

Effects of alternative feeding strategies for feedlot cattle on meat quality

by

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B.S., North Dakota State University, 2012

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry  
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## Abstract

American beef producers use a multitude of production regimens, with new products constantly becoming available to producers that could ultimately produce beef that fits niche markets. Additionally, U.S. producers employ the use of two exogenous growth promotants (**ExGP**), anabolic implants and  $\beta$ -adrenergic agonists, to maximize production efficiency. This body of work examined effects of different production strategies on beef quality. In the first study, steers were fed a conventional diet or a diet containing two supplements of the Programmed Nutrition Beef Program (**PN**) and each diet was fed with or without ExGP. There were no adverse effects on color, but use of ExGP negatively impacted tenderness of steaks. However, the inclusion of the PN supplements decreased purge loss of loins during aging and decreased cook loss of beef steaks. The decrease in purge and cook loss may be intriguing for retailer who purchase-in and cook products as they could specify a demand for beef from animals in this program to potentially save on product losses. Researchers have examined strategies to increase omega-3 fatty acids within beef, as omega-3 fatty acids are health beneficial. The second study examined impacts of feeding increasing levels of a docosahexaenoic acid (**DHA**)-rich microalgae to heifers on fatty acid profiles, color stability, and palatability of the LM and color and . Feeding increasing levels of microalgae meal quadratically increased total omega-3 PUFA, with increases in DHA content up to 850% and eicosapentaenoic acid (**EPA**) up to 340% at the greatest feeding level. Although feeding microalgae changed fatty acid profiles to be more health beneficial, color and flavor were adversely affected. At the end of display, steaks from heifers fed the greatest amount of microalgae had reduced  $a^*$  (redness) values and increases in surface metmyoglobin (discoloration) formation. Panelists detected more off-flavors as the level of microalgae meal increased in the diet. Poor color stability and

increases in off-flavors were due to increased oxidation products in these steaks, but problems could be mitigated by inclusion of antioxidants in the diet. The third study presented examined effects of feeding antioxidants to steers fed microalgae meal on color and palatability of *Longissimus lumborum* steaks. Steers were fed vitamin E at a level over their nutritional need and a selenium-yeast product in addition to feeding microalgae. Again, feeding microalgae without antioxidants in the diet negatively impacted color during display, but feeding antioxidants significantly improved the color stability. There were no off-flavor differences between steaks from steers fed the diet containing only microalgae and diet containing microalgae with antioxidants. Increasing the antioxidant content of the finishing diet when microalgae was fed is a feasible way to increase the color stability of steaks and decrease off-flavors of *Longissimus lumborum* steaks.

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Approved by:

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American beef producers use a multitude of production regimens, with new products constantly becoming available to producers that could ultimately produce beef that fits niche markets. Additionally, U.S. producers employ the use of two exogenous growth promotants (**ExGP**), anabolic implants and  $\beta$ -adrenergic agonists, to maximize production efficiency. This body of work examined effects of different production strategies on beef quality. In the first study, steers were fed a conventional diet or a diet containing two supplements of the Programmed Nutrition Beef Program (**PN**) and each diet was fed with or without ExGP. There were no adverse effects on color, but use of ExGP negatively impacted tenderness of steaks. However, the inclusion of the PN supplements decreased purge loss of loins during aging and decreased cook loss of beef steaks. The decrease in purge and cook loss may be intriguing for retailer who purchase-in and cook products as they could specify a demand for beef from animals in this program to potentially save on product losses. Researchers have examined strategies to increase omega-3 fatty acids within beef, as omega-3 fatty acids are health beneficial. The second study examined impacts of feeding increasing levels of a docosahexaenoic acid (**DHA**)-rich microalgae to heifers on fatty acid profiles, color stability, and palatability of the LM and color and . Feeding increasing levels of microalgae meal quadratically increased total omega-3 PUFA, with increases in DHA content up to 850% and eicosapentaenoic acid (**EPA**) up to 340% at the greatest feeding level. Although feeding microalgae changed fatty acid profiles to be more health beneficial, color and flavor were adversely affected. At the end of display, steaks from heifers fed the greatest amount of microalgae had reduced  $a^*$  (redness) values and increases in surface metmyoglobin (discoloration) formation. Panelists detected more off-flavors as the level of microalgae meal increased in the diet. Poor color stability and

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## **Dedication**

This document is dedicated to those who have believed in and encouraged me during my time as a graduate student, especially to my parents, Rick and Barb, and my husband Kendall.

## Chapter 1 - Introduction

Antibiotics, ionophores, implants, and beta-adrenergic agonists are used routinely in conventional systems of feedlot cattle production. When used, feed additives such as monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and tylosin (Tylan, Elanco Animal Health) improve feedlot performance. Monensin, an ionophore that also is fed to prevent and control coccidiosis, improves feed efficiency while decreasing feed intake (Potter et al., 1985). Feeding tylosin greatly reduces the incidence of liver abscesses (Potter et al., 1985), which when present in an animal can decrease performance and dressing percentage. When fed in combination, monensin and tylosin increase ADG and feed efficiency by 3% and 4 %, respectively (Stock et al., 1995).

Aside from feed additives like monensin and tylosin, exogenous growth promotants such as hormonal implants and beta-adrenergic agonists (**BAA**) also improve efficiency of production in commercial feedlots. Over 97% of cattle entering commercial feedlots are implanted at least once (Duckett and Andrae, 2001), which can increase ADG and improve feed efficiency by 18% and 8%, respectively (Duckett et al., 1996). Implanting feedlot cattle also increases HCW and loin muscle area (**LMA**) (Duckett and Andrae, 2001). Beta-adrenergic agonists, such as ractopamine hydrochloride (**RAC**; Optaflexx®, Elanco Animal Health) are a class of compounds that act as repartitioning agents. These compounds have received this classification because their mode of action improves feed efficiency and lean tissue deposition during the final days of finishing by directing nutrients to muscle accretion at the expense of adipose tissue (Mersmann, 1998). Feeding a beta-agonist for the final 20 to 30 days of finishing greatly improves feed efficiency, ADG, HCW, and yield grades of carcasses (Montgomery, et al., 2009a). Advantages in live performance that are attributed to exogenous steroids and BAA often are accompanied by

deleterious impacts on carcass quality and meat tenderness. Mader et al. (1999) reported that implanted cattle produce carcasses with lower marbling scores, while Roeber et al. (2000) found that steaks from implanted cattle had greater Warner-Bratzler shear force (**WBSF**) scores than cattle that were not implanted. Also, Kellermeier et al. (2009) reported increased WBSF scores and decreased marbling scores for cattle supplemented zilpaterol hydrochloride with or without implants.

In addition to conventional finishing strategies, producers can choose from a plethora of feedstuffs and supplements to feed to cattle in order to potentially market them to specific markets. One specific thing producers can do is feed supplements to manipulate the fatty acid profile of beef which typically contains greater amounts of saturated fatty acids that could lead to health problems. Many researchers have explored ways to increase omega-3 fatty acids in beef products to make beef more health beneficial. Omega-3 fatty acids are a family of PUFA that provide numerous health benefits including reduced risk of CVD, type-2 diabetes, and cancer (Ruxton et al., 2004; Calder, 2014). In particular, long chain omega-3 fatty acids including eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) are the most functionally active within the body (Calder, 2014). Both EPA and DHA are found in copious amounts in fatty fish such as salmon and trout, but are found in marginal amounts in beef products. Because most Americans do not consume adequate amounts of fatty fish, some research has focused on manipulating the fatty acid profile of beef to contain greater amounts of omega-3 fatty acids. The main strategy used to manipulate the fatty acid profile of beef is through the dietary supplementation of oilseeds, plant oils, fish oil, marine algae, and fat supplements (Woods and Fearon, 2009). Supplementation of flaxseed and fish oil increases omega-3 content of beef derived from forage- and grain-fed cattle (Vatansever et al., 2000; Wistuba et al., 2006; LaBrune

et al., 2008, Kronberg et al., 2011). Although the increase in omega-3 fatty acid content can make beef products healthier, there is concern over the effect that increasing PUFA of beef will elicit on meat quality. Increasing omega-3 fatty acid content of beef can decrease color stability during display (LaBrune et al. 2008; Kronberg et al., 2011) and increase off-flavors of cooked product (Vatansever et al., 2000; Wistuba et al., 2006; LaBrune et al., 2008).

Variations in finishing strategy for feedlot cattle, leads to variability in palatability of beef products. Since the 1980s, the beef industry has conducted several audits of the beef industry to evaluate if beef products produced are satisfactory for consumers. According to interview results from the 2010 Beef Quality Audit, nearly 30% of retailers, 24% of food service and government sectors, and 20% of packers reported eating satisfaction as the most important concern for the beef industry. When interviewees were further asked to describe factors impacting eating satisfaction, 64% of government entities, 44% of feeders, 65% of packers, 52% of food service entities, and 67% of retailers considered tenderness to be the most important factor for consumer eating satisfaction (Igo, et al. 2013). Additionally, consumers are willing to pay premiums for products that are guaranteed tender. Lusk et al. (2001) found 51% of consumers were willing to pay a \$1.84 per 0.45 kg premium for beef that carried a tenderness guarantee. Since tenderness is very important in determining consumer eating satisfaction and consumers are willing to pay for guaranteed products, USDA has implemented Certified Tender and Very Tender programs (ASTM, 2011). The first National Consumer Retail Beef Study was conducted in 1985 (Savell et al., 1987, 1989) with other surveys occurring in 1993 (Neely et al. 1998), 1998 (Brooks et al., 2000), 2006 (Voges et al., 2007), and 2010 (Guelker et al., 2013). Each study was designed in order to examine the impact of quality grade which is used to sort

carcasses based on potential eating quality (USDA, 1997), and examine the palatability of different steaks that are available for retail or foodservice for purchase by consumers.

## **NATIONAL CONSUMER RETAIL BEEF STUDY**

In Phase I of the National Consumer Retail Beef Study, Savell et al. (1987) evaluated the impact of USDA quality grade on the palatability of top loin steaks. Strip loins from seven marbling levels were selected and shipped to consumers in Philadelphia, PA, Kansas City, MO, and San Francisco, CA, where panelists cooked steaks in the way they would normally prepare them (both cooking method and degree of doneness) and then evaluate the top loin steaks for overall desirability. Marbling was highly significant in all 3 cities. Consumers in Kansas City and San Francisco rated the overall desirability of top loin steaks in each marbling category similar. Consumers in Philadelphia rated steaks at the greater marbling categories similar to those in San Francisco and Kansas City, but had decreased ratings for the Upper Slight, Lower Slight, and Traces marbling categories. Consumers in Philadelphia cooked steaks to greater degrees of doneness compared to those in Kansas City and San Francisco. In Philadelphia, 36% of households cooked their steaks well done compared to 25% in San Francisco which could be a reason for divergences in overall desirability ratings. Also, differences in overall desirability ratings could be because Philadelphia was a “Choice” grade market whereas San Francisco was a “Select” market. Overall, this study demonstrated consumers in different regions respond to different marbling levels, which provided information in how to potentially market beef by region.

Phase II of the National Consumer Retail Beef Study was designed to evaluate the interaction of trim level, price, and grade on consumer acceptance of beef steaks and roasts (Savell et al., 1989). Consumers listed taste as the top reason for purchasing roasts and steaks

respectively. Other top reasons for purchasing beef roasts and steaks included value for money, ease of preparation, and nutritional value. Things that hindered purchasing roasts and steaks included price, how well they were trimmed, and the appearance of waste. Overall, taste was a positive influencer of purchasing decisions for consumers, but price and fat trim level were negative towards purchase decisions.

After purchase, consumers in both cities reported premium priced or completely trimmed cuts to be more appetizing, better tasting, less wasteful, and lower in cholesterol than regularly trimmed products. Regular trimmed products were wasteful and had a negative connotation for taste and healthfulness of beef. Consumers observed no visual difference between grades when external fat was the same, and they were also unaware two grades were available for purchase even though packages clearly stated the grade. Philadelphia consumers who purchased cuts that were trimmed more were less satisfied than if they had purchased regularly trimmed cuts. The main objections stated were the products were tougher and drier, this occurred even if products were in the same grade. Similar to Phase I of the study, it appeared degree of doneness and cooking method influenced these outcomes as consumers in Philadelphia cooked their products to greater degrees of doneness. Consumers in San Francisco were very satisfied with roasts and steaks with no external fat compared to those with some fat trim left assigning similar ratings for leanness, tenderness, juiciness, and taste. Consumers in both cities preferred the taste of Choice over Select Beef likely because they rated Select products as tougher. Overall, in Philadelphia, 62% of Choice and 66% of Select consumers would re-purchase product. Whereas in San Francisco only 48% of Select and 44% of Choice consumers indicated they would re-purchase the product. With extended use of the more trimmed products, satisfaction of consumers in Philadelphia improved from 63 to 78% and taste ratings increased from 51 to 69%. In San

Francisco, overall satisfaction level increased to 100% for all product categories. Examining the impact of grade with extended use indicated the satisfaction for Select beef in Philadelphia decreased from 72 to 53%, causing re-purchase to fall from 66 to 61%. Because satisfaction remained high in San Francisco the willingness to purchase Choice products increased from 44 to 59%. Phase II of the National Consumer Retail Beef Study clearly indicated regional differences for marbling and trim levels of beef steaks and roasts.

### **BEEF CUSTOMER SATISFACTION PROJECT**

A second market basket survey, the Beef Customer Satisfaction Project, was conducted in 1993 to evaluate top loin, top sirloin, and top round steaks from carcasses of five different quality grades and then distributed them to consumers in San Francisco, Houston, Chicago, and Philadelphia for in-home consumer testing.

Consumers in Chicago and Philadelphia rated Top Choice steaks greater for tenderness than the other quality grades, but Houston and San Francisco consumers were not able to perceive differences in tenderness of all grades. Consumers in all cities rated steaks top loin steaks greater for tenderness than top sirloin and top round steaks, and top sirloin steaks greater than top sirloin steaks. Juiciness ratings for Top Choice steaks were greater in Chicago and Philadelphia than the other quality grades in those cities, and in Houston and San Francisco all steaks from both Top Choice and Low Choice were rated greater for juiciness than Low Select steaks. Juiciness scores were related to USDA quality grade, but the greatest differences occurring seemed to be driven by the grade history of each city, with Chicago and Philadelphia being “Choice” cities and Houston and San Francisco being “Select” cities. Consumers in Chicago and Philadelphia rated Top Choice steaks greater for flavor desirability and intensity than High and Low Select. In contrast, Houston and San Francisco consumers rated Top Choice,

Low Choice, High Select similar for both flavor desirability and intensity. Across all grades and cities, top loin steaks were rated greater than both top sirloin and top round steaks, and top sirloin steaks received greater overall liking scores than top round steaks. Grade impacted top loin steaks the greatest with Top Choice rated greater than all other grades, High Select was rated similar to Low Choice and Low Select, and Low Choice greater than Low Select top loin steaks. In contrast, Grade had no impact on consumer overall liking of top sirloin steaks, but Top Choice top round steaks received greater overall liking than all the other grades of top round steaks.

In a second phase of the Beef Customer Satisfaction Project, the impact of cooking method and degree of doneness of top loin (Lorenzen et al. 1999), top sirloin (Savell et al., 1999), and top round (Neely et al., 1999) steaks on consumer palatability ratings were evaluated utilizing information from phase one. Overall, consumers in San Francisco and Philadelphia cooked top loin steaks to lower degrees of doneness (medium rare or less) when compared to Houston and Chicago consumers who cooked top loin steaks to medium well and well done more frequently (Lorenzen et al., 1999). In contrast, top sirloin steaks were cooked to well done or greater most frequently by consumers in all cities (Savell et al., 1999), and the medium degree of doneness was used more than medium rare or less. Similar to the top loin steaks, consumers in Houston tended to cook top sirloin steaks to greater degrees of doneness, whereas consumers in San Francisco cooked steaks to medium rare or less. Across all cities, consumers most frequently cooked top round steaks to well done or greater (Neely et al., 1999). When looking at cooking method, outdoor grilling was the most common method for cooking top loin steaks followed by broiling, pan frying, and indoor grilling (Lorenzen et al., 1999). Similarly, consumers in all cities utilized outdoor grill as the most common method to cook top round steaks, followed by broiling and pan-frying (Savell et al. 1999). Consumers in Philadelphia cooked top round steaks most

often by outdoor grilling, and Chicago consumers used simmer and stew. Also, consumers in San Francisco most often pan-fried top round steaks (Neely et al., 1999). Small differences were observed for palatability when looking across cooking method and degree of doneness of all steaks in each city.

## **NATIONAL BEEF TENDERNESS SURVEYS**

These early market studies provided an excellent platform for future national surveys. Instead of conducting separate market basket surveys, consumer palatability evaluations were conducted in accordance with the National Beef Tenderness Surveys (**NBTS**; Brooks et al., 2000; Voges et al., 2007, Guelker et al., 2013). While these surveys were designed to evaluate tenderness of beef steaks, consumer were also asked to evaluate juiciness, flavor, and overall liking. The reason for more closely examining the other palatability attributes is because Neely et al. (1998) found that in addition to tenderness ( $r = 0.85$ ), overall liking of products to be moderately to highly correlated with juiciness ( $r = 0.77$ ), flavor desirability ( $r = 0.85$ ), and flavor intensity ( $r = 0.79$ ). These data indicated flavor desirability and juiciness may be just as important as tenderness for consumer palatability.

### ***Tenderness***

In the 1998 NBTS (Brooks et al., 1998) found there was only trend for quality grade to impact consumer tenderness of retail steaks. Also, tenderness ratings for retail top sirloin, clod, and top round steaks was not different. There were no tenderness differences across quality grade groups for foodservice ribeye, top loin, and top sirloin steaks. Retail bone-in top loin, boneless top loin, ribeye, T-bone, and porterhouse steaks received the greatest consumer overall tenderness scores whereas round steaks received the lowest overall tenderness scores in the 2006 NBTS (Voges et al., 2007). Also, Voges et al. (2007) reported foodservice ribeye and top loin

steaks had greater consumer ratings for tenderness than top sirloin steaks, but consumers did not rate foodservice steaks different for tenderness across quality grades. In the 2010 NBTS, Guelker et al. (2013) reported retail top blade steaks received the greatest tenderness and tenderness liking scores compared to the other retail steaks. Also, steaks from the sirloin and round received lower tenderness and tenderness liking scores compared to steaks from the rib, loin, and chuck. Prime foodservice ribeye steaks received greater tenderness and tenderness liking compared to steaks from the other grades. Foodservice top loin steaks received greater tenderness and tenderness liking scores compared to ribeye and top sirloin steaks, but quality grade did not impact consumer tenderness of these steaks.

### ***Juiciness***

In contrast to Neely et al. (1998), the 1998 NBTS (Brooks et al., 2000) reported across all grades there were no differences in juiciness for retail ribeye, top loin, top sirloin, top round, and clod steaks or foodservice ribeye, top loin, and top sirloin steaks. Voges et al. (2007) reported retail bone-in top loin steaks received the greatest consumer juiciness scores compare to the other cuts and retail round steaks received the lowest scores. Also foodservice ribeye and top loin steaks had greater consumer ratings for juiciness than top sirloin steaks, but quality grade did not affect consumer juiciness ratings of these steaks. In the 2010 NBTS, retail top blade steaks received greater juiciness and juiciness liking scores than the other retail steaks. Also, steaks from the rib, loin, and chuck had greater juiciness and juiciness liking scores than steaks from the sirloin and round. Similar to tenderness, foodservice top loin steaks in the 2010 NBTS received greater juiciness and juiciness liking scores than ribeye and top sirloin steaks, but quality grade did not impact top loin juiciness. Within top sirloin steaks, ungraded steaks received greater

juiciness and juiciness liking scores compared top sirloin steaks from other grades which may be attributed to enhancement.

### ***Flavor***

In the 1998 NBTS, Brooks et al. (2000) found only retail ribeye steaks to be affected by grade for overall flavor scores. Prime steaks received the greatest overall flavor scores and Low Choice steaks received the least overall flavor scores compared to the other quality grades. However, there were no differences for sensory flavor ratings for retail top loin, top sirloin, and top round steaks, and no differences for foodservice ribeye, top loin, and top sirloin steaks. The 2006 NBTS (Voges et al., 2007) reported retail boneless ribeye, bone-in ribeye, boneless top loin, bone-in top loin, T-bone, and porterhouse steaks received the greatest beef flavor and flavor liking scores whereas round steaks received the lowest beef flavor and flavor like scores. Additionally, Voges et al. (2006) reported no differences in flavor scores for foodservice ribeye, top loin, and top sirloin steaks. Retail top blade and boneless ribeye steaks received greater flavor liking scores than the other retail steaks in the 2010 NBTS (Guelker et al., 2013). Also, round steaks received lower flavor liking and flavor intensity scores compared to retail steaks from the rib, loin, and chuck. Similar to both tenderness and juiciness, foodservice top loin steaks received greater flavor and flavor liking scores than ribeye and top sirloin steaks, but quality grade did not impact consumer flavor ratings of top loin steaks. Like juiciness, ungraded top sirloin steaks received greater flavor and flavor like scores compared to graded top sirloin steaks, again which is liking attributed to enhancement.

### ***Overall liking***

The 1998 NBTS (Brooks et al., 2000) reported retail ribeye steaks were impacted by grade for overall liking scores. Prime, ribeye steaks received greater overall liking scores than

ribeye steaks from the other quality grades, but these quality groups did not differ in overall liking scores. Across grade, there were no differences in overall liking scores for top loin, top sirloin, or top round steaks. Also, there were no overall liking differences for foodservice ribeye, top loin, and top sirloin steaks. The 2006 NBTS (Voges et al., 2007) reported retail bone-in top loin, boneless top loin, ribeye, T-bone, and porterhouse steaks received the greater consumer overall liking scores compared to retail round steaks. Similarly, foodservice ribeye and top loin steaks were given greater overall liking scores than top sirloin steaks. In the 2010 NBTS, Guelker et al. (2013) reported retail top blade steaks received greater overall liking scores compared to the other retail steaks and round steaks received the lower overall liking scores compared to the other steaks. Foodservice Choice and No-Roll ribeye steaks received lower ratings for overall liking than ribeye steaks in the other grades, and Prime ribeye steaks received greater overall liking scores than ribeye steaks in the other grades. Interestingly, foodservice top sirloin steaks had greater overall liking scores than ribeye and top loin steaks, which is possibly due to the application of enhancement to these steaks.

## **CONCLUSION**

The early consumer market basket surveys demonstrated consumers in different regions of the country have different perceptions of beef palatability. They also found flavor and juiciness to be strongly correlated with overall palatability/desirability of steaks. The NBTS indicated retail steaks from the rib, loin, and chuck are perceived as more tender, juicier, and flavorful than steaks from the sirloin and round which lead to greater overall liking scores. However, in the 2010 NBTS, foodservice top sirloin steaks were given greater juiciness, flavor, and overall liking scores than ribeye and top loin steaks, which was likely due to the application of enhancement. Overall, palatability of steaks from the rib, loin, and chuck have remained the

same or improved over time, but more attention to sirloin and round steaks are needed to increase palatability ratings. Research needs to continually evaluate the impact of finishing strategies on beef quality in order to continue to provide beef products that are satisfactory to consumers. This body of work describes three separate finishing strategies for feedlot cattle and their impact on beef color and palatability.

## **Chapter 2 - Effects of the Programmed Nutrition Beef Program on meat quality characteristics**

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### **ABSTRACT**

The objective of this study was to examine the effects of alternative finishing strategies on beef steak color and cooked meat characteristics. Beef steers (n = 64 pens; 8 steers/pen) were allocated to a randomized complete block design with a 2 × 2 factorial treatment arrangement and initial body weight serving as the blocking factor. Factor 1 consisted of dietary treatment with cattle either being fed a conventional feedlot diet (CON) or a diet that included Programmed Nutrition Beef Program supplements. Cattle in the Programmed Nutrition (PN) treatments were fed in two-stages: 1) the basal diet with Programmed Nutrition Beef Receiver from d 1 to 20 and the basal diet with Programmed Nutrition Beef Finisher from d 21 to harvest. Factor 2 consisted of the inclusion (EGP+) or absence (EGP-) of an exogenous growth promoting program. Steers in the EGP+ treatments were implanted initially with Component E-S, reimplanted with Component TE-IS, and fed 400 mg·d<sup>-1</sup>·steer<sup>-1</sup> of ractopamine hydrochloride for the final 28 d before harvest. Steers were harvested on d 175 of feeding and 1 strip loin was removed from 2 carcasses selected at random from each pen for transport to Kansas State University. After 14 d of aging, loins were fabricated into 2.54-cm thick steaks for objective and trained sensory panel measurement of cooked meat characteristics and objective color

measurements during 7 d retail display. There were no interactions ( $P > 0.10$ ) between feeding strategy and exogenous growth promotants for all objective measures of color and cooked meat characteristics. Throughout the display period, PN steaks were darker ( $P = 0.02$ ) than CON steaks, but surface percentages of oxymyoglobin and metmyoglobin and metmyoglobin reducing ability were unaffected by feeding strategy ( $P > 0.10$ ). Loins and steaks from PN cattle possessed decreased moisture loss during aging and cooking ( $P < 0.01$ ). Trained sensory panel evaluation of cooked meat revealed a dietary program  $\times$  growth promotant interaction for myofibrillar tenderness, connective tissue amount, and overall tenderness ( $P = 0.01$ ). Compared to the CON/EGP– and PN/EGP– treatments, steaks from the CON/EGP+ and PN/EGP+ treatments were evaluated by panelists as being less myofibrillar and overall tender ( $P < 0.05$ ). The alternative feeding strategies presented in this study can favorably impact water-holding capacity without negatively compromising retail display discoloration.

## INTRODUCTION

In an eloquent review of global food security, Godfray et al. (2010) reported that through the middle of the century, the global population will increase and peak at 9 billion people, which will trigger an increase in the global demand for food. The authors noted that to meet this increase in demand, production efficiency must be elevated, but overcoming Earth's limited resources serve as a major hurdle. To maximize production efficiency, U.S. beef producers currently use a multitude of production regimens that use feed additives such as monensin (Rumensin; Elanco Animal Health, Greenfield, IN) or tylosin (Tylan; Elanco Animal Health) and exogenous growth promotants (**ExGP**).

When fed in combination, monensin and tylosin increase ADG and feed efficiency by 3 and 4%, respectively (Stock et al., 1995). Producers will use implant programs and feed  $\beta$ -

adrenergic agonists (**BAA**) due to their ability to enhance feed efficiency, ADG, HCW, and yield grades of carcasses (Duckett et al., 1996; Gruber et al., 2007; Winterholler et al., 2008). While monensin and tylosin do not negatively affect LM tenderness or color characteristics (Hilton et al., 2009), numerous studies indicate both implants and BAA negatively affect LM tenderness (Roeber et al., 2000; Schroeder et al., 2004). The Programmed Nutrition Beef Program (**PN**; Alltech Inc., Nicholasville, KY) consists of 2 products that are designed to replace components of the conventional feedlot diet. The Programmed Nutrition Beef Receiver is intended to be fed during the step up period of feeding at a rate of  $14 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$ , while Programmed Nutrition Beef Finisher is intended to be fed during the remainder of finishing period at a rate of  $20 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$ . Since both products are new feed alternatives, the objective of this study was to demonstrate the effect the Programmed Nutrition Beef Program when combined with or without ExGP elicits on fresh beef color characteristics and tenderness.

