioxidant capacity of ethoxyquin. Presently the product is commercially available in two forms. The most concentrated (90%) form is a clear, strongly refractive oily liquid (base); when it is oxidized by air exposure, it changes from clear to an opaque reddish-black color. The product also is available in a salt form (25 - 66 2/3 % pure) that is derived from further processing of the base. This salt form ranges in color from a fine white crystal to a light tan crystalline substance. A powder form containing the chemical and a carrier (60% active ingredient) has the consistency of a fine, free flowing powder much like that of Talcum powder. This powder form was used in all our trials. Although normally insoluble in water, some formations of ethoxyquin can be maintained in a water solution up to 75 ppm. Precautions should be taken when handling ethoxyquin, like other chemicals, because repeated skin exposure to high concentrations can cause dermatitis.

Applications

Ethoxyquin was first used extensively as a preservative of carotenes and vitamin E in dehydrated alfalfa meal. Since then, ethoxyquin has been used for multifarious stabilization purposes both by the human food and animal feed industries. In foods for human consumption, the compound is used to preserve the color of paprika and chili powder. The fruit industry utilizes this product to prevent scab and scald on apples and pears. For animal feeds, ethoxyquin typically is added at the maximum level approved by the US Food and Drug Administration (150 ppm or .015%) to reduce lipid oxidation and preserve vitamins A and E both in premixes and in manufactured feeds. Jones et al. (1986) reported that addition of ethoxyquin to a vitamin premix prevented degradation of vitamin A in pelleted broiler feeds subjected to outside storage (in metal containers with tight fitting lids) for 30 days. Poultry producers typically include ethoxyquin at .0125% (125 ppm) in the diet to enhance pigmentation of the skin (Bailey et al., 1996). The pet food industry has utilized ethoxyquin to prevent rancidity of fats in extruded pet foods. Addition of ethoxyquin and butylated hydroxyanisole (BHT) prevents peroxide formation during 16 weeks of storage (Gross et. al., 1994). The maximum recommended level for dog food is 75 ppm. The addition of ethoxyquin at .015% of dry matter intake for seven days increased the oxidative stability of milk and appeared to increase tocopherol levels; responses were even more dramatic when ethoxyquin was added at .15% (1500 ppm) of diet dry matter. Whether ethoxyquin when fed at 150 ppm is

absorbed and deposited in meat or milk or action is indirect through increasing concentrations of other antioxidants is not fully clear. A subsequent study of milk found no increase in tocopherol levels in milk but presence of a second foreign compound in milk was detected (Dunkley et al., 1966,1967). With a zero tolerance for feed additives in milk, this finding, that remains to be confirmed with modern analytical techniques, indicates that ethoxyquin should not be fed to lactating cows. Work with 1 week old lambs, fed for 12 weeks, suggests that ruminal metabolism is insignificant; tissue concentration of a compound presumed to be ethoxyquin reached .0053 and .0329 ppm in triceps muscle when ethoxyquin was fed at .01 and .1% of a pelleted diet for 12 weeks (Demille et al., 1972).

Biological *effects*

Glutathione, a tripeptide comprised of glycine, glutamate, and cysteine acts as a redox buffer in cells. It maintains the sulfhydryl groups of proteins in a reduced chemical form and the iron of heme in the ferrous form. Through a redox reaction catalyzed by glutathione peroxidase, glutathione can remove toxic peroxides that form under aerobic oxidation during growth and metabolism (Lehninger et al., 1994). Since the ethoxyquin concentration is difficult to measure in the cells of animals and because it is rapidly excreted in feces, measurement of glutathione often has been used to determine biological effects of ethoxyquin. Other methods to determine ethoxyquin concentrations include direct measurement with high pressure chromatography (HPLC); effects often are

monitored through measurement of responses in concentrations of thiobarbituric acid (TBA), an index of malonaldehyde accumulation that appears during lipid oxidation, and glutathione. Radioactively labeled ethoxyquin (^{14}C) has been used to monitor its metabolism. Radioactivity was greater in vital organs; this raised questions about the safety of individuals exposed to ethoxyquin. However, the chemical nature of the form found in tissue has not been identified. Extensive studies of safety have been conducted both when the compound was first approved by the FDA and later due to concerns expressed by pet owners.

To evaluate the safety of ethoxyquin, numerous metabolism and residue studies have been conducted. Toxicity to man has not been determined but a tolerance factor for man has been calculated based on studies of the most sensitivity animal species, the dog. In 1996 the Center for Food Safety and Applied Nutrition, a division of the U.S. Food and Drug Administration, estimated the potential exposure to ethoxyquin in human food. The maximum safe level for human consumption was calculated based on two scenarios; "worst case" exposure assessment and most probable exposure assessment. Worst case exposure used information found in the "Food Consumption, Prices, and Expenditures, 1996 Annual Data, 1970-94" published by the USDA Economic Research Service (Statistical Bulletin Number 928). Per capita consumption is actually overestimated in this source as no waste or other losses are accounted for in the calculations; only "disappearance" or "availability" is calculated from agricultural statistics. Actual analytical analysis of food residues were not used. "Most probable exposure" assessment was calculated using "Results from

USDA's 1994 Continuing Survey of Food Intakes by Individuals and 1994 Diet and Health Knowledge Survey." This source used actual food consumption data and derived from 1 day food intake records of over 5,500 individuals. Analytical data, when available, where also used to make calculations. This method of estimating ethoxyquin intake is more realistic since actual consumption data is used versus maximum permissible ethoxyquin residues used in "worst case" calculations. "Worst case assessment" calculated to be 3.93 ug/kg/day and "most probable exposure" at 1.29 ug/kg/day; when compared to the ADI of 30 ug/kg/day ethoxyquin has a large margin of safety. When exposure from use as a direct human additive in certain spices (0.188 ug/kg/day consumption) was compared to the 1982 Redbook Safety Assessment: Levels of Concern, ethoxyquin was below the "concern level" of 0.31 ug/kg/day ("worst case" structure "C" category). The U.S. Food and Drug Administration concluded, based on exposure assessments, ethoxyquin has a large margin of safety for potential human exposure in food residues (Samuels, W., personal communication).

Workers handling ethoxyquin when preparing feed have exhibited allergic reactions resulting in dermatological symptoms. Eczema (red, itchy blisters) has been reported by workers who have handled the substance for years without protective clothing. When ethoxyquin was fed to 60 to 100 kg cattle at 250 mg/kg body weight, an intake that equates 1% or 10,000 ppm in a diet consumed at 2.5% of body weight, peripheral cell necrosis and midzone vacuolation of the liver occurred within 48 hours. When these calves were fed .68 and 6.8 grams of ethoxyquin daily for 109 days, an intake equivalent to 270 and 2,700 ppm in a diet

consumed at 3% of body weight, no adverse effects were detected. The calves consuming the ration with 6.8 grams eventually refused to feed, probably due to the bitter taste of ethoxyquin. When fed to poultry at 2 g / kg of body weight, an intake equal to 3,333 ppm at a feed intake of 6% of body weight, fed intake was reduced for 12 hours but returned to normal after 48 hours. Tissue concentrations of ethoxyquin in poultry tissues fed these high levels of ethoxyquin were minimal. This suggests that poultry are more tolerant to high levels of ethoxyquin than mammals (W. Samuels, personal communication).

Research using rats and mice has revealed both deleterious and advantageous properties of ethoxyquin. When added to whole homogenates of mitochondria extracted from the renal cortex of adult male Wistar rats at 0.1, 0.5, 1.0, and 1.5 mM concentrations in ethanol, ethoxyquin inhibited oxygen uptake when glucose was used as a substrate but not when succinate was used as the substrate. A study with beef heart sub-mitochondrial particles revealed a similar concentration dependency when measured by NADH oxidation with ferricyanide as an artificial electron acceptor (Reyes et al., 1995). These studies were conducted *in vitro* and may not reflect *in vivo* metabolism of ethoxyquin. In vivo, Kim (1991) found that feeding 0.125% and 0.5% of ethoxyquin HCl raised the hepatic glutathione level in female ICR mice and increased liver weight.

To understand the metabolism and disposition of this substance , F344 rats and B6C3F_1 mice were dosed with 3-^{14}C ethoxyquin either orally or intravenously. Dosage rates at 2.5, 25, and 250 mg/kg body weight (by gavage or dosed directly in heart by a bolus via cannula), as a single dose or 6

consecutive daily doses showed that ethoxyquin was readily absorbed from the gut in both species at 25 mg/kg body weight. However, excretion in both urine and feces was rapid. Rats excreted less labeled carbon in the feces when dosed at the higher level (250 mg/kg body weight) or consecutively dosed at lower levels than did the mouse. This was attributed to a survival reflex that delays gastric emptying of the rat and minimizes absorption of irritating compounds by the intestinal tract. Tissue accumulation was greatest in the rat liver (125 μg/g), kidney (123.3 μg/g), and adipose (263.9 μg/g) tissue when dosed (by gavage) at 250 mg/kg body weight for 6 consecutive days. Rapid removal of the parent material was exhibited in these same tissues. Muscle was not a major depot (Sanders et al., 1996). These results are in agreement with a feeding study conducted with female ICR mice (Kim, 1991). Although the parent material was excreted, metabolites were detected in a subsequent study; Burka et al. (1996) suggested that ethoxyquin was conjugated with glutathione. When comparing ethoxyquin and ethoxyquin HCl metabolism , Kim et al. (1992) found metabolites in the urine of Wistar rats and Rambouillet x Suffolk sheep. After 6 days of oral dosing (0.08 g/d), rat urine contained ethoxyquin in the hydroxylated and the dihydroxylated form. In contrast, sheep urine contained only ethoxyquin and hydroxylated ethoxyquin when fed a diet containing 0.5% (5000 ppm) ethoxyquin or ethoxyquin HCl in the diet for 12 days. This suggests that ruminant metabolism of ethoxyquin differs from metabolism in monogastrics. Since ethoxyquin is removed rapidly from the body and muscle is not a major

depot, one could conclude that ingestion of meat from animals that have consumed diets containing 150 ppm ethoxyquin should not have any deleterious effects on humans. In fact, diets containing ethoxyquin can reduce effects of certain toxins on rats and sheep.

Aflatoxin B_1 (AFB_1), a mycotoxin produced by the *Aspergillus flavus* mold, is found in improperly stored grains and can induce necrotic and carcinogenic effects in the liver when ingested by animals and humans. Fischer rats consuming a diet containing ethoxyquin (concentration not stated) for five days showed resistance to this mycotoxin due to a novel alpha-class glutathione S-transferase subunit, Yc_2. This subunit also was found in pre-neoplastic nodules of rat livers after ingesting Aflatoxin B_1 for 1 year. Livers from rats consuming a normal diet do not contain Yc_2; this suggests that ethoxyquin induces synthesis of this detoxifying subunit. Ethoxyquin also has a dramatic effect on hepatic glutathione S-transferases (Hayes et al. 1991). Hayes et al. (1993) found that adding 0.5% ethoxyquin to powdered diets for 5 consecutive days also induced synthesis of an aldehyde reductase that protects rats against Aflatoxin B_1 toxicity. This reductase is a member of the subfamily of aldo-keto reductases (Ellis et al., 1993).

Bitterweed increases thiol levels in the body, has adverse effects on the liver, and is toxic. When ethoxyquin HCl was added at 0.5% to the diets of crossbred lambs 9 days prior to bitterweed administration, blood thiol levels were decreased. Lambs were fed diets containing 10 or 20% crude protein or 20% crude protein plus 0.5% ethoxyquin HCl. When bitterweed was dosed into the

rumen, extracellular blood and acid soluble liver thiol concentrations were lower in the challenged lambs consuming the 20% CP + ethoxyquin diets. The unchallenged lambs had the greatest liver weights (Calhoun et al., 1989).

Santoquin fed at 2 g/kg (2,000 ppm) to partially hepatectomized rats, liver regeneration rate was increased. Sprague-Dawley rats received Santoquin in the diet for 6.5 to 10 weeks prior to partial removal of the liver. Pentane production, a measure of *in-vivo* lipid peroxidation in rats, was lower 3 and 6 days after surgery suggesting that Santoquin was having antioxidant effects. This was substantiated by lower thiobarbituric acid reactants (TBA) 6 days post surgery for santoquin-fed rats than both vitamin E supplemented (40 mg/kg in diet) rats and control rats. Liver sulfhydryl levels were higher for the rats fed Santoquin during this same time period. The effect of lower lipid peroxidation of Santoquin fed rats resulted in liver weights of 3.03 and 3.12 g / 100 g body weight 3 and 6 days post surgery compared to 2.41 and 2.31 g / 100 g body weight in controls (Gavino et al., 1985).

The literature concerning *in vivo* effects ethoxyquin reveals numerous intracellular effects in animals whose degree is controlled by the concentration of ethoxyquin introduced to the system. *In vitro* incubation and parenteral dosing studies also show cellular activity but these presumably are more extreme than effects noted from *in vivo* oral dosing or feeding because of metabolism in the gastrointestinal tract and limited absorption. Nevertheless, through acting as a antioxidant when ingested by animals ethoxyquin can have an advantageous effects on health, metabolism, and production of animals.

Chapter III

Autoxidation of myoglobin and lipid

To sell, fresh meat must have a desirable appearance in the retail case. Between harvesting the animal and the placing retail cuts in the case for sale, many factors may render the product unacceptable to consumers. Color is the most important factor in the purchasing decision of fresh beef (Faustman and Cassen, 1990; Kropf, 1980). Factors that can affect color of fresh beef include bacterial load, lighting, aging time, storage temperature, muscle fiber type, oxidation of myoglobin, and lipid peroxidation. These factors Individually or in combination can cause fresh beef to become discolored. Importance of these discoloration factors vary with meat processing. Ground or minced meat is more susceptible to discoloration due to the greater surface area created by grinding. Sanitation during harvesting and processing can help minimize microbial contamination. Ironically, sanitation during processing has very limited affect on psychrotrophic bacterial loads of ground beef. In contrast, microbial contamination during harvesting or processing was correlated closely with surface discoloration (Greer et al., 1980). Comparing sanitation effects of a

modern abattoir and local retail shops in India, Rao and Ramesh (1988) found that processing sanitation can affect shelf life of minced sheep meat. Temperatures above 30° C promoted microbial spoilage. When storing meat in an oxygen free atmosphere, simply raising the temperature from -1 ° C to 0 ° C speeded surface discoloration (O'Keefe and Hood, 1980). Lighting of the retail case and the temperature at which meat is displayed for sale can affect the longevity of fresh meat (Kropf, 1980). Even location within a retail case can affect surface temperature and microbial growth (Greer et al., 1994). Aging increases tenderness and produces a brighter red color in fresh beef (Hood, 1980). Although important to the preservation of meat quality, antioxidants cannot alter any of these factors. Instead, antioxidants can reduce myoglobin oxidation and lipid peroxidation; these effects will be my next focus.

Myoglobin, a globular oxygen-binding protein found in muscle cells, contains a single heme group (iron) that normally exists in one of three states and is responsible for the bright cherry red or brown color in the exposed surface of fresh meat. Deoxymoglobin (Mb), the reduced or anaerobic state of myoglobin, is purple in color, It is found in deep muscles and in vacuum packaged meat. Oxymyoglobin (MbO_2), prevalent when myoglobin is exposed to oxygen, gives meat a "bright cherry red" color and the "bloom" when beef carcasses are ribbed before grading. Metmyoglobin (MetMb), produced when muscle tissue is exposed to oxygen for a long time period, is a more oxidized form of myoglobin. Formation of metmyoglobin depends on the reducing capacity of muscle, oxygen availability, and myoglobin autoxidation rate. It is responsible for the brown color

of fresh meat displayed in a retail case (Renerre, 1990). Oxidation rate of myoglobin, when exposed to oxygen, can be regulated by factors previously stated varies with the specific muscle being tested. Using the hindquarter of yearling heifers, Hood (1980) found that muscle type alone accounted for 45% of the variation in discoloration while temperature was responsible for 32.5% of metmyoglobin formation. Longissimus muscle was the most stable (least rapidly discolored) whereas the semimembranosus muscle was the least stable (most rapidly discolored). Psoas major muscle, also very unstable when exposed to oxygen, provides one of the most expensive retail cuts of beef, Tenderloin. Variation among individual muscles can explain why ground beef is more susceptible to discoloration. Besides the increased surface area, less stable muscle tissue is being combined with more stable muscle tissue.

