EFFECTS OF ZILPATEROL HYDROCHLORIDE FEEDING DURATION ON COLOR OF BEEF AND HOLSTEIN SEMIMEMBRANOSUS STEAKS PACKAGED IN PVC AND MAP SYSTEMS

by

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Abstract

The objective of this study was to determine the effects of Zilmax® feeding duration (7.56 g/ton Zilmax for 0, 20, 30, or 40 d) on color development and stability of crossbred beef (B) and Holstein (H) *semimembranosus* (SM) steaks packaged in polyvinyl chloride overwrap (PVC), high-oxygen (80% O_2, 20% CO_2, HiOx) modified atmosphere packaging (MAP), and low-oxygen (0.4% CO, 30% CO_2, 69.6% N_2, LoOx) MAP. A 7.62-cm thick portion from beef and Holstein SM muscles (n = 120 total, 30 from each feeding duration) was removed, vacuum packaged, and stored until d 21 when two, 2.54-cm thick steaks were cut, overwrapped with PVC, and placed into retail display for 0 or 3 d. On d 10, the remaining muscle of the SM was enhanced, and five, 2.54-cm thick steaks were cut and assigned to 0, 3, or 5 d (HiOx) and 0 or 9 d (LoOx) of display. Measurements taken on the deep (DSM) and superficial (SSM) portions were: pH, L*, a*, b*, hue angle, and saturation indices, initial color, display color, and discoloration. No Diet × Display Day ($P > 0.05$) interaction occurred for display color or discoloration scores of B steaks in PVC. On d 1, PVC 30 d H steaks were brighter ($P < 0.05$) than 40 d H steaks in PVC; no differences ($P > 0.05$) in H PVC display color due to diet occurred on d 0, 2, and 3. For steaks in HiOx, the DSM of 20 and 30 d B steaks on d 4 and the DSM of 20 d B steaks on d 5 was brighter ($P < 0.05$) red than 40 d Zilmax B DSM. HiOx 20 d H steaks were darker red ($P < 0.05$) on d 5 of display and more discolored ($P < 0.05$) on d 3 to 5 than HiOx H steaks from all other diet regimens. For LoOx, 30 d B steaks were brighter ($P < 0.05$) red than 0 or 40 d steaks on d 0 and 9 of display. The DSM and SSM of LoOx H steaks from cattle fed Zilmax tended ($P > 0.05$) to be brighter red than control H steaks through 9 d of display. In conclusion, both HiOx and LoOx minimized color differences due to SM muscle areas. Feeding B cattle Zilmax for 20 or 30 d yields steaks equal to or better in color traits than cattle fed 0 or 40 d when packaged in PVC, HiOx, or LoOx. H steaks in LoOx had slight benefits in color stability when cattle were fed Zilmax for any duration, whereas H steaks in PVC had color advantages with only the 20 and 30 d durations. Packaging 20 d H steaks in HiOx resulted in less desirable color characteristics than all other feeding treatments.

Keywords: zilpaterol, beef, Holstein, display color, modified atmosphere packaging, semimembranosus

Table of Contents

List of Figures

List of Tables

List of Abbreviations

B = crossbred beef steer

DSM = deep portion of the *semimembranosus*

H = Holstein beef steer

HiOx = High oxygen modified atmosphere (80% oxygen, 20% carbon dioxide)

LoOx = Lo oxygen/carbon monoxide modified atmosphere (0.4% carbon monoxide, 30% carbon dioxide, 69.6% nitrogen)

MAP = modified atmosphere packaging

PVC = polyvinyl chloride film used in overwrap packaging

SM = *semimembranosus*

SSM = superficial portion of the *semimembranosus*

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Dedication

I would like to dedicate this thesis to my parents, Jim and Pat, and my brother Matt, and his fiancé Mitch. Words cannot say enough how much your love, support, and guidance has meant to me on the journey through my graduate program. Your wisdom through all of the happiness and the tears meant the world to me. Thank you for all you've done for me!