## **MATERIAL AND METHODS**

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee, and the Kansas State University Institutional Review Board approved procedures for the use of human subjects in sensory panel evaluations.

### ***Animals***

Feedlot steers (initial BW  $383 \text{ kg} \pm 30$ ;  $n = 64$  pens; 16 pens/treatment; 8 steers/pen) were blocked by weight and assigned, within strata, to 1 of 4 treatments in a  $2 \times 2$  factorial arrangement. Factors consisted of feeding program and the use of ExGP. For the dietary program factor, steers were separated into a conventional finishing program treatment (**CON**) or Alltech

Programmed Nutrition Beef Program treatment (**PN**; Table 3.1). Conventional diets included vitamin A at 2,200 IU/kg, vitamin E at 22 IU/kg, copper sulfate to provide 10 mg/kg Cu, cobalt carbonate to provide 0.15 mg/kg Co, ethylenediamine dihydriodide to provide 0.5 mg/kg I, manganous sulfate to provide 60 mg/kg Mn, sodium selenite to provide 0.3 mg/kg Se, and zinc sulfate to provide 60 mg/kg Zn on a DM basis as well as 300 mg·d<sup>-1</sup>·steer<sup>-1</sup> of monensin and 90 mg·d<sup>-1</sup>·steer<sup>-1</sup> of tylosin (Elanco Animal Health). The components of the Alltech Programmed Nutrition Beef Program diet were premixed into a ground corn carrier and subsequently blended into the total mixed ration. Both supplements contained a proprietary blend of organic trace elements, ascorbic acid, *Lactobacillus acidophilus* fermentation product, *Enterococcus faecium* fermentation product, and selenium yeast. Additionally, Programmed Nutrition Beef Receiver included *Aspergillus niger* fermentation extract and Programmed Nutrition Beef Finisher included *Aspergillus oryzae* fermentation extract. The Programmed Nutrition Beef Receiver portion of the diet was included in the total mixed ration for the first 21 d at a rate of 14 g·d<sup>-1</sup>·steer<sup>-1</sup>. The Programmed Nutrition Beef Finisher was included in the total mixed ration at a rate of 20 g·d<sup>-1</sup>·steer<sup>-1</sup> for the final 154 d of the feeding period. Each diet was fed in conjunction with (**EGP+**) or in the absence of (**EGP-**) exogenous promotants. Steers receiving growth ExGP were administered a Component E-S implant on d 1 of the study, reimplanted with Component TE-IS (Elanco Animal Health) on d 94, and fed ractopamine hydrochloride (**RAC**; Elanco Animal Health) at a rate of 400 mg·d<sup>-1</sup>·steer<sup>-1</sup> the final 28 d before harvest.

### ***Loin Collection***

On d 175 of the experiment, steers were shipped 430 km to a commercial abattoir for harvest (Tyson Fresh Meats, Holcomb, KS). Strip loins (Institutional Meat Purchase Specifications 180) were removed from the left side of 2 carcasses selected at random from each

pen and were transported back to Kansas State University Meats Laboratory. Upon arrival, a 1.27-cm thick steak was removed from the 13th rib of each loin perpendicular to the orientation of muscle fibers for histological measurements. The remaining portion of each loin was weighed, vacuum packaged, and stored for 14 d at  $2 \pm 1^\circ\text{C}$ . On d 14, loin packages were opened, patted dry, and reweighed. Purge loss was calculated as  $[(\text{initial weight} - \text{final weight})/\text{initial weight}] \times 100$ . Additionally, ultimate pH was measured using a meat pH meter (model HI 99163; Hanna Instruments, Smithfield, RI). Five 2.54-cm thick steaks were fabricated from the anterior portion of the loin for 7 d simulated retail display, Warner-Bratzler shear force (**WBSF**), and sensory panel. Steaks 1 and 2 were used for metmyoglobin reducing ability (**MRA**) analysis on d 0 and 3 of display, respectively. Steak 3 was displayed under simulated retail conditions for 7 d and used for MRA analysis on d 7. Steaks 4 and 5 were used for WBSF and sensory panel analysis, respectively. After cutting, steaks for WBSF and sensory analysis were frozen at  $-40^\circ\text{C}$  until analysis.

### ***Immunohistochemistry***

One, 1 by 1 by 1.27 cm sample was collected from the geometric center of the medial, medial-lateral, and lateral areas of the *longissimus lumborum* (**LL**;  $n = 3$  per steak; Figure 3.1). Samples were placed in optimum cutting temperature tissue freezing medium (Fisher Scientific, Pittsburgh, PA), frozen using liquid nitrogen cooled 2-methyl-butane (Fisher Scientific), and stored at  $-80^\circ\text{C}$  until analysis. For each sample, one 5- $\mu\text{m}$  cryosection was collected on frost resistant slides and nonspecific antigen sites were blocked with 10% horse serum and 0.2% TritonX-100 in PBS. Cryosections were then incubated in the following primary antibodies for 1 h at room temperature: 1:50  $\alpha$ -dystrophin (Thermo Scientific, Waltham, MA); 1:10 supernatant antimyosin heavy chain (**MHC**), slow, IgG2b (BA-D5; Developmental Studies Hybridoma

Bank, University of Iowa, Iowa City, IA); and supernatant anti-MHC all but type IIX, IgG1 (BF-35, Developmental Studies Hybridoma Bank). After washing with PBS, sections were incubated with the following secondary antibodies for 30 min at room temperature: goat anti-mouse IgG1 Alexa-Fluor 488 (Invitrogen, Grand Island, NY), goat anti-mouse IgG2 Alexa Fluor 633 (Invitrogen), and goat anti-rabbit H & L Alexa Fluor 594 (Invitrogen). After a final wash with PBS, slides were cover-slipped and photomicrographs were captured using a Nikon Eclipse TI-U inverted microscope equipped with a DS-QiMC digital camera at a 10× working distance magnification Nikon Instruments Inc., Melville, NY).

Photomicrographs were analyzed for MHC type distribution and individual muscle fiber cross-sectional area (CSA) using NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments, Inc.). For each steak location area, a minimum of 500 fibers and 2 photomicrographs were analyzed. The area constrained by  $\alpha$ -dystrophin immunostaining defined individual fibers for CSA measurements. Fibers that stained positive for the BA-D5 antibody were classified as type I fibers. Fibers that stained positive for BF-35 but that were negative for BA-D5 were classified as type IIA fibers. All fibers that were negative for the BF-35 antibody were classified as type IIX fibers (Moreno-Sanchez et al., 2008; Schiaffino et al., 1989).

### ***Simulated Retail Display***

Steaks used for simulated retail display were placed on white 17S polystyrene foam trays with a Dri Loc (Dri-Loc50, Cryovac Sealed Air Corporation, Duncun, SC) absorbent pad and overwrapped with Poly-Vinyl Chloride film (AEP Industries, South Hackensack, NJ) possessing an oxygen transmission rate of  $1,450 \text{ cm}^{-3} \cdot 645.2 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$ . All steaks were orientated with the posterior portion facing up and medial portion of the steak placed on the left side of the tray (Figure 3.1). Steaks were displayed in coffin-style retail cases (Model DMF 8; Tyler

Refrigeration Corporation, Niles, MI) set to run at  $3 \pm 2^\circ\text{C}$  and defrost twice daily (morning and evening) at  $11^\circ\text{C}$  for 30 min. Case temperature was monitored using a Thermocon iButton (Maxim Integrated Products, Sunnyvale, CA). Cases were illuminated by fluorescent lights (32 W Del-Warm White  $3000^\circ\text{K}$ ; Philips Lighting Company, Somerset, NJ) that emitted a constant 24-h case average intensity of  $2,143 \pm 113$  lx. Every 12 h, steaks were rotated from left to right and front to back in the cases to account for variation in temperature and light intensity. Readings for CIE  $L^*$ ,  $a^*$ , and  $b^*$  and reflectance from 400 to 700 nm were taken at 3 locations on each steak on d 0, 2, 4, 5, 6, and 7 using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54-cm diameter aperture,  $10^\circ$  observer; Hunter Associates Laboratory, Reston, VA). Values from the 3 scans were averaged for  $L^*$ ,  $a^*$ , and  $b^*$  values. Additionally, readings were averaged for reflectance at 473, 525, 572, and 700 nm, which were used to calculate surface percentages of metmyoglobin and oxymyoglobin using equations from Krzywicki (1979) as published in the American Meat Science Association color guidelines (AMSA, 2012).

### ***Metmyoglobin Reducing Ability***

The procedures from Gonzalez et al. (2009) were followed for MRA with modifications. Steaks were cut into medial, mid-lateral, and lateral sections according to their anatomical location within the live animal and orientation of display on the polystyrene foam tray. Each section was placed in a 400 mL beaker and oxidized in 100 mL of 0.3% sodium nitrite at  $25 \pm 2^\circ\text{C}$  for 20 min. Afterwards, samples were blotted of excess solution and vacuum packaged in 25.4 by 30.5 cm vacuum bags (3 mil standard barrier, Prime Source Vacuum Pouches; Bunzl Processor Division, Koch Supplies, Kansas City, MO) that possessed an oxygen transmission rate of  $4.5 \text{ cm}^3 \cdot 100 \text{ cm}^2 \cdot 24 \text{ h}^{-1}$  at  $23^\circ\text{C}$  and 65% relative humidity. Reflectance measurements were taken from 400 to 700 nm at 0 and 2 h using a Hunter Lab Miniscan EZ spectrophotometer

(Illuminant A, 2.54 cm diameter aperture, 10° observer; Hunter Associates Laboratory).

Readings from the medial, mid-lateral, and lateral sections of each steak were averaged and spectral data at 525, 572, and 700 nm were used to calculate metmyoglobin percentages at 0 and 2 h using equations from Krzywicki (1979) as published in the meat color guidelines (AMSA, 2012). Metmyoglobin reducing ability was calculated as (observed decrease in metmyoglobin concentration/initial metmyoglobin concentration) × 100.

### ***Warner-Bratzler Shear Force and Sensory Panel***

Warner-Bratzler shear force procedures were conducted according to the meat cookery and sensory guidelines (AMSA, 1995). Steaks were thawed on trays at  $7 \pm 1^\circ\text{C}$  for 24 h before cooking. Before cooking, steaks were weighed and a thermocouple wire (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak for internal temperature monitoring using a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Steaks were cooked on electric, open-hearth Farberware grills (Model 450-A; Yonkers, NY), turned once at  $40^\circ\text{C}$ , and removed from grills at  $70^\circ\text{C}$ . Steaks were weighed following cooking for calculation of the percent moisture lost during cooking. After a 24-h chill period at  $7 \pm 1^\circ\text{C}$ , eight 1.27-cm cores were removed from each steak parallel to the muscle fiber and were sheared once through the center using an INSTRON Model 5569 testing machine (Instron, Canton, MA) with a Warner-Bratzler shear head attached (100 kg compression load cell and crosshead speed of 250 mm/min).

For sensory panel analysis, steak thawing, cooking, and temperature monitoring mirrored the procedures outlined for WBSF. After cooking, steaks were cut into cubes (1.27 by 1.27 by 2.54 cm) and presented to a 6- to 9-member trained sensory panel. Panelists were selected from a

larger pool of candidates that were screened and trained according to American Meat Science Association (AMSA, 1995) guidelines. Before initiation of the panel, panelists were also oriented to evaluating strip steaks during 4 sessions. Panelists were seated at individual cubicles in a room designed for subjective meat sensory panels. At each panel, 8 steaks ( $n = 2$  per treatment) were evaluated and panelists evaluated 2 cubes from each steak for myofibrillar tenderness, juiciness, beef flavor intensity, connective tissue amount, overall tenderness, and off-flavor intensity using an 8-point scale (1 = extremely tough, extremely bland, abundant, extremely tough, or abundant; 8 = extremely tender, extremely juicy, extremely intense, none, extremely tender, or none). A total of 15 separate panels were conducted to analyze all the samples.

### *Statistical Analysis*

Data were analyzed as a randomized complete block design with a  $2 \times 2$  factorial arrangement. Dietary program and ExGP served as the main effects and weight block served as the random effect. For retail shelf life data, data were analyzed as a randomized complete block design with a  $2 \times 2$  factorial arrangement with repeated measures. Day served as the repeated measure with steak (observational unit) as the subject and compound symmetry as the covariance structure. The PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used and pairwise comparisons between the least square means of the factor levels, including planned interaction comparisons, were computed using the PDIFF option of the LSMEANS statement. Differences were considered significant at  $\alpha \leq 0.05$  and tendencies at  $\alpha \leq 0.10$ .

## RESULTS

### *Muscle Fiber Type and Cross-Sectional Area*

Using immunohistological techniques, we evaluated the effect of our treatments on MHC isoform distribution (Figure 3.2) and muscle fiber hypertrophy (Table 3.2) of the LL. There was no program  $\times$  ExGP interaction for percentages of type IIA and type IIX fibers ( $P > 0.10$ ); however, there was a program  $\times$  ExGP interaction on the percentage of type I fibers ( $P < 0.0001$ ) in the LL. Muscle from CON/EGP+ and PN/EGP- treatments did not differ in type I fiber percentage ( $P > 0.10$ ), but both contained a greater percentage of type I fibers than the CON/EGP- and PN/EGP+ treatments ( $P < 0.05$ ). Dietary program influenced the percentage of type IIA and type IIX fibers ( $P < 0.05$ ), but the use of ExGP did not elicit an effect on any of the fiber types ( $P > 0.10$ ). When compared to the CON diet, the PN program decreased ( $P = 0.0041$ ) the percentage of type IIA fibers and increased ( $P = 0.0016$ ) the percentage of type IIX fibers.

There was no program  $\times$  ExGP interaction for the CSA of IIA or IIX fibers ( $P > 0.10$ ), but we did detect a program  $\times$  ExGP interaction for the CSA of type I fibers ( $P < 0.0439$ ). Type I fiber CSA of the PN/EGP- treatment was smaller than all other treatment groups ( $P < 0.02$ ). Use of ExGP tended to increase ( $P = 0.07$ ) the CSA of type IIA fibers and increased ( $P < 0.0007$ ) the CSA of IIX fibers. Dietary program did not affect type IIX fiber CSA ( $P > 0.10$ ), but the CSA of type IIA fibers from PN treatment were smaller than type IIA fibers from CON treatment ( $P = 0.05$ ).

### *Simulated Retail Display and Metmyoglobin Reducing Ability*

Strip steaks were displayed under simulated retail conditions to examine the effect of the treatments on strip loin shelf life and color stability. For  $L^*$ ,  $a^*$ , and  $b^*$  values (Table 3.3), there were no 3-way interactions between day of display, dietary program, and ExGP ( $P > 0.10$ ).

Additionally, there were no 2-way interactions between dietary program and ExGP ( $P > 0.10$ ). Independent of treatment group, day of display ( $P < 0.01$ ) affected  $L^*$ ,  $a^*$ , and  $b^*$  values. Compared to d 0 values, steak  $L^*$  values were decreased ( $P < 0.05$ ) on d 2, 4, 5, and 6, indicating steaks were darker. On d 7 of display, steak  $L^*$  values were similar ( $P > 0.10$ ) to d 0 values. Dietary program influenced ( $P = 0.02$ )  $L^*$  values over the 7 d display period, with PN steaks measuring darker than CON steaks. For  $a^*$  values, steaks became redder ( $P < 0.05$ ) on d 2 when compared to d 0. The remainder of the display period, steaks became less red ( $P < 0.05$ ). Blueness values ( $b^*$ ) followed the same trend as  $L^*$  values, becoming more yellow d 2, 4, 5, and 6 but returning to d 0 blueness values on d 7. Dietary program did not impact  $a^*$  and  $b^*$  values ( $P > 0.10$ ), and use of ExGP did not influence  $L^*$ ,  $a^*$ , and  $b^*$  values ( $P > 0.10$ ).

Similar to the previous objective color data, there were no 3-way or 2-way interactions for steak surface oxymyoglobin percentage, surface metmyoglobin percentage, and MRA ( $P > 0.10$ ; Figure 3.3 and 3.4). Dietary program tended to affect ( $P = 0.09$ ) steak surface oxymyoglobin percentage with PN steaks possessing an average of 0.7% less oxymyoglobin than CON steaks over the entire display period. As expected, both redox forms of myoglobin were affected by day of display ( $P < 0.0001$ ), with surface oxymyoglobin decreasing in percentage and metmyoglobin increasing in percentage. Similarly, MRA decreased ( $P < 0.001$ ) as day of display increased, decreasing from 53% reducing ability at d 0 to 32% on d 3 and 9% on d 7.

#### ***Warner-Bratzler Shear Force and Sensory Panel***

The effects of treatments on ultimate pH and purge loss during aging and moisture loss during cooking are displayed in Table 3.4. There was no dietary program  $\times$  ExGP interaction for pH, purge loss, or cook loss ( $P > 0.10$ ). Dietary program did elicit an effect on purge loss, with loins from steers fed the CON program losing 0.14% more ( $P = 0.004$ ) moisture than the PN

program loins during the 14-d aging period. Dietary program also had an effect on weight loss during cooking, with steaks from steers fed the CON program losing a greater ( $P = 0.01$ ) percentage of moisture (26.05%) than the PN program steaks (24.77%) during the cooking process. Use of ExGP did not affect ( $P > 0.10$ ) purge loss, but EGP+ steaks lost 2.4% more moisture ( $P = 0.001$ ) during cooking than the EGP- steaks. Objective tenderness (Table 3.4) measured by WBSF was unaffected by the dietary program  $\times$  ExGP interaction ( $P = 0.21$ ) or the dietary program main effect ( $P = 0.77$ ). As expected, ExGP increased ( $P = 0.003$ ) EGP+ steak WBSF by 0.38 kg when compared to EGP- steaks.

For subjective sensory attributes of cooked meat characteristics (Table 3.4), there was a program  $\times$  ExGP interaction for myofibrillar tenderness, connective tissue amount, and overall tenderness ( $P < 0.05$ ). There was no program  $\times$  ExGP interaction for juiciness, beef flavor intensity, and off-flavor intensity ( $P > 0.10$ ). For myofibrillar tenderness, steaks from the PN/EGP+ treatment were less tender ( $P < 0.05$ ) when compared to the other 3 treatment groups. Panelists also scored steaks from the CON/EGP+ treatment as less tender than steaks from the CON/EGP- and PN/EGP- treatments. Also, PN/EGP- steaks tended to receive greater ( $P = 0.086$ ) myofibrillar tenderness scores than the CON/EGP- steaks. Panelists indicated that the PN/EGP+ steaks contained more ( $P < 0.05$ ) connective tissue than steaks from the other 3 treatment groups, while the PN/EGP- treatment group steaks had the least ( $P < 0.05$ ) detectable amount of connective tissue. For overall tenderness, our data indicated that all 4 treatment groups differed ( $P < 0.05$ ) in tenderness from one another with panelists scored PN/EGP- steaks as the most tender followed by CON/EGP-, CON/EGP+, and PN/EGP+ steaks. Dietary program had no describable effects on sensory attributes ( $P > 0.10$ ); however, panelists indicated that EGP+

steaks were less ( $P < 0.01$ ) myofibrillar tender, were less ( $P = 0.003$ ) juicy, had more ( $P < 0.01$ ) connective tissue, and were less ( $P < 0.01$ ) tender overall than EGP– steaks.

## DISCUSSION

When evaluating finishing programs for cattle, potential impacts on meat attributes including retail shelf life color stability and tenderness should be considered. Consumer perception of meat color is an important consideration for commercial retailers as it is the single most important attribute that will determine if a customer will purchase a product (Kropf, 1980; Hedrick et al., 1994). At present time, few simulated retail display studies have been conducted to evaluate the effect different finishing strategies and the use of ExGP elicit on objective meat color characteristics. The only objective color measurements influenced by dietary program, use of ExGP, or their interaction were  $L^*$  values and steak surface oxymyoglobin percentage. Objective lightness values indicated that PN steaks were darker and tended to possess less oxymyoglobin than CON steaks throughout the entire display period. These findings occurred independently of differences in pH since all treatments possessed similar pH values. The differences observed in  $L^*$  values in the current study may be due to reduction in the CSA of type I and IIA fibers catalyzed by the PN treatment. Type I and IIA fiber types rely heavily on aerobic metabolism and possess the greatest content of mitochondria (Aberle et al., 2003). Since the fibers from the PN treatment were smaller, we hypothesize that the mitochondrial density was higher for those treatments. Tang et al. (2005) demonstrated that increased mitochondrial density increases oxygen consumption rate and increases deoxymyoglobin formation. The PN muscles maintaining a deoxygenated state would result in decreased, bright “cherry red,” oxymyoglobin formation and lower  $L^*$  values (Sammel et al., 2002). While the

spectrophotometer was able to detect these slight differences, we doubt the human eye would be able to detect the 1 unit difference in L\* value or the small difference in oxymyoglobin content.

Since the PN diet lacked vitamin E in the ration, and a large body of literature reports vitamin E inhibits the formation of surface metmyoglobin (Liu et al., 1995; Faustman et al., 1998), we were concerned that the removal of this potent antioxidant would be detrimental to retail color stability. We hypothesized the inclusion of selenium, another antioxidant, may be able to elicit similar color stability effects. Gerloff (1992) reported that selenium can serve as an antioxidant during retail display. Additionally, the use of selenium as a replacement for vitamin E in the diet was demonstrated by several studies that reported no differences in color attributes when selenium was the primary antioxidant in the diet (O'Grady et al., 2001; Lawler et al., 2004; Skrivanova et al., 2007). At this time, we conclude that the lack of large dietary treatment effects on objective color characteristics may be due to the inclusion of selenium in the diet.

In agreement with the majority of BAA data (Gonzalez et al., 2009; Gunderson et al., 2009; Rogers et al., 2010), our color data indicated there was the lack of an ExGP effect on the objective color attributes of the steaks. Commonly in pork, the addition of RAC to diets will affect both L\* and a\* values where chops are lighter and less red than non-RAC chops (Armstrong et al., 2004; Apple et al., 2008; Leick et al., 2010). This phenomenon seems restricted to pork, since BAA increase the percentage of type IIB fibers in the muscle of this species (Depreux et al., 2002; Gunawan et al., 2007) and cattle do not possess this MHC isoform. Another reason for the lack of an ExGP effect in the present study could stem from the inability of our ExGP treatments to shift muscle fiber type among the 3 MHC forms. Since the combined effect of both ExGP can increase MHC type II percentage in the LM (Gonzalez et al., 2007), we were concerned with the effect of this shift on MRA and color stability. Our concerns stem from

the fact that a type II fiber shift is associated with a reduction in muscle mitochondria content (Aberle et al., 2003). This in turn reduces the amount of NADH in the muscle (Howlett and Willis, 1998), and since NADH is important in catalyzing the MRA reaction, less NADH content decreases the MRA of muscles (Mancini and Hunt, 2005). Use of ExGP in our study did not affect the MHC distribution in the muscle, which could account for the lack of response in all objective color attributes. In agreement with our results, Gonzalez et al. (2009) reported that steaks from steers supplemented RAC did not differ in the same objective color measurements; however, the authors did detect a RAC-induced 2% decrease in type I fiber percentage in the LL. While the authors did not distinguish between type IIA and IIX fibers in the study, the lack of a RAC effect on MRA and color indicates that a type I MHC isoform shift of that magnitude is not enough to affect color stability.

Another important consideration that producers must account for when selecting dietary finishing programs and ExGP technologies is the impact of each on water holding capacity and meat palatability. We did not find a program  $\times$  ExGP interaction for purge loss, cook loss, or sensory panel juiciness scores; however, our study found that steaks from the PN treatment retained 0.14 and 1.3% more moisture during aging and cooking, respectively. Although this did not translate to increased juiciness scores in the sensory analysis, the finding that the PN loins and steaks hold more moisture during aging and cooking could be significant for large retailers or meat processors who sell large amounts of raw and cooked products. Use of ExGP increased the loss of moisture during cooking by 1.6%, which sensory panelists were able to detect by indicating differences in juiciness scores between the EGP+ and EGP- treatments. This finding is quite unique since most of the literature documents numerous studies that report no implant or BAA effect on drip loss during cooking (Scheffler et al., 2003; Quinn et al., 2008; Boler et al.,

2012); however, dietary composition may influence the implant effect on drip loss as indicated by Faucitano et al. (2008), who found that implants increased drip loss when they were combined with high-silage diets.