One important reaction that forms metmyoglobin from myoglobin is 'autoxidation'. Oxygen, as a reducing agent, oxidizes the ferrous form of iron in myoglobin. This reaction is a nonenzymatic spontaneous oxidation that causes discoloration (Renerre, 1990). Through action as an oxygen scavenger, antioxidants, such as ethoxyquin, could reduce the rate of autoxidation. Ascorbic acid can prevent metmyoglobin formation in this manner (Renerre, 1990). Metmyoglobin formation and lipid oxidation are closely interrelated processes; it has been suggested that oxidation of heme and non-heme iron catalyzes lipid oxidation (Lui and Watts, 1970). However, which reaction is a catalyst for the other still is unknown.

Autoxidation that occurs in lipids exposed to atmospheric oxygen results in oxidative rancidity. This reaction is catalyzed by pro-oxidants including heat and light. The distinctive taste and smell of rancid fats are due to aldehydes, ketones, and acids formed during decomposition of fatty acids (Forrest et al., 1975). Unsaturated fatty acids are more susceptible to oxidation than saturated fat; this causes pant fat to oxidize quicker than animal fat. Lipid oxidation is more severe in ground meat than steaks or roast because grinding causes the loss of intracellular reductants and exposes meat protein to intracellular phospholipids and polyunsaturated fatty acids (Benedict et al., 1975). The use of antioxidants can reduce or retard both lipid and myoglobin oxidation thus increasing case life of meat.

Addition of ascorbic acid or ascorbyl palmitate to broilers consuming a diet containing an unsaturated vegetable oil (soybean) failed to stabilize either abdominal fat or thigh muscle as measured by the increase in TBA values over time. Nevertheless, addition of either ethoxyquin or alpha-tocopherol acetate to this same diet stabilized these tissues (Bartov, 1977). When broilers where fed a diet without added unsaturated fat, the supplementation of ethoxyquin or alpha-tocopherol still stabilized meat and fat tissue (Bartov and Bornstein, 1977). Laying hens consuming diets supplemented with 125 ppm ethoxyquin for 24 weeks had increased plasma vitamin E activity; a subsequent study showed that the meat from these birds had reduced TBA values after 90 days of storage at -20° C (Combs and Regenstein, 1980). Antioxidants (natural and synthetic)

added to the microsomal fractions of catfish muscles completely inhibited lipid peroxidation. Ethoxyquin added at 100 ppm also inhibited lipid oxidation.

Autoxidation of myoglobin and lipid is a major promoter of fresh meat discoloration and a costly concern to the meat industry. This concern can be reduced by addition of antioxidants either directly to the meat or, with some antioxidants, to the diet. Antioxidant use definitely can be beneficial to the meat industry.

Chapter IV

Antioxidant benefits on animal health

With the burgeoning world population and development in third world countries, demand for meat protein should increase. Animal production systems already are developed for efficient and profitable meat production on a large scale. Genetic selection of animals for rapid accretion of lean in a short time has resulted in animals with tremendous growth potential. This growth potential places additional stress on domesticated animals raised for human consumption. The key to making any animal production system profitable and efficient is keeping animals on a positive growth curve. This becomes difficult when unacquainted animals are penned or housed together. Intermingling unfamiliar animals, common during both receiving and marketing, adds to the stress already present during these periods. Generally, cattle are bought from several locations, loaded on a semi-trailer and transported some distance to the feedlot. During this time feed and water is not available. Upon the arrival at the feeding facility the animal, being stressed, is susceptible to disease due to a non-functioning rumen and a depressed immune system. Not all cattle arrive under extreme stress but merely removing animals from their native environment and

translocating them to a confined facility can induce stress. During stress, cattle often refuse feed or reduce feed consumption further complicating the problem. Norepinephrine and epinephrine are released under stressful situations. Epinephrine induced reactions lead to formation of free radicals that cause lipoperoxidation (Nockels et al., 1996) that is detrimental to the immune system. With stress and feed refusal, morbidity, mortality, and poor performance become paramount concerns for managers receiving cattle.

Supplementing the diet with antioxidants, such as vitamin E and ethoxyquin, has been one method proposed to combat poor performance, mortality, and morbidity of stressed cattle. In a review of 5 published articles, Secrist et al. (1996) found that supplementation of vitamin E increased average daily gain and feed:gain in receiving cattle subjected to transport stress. Morbidity tended to be reduced with vitamin E supplementation. Creatine kinase, a measure of cellular damage, increases with added stress. When vitamin E was topdressed (1000 IU/d) 28 days prior to being subjected to stress and injected with ACTH, the increase in creatine kinase was reduced in Charolais heifers; liver alpha-tocopherol levels also were higher than in non-supplemented vitamin E heifers (Nockels et al., 1996). Accumulation of vitamin E in the liver could help sustain health of receiving cattle by reducing lipid peroxidation in this vital organ. If high rates of supplementation increase the muscle tissue concentrations of this vitamin, its antioxidative properties may prove beneficial. Leghorn x Rhode Island Red laying hens (34 weeks old) supplemented with 125 mg/kg vitamin E or 250 mg/kg ethoxyquin had lower mortality during a Newcastle Disease

outbreak (Bartov et al., 1991). Although antioxidant use for stressed animals may prove advantageous, further research is needed to explain how antioxidants might increase the immune responsiveness by stressed animals.

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Chapter V

Agrado™ for Finishing Cattle: Effects on Performance, Carcass Measurements and Meat Quality

C. L. Krumsiek and F. N. Owens
Oklahoma State University, Stillwater, OK 74078

Abstract

Seventy-five feedlot cattle in 15 pens were fed high concentrate diets supplemented with either 0 or 136 ppm Agrado (30 and 45 cattle, respectively) for 28 days prior to harvest. Added Agrado had little impact on performance although rate and efficiency of gain tended to be improved slightly by supplemental Agrado. Feces were examined for odor potency and offensiveness. Feeding Agrado reduced offensiveness and tended to reduce potency of odor of cattle feces at 6 hours but not at 24 h after defecation. For carcasses, lean maturity, an indicator of darkness of ribeye color, was reduced while USDA yield grade was increased slightly by feeding Agrado. Shelf life of both ground beef and ribeye steaks that had been aged for 13 days was determined during display for 10 days in a simulated meat counter; samples were appraised visually by a panel of 6 people, electronically with a color reflectance meter, and chemically by measuring thiobarbituric acid equivalents (ground beef only). According to visual estimates, shelf life was extended (6

versus 2 days for ground beef; 4 versus 3 days for ribeye steaks) for beef samples obtained from cattle that had been fed Agrado. Electronic measurements and thiobarbituric acid assays confirmed these visual differences. Eight members of an untrained taste panel were each given one steak from a control animal and one steak from an Agrado-fed animal. All steaks had been aged for 13 days prior to being vacuum packaged and frozen for 3 weeks prior to being provided to each panel member to cook at home. No differences in color, flavor, tenderness, juiciness, and overall acceptability were detected between steaks from cattle fed or not fed Agrado.

(Key words: Cattle, Shelf life, Agrado, Rancidity, Case life)

Introduction

Feeding vitamin E, an antioxidant, at concentrations 10 to 100 times the amounts required nutritionally has resulted in steaks that exhibited superior lean color, less surface discoloration, more desirable overall appearance, and less lipid oxidation during retail display (Sanders et al., 1997). Work from Wisconsin indicated that higher levels of E (500 IU/day for 100 days or 1000 IU/day for 50 days) were needed to prolong shelf-life. Unfortunately, vitamin E is rather costly; cost for supplementation is between $3 and $4 per animal. Whether other more economical antioxidants would improve shelf life of beef similarly has received limited attention. Infusing vitamin C (up to 100 g per animal) into the blood immediately before slaughter showed little benefit according to Schaefer

(personal communication). But with broilers (Bartov and Bornstein, 1977) and rat (Gavino et al., 1985), tissue or blood antioxidant activity was increased when Agrado was fed. In one study, lambs were fed diets containing 0, .01 and .1% Agrado (DeMille et al., 1972); tissue concentrations of a compound presumed to be Agrado reflected the amounts being fed. When Agrado was fed to lactating dairy cows, milk contained elevated concentrations of a compound not separable from vitamin E. So whether Agrado fed to animals acts directly as an antioxidant in tissue, acts in the digestive tract to reduce either production or absorption of oxidized products, or enhances absorption, activity, or stability of vitamin E and other antioxidants is not certain. Agrado is used commonly as an antioxidant additive for fish meal, fat, and alfalfa meal; maximum permitted concentration to be added is 150 ppm (except for dog food where the maximum suggested concentration is 75 ppm). If Agrado is present in tissue and acts directly or indirectly as an antioxidant, it should reduce metmyoglobin formation and extend the shelf-life of meat products like beef. The objective of this study was to determine the impact of feeding Agrado at approximately 150 ppm on performance, carcass, and meat characteristics of feedlot cattle.

Materials and Methods

Treatments. A 121 day feeding study was conducted at the Progeny Test Barn at Oklahoma State University located near Stillwater, OK; Agrado treatments were imposed only during the final 28 days of this trial. Cattle that had been fed

a high concentrate diet for 92 days previously and having an average full weight of 506 kg were divided into two groups. Each pen in one of these groups (5 cattle per pen) received no supplemental Agrado whereas the other group received 13 g top-dressed on their daily diet once daily; this was calculated to provide 1.7 g Agrado per animal daily for 28 days prior to marketing. For pens receiving Agrado, preweighed plastic bags containing Agrado premix were poured onto and mixed with the fresh feed in the feed bunks once each day. On chemical analysis by Covance Laboratories, Madison, WI, the dry Agrado premix purchased from Nutra Blend Corp., Neosho, MO, listed to contain 66% Agrado, actually contained 52.8% Agrado. This means that Agrado intake averaged 1.37 g daily per animal. Because feed intake for the final 35 days of the trial averaged 22.3 lb. (10.1 kg), Agrado concentration, expressed as parts per million of diet dry matter, was 136 ppm for cattle receiving the Agrado supplement. Other diet ingredients are listed in Table 1. Cattle received fresh feed once each day at 0830. For compiling the corn grain, alfalfa meal pellets and the pellet supplement, a Data Ranger feed delivery system was used. Based on the amount of feed remaining in the bunk from the previous day, the amount of fresh feed to add was adjusted each day to keep excess below 5% but yet avoid bunks from being empty. Feed weights were recorded daily and summarized across each weighing period.

Table 1. Diet composition (DM basis)

Ingredient	Percentage (DM basis)
Corn grain, ground	82.0
Alfalfa meal pellets	10.2
Pelleted supplement	7.8
Cottonseed meal	4.61
Limestone, 38% Ca	1.11
Soybean meal	.91
Urea	.50
Salt	.30
Cane molasses	.18
Potassium chloride	.15
Manganese oxide	.0062
Zinc oxide	.0047
Vitamin A, 30000 IU/g	.0010

Cattle Management and Weighing. In this study, 75 cattle, predominately of British breeding from the Oklahoma State University cattle herd, were used; 45 cattle (9 pens with 5 animals in each pen) received the Agrado supplement while 30 cattle (6 pens with 5 animals in each pen) received no Agrado. Cattle were housed in partially covered pens (3.8 m by 16 m) equipped with automatic waterers and fence-line cement feed bunks with 38 inches of linear bunk space per animal. Cattle were weighed individually at approximately 28 day intervals and 2 days prior to harvest. For calculation purposes, final body weight was calculated as hot carcass weight assuming that dressing percentage was 62%. This calculation helps to adjust for individual differences in gut fill and water intake and more precisely estimates sellable product.

Fecal Odor Evaluation. Herdsmen feeding the cattle noted that "The barn smells differently since we started feeding 'Coenzyme Q'," our code name for

Agrado. Consequently, potency and offensiveness of odors were evaluated using an untrained smell panel of 12 graduate students, technicians and one faculty member. Freshly voided fecal samples were collected at 0800 from cattle in 3 randomly selected pens receiving each treatment; samples, taken only from freshly voided feces, were obtained from 2 or 3 cattle per pen. A fecal composite within each pen was obtained by mixing equal weights of fresh feces from each animal sampled in that pen. This yielded a total of 6 composite samples, one from each of 6 pens, for odor evaluation. The 12-member untrained smell panel was recruited or coerced to scale these 6 randomly labeled samples for the 1) strength of odor and 2) offensiveness of the odor. Panel members were instructed to evaluate the samples using a "whiff" method whereby the evaluator held the sample at a distance but used their free hand to create a draft toward their nose. The scoring scale for both presence and offensiveness was based on a ten point marked scale with 0 being equivalent to "No Odor" or "Not Offensive" and 10 being equivalent to "Very Strong Odor" or "Very Offensive." The samples were located randomly under separate vented hoods; after appraising three samples, each evaluator took a ten minute break before appraising the remaining three samples. Odor evaluations were conducted 6 hours after samples were collected; the test was repeated using these same samples 24 hours post collection.

Carcass Measurements and Sampling. On June 17, 1997 all cattle were loaded onto two semi-trailer trucks and transported to Excel Inc., Dodge City, KS

and harvested. Following exsanguination and hide removal, each animal's identification tag was transferred from the ear to the carcasses to maintain identity of each carcass in the meat cooler. Each liver was examined for presence and severity of abscesses. After carcasses were held at 4 C for 36 hours, each carcass was ribbed (sliced at the 12th rib); quality grade, yield grade, ribeye area, marbling score, skeletal maturity, kidney-heart-pelvic fat percentage, and maturity of lean and bone were determined for each carcass. A 3 inch section from the posterior end of the Longissimus muscle (Ribeye) was removed, deboned, and trimmed of excess external fat to form steaks for case life evaluation. This ribeye section, identified with the animal's slaughter order number, was vacuum packaged and transported under refrigeration to Oklahoma State University for processing. These samples were aged at 4 C in the OSU Food Technology and Processing Center for 13 days after slaughter prior to preparation of meat cuts.

Meat Preparation. On June 30, 1997, the rib sections were processed for evaluation of case life. Two steaks 2.5 cm thick were sliced from each rib section; one of these was packaged in a 17s Grace Styrofoam meat tray containing an absorption pad. Each steak was covered with oxygen permeable, clear wrap. The remaining portion of the ribeye section was ground twice with a mechanical meat grinder and composited with ground beef from all other cattle receiving the same treatment. This ground beef was formed into 114 gram patties and wrapped in the same manner as the ribeye steaks. Eight patties

from each treatment were prepared for the case life study. An additional ten patties were packaged from each treatment for chemical and microbial analysis; these also were vacuum packaged but were removed from display and frozen each day. Subsequently, these samples were assayed for rancidity by the thiobarbituric acid (TBA) procedure and for total microbial counts.

Shelf Life Measurement. The steaks and ground beef were displayed continuously for 10 days in an environment simulating a commercial meat case. This consisted of a continuously lighted cold room. Meat packages were placed on two 1.2 x 1.8 m meat cutting tables. For lighting, eight 3000 Kelvin fluorescent bulbs were held in fixtures suspended above the tables at a distance of about .6 m to provide precisely 150 lumens at the meat surface on all tables. Ambient temperature, monitored continuously, was maintained at 1.1 C except during each defrost cycle that occurred every eight hours during which temperature was allowed to rise to 4.4 C for about 5 minutes. Each day, colors of steaks and ground beef were monitored visually and electronically. For visual appraisal, lean color and discoloration were monitored daily by a six member, semi-trained panel between 0800 and 0900 and by three panel members again between 1600 and 1700. Lean color and percent discoloration of the ribeyes and ground beef patties were evaluated independently. Steaks were evaluated for color by each panel member independently using an 8 point discrete scale; steps in this scale represented 1) Extremely Dark Brown or Green, 2) Very Dark Brown, 3) Brown, Dark Red or Brown, 4) Moderately Dark Red, 5) Slightly Dark

Red, 6) Cherry Red, 7) Moderately Bright Cherry Red, and 8) Bright Cherry Red. Ground beef was evaluated for percent of the surface that was discolored. Percent discoloration was evaluated using a 7 point discrete scale of 1) Completely Discolored, 2) 76-99% Discolored, 3) 51-75% Discolored, 4) 26-50% Discolored, 5) 11-25% Discolored, 6) 1-10% Discolored, and 7) No Discoloration. Colors were evaluated initially when processing and packaging had been completed (Day 0) and daily for the next ten consecutive days. Location of each sample on the table was changed each day to avoid panelists from identifying sample by location. For electronic appraisal of color, light reflectance measurements also were taken each morning using a hand-held Minolta Color Monitor; three readings were taken across the face of each sample each day. Output values (L, a*, and b*) were recorded; these measurements were used to calculate hue and chroma for each day as described by Liu et al (1996). One extra ground beef patty from each treatment was removed from display each day, vacuum packaged, and frozen for further analysis. On day 11, the 75 steaks and 10 beef patties, being quite deteriorated, were fed to locally prevalent wild coyotes (*Canine pesticus* var. *howlee*) in hopes of inducing liver damage. Unfortunately, no changes in health or behavior were detected.