CHAPTER 1 - Introduction

With the recent approval (2006) of the beta-adrenergic agonist zilpaterol hydrochloride (marketed under the name Zilmax®, Intervet, Millsboro, DE) for use in fed cattle in the United States, a need arose for research regarding nearly all aspects of production and meat quality of cattle fed Zilmax. Intervet funded a multi-university study to evaluate attributes of carcasses, cuts, and individual muscles. Numerous qualitative and quantitative characterizations were done including carcass weight cut-outs, muscle tenderness, sensory attributes, meat color, and muscle/steak yields.

One specific aspect of this research was to analyze differences in feeding Zilmax to commercial crossbred beef steers versus Holstein steers. Cattle of varying genetic backgrounds may respond differently to Zilmax, resulting in the potential for muscle variation postmortem. Using Zilmax in feeding rations could be more beneficial for one type of cattle over another.

This thesis focused on meat color and pigment chemistry of the *semimembranosus* muscle using various packaging systems (traditional over-wrap and modified atmosphere packaging). This muscle is unique, as it typically varies from deep to superficial based primarily on how the muscle chills after slaughter prior to fabrication. Therefore, each region (deep or superficial) may respond in a different manner based upon its environment post-fabrication. Similar data were collected for other muscles or ground beef at Oklahoma State University, Texas Tech University, and the University of Illinois.

The objective of this study was to determine the effects of Zilmax feeding duration on the development of color, color stability, and purge of crossbred beef and Holstein *semimembranosus* steaks packaged in traditional or modified atmosphere packaging systems for various storage times.

CHAPTER 2 - Review of Literature

Beta-Adrenergic Agonists

Metabolic modifiers consist of steroid hormones or β-adrenergic agonists (β-AA) administered to livestock that enhance the efficiency of animal production without greatly compromising meat quality (Anderson & Johnson, 2004). Specifically, β-AA are feed additives incorporated into the diet for several weeks before the animals are harvested. According to Beermann (2004), altering the metabolic status of the animal results in profound physical changes regulated by the dosage and duration of β-AA supplementation in the diet.

Chemical Structure

β-adrenergic compounds, found in nature or synthetically produced, are chemically grouped as phenethanolamines (Beermann, 2004). The phenyl ring and ethanol-amine groups are defining structures of the compound, and different compounds attached determine the activity of the molecule. Epinephrine and norepinephrine are β-AA found naturally in the body, but they do not affect muscle mass or fat deposition in the same manner (Beermann, 2004), probably due to their short half-life in the body.

Mode-of-Action

Sometimes referred to as “repartitioning agents” (Anderson & Johnson, 2004; Ricks, Dalrymple, Baker, & Ingle, 1984), β-AA move energy consumed in feed toward the production of muscle, while simultaneously encouraging lipid degradation (Beermann, 2004). Ricks et al. (1984) also proposed a similar mechanism (Figure 2.1). As the animal consumes energy through the diet, most of the nutrients are directed toward muscle development. Depending on the type of β-AA, protein synthesis and/or degradation may be altered. At the same time energy is being directed away from fat deposition, adipose tissue is being broken down. Overall, this results in better feed conversion rates and more efficiency in livestock production (Anderson & Johnson, 2004; Beermann, 2004; Dunshea, D'Souza, Pethick, Harper, & Warner, 2005). The alteration of musculature in animals occurs through an increase in the length and diameter of muscle fibers (hypertrophy), not by an increase in the number of fibers (hyperplasia) (Beermann, 2004). This change in muscle structure is not the result of added DNA to muscle via satellite cells. Without added DNA, changes in muscle that occur as a result of β-AA supplementation are only temporary and cannot be sustained over a long period of time (Anderson & Johnson, 2004).

Bergen et al. (1989) fed pigs the β-AA ractopamine for 14, 28, or 42 days (d) and measured weights, protein, and DNA content of the *semitendinosus* (ST) muscle. The ST was significantly heavier (d 28) and had a higher protein concentration (d 42) from ractopamine fed pigs in comparison to controls; however, no difference occurred in DNA content between diet treatments on d 14, 28, or 42. The authors concluded that the increase in ST weight and size was not due to increased DNA content of muscle (Bergen et al., 1989).