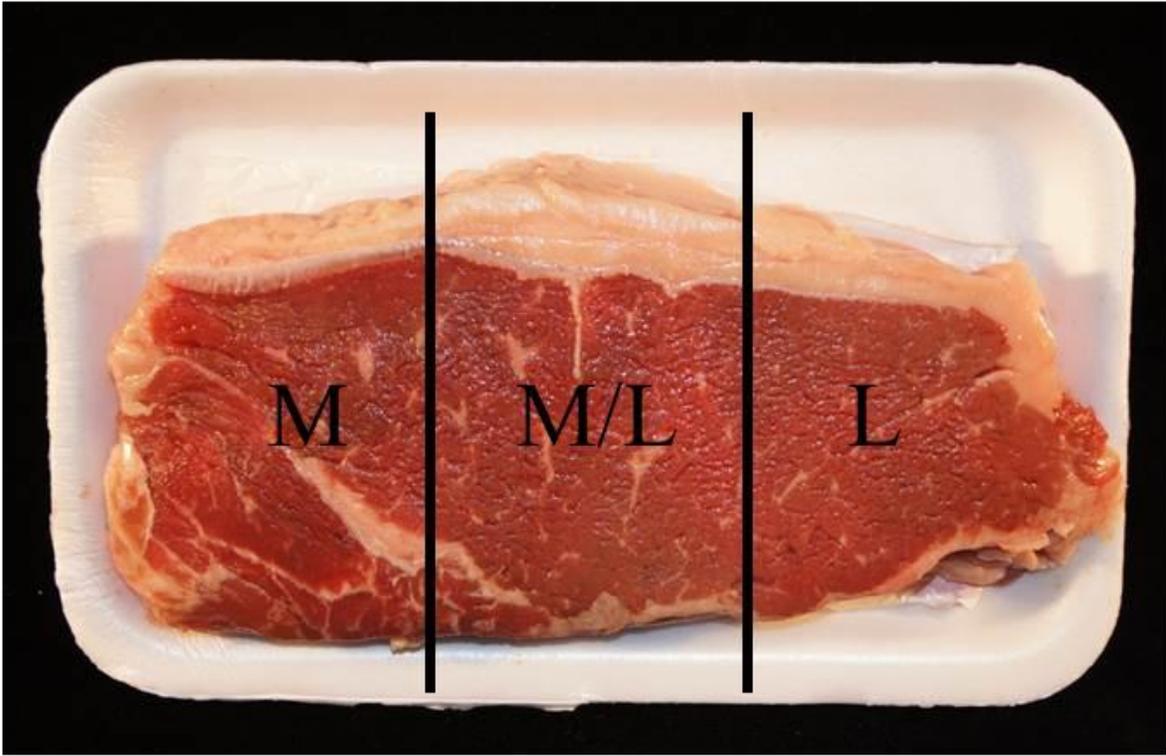
Tenderness constitutes the most important factor consumers evaluate when determining the acceptability of their beef eating experience (Beermann, 2009). This attribute is so important that consumers are willing to pay more for guaranteed tender beef (Boleman et al., 1997). Our results indicate that there was no dietary program  $\times$  ExGP interaction for objective tenderness as measured by WBSF. For the trained panel evaluation of cooked meat characteristics, panelists did detect a program  $\times$  ExGP interaction for myofibrillar tenderness, connective tissue amount, and overall tenderness. Steaks from the PN/EGP+ and CON/EGP+ were the toughest for the myofibrillar and overall tenderness attributes. Interestingly, the PN/EGP+ steaks were the toughest in both categories when compared to the other 3 treatments. We hypothesize these results are the function of 3 attributes associated with the biology within the muscle. First, the ExGP influence on reducing meat tenderness is well documented when considering the effect BAA elicit on postmortem calpastatin activity. When lambs and steers were fed the BAA L644,969 at 4 and 3 mg/kg, respectively, calpastatin activity was increased (Kretchmar et al., 1990; Wheeler and Koohmaraie, 1992). Strydom et al. (2009) found that supplementing steers with 30 mg/kg of RAC increased LM calpastatin activity by 49% when compared to non-BAA supplemented animals. Previous trained sensory panel scores demonstrate that BAA can negatively affect tenderness scores (Gruber et al., 2008; Hilton et al., 2009; Leheska et al., 2009). When RAC is supplemented at higher rates (300 mg/animal daily), studies have reported decreased tenderness of steaks (Schroeder et al., 2004; Avendano-Reyes et al., 2006). The PN dietary program shift on MHC type could have also affected postmortem proteolysis. Literature

indicates that increasing the percentage of type IIX fibers while reducing the percentage of type IIA fibers can influence the tenderness of steaks since slower and more oxidative type fibers contain more calpains (Ouali and Tulmant, 1990). Because PN muscles contained more type IIX fibers, they could have had less calpain activity than the CON muscles postmortem. Therefore, when they were combined with the EGP+ treatment, the possible increased calpastatin activity associated with these treatments could have overwhelmed the reduced calpain pool to minimize postmortem tenderization of the steaks originating from these treatments.

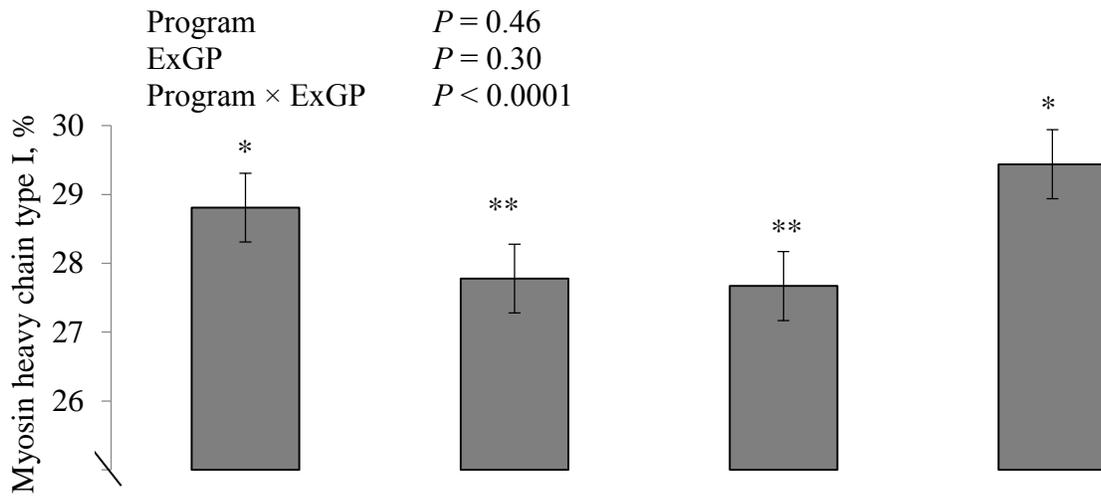
The second attribute of the muscle that may have affected tenderness involves the ability of the EGP+ treatment to increase the size (CSA) of the type IIA and IIX muscle fibers. Increases in muscle fiber hypertrophy as indicated by larger muscle fiber CSA contributes to reductions in tenderness (Renand et al., 2001). Herring et al. (1965) reported that differences in muscle fiber diameter were highly correlated to WBSF ( $r = 0.73$ ). Implant programs have also been found to reduce WBSF and trained and consumer sensory panelists' tenderness scores (Roeber et al., 2000; Barham et al., 2003; Platter et al., 2003; Boles et al., 2009). Currently, researchers have not identified a singular mechanism responsible for this influence, but many hypothesize increases in type IIA and IIX fiber CSA of cattle subjected to ExGP are partly responsible for decreased tenderness.

The final biological attribute that may have affected tenderness ratings involved the increase in the amount of connective tissue detected by panelists. In our study, panelists scored steaks that were also scored the most myofibrillar and overall tough as possessing the most connective tissue. In the literature mentioned previously, panelists were not asked to rate connective tissue content of the meat. Additionally, Bloomberg et al. (2013) reported Zilpaterol did not affect sensory panel connective tissue score, so our findings are quite novel. A small

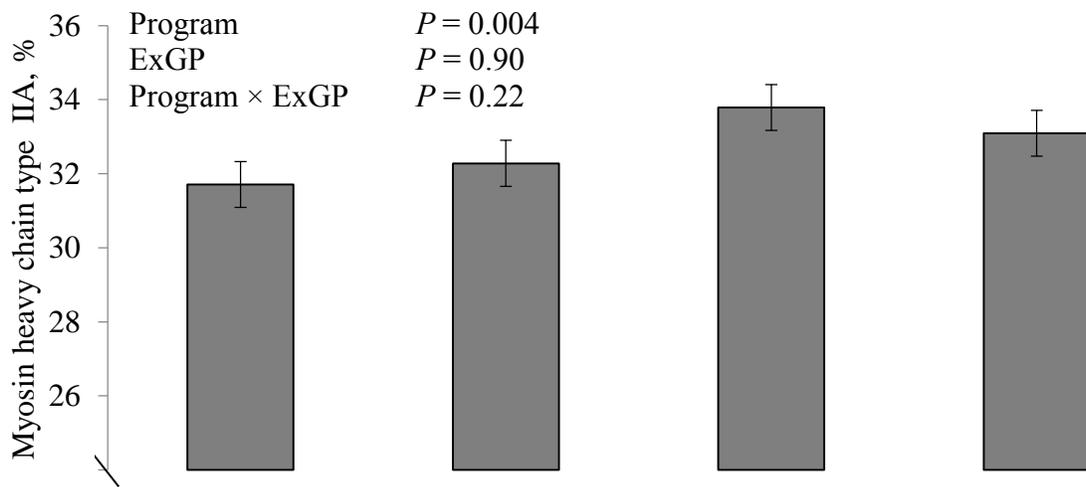
number of studies examine the effect of ExGP on connective tissue characteristics. Huffman et al. (1991) found implants had no effect on sensory panel connective tissue scores, while Strydom et al. (2009) also reported that RAC had no effect on total collagen or solubility in the LM or semitendinosus muscle. Additionally, Girard et al. (2011) found that total collagen and solubility collagen content were unaffected by RAC or implants. If collagen contains a high percentage of heat-resistant intermolecular bonds (crosslinks), the collagen will not gelatinize during the application of heat (Light et al., 1985), thus resulting in a less tender cooked meat product. The formation of crosslinks is dependent on the rate at which collagen protein is turned over (Purslow, 2005). For our study, we hypothesize that since ExGP stimulate muscle hypertrophy by reducing protein degradation, they also may be reducing the rate at which collagen is turning over. Therefore, this could enable the collagen an additional period to crosslink and become more insoluble, which could have contributed to the observed decrease in overall tenderness indicated by the trained panelists.



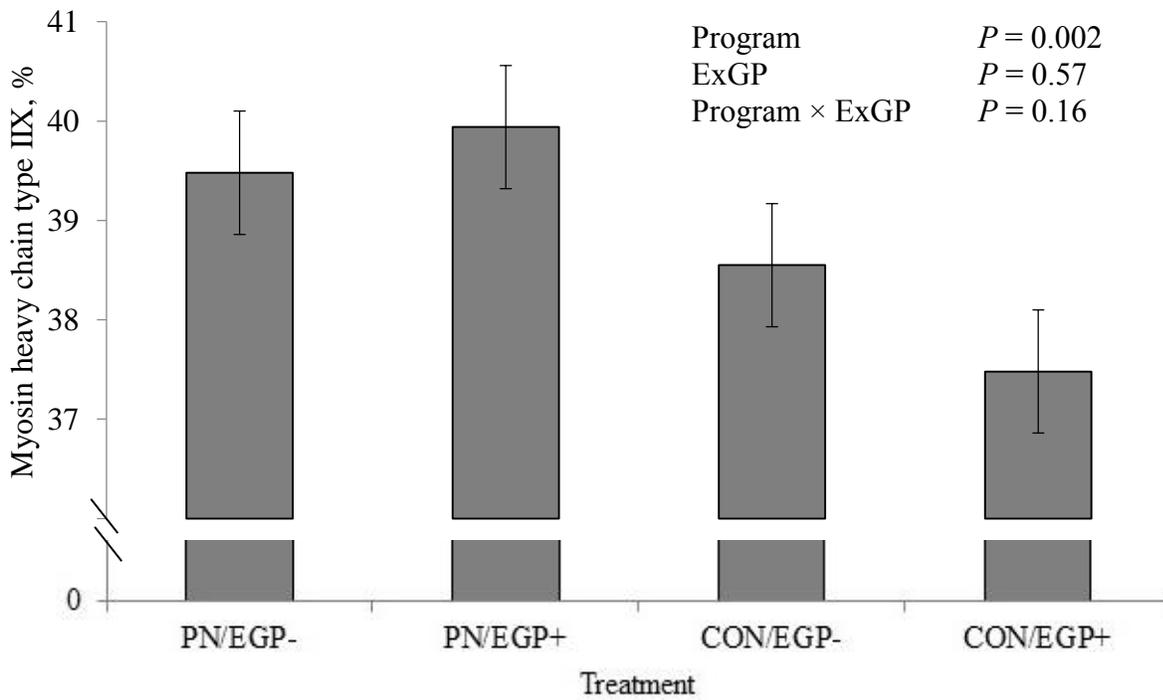
**Figure 2.1** Representative photograph depicting the orientation of a strip loin steak as it was presented on the polystyrene foam tray. The steak was presented with the posterior portion of the cut facing up and the medial portion of the steak placed on the left side of the tray. For  $L^*$ ,  $a^*$ ,  $b^*$ , and spectral color readings, readings were taken on the medial (M), medial/lateral (M/L), and lateral (L) portions of the steak. These values were averaged to calculate the average spectral readings.



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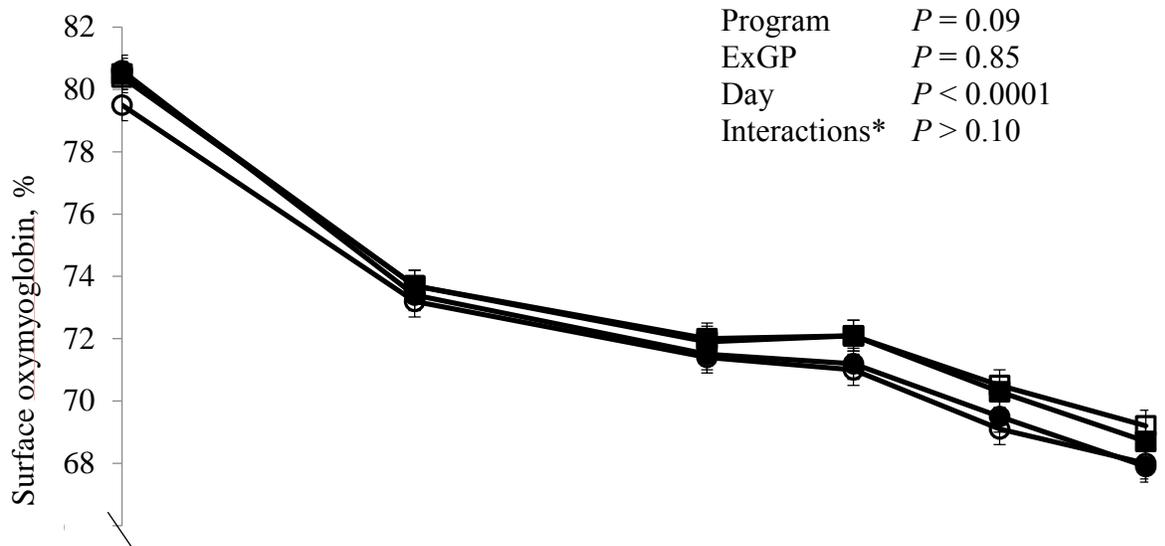
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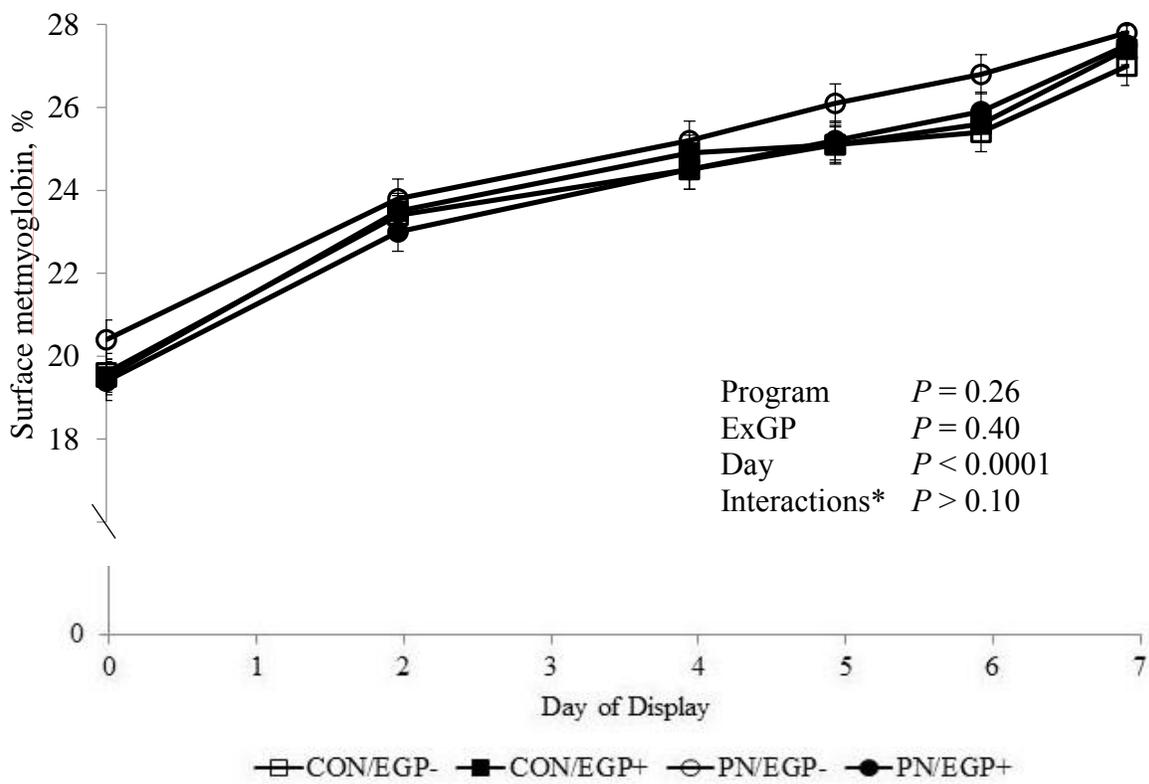
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7 **Figure 2.2** Muscle fiber type distribution of the *Longissimus lumborum* muscle from steers fed two  
8 dietary programs with (EGP+) and without (EGP-) the use of exogenous growth promotants (ExGP).  
9 Steers were fed the experimental diets for 175 d prior to slaughter. Alltech Programmed Nutrition steers  
10 were fed the basal diet and supplemented with Programmed Nutrition Beef Receiver from d 1 to d 20 at  
11  $14 \text{ g}\cdot\text{d}^{-1}\cdot\text{steer}^{-1}$  and Programmed Nutrition Beef finisher from d 21 to harvest at  $20 \text{ g}\cdot\text{d}^{-1}\cdot\text{steer}^{-1}$ .  
12 Conventional fed steers were fed the basal diet and were supplemented with vitamin A, inorganic  
13 minerals, monensin and tylosin. Steers receiving exogenous growth promotants were implanted on d 1 of  
14 the experiment with Component E-S, reimplanted with Component TE-IS on day 94, and fed  $400 \text{ mg}\cdot\text{d}^{-1}\cdot\text{steer}^{-1}$   
15 of ractopamine-hydrochloride during the final 28 days of finishing. Fibers that stained positive  
16 for the BA-D5 antibody were categorized as type I fibers. Fibers that stained positive for BF-35, but were  
17 negative for BA-D5 were categorized as type IIA fibers. All fibers that were negative for the BF-35  
18 antibody were categorized as type IIX fibers (Moreno-Sanchez et al., 2008; Schiaffino et al., 1989).  
19 \*,\*\*Treatments with different asterisk are significantly different ( $P < 0.05$ ).



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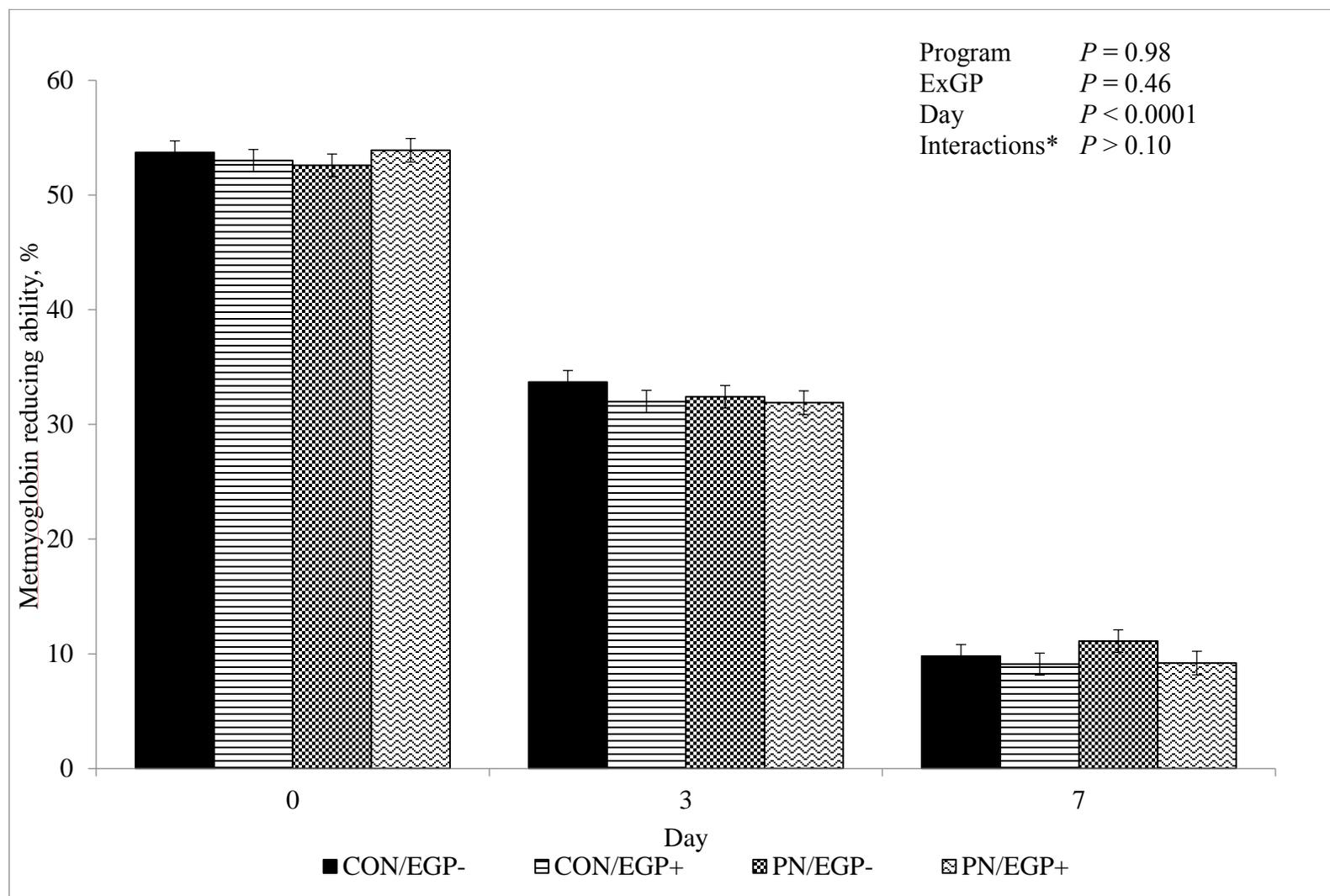
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**Figure 2.3** *Longissimus lumborum* steak surface myoglobin redox percentages from steers fed two dietary programs with or without the use of exogenous growth promotants. Steers were fed the experimental diets for 175 d prior to slaughter. Alltech Programmed Nutrition steers were fed the basal diet and supplemented with Programmed Nutrition Beef Receiver from d 1 to d 20 at

32 14 g·d<sup>-1</sup>·steer<sup>-1</sup> and Programmed Nutrition Beef finisher from d 21 to harvest at 20 g·d<sup>-1</sup>·steer<sup>-1</sup>.  
33 Conventional fed steers were fed the basal diet and were supplemented with vitamin A, inorganic  
34 minerals, monensin and tylosin. Steers receiving exogenous growth promotants were implanted  
35 on d 1 of the experiment with Component E-S, reimplanted with Component TE-IS on day 94,  
36 and fed 400 mg·d<sup>-1</sup>·steer<sup>-1</sup> of ractopamine-hydrochloride during the final 28 days of finishing.  
37 Steaks were displayed under simulated retail display for 7-d and percent of surface  
38 metmyoglobin was calculated using the equations of Krzywicki (1979).



40

41 **Figure 2.4** *Longissimus lumborum* steak metmyoglobin reducing ability from steers fed two dietary programs with and without the  
 42 use of exogenous growth promotants. Steers were fed the experimental diets for 175 d prior to slaughter. Alltech Programmed

43 Nutrition steers were fed the basal diet and supplemented with Programmed Nutrition Beef Receiver from d 1 to d 20 at  $14 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$   
44 <sup>1</sup> and Programmed Nutrition Beef finisher from d 21 to harvest at  $20 \text{ g} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$ . Conventional fed steers were fed the basal diet and  
45 were supplemented with vitamin A, inorganic minerals, monensin and tylosin. Steers receiving exogenous growth promotants were  
46 implanted on d 1 of the experiment with Component E-S, reimplanted with Component TE-IS on day 94, and fed  $400 \text{ mg} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$   
47 of ractopamine-hydrochloride during the final 28 days of finishing. Metmyoglobin reducing ability was measured on d 0, 3 and 7 of  
48 display. Percent of surface metmyoglobin was measured at 0 and 2 h post induction of surface metmyoglobin and was calculated using  
49 the equations of Krzywicki (1979). Reducing ability was calculated as (observed decrease in metmyoglobin concentration  $\div$  initial  
50 metmyoglobin concentration)  $\times$  100.

**Table 2.1** Diets (dry basis) for steers fed conventional feedlot diets<sup>†</sup> or Alltech Programmed Nutrition program<sup>‡</sup>

Ingredient, %	Conventional	Alltech
Wet corn gluten feed	35.00	35.00
Steam-flaked corn	53.55	53.56
Ground wheat straw	7.00	7.00
Feed additive premix	2.16	-
Mineral/vitamin supplement	2.29	2.23
PN supplement	-	2.21

<sup>†</sup>Conventional diets included vitamin A at 2,200 IU/kg; vitamin E at 22 IU/kg; copper sulfate to provide 10 mg/kg Cu; cobalt carbonate to provide 0.15 mg/kg Co; ethylenediamine dihydriodide to provide 0.5 mg/kg I; manganous sulfate to provide 60 mg/kg Mn; sodium selenite to provide 0.3 mg/kg Se; zinc sulfate to provide 60 mg/kg Zn on a dry matter basis; as well as 300 mg·d<sup>-1</sup>·steer<sup>-1</sup> of monensin and 90 ·d<sup>-1</sup>·steer<sup>-1</sup> of tylosin (Elanco Animal Health; Greenfield, IN).

<sup>‡</sup>The Alltech diet included Programmed Nutrition Receiver in the total mixed ration for the first 21 days at the rate of 14 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained: zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus oryzae* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Thereafter, PN Finisher was included in the total mixed ration at the rate of 20 g·d<sup>-1</sup>·steer<sup>-1</sup>: 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus niger* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Both supplements were premixed into a ground corn carrier and subsequently blended into the total mixed ration.

**Table 2.2** Interaction least squares means of muscle fiber cross-sectional area of the Longissimus lumborum muscle of steers fed conventional diets<sup>†</sup> or Alltech Programmed Nutrition<sup>‡</sup> program with and without exogenous growth promotants (ExGP)<sup>§</sup>

Cross-sectional area, $\mu\text{m}^2$	Conventional		Alltech PN		SEM	P-value		
	EGP-	EGP+	EGP-	EGP+		Program	ExGP	Program $\times$ ExGP
Type I	2928.12 <sup>a</sup>	2896.00 <sup>a</sup>	2629.94 <sup>b</sup>	2862.32 <sup>a</sup>	83.6	0.011	0.12	0.04
Type IIA	3109.37	3194.18	2946.80	3098.82	83.7	0.05	0.07	0.62
Type IIX	4405.04	4629.08	4210.12	4587.81	104.6	0.18	0.0007	0.39

<sup>a,b,c</sup> Values within a row with different letters differ significantly ( $P < 0.05$ ).