TBA Measurements. Following the procedures of Witte et al. (1970), concentrations of thiobarbituric acid-like substances in the ground beef were measured. Two samples were obtained from each beef patty that had been held frozen after being displayed for each of the 10 days of the shelf life study.

Following color development, optical density measurements at 533 nm were compared to TBA standards.

Total Microbial Counts. The same frozen meat patty samples used for TBA analysis were extracted with water. This extract, at dilutions of 10^1 to 10^5,were incubated with petri film media for 24 h at 38 C. Colonies formed were counted to estimate most probable numbers of bacteria.

Taste Panel Evaluation. Frozen steaks labeled only with the animal's identification number were transported to St. Louis by air and distributed in pairs (one steak from an animal fed Agrado and a second steak from an animal that had not been fed supplemental Agrado) to untrained panelists in a single blind experiment. Each panelist was asked to cook the two steaks simultaneously and to rank these two steaks for color (after thawing but before cooking), and, after cooking, for tenderness, juiciness, and flavor using a double bar 0 to 5 scale for preference. Degree of preference was evaluated by measuring the location of the mark on the scaling bar. Animal numbers were matched with treatments to calculate preference rankings.

Statistical Analysis. Data were analyzed as a completely randomized design with 2 treatments; pen means were used for analysis of performance data and olfactory measurements while means from individual animals (or replicate samples of ground beef) were used for statistical analysis of carcass and shelf

life data. For shelf life and taste panel data, measurements on individual meat patties or steaks were averaged across panelists for statistical analysis. This means that variation among steaks or beef patties within treatment served as the error term. For all shelf life data, statistical comparisons were calculated within each display day. To appraise relative shelf life, plots of mean values against display day were evaluated visually to approximate the time lag that would cause the two curves to be overlaid. Electronic color measurements also were averaged across the three sites within each steak or each meat patty for statistical analysis within each display day. In cases where repeat observations were not available (TBA values and microbial counts for ground beef), values were regressed across display day; slopes and intercepts of regression lines were compared to appraise treatment effects.

Results and Discussion

Cattle Performance. Cattle weights, feed intakes and feed efficiencies are presented in Table 2. Performance measurements were taken on days that did not correspond precisely with the 28 day period when Agrado was fed. The weigh day nearest to the day that Agrado was first fed (day 92) was day 84. Because Agrado was fed for 28 of the 37 days of this final period, responses during this time period were attributed to added Agrado. Live and carcass weights were not significantly altered by Agrado supplementation although daily gain tended to be slightly greater (about 5%) for cattle fed Agrado during this final 37 day period. No sorting or rejection of the small particles carrying Agrado

was detected. Dry matter intakes were not different; consequently, the amount of feed required per unit of gain either on a live basis (119 day values) or a carcass adjusted basis (121 day values) slightly favored (7 to 10%) cattle receiving Agrado during this final period. Feed energy value, calculated from gain, mean weight, and feed intake, again tended to favor cattle fed Agrado during this period. However, none of these performance differences was significant statistically due to the small number of pens of cattle in this experiment.

Table 2. Effects of adding 150 ppm Agrado for the final 28 days on performance of finishing beef cattle. (Agrado was fed only from 5/20 to 6/17/97)

Agrado added, ppm	0	150	Effect, %	Probability P<
Cattle, number	30	45		
Weights, lb.				
Live, Day 0 (2/17/97)	729	716	-1.8	0.27
Live, Day 84 (5/12/97)	1125	1109	-1.4	0.50
Live, Day 119 (6/16/97)	1238	1229	-0.7	0.80
Carcass, Day 121 (6/18/97)	760	756	-0.5	0.84
Daily gain, lb./day				
0-119 d (live weights; 5% shrink)	3.76	3.79	0.8	0.87
0-121 d (carcass weight/dress %)	4.19	4.24	1.2	0.75
84-119 d (Agrado began day 92)	3.09	3.26	5.2	0.64
84-121 d (Agrado began day 92)	4.51	4.75	5.1	0.45
Dry matter intake, lb./day				
0-120 d	21.41	21.23	-0.8	0.80
84-120 d	22.72	22.26	-2.1	0.67
Feed/gain				
0-119 d	5.72	5.62	-1.8	0.61
0-121 d	5.12	5.02	-2.0	0.42
84-119 d	7.65	6.92	-10.5	0.19
84-121 d	5.06	4.72	-7.2	0.17
Diet Metabolizable Energy, Mcal/kg				
0-121 d	2.92	2.94	0.7	0.71
84-121 d	2.76	2.87	3.8	0.22

Fecal Odor Evaluation. Results of the panel evaluation of odor of fecal samples are presented in Table 3. Differences were not evident 24 hours after the feces had been produced, but at 6 hours after feces were collected, strength of odor tended to be lower ($P < .08$) and odor offensiveness was lower ($P < .03$) for feces from cattle fed Agrado. Components responsible for potency of odor of feces include ammonia and fermentation products derived from tryptophan and sulfur-amino acids that are generated by facultative or anaerobic microbes in the large intestine or feces. Feeding Agrado may have decreased the oxygen availability for facultative microbes within the gut or in fecal material and thereby altered fermentation.

Table 3. Results of fecal odor evaluation based on 12 panelists.

Measurement	Control pens n = 3	Agrado-fed pens n=3	Probability, P <
Odor strength			
6 hr	5.17	4.53	.08
24 hr	4.98	5.31	.34
Odor offensiveness			
6 hr	5.18	4.19	**.03**
24 hr	5.00	5.17	.65

Carcass Differences. Effects of Agrado supplementation on carcass measurements are presented in Table 4. Only two differences proved to be significant statistically. First, maturity as judged by lean color was lower (lean color was brighter red) for cattle fed Agrado; skeletal maturity, an index of calcification of spinus processes, was not altered by Agrado feeding. Brighter

lean color 48 hours after harvest probably reflects an alteration in oxygen uptake or holding capacity by ribeye muscle tissue.

USDA Yield Grade, an index of carcass fatness that is dependent largely on fat thickness over the ribeye muscle, was greater for cattle fed Agrado. This means that cattle fed Agrado were slightly fatter than control cattle were. Likewise, a lower percentage of cattle fed Agrado fell in the leanest yield grade (1) while several more cattle fell in the less desirable and discounted yield grades (4 and 5). No explanation for this difference is apparent. The incidence of liver abscesses was very low for all cattle but numerically was lower for cattle receiving Agrado. The USDA grader that examined each liver for presence of abscesses, flukes, and indicated that healthfulness of all the cattle was very good with no apparent discoloration or defects. Studies with rats has indicated that rate of liver regeneration was greater for rats fed Agrado. In contrast, dog fanciers have proposed that Agrado is responsible for liver malfunction in certain breeds of dogs. However, dogs would consume much more Agrado than cattle because dogs have much higher feed intakes (over 4% versus 2% of body weight intake per day) and consume fortified diets for a much longer time period (lifetime versus 28 days) than the cattle used in this experiment.

Table 4. Effects of adding 150 ppm Agrado for the final 28 days on carcass characteristics of finishing beef cattle. (Agrado was fed only from 5/20 to 6/17/97)

Agrado added, ppm	0	150	Effect, %	Probability P<
Dressing % (carcass wt/live wt*100)	61.4	61.5	0.2	0.82
Skeletal maturity	153	153	0.0	0.96
Lean maturity	***153***	***142***	***-7.7***	***0.02***
Overall maturity	153	148	-3.4	0.24
Marbling (400 = choice)	285	341	16.4	0.40
Quality score	1.33	1.47	9.5	0.46
Prime, %	0	6.7		0.16
High choice, %	6.7	4.4		0.75
Choice, %	13.3	4.4		0.12
Select, %	73.3	71.1		0.88
Percent above choice	26.7	28.9		0.88
Rib eye area, sq. in.	13.9	13.6	-2.2	0.34
Fat thickness, in.	0.43	0.5	14.0	0.16
Adjusted fat thick, in.	0.51	0.57	10.5	0.26
Kidney, heart, pelvic fat, %	2.17	2.26	4.0	0.36
USDA Yield grade	***2.27***	***2.67***	***15.0***	***0.04***
Final yield grade	2.64	2.88	8.3	0.17
Yield grade 1, %	16.7	6.7		0.13
Yield grade 2, %	53.3	48.9		0.74
Yield grade 3, %	30	40		0.49
Yield grade 4, %	0	4.4		0.27
Liver abscesses, %	6.7	4.4	-52.3	0.79

Shelf Life Measurements. Color scores of steaks and ground beef from cattle receiving 0 or 150 ppm Agrado are presented in Table 5. On the first day after display began, both steaks and ground beef from cattle fed Agrado had less bright red color than steaks from cattle not fed Agrado. But thereafter, color scores were higher (brighter red or less brown) for meat from cattle fed Agrado. For steaks, the reduction in color score was delayed by about 1 day for steaks from cattle fed Agrado. This differences was much more dramatic for ground beef than for steaks; for ground beef, color deterioration was delayed by 4 to 5 days for cattle fed Agrado (Figure 1). The duller red values initially followed by

brighter red values later would suggest that Agrado present in the tissues is delaying oxygen uptake and thereby reducing the oxygenation of myoglobin; this in turn could delay deterioration of oxymyoglobin to metmyoglobin and browning. With the ground beef, browning typically began in very small area that spread gradually over the total surface. These darkened areas became apparent much sooner and spread was much faster within ground beef patties from steers fed the control diet than for steers receiving Agrado.

Table 5. Mean color scores by 6 panelists evaluating steaks and ground beef from cattle fed or not fed 150 ppm Agrado for 28 days.

	Steak color score			*Ground Beef color score*		
Display time, days	Control n = 30	Agrado n = 45	Probability	Control n = 8	Agrado n = 8	Probability
0	7.04	6.99	0.57	6.50	6.50	1.0000
1	7.12	6.94	**0.06**	7.29	6.58	**0.0002**
2	5.94	6.20	**0.03**	4.54	6.21	**0.0001**
3	5.79	6.02	0.08	4.77	6.09	**0.0001**
4	5.45	5.74	0.07	3.98	5.67	**0.0001**
5	5.16	5.58	**0.01**	3.50	5.63	**0.0001**
6	4.97	5.33	**0.04**	3.02	5.25	**0.0001**
7	4.62	4.99	**0.04**	2.33	5.17	**0.0001**
8	4.13	4.59	**0.05**	1.52	4.65	**0.0001**

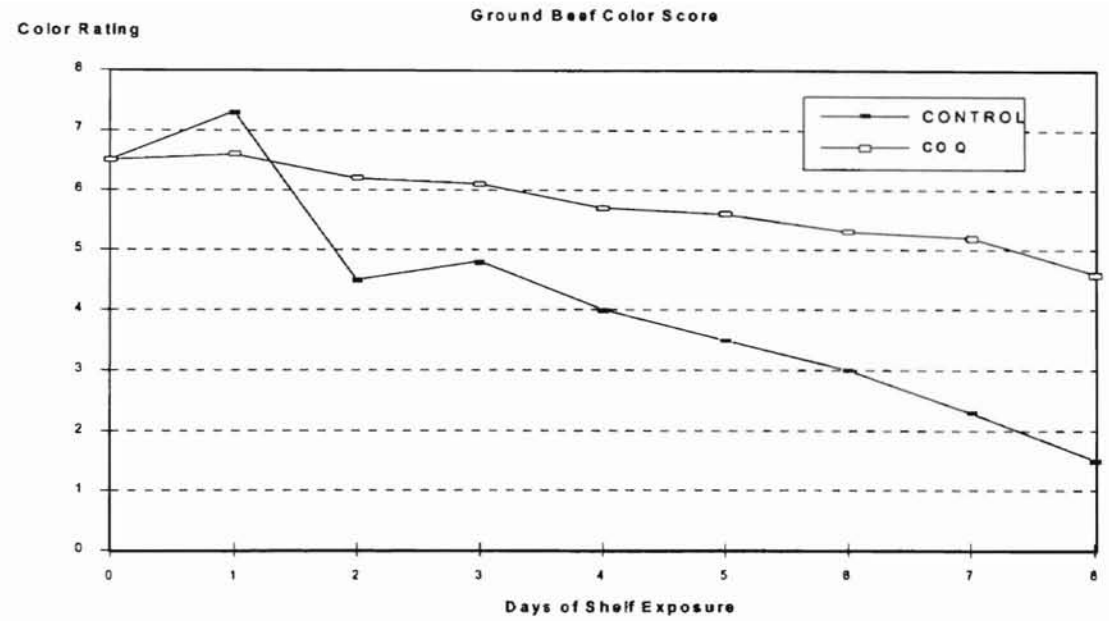

Figure 1.

Electronically measured color indicators (L, a, b, hue, chroma), averaged across three points on each steak (or hamburger patty) each day and averaged for cattle fed control or Agrado supplemented diets are presented in Tables 6 and 7. Statistical probabilities for difference between the two dietary treatments are denoted in the columns marked "Prob". The 'L' value indicates color brightness or grayness, the 'a' value indicates red intensity, the 'b' value indicates yellow intensity, the 'hue angle' value indicates proportion of redness and yellowness, and the 'chrom' value indicate color saturation of each sample. For ground beef patties, both 'a' and 'chrom' values were lower for ground beef samples from those cattle fed Agrado on day 0; thereafter, these values were greater for meat samples from cattle fed Agrado. In contrast, 'b' values were greater for ground beef samples on day 1 but less on subsequent days. 'Hue' values consistently were lower after day 1 for beef samples from cattle fed

Agrado. The 'a' and 'chrom' values match with visual estimates for treatment effects quite closely.

Table 6. Minolta color readings (L, a, b, hue, and chrom) for ground beef on consecutive days of display averaged across ground beef samples from Agrado-fed (8 samples) and control cattle (8 samples).

Day	L-con	L-Agra	Prob	a-con	a-Agra	Prob	b-con	b-Agra	Prob	Hue-c	Hue-e	Prob	Chrom-c	Chrom-e	Prob
0	47.00	47.04	0.94	23.01	22.19	**0.02**	8.45	8.00	0.09	20.14	19.80	0.39	24.51	23.60	**0.03**
1	45.36	46.40	0.06	17.70	19.64	**0.01**	6.61	7.23	**0.01**	20.53	20.21	0.60	18.90	20.94	**0.01**
2	45.64	46.69	0.15	15.44	18.09	**0.01**	6.55	6.27	0.29	23.10	19.11	**0.01**	16.78	19.16	**0.01**
3	45.17	46.19	0.10	13.71	17.17	**0.01**	7.39	6.45	**0.01**	28.63	20.61	**0.01**	15.61	18.34	**0.01**
4	45.18	46.16	0.12	13.70	16.84	**0.01**	7.39	6.32	**0.01**	28.63	20.56	**0.01**	15.61	17.99	**0.01**
5	45.56	46.44	0.17	9.98	15.18	**0.01**	7.74	6.48	**0.01**	37.95	23.18	**0.01**	12.64	16.52	**0.01**
6	46.85	46.17	0.30	8.55	14.36	**0.01**	8.15	6.55	**0.01**	43.72	24.68	**0.01**	11.83	15.81	**0.01**
7	41.20	45.79	0.34	9.70	13.52	**0.05**	8.47	6.84	**0.01**	43.59	26.87	**0.01**	13.15	15.16	0.20
8	44.78	42.74	0.26	7.01	12.32	**0.01**	9.76	8.60	**0.01**	54.47	35.02	**0.01**	12.05	15.06	**0.01**
9	45.65	44.23	**0.04**	5.88	10.42	**0.01**	10.04	9.02	**0.01**	59.64	40.96	**0.01**	11.66	13.81	**0.01**
10	45.62	44.78	0.32	5.67	8.19	**0.01**	10.39	9.42	**0.01**	61.43	49.00	**0.01**	11.84	12.49	**0.04**

Color readings for ribeye steak muscles are presented in Table 7. In general, trends were the same as noted for ground beef samples above; the only significant differences were in 'L' values that were greater for steaks from cattle fed Agrado on days 6, 8 and 10. Similar to visual measurements, treatment differences were less consistent for steaks than for ground beef. This probably is because ground beef samples were replicates from a single batch of ground beef from cattle fed each of the diets whereas steaks were samples from individual animals within each treatment and thereby exhibited much greater variation. In practice, ground beef normally would represent a composite across a number of cattle whereas steaks would be from individual animals.