Figure 2.1 Possible β-adrenergic agonist mode-of-action (from Ricks et al., 1984)

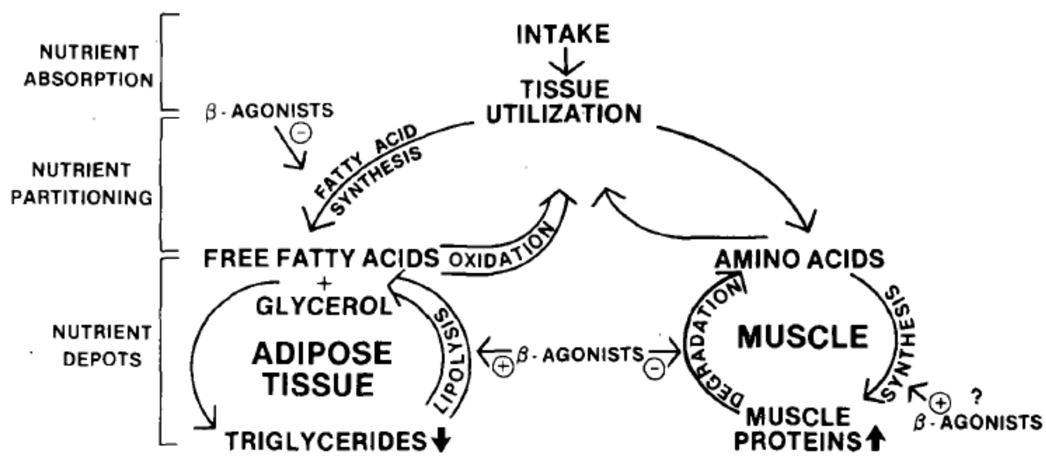


Figure 2. Proposed mode of action of β-agonists in altering muscle and fat deposition. + = stimulation, − = inhibition, ? = speculated.

To date, β-1, β-2, and β-3 are the only known membrane-bound β-AA receptor sites. β-1 agonists promote alterations in muscle through increased protein synthesis; however, β-2 agonists enhance protein synthesis and slow degradation (Anderson & Johnson, 2004). β-3 receptors are not known to influence changes in muscle mass. The composition of β-AA receptors in cattle is predominately β-2 (Mersmann, 1998), but the mixture of receptor sites on cells is unknown as most cells possess a combination of receptor types.

Effects on Meat Quality

Depending upon the species, β-AA have different effects on carcass composition and meat quality. Dunshea et al. (2005) reviewed multiple studies and concluded that ractopamine fed to pigs decreases fat deposition in all locations except for backfat, whereas cattle experience large adipose tissue reductions in all depots. Focusing specifically on carcass traits, Dikeman (2007) noted minimal to slight benefits in meat color and certain sensory attributes of beef or pork from livestock supplemented with β-AA; tenderness is still a concern.

Wheeler & Koohmaraie (1992) studied the effects on the calpain/calpastatin system and tenderness of steaks from beef steers fed L-644,969 for six weeks. While calpain activity was normal early postmortem, calpastatin activity was significantly greater (60%) in β-AA supplemented *longissimus* muscle. Steaks from cattle fed L-644,969 (a β-2 agonist) were significantly tougher (higher Warner-Bratzler shear force, WBSF) than control steaks on d 7 and 14 postmortem. In conclusion, the authors attributed the increased toughness of steaks from cattle fed L-644,969 to the higher activity of calpastatin early postmortem in supplemented cattle (Wheeler & Koohmaraie, 1992).

A recent study analyzed the effects of feeding 200 mg of ractopamine for 28 d to various breeds of beef steers on WBSF, slice shear force, and sensory characteristics (Gruber et al., 2008). Ractopamine supplementation resulted in significantly higher WBSF and slice shear force values as well as decreased tenderness and juiciness of *longissimus* steaks in comparison to controls. The authors hypothesized that the resulting negative changes in tenderness and sensory from feeding ractopamine, a β-1 agonist, was from an alteration in muscle fiber size, not inhibition of the calpain system (Gruber et al., 2008). For dairy cattle, Holsteins fed 200 or 300 mg/hd/d of ractopamine exhibited increased hot carcass weights (10.3% and 11.1%) and larger ribeyes [78.7 cm^2 (12.2 in^2) and 80 cm^2 (12.4 in^2)],respectively, compared with non-supplemented cattle (Duff & McMurphy, 2007).