<sup>†</sup>Conventional diets included vitamin A at 2,200 IU/kg; vitamin E at 22 IU/kg; copper sulfate to provide 10 mg/kg Cu; cobalt carbonate to provide 0.15 mg/kg Co; ethylenediamine dihydriodide to provide 0.5 mg/kg I; manganous sulfate to provide 60 mg/kg Mn; sodium selenite to provide 0.3 mg/kg Se; zinc sulfate to provide 60 mg/kg Zn on a dry matter basis; as well as 300 mg·d<sup>-1</sup>·steer<sup>-1</sup> of monensin and 90 mg·d<sup>-1</sup>·steer<sup>-1</sup> of tylosin (Elanco Animal Health; Greenfield, IN).

<sup>‡</sup> The Alltech diet included Programmed Nutrition Receiver in the total mixed ration for the first 21 days at the rate of 14 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus oryzae* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Thereafter, Programmed Nutrition Finisher was included in the total mixed ration at the rate of 20 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus niger* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Both supplements were premixed into a ground corn carrier and subsequently blended into the total mixed ration.

<sup>§</sup> Exogenous growth promotants included Component ES implant administered on d 1 of the experiment, Component TE-IS at reimplant, and ractopamine hydrochloride (Optaflexx) fed at 400 mg/animal daily for the final 28 d before harvest (all products from Elanco Animal Health).

**Table 2.3** Interaction least-squares means of instrumental L\*, a\*, and b\* values<sup>†</sup> for steaks from steers fed conventional diets<sup>‡</sup> or Alltech Programmed Nutrition<sup>§</sup> program diets with or without exogenous growth promotants (**ExGP**)<sup>#</sup> displayed under simulated retail display conditions for 7 d.

Item	Day	Conventional		Alltech PN		SEM	P-value		
		EGP-	EGP+	EGP-	EGP+		Program	ExGP	Day
L*	0	46.6	45.5	45.4	45.0	0.53	0.02	0.27	< 0.01
	2	46.1	45.3	44.5	44.8				
	4	44.5	43.8	43.1	43.4				
	5	45.2	44.0	43.3	43.4				
	6	44.9	43.8	43.5	43.6				
	7	46.7	45.2	44.4	44.7				
	a*	0	30.3	30.5	31.4				
2		32.3	32.3	32.8	32.5				
4		31.0	30.9	31.0	30.8				
5		31.0	31.3	31.1	30.9				
6		31.4	31.2	30.8	30.3				
7		29.2	28.6	28.8	28.0				
b*		0	23.3	23.4	24.1	22.8	0.45	0.60	0.21
	2	25.9	25.9	26.3	26.0				
	4	25.0	25.0	25.1	24.8				
	5	25.9	26.4	26.3	25.9				
	6	26.3	26.1	25.8	25.2				
	7	23.9	23.1	23.4	22.7				

<sup>†</sup>L\* = Lightness (0 = black, 100 = white); a\* = Redness (-60 = Green, 60 = Red); b\* = Blueness (-60 = Blue, 60 = Yellow).

<sup>‡</sup>Conventional diets included vitamin A at 2,200 IU/kg; vitamin E at 22 IU/kg; copper sulfate to provide 10 mg/kg Cu; cobalt carbonate to provide 0.15 mg/kg Co; ethylenediamine dihydriodide to provide 0.5 mg/kg I; manganous sulfate to provide 60 mg/kg Mn; sodium selenite to provide 0.3 mg/kg Se; zinc sulfate to provide 60 mg/kg Zn on a dry matter basis; as well as 300 mg·d<sup>-1</sup>·steer<sup>-1</sup> of monensin and 90 mg·d<sup>-1</sup>·steer<sup>-1</sup> of tylosin (Elanco Animal Health; Greenfield, IN).

<sup>§</sup> The Alltech diet included Programmed Nutrition Receiver in the total mixed ration for the first 21 days at the rate of 14 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus oryzae* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Thereafter, Programmed Nutrition Finisher was included in the total mixed ration at the rate of 20 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus niger* fermentation product, *Lactobacillus acidophilus*

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fermentation product, and *Enterococcus faecium* fermentation product. Both supplements were premixed into a ground corn carrier and subsequently blended into the total mixed ration.

# Exogenous growth promotants included Component ES implant administered on d 1 of the experiment, Component TE-IS at reimplant, and ractopamine hydrochloride (Optaflexx) fed at 400 mg·d<sup>-1</sup>·steer<sup>-1</sup> for the final 28 d before harvest (Elanco Animal Health, Greenfield, IN).

**Table 2.4** Interaction least squares means of objective and subjective cooked meat attributes of steaks from steers fed conventional diets<sup>†</sup> or Alltech Programmed Nutrition<sup>‡</sup> program with and without exogenous growth promotants (**ExGP**)<sup>§</sup>

Item	Conventional		Alltech PN		SEM	P-value		
	EGP-	EGP+	EGP-	EGP+		Program	ExGP	Program × ExGP
Objective measures								
Ultimate pH	5.61	5.62	5.61	5.61	0.008	0.64	0.18	0.37
Purge loss, %	0.65	0.62	0.50	0.50	0.04	0.004	0.69	0.79
Cook loss <sup>#</sup> , %	25.6	26.5	23.6	25.9	0.52	0.01	0.001	0.19
WBSF, kg	3.20	3.42	3.00	3.55	0.13	0.77	0.003	0.21
Subjective measures <sup>  </sup>								
Myofibrillar tenderness	5.59 <sup>a,y</sup>	5.36 <sup>b</sup>	5.77 <sup>a,x</sup>	5.03 <sup>c</sup>	0.09	0.30	< 0.01	0.01
Juiciness	5.21	5.02	5.12	4.97	0.07	0.18	0.003	0.70
Beef flavor intensity	5.28	5.30	5.23	5.26	0.05	0.39	0.62	0.96
Connective tissue amount	6.51 <sup>a</sup>	6.44 <sup>a</sup>	6.71 <sup>b</sup>	6.18 <sup>c</sup>	0.08	0.75	< 0.01	0.01
Overall tenderness	5.59 <sup>a</sup>	5.37 <sup>b</sup>	5.80 <sup>c</sup>	5.00 <sup>d</sup>	0.09	0.27	< 0.01	< 0.01
Off flavor intensity	7.70	7.68	7.65	7.61	0.05	0.24	0.57	0.84

<sup>a,b,c,d</sup>Values within a row with different letters are significantly different ( $P < 0.05$ ).

<sup>x,y</sup>Values within a row with different letters tend to be different ( $P < 0.10$ ).

<sup>†</sup>Conventional diets included vitamin A at 2,200 IU/kg; vitamin E at 22 IU/kg; copper sulfate to provide 10 mg/kg Cu; cobalt carbonate to provide 0.15 mg/kg Co; ethylenediamine dihydriodide to provide 0.5 mg/kg I; manganous sulfate to provide 60 mg/kg Mn; sodium selenite to provide 0.3 mg/kg Se; zinc sulfate to provide 60 mg/kg Zn on a dry matter basis; as well as 300 mg·d<sup>-1</sup>·steer<sup>-1</sup> of monensin and 90 mg·d<sup>-1</sup>·steer<sup>-1</sup> of tylosin (Elanco Animal Health; Greenfield, IN).

<sup>‡</sup>The Alltech diet included Programmed Nutrition Receiver in the total mixed ration for the first 21 days at the rate of 14 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus oryzae* fermentation product, *Lactobacillus acidophilus* fermentation product, and *Enterococcus faecium* fermentation product. Thereafter, Programmed Nutrition Finisher was included in the total mixed ration at the rate of 20 g·d<sup>-1</sup>·steer<sup>-1</sup> which contained zinc proteinate to provide 10.7 mg/kg Zn; manganese proteinate to provide 7.1 mg/kg manganese; cobalt proteinate to provide 1.2 mg/kg cobalt; copper proteinate to provide 2.9 mg/kg copper; calcium iodate to provide 0.6 mg/kg iodine; selenium yeast to provide 0.31 mg/kg selenium on a dry matter basis; as well as ascorbic acid, *Aspergillus niger* fermentation product, *Lactobacillus acidophilus* fermentation product, and

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*Enterococcus faecium* fermentation product. Both supplements were premixed into a ground corn carrier and subsequently blended into the total mixed ration.

§ Exogenous growth promotants included Component ES implant administered on d 1 of the experiment, Component TE-IS at reimplant, and ractopamine hydrochloride (Optaflexx) fed at  $400 \text{ mg} \cdot \text{d}^{-1} \cdot \text{steer}^{-1}$  for the final 28 d before harvest (Elanco Animal Health, Greenfield, IN).

# Measured as  $[(\text{raw weight} - \text{cooked weight}) / \text{raw weight}] \times 100$ .

|| Myofibrillar Tenderness (1 = extremely tough, 8 = extremely tender); Juiciness (1 = extremely dry, 8 = extremely juicy); Beef Flavor Intensity (1 = extremely bland, 8 = extremely intense); Connective Tissue Amount (1 = abundant, 8 = none); Overall Tenderness (1 = extremely tough, 8 = extremely tender); Off Flavor Intensity (1 = abundant, 8 = none).

## **Chapter 3 - Feeding microalgae meal (All-G Rich™; *Schizochytrium limacinum* CCAP 4087/2) to beef heifers. I: Effects on longissimus lumborum steak color and palatability**

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### **ABSTRACT**

The objective of this study was to examine effects of 4 levels of microalgae meal (All-G Rich, *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY) supplementation to the diet of finishing heifers on longissimus lumborum (LL) steak PUFA content, beef palatability, and color stability. Crossbred heifers (n = 288; 452 ± 23 kg initial BW) were allocated to pens (36 pens and 8 heifers/pen), stratified by initial pen BW (3,612 ± 177 kg), and randomly assigned within strata to 1 of 4 treatments: 0, 50, 100, and 150 g·heifer<sup>-1</sup>·d<sup>-1</sup> of microalgae meal. After 89 d of feeding, cattle were harvested and LL were collected for determination of fatty acid composition and Warner–Bratzler shear force (WBSF), trained sensory panel evaluation, and 7-d retail color stability and lipid oxidation analyses. Feeding microalgae meal to heifers increased (quadratic,  $P < 0.01$ ) the content of 22:6n-3 and increased (linear,  $P < 0.01$ ) the content of 20:5n-3. Feeding increasing levels of microalgae meal did not impact total SFA or MUFA ( $P > 0.25$ ) but tended ( $P = 0.10$ ) to increase total PUFA in a

quadratic manner ( $P = 0.03$ ). Total omega-6 PUFA decreased (linear,  $P = 0.01$ ) and total omega-3 PUFA increased (quadratic,  $P < 0.01$ ) as microalgae meal level increased in the diet, which caused a decrease (quadratic,  $P < 0.01$ ) in the omega-6:omega-3 fatty acid ratio. Feeding microalgae meal did not affect WBSF values or sensory panel evaluation of tenderness, juiciness, or beef flavor scores ( $P > 0.16$ ); however, off-flavor intensity increased with increasing concentration of microalgae meal in the diet (quadratic,  $P < 0.01$ ). From d 5 through 7 of retail display, steaks from heifers fed microalgae meal had a reduced  $a^*$  value and oxymyoglobin surface percentage, with simultaneous increased surface metmyoglobin formation (quadratic,  $P < 0.01$ ). Lipid oxidation analysis indicated that at d 0 and 7 of display, as the concentration of microalgae meal increased in the diet, the level of oxidation increased (quadratic,  $P < 0.01$ ). Muscle fiber type percentage or size was not influenced by the inclusion of microalgae meal in diets ( $P > 0.19$ ); therefore, the negative effects of microalgae on color stability were not due to fiber metabolism differences. Feeding microalgae meal to finishing heifers improves PUFA content of beef within the LL, but there are adverse effects on flavor and color stability.

## INTRODUCTION

In a review of the role of meat in a healthy human diet, Givens et al. (2006) stated that as economies become more developed, the amount of animal-derived foods increases. Omega-3 fatty acids are a family of PUFA that provide numerous health benefits, including reduced risks of cardiovascular disease, type 2 diabetes, and cancer (Ruxton et al., 2004; Calder, 2014). In particular, the long-chain omega-3 fatty acids eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) are the most functionally active within the body (Calder, 2014). Both EPA and DHA are found in copious amounts in fatty fish such as salmon and trout but are relatively absent in beef products due to biohydrogenation of dietary PUFA in the rumen (Harfoot, 1978). Because

most Americans do not consume adequate amounts of omega-3 PUFA (USDA and U.S. Department of Health and Human Services, 2010), research has focused on manipulating the fatty acid profile of beef as an alternative source of omega-3 fatty acids. The main strategy used to manipulate fatty acid profiles of beef has been through feeding oilseeds, plant oils, fish oil, marine algae, and fat supplements (Woods and Fearon, 2009). Supplementation of flaxseed and fish oil has increased the omega-3 content of beef derived from forage- and grain-fed cattle (Vatansever et al., 2000; Wistuba et al., 2006; Kronberg et al., 2011).

Increasing the omega-3 fatty acid content of beef may be appealing for some consumers, but polyunsaturated fats are susceptible to oxidation and, therefore, may cause adverse effects on meat quality. Increasing the omega-3 fatty acid content of beef has decreased color stability during display (LaBrune et al., 2008; Kronberg et al., 2011) and has increased the off-flavors of cooked product (Vatansever et al., 2000; Wistuba et al., 2006; LaBrune et al., 2008). Therefore, the objective of this study was to evaluate effects of feeding a microalgae meal during the finishing phase on LM fatty acid profiles and fresh meat color stability and palatability.

## **MATERIALS AND METHODS**

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee, and the Kansas State University Institutional Review Board approved procedures for the use of human subjects in sensory panel evaluations.

### ***Heifer Management***

Crossbred feedlot heifers ( $452 \pm 23$  kg initial BW) were housed in partially covered, concrete-surfaced pens (4.2 by 8.4 m; 36 pens and 8 heifers/pen) that provided 3.4 m of linear bunk space and were equipped with watering fountains between adjacent pens. Heifers were

predominantly black hided (80%), consisting mostly of Black Angus and Black Angus crossbreds, with lesser numbers of gray- and red-hided heifers having phenotypes consistent with Charolais crossbreds and Red Angus. Prior to the start of the experiment, pens were blocked by initial pen BW ( $3,612 \pm 177$  kg) and assigned within strata to 1 of 4 treatments. Treatments consisted of 0, 50, 100, and 150 g·heifer<sup>-1</sup>·d<sup>-1</sup> of supplemental microalgae meal (All-G Rich™; *Schizochytrium limacinum* CCAP 4087/2; Alltech Inc., Nicholasville, KY). All treatment groups were fed a similar basal diet, but the feed additive premix for each treatment group was formulated to provide the appropriate amount of microalgae meal by substituting it for ground corn. Additionally, supplemental vitamin E was included in all rations at 22 IU/kg of feed. Ractopamine hydrochloride (Optaflexx; Elanco Animal Health, Greenfield, IN) was supplemented for the final 28 d of the experiment at the rate of 400 mg·heifer<sup>-1</sup>·d<sup>-1</sup>. Heifers were fed once daily in fence line feed bunks that provided 28 linear centimeters of bunk space per animal. Daily rations were presented in quantities estimated to result in 227 g of unconsumed feed on the following day.

### ***Loin Collection and Processing***

At the completion of the 89-d feeding trial, a subset of black-hided heifers ( $527.5 \pm 8.4$  kg final BW; 3/pen) were randomly selected and transported to a commercial abattoir for harvest (Creekstone Farms, Arkansas City, KS). After a 48-h refrigeration period, strip loins (Institutional Beef Purchase Specifications number 180; NAMP, 2010) were removed from the left side of each carcass, vacuum packaged, and transported to the Kansas State University Meats Laboratory (Manhattan, KS) for processing. Twelve hours after arrival (72 h postmortem), two 1.27-cm thick steaks were removed from the anterior end of each loin for fatty acid and immunohistochemistry analyses. The remaining portion of each loin was weighed, vacuum

packaged, and stored at  $2 \pm 1^\circ\text{C}$  until 14 d postmortem. Following 14 d of refrigerated storage, loins were reweighed and pH was measured at the geometric center of each loin using a meat pH meter (model HI 99163; Hanna Instruments, Smithfield, RI). Purge loss was calculated using the equation  $[(\text{initial weight} - \text{final weight})/\text{initial weight}] \times 100$ . Subsequently, four 2.54-cm thick steaks were fabricated from each loin, starting at the anterior end. Steak 1 was used for display and 0 lipid oxidation analysis and steak 2 was displayed under simulated retail conditions for 7 d before being used for lipid oxidation analysis. Steaks 3 and 4 were vacuum packaged and frozen at  $-40^\circ\text{C}$  and subsequently used for Warner–Bratzler shear force (**WBSF**) and trained sensory panel analysis, respectively.

#### ***Fatty Acid Methyl Ester Analyses***

Determination of fatty acid methyl esters (**FAME**) of ground beef samples was performed using a 1-step extraction/transesterification method as described by Sukhija and Palmquist (1988). Two hundred milligrams of freeze-dried ground beef was extracted and transesterified in methanol:benzene:acetyl chloride (20:27:3, vol/vol) and 2 mL of internal standard (methyl tridecanoate; 2.0 mg 13:0/mL of benzene). Samples were heated for 2 h at  $70^\circ\text{C}$  in a 70-mL sealed screw-capped tube. After cooling, 5 mL potassium carbonate and 2 mL benzene were added. The tubes were then vortexed and centrifuged for 5 min at  $500 \times g$  at  $25^\circ\text{C}$  to allow separation, and the FAME in the solvent were transferred to 2-mL vials. The FAME was analyzed using an Agilent Gas Chromatograph (model 7890A; Agilent Technologies, Inc., Santa Clara, CA). Separation of FAME was accomplished on a fused silica capillary column HP-88 (30 m by 0.25 mm by  $0.20 \mu\text{m}$ ; Agilent Technologies, Inc.), with hydrogen as the carrier gas (35 mL/min flow rate and 100:1 split ratio). The initial oven temperature was  $80^\circ\text{C}$ , which was held 1 min; then, the oven temperature was increased at  $14^\circ\text{C}/\text{min}$  to  $240^\circ\text{C}$  and held 3 min. Injector

and detector temperatures were at 280 and 300°C, respectively. Individual fatty acids were identified by comparing retention times using genuine external standard Supelco 37 (47885-U Supelco; Sigma-Aldrich, St. Louis, MO). Individual FAME were quantified as a percentage of total FAME analyzed.

### ***Warner–Bratzler Shear Force and Sensory Analyses***

Warner–Bratzler shear force and sensory analyses were conducted according to procedures outlined in the American Meat Science Association (AMSA) meat cookery and sensory guidelines (AMSA, 2015). Twenty-four hours prior to cooking, steaks were thawed on trays at  $2.7 \pm 0.9^\circ\text{C}$ . Before cooking, steaks were weighed and a thermocouple (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak. Steaks were cooked on clam-style grills (Cuisinart Griddler; Cuisinart, Stamford, CT) using the ribbed grill plate side set to a surface temperature of  $232^\circ\text{C}$  and removed from grills at  $70^\circ\text{C}$ . Following cooking, steaks were reweighed and the cook loss was determined using the equation  $[(\text{initial weight} - \text{cooked weight})/\text{initial weight}] \times 100$ . After a 24-h chill period, six 1.27-cm cores were removed from each steak parallel to the muscle fiber and sheared once through the center using an Instron model 5569 testing machine (Instron, Canton, MA) with a Warner–Bratzler shear head attached (100 kg compression load cell and crosshead speed of 250 mm/min).

Sensory panel steaks were cooked according to procedures described for WBSF analyses. After cooking, steaks were cut into 1.27 by 1.27 by 2.54 cm pieces and presented to a 6- to 9-member trained sensory panel. Panelists were selected from a pool of 25 candidates from Kansas State University's Animal Sciences and Industry Department (Manhattan, KS), and panelists were screened and trained according to the AMSA meat cookery and sensory guidelines (AMSA, 2015).

Selected panelists were oriented to strip loin steak evaluation procedures over 4 training sessions prior to initiation of panels by evaluating commodity beef steaks. Panelists were presented 2 pieces from each of 8 steaks ( $n = 2$  per treatment) under low intensity ( $<107.64$  lumens) red incandescent lighting. Panelists evaluated samples for myofibrillar tenderness, juiciness, beef flavor intensity, connective tissue amount, overall tenderness, and off-flavor intensity using an 8-point scale (1 = extremely tough, extremely dry, extremely bland, abundant, extremely tough, or abundant, respectively, and 8 = extremely tender, extremely juicy, extremely intense, none, extremely tender, or none, respectively). A total of 12 separate panels were conducted to analyze all samples.

### ***Simulated Retail Display***

Steaks used for the 7-d simulated retail display were placed on 17S polystyrene foam trays with a Dri-Loc (Dri-Loc 50; Cryovac Sealed Air Corp., Duncan, SC) absorbent pad and overwrapped with polyvinyl chloride film (AEP Industries Inc., South Hackensack, NJ) with an oxygen transmission rate of  $1,450 \text{ cm}^{-3} \cdot 645.2 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$ . Steaks were orientated on trays so the posterior end faced up and the medial portion of the steak was on the left side of the tray, as viewed from above. Steaks were displayed in coffin-style retail cases (model DMF 8; Tyler Refrigeration Corp., Niles, MI) under fluorescent lights (32 W Del-Warm White 3000° K; Philips Lighting Co., Somerset, NJ) that emitted a constant 24-h case average intensity of  $2,230 \pm 34 \text{ lx}$ . Case temperature was monitored using a Thermochron iButton (Maxim Integrated Products, Sunnyvale, CA). Average temperatures of the cases at steak package surfaces were  $0.26 \pm 0.95^\circ\text{C}$  and the cases were defrosted twice daily (morning and evening) at  $11^\circ\text{C}$  for 30 min. Every 12 h, steaks were rotated in the cases from left to right and front to back to account for variation in temperature and light intensity within the cases. Readings for CIE  $L^*$ ,  $a^*$ , and  $b^*$

and reflectance from 400 to 700 nm were taken at the 3 steak locations on each day of display using a HunterLab Miniscan EZ spectrophotometer (Illuminant A, 2.54-cm diameter aperture, 10° observer; Hunter Associates Laboratory, Reston, VA) every 24 h. Surface reflectance values at 473, 525, 572, and 700 nm were used to calculate surface percentages of metmyoglobin and oxymyoglobin using equations from Krzywicki (1979) as published in the *AMSA Meat Color Measurement Guidelines* (AMSA, 2012). Values from the 3 scans were used to calculate an average value for each steak.

### ***Thiobarbituric Acid Reactive Substances***

The extent of lipid oxidation during simulated retail display was assessed using the thiobarbituric acid reactive substances (**TBARS**) assay using procedures first described by Buege and Aust (1978) published in the *AMSA color guidelines* (AMSA, 2012). Briefly, steaks were cut into 1.27 by 1.27 by 2.54 cm pieces, frozen in liquid nitrogen, and pulverized using a Waring blender (Waring Products Division, Hartford, CT). Duplicate 0.5-g subsamples of each steak were weighed into 15-mL conical tubes and stored at  $-80^{\circ}\text{C}$  until analysis. Samples were heated in 2.5 mL of thiobarbituric acid stock solution (0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 *N* HCl) in a  $100^{\circ}\text{C}$  water bath for 10 min. Samples were cooled in room temperature water for 5 min and centrifuged at  $4^{\circ}\text{C}$  and  $5,000 \times g$  for 10 min. One milliliter of the resulting supernatant was transferred to a cuvette and absorbance was read at 532 nm (Eon Microplate Spectrophotometer; BioTek Instruments, Inc., Winooski, VT). Values were expressed as milligrams malonaldehyde/kilogram of muscle calculated using the equation published in the *AMSA Meat Color Measurement Guidelines* (AMSA, 2012).

### ***Immunohistochemistry***

Analyses of muscle fiber characteristics were conducted following procedures described by Phelps et al. (2014). Briefly, one 1 by 1 by 1.27 cm sample was collected from the geometric center of the medial, midlateral, and lateral portions of the longissimus lumborum (**LL**;  $n = 3/\text{steak}$ ). Five-micrometer cryosections were cut using a Microm 550 cryostat (Thermo Fisher Scientific Inc., Waltham, MA). Cryosections were blocked using 10% horse serum in PBS. Afterwards, cryosections were incubated in the following primary antibodies diluted in blocking solution for 1 h: anti-dystrophin (Thermo Fisher Scientific Inc.), anti-slow myosin heavy chain (BA-D5; Developmental Studies Hybridoma Bank, Iowa City, IA), and anti-myosin heavy chain all but IIX (BF-35; Developmental Studies Hybridoma Bank). Following a wash step, cryosections were incubated in secondary antibodies (AlexaFluor 594, 633, and 488 for anti-dystrophin, BA-D5, and BF-35, respectively; Thermo Fisher Scientific Inc.) in blocking solution for 45 min. Cryosections were cover slipped and photomicrographs were captured using a Nikon Eclipse TI-U inverted microscope with an attached DS-QiMC digital camera at a 100x magnification (Nikon Instruments Inc., Melville, NY).

For each steak location, a minimum of 500 fibers were analyzed for myosin heavy chain type and muscle fiber cross-sectional area (**CSA**) using NIS-Elements software (Basic Research 3.3; Nikon Instruments, Inc., Lewisville, TX). The area constrained by  $\alpha$ -dystrophin immunostaining defined individual fibers for CSA measurements. Fibers that stained positive for the BA-D5 antibody were classified as type I fibers. Fibers that stained positive for BF-35 but that were negative for BA-D5 were classified as type IIA fibers. All fibers that were negative for BF-35 were classified as type IIX fibers (Schiaffino et al., 1989; Moreno-Sánchez et al., 2008).

### *Statistical Analyses*

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) with pen as the experimental unit and animal as the observational unit. Treatment was the fixed effect and initial BW block was the random effect. For retail shelf life data, data were analyzed as a randomized complete block design with repeated measures. Day of display served as the repeated measure with steak (observational unit) as the subject and compound symmetry as the covariance structure. Preplanned linear and quadratic contrasts were tested for all data and within each day of display for the color data. Differences were considered significant at  $P \leq 0.05$  and regarded as tendencies at  $0.05 > P \leq 0.10$ .

## **RESULTS**

### *Longissimus Lumborum Fatty Acid Content*

Fatty acid profiles of LL steaks from the 4 treatments are presented in Table 4.1. As the amount of microalgae meal in the diet increased, the amount of 18:1 *trans*-11 increased (quadratic,  $P < 0.01$ ) and the amount of 18:2 $n$ -6 *cis* and 20:3 $n$ -6 decreased (linear,  $P < 0.01$ ). As the amount of microalgae meal in the diet increased, the content of CLA *cis*-9, *trans*-11 increased (quadratic,  $P < 0.01$ ). Also, as the amount of microalgae meal in the diet increased, the amount of 24:1 decreased (quadratic,  $P < 0.01$ ). The amount of 20:5 $n$ -3 and 22:6 $n$ -3 increased at a greater rate (quadratic,  $P < 0.01$ ) as the microalgae meal content of the diet was increased. Feeding microalgae meal affected ( $P = 0.01$ ) 22:5 $n$ -3 content, but no linear or quadratic responses were found ( $P > 0.19$ ). Feeding increasing levels of microalgae meal did not impact total SFA or MUFA ( $P > 0.25$ ) but tended ( $P = 0.10$ ) to increased total PUFA in a quadratic manner ( $P = 0.03$ ). Total omega-6 PUFA decreased (linear,  $P = 0.01$ ) and total omega-3 PUFA

increased (quadratic,  $P < 0.01$ ) as microalgae meal level increased in the diet, which caused a decrease (quadratic,  $P < 0.01$ ) in the omega-6:omega-3 fatty acid ratio.