Table 7. Minolta color readings (L, a, b, hue, and chrom) for steaks on consecutive days of display averaged across ribeye steaks from Agrado-fed (45 steaks) and control cattle (30 steaks).

Day	L-con	L-Agra	Prob	a-con	a-Agra	Prob	b-con	b-Agra	Prob	Hue-c	Hue-e	Prob	Chrom-c	Chrom-e	Prob
0	41.54	42.14	0.17	18.40	18.61	0.45	6.15	6.36	0.36	18.41	18.80	0.39	19.41	19.67	0.41
1	43.38	43.99	0.19	17.62	17.85	0.41	5.42	5.40	0.90	17.12	16.81	0.53	18.44	18.66	0.47
2	43.05	43.48	0.34	17.48	17.90	0.15	5.35	5.51	0.35	17.00	17.09	0.82	18.28	18.75	0.16
3	42.55	43.20	0.08	17.39	17.80	0.19	5.77	5.92	0.28	18.34	18.42	0.80	18.66	18.77	0.19
4	43.46	44.09	0.08	16.57	17.12	0.13	5.11	5.28	0.06	17.25	17.20	0.87	17.34	17.92	0.12
5	43.45	43.06	0.74	16.09	16.69	0.17	5.28	5.47	0.15	18.23	18.23	0.99	16.94	17.57	0.16
6	43.16	44.18	**0.05**	15.68	16.33	0.08	5.28	5.40	0.19	18.70	18.42	0.54	16.55	17.20	0.07
7	42.21	42.61	0.38	15.58	16.39	0.08	5.71	5.89	0.36	20.21	19.87	0.56	16.61	17.43	0.08
8	41.44	42.26	**0.04**	15.30	15.92	0.25	6.53	6.56	0.80	23.42	22.86	0.53	16.66	17.26	0.22
9	41.96	42.59	0.10	13.99	14.61	0.35	6.47	6.45	0.86	25.36	24.80	0.70	15.46	16.06	0.30
10	41.88	42.73	**0.02**	12.38	13.25	0.24	6.49	6.49	0.97	28.74	27.45	0.47	14.07	14.87	0.20

TBA Assays. Another index of fat stability of ground beef is concentration of thiobarbituric acid-like substances; values are shown in Table 8 (Figure 2). Higher concentrations reflect higher amounts of peroxides derived from degradation of fatty acids. The TBA values were higher on every day of display for ground beef from cattle that had not been fed Agrado. Furthermore, during display in simulated meat cases, TBA values increased 3 times faster for ground beef from control cattle than from cattle fed diets supplemented with Agrado. The lower initial TBA values for ground beef from cattle fed Agrado and the delay in the increase in TBA values over time were even more dramatic than the differences noted for color. Because these TBA values should reflect lipid rancidity, higher and increasing values should mirror presence of compounds that give meat an undesirably rancid taste. No taste panel measurements were conducted with these samples. This is because after 2 days of display for

ground beef from control cattle and 6 or 7 days of display for ground beef from cattle fed Agrado, samples had an undesirable color.

Table 8. Thiobarbituric acid equivalent values of ground beef at various display times.

Display Day	Control	Agrado
	Thiobarbituric acid equivalents	
0	1.640	0.647
1	1.482	0.559
2	2.338	0.862
3	2.748	0.915
4	3.366	0.880
5	5.093	1.808
6	5.678	1.470
7	4.993	1.663
8	4.063	1.543
9	4.568	1.351
10	5.336	1.919
Intercept	1.809	0.617
Slope	0.389	0.124

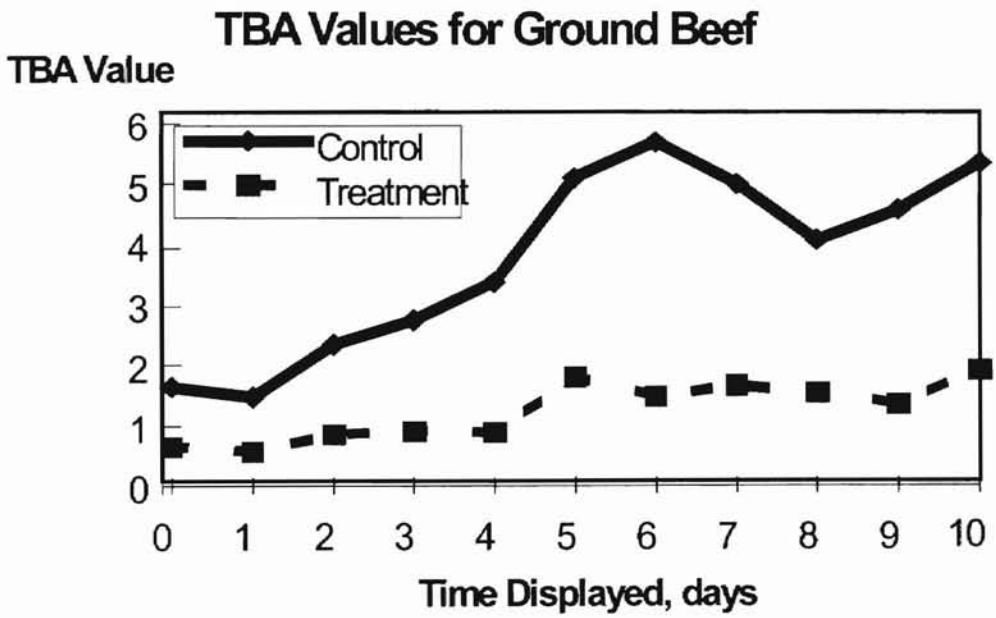

Figure 2.

Microbial Counts. Total microbial counts on fluid extracts from ground beef samples frozen after being on display for various time periods all were very low, under 10^2 colonies per gram. Values probably were low because of bacteriocidal effects of freezing and thawing these meat samples. For more reliable microbial counts, samples should be analyzed fresh on each day that they are removed from display.

Taste Panel Evaluation. To determine relative acceptability of steaks from cattle fed control and Agrado-supplemented diets, members of an untrained taste panel were given two steaks each, one being from an animal fed the control diet and the second being from an animal fed a diet containing supplemental Agrado. Mean rankings provided by 13 evaluators for meat color, flavor, juiciness, tenderness, and overall acceptability are presented in Table 9. Values less than 0 represent a preference for steaks from control cattle whereas values greater than 0 represent a preference for steaks from cattle supplemented with Agrado. In color of uncooked but thawed steaks, control steaks were slightly preferable; other responses tended to favor steaks from cattle supplemented with Agrado. However, none of these differences was significant statistically due to the large variation among individual panel members. Nevertheless, one might conclude that feeding Agrado did not adversely alter consumer acceptability of steaks.

Table 9. Taste panel preferences (n = 13) among steaks from cattle fed or not fed Agrado.

Response	Mean score[a]	Standard deviation of score
Uncooked color	-.13	.61
Juiciness	.26	.85
Flavor	.32	1.01
Tenderness	.32	.88
Overall acceptability	.06	1.14

[a] A score of 0 represents equal preference whereas scores greater than 1 equate to a preference for steaks from cattle fed Agrado.

Implications

Including Agrado at 138 ppm of the diet for 28 days prior to harvesting feedlot cattle increased rate and efficiency of gain slightly; it reduced lean maturity (ribeye color was brighter) but it increased carcass fatness slightly. Shelf life, the time period before discoloration of steaks and ground beef displayed in a commercial meat case, was extended by adding Agrado to the diet as measured visually by an untrained panel, electronically as measured with a color reflectance meter, and chemically as measured by concentrations of thiobarbituric acid-like substances. Flavor, tenderness, juiciness, and overall acceptability of steaks aged 13 days and frozen prior to sampling were not altered by feeding Agrado. Because its cost is lower than vitamin E, Agrado appears to be a viable alternative for cattle producers to feed to improve the shelf life of beef. By extending the shelf life of ground beef, Agrado should enlarge the time window during which beef will retain desirable visual and olfactory appeal for the consumer.

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Chapter VI

Agrado™ for Finishing Cattle: Effects on Performance, Carcass Measurements and Meat Quality when Fed for Various Time Intervals.

C. L. Krumsiek and F. N. Owens

ABSTRACT

One hundred feedlot steers in 20 pens were fed high concentrate diets supplemented with no antioxidant, 148 ppm Agrado, or 1000 IU vitamin E. Agrado was supplemented for either 7, 28, or 66 days prior to harvest (20 cattle each). Vitamin E was supplemented only for the last 66 days (20 cattle). Remaining cattle received no supplement (20 cattle). Cattle supplemented with Agrado for the final 28 and 66 days had slightly (5 and 2.5 %, respectively) greater ADG than control steers. Carcasses from cattle fed Agrado had less subcutaneous fat than non-Agrado supplemented carcasses; steers fed Agrado for 28 days produced leaner carcasses with a higher percentage of desirable carcasses than steers on other treatments. Using a three point scale, liver abscesses were analyzed. Steers fed Agrado for 66 days tended ($P < .10$) to have fewer liver abscesses than control steers. Case life of steaks from the strip loin and ground beef from the knuckle was measured visually and electronically for 5 days of display. No significant response in case life was detected for

addition of either antioxidant to the diet, perhaps due to the long time period (28 days) of aging prior to display.

INTRODUCTION

Loss of the bright cherry red color from beef is very expensive to retailers in the US and abroad. Consumers equate a bright-cherry red color to the freshness and palatability of beef and any deviation from this color (discoloration) affects buying decisions of consumers (Kropf, 1980). Fresh beef discoloration is attributed primarily to oxidation of lean and lipid tissues. Feeding vitamin E, an antioxidant, at 10 to 100 times its nutritional requirement, results in steaks that exhibit superior lean color, less surface discoloration, less lipid oxidation, and more desirable overall appearance (Sanders et al., 1997). Sherbeck et al. (1995) found that supplementing cattle with 500 IU of vitamin E per animal daily for a total cost of $3 to $4 per animal extended the case life of beef. In an effort to find a less expensive antioxidant that might produce comparable results, Agrado, a synthetic antioxidant produced by Solutia, Inc., St. Louis, MO containing ethoxyquin and other chemicals, was fed to feedlot steers. In previous studies, when ethoxyquin was fed to broilers (Bartov and Bornstein, 1977) and to rats (Gavino et al., 1985), antioxidant activity of tissue or blood was increased. In one study, lambs were fed diets containing either 0, .01, or .1% ethoxyquin (DeMille et al., 1972); tissue concentrations of a compound presumed to be ethoxyquin increased in parallel with amount fed. When

ethoxyquin was fed to lactating dairy cows, milk contained elevated concentrations of a compound the authors could not separate from vitamin E. Whether ethoxyquin is deposited in tissue and therein acts directly as an antioxidant or it acts indirectly enhancing absorption, activity, or stability of vitamin E and(or) other antioxidants is not known. However, ethoxyquin is used widely commercially as an antioxidant for fish meal, fat and alfalfa meal (vitamin A stabilizer); its use in feeds at a maximum concentration of 150 ppm is sanctioned by the US Food and Drug Administration. Recently, because of concerns about liver health of dogs, the maximum level suggested for dog food was decreased to 75 ppm . Whether present in tissue and acting directly or acting indirectly through enhancing concentrations of other tissue antioxidants, ethoxyquin should reduce metmyoglobin formation and extend the case life of beef. In a previous experiment, feeding Agrado to steers for their final 28 days on feed, slightly improved rate and efficiency of gain and markedly extended shelf life of ground beef (Krumsiek et al., 1998). This experiment was conducted to determine the impact of feeding ethoxyquin at 150 ppm for various time periods prior to harvest on performance, carcass, and meat characteristics of feedlot cattle.

MATERIALS AND METHODS

Treatments. A 118 day feeding study was conducted at the Progeny Test Barn of Oklahoma State University located near Stillwater, OK. For the first four

treatments, Agrado was supplemented during either the final 0, 7, 28, or 66 days of feeding. In the fifth treatment, vitamin E was supplemented at 1,000 IU daily for the final 66 days of the trial. Steers previously divided into weight groups and had been fed their high concentrate diet for 52 days prior to imposition of these treatments. At the start of the 118 day trial, steers had a mean weight of 421 kg. For this trial, each pen contained 5 animals with 4 pens being randomly assigned to each of the five treatments. Agrado was top-dressed onto the daily diet each day when feed was provided at 0800 each day as 12 g of Agrado™ premix per pen; this premix was assayed by Covance Laboratories, Madison, WI to contain 56% Agrado yielding a mean intake of 1.34 g Agrado daily. With mean daily dry matter intake for the final 66 days averaging 20 pounds per steer, this equals a dietary concentration of 148 ppm. Vitamin E also was top-dressed onto the diet at a rate of 2 g of a vitamin E premix guaranteed to contain 500 IU dl-alpha tocopherol/g premix; this yielded a daily intake of 1000 IU vitamin E. Animals received fresh feed each day at 0800. The diet contained whole shelled corn, pelleted alfalfa meal, cottonseed hulls, and a pelleted premix (Table 1) that was compiled and mixed each day in a Data Ranger feed delivery system.

Table 1. Diet Composition (DM calculated)

Ingredient	Percentage (DM basis)
Whole corn grain	86.74
Cotton seed hulls	5.09
Pelleted supplement	8.41
Cottonseed meal	5.13
Soybean hulls	.82
Urea	.67
Salt	.323
Limestone, 38% Ca	1.24
Potassium chloride	.17
Zinc sulfate	.005
Manganese oxide	.0042
Vitamin A, 30,000 IU/g	.0109
Rumensin, 80g/lb.	.019
Tylan, 40g/lb.	.009

Cattle Management and Weighing: One hundred steers, predominately of British breeding provided by a cattle feeder, were used for this study. These steers were blocked by initial weight (light, medium light, medium heavy, heavy) and assigned randomly to 20 pens of 5 steers each and fed for 56 days prior to imposing the dietary treatments. Twenty steers from each block were randomly assigned to vitamin E or Agrado treatments. The vitamin E steers and one group from the Agrado treatment were fed for 66 days; the remaining Agrado steers had their diets fortified for 28 or 7 days. The steers not assigned to these treatments served as controls. The steers were housed in partially covered pens (3.8 m by 16 m) equipped with automatic waterers and fence-line cement feed bunks with 38 inches of linear bunk space per animal. Steers were weighed at approximately 28 day intervals and again 2 days prior to harvest. For calculation purposes, final body weight was calculated as hot carcass weight assuming that

dressing percentage was 62%. This calculation helps to adjust for individual differences in gut fill and water intake and estimates sellable product more precisely than live body weight.

Carcass Measurements and Sampling. On November 11, 1997 all steers were transported by semi-trailer trucks to Excel Inc., Dodge City, KS and harvested. Following exsanguination and hide removal, the each animal's identification tag was transferred from the ear to the carcasses to maintain identity of each carcass in the meat cooler. Each liver was examined for presence and severity of abscesses. Each lung was examined for signs of lesions indicative of past or present pneumonia. After carcasses were held at for 36 h at 4 C, each carcass was partially separated between the 12^{th} and 13^{th} rib; quality grade, yield grade, ribeye area, marbling score, skeletal maturity, kidney-heart-pelvic fat percentage, and maturity of lean and bone were visually appraised for each carcass. The caudal section of the Longissimus muscle including the 13^{th} rib (strip loin) was removed, deboned and trimmed of excess external fat for fabrication into steaks. The Sartorius, Vastus medialis, Vastus intermedius, Vastus lateralis, Rectus femoris and tensor fasciae latae muscles of the round including the patella (knuckle) were retained for processing into ground beef. Each cut was removed from the left half of the carcass. These cuts, along with the animal's identification tag, were individually vacuum packaged and transported under refrigeration to Oklahoma State University for further

processing. These samples were aged at 3.3 C for 28 days in the OSU Food Technology and Processing Center prior to preparation for case life study.