In regards to meat color, little research exists that has studied the effects of β-AA supplementation on color, especially color stability during display. Geesink, Smulders, Vanlaack, Vanderkolk, Wensing, & Breukink (1993) fed veal calves clenbuterol for 42 d prior to withdrawal times of 2, 4, or 8 d before slaughter. *Longissimus* and *semimembranosus* steaks were significantly lighter (higher L* values) for calves from the 4 and 2 d withdrawal times and the 2 d withdrawal time, respectively in comparison to control steaks. No significant differences in redness (a*) or yellowness (b*) occurred. Quinn et al. (2008) reported no difference in lightness, redness, or yellowness of *longissimus* steaks from beef heifers fed ractopamine for 28 d or no β-AA.

With regards to dairy cattle, Moloney, Allen, Joseph, Tarrant, & Convey (1994) fed Friesian steers various levels (0, 0.25, 1.0, or 4.0 ppm) of L-644,969 and collected instrumental color data on d 2, 3, and 6 from *longissimus* steaks. No significant difference occurred in lightness (L*) on all d of display for all supplementation levels. No difference in hue angles occurred on d 3 or 6; however, steaks became more discolored (lower hue angle) with increasing level of L-644,969. On all d of display, steaks were less vivid (lower saturation indices) with increased level of β-AA fed. The authors concluded that color changes due to feeding various levels of L-644,969 would not significantly impact meat from dairy cattle fed this β-AA (Moloney et al., 1994).

β-AA are very effective at improving the efficiency of livestock production. Several different types are available, and the receptor they target affects how β-AA are active within the animal. Variations in tenderness may be attributed to the type of β-AA fed and which type of receptor they target. Research regarding meat color yields inconsistent results; although most authors agree that the effect of β-AA supplementation on color traits is minimal.

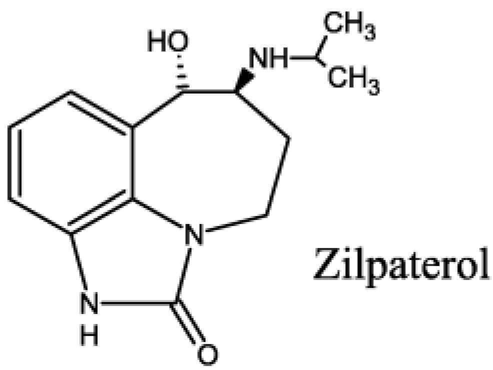
Zilpaterol Hydrochloride

β-AA Classification

Little is known about the mode-of-action of zilpaterol hydrochloride (ZH). All β-AA activate receptors in a similar manner. Mersmann (1998) outlined the series of events surrounding the activation of β-AA receptors. When a β-AA attaches to an available receptor, the G_S protein activates adenylyl cyclase, producing cyclic adenosine monophospate (cAMP). Protein kinase A, activated by cAMP, allows the activation of various enzymes inside the cell that affect the degradation and synthesis of proteins and lipids (Mersmann, 1998).

Verhoeckx, Doornbos, Van der Greef, Witkamp, & Rodenburg (2005) conducted a study evaluating the possibility of ZH activity towards β-1 or β-2 receptors since the chemical structure of ZH is different than other β-2 agonists (Figure 2.2). Data indicated that the production of cAMP was regulated more by β-2 than β-1 receptors, and that ZH bound to more β-2 than β-1 receptors. Verhoeckx et al. (2005) concluded that ZH was a β-AA with high affinity for β-2 receptors.