### ***Warner–Bratzler Shear Force and Sensory Analyses***

There were no treatment effects on ultimate pH or purge loss from loins stored 14 d postmortem ( $P > 0.20$ ; Table 4.2). Increasing microalgae meal in the diet did not affect cook loss or WBSF ( $P > 0.15$ ). Trained sensory panelists detected no differences among treatments for myofibrillar tenderness, juiciness, beef flavor intensity, connective tissue amount, or overall tenderness ( $P > 0.16$ ). Off-flavor intensity increased (quadratic,  $P < 0.01$ ) as the microalgae meal concentration of the diet increased.

### ***Simulated Retail Display***

As expected, day of display impacted all color measurements in a manner consistent with color deterioration during retail display ( $P < 0.01$ ). Additionally, there were treatment  $\times$  day interactions for all color attributes and TBARS values ( $P < 0.01$ ). Therefore, within each day of display, linear and quadratic contrasts of microalgae meal treatments were analyzed. From the outset of the display period through d 2 there was a decrease in  $L^*$  value as the concentration of microalgae meal in the diet was increased (linear,  $P < 0.03$ ; Figure 4.1). This was also seen on d 4 of display (linear,  $P < 0.01$ ). On d 3, 5, and 6 of display, the magnitude of the decrease in  $L^*$  due to increasing the microalgae meal content of the diet tended to decline (quadratic,  $P < 0.07$ ) and continued to decline through d 7 of display (quadratic,  $P = 0.05$ ).

In contrast to the  $L^*$  value, there was no effect of treatment on  $a^*$  and  $b^*$  values on d 0 of display ( $P > 0.38$ ; Figure 4.1); however, on d 1, treatment affected ( $P < 0.03$ )  $a^*$  and  $b^*$  values of steaks. On d 2, 3, and 4 of display, as the content of microalgae meal was increased in the

diet, there were decreases in  $a^*$  and  $b^*$  values (linear,  $P < 0.04$ ). On d 5, 6, and 7, the magnitude of decreases in  $a^*$  and  $b^*$  values increased as the concentration of microalgae meal in the diet increased (quadratic,  $P < 0.03$ ).

The discoloration patterns observed for  $a^*$  and  $b^*$  measurements were also seen for steak surface oxymyoglobin and metmyoglobin accumulation (Figure 4.2). From d 0 through 4 of display, increasing the amount of microalgae meal in the diet increased (linear,  $P = 0.04$ ; quadratic,  $P = 0.06$ ) the percentage of surface metmyoglobin. On d 0 of display, increasing microalgae meal in the diet decreased (linear,  $P < 0.01$ ) the percentage of surface oxymyoglobin, but from d 1 to 2, treatment only tended to affect the surface oxymyoglobin percentage ( $P < 0.06$ ). During d 3 and 4 of display, the surface oxymyoglobin percentage decreased as microalgae meal in the diet increased (linear,  $P < 0.01$ ) whereas the surface metmyoglobin percentage linearly increased ( $P < 0.01$ ) on d 3 and tended to increase in a quadratic fashion ( $P = 0.08$ ) on d 4. By d 5 and through d 7 of the display period, the decrease in the surface oxymyoglobin percentage and subsequent increase in the metmyoglobin percentage became more severe as the concentration of microalgae meal in the diet was elevated (quadratic,  $P < 0.04$ ).

### ***Thiobarbituric Acid Reactive Substances***

The extent of lipid oxidation during retail display was measured on d 0 and 7 of display (Figure 4.3). There was a treatment  $\times$  day interaction ( $P < 0.01$ ); therefore, contrasts were conducted within each day of display. On both days of display, feeding increasing concentrations of microalgae meal in the diet increased lipid oxidation (quadratic,  $P < 0.01$ ).

### ***Immunohistochemistry***

There were no treatment effects on the distribution of type I, IIA, and IIX fibers within the LL ( $P > 0.30$ ; Figure 4.4). Additionally, there were no treatment effects on the CSA of each fiber type within the LL ( $P > 0.16$ ; Table 4.3).

## **DISCUSSION**

In the United States, meat contributes more than 40% of daily protein intake and 20% of daily fat intake (Daniel et al., 2011). Beef is one major meat protein source in the United States but is regarded as having relatively high concentrations of SFA compared with other protein sources. The intake of fat from meat has been a public health discussion for over 60 yr following the first recommendations of the American Heart Association to reduce intake of dietary cholesterol, saturated fat, and total fat to prevent cardiovascular disease (Eckel et al., 2013). According to the 2015 *Dietary Guidelines for Americans*, 20 to 35% of a person's daily calories should originate from fat but less than 10% should be from saturated fats and the remainder from unsaturated fats (U.S. Department of Health and Human Services and USDA, 2015). Due to the undesirable fatty acid profile of beef and changes in dietary recommendations over time, researchers have explored nutritional regimens that increase the PUFA content of meat. Although feeding microalgae meal only tended to increase total PUFA, it reduced the amount of omega-6 PUFA and increased the amount of omega-3 PUFA, leading to a reduction in the omega-6:omega-3 ratio. The reduction of the omega-6:omega-3 ratio from 7:1 for steaks from heifers fed 0 g of microalgae/d (**Algae0**) to 3.4:1 for steaks from heifers fed 100 g of microalgae/d (**Algae100**) and 150 g of microalgae/d (**Algae150**) meets dietary recommendations for consuming foods with an omega-6:omega-3 ratio below a 4:1 for health benefits

(Simopoulos, 2002). In other studies, Mandell et al. (1997) reported that feeding steers a diet containing 10% fish meal for 168 d reduced the omega-6:omega-3 ratio from 5.6 to 1.72 and Dunne et al. (2011) reported that supplementing heifers up to 275 g of rumen-protected fish oil reduced the ratio from 4.3 to 2.04.

Givens et al. (2000) concluded that fish oil/meal studies demonstrate the greatest ability to increase the DHA and EPA content of beef; however, questions about maintaining sustainable sources of fish oil/meal that are consistent in quality remain. Therefore, Givens et al. (2000) identified microalgae as an alternative to fish oil/meal. In the current study, as the microalgae content of the diet of heifers increased, EPA and DHA content of the LL increased. Compared with steaks from heifers fed no microalgae meal in the diet, steaks from heifers fed 150 g·heifer<sup>-1</sup> · d<sup>-1</sup> of microalgae meal had 850 and 340% greater DHA and EPA concentrations, respectively. The DHA finding is in agreement with Franklin et al. (1999), who found a similar increase in the DHA content of milk from cows fed 910 g of protected microalgae. When compared with literature on fish oil, elevation of DHA and EPA in the current study are greater than increases reported by Vatansever et al. (2000), Scollan et al. (2001), and Wistuba et al. (2007); however, Mandell et al. (1997) reported similar increases in DHA and EPA when fish meal was included at 10% of the diet. Dunne et al. (2011) found that increasing the content of ruminally protected fish oil produced the same quadratic increase in DHA content of neutral lipids but saw no response for EPA in neutral lipids.

Although there are few studies documenting the impact of microalgae on beef fatty acid profiles, there are 2 studies that use lamb models. Cooper et al. (2004) reported that adding microalgae to diets also containing fish oil increased lamb LM EPA and DHA phospholipids by 127 and 39%, respectively, compared with diets containing linseed oil. Also, Cooper et al.

(2004) reported that adding microalgae to diets containing protected lipid supplement increased lamb LM EPA and DHA content of phospholipids by 329 and 377%, respectively, compared with diets containing linseed oil. In the neutral lipid fraction, including microalgae in diets containing fish oil increased EPA and DHA by 125 and 575%, respectively, compared with diets containing linseed oil. Additionally, including microalgae in diets containing a protected lipid supplement substantially increased EPA and DHA in the neutral lipid fraction compared with diets containing linseed oil. Using the same treatments as Cooper et al. (2004), Nute et al. (2007) reported that adding microalgae to diets containing fish oil increased EPA and DHA by 127 and 25%, respectively, in the phospholipid fraction compared with diets containing linseed oil. Also, Nute et al. (2007) reported that adding microalgae to diets containing a protected lipid supplement increased EPA and DHA by 373 and 377%, respectively, in the phospholipid fraction compared with diets containing linseed oil. Therefore, these findings would indicate that microalgae may serve as a better source of dietary DHA and EPA for ruminant animals than fish oil or fish meal.

The results of fish oil and microalgae studies are encouraging for improving the fatty acid profile of animal products, but some of the same studies indicate there may be negative effects on both palatability and shelf life. Tenderness and flavor are the 2 most important attributes consumers evaluate when determining the quality of their eating experience (Beermann, 2009; O'Quinn et al., 2012). In the current study, feeding microalgae did not influence measures of objective and subjective tenderness. Because muscle fiber CSA can influence cooked meat tenderness (Crouse et al., 1991; Ebarb et al., 2016), the absence of an effect of microalgae feeding on CSA helps explain the lack of differences in tenderness. In agreement, Nute et al. (2007) reported that sensory panel tenderness and juiciness scores were not influenced by

feeding microalgae to lambs. Wistuba et al. (2006) reported that supplementing steers 3% fish oil during the finishing phase did not affect WBSF or steak cook loss. Using 2 separate sensory panels, Vatanserver et al. (2000) found that sirloin steaks from steers fed fish oil had slightly poorer tenderness scores when panelists evaluated the steaks on an 8-point scale but there were no differences when panelists used a 100-mm line scale.

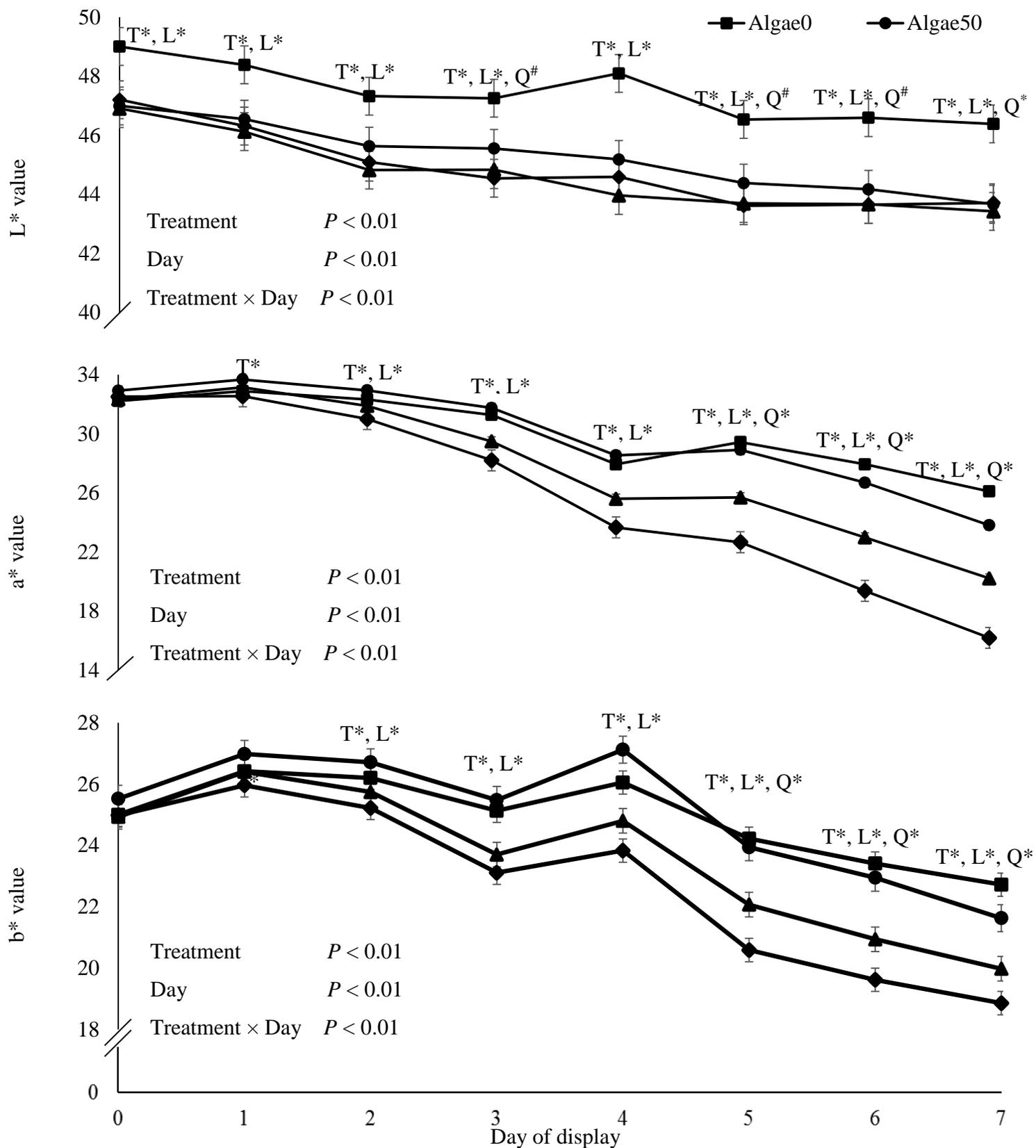
The adverse impact of microalgae feeding on sensory panel off-flavor ratings of LL steaks could be a concern for retailers. When examining off-flavor descriptors recorded by panelists in this study, oxidized, grassy, and fishy were the 3 most commonly recorded off-flavors. An increase in off-flavor intensity of products with increased levels of omega-3 fatty acids is commonly reported because these fatty acids are more susceptible to lipid oxidation (Jacobsen, 2008). In contrast to the present study but analyzing a different animal product, Franklin et al. (1999) found that microalgae did not alter the flavor of milk. Vatanserver et al. (2000) reported that supplementing fish oil to steers increased fishy and rancid flavors and increased the intensity of abnormal flavors in longissimus dorsi steaks. Similarly, Wistuba et al. (2006) reported that supplementing fish oil elevated fishy flavors in LL steaks. In lambs, sensory panelists rated chops from microalgae meal-supplemented lambs as having the most “rancid” off-flavors (Nute et al., 2007). These chops had the greatest proportion of DHA in the muscle, which correlated with reductions in lamb flavor and increases in abnormal lamb, rancid, and fishy flavors. Additionally, muscle EPA content correlated to fishy off-flavors more than DHA content. Nute et al. (2007) concluded that the rancid flavors detected by panelists were due to the greater PUFA content of the microalgae meal and fish oil supplemented meat, which possessed more oxidation-susceptible double bonds.

Color is the most important attribute that consumers evaluate when making purchasing decisions (Mancini and Hunt, 2005). Faustman et al. (1998) reported that consumers prefer bright, cherry red-colored steaks; therefore, maintaining the redness of steaks during display will help keep products available for purchase longer. In the current study, differences in the color of steaks from each treatment group are depicted in Fig. 5. All measures of surface color followed the typical patterns associated with steak discoloration; however, steaks of microalgae-fed heifers had accelerated discoloration in all measures. This is especially true for the accelerated decreases in surface oxymyoglobin percentage and  $a^*$  and the simultaneous increase in metmyoglobin percentage as concentration of microalgae fed and display time were increased. In agreement, color deteriorated quicker during the final 4 d of an 11-d retail display study when lambs were fed a fish oil/microalgae-supplemented diet (Nute et al., 2007). In minced beef models, feeding fish oil adversely affected color saturation (Vatansever et al., 2000) and surface metmyoglobin formation (Daly et al., 2007). Vatansever et al. (2000) also demonstrated the accelerated increase in metmyoglobin formation with fish oil inclusion compared with other treatments as display time increased. In contrast to these studies, minced neck muscle from heifers supplemented ruminally protected fish oil did not exhibit differences in  $L^*$ ,  $a^*$ , and oxymyoglobin and metmyoglobin formation (Dunne et al., 2011). The authors hypothesized that the difference in their results compared with other studies were due the neck muscle being used and muscles from this from this area having muscle fibers that are more oxidative and smaller, which leads to greater antioxidant capability.

A muscle's fiber composition has an effect on its postmortem metabolic properties that impact color such as oxygen consumption rate and metmyoglobin reducing ability (Mancini and Hunt, 2005). Because muscle fiber type percentage was not altered by microalgae inclusion, a

change in the metabolism of the muscle is not responsible for the rapid color deterioration demonstrated in the current study. Therefore, the color deterioration observed in the current study is likely due to the shift to a more unsaturated fatty acid profile and increased oxidation of these fatty acids. Beef products with greater PUFA content are susceptible to oxidation during display (Yang et al., 2002). At both the beginning and end of display, lipid oxidation as measured by TBARS increased with increasing microalgae meal concentrations of the diet. Also of importance is the amount of oxidation detected on d 0 of display in relation to the flavor problems noted above. Younathan and Watts (1959) stated that a TBARS value of 1 indicated a level of lipid oxidation at which consumers detected rancidity. On d 0 of display, which corresponds to d 14 of aging, both the Algae100 and Algae150 treatments displayed TBARS values greater than 1. Because lipids having more unsaturated fatty acids are more susceptible to oxidation, they can have greater TBARS values (Jacobsen, 2008). Because Algae100 and Algae150 treatments had greater unsaturated fatty acid content, this may indicate why sensory panelists detected more off-flavors in steaks from these treatments. Vatansever et al. (2000) reported that steaks from steers fed fish oil had increased TBARS values on d 4, 8, and 11 of display. Additionally, steaks from fish oil-supplemented steers had values over 1 on d 8 and 11 of display, whereas the other treatments never achieved this value. Nute et al. (2007) reported that chops from lambs supplemented fish oil/microalgae had greater TBARS value than those from lambs supplemented linseed oil and protected lipid supplement. Interestingly, the fish oil/microalgae steaks also had reduced vitamin E content than these 2 treatments. Dunne et al. (2011) also found in their dose titration study that the greatest level of fish oil supplemented caused minced beef to have the greatest TBARS value at d 10 of display; however, there were no differences in surface color properties. The authors speculated that their muscle had a high  $\alpha$ -

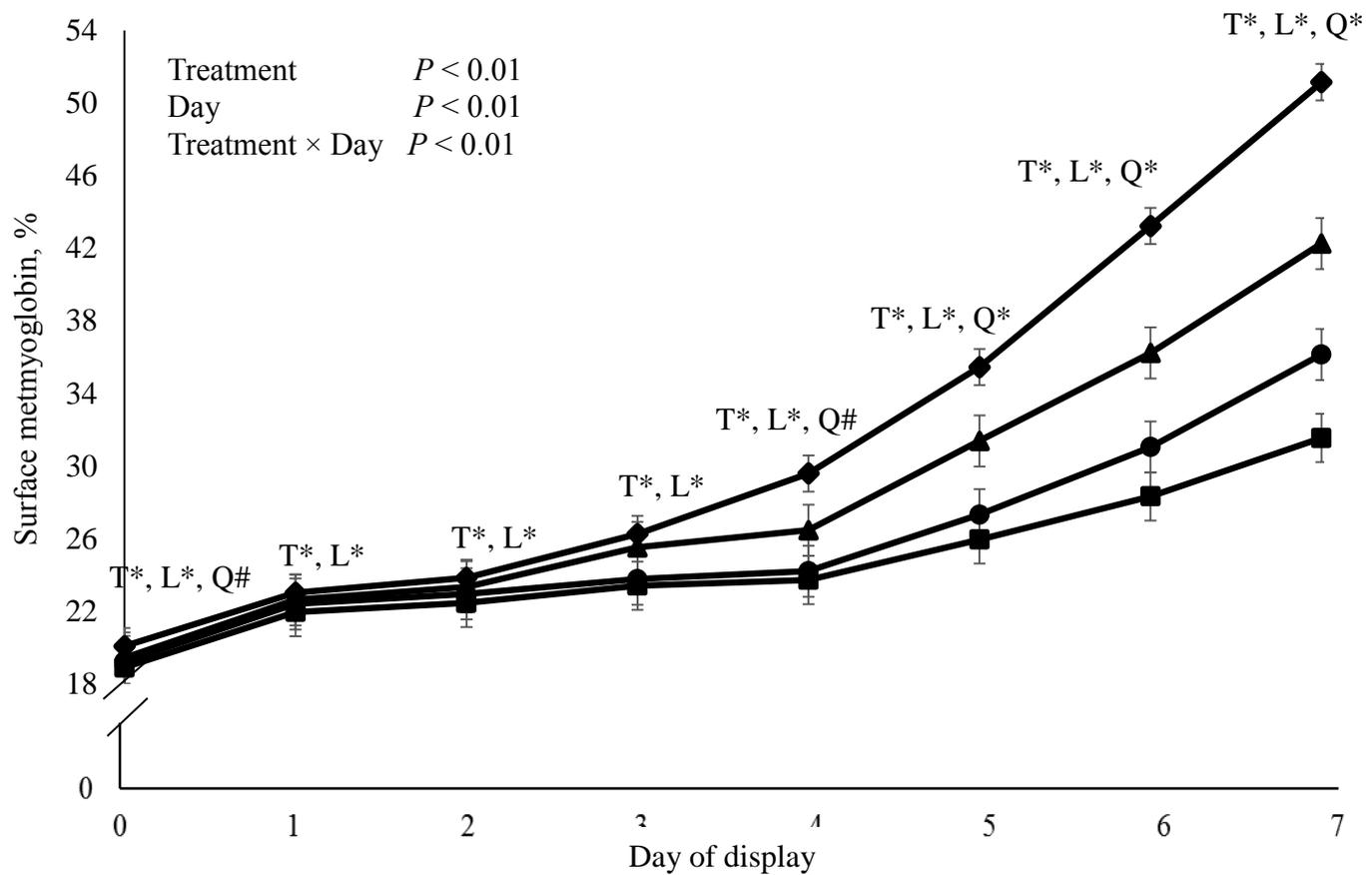
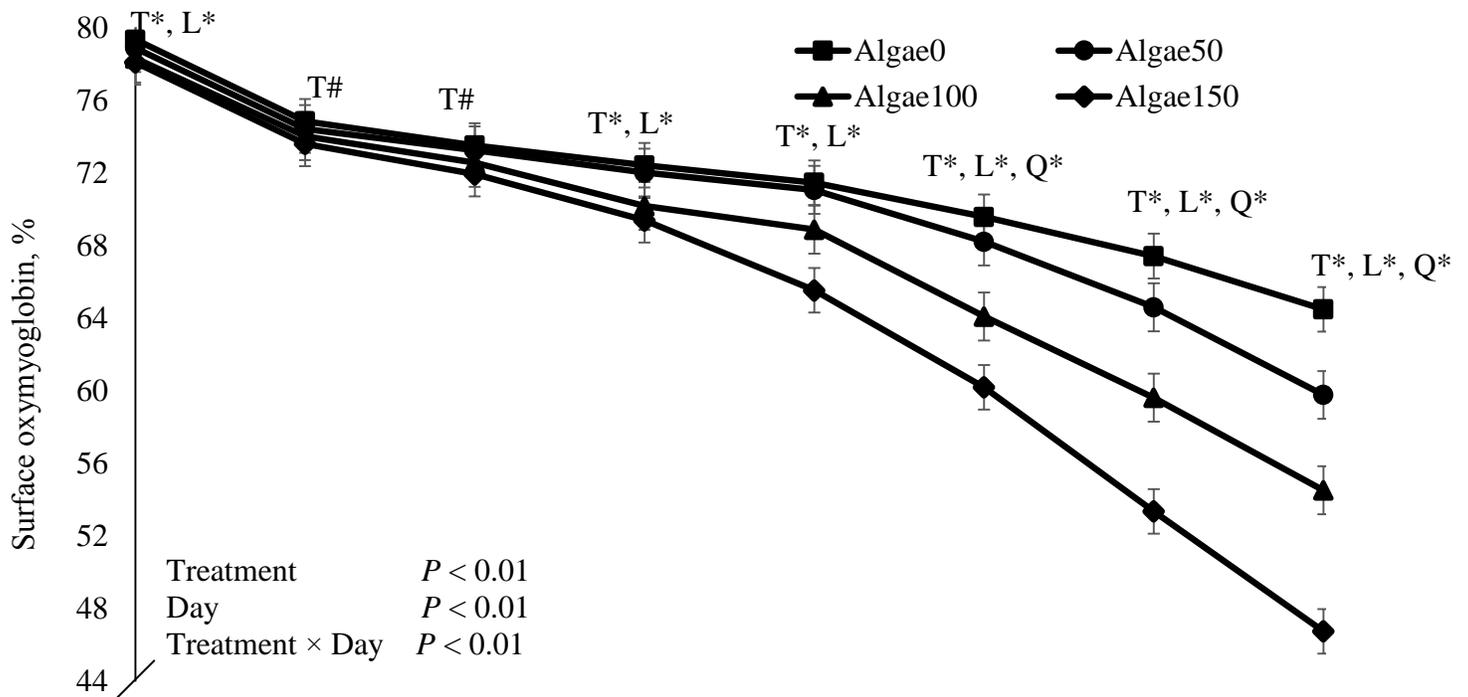
tocopherol content that inhibited treatment differences. Feeding elevated levels of vitamin E during the finishing phase can help mitigate lipid oxidation and color deterioration during display (Yang et al., 2002; Gobert et al., 2010). In the current study, the antioxidant content of the diet was not adjusted to account for potential increases in PUFA due to the inclusion of microalgae. Future studies using this microalgae product should focus on altering the antioxidant content of the diet to prevent abnormal flavors of beef steaks and reduction in color stability.



**Figure 3.1** Longissimus lumborum steak L\* (lightness: 0 = black, 100 = white), a\* (redness: -60 = green, 60 = red), and b\* (blueness: -60 = blue, 60 = yellow) values from heifers supplemented 0 (Algae0), 50 (Algae50),

100 (Algae100), and 150 (Algae150) g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Steaks were displayed under simulated retail conditions for 7-d. A T indicates a treatment effect, L indicates a linear effect of algae, and Q indicates a quadratic effect of algae. \* indicates a significant effect ( $P \leq 0.05$ ) and # indicates a tendency ( $P \leq 0.10$ ).