Meat Preparation. On December 8, 1997, the Strip loin sections were processed for evaluation of case life in a sanitized cutting room. Using a sanitized knife, a 2.5 cm steak was sliced from the posterior end of each loin and packaged in a 17s Styrofoam meat tray containing an absorption pad. Each steak was covered with clear, oxygen permeable film (PVC Wrap, Wilco, Aurora, Ohio). After the steaks had been prepared, the knuckles were sorted by treatment and pen and processed as a pen. Each knuckle was sliced vertically with a smooth cut along the midline with a sanitized knife producing 2 halves which were designated left and right. The right half was identified and vacuum packaged for continued aging. To prepare a ground beef sample representing the composite for each pen, the left half of the knuckle of each animal each of the 20 pens was ground using a 3/8 inch die; ground knuckles were combined and reground using a ¼ inch die. These ground beef pen composites were subdivided into 9 packages each containing about 1 pound. These ground beef packages were weighed by placing the ground beef on a premarked Styrofoam tray using a gloved hand; packages were covered with a clear, oxygen permeable wrap. These 9 packages from each of the 20 pens were utilized to evaluate color (2), microbial numbers (6), and effect of freezing on color (1). The meat grinder was completely cleaned and sanitized between each treatment. Each member of the fabricating team changed rubber gloves between

treatments. The 6 packages for microbial evaluation were displayed in the same manner as the 2 samples used for color evaluation. One ground beef sample representing each pen of cattle was removed from display each day for counting total culturable bacteria and coliform bacteria. The portion not used for microbial counting was vacuumed packaged each day and frozen for later assay for rancidity using the thiobarbituric acid (TBA) procedure.

Shelf Life Measurements. The steaks and ground beef were displayed continuously for 5 days in an environment simulating a commercial meat case consisting of a continuously lighted cold room. Meat packages were placed on two 1.2 x 1.8 m meat cutting tables. For lighting, eighteen 3000 Kelvin fluorescent bulbs held in covered fixtures were suspended above the tables at a distance of about .6 m to provide approximately 170 lumens at the meat surface of each table. Ambient temperature, monitored continuously, was maintained at 2.2 C except during the defrost cycle that occurred every eight hours during which temperature rose to 5.5 C for about 5 minutes. Each day, the colors of steaks and ground beef were monitored both visually and electronically. For visual appraisal, lean color and percent of the surface that was discolored were evaluated daily by a eight member, trained panel between 1400 and 1430. Lean color and percent discoloration of the loin steaks and ground beef were evaluated independently on the day of processing (day 0) and daily for the next five days. Each panelist used an eight point discrete scale to rate the meat in each package with one of the following shades of color; 1) Extremely Dark

Brown or Green, 2) Very Dark Brown, 3) Dark Red or Brown, 4) Moderately Dark Red, 5) Slightly Dark Red, 6) Cherry Red, 7) Moderately Bright Cherry Red, 8) Bright Cherry Red. Location of each meat package was changed each day using randomly generated locations to balance for location effects. For electronic appraisal of color, light reflectance measurements were taken each morning using a hand held Minolta Color Monitor; three readings were taken across the face of each sample each day. Output values (L, a*, b*) were recorded. On day 6, the steaks were consumed by the graduate students on the panel; no ill effects were reported. Ground beef samples were discarded.

Visual Panel Training. Eight panelist (7 males, 1 female) were trained with aged ground beef to evaluate color and discoloration. Past experience with meats evaluation ranged from serving as a meats judging coach to a newly arrived research assistant. To train the panel, two 1 pound packages of ground beef were purchased at a local retail store on each of the five days preceding the training period with the last two samples being purchased on the day of training. These samples were displayed as described above until training of the panel began. The panelists were shown the color and discoloration of these ground beef samples of known age so they could visualize to various shades of red and green to expect during the study. Discoloration also was discussed. A color photo of these samples plus various shades of red and discoloration was made and kept available for reference during the trial.

TBA Measurements. Following the procedures of Witte et al. (1970), concentrations of thiobarbituric acid-like (TBA) substances in the ground beef were measured. A ten gram sample, taken after thoroughly mixing the larger sample, was obtained from each ground beef package that previously had been displayed for 0 to 5 days and had been used for microbial analysis. This 10 g sample was homogenized first for 60 seconds with 50 ml deionized water then homogenized an additional 15 seconds after adding 50 ml of a chilled TCA-H_3PO_4 solution. From this solution, 30 ml was centrifuged for 30 minutes at 3000 x g and filtered. To 2 ml of filtrate in a 16 x 100 mm test tube, 2 ml of TBA solution and the mixture was incubated for 15 hours in the dark at room temperature for color development. Optical density measurements at 533 nm were compared to those obtained from standard solutions of TBA.

Total Microbial Counts. Microbial analysis was performed at the OSU Food and Technology Center using samples of unfrozen ground beef. This extract, at dilutions of 10^1 to 10^{10}, were incubated in duplicate with agar media and petri film media for 24 h at 38 C. Colonies that grew were counted to estimate the most probable number of bacteria. Coliforms were determined with petri film media with Escherichia coli being specifically identified by presence of a gas pocket around the coliform culture.

Statistical Analysis. Data were analyzed as a completely randomized design with 5 treatments; pen means were used for analysis of performance data while means from individual animals (or pen replications for ground beef) were used for statistical analysis of carcass and case life data. For case life estimation, scores by panelists were averaged each day for each individual ground beef package or steak; these means were used for statistical analysis. This means that the variation among steaks and ground beef packages within a treatment (individual cattle for steaks and pens of cattle for ground beef) served as the error term. Electronic color measurements also were averaged across the three sites within each steak or ground beef package for statistical analysis within each display day.

Results and Discussion

Cattle Performance. Cattle weights, feed intakes, and feed efficiencies are presented in Table 2. Animal and feed weights were taken on days that did not correspond precisely with the specific days that the treatments were imposed. The weigh date nearest the first day Agrado and vitamin E (66 day treatment) were first fed (day 56) was day 49. The 28 day Agrado treatment was imposed three days after the third weigh date (day 84). The 7 day Agrado treatment began seven days before slaughter (day 118). Although the initiation of the treatments did not coincide precisely with these weigh dates, the values listed in Table 2 present cattle performance between respective weigh dates. Live and

carcass weights were not significantly altered by treatments although the steers fed Agrado for 28 and 66 days tended to yield the heaviest live and carcass weights. Steers on the 28 day treatment, imposed on day 87 of the feeding trial, gained 10 pounds more than in the previous weigh period. Furthermore, these steers had the heaviest carcass weights and the best overall feed to gain ratio. Compared to the control steers, average daily gain (ADG) calculated to be about a 5% greater for cattle fed Agrado for the final 28 days. (This increase closely matches the increased ADG of 5% noted with feeding of Agrado the final 28 days noted by Krumsiek et al., 1998 in a previous study in the same facility.) For steers fed Agrado for the final 66 days of the trial, ADG for the total trial was increased by 2.5% and feed to gain ratio was 3.7% greater than for steers not fed Agrado. When compared to the steers fed vitamin E the final 66 days of the study, steers fed Agrado had 5% greater ADG and a 2.5% advantage in feed efficiency. Feeding Agrado for 7 days did not alter gain or efficiency. None of these differences in performance were significant statistically due to the small number of pens of cattle within each treatment. Nevertheless, these numerical differences, if observed in practice, would be very beneficial economically to the cattle feedlot industry.

Table 2. Effects of adding 150 ppm Agrado ™ (AG) for the final 0 to 66 days on performance of finishing beef cattle.

Compound added Additive provided, final number of days	AG 0	AG 7	AG 28	AG 66	Vit E 66	Prob. 28 vs 0; P<	AG vs Vit E; P<
Cattle, number	20	20	20	20	20		
Weights, lb.							
Live, day 0 (8/17/97)	789	796	807	798	796	.54	.94
Live, day 28 (11/16/97)	886	883	905	887	884	.54	.92
Live, day 56 (11/16/97)	958	959	979	972	959	.63	.80
Live, day 84 (11/16/97)	1031	1034	1056	1049	1032	.60	.76
Live, day 115 (11/16/97)	1114	1116	1144	1121	1108	.56	.82
Carcass, day 118 (11/18/97)	683	680	704	693	682	.53	.76
Adjusted live, day 118 (11/16/97)	1071	1066	1104	1086	1070	.54	.76
Daily gain, lb./day							
0-28 d (live weights; 5% shrink)	2.19	1.83	2.18	1.93	1.89	.98	.88
28-56 d (live weights; 5% shrink)	2.49	2.60	2.56	2.90	2.63	.90	.72
56-84 d (live weights; 5% shrink)	2.49	2.56	2.64	2.63	2.49	.69	.77
84-115 d (live weights; 5% shrink)	2.55	2.54	2.71	2.24	2.32	.77	.89
0-56 d (live weights; 5% shrink)	2.34	2.21	2.37	2.42	2.24	.91	.66
0-115 d (live weights; 5% shrink)	2.43	2.39	2.53	2.42	2.43	.62	.99
0-118 d (adjusted weights; 5% shrink)	2.39	2.29	2.51	2.45	2.33	.56	.60
Dry matter intake, lb./day							
0-56 d	17.23	16.87	16.97	17.06	16.6	.79	.72
57-118 d	20.41	19.58	20.54	20.18	19.3	.93	.63
0-118 d	18.86	18.26	18.80	18.66	18.0	.96	.66
Feed/gain							
0-56 d	7.45	7.71	7.27	7.20	7.72	.78	.56
57-118 d	8.15	7.90	7.91	8.41	8.09	.83	.72
0-115 d	7.74	7.72	7.47	7.73	7.80	.54	.87
0-118 d	7.93	8.07	7.51	7.63	7.83	.40	.62

Carcass Effects. Results from Agrado supplementation on carcass characteristics of the cattle are presented in Table 3. Only two carcass traits were significantly altered by feeding Agrado in this study. Fat thickness over the 12th rib, an index of subcutaneous fat deposition and a key factor in calculating USDA yield grades, was lower for cattle fed Agrado. A higher preliminary yield

grade reflects greater fat thickness over the rib eye. This value is "adjusted" up or down depending on fat thickness over the round and chuck by the USDA meat quality inspector to produce Adjusted Fat Thickness. Cattle fed Agrado had lower adjusted yield grades and less fat thickness over the rib than cattle not fed Agrado. This indicates that carcasses of steers fed Agrado had less subcutaneous fat at slaughter than steers not fed the compound. Fat thickness was significantly lower for the 7, 28, and 66 day carcasses vs the controls ($P<.03,.03,.01$; Table 4). This finding contrasts with measurements from a the previous study (Krumsiek et al., 1998) in which fat thickness tended to be greater and yield grade was significantly greater for cattle fed Agrado. In this study, steers fed the compound for 7 and 28 days had numerically more carcasses in the leanest yield grade (1) and steers on all treatments receiving Agrado steers had a numerically higher percentage of carcasses in yield grades 1 and 2 than other treatment groups. Steers fed Agrado for 28 days yielded the higher percentage of carcasses in these two yield grades. When compared to the carcasses of control cattle, steers fed Agrado for 28 days had ($P < .06$) more carcasses in yield grade 2. For yield grade 3, steers fed Agrado for 28 days again had a statistical advantage over steers on other treatments. Although the steers fed Agrado for 7 days produced fewer yield grade 4 carcasses than those fed Agrado for 28 days or those not fed Agrado. In summary, the steers fed Agrado for 28 days were leaner and produced a higher percentage of desirable carcasses than steers in other treatment groups and compared to control cattle, all steers fed Agrado produced more desirable carcasses. No significant

statistical differences in carcass characteristics were detected between steers fed Agrado and those fed supplemental vitamin E for 66 days.

Liver abscesses were analyzed using a three point scale; 0 = None, 1 = Minor, 2 = > 2 moderately sized, and 3 = Severe or open abscesses. Steers supplemented with Agrado for 66 days had significantly lower liver abscess mean scores and a lower incidence of abscesses than control steers had. Steers fed Agrado for 7 or 28 days tended to have a lower frequency of liver abscesses than control steers. Coupling the above information with the performance data might imply that supplementing Agrado for 28 to 66 day might increase performance due to a decreased incidence of liver abscesses. Note that these diets all contained tylosin, an antibiotic known to reduce the incidence of liver abscesses. Rate of liver regeneration was found to be greater for rats fed the active ingredient found in Agrado (Gavino et al., 1985). Because severe liver abscesses are associated with reduced rates of gain and lower dressing percentages, probably due to liver adhesions (Brink et al., 1990), decreasing the incidence of severe liver abscesses should be of economic interest to feedlot cattle producers.

Table 3. Effects of adding various antioxidants on carcass measurements of finishing beef cattle.							
Compound added Additive provided, final number of days	AGR 0	AGR 7	AGR 28	AGR 66	Vit E 66	Probability 28 vs 0; P<	Probability AGRADO ™ vs Vit E; P<
Dressing % (carcass wt/live wt*100)	63.9	63.5	64.1	64.5	64.1	.69	.34
Skeletal maturity	235	208	214	205	241	.43	.15
Lean maturity	185	170	174	169	174	.49	.66
Overall maturity	210	189	194	187	207	.40	.18
Marbling (400 = choice)	450	474	440	428	438	.80	.82
Quality score							
Choice, %	75	70	55	50	60	.21	.36
Select, %	20	25	45	45	40	.13	.62
Standard, %	5	5	0	5	0	.43	.36
Rib eye area, sq. in.	11.5	11.6	12.0	11.7	12.0	.29	.58
Fat thickness, in.	**$.70^{a}$**	**$.54^{b}$**	**$.58^{ab}$**	**$.62^{ab}$**	.60	**.10**	.73
Adjusted fat thick, in.	**$.76^{a}$**	**$.66^{b}$**	**$.66^{b}$**	**$.64^{a}$**	.64	**.03**	1.00
Kidney, heart, pelvic fat, %	2.50	2.35	2.35	2.30	2.40	.26	.62
Final yield grade	3.66	3.19	3.24	3.40	3.25	.12	.56
Yield grade 1, %	0	5	5	0	10	.33	.13
Yield grade 2, %	20^{b}	35^{ab}	50^{a}	35^{ab}	35	.06	1.00
Yield grade 3, %	**55^{a}**	**55^{a}**	**15^{b}**	**40^{a}**	30	**.01**	.13
Yield grade 4, %	**35^{a}**	**5^{b}**	**30^{a}**	**25^{ab}**	25	.66	1.00
Lung lesion, mean score	45	45	45	50	25	1.00	.17
Lung lesion incidence, % of cattle	40	35	25	40	25	.34	.35
Liver abscesses, mean score	**$.65^{a}$**	**$.20^{ab}$**	**$.30^{ab}$**	**$.10^{b}$**	.30	.18	.27
Liver abscess incidence, % of cattle	**35^{a}**	**15^{ab}**	**20^{ab}**	**5^{b}**	25	.23	.11

Table 4. Backfat and Adjusted Backfat Least Square Means and Probabilities.

Treatment	LS Means Backfat	Probability, P< AGRADO 0 vs	LS Means Adj. Backfat	Probability, P< AGRADO 0 vs
AGRADO 0	$.70^{a}$	.	$.76^{a}$	.
AGRADO 7	$.54^{b}$	.035	$.66^{b}$	.0334
AGRADO 28	$.58^{ab}$	.10	$.66.^{b}$	.0334
AGRADO 66	$.62^{ab}$	.2577	$.64^{b}$	.0138

Shelf Life Measurements. Results from visual color evaluation of the ground beef (Table 5) and steaks (data not presented) did not match those detected in a previous study (Krumsiek et al., 1998). On day 0, no statistical significance was observed across the treatments although ground beef from carcasses of steers fed Agrado for 66 days tended ($P < .09$) to be more desirable in color (higher L value) than ground beef from carcasses of steers fed vitamin E for 66 days. However, this trend did not continue throughout the entire case life study. When contrasted using pen within treatment as error term, ground beef from steers fed vitamin E had brighter ($P < .04$) color than ground beef from control steers. On the third day, ground beef from control steers had brighter color ($P < .03$) than ground beef from steers fed Agrado for 66 days. No contrasts were significant on day 3, but again on day 4, color was more favorable for ground beef from control steers when compared with various Agrado feeding times (7 days, $P<.02$.; 28 days, $P<.03$; 66 days, $P<.05$). Compared to the mean of ground beef from steers fed Agrado, ground beef from control steers was brighter ($P < .01$) on day 4. On the fifth and final day of display, all ground beef samples were discolored; no statistical differences in lean color score were detected.

Results were similar when animal rather than pen means were used to compare color of ground beef. Contrasts also were used to compare the mean of all Agrado treatments against the controls and to compare samples from steers fed vitamin E against those from steers fed Agrado for 66 days or never fed Agrado. On the initial day, ground beef samples from steers fed Agrado for 66 days were brighter in color ($P < .03$) in color than samples from steers fed

vitamin E although the numerical difference was very small. The reverse was true (P>.008) on day 3 and this numerical but non-statistical difference remained on day 4. No statistical difference was detected on day 5 although a slight numerical advantage remained for ground beef from steers fed vitamin E. Compared to the average for ground beef from steers fed Agrado, ground beef from control steers was brighter on day 2 (P>.01), this advantage remained on days 3 (P>.05) and 4 (P>.0004) but disappeared on day 5. Compared to ground beef from steers fed vitamin E, ground beef from control steers was slightly brighter (P < .07) on day 0 but thereafter the difference that remained was numerical but not significant statistically.

Table 5. Ground Beef Visual Least Square Means and Probabilities.
A Score of '8' is Optimum.

Day	Treatment	LS Mean	Prob., P<	Day	Treatment	LS Mean	Prob., P<
0	0	7.91	.	3	0	5.5	.
	Q7	7.89	.85		Q7	5.18	.56
	Q28	7.83	.39		Q28	5.20	.58
	Q66	7.94	.77		Q66	4.34	**.03**
	E66	7.78	.16		E66	5.29	.73
1	0	7.13	.	4	0	5.83	.
	Q7	7.16	.94		Q7	4.41	**.02**
	Q28	7.30	.48		Q28	4.58	**.04**
	Q66	7.06	.75		Q66	4.68	**.05**
	E66	7.04	.68		E66	5.08	.19
2	0	6.83	.	5	0	4.25	.
	Q7	6.33	.17		Q7	4.35	.92
	Q28	6.39	.23		Q28	3.16	.27
	Q66	6.20	.09		Q66	3.68	.56
	E66	6.5	.35		E66	3.94	.75

TBA Measurements. All treatment groups had lower TBA values when compared to the controls. Agrado 66 samples exhibited the lowest value on day 0 among all treatments; Agrado 66 ended the study with the lowest TBA value among all Agrado treatments. The 28 day treated samples showed a steady increase in value and had a higher rancidity value than the controls. Samples treated for 7 days with Agrado had higher values on days 1, 3, than the controls and ended the study as the most rancid among all treatments. Vitamin E samples consistently had low TBA values and were the least rancid on day 5 compared to all treatments.

Table 6. TBA Values for Ground Beef

	Day of Display					
TRT	**0**	**1**	**2**	**3**	**4**	**5**
0	0.20393	0.37877	0.39124	0.58968	0.74214	0.605155
7	0.10289	0.40497	0.34757	0.62570	0.66771	0.752431
28	0.13693	0.40122	0.48731	0.63290	0.67612	0.682299
66	0.08162	0.26273	0.37502	0.41921	0.76255	0.511178
E66	0.09226	0.24651	0.21408	0.32918	0.39040	0.361097

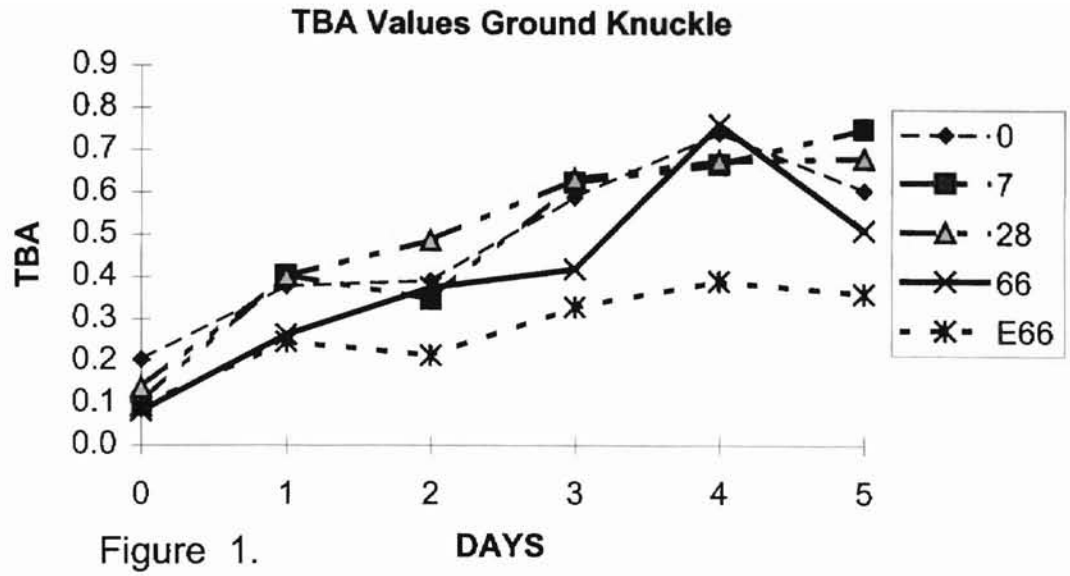

Figure 1.

Minolta Readings. Ground beef samples and loin steaks were evaluated with a Minolta color monitor using the L, a*, b* setting. The L value depicts the brightness of the color whereas the a* value measures the degree of redness and the b* value detects the yellow color of the spectrum. No significant treatment effects were noted with either the ground beef or the loin steaks using these values during this study.

Microbial Counts. On the day of fabrication, day 0, total microbial counts and total coliforms in ground beef samples were lower for control steers and steers fed Agrado for 28 days than from steers fed Agrado for 66 days ($P < .05,.05$). A consistent but non-significant trend continued for lower coliforms counts for ground beef from steers fed Agrado for 28 days than for steers on other treatments. When log transformed prior to statistical analysis, a standard

microbiological procedure, these numerical differences become significant statistically. Significance was seen on days 0 (P<.025, .031) when compared to 7day and vitamin E; Day 4 (P<.047 and .021) and Day 5 (P<.06) when compared to vitamin E. Treatment differences in total plate counts on both absolute and a log transformed basis were not detected either statistically or numerically during the study.

Discussion

Although this study was conducted to expand on observations from a previous study in which Agrado was fed to steers for 28 days prior to harvest (Krumsiek et al., 1998), several factors were different for this study due to logistical reasons. In this trial, all cattle were steers whereas both steers and heifers were used in the first study. Although the diet was similar in ingredients, grain form differed, being ground corn in the former study and whole corn in the second study. Furthermore, the diet in the second study contained Rumensin™ and Tylan™, to improve efficiency and reduce the incidence of liver abscesses, respectively. As these compounds are present in nearly all feedlot diets fed to feedlot cattle in the US, new compounds must have effects that are equal to or additive with these feed additives. Differences on grain form or presence of these feed additives may have altered the amount of ethoxyquin or antioxidants being available to tissue. However, the primary differences of concern are the time and method of meat fabrication. In the first trial, steaks (individually) and

ground beef meat (en mass) were aged for 13 days and fabricated on day 13 following harvest whereas in the second trial, steaks (individually) and ground beef (pen composites) were aged for 28 days and fabricated on day 28 following harvest. Each week that aging time is increased will reduce shelf life; absolute values of decreased shelf life is muscle dependent (O'Keefe and Hood, 1980). In addition, to increase precision in the second trial, exactly 454 g of ground beef samples were placed in each package. Although meat was handled with gloved hands, the meat was shaped into "bricks" before it was covered by plastic film. This may have spread bacteria across the face of the ground beef sample and increased contact between the meat and the plastic film that increased rate of discoloration. Indeed, all ground beef samples began to discolor by day 1 of the second trial. In contrast, loin steaks did not exhibit abnormal discoloration and shelf life of steaks remained similar to that measured in the first study.

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Chapter VII

Agrado™ For Finishing Cattle: Effects on Meat Quality

C. L. Krumsiek and F. N. Owens
Oklahoma State University, Stillwater, OK 74078

Abstract

Twenty feedlot cattle in 10 pens were fed high concentrate diets supplemented with either 0 or 148 ppm Agrado (10 cattle per treatment) for 28 days prior to harvest. Rate and efficiency of gain were increased slightly (3%) for these limit fed steers by addition of Agrado to the diet. Feeding Agrado tended to reduce offensiveness and potency of odor 6 hours but not at 24 hours after defecation. Case life of ground knuckle, ground ribeye, and ribeye steaks that had been aged an average of 7 days was studied in both a simulated meat counter and in a retail meat case; samples were appraised visually by a panel of 8 people, electronically with a color reflectance meter, and chemically by measuring thiobarbituric acid equivalents (ground knuckle only). According to visual estimates, no difference in case life due to feeding of Agrado was detected; electronic measurements confirmed these estimates. Thiobarbituric acid analyses of ground knuckle samples indicated that rancidity was decreased by feeding for the Agrado to steers. Loss of red coloration tended to be greater

in a cooler designed to simulate conditions in a display case but percentage of cut discolored was greater for cuts maintained in a commercial display case. (Key words: Case life, Agrado, Rancidity, Cattle)

INTRODUCTION

Loss of the bright cherry red color from beef is very expensive to retailers in the US and abroad. Consumers equate the bright-cherry red color of beef to the freshness and desired taste; any deviation from this color (discoloration) affects buying decisions of consumers (Kropf, 1980). Fresh beef discoloration is attributed primarily to oxidation of lean tissues. Feeding vitamin E, an antioxidant, at 10 to 100 times its nutritional requirement, results in steaks that exhibit superior lean color, less surface discoloration, less lipid oxidation, and more desirable overall appearance (Sanders et al., 1997). Sherbeck et al. (1995) found that supplementing cattle with 500 IU of vitamin E per animal daily at a total cost of $3 to $4 per animal extended the case life of beef. In an effort to find a less expensive antioxidant that might produce comparable results, Agrado, a synthetic antioxidant produced by Solutia, Inc., St. Louis, MO containing ethoxyquin and other chemicals, was fed to feedlot steers. In past studies, ethoxyquin was fed to broilers (Bartov and Bornstein, 1977) and to rats (Gavino et al., 1985); antioxidant activity of tissue or blood was increased. In one study, lambs were fed diets containing either 0, .01, or .1% ethoxyquin (DeMille et al., 1972); tissue concentrations of a compound presumed to be

ethoxyquin increased in parallel with amount fed. When ethoxyquin was fed to lactating dairy cows, milk contained elevated concentrations of a compound the authors could not separate from vitamin E. Ethoxyquin may be deposited in tissue and therein acts directly as an antioxidant; alternatively, it may acts indirectly through enhancing absorption, activity, or stability of vitamin E and(or) other antioxidants. Nevertheless, ethoxyquin is used widely as a commercial antioxidant for fish meal, fat and alfalfa meal (vitamin A stabilizer); its use in feeds at a maximum concentration of 150 ppm is sanctioned by the US Food and Drug Administration. Recently, because of concerns about liver health of dogs, the maximum level suggested for dog food was decreased to 75 ppm . Whether present in tissue and acting directly or acting indirectly through enhancing concentrations of other tissue antioxidants, ethoxyquin should reduce metmyoglobin formation and extend the case life of beef. In one previous experiment, including Agrado at 150 ppm in diets of steers for their final 28 days on feed slightly improved rate and efficiency of gain and markedly extended shelf life of ground beef (Krumsiek et al., 1998). This experiment was conducted to determine the impact of feeding ethoxyquin at 150 ppm of the diet on odor of feces and of meat characteristics of feedlot cattle.

Materials and Methods

Treatments. Twenty steers were fed diets containing either no added Agrado or approximately 150 ppm Agrado for 29 days prior to harvest. These steers, that had been maintained on a high concentrate diet and had a starting unshrunk weight of 493 kg, were blocked by weight into two groups and randomly assigned within weight block to either 0 or 150 ppm Agrado treatment with five pens with 2 cattle per pen receiving supplemental Agrado and 5 additional pens receiving the identical diet without added Agrado. Agrado was supplemented by top-dressing 6 grams of the Agrado premix onto the diets that were fed once daily at 0900. The premix, purchased from Nutra Blend Corp., Neosho, MO that supposedly contained 60% ethoxyquin, assayed to contain 56% ethoxyquin by Covance Laboratories, Madison, WI. When fed at a rate of 3 grams per animal daily, this premix provided 1.68 g Agrado per animal daily which, when diluted with 11.35 kg of feed per steer daily, yields a diet that contains 148 parts per million (ppm) of ethoxyquin. Supplementation with Agrado was staggered as related to harvest date of the cattle, with Agrado being fed the final 29 days prior to harvest. For compiling the diet ingredients (Table 1), a Data Ranger feed delivery system was used. Daily feed intake was limited to 22.7 kg per pen each day.

Table 1. Diet Composition (DM basis)

Ingredient	Percentage (DM basis)
Corn grain, cracked	62.80
Cottonseed Hulls	14.29
Alfalfa Meal Pellets	6.12
Cane Molasses	4.25
Soybean Meal, 44%	10.21
Dicalcium Phosphate	.55
Limestone, 38%	.55
Salt	.55
Urea	.11
Potassium Chloride	.56

Cattle Management and Weighing. Cattle were housed in partially covered pens (3.8 m by 16 m) equipped with automatic waterers and fence-line cement feed bunks with 190 cm (75 linear inches) of bunk space per animal. Steers were weighed individually at the beginning of the study and on the day of harvest. Feed was withdrawn 24 hours prior to transport to harvest.

Fecal Odor Evaluation. In a previous study that Krumsiek and co-workers (1998) conducted at the same facility, odor of cattle feces was less offensive 6 hours after defecation when obtained from cattle consuming the diet with added Agrado. To test repeatability of this observation, fecal odors were evaluated using 12 of these steers. Three fecal samples were collected from each treatment group and evaluated for odor intensity and offensiveness at 4 and 24 hours after the sample was produced as described in the previous study. In this trial, 10 rather than 12 untrained panelist were used.

Carcass Sampling. At 1400 on January 11, 1998, 1 block of animals, 2 that had been fed Agrado and 2 that had been fed the control diet, were weighed and transported to a local harvesting facility (Ralph's Packing, Perkins, OK) for processing at 0700 the following morning. On the next four consecutive mornings, blocks of cattle were similarly transported and harvested. Following exsanguination and hide removal, individual carcasses were weighed and identified. After being held for approximately 36 hours at 4° C, the Longissimus muscle extending from the 6^{th} to the 12^{th} thoracic vertebrae (Ribeye) was removed and deboned for fabrication into steaks and ground beef. The Sartorius, Vastus medialis, Vastus intermedius, Vastus lateralis, Rectus femoris and tensor fasciae latae muscles of the round excluding the patella (knuckle) also were recovered. Each of these cuts was removed from both right and left half of each carcass. These cuts, along with the animals identification number and side designation, were individually vacuum packaged and transported to Oklahoma State University for further processing. Samples were aged until January 19, 1998 (from 4 to 9 days after harvest) at 3.3° C in the OSU Food Technology and Processing Center prior to preparation for case life study.