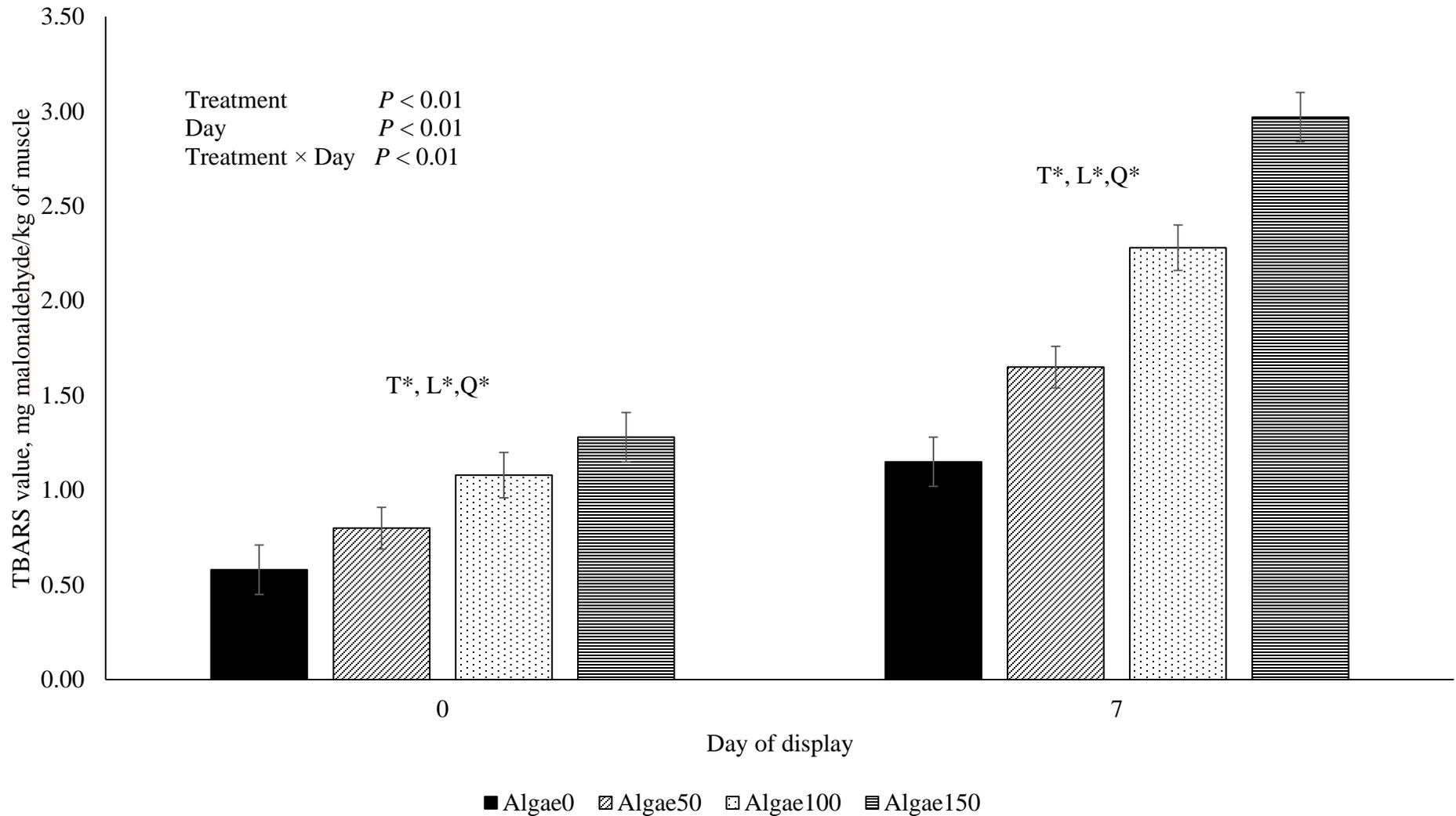
**Figure 2.**



**Figure 3.2** Longissimus lumborum steak surface oxymyoglobin and metmyoglobin percentage from heifers supplemented 0 (Algae0), 50 (Algae50), 100 (Algae100), and 150 (Algae150)  $\text{g} \cdot \text{heifer}^{-1} \cdot \text{d}^{-1}$  of microalgae meal

(All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Steaks were displayed under simulated retail conditions for 7-d and percent oxymyoglobin and metmyoglobin were calculated using the equations of Krzywicki (1979). A T indicates a treatment effect, L indicates a linear effect of algae, and Q indicates a quadratic effect of algae. \* indicates a significant effect ( $P \leq 0.05$ ) and # indicates a tendency ( $P \leq 0.10$ ).

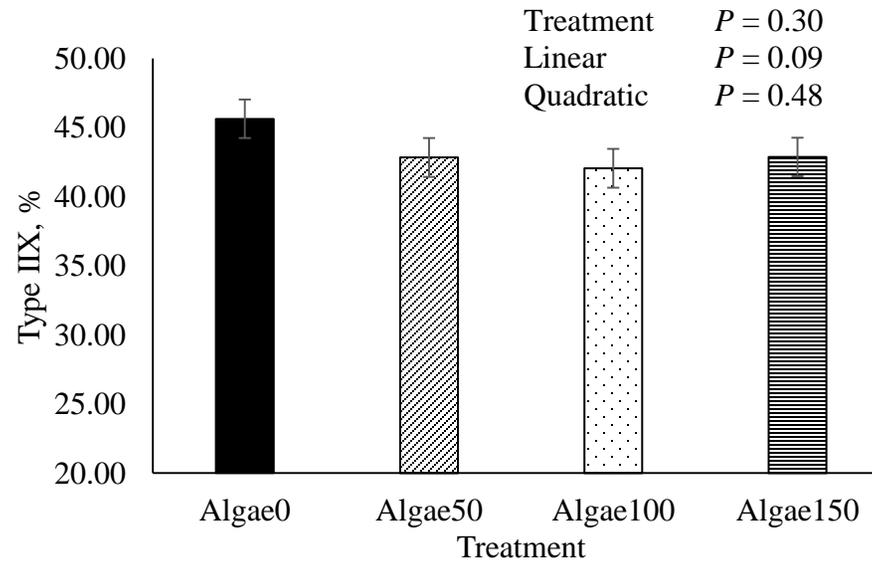
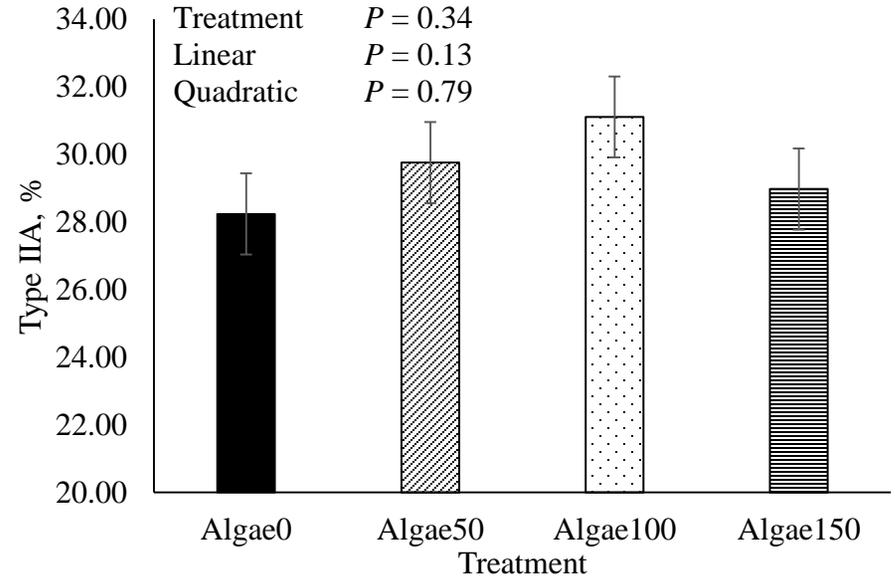
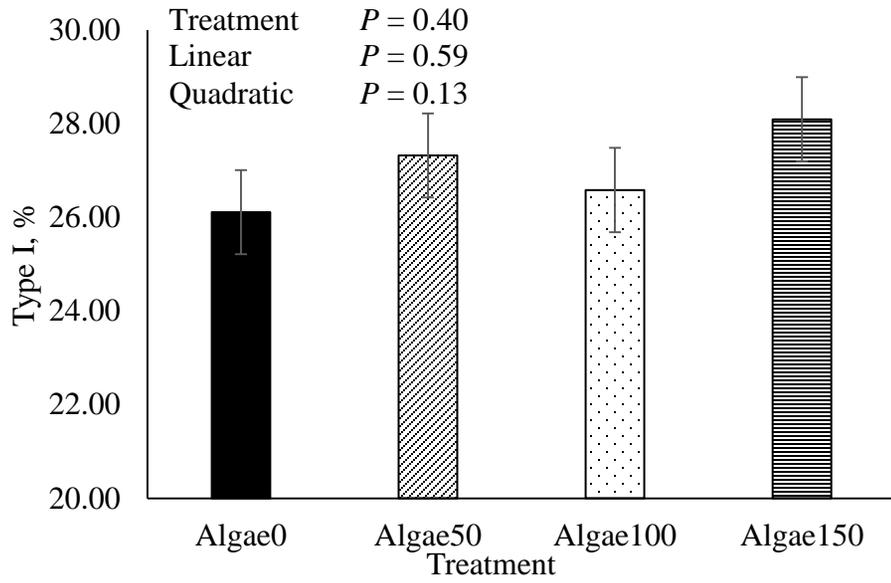
Figure 3.



**Figure 3.3** Day 0 and 7 Longissimus lumborum steak lipid oxidation from heifers supplemented 0 (Algae0), 50 (Algae50), 100 (Algae100), and 150 (Algae150) g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Thiobarbituric acid reactive substances (TBARS) assay was conducted according procedures outlined in the AMSA Color Guidelines (AMSA,

2012). Values were expressed as a TBARS expressed in mg malonaldehyde (**MDA**)/kg of muscle. T indicates a treatment effect, L indicates a linear effect of algae, and Q indicates a quadratic effect of algae. \* indicates a significant effect ( $P \leq 0.05$ ).

**Figure 4.**



**Figure 3.4** Muscle fiber type distribution of the Longissimus lumborum from heifers supplemented 0 (Algae0), 50 (Algae50), 100 (Algae100), and 150 (Algae150) g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). The methods of Phelps et al. (2014) were followed for immunostaining. Fibers that stained positive for the BA-D5 antibody were categorized as type I fibers. Fibers that stained positive for BF-35, but were negative for BA-D5 were categorized as type IIA fibers. All fibers that were negative for the BF-35 antibody were categorized as type IIX fibers (Moreno-Sanchez et al., 2008; Schiaffino et al., 1989).



Algae0



Algae50



Algae100



Algae150

**Figure 3.5** Day-7 photographs of representative steaks from heifers supplemented 0 (Algae0), 50 (Algae50), 100 (Algae100), and 150 (Algae150)  $\text{g}\cdot\text{heifer}^{-1}\cdot\text{d}^{-1}$  of microalgae meal (All-G Rich<sup>TM</sup>, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY). Steaks were placed on 17S Styrofoam trays with absorbent pads, overwrapped with polyvinyl chloride film, and displayed under simulated retail conditions for 7 d.

**Table 3.1** Least squares means of fatty acid profiles of longissimus lumborum steaks from heifers fed 0, 50, 100, or 150 g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Fatty acid methyl ester <sup>2</sup>	Algae, g•heifer <sup>-1</sup> •d <sup>-1</sup>				SEM	P – value <sup>1</sup>		
	0	50	100	150		Algae	Linear	Quad
14:0	1.79	1.94	1.66	1.98	0.18	0.51	0.62	0.15
14:1	0.48	0.55	0.44	0.56	0.06	0.51	0.46	0.19
15:0	0.25	0.25	0.22	0.27	0.02	0.40	0.32	0.22
15:1	0.04	0.04	0.04	0.04	0.01	0.14	0.04	0.54
16:0	16.13	17.51	14.72	17.79	1.40	0.33	0.50	0.09
16:1	2.09	2.29	1.77	2.13	0.20	0.25	0.16	0.12
17:0	0.69	0.69	0.59	0.75	0.07	0.31	0.34	0.20
17:1	0.56	0.55	0.45	0.56	0.04	0.22	0.09	0.21
18:0	7.87	8.01	6.61	7.68	0.64	0.38	0.13	0.31
18:1 <i>cis</i> -9	22.57	24.00	18.60	22.42	1.96	0.22	0.10	0.15
18:1 <i>trans</i> -11	1.74	2.44	2.50	3.61	0.26	<0.01	<0.01	<0.01
18:1 <i>cis</i> -11	0.86	0.93	0.75	0.88	0.06	0.22	0.16	0.10
18:2n-6 <i>cis</i>	2.36	2.34	1.96	2.24	0.12	0.03	0.01	0.20
18:2n-6 <i>trans</i>	0.04	0.04	0.04	0.05	0.01	0.14	0.64	0.13
18:3n-6 <i>cis</i>	0.02	0.01	0.02	0.02	0.001	<0.01	0.99	0.05
18:3n-3	0.19	0.20	0.15	0.19	0.02	0.13	0.09	0.08
20:0	0.04	0.04	0.03	0.04	0.01	0.31	0.08	0.40
20:1	0.09	0.10	0.07	0.09	0.01	0.29	0.11	0.32
20:2	0.07	0.07	0.07	0.08	0.01	0.27	0.84	0.06
20:3n-6	0.15	0.13	0.13	0.13	0.01	<0.01	<0.01	0.10
20:4n-6 and 22:1n-9 <sup>3</sup>	0.41	0.41	0.41	0.41	0.01	0.92	0.93	0.48
20:5n-3	0.05	0.09	0.17	0.22	0.01	<0.01	<0.01	<0.01
22:5n-3	0.12	0.10	0.12	0.12	0.01	0.01	0.19	0.34
22:6n-3	0.02	0.13	0.19	0.19	0.01	<0.01	<0.01	<0.01
23:0	0.02	0.02	0.02	0.02	0.01	0.72	0.90	0.26
24:0	0.04	0.04	0.04	0.05	0.01	0.25	0.98	0.22
24:1	0.04	0.03	0.02	0.02	0.01	<0.01	<0.01	<0.01
CLA <i>cis</i> -9, <i>trans</i> -11	0.16	0.21	0.17	0.23	0.02	0.07	0.72	0.01
CLA <i>trans</i> -10, <i>cis</i> -12	0.02	0.02	0.02	0.02	0.01	0.35	0.51	0.12
CLA <i>trans</i> -9, <i>trans</i> -11	0.07	0.09	0.08	0.11	0.01	0.01	0.18	0.02
Total SFA <sup>4</sup>	26.84	28.51	23.88	28.56	2.27	0.39	0.35	0.13
Total MUFA <sup>5</sup>	28.48	30.94	24.65	30.25	2.53	0.25	0.25	0.09
Total PUFA <sup>6</sup>	3.20	3.37	3.02	3.53	0.17	0.10	0.59	0.03
Total omega-6 PUFA <sup>7</sup>	2.57	2.53	2.13	2.44	0.12	0.03	0.01	0.23
Total omega-3 PUFA <sup>8</sup>	0.37	0.52	0.62	0.73	0.02	<0.01	<0.01	<0.01
PUFA:SFA <sup>9</sup>	0.13	0.13	0.14	0.13	0.01	0.40	0.10	0.56
Omega-6:omega-3 <sup>10</sup>	7.02	4.84	3.42	3.37	0.18	<0.01	<0.01	<0.01
Total fatty acids	59.09	63.41	52.13	62.94	4.91	0.30	0.30	0.10

<sup>1</sup>Probability values for overall F-test, as well as contrasts for linear and quadratic effects of algae.

<sup>2</sup>mg/g wet tissue.

<sup>3</sup>Fatty acids 20:4n-6 and 22:1n-9 eluted together.

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<sup>4</sup>Total SFA= 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 +24:0

<sup>5</sup>Total MUFA= 14:1 + 15:1 + 16:1 + 18:1 *cis*-9 + 18:1 *cis*-11 + 18:1 *trans*-11 + 20:1 + 24:1

<sup>6</sup>Total PUFA= 18:2n-6 *cis* + 18:2n-6 *trans* + 18:3n-6 *cis* + 18:3n-3 + 20:2 + 20:3n-6 + 20:4n-6 and 22:1n-9 + 20:5n3 + 22:5n-3 + 22:6n-3 + CLA *cis*-9, *trans*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-9, *trans*-11

<sup>7</sup>Total omega-6 PUFA= 18:2n-6 *cis* + 18:2n-6 *trans* + 18:3n-6 *cis*

<sup>8</sup>Total omega-3 PUFA =18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3

<sup>9</sup>PUFA:SFA= (18:2n-6 *cis* + 18:2n-6 *trans* + 18:3n-6 *cis* + 18:3n-3 + 20:2 + 20:3n-6 + 20:4n-6 and 22:1n-9 + 20:5n3 + 22:5n-3 + 22:6n-3 + CLA *cis*-9, *trans*-11 + CLA *trans*-10, *cis*-12 + CLA *trans*-9, *trans*-11)/(14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0 +24:0)

<sup>10</sup>n-6:n-3: (18:2n-6 *cis* + 18:2n-6 *trans* + 18:3n-6 *cis*)/(18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3)

**Table 3.2** Least square means of objective and subjective cooked meat attributes of *Longissimus lumborum* steaks from heifers fed 0, 50, 100, or 150 g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Item	Algae, g•heifer <sup>-1</sup> •d <sup>-1</sup>				SEM	P – value <sup>1</sup>		
	0	50	100	150		Treatment	Linear	Quadratic
Objective measures								
Ultimate pH <sup>2</sup>	5.65	5.62	5.62	5.64	0.03	0.20	0.27	0.77
Purge loss <sup>3</sup> , %	1.48	1.30	1.19	1.16	0.15	0.34	0.11	0.46
Cook loss <sup>4</sup> , %	23.08	23.68	22.22	22.85	0.47	0.15	0.09	0.16
WBSF <sup>5</sup> , kg	3.48	3.47	3.63	3.35	0.16	0.65	0.63	0.36
Trained sensory panel measures <sup>6</sup>								
Myofibrillar tenderness	5.85	5.92	5.77	5.92	0.12	0.79	0.67	0.35
Juiciness	4.99	5.16	5.08	5.17	0.10	0.49	0.48	0.18
Beef flavor intensity	5.07	5.08	5.11	4.93	0.06	0.16	0.58	0.34
Connective tissue	6.71	6.61	6.51	6.63	0.10	0.50	0.17	0.89
Overall tenderness	5.96	5.97	5.83	6.03	0.12	0.64	0.50	0.38
Off flavor intensity	7.55	7.48	7.19	6.66	0.10	<0.01	<0.01	<0.01

<sup>1</sup>Probability values for overall F-test, as well as contrasts for linear and quadratic effects of algae.

<sup>2</sup>Ultimate pH was recorded at d14 postmortem at the geometric center of each loin.

<sup>3</sup>[(initial weight-stored weight)/initial weight] × 100.

<sup>4</sup>[(pre-cooked weight- cooked weight)/pre-cooked weight] × 100.

<sup>5</sup>WBSF = Warner Bratzler shear force.

<sup>6</sup>Myofibrillar tenderness: 1 = extremely tough and 8 = extremely tender; juiciness: 1 = extremely dry and 8 = extremely juicy; beef flavor intensity: 1 = extremely bland and 8 = extremely intense; connective tissue amount: 1 = abundant and 8 = none; overall tenderness: 1 = extremely tough and 8 = extremely tender; off-flavor intensity: 1 = abundant and 8 = none.

**Table 3.3** Least square means of *Longissimus lumborum* type I, IIA, and IIX muscle fiber cross-sectional area from heifers fed 0, 50, 100, or 150 g•heifer<sup>-1</sup>•d<sup>-1</sup> of microalgae meal (All-G Rich™, *Schizochytrium limacinum* CCAP 4087/2, Alltech Inc., Nicholasville, KY)

Cross-sectional area, μm <sup>2</sup>	Algae, g•heifer <sup>-1</sup> •d <sup>-1</sup>				SEM	P – value <sup>1</sup>		
	0	50	100	150		Trt <sup>2</sup>	Linear	Quadratic
Type I	2,601	2,321	2,289	2,521	119	0.19	0.17	0.84
Type IIA	2,794	2,692	2,710	2,676	139	0.93	0.67	0.62
Type IIX	4,171	3,809	3,954	4,020	181	0.56	0.59	0.41

<sup>1</sup>Probability values for overall F-test, as well as contrasts for linear and quadratic effects of algae.

<sup>2</sup>Treatment

## **Chapter 4 - Effects of feeding antioxidants to steers fed microalgae (*Aurantiochytrium limacinum* CCAP 4087/2) on color stability and palatability of *Longissimus lumborum* steaks**

### **ABSTRACT**

Objectives of this study were to evaluate effects of feeding antioxidants to steers fed microalgae (MA; *Aurantiochytrium limacinum* CCAP 4087/2, Alltech, Inc., Nicholasville, KY) on color stability and palatability of *Longissimus lumborum* (LL) steaks. Steers ( $n = 40$ ) were blocked by initial BW ( $638 \pm 29$  kg) and assigned to one of four dietary treatments: conventional finishing diet with 10% flaxseed (FLAX), FLAX diet plus  $100 \text{ g} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$  MA (ALGAE), ALGAE diet plus  $100 \text{ g} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$  MA plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc. to provide 0.3 mg/kg Se) fed throughout feeding (AOX), and FLAX diet for 35 d and ALGAE diet fed for the final 10 d of feeding (LATE). On d-45 steers were harvested, LL were removed 48 h postmortem, and aged 14 d. After aging, each LL was fabricated into steaks for simulated retail display and trained sensory panel analyses. There were treatment  $\times$  day interactions for all retail display measures ( $P < 0.01$ ) except  $L^*$ , MRA, and OC ( $P > 0.08$ ). From d 0-4 there were no treatment differences for  $a^*$ ,  $b^*$  or surface oxymyoglobin ( $P > 0.08$ ), and from d 0-5 there no treatment differences for surface metmyoglobin and panel discoloration ( $P > 0.10$ ). On d 6 of display, steaks from ALGAE steers had smaller  $a^*$  and  $b^*$  values, less oxymyoglobin, more metmyoglobin, reduced redness scores, and increased discoloration scores than steaks from AOX and LATE steers ( $P < 0.01$ ). From d 7 until the end of display, steaks from ALGAE steers had reduced  $a^*$  and  $b^*$  values, less oxymyoglobin, and more metmyoglobin, reduced panel redness and increased discoloration scores compared to steaks from the other three

treatments ( $P < 0.02$ ). From d 7 until the end of display, AOX steaks had greater  $a^*$  and  $b^*$  values, more oxymyoglobin, and less metmyoglobin, reduced redness and discoloration scores than steaks from FLAX steers ( $P < 0.04$ ). Treatment did not affect steak sensory characteristics ( $P > 0.15$ ), except off-flavor intensity ( $P < 0.01$ ). Steaks from FLAX steers had decreased off-flavor scores compared to steaks from ALGAE and AOX steers ( $P < 0.03$ ), but did not differ ( $P = 0.07$ ) from LATE steaks. Off-flavor of steaks from ALGAE steers were not different from steaks from AOX and LATE steers ( $P > 0.10$ ), but steaks from AOX steers had greater ( $P = 0.04$ ) off-flavor scores than steaks from LATE steers. When antioxidants were supplemented in diets containing microalgae, color of LL steaks was improved, but antioxidants did not prevent off-flavors in cooked steaks.

## INTRODUCTION

According to the *2015 Dietary Guidelines for Americans*, consumption of the long chain omega-3 fatty acids eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), both found in fatty fish and other seafood, are recommended as they can reduce risk factors for cardiovascular disease, type 2 diabetes, and cancer (Ruxton, 2004; Calder, 2014). Americans consume 76 g of seafood weekly (USDA ERS, 2014), which is well below the recommended 227 g needed to intake adequate amounts of EPA and DHA (USDA, 2015). Because of this, research has focused on manipulating the fatty acid profile of beef to achieve greater amounts of omega-3 fatty acids. The main strategy used to manipulate the fatty acid profile of beef is through the dietary supplementation of oilseeds, plant oils, fish oil, marine algae, and fat supplements (Woods and Fearon, 2009).

Increasing omega-3 fatty acids in beef may make products healthier, but PUFAs are susceptible to oxidation, and thus may elicit adverse effects on meat quality. Previously, it was

demonstrated that feeding increasing levels of microalgae (previously *Schizochytrium limacinum*, reclassified as *Aurantiochytrium limacinum* CCAP 4087/2) in a feedlot diet, increased DHA and EPA quadratically (Phelps et al., 2016), leading to accelerated color deterioration during display and increased product off-flavors. Studies report feeding elevated levels of the antioxidant vitamin E, during the finishing phase helped mitigate lipid oxidation and color deterioration during display (Yang et al., 2002; Gobert et al., 2010). Selenium has also been considered for use as a muscle antioxidant, but current data suggests selenium supplementation has mixed impacts color (O'Grady et al., 2001; Taylor et al., 2008; Jose et al., 2010). The objective of this study was to evaluate effects supplementing microalgae (*Aurantiochytrium limacinum* CCAP 4087/2), elevated vitamin E, and selenium yeast to feedlot steers on display color and palatability of Longissimus lumborum (**LL**) steaks.

## **MATERIALS AND METHODS**

All animal experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee and the Kansas State University Institutional Review Board approved procedures for the use of human subjects for sensory panel evaluations.

### ***Animals***

Black-hided crossbred feedlot steers ( $n = 40$ ;  $638 \pm 29$  kg initial BW) were blocked by initial BW and assigned within strata to one of four dietary treatments (Table 5.1). Dietary treatments were a conventional feedlot diet containing 10% ground flaxseed (**FLAX**), FLAX diet plus  $100 \text{ g} \cdot \text{steer}^{-1} \cdot \text{d}^{-1}$  supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc. to provide 0.3 mg/kg Se) fed throughout feeding (**AOX**), and the FLAX diet fed for the first 35 d of finishing and the AOX diet fed for the final 10 d of finishing (**LATE**). Steers were housed in uncovered,

dirt surfaced pens (10 × 19.8 m; 4 pens; 10 steers/pen) that provided 10 m of linear bunk space and were equipped with watering fountains between adjacent pens. All experimental diets were fed for 45 d prior to harvest.

### ***Loin Collection and Processing***

Steers were transported 262 km to a commercial abattoir for harvest (Creekstone Farms; Arkansas City, KS). After a 48-h chilling period, strip loins (Institutional Meat Purchase Specifications #180; NAMP, 2010) were removed from the right side of each carcass, vacuum packaged, and transported to the Kansas State University Meats Laboratory where they were stored until d 14 postmortem. Following storage, packages were opened and five, 2.54-cm thick steaks were fabricated from each loin. Steak 1 was utilized for long-chain fatty acid analyses, steak 2 was utilized for d 0 metmyoglobin reducing ability (**MRA**) and oxygen consumption rate (**OCR**) analyses, steak 3 was used for d 5 of display MRA and OCR analyses, and steak 4 was used for daily objective and subjective color measurement and d 10 of display MRA and OCR analyses. Steak 5 was vacuum packaged, frozen at -40°C, and ultimately utilized for trained sensory panel analysis.