Meat Preparation. On January 19, 1998 the knuckles were processed for case life in a sanitized cutting room. Knuckles were sorted by treatment and animal identification; treatments were processed as individual groups but animals within a treatment were randomly processed. Left and right knuckles of each animal

were ground using a 3/8 inch die; ground knuckles were reground using a ¼ in die. This ground beef animal composite was subdivided into 9 packages each containing about 1 pound. Ground beef was placed on pre-marked Styrofoam trays directly from the meat grinder. To ensure consistency, one person filled all the trays after practicing for 30 min using knuckles from a previous study. Each package was covered with clear, oxygen permeable wrap (PVC wrap, Wilco, Aurora, Ohio). The 9 packages from each of the 20 animals were utilized to evaluate color (2 cooler, 1 case), microbial numbers (4 cooler, 1 case), and effect of freezing on color (1). Between treatment groups, the meat grinder was completely cleaned and sanitized; between individual animals excess ground meat was removed and the first several inches of ground product, flowing from grinder, was discarded before catching sample on Styrofoam tray. Microbial packages were displayed in the same manner as the samples being evaluated for color. The 4 microbial packages displayed in the cooler were analyzed for total culturable bacteria and coliform bacteria on days 0,1, 3, and 5; a sample representing each individual animal was remove from display on each of these days and transported to the microbiological laboratory for analysis.

On January 20, 1998 ribeye sections were processed using the same sanitized cutting room. Ribeye sections were sorted by treatment and animal. Meat from each animal within each treatment was processed separately with animals being processed from the control treatment being processed first and animal order within treatment being random. Two 2.5 cm steaks were cut from the anterior end of the left and right rib sections from each animal, and packaged

in a pre-marked 17s Styrofoam tray containing an absorption pad. Each steak was covered with clear, oxygen permeable wrap. The first steak from the left and right side from each animals was displayed in the simulated retail case environment (cooler) whereas the second steak cut from the left side was displayed in a retail case and the second steak from the right side was frozen. After cutting the second steak, the remaining portion of the ribeyes (left and right of individual animal) were combined and ground first using a 3/8 in die secondly using a ¼ inch die. This resulted in a single composite of ground ribeye for each animal. Ribeye ground beef was placed in pre-marked 17s Styrofoam trays in the same manner in two ways - first as described for knuckle ground beef and secondly after being formed into round, 113 gram patties. The remaining ground beef each animal was combined with ground beef from other animals receiving the same treatment and ground twice using a ¼ in die (to ensure mixing) to produce a treatment composite. Two 113 g patties and 2-one pound packages were obtained from each treatment composite. All samples were covered with clear, oxygen permeable wrap and displayed in a simulated retail case.

Shelf Life Measurements. The steaks and ground beef were displayed continuously for 5 days in the retail case or the cooler which simulated a retail meat case but consisted of a continuously lighted cold room. Retail case temperatures were adjusted to 2 - 4 C except during an hourly defrost cycle during which temperature rose to 7 C for about 5 minutes; lighting was provided by four 3000 Kelvin bulbs enclosed in light fixtures that provided 170 lumens at

the meat surface. Meat packages in the simulated environment (cooler room) were placed on two 1.2 x 1.8 m meat cutting tables. For lighting, eighteen 3000 Kelvin fluorescent bulbs held in covered fixtures were suspended above the tables at a distance of about .6 m to provide 170 lumens at the meat surface of each table. Ambient temperature, monitored continuously, was maintained at 2.2 C except during the defrost cycle that occurred every eight hours during which temperature rose to 5.5 C for about 5 minutes. Each day, the colors of steaks and ground beef were monitored both visually and electronically. For visual appraisal, both lean color and percent of the surface that was discolored were evaluated daily by a eight member, trained panel between 0800 and 0900. Lean color and percent discoloration of the loin steaks and ground beef were evaluated independently on the day of processing (day 0) and daily for the next five days. Each panelist used an eight point discrete scale to rate the meat in each package with one of the following shades of color; 1) Extremely Dark Brown or Green, 2) Very Dark Brown, 3) Dark Red or Brown, 4) Moderately Dark Red, 5) Slightly Dark Red, 6) Cherry Red, 7) Moderately Bright Cherry Red, 8) Bright Cherry Red. Discoloration was measured as a percentage of the total surface; panelist recorded their trained opinion. Location of each meat package was changed each day using randomly generated locations to balance for location effects. For electronic appraisal of color, light reflectance measurements were taken each morning using a hand held Minolta Color Monitor; two readings were taken across the face of each sample, in the same

location, each day. Output values (L, a*, b*) were recorded. On day 6, all meat was discarded.

Visual Panel Training. Eight panelist (7 males, 1 female) were trained to evaluate color and discoloration of ground beef. Past experience with meats evaluation of panelists ranged from serving as a meats judging coach to a newly arrived research assistant. To train the panel, two 1 pound packages of ground beef were purchased at a local retail store daily on each of the five days preceding the training period; the two final samples being purchased on the day of training. These samples were displayed as described above until training of the panel began. The panelists were shown the color and discoloration of these ground beef samples of known age so that they could visualize to various shades of red and green to expect during the study. Discoloration also was discussed. A color photo of these samples plus a sheet depicting various shades of red and discoloration was made and kept available for reference during the trial.

TBA Measurements. Ground knuckles displayed in the cooler were used for this analysis. These samples had been frozen following sub-sampling for microbial analysis. These ground knuckle samples were allowed to defrost for 24 h at about 4 C prior to analysis. Following the procedures of Witte et al. (1970), concentrations of thiobarbituric acid-like (TBA) substances in the ground beef were measured. A ten gram sample, taken after thoroughly mixing the

larger sample, was obtained from each ground beef package that previously had been on displayed for up to 5 days. This 10 g sample was homogenized for 60 seconds first with 50 ml deionized water then homogenized an additional 15 seconds after adding 50 ml of a chilled TCA-H_3PO_4 solution. Following blending, 30 ml of the supernatant was centrifuged for 30 minutes at 3000 x g and filtered. To 2 ml of filtrate in a 16 x 100 mm test tube, 2 ml of TBA solution were added and the mixture was incubated for 15 hours in the dark at room temperature for color development. Optical density measurements at 533 nm were compared to those obtained from standard solutions of TBA.

Total Microbial Counts. Microbial analysis was performed at the OSU Food and Technology Center using samples of unfrozen ground beef. Water extracts, at dilutions of 10^1 to 10^{10}, were incubated in duplicate with agar media and petri film media for 24 h at 38 C. Colonies that grew were counted to estimate the most probable number of bacteria. Coliforms were determined with petri film media with Escherichia coli being specifically identified by presence of a gas pocket around the coliform culture.

Statistical Analysis. For color evaluations, data were analyzed as factorial design to test main effects and interactions with repeated measures being analyzed as a split plot in time. The experiment was analyzed as a 2 (rib vs ground beef) by 2 (cooler vs case) by 2 (Agrado vs control) by 5 (days of aging prior to fabrication) factorial with repeated measurements over time (5 days on

display). Each animal served as an experimental unit with color appraisals from multiple evaluators or multiple Minolta color readings within a meat sample and day being averaged prior to data analysis. Main effects and interactions were tested by the 4-factor interaction. Effects of display day and interactions of days of day with the factors above was considered to be a subplot and was tested by the residual error term after including each factor and interaction in the total model.

Results and Discussion

Animal weights, gains, carcass weights, and dressing percentages are presented in Table 2. Initial and final live weight and carcass weight were greater for steers fed the control diet than for those fed the diet supplemented with Agrado. However, ADG and feed efficiency slightly favored (3 and 3%, respectively) steers supplemented with Agrado.

TABLE 2. Performance and carcass weights of cattle fed test diets for 29 days.

Measurement	Treatment		Probability, P <
	Control	Agrado	
Steers, no.	10	10	
Initial weight, kg.	519	489	.02
Final weight, kg.	565	536	.03
Daily gain, kg	1.48	1.52	.89
Feed intake, kg	10.22	10.22	.99
Feed/gain	6.91	6.72	.89
Carcass weight, kg.	352	332	.06
Dressing percentage	62.43	62.02	.65

Shelf Life Measurements. Knuckle ground beef (knuckle) and ribeye steaks (steaks) both were displayed in a simulated retail case environment (cooler) and a retail case (case) with additional samples of ground ribeye being displayed as 113 g patties or a 1 pound package (chub) in the cooler. Effects of cut (ground beef vs steaks), location (cooler vs case), aging (4 to 9 days from harvest until processing), and diet (with vs without 150 ppm added Agrado), and the two and three way interactions between these effects on visual color and discoloration scores and Minolta readings are presented from the full analyses in Table 3. Effects of display time or shelf life will be discussed later.

TABLE 3. Significance of main treatment effects and interactions.

Effect	Visual score	Discolor %	Minolta L value	Minolta a value	Minolta b value
Cut	.01	.01	.01	.01	.01
Aging days	.02	.13	.29	.01	.01
Cooler vs case	.99	.37	.89	.01	.01
Agrado	.70	.78	.05	.14	.75
Cut*age	.19	.08	.96	.03	.10
Cut*cooler vs case	.04	.17	.01	.01	.10
Cut*Agrado	.25	.86	.82	.01	.07
Age*cooler vs case	.48	.15	.90	.02	.15
Agrado*age	.08	.03	.03	.01	.80
Agrado*cooler vs case	.21	.22	.68	.27	.34
Cut*age*cooler vs case	.44	.15	.75	.05	.40
Cut*age*Agrado	.22	.09	.86	.06	.42
Age*Agrado*cooler vs case	.80	.82	.26	.06	.66

Means for knuckle and ribeye are presented in Table 4. On the average, brightness of lean was less for ribeye steaks than ground knuckles visually

although based on Minolta a values, ribeyes were less red. Percent discoloration averaged across the 5 days was greater for ground beef samples than for ribeye samples. Cuts that had aged longer prior to processing were brighter red by both visual and colorimetric measurements with an increase that was almost linear for both measurements up to an aging time of 8 days.

TABLE 4. Mean values for various main effects.

Effect	Visual score	Discoloration percentage	Minolta a value
Knuckle	6.35	10.49	18.94
Ribeye	6.00	6.21	17.78
Probability, P <	**.01**	**.01**	**.01**
Aging days			
4 days	6.05	8.86	17.93
5 days	5.99	8.08	17.84
6 days	6.34	6.45	18.30
7 days	6.29	9.82	18.57
8 days	6.18	8.53	19.16
Probability, P <	**.02**	.13	**.01**
Case	6.17	8.69	18.95
Cooler	6.17	8.01	17.77
Probability, P <	.99	.37	**.01**

No significant differences between samples displayed in the case versus those in the cooler were detected visually or as percent discoloration, but meat cuts on the average stayed brighter in the case than in the cooler. An interaction between cut and cooler vs case was detected. This effect is shown in Table 5. Visual scores were higher in the cooler than in the case for ribeye steaks while the opposite was true for knuckles. In contrast, mean Minolta a values (redness)

was less for both meat cuts in the cooler than in the case but the difference between locations was greater for knuckles than for ribeye steaks.

No main effect of Agrado was detected in this trial although interactions between cut and Agrado and between aging and Agrado supplementation were detected. Mean Minolta a value (red coloration) was greater in ribeye steaks from cattle fed Agrado than cattle fed the control diet while no difference was detected with ground beef.

TABLE 5. Interactions among main effects.

Effect	Visual score	Minolta a value
Ribeye in cooler	6.07^{c}	17.40^{c}
Ribeye in case	5.93^{d}	18.15^{b}
Knuckle in cooler	6.28^{b}	18.13^{b}
Knuckle in case	6.41^{a}	19.74^{a}
Ribeye - control diet	5.98	17.45^{c}
Ribeye - Agrado diet	6.02	18.11^{b}
Knuckle - control diet	6.38	19.10^{a}
Knuckle - Agrado diet	6.30	18.78^{a}

The impact of days on display and interactions between display time and main effects are presented in Table 6. With longer display, cuts lost red color as measured visually or with the colorimeter and discoloration increased. However, cuts responded differently with color scores for knuckles dropping faster and discoloration increasing faster than for ribeye steaks. Discoloration increased faster for carcasses than had been aged longer prior to processing and Minolta a values decreased faster during display in the cooler than in the case.

TABLE 6. Significance (P <) of the impacts of duration of display and interactions of main effects with display days on color and discoloration of steaks and ground beef.

Effect	Visual score	Discoloration percentage	Minolta a value
Days of display	**.01**	**.01**	**.01**
Day within cut	**.01**	**.01**	**.01**
Day within carcass aging	.20	**.01**	.19
Day within case vs cooler	.07	.15	**.01**
Day within Agrado	.57	.54	.36
Day within cut*carcass aging	.53	**.01**	.49
Day within cut*cooler vs case	**.01**	.64	**.01**
Day within cut*Agrado	.95	.97	.99
Day within aging*cooler vs case	.59	**.01**	.77

As an example of the effects of display time on color as appraised visually and electronically, effects of feeding Agrado on scores for knuckles in the cooler are presented in table 7. On the day of processing (day 0), both the controls and Agrado treated knuckle had slight, bright cherry red color with no discoloration. Visual appraisal on day 1 found a numerically small difference in color; this was supported by Minolta a* values, but knuckles from steers fed the diet containing Agrado had a numerically higher percentage discoloration; both treatments had began to discolor. By day 2, visual color scores for both treatments had decreased from bright cherry red to cherry red with no statistical difference due to Agrado feeding although Minolta a* values were greater (P<.03) for knuckles from cattle fed the control than for those fed the Agrado diet. Beyond 4 days of display, cattle fed both diets had similar color readings but discoloration percentage continued to increase.

TABLE 7. Ground Knuckle Visual and Minolta a* Least Square Means: Probabilities (4 Days of Display)

Visual (8 is Optimum)				**Minolta a*** (High Value Optimum)			
Day	Treatment	Mean	Prob. <	Day	Treatment	Mean	Prob. <
0	0	7.77	.96	0	0	23.51	.16
	29	7.77			29	22.76	
1	0	7.68	.63	1	0	20.00	.24
	29	7.61			29	19.14	
2	0	6.66	.52	2	0	19.80	**.03**
	29	6.54			29	18.63	
3	0	6.06	.85	3	0	18.30	.86
	29	6.10			29	18.19	
4	0	5.17	.55	4	0	15.45	.72
	29	5.29			29	15.68	

Effects of feeding Agrado on color of ribeye steaks displayed in the cooler are presented in Table 8. Steaks had relatively similar color readings both visually and mechanically of only moderately cherry red. This suggests that these steaks did not fully "bloom" to becoming a bright cherry red color. During the first day of display, steaks from cattle fed both diets began to decline in color and discoloration began. Although control steaks had no color advantage on day 2, steaks from cattle fed Agrado had greater ($P<.04$) in discoloration (2.1 vs .8%). This advantage continued on day 3 ($P<.02$) with values of 6.5% and 3.8% discoloration but yet no color differences were detected. By day 4, all steaks were quite severely discolored but no difference in percentage discoloration was detected. The color decline and discoloration continued on day 5 for cattle fed both diets.

Table. 8 Cooler Ribeye Steaks Visual and Discoloration Least Square Means: Probabilities

Visual (8 is Optimum)				**Discoloration** (%)			
Day	Treatment	Mean	Prob. <	Day	Treatment	Mean	Prob. <
0	0	7.06	.30	0	0	0.00	
	29	6.93			29	0.00	
1	0	6.64	.36	1	0	.14	.33
	29	6.52			29	.36	
2	0	6.54	.79	2	0	.80	**.04**
	29	6.50			29	2.10	
3	0	5.63	.55	3	0	3.84	**.02**
	29	5.74			29	6.52	
4	0	5.38	.70	4	0	9.09	.28
	29	5.31			29	11.17	
5	0	4.80	.73	5	0	16.67	.67
	29	4.87			29	18.03	

Steaks maintained in the meat display case began the study with a moderately bright cherry red color, visually, and only a small difference in Minolta a* values due to Agrado feeding. Unexpectedly, both treatments began to discolor on the first day but no treatment effect was detected. The color decline continued on day 3; at this time, steaks from steers fed Agrado tended to be more discolored ($P < .08$) than steaks from steers fed the control diet (9 vs 6.5%). However, by the fourth day of display, Minolta a* values favored the Agrado steaks ($P<.07$). Even though all steaks had discolored and visual scores had declined, the Agrado steaks by day 5 had more desirable Minolta a* value ($P<.05$). However, due to loss of the bright red color and areas of severe discoloration, the meat manager of a retail store probably would have discarded all these steaks after day 1 of display.