### ***Simulated Retail Display***

Steaks used for d-5 and d-10 simulated retail display were placed on 17S polystyrene foam trays with a UltraZap-40 absorbent pad (Paper-Pak Industries Inc., La Verne, CA) and overwrapped with poly-vinyl chloride film (AEP Industries, South Hackensack, NJ) possessing an oxygen transmission rate of  $1,450\text{-cm}^{-3}\cdot 645.2\text{-cm}^{-2}\cdot 24\text{-h}^{-1}$ . Steaks were orientated on trays with the medial portion on the left and posterior end facing up with respect to anatomical location in the animal. Steaks were divided among two coffin-style retail cases (Model DMF 8; Tyler Refrigeration Corporation, Niles, MI) ensuring treatments were represented equally in each

case. Steaks were displayed under fluorescent lights (32 W Del-Warm White 3000°K; Philips Lighting Company, Somerset, NJ) that emitted a constant 24-h case average intensity of  $2,190 \pm 34$  lx. Cases were set to operate at  $2 \pm 1^\circ\text{C}$  and defrosted every 12 h at  $13^\circ\text{C}$  for 30 min. Case temperatures were monitored using a Thermochron iButton (Maxim Integrated Products, Sunnyvale, CA) and average recorded temperature of the cases was  $1.94 \pm 0.95^\circ\text{C}$ . Every 12 h, steaks were rotated in the cases from left to right and front to back to account for variation in temperature and light intensity within the cases.

### ***Objective color collection***

Every 24-h on d-10 steaks, surface color readings including CIE  $L^*a^*b^*$  and reflectance from 400 to 700 nm were collected at three locations on each steak using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54-cm diameter aperture,  $10^\circ$  observer; Hunter Associates Laboratory, Reston, VA). Surface reflectance values at 473, 525, 572, and 700 nm were used to calculate surface percentages of metmyoglobin and oxymyoglobin using equations from Krzywicki (1979) as published in the American Meat Science Association's (AMSA) Meat Color Measurement Guidelines (AMSA, 2012). Values from the three scans were averaged to yield a steak average.

### ***Subjective color collection***

In addition to objective color data collection, subjective redness and surface discoloration were assessed by a trained visual panel daily. A total of 12 panelists were oriented to redness and discoloration evaluation over 4 training sessions. Utilizing continuous 100-mm line scales on paper ballots (Figure 5.1), panelists evaluated redness using 6 anchors points where 0 mm = 1 or light-pinkish red and 100 mm = 6 or very dark red (Figure 5.1), with anchors spaced every 20 mm. Additionally, panelists assessed surface discoloration as a percentage of the LM area also

on 100 mm continuous line scales with anchors at 0 = no discoloration and 100= 100% discoloration. Eight panelists participated in the panel daily panelist responses (distance from 0 mm for each attribute) were measured using a DrawingBoard VI (GTCO Calcomp, Turning Technologies, Scottsdale, AZ) and TabletWorks Software (Version 10.10; GTCO Calcomp, Turning Technologies).

### ***Metmyoglobin Reducing Ability and Oxygen Consumption Rate Analyses***

At the time of analysis (d 0, 5, and 10), each steak was split laterally into equal 1.27-cm thick steaks. The display portion was utilized for MRA analysis and the freshly cut portion was utilized for OCR. The procedures of Phelps et al. (2014) were followed for MRA. Briefly, steaks were cut into 3 locations (medial, middle, and lateral locations) to represent the entire steak. Each section was placed in a beaker and oxidized in 100 mL of 0.3% sodium nitrite at  $25 \pm 2^\circ\text{C}$  for 20 min. Following incubation, samples were blotted of excess solution and vacuum packaged in  $25.4 \times 30.5$  cm vacuum bags (3 mil standard barrier, Prime Source Vacuum Pouches; Bunzl Processor Division, Koch Supplies, Kansas City, MO) that possessed an oxygen transmission rate of  $4.3 \text{ cm}^3 \cdot 100 \text{ cm}^{-2} \cdot 24 \text{ h}^{-1}$  at  $23^\circ\text{C}$  and 65% relative humidity. Reflectance measurements were taken from 400 to 700 nm at 0 and 2 h using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture,  $10^\circ$  observer; Hunter Associates Laboratory). Spectral readings at 525, 572, and 700 nm used to calculate metmyoglobin percentages at using equations described previously. Metmyoglobin percentages from the three locations were averaged and metmyoglobin reducing ability was calculated as (observed decreased in metmyoglobin concentration/initial metmyoglobin concentration)  $\times 100$ .

Oxygen consumption analysis was conducted as described in the AMSA Meat Color Measurement Guidelines (AMSA, 2012). The bottom portion of the steak fabricated for OCR

was allowed to oxygenate for 2 h at 4°C covered with poly-vinyl chloride film (AEP Industries) possessing an oxygen transmission rate of  $1,450\text{-cm}^3\cdot645.2\text{-cm}^{-2}\cdot24\text{-h}^{-1}$ . Following oxygenation, steaks were cut into the 3 sub-locations, vacuum packaged in  $25.4 \times 30.5$  cm vacuum bags described previously, and incubated at 25°C for 40 min. Reflectance measurements from 400 to 700 nm were collected immediately following packaging and after the 40 min incubation. Spectral readings at 473, 525, 572, and 700 nm were averaged across the three location to calculate surface oxymyoglobin percentage use equations described above. Oxymyoglobin consumption was calculated as (observed decreased in oxymyoglobin concentration/initial oxymyoglobin concentration)  $\times 100$  and OCR was calculated as oxymyoglobin consumed/time.

### ***Sensory Analyses***

Sensory analyses were conducted according to procedures outlined in the AMSA Meat Cookery and Sensory Guidelines (AMSA, 2015). Twenty-four hours prior to cooking, steaks were thawed on trays at  $2.7 \pm 0.9^\circ\text{C}$ . Before cooking a thermocouple (30 gauge copper and constantan, Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak. Steaks were cooked on clam-style grills (Cuisinart Griddler; Cuisinart, Stamford, CT) set to  $177^\circ\text{C}$  and removed from grills at  $70^\circ\text{C}$ . After cooking, steaks were cut into  $1.27 \times 1.27 \times 2.54$  cm cubes and presented to an 8 member trained sensory panel. Panelists were selected from a larger pool of candidates trained according the AMSA Meat Cookery and Sensory Guidelines (AMSA, 2015). Selected panelists were oriented to strip loin steak evaluation over 4 training sessions prior to the initiation of panels. At each panel, panelists were seated at individual cubicles in a room designed for subjective sensory panel analysis and were presented two cubes from each of 8 steaks ( $n = 2$  per treatment/panel). Panelists evaluated the two cubes for initial juiciness, sustained juiciness, myofibrillar tenderness, connective tissue amount, overall

tenderness, beef-flavor identity, beef-flavor intensity, and off-flavor intensity using 100-mm continuous line scales on paper ballots (Figure 5.2). The 0-mm anchors were: extremely dry, extremely dry, extremely tough, none, extremely tough, extremely unbeef-like, extremely bland, and extremely bland. Conversely, the 100-mm anchors were: extremely juicy, extremely juicy, extremely tender, abundant, extremely tender, extremely beef-like, extremely intense, and extremely intense. Also, there were midpoint (50%) labels for the initial juiciness, sustained juiciness, myofibrillar tenderness, overall tenderness, and beef identity scales. These anchors were labeled as neither dry nor juicy, neither dry nor juicy, neither tough nor tender, neither tough nor tender, and neither unbeef-like nor beef-like. A total of 5 separate panels were conducted to analyze all the samples. For analysis of ballots, panelist responses were measured using a DrawingBoard VI (GTCO Calcomp, Turning Technologies, Scottsdale, AZ) and TabletWorks Software (Version 10.10; GTCO Calcomp, Turning Technologies). Data is expressed as the distance on the scale away from 0 mm.

### *Statistical analysis*

Data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc, Cary, NC) with steer/loin as the experimental unit and steak as the observational unit. For fatty acid and sensory data, treatment served as the fixed effect and initial BW block served as the random effect. Retail shelf-life data were analyzed as a randomized complete block design with repeated measures. Treatment, day, and the interaction of treatment and day served as the fixed effects and initial BW block was the random effect. Day of display served as the repeated measure with steak (observational unit) as the subject and compound symmetry as the covariance structure. For sensory data, pairwise comparisons were computed using the PDIFF option of the LSMEANS statement. For retail shelf-life data,

pairwise comparisons were computed using the PDIFF option of the LSMEANS statement within each day of display. Differences were considered significant at  $P \leq 0.05$ .

## RESULTS

### *Objective display color*

To assess changes in color, steaks from all treatments were displayed under simulated retail conditions for 10 d. As expected, day of display impacted  $L^*$ ,  $a^*$ , and  $b^*$  values of steaks (Figure 5.3;  $P < 0.01$ ), and surface oxymyoglobin and metmyoglobin (Figure 5.4;  $P < 0.01$ ) that were consistent with discoloration patterns seen during retail display.

There was no treatment  $\times$  day interaction ( $P = 0.08$ ) for steak  $L^*$  value, but there were treatment  $\times$  day interactions for steak  $a^*$  and  $b^*$  values ( $P < 0.01$ ). Therefore, treatment means for these two attributes were compared within each day of display. From d 0 to 4 there were no treatment differences for  $a^*$  or  $b^*$  values ( $P > 0.08$ ). On d 5 of display,  $a^*$  value of steaks from ALGAE steers were smaller ( $P = 0.05$ ) than steaks from AOX steers, but were similar to FLAX and LATE steaks ( $P > 0.14$ ). Steaks from FLAX, LATE, and AOX also had similar  $a^*$  values on d 5 of display ( $P > 0.18$ ). On d 6 of display, steaks from ALGAE steers possessed smaller  $a^*$  values than steaks from AOX and LATE steers ( $P < 0.01$ ), which did not differ from each other ( $P = 0.75$ ). Also on this d of display, steaks from FLAX steers had a similar  $a^*$  values than steaks from all the other treatment groups ( $P > 0.18$ ). From d 7 throughout the remainder of the display period, steaks from ALGAE steers had reduced  $a^*$  values compared to steaks from the other three treatments ( $P < 0.02$ ). Also,  $a^*$  values of steaks from AOX steers were similar to steaks from LATE steers ( $P > 0.06$ ), but AOX steaks had greater  $a^*$  values than steaks from FLAX steers ( $P < 0.04$ ). Finally during this time period,  $a^*$  values of steaks from FLAX steers were similar to values of steaks from LATE steers ( $P > 0.19$ ).

On d 5 of display, steaks from ALGAE steers had a decreased ( $P = 0.03$ )  $b^*$  value than steaks from AOX steers, but were similar to steaks from FLAX and LATE steers ( $P > 0.12$ ). Steaks from FLAX, AOX, and LATE steers did not possess different  $b^*$  values on d 5 of display ( $P > 0.06$ ). On d 6, ALGAE steaks has smaller  $b^*$  values than AOX and LATE steaks ( $P < 0.01$ ), but did not differ ( $P = 0.27$ ) from FLAX steaks. Similar to d 5 of display, steaks from FLAX, AOX, and LATE steers had similar  $b^*$  values ( $P > 0.08$ ). On d 7 and throughout the rest of display, steaks from ALGAE steers possessed smaller  $b^*$  values than steaks from the other three treatments ( $P < 0.01$ ). Steaks from FLAX steers had decreased ( $P < 0.01$ )  $b^*$  values than steaks from AOX steers, but had similar ( $P = 0.14$ )  $b^*$  values than LATE steaks. Finally,  $b^*$  values of steaks from AOX and LATE steers were similar ( $P > 0.06$ ).

There was no treatment effect ( $P = 0.15$ ) for steak  $L^*$  value, but treatment impacted both  $a^*$  and  $b^*$  values ( $P < 0.01$ ) over the entire display period. The  $a^*$  value of steaks from ALGAE steers were smaller than values of steaks from the other three treatments ( $P < 0.05$ ), while the other three treatments did not differ ( $P > 0.06$ ). Steaks from ALGAE steers had a similar ( $P = 0.16$ )  $b^*$  value as steaks from FLAX steers, but had smaller values than steaks from AOX and LATE steers ( $P < 0.01$ ). Also, steaks from AOX steers had a similar ( $P = 0.24$ )  $b^*$  values as steaks from LATE steers, but possessed greater ( $P = 0.02$ ) values than steaks from FLAX steers. Finally, steaks from FLAX and LATE steers had similar ( $P = 0.26$ )  $b^*$  values.

There were treatment  $\times$  day interactions for both steak surface metmyoglobin and oxymyoglobin percentage (Figure 5.4;  $P < 0.01$ ). From d 0 through d 4 there were no treatment differences in steak surface oxymyoglobin percentage ( $P > 0.16$ ). On d 5, steaks from ALGAE steers had less surface oxymyoglobin ( $P = 0.03$ ) than steaks from AOX steers, but had similar surface oxymyoglobin as steaks from FLAX and LATE steers ( $P > 0.20$ ). Additionally, steaks

from FLAX, AOX, and LATE steers had similar surface oxymyoglobin on d 5 of display ( $P > 0.13$ ). On d 6 of display, steaks from ALGAE steers had a similar ( $P = 0.15$ ) amount of surface oxymyoglobin as steaks from FLAX steers, but AGLAE steaks also had less surface oxymyoglobin than steaks from AOX and LATE steers ( $P < 0.03$ ). Steaks from AOX, LATE, and FLAX steers all had the same amount of surface oxymyoglobin on this d of display ( $P > 0.13$ ). From d 7 through d 9 of display, steaks from ALGAE steers had less surface oxymyoglobin than the other three treatments ( $P < 0.01$ ), steaks from AOX steers had more ( $P < 0.04$ ) surface oxymyoglobin than steaks from FLAX steers, and steaks from LATE steers had a similar amount of surface oxymyoglobin as steaks from ALGAE and FLAX steers ( $P > 0.27$ ). On d 10 of display, steaks from ALGAE steers had less oxymyoglobin than all other treatments ( $P < 0.01$ ), and steaks from LATE and FLAX steers had less oxymyoglobin than AOX steaks ( $P < 0.02$ ), but did not differ ( $P = 0.27$ ) from each other.

Unlike surface oxymyoglobin percentage, treatments did not differ in surface metmyoglobin percentage through d 5 of display ( $P > 0.10$ ). On d 6 of display, steaks from ALGAE steers had more surface metmyoglobin than steaks from AOX and LATE steers ( $P < 0.05$ ), but had a similar ( $P = 0.18$ ) amount of metmyoglobin as FLAX steaks. Steaks from AOX, LATE, and FLAX steers had the same amount of metmyoglobin on this d ( $P > 0.29$ ). From d 7 through the remainder of display, steaks from ALGAE steers had more metmyoglobin than steaks from the other three treatments ( $P < 0.02$ ). From d 7 through 9 steaks from AOX steers had a similar amount of surface metmyoglobin as steaks from LATE steers ( $P > 0.14$ ), but had less ( $P = 0.04$ ) metmyoglobin on d 10. Steaks from FLAX and LATE steers had a similar amount of surface metmyoglobin from d 7 through the end of display ( $P > 0.25$ ). Over the entire display period, treatment affected formation of surface metmyoglobin and oxymyoglobin ( $P <$

0.01). Steaks from ALGAE steers had less surface oxymyoglobin and more surface metmyoglobin than the steaks from the other three treatments ( $P < 0.03$ ), but these treatments were similar to each other ( $P < 0.06$ ).

### ***Subjective display color***

There were treatment  $\times$  day interactions for visual panel redness and surface discoloration scores (Figure 5.5;  $P < 0.01$ ). From d 0 to 2 of display there were no treatment differences for visual panel redness ( $P > 0.13$ ). On d 3 of display ALGAE steaks had similar redness scores to AOX and FLAX steaks ( $P > 0.25$ ), but ALGAE steaks had a greater ( $P = 0.04$ ) redness score than LATE steaks. Steaks from FLAX, AOX, and LATE steers do not differ in redness scores on d 3 ( $P > 0.26$ ). At d 4 of display, steaks from ALGAE steers had greater redness scores than steaks from the other three treatments ( $P < 0.01$ ), and steaks from FLAX, AOX, and LATE steers had similar redness scores on d 4 ( $P > 0.31$ ). From d 5 to 9 of display, steaks from ALGAE steers had darker redness scores than steaks from the other three treatments ( $P < 0.01$ ), but on d 10 redness of steaks from ALGAE steers was not different ( $P = 0.31$ ) from steaks from FLAX steers. Steaks from AOX and LATE steers had similar redness scores from d 5 until the end of display ( $P > 0.10$ ). Additionally from d 5 until the end of display steaks from FLAX steers had similar redness scores as steaks from LATE steers ( $P > 0.07$ ). Steaks from AOX steers had decreased redness scores compared to steaks from FLAX steers from day 5 until the end of display ( $P < 0.05$ ). Day of display impacted redness scores, with scores increasing over the display period ( $P < 0.01$ ). Over the entire display period, steaks from ALGAE steers had greater redness scores than steaks from AOX and LATE steers ( $P < 0.01$ ), but did not differ ( $P > 0.07$ ) from steaks from FLAX steers. Finally, over the entire display period visual panel redness scores of steaks from FLAX, AOX, and LATE steers did not differ ( $P > 0.16$ ).

From d 0 to 5 of display, there were no treatment difference for panel surface discoloration ( $P > 0.39$ ). Steaks from ALGAE steers had a great discoloration score from d 6 until the end of display than steaks from the other three treatments ( $P < 0.03$ ). From d 6 through d 8 steaks from FLAX, AOX, and LATE steers did not differ in surface discoloration score ( $P > 0.08$ ). On d 9 and 10 steaks from FLAX steers had greater discoloration scores than AOX steers ( $P < 0.04$ ), but did not differ from steaks from LATE steers ( $P > 0.58$ ). Finally, on d 9 and 10 of display steaks from AOX and LATE steers did not differ in discoloration score ( $P > 0.06$ ).

### ***Metmyoglobin reducing ability and oxygen consumption***

There were no treatment  $\times$  day interactions or treatment effects for MRA, oxygen consumption, and OCR (Table 5.2;  $P > 0.09$ ). As expected, MRA decreased ( $P < 0.01$ ) with MRA of steaks greater on d 0 than d 5 and 10 ( $P < 0.01$ ) of display, and MRA of steaks on d 5 of display was greater ( $P < 0.01$ ) than d 10 day of display. Similarly, oxygen consumption and OCR decreased with day of display ( $P < 0.01$ ). Oxygen consumption and OCR on d 0 was greater than on d 5 and 10 of display ( $P < 0.01$ ), but oxygen consumption and OCR did not differ on those d 5 and 10 of display ( $P = 0.21$ ).

### ***Sensory analysis***

There were no treatment differences observed for initial or sustained juiciness, myofibrillar tenderness, connective tissue amount, and overall tenderness of LL steaks (Table 5.3;  $P > 0.21$ ). Treatments did not affect beef flavor identity or beef flavor intensity ( $P > 0.15$ ), but off-flavor intensity differed ( $P < 0.01$ ) among treatments. Steaks from FLAX steers had less off-flavor intensity than steaks from ALGAE and AOX steers ( $P < 0.03$ ), but did not differ ( $P = 0.07$ ) in intensity when compared to LATE steaks. Steaks from ALGAE steers had similar off-

flavor intensity scores than steaks from AOX and LATE steers ( $P > 0.10$ ), but steaks from AOX steers had greater ( $P = 0.04$ ) off-flavor intensity scores than steaks from LATE steers.

## DISCUSSION

In the U.S., meat contributes more than 40% of daily protein intake and 20% of daily fat intake (Daniel, 2011). Beef is a major meat protein source in the U.S., but regarded as having relatively high concentrations of saturated fatty acids. Seafood, which is high in the omega-3 fatty acids EPA and DHA, is consumed at much lower than recommend levels (USDA ERS, 2016). Researchers have examined ways to increase omega-3 fatty acids in beef, and the main strategies used to manipulate the fatty acid profile of beef is through the dietary supplementation of oilseeds, plant oils, fish oil, marine algae, and other fat supplements (Woods and Fearon, 2009). Previously, it was demonstrated feeding increasing levels of microalgae in a feedlot diet, increased DHA and EPA up to 850 and 340%, respectively (Phelps et al., 2016), but this lead to color deterioration during display and increased steak off-flavors detected by trained sensory panelists. It was hypothesized the inclusion of elevated levels of dietary antioxidants during the supplementation of microalgae would mitigate color problems during display and prevent production of off-flavors during cooking.

Myoglobin oxidation which leads to poor color stability during display is dependent on a multitude of factors including, temperature, pH, MRA, oxygen consumption and lipid oxidation (Faustman et al., 2010). Dietary treatments did not impact steak MRA or oxygen consumption which indicate color differences during display were more likely due to antioxidant status and lipid oxidation. Lipid oxidation can enhance meat discoloration, and PUFA are more susceptible to oxidation due to the increase in double bonds in the carbon chains of those fatty acids (Jacobson, 2008). Supplementing products to cattle or other livestock that have the potential to

increase omega-3 content of tissues can adversely impact color stability of products. Compared to the 100 g/d level supplemented in Phelps et al. (2016), a\* value of ALGAE treatments in the current study was similar on d 5 and slightly greater (3-4 units) on d 6 and 7 of display. Additionally, surface oxymyoglobin percentage was greater and metmyoglobin percentage was lower by 3 to 4% on d 5, 6, and 7 for the ALGAE treatment compared to Phelps et al. (2016) when the microalgae was supplemented at 100 g/d. When examining differences in color saturation, which is a measure of metmyoglobin formation, Vatansever et al. (2000) reported no differences in color saturation values of LL steaks from steers fed linseed (flaxseed) and linseed/fish oil indicating similar amounts of metmyoglobin formed. However, in lamb, color saturation of chops was reduced (more metmyoglobin formed) when marine algae or fish oil was supplemented compared to linseed oil supplementation (Nute et al., 2007).

One way to preserve color stability of beef products is through the use of antioxidants such as vitamin E or selenium. When 103 IU of vitamin E and selenium yeast were added to diets, color attributes, especially a\* (redness) and surface oxymyoglobin and metmyoglobin percentage were markedly improved compared to ALGAE treatment. The a\* values, surface oxymyoglobin and metmyoglobin on d 5, 6, and 7 for the AOX treatment were similar to the 0 g/d microalgae level in Phelps et al. (2016), indicating adding vitamin and selenium-yeast to diets containing microalgae would produce steaks with a similar color shelf-life as something from an animal finished on a diet without microalgae. Zakrys et al. (2008) found decreases in oxymyoglobin and a\* value were strongly correlated with TBARS value, a measurement of oxidation. Phelps et al. (2016) demonstrated increased TBARS values as microalgae supplementation increased in the diet and as display increased. Although TBARS were not

measured it is likely AOX and LATE steaks had reduced lipid oxidation than steaks from the FLAX and ALGAE treatments which translated to better color stability during display.

Many researchers have demonstrated supplementing vitamin E over the nutritional requirement improves steak redness and stability of color during display (Faustman et al. 1989; Arnold et al, 1993; Lynch et al. 1999). Utilizing linseed (flaxseed) as source for the omega-3 fatty acid alpha-linoleic acid, Alberti et al. (2014) found no difference in surface metmyoglobin or oxymyoglobin on steaks from Pirenaica bulls supplemented 5% linseed alone or 5% linseed with 200 IU vitamin E, indicating no advantage to adding vitamin E to diets containing 5% flaxseed. When comparing diets containing 10% flaxseed, Juarez et al. (2012) reported no difference in steak lightness when vitamin E was supplemented at 451 IU/d (control level) and when vitamin E was supplemented at 1051 IU/d (high vitamin E). When compared to the current study, lack of difference may be because the control level in the Juarez et al. (2012) study is four times greater than the elevated level in this study. Adding of vitamin E (1,051 IU/d) to diets of feedlot steers containing 10% flaxseed reduced calculated hue angle which indicates formation of less metmyoglobin compared to diets with 10% flaxseed and 451 IU/d vitamin E (Juarez et al., 2012). In a lamb model, Ponnampalam et al. (2016) supplemented 10.7% and 1.8% flax flake and algae (*Schizochrytrium* sp.), respectively to lambs on pasture separately and in combination. Animals fed pasture can have a greater muscle vitamin E content (Scollan et al., 2014). Ponnampalam observed there was no statistical difference in Longissimus lumborum vitamin E muscle content across treatments, but supplementing algae alone or in combination with flax flake numerically decreased vitamin E in the LL. However, authors observed no color differences in L\*, a\*, b\*, or metmyoglobin formation during a 4-d display, indicating the vitamin

E levels in LL were adequate to prevent color deterioration during display when flax flake, algae, or a combination were fed in diets.

Researchers have also investigated selenium as source of antioxidants in meat systems; however, the impact on meat color is mixed. O'Grady et al. (2001) reported more oxymyoglobin formed on minced LL muscle at the end of a 14 d display in 80% oxygen modified atmosphere packaging when an organic selenium product and 300 IU of vitamin E was supplemented compared to a control in which 20 IU of vitamin E and inorganic selenium was fed. Taylor et al. (2008) reported no difference in L\*, a\*, or b\* of LL steaks from animals fed a selenium enriched diet compared to a non-selenium enriched diet. Jose et al. (2010) reported supplementation of selenium to lambs did not improve oxymyoglobin formation on the surface of LL and Semimembranosus steaks. Because vitamin E and selenium were supplemented together it is impossible to discern which contributed more to the improved color. In a lamb model, Ripoll et al. (2010) examined the supplementation of vitamin E alone, selenium selenite alone, or in combination on color stability of the LL. Loins from lambs of supplementation of selenium selenite alone had increase metmyoglobin formation compared to vitamin E alone or when the two were fed in combination This may suggest, vitamin E is more important for color shelf-life than selenium, but in the current study it is impossible to discern which contributed more because they were fed in combination.