Ribeye patties had bright cherry red color on the initial day of display in the cooler. Discoloration began on day 1 in both treatments although color was maintained; this is contradictory to results found in a previous study conducted in the same facility where no discoloration appeared on Agrado treated patties until about the fourth day of display (Krumsiek et al., 1998). Patties from steers fed both the Agrado and the control diet discolored and declined in color desirability at similar rates during the remainder of the 5 day case life study. Upon completion of the study, all patties had greater than 50% discoloration and an undesirable color.

Ground ribeye chubs when initially displayed in the cooler had a moderately cherry red color and similar Minolta a* values. A moderate decline in color was exhibited on day 1 with discoloration beginning across the entire surface of both treatments. By day 2, discoloration was greater ($P<.02$) for chubs from steers fed Agrado (2.1% vs .8%) although color evaluations showed no difference. While color decline was constant between treatments, discoloration was inconsistent on day 3. The mean percentage of area that had discolored remained greater ($P < .02$) for chubs obtained from cattle fed Agrado (6.5% vs 3.8%). This difference decreased on day 4 as chubs from control cattle rapidly discolored; color scores were the same for both treatments. Chubs from cattle on both diets had a slightly dark red color and averaged 15% discoloration on day 5. As with the patties, partially discolored chubs probably would have been discarded on day 1 in a retail store.

TBA Results. Thiobarbituric acid test (Fig. 1) results indicate that the ground knuckles displayed in the cooler had less malonaldehyde on the first day and throughout the display time for meat samples obtained from cattle fed Agrado than from cattle fed the control diet. This suggests that Agrado is protecting against rancidity. This observation agrees with results of a previous study conducted in the same facility (Krumsiek et al. 1998).

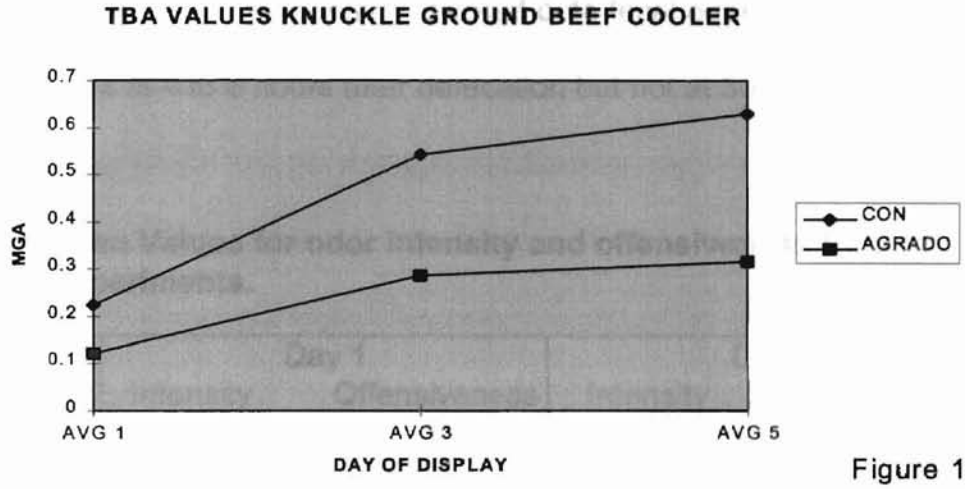

Figure 1

Microbial Counts. When log transformed prior to statistical analysis, a standard microbiological procedure, no statistical significance between dietary treatments was detected in coliform count. For total plate counts were made, least square means were greater ($P < .05$) for ground knuckle from cattle fed Agrado than from cattle fed the control diet (3.44 vs 3.18) on day 1. No differences were detected on subsequent days.

An impact of length of aging on microbial counts was detected. The longer the aging time prior to processing, the greater the microbial counts. This suggests that for low microbial counts, shorter aging times would be preferable.

Fecal Odor. Mean values for odor and offensiveness for both studies are presented in table 9. Results were similar in direction to those from our previous study. When findings from the two studies were merged, results indicate that feeding of Agrado to feedlot cattle lowered both fecal odor intensity and offensiveness at 4 to 6 hours after defecation but not at 30 hours after defecation.

Table 9. Mean Values for odor intensity and offensiveness for both experiments.

	Day 1		Day 2	
Experiment	Intensity	Offensiveness	Intensity	Offensiveness
I C	5.17	5.18[a]	4.95	5.00
I A	4.53	3.98[b]	5.53	5.17
II C	4.82	5.17	4.65	4.01
II A	4.16	4.28	5.19	4.54
I and II	4.99[c]	5.19[a]	4.80	4.51
I and II	4.35[d]	4.12[b]	5.36	4.85

[a b] Means within a square with different superscripts differ (p<.01).
[c d] Means within a square with different superscripts differ (p<.05).

Case versus Simulated Case Comparison

Previous shelf life studies were conducted in a simulated meat case, not a commercial retail case. This may have altered the environmental stresses on the meat samples. In this study, identical beef retail cuts were displayed in both

a retail case and the cooler which was designed to simulate a retail case. Values for steaks and ground knuckle displayed under the two conditions are presented in table 4. Color differences between ribeyes and ground beef were significant each of the 5 display days. Compared to meat cuts in the case, cuts in the cooler generally had lower visual scores though the visual difference on any single day was never significant. Minolta a values were significantly lower for cuts in the cooler than cuts in the case on days 0, 1, 4, and 5. In contrast, meat degradation appraised by the percentage of each cut that was discolored on any day was judged visually to be lower for cuts in the cooler than in the case. This suggests that percentage discoloration may not necessarily parallel loss of red coloration of the cut. Some interactions between cut and location were detected although those differences are difficult to explain. Minolta a* values generally seemed more sensitive at detecting smaller color differences than the color evaluation panel suggesting that both visual and mechanical measurements should be utilized when evaluating meat discoloration.

Implications

Supplementing Agrado in the diet of feedlot cattle for the final 28 days of feeding had no detectable or consistent impact on case life of beef retail cuts in this trial. This finding does not match the results of our first study with this product. Although cuts from cattle fed Agrado had lower malonaldehyde concentrations, no advantage in color or degree of discoloration was detected

with either ground beef knuckles or ribeye steaks. In this study, supplementing with Agrado tended again to slightly increase daily gain and feed efficiency. Only slight differences between meat cuts maintained in a commercial meat display case and cuts maintained in a cold room with lighting to simulate the display case were detected except that loss of color appeared greater for cuts in the cold room while percentage of the cut discolored frequently was greater for cuts in the display case.

Table 10. Cut by Location and Case vs Cooler Visual, Discoloration, Minolta Readings, Least Square Means Comparison and Interaction.

Group Least Squares Means						Prob., P<	
Display Day	GB-Case	GB-Cooler	RE-Case	RE-Cooler	GB vs RE	Case vs Cooler	Interaction
Visual Score							
0	7.78	7.79	7.24	7.06	**0.01**	0.11	0.07
1	7.53	7.66	6.62	6.60	**0.01**	0.46	0.28
2	6.90	6.64	6.06	6.60	**0.01**	0.07	**0.01**
3	5.94	6.12	5.69	5.83	**0.03**	0.07	0.83
4	5.57	5.29	5.01	5.45	**0.02**	0.4	**0.02**
5	4.85	4.40	4.92	4.91	**0.03**	0.09	0.12
Discoloration, %							
0	0.00	0.00	0.00	0.00			
1	0.67	0.69	0.24	0.17	**0.02**	0.9	0.64
2	4.30	4.60	3.40	0.90	**0.01**	**0.02**	**0.03**
3	14.70	12.10	7.60	4.60	**0.01**	**0.01**	0.87
4	13.30	13.70	13.10	9.00	0.15	0.27	0.19
5	27.10	30.80	17.60	16.90	**0.01**	0.6	0.44
Minolta L value							
0	46.47	45.99	39.11	39.84	**0.01**	0.8	0.22
1	44.40	42.93	39.41	39.43	**0.01**	0.04	**0.04**
2	45.11	44.34	40.12	39.96	**0.01**	**0.1**	0.28
3	45.02	43.45	39.29	41.36	**0.01**	0.37	**0.01**
4	45.24	45.28	39.45	40.94	**0.01**	0.06	**0.01**
5	45.30	44.96	40.31	41.44	**0.01**	**0.2**	**0.02**
Minolta a value							
0	24.05	23.18	18.77	18.16	**0.01**	**0.01**	0.62
1	21.41	19.76	18.74	18.10	**0.01**	**0.01**	0.12
2	19.23	19.36	18.27	17.67	**0.01**	0.39	0.19
3	18.99	18.34	18.03	17.04	**0.02**	0.06	0.55
4	17.85	15.73	17.76	17.09	0.06	**0.01**	**0.04**
5	17.20	13.25	17.30	16.41	**0.01**	**0.01**	**0.03**
Minolta b value							
0	12.33	11.55	9.61	8.51	**0.01**	**0.01**	0.32
1	11.60	10.70	8.92	8.72	**0.01**	**0.01**	0.09
2	10.51	10.67	7.74	8.26	**0.01**	**0.02**	0.24
3	9.54	10.20	8.17	7.54	**0.01**	0.9	**0.02**
4	9.92	8.29	8.35	7.83	**0.01**	**0.01**	0.06
5	9.96	8.12	7.90	7.66	**0.01**	**0.01**	0.01

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Chapter VIII

Agrado™ for Receiving Cattle: Effects on Performance and Morbidity.

C. L. Krumsiek[1], F.N. Owens[1], S. A. Silzell[2], E. B. Kegley[2], D. H. Hellwigh[2], D. R. Gill[1], and W. A. Samuels[3] , [1]Oklahoma State University, Stillwater, 74078, [2]University of Arkansas, Fayetteville 72701, and [3]Solutia Inc., St. Louis, MO 63166.

Abstract

Ninety-six crossbred newly received heifers averaging 206 kg were fed 0 or 150 mg/kg Agrado (in diet) for 42 days following transport while being monitored for feed efficiency, rate of gain, and morbidity from bovine respiratory disease. The diet, consisting of 53% cracked, corn grain, 30% cottonseed hulls, and 11% soybean meal, was fed as a total mixed ration. Heifers were blocked by weight into 8 groups and assigned randomly within weight block to pen resulting in 8 pens (48 heifers) per treatment. Weights were measured initially, 14, 28, and 42 days later; no significant treatment effect on weight or ADG was detected. However, Agrado reduced (P<.02) the number of heifers that were diagnosed as morbid which reduced (P<.05) medical cost from $8.45 to $5.75 per head during the 42 day receiving study. Adding Agrado to the receiving ration of calves appears to have an early antioxidant effect thus reducing morbidity.

Introduction

The key to making an animal production system profitable and efficient is to keep animals growing. This becomes difficult when unacquainted animals are stressed, deprived of feed and water, transported to unfamiliar surroundings and penned or housed together. Intermingling animals, common during both receiving and marketing, adds to the stress already present during these periods. Generally, cattle are bought from several locations, loaded on a semi-trailer and transported some distance to a feedlot. During this time feed and water is not available. Upon the arrival at the feeding facility the animal is susceptible to disease due to a non-functioning rumen and a depressed immune system. Although not all cattle arrive under stress, merely removing animals from their native environment and translocating them to a confined facility can induce stress. During stress, cattle often refuse to eat or eat less complicating the problem. The adrenal hormones norepinephrine and epinephrine are released under stressful situations. Epinephrine induced reactions lead to formation of free radicals that cause lipoperoxidation (Nockels et al., 1996); these in turn are detrimental to the immune system. With stress and feed refusal, the problems of morbidity, mortality, and poor performance become paramount concerns for managers receiving cattle.

Supplementing the diet with antioxidants, may combat poor performance, mortality, and morbidity of stressed cattle. In a review of 5 published articles, Secrist et al. (1996) found that supplementation of the diet with vitamin E

increased average daily gain and feed:gain in receiving cattle subjected to transport stress. Morbidity tended to be reduced with vitamin E supplementation. Creatine kinase, a measure of cellular damage, typically increases during stress. When vitamin E was topdressed (1000 IU/d) 28 days prior to subjecting cattle to stress and injecting them with ACTH, the increase in creatine kinase was reduced in Charolais heifers; liver alpha-tocopherol levels also were higher than in non-supplemented vitamin E heifers (Nockels et al., 1996). Accumulation of vitamin E in the liver could help sustain health of receiving cattle by reducing lipid peroxidation in this vital organ. If high rates of supplementation increase the muscle tissue concentrations of this vitamin, its antioxidative properties may prove beneficial for poultry as well. Leghorn x Rhode Island Red laying hens (34 weeks old) supplemented with either 125 mg/kg vitamin E or 250 mg/kg ethoxyquin had lower mortality during a Newcastle Disease outbreak (Bartov et al., 1991). Although antioxidant use for stressed animals may prove advantageous, further research is needed to determine the relative value of antioxidants other than vitamin E and to test the impact of the antioxidant Agrado on health, growth, and performance of stressed animals.

Materials and Methods

Cattle Management. Ninety - six crossbred heifer calves having an average weight of 206 kg were blocked by weight and randomly assigned within each of 8 weight blocks to one of 16 pens containing 6 heifers each. Cattle arrived at the

University of Arkansas research facility as one group after being procured from several different sale barns. Heifers were fed and health was monitored for 42 days (Dec. 4, 1997 - Jan. 15, 1998) with either 0 or 150 ppm Agrado, added to the diet, to examine the effects of Agrado on growth, feed efficiency, and incidence of morbidity. The receiving diet consisted of a totally mixed ration of 53% cracked corn grain, 30% cottonseed hulls, and 11% soybean meal (as fed basis). Heifers were weighed initially and every 14 days thereafter. Heifers were housed in partially covered pens with fence line bunks and observed daily for signs of morbidity.

Statistical Analysis. General Linear Models Procedure of SAS was used for statistical analysis with weight block and Agrado concentration included as class variables. Pen means were used in all calculations.

Results and Discussion

Initial and interim weights and ADG of heifers were not significantly due to treatment (Table 1). Vitamin E, in contrast, often has increased ADG of newly received cattle (Secrist et al., 1996). The number of animals repulled to be medicated and the date animals were pulled for first treatment was not altered by Agrado. However, heifers fed Agrado exhibited less morbidity ($P<.02$) that in turn reduced($P<.05$) medical cost. The average cost of medication was $8.45 and $5.75 per head for the controls and Agrado heifers, respectively, the 42 day

study. These findings suggests that Agrado, probably through acting as an antioxidant, may enhance health status and lower the incidence of disease of newly arrived cattle.

Table 1. Initial and Interim Weights; Average Daily Gain.

	Weights (lb.)				
Day	CONTROL	AGRADO	Day	CON	AGRADO
Initial	431	436			
14	454	454	0-14	-.01	.05
28	457	458	0-26	1.78	1.97
42	503	503	0-42	1.88	1.96

Implications

Supplementing Agrado in the diet of newly arrived cattle could reduce medical cost during the receiving phase. This reduction in initial cost could benefit the cattle feeder by increasing the net return on investment.

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VITA

Charles L. Krumsiek

Candidate for the Degree of

Master of Science

Thesis: AGRADO™ FOR FINISHING CATTLE: EFFECTS ON PERFORMANCE, CARCASS MEASUREMENTS, AND MEAT QUALITY

Major Field: Food Science

Biographical:

Education: Graduated from Charles Page High School, Sand Springs, Oklahoma in May 1984; received Bachelor Of Science Degree in Animal Science / Agricultural Education from Oklahoma State University in December 1988. Completed the requirements for the Master of Science Degree with a Major in Food Science at Oklahoma State University in May 1998.

Experience: Owned a purebred Duroc swine operation in partnership with my Brother and Father in conjunction with being employed as Sheet Metal Fabricator and Welder during high school. Worked at University Feed Mill for the first year of college. Territory Manager for Manna Pro Corp. in Tampa, Florida for two years; Livestock Production Specialist for Farmland Industries in McGregor Texas. Mineral and protein Territory Sales Manager for P.M. Ag. Products in Montgomery, Alabama. Feed Department Manager for Lenora Mercantile located in Lenora, Kansas. Currently employed as a Research Assistant for the Animal Science Department at Oklahoma State University, Stillwater, Oklahoma.