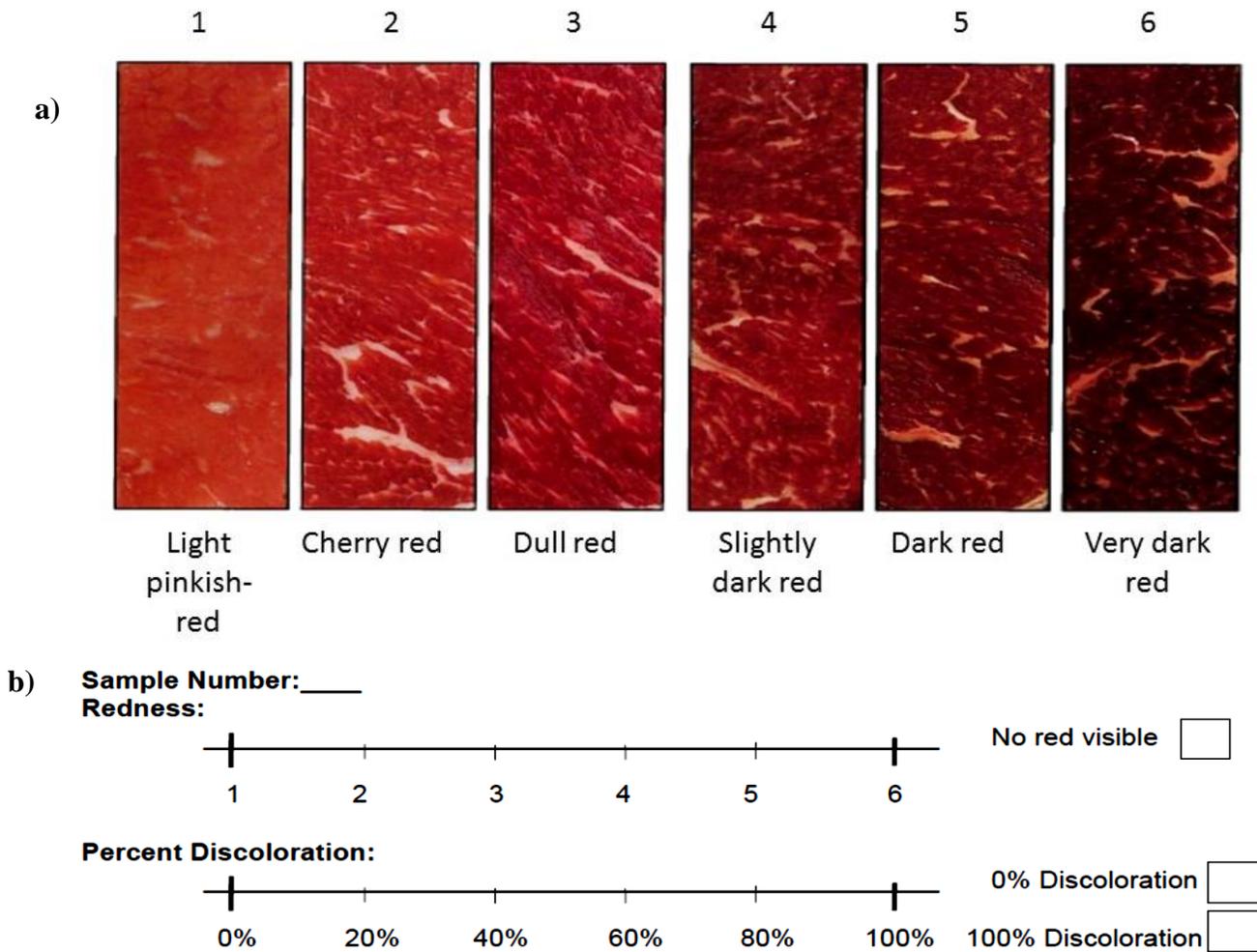
Previously when feeding increased levels of microalgae meal panelists detected an increase in off-flavors, by 5% when the microalgae was supplemented to heifers at 100 g/day, but tenderness, juiciness, beef flavor were not impacted by feeding microalgae (Phelps et al., 2016). Again, there were no tenderness, juiciness, or beef flavor differences, but supplementing microalgae increased off-flavors in cooked steaks as both the ALGAE and AOX had more off-

flavors than the FLAX treatment by 3 and 5% respectively, which is similar to the increase observed previously when microalgae was supplement at 100 g/day. When the microalgae was only supplemented the last 10 d of finishing there were no off-flavor differences when compared to the FLAX treatment, indicating duration of microalgae supplementation may influence off-flavors. The differences detected span less than 10% of the scale it is possible these differences may not be detectable my consumers. It is important to acknowledge long-chain PUFA are overall more susceptible to oxidation and increasing their level within the products is going increase oxidation overall (Jacobsen et al., 2008) which is likely why panelists detected small levels of off-flavors. In contrast to our study, Vatansever et al. (2000) reported no differences for sensory panel tenderness, juiciness, or flavor between sirloin steaks from steers fed linseed or linseed/fish oil. For lamb, supplementing marine algae and a protected oil containing linoleic and linolenic acid increased abnormal flavors of lamb chops compared to supplementing linseed oil alone (Nute et al., 2007). Because antioxidants inhibit lipid oxidation, they may reduce off-flavors in cooked products that occur because of oxidized lipids; however, sensory evaluation of beef from cattle supplemented sources of omega-3 fatty acids and antioxidants is limited. Juarez et al. (2012) reported no differences in sensory panel tenderness, juiciness, or flavor measures for Longissimus thoracis steaks when 1051 IU of vitamin E was added to diets containing 10% flaxseed compared to diets containing 10% flaxseed and 451 IU of vitamin E.

## **CONCLUSION**

The color shelf-life of LL steaks from steers receiving microalgae was improved when the antioxidants of over-nutritional vitamin E and selenium-yeast were supplemented. This was true when the antioxidants and microalgae were supplemented only the last 10 d of finishing. It is impossible to discern whether the over-nutritional vitamin or selenium-yeast had a bigger

impact on color. Further research is needed to compare differences in supplementing vitamin E and selenium-yeast in diets containing microalgae on steak color. There was no effect of dietary treatments on palatability of LL steaks except off-flavors. These differences were minute and further research is necessary to examine if these off-flavors would be detectable by consumers.



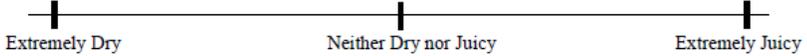
**Figure 4.1** Redness scale (a) and 100- mm continuous line scales (b) utilized for visual panel. 0 mm = 1 (light pinkish-red) and 100 mm = 6 (very dark red). If there was no visible red on the surface of the Longissimus lumborum, panelists marked the “no red visible” box. b) Example of the 100 mm continuous line scales.

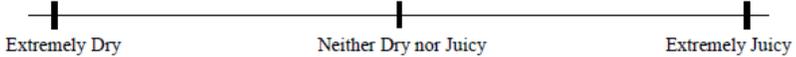
## Trained Sensory Evaluation Form

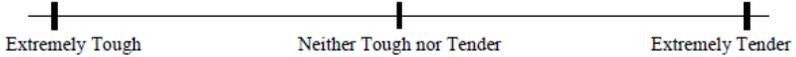


Panelist ID: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_

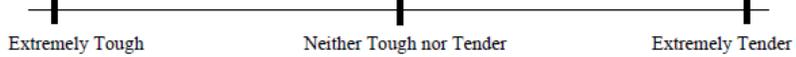
Sample ID: \_\_\_\_\_

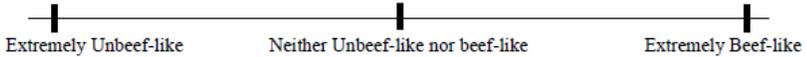
**Initial Juiciness:**      

**Sustained Juiciness:**      

**Myofibrillar Tenderness:**      

**Amount of connective tissue**      

**Overall Tenderness:**      

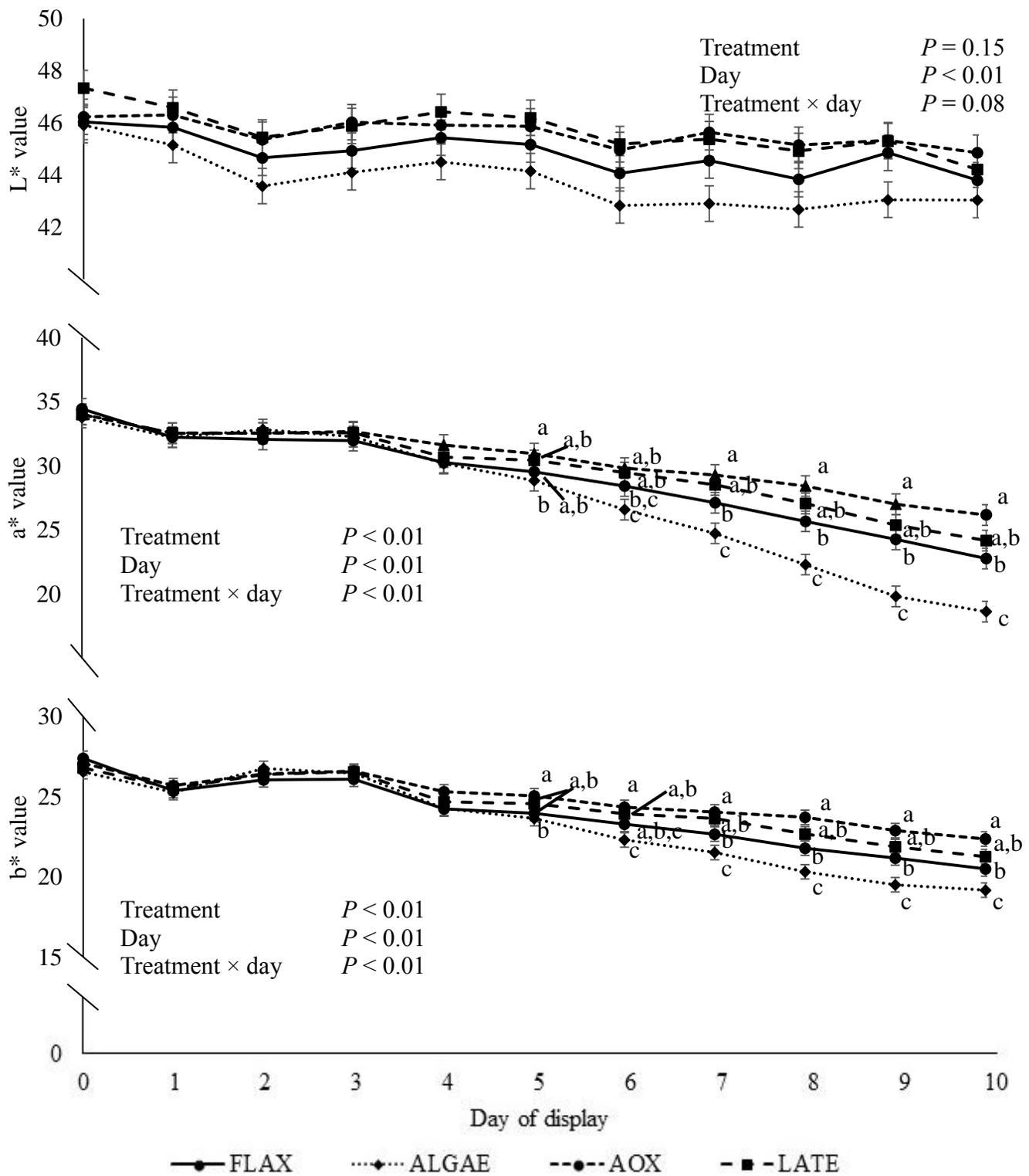
**Beef Flavor Identity:**      

**Beef Flavor Intensity:**      

**Off-Flavor Intensity:**      

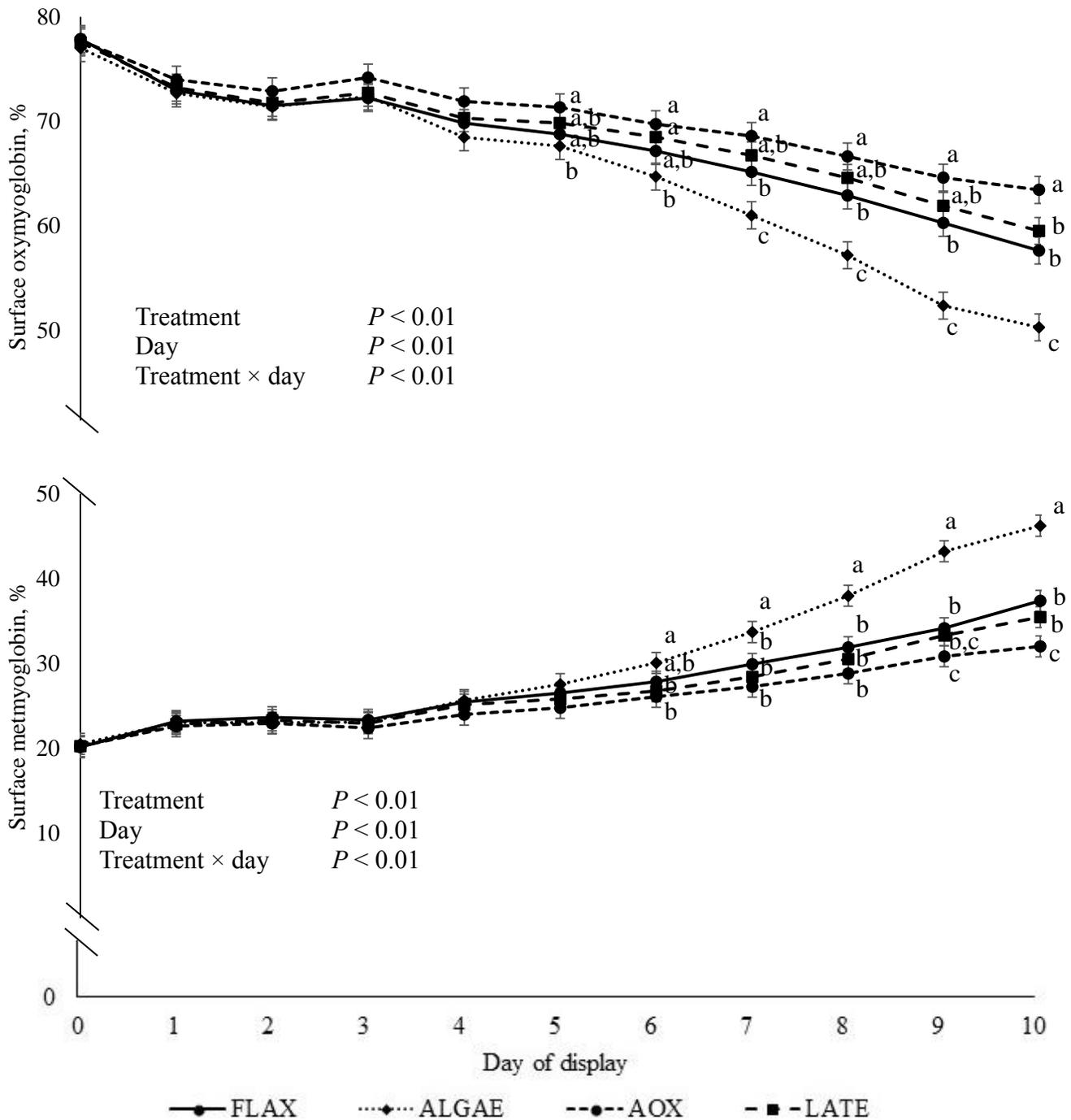
**Off-Flavor Description:**       (Please list if present) \_\_\_\_\_  
None Present

**Figure 4.2** Example of 100-mm continuous line scales utilized for trained sensory panel analysis.



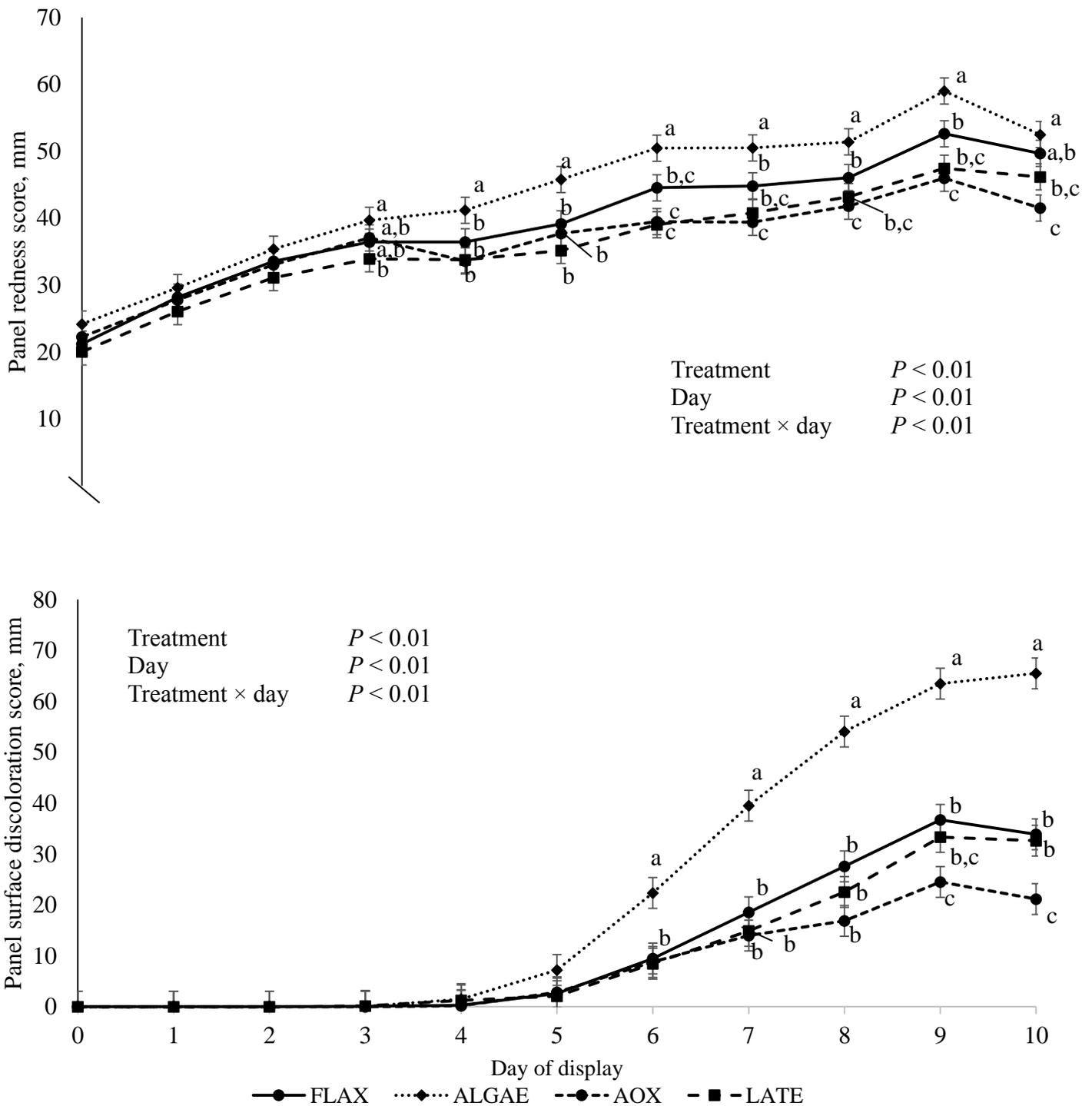
**Figure 4.3** Longissimus lumborum steak L\* (Lightness: 0 = black, 100 = white), a\* (Redness: -60 = Green, 60 = Red), and b\* values (Blueness: -60 = Blue, 60 = Yellow) from steers fed four different finishing diets. A conventional finishing diet with 10% flaxseed (**FLAX**) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed throughout feeding (**AOX**), and FLAX diet the first 35 d of finishing and the

AOX diet fed for the final 10 d of finishing (**LATE**).<sup>a,b,c</sup> Within a day, means without common superscripts differ ( $P < 0.05$ ).



**Figure 4.4** Surface percentages of oxymyoglobin and metmyoglobin of *Longissimus lumborum* steaks from steers fed from steers fed four different finishing diets. A conventional finishing diet with 10% flaxseed (FLAX) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (ALGAE; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed

throughout feeding (**AOX**), and **FLAX** diet the first 35 d of finishing and the **AOX** diet fed for the final 10 d of finishing (**LATE**). <sup>a,b,c</sup> Within a day, means without common superscripts differ ( $P < 0.05$ ).



**Figure 4.5** Visual panel redness and surface discoloration score of *Longissimus lumborum* steaks from steers fed from steers fed four different finishing diets. A conventional finishing diet with 10% flaxseed (**FLAX**) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed throughout finishing (**AOX**), and FLAX diet the first 35 d of finishing and the AOX diet fed for the final 10 d of finishing (**LATE**). Utilizing continuous 100-mm line scales on paper ballots, panelists evaluated redness using 6 anchors points where 0 mm

= 1 or light-pinkish red and 100 mm = 6 or very dark red with anchors spaced every 20 mm. Additionally, panelists assessed surface discoloration as a percentage of the LM area also on 100-mm continuous line scales with anchors at 0 = no discoloration and 100= 100%. <sup>a,b,c</sup> Within a day, means without common superscripts differ ( $P < 0.05$ ).

**Table 4.1** Calculated diets (DM basis) of steers fed four different finishing diets.

Ingredient, %	Treatment <sup>1</sup>			
	FLAX	ALGAE	AOX	LATE
Steam-flaked corn	46.72	45.97	45.91	45.91
Wet-corn gluten feed	30.00	30.00	30.00	30.00
Corn silage	10.00	10.00	10.00	10.00
Ground flax	10.00	10.00	10.00	10.00
Monensin and tylosin premix <sup>2</sup>	1.35	1.35	1.35	1.35
Mineral/vitamin supplement <sup>2</sup>	0.05	0.05	-	-
Mineral/vitamin supplement- no selenium selenite <sup>4</sup>	-	-	0.01	0.01
Microalgae meal supplement <sup>5</sup>	-	0.75	0.69	0.69
Ground limestone	1.59	1.59	1.59	1.59
Salt	0.25	0.25	0.25	0.25
Vitamin A <sup>6</sup> , 30,000 IU/g	0.007	0.007	0.007	0.007
Vitamin E <sup>6</sup> , 44,092 IU/g	0.03	0.03	0.14	0.14

<sup>1</sup> A conventional finishing diet with 10% flaxseed (**FLAX**) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed throughout feeding (**AOX**), and FLAX diet the first 35 d of finishing and the AOX diet fed for the final 10 d of finishing (**LATE**).

<sup>2</sup> Provided 300 mg·steer<sup>-1</sup>·d<sup>-1</sup> of monensin and 90 mg·steer<sup>-1</sup>·d<sup>-1</sup> of tylosin (Elanco Animal Health, Greenfield, IN) in a ground corn carrier.

<sup>3</sup> Diets included CuSO<sub>4</sub> to provide 10 mg/kg Cu, CoCO<sub>3</sub> to provide 0.15 mg/kg Co, ethylenediamine dihydriodide to provide 0.5 mg/kg I, MnSO<sub>4</sub> to provide 60 mg/kg Mn, Na<sub>2</sub>SeO<sub>3</sub> to provide 0.3 mg/kg Se, and ZnSO<sub>4</sub> to provide 60 mg/kg Zn in the total mixed ration on a DM basis.

<sup>4</sup> Diets included CuSO<sub>4</sub> to provide 10 mg/kg Cu, CoCO<sub>3</sub> to provide 0.15 mg/kg Co, ethylenediamine dihydriodide to provide 0.5 mg/kg I, MnSO<sub>4</sub> to provide 60 mg/kg Mn, ZnSO<sub>4</sub> to provide 60 mg/kg Zn, and an organic yeast (Sel-Plex, Alltech Inc., Nicholasville, KY) to provide 0.3 mg/kg Se in the total mixed ration on a DM basis.

<sup>5</sup> Provided 100 g·steer<sup>-1</sup>·d<sup>-1</sup> of microalgae (*Aurantiochytrium limacinum* CCAP 4067/2; Alltech, Inc.).

<sup>6</sup> Added 2,205 IU/kg vitamin A to all diets, 22 IU/kg vitamin E to CON and ALGAE diets, and 103 IU/kg vitamin E to AOX and LATE diets.

**Table 4.2** Metmyoglobin reducing ability (**MRA**), oxygen consumption (**OC**), and oxygen consumption rate (**OCR**) of *Longissimus lumborum* steaks from steers fed four different finishing diets.

Item	Treatment <sup>1</sup>				SEM	P - value		
	CON	ALGAE	AOX	LATE		Treatment	Day	Treatment × Day
MRA <sup>2</sup> , %					2.11	0.75	< 0.01	0.63
d 0	48.02	50.06	48.75	48.56				
d 5	22.67	25.64	27.95	24.95				
d 10	4.19	3.72	4.37	5.87				
OC <sup>3</sup> , %					3.79	0.15	< 0.01	0.96
d 0	53.18	59.46	55.49	48.51				
d 5	11.12	19.19	16.79	12.23				
d 10	9.75	16.07	11.44	10.15				
OCR <sup>4</sup> , %/min					0.09	0.15	< 0.01	0.96
d 0	1.33	1.49	1.39	1.21				
d 5	0.28	0.48	0.42	0.31				
d 10	0.24	0.40	0.29	0.25				

<sup>1</sup>A conventional finishing diet with 10% flaxseed (**FLAX**) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed throughout feeding (**AOX**), and FLAX diet the first 35 d of finishing and the AOX diet fed for the final 10 d of finishing (**LATE**).

<sup>2</sup>Metmyoglobin reducing ability was calculated as (metmyoglobin observed decreased in metmyoglobin concentration/initial metmyoglobin concentration) × 100.

<sup>3</sup>Oxygen consumption was calculated as (observed decreased in oxymyoglobin concentration at 40 min/initial metmyoglobin concentration) × 100.

<sup>4</sup>Oxygen consumption rate was calculated as oxymyoglobin consumed at 40 min/time.

**Table 4.3** Trained sensory panel evaluation of Longissimus lumborum steaks from steers fed four different finishing diets.

Attribute <sup>2</sup>	Treatment <sup>1</sup>				SEM	P – value
	FLAX	ALGAE	AOX	LATE		
Initial juiciness	65.34	60.94	65.98	67.28	2.66	0.38
Sustained juiciness	56.25	49.09	55.15	57.70	3.00	0.21
Myofibrillar tenderness	65.89	64.76	67.62	68.47	3.28	0.85
Connective tissue	20.16	18.70	16.46	19.28	2.35	0.70
Overall tenderness	60.60	60.15	63.12	62.78	3.40	0.90
Beef flavor identity	60.59	54.17	57.88	55.93	2.00	0.15
Beef flavor intensity	45.80	40.26	43.54	40.10	2.53	0.33
Off flavor intensity	1.68 <sup>c</sup>	4.65 <sup>a,b</sup>	6.82 <sup>a</sup>	4.11 <sup>b,c</sup>	0.97	< 0.01

<sup>a,b,c</sup>Within a row, means without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>A conventional finishing diet with 10% flaxseed (**FLAX**) or FLAX diet plus 100 g·steer<sup>-1</sup>·d<sup>-1</sup> supplemental microalgae meal (**ALGAE**; Alltech Inc., Nicholasville, KY), ALGAE diet plus antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed throughout feeding (**AOX**), and FLAX diet the first 35 d of finishing and the AOX diet fed for the final 10 d of finishing (**LATE**).

<sup>2</sup>0 = extremely dry, extremely dry, extremely tough, none, extremely tough, extremely unbeef-like, extremely bland, and extremely bland; 100 = extremely juicy, extremely juicy, extremely tender, abundant, extremely tender, extremely beef-like, extremely intense, and extremely intense.

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