Figure 2.2 Chemical structure of the β-adrenergic agonist zilpaterol hydrochloride (adapted from Verhoeckx et al., 2005)



Effects on Carcass Composition

ZH is currently approved for use as a β-AA for livestock in South Africa, Mexico, and most recently, the United States (Vasconcelos et al., 2008). Limited research exists regarding the effects this specific β-AA has on carcass yields. Plascencia, Torrentera, & Zinn (1999) fed crossbred steers 0 or 6 mg/kg of ZH for 42 d and found significantly increased carcass weights, dressing percentages, and loin eye areas for the ZH treatment over controls. Feeding ZH also resulted in increased weights of bone-in rounds and top rounds in comparison to their control wholesale and subprimal counterparts.

Another study supplemented Sussex × Brahman steers with or without 0.15 mg/kg of ZH for 49 d (Casey, Webb, & Maritz, 1997). No significant differences in hot carcass weight, dressing percentage, or subcutaneous fat occurred between the treatments. This may be due in part to the lower dosage of ZH (0.15 mg/kg) in comparison to the Plascencia et al., (1999) study (6 mg/kg). Avendaño-Reyes, Torres-Rodríguez, Meraz-Murillo, & Pérez-Linares (2006) reported significantly higher carcass yields and larger *longissimus* muscle areas in ZH-fed beef steers than control cattle.

A recent study looked at the effects of ZH feeding duration (8.33 mg/kg for 0, 20, 30, or 40 d) on carcass composition of beef steers (Vasconcelos et al., 2008). Regardless of feeding duration, ZH-fed steers yielded significantly heavier carcasses, larger dressing percentages and ribeye areas, and lower yield grades than control carcasses. Quality grade was also affected by ZH; significantly more control carcasses graded Choice or higher than those from ZH-fed steers. A higher percentage of Select quality grades occurred with increased ZH feeding duration in comparison to their control counterparts. Vasconcelos et al. (2008) concluded that no added benefit existed in carcass yields to merit feeding ZH for more than 20 d before slaughter.

Effects on Tenderness

Few studies have been conducted looking at the effects of ZH supplementation and/or feeding duration on meat quality. Concerning tenderness, both Avendaño-Reyes et al. (2006) and Strydom, Frylinck, & Marais (2007) found significantly tougher *longissimus* steaks (higher WBSF values) for beef cattle supplemented 60 mg/head/d for 33 d or 0.15 mg/kg for 30 d, respectively, in comparison to steaks from control cattle. Strydom, Osler, Leeuw, & Nel (1999) reported significantly lower tenderness scores for *longissimus* steaks from cattle fed 45 d of ZH in comparison to the controls. In contrast, Casey et al. (1997) had no difference in shear force values of steaks from steers fed 0.15 mg/kg of ZH for 0 or 49 d.

In an earlier study, Strydom, Osler, Nel, & Leeuw (1998) fed beef steers ZH for 0, 15, 30, or 45 d before *longissimus* (LT) and *semitendinosus* (ST) steaks were tested for WBSF or sensory attributes by panelists. No differences in WBSF occurred for the ST; however, cattle fed ZH for 45 d had significantly tougher LT steaks than control cattle. LT steaks from the 45 d-ZH treatment were less tender, had less connective tissue, and less sustained juiciness than control steaks. Differences for ST steaks were primarily within ZH feeding durations, as the 45 d LT steaks had lower initial juiciness and first bite scores than 30 d steaks and lower sustained juiciness scores than 15 d LT steaks (Strydom et al., 1998). While most of the data agree that supplementation of ZH for extended periods of time has a detrimental effect on tenderness, variation between studies may be due to ZH dosage level or duration, as well as genetic variation in the cattle used.

Effects on Meat Color

Even fewer studies have looked at the effects of ZH supplementation on color attributes. Strydom et al. (2007) reported that *longissimus* steaks from bulls fed 30 d ZH were significantly lighter (higher L* value) and more vivid (higher saturation indices) than control steaks. In an earlier study, Strydom, Buys, & Strydom (2000) had panelists evaluate the acceptability of *longissimus* and *gluteus medius* steaks from steers fed ZH for 0, 30, or 50 d. Percentage metmyoglobin was also measured and found to be lower for steaks from either ZH treatment in comparison to control *longissimus* and *gluteus medius* steaks. The authors concluded that display color throughout storage was improved with either level of ZH supplementation.

Avendaño-Reyes et al. (2006) reported similar values for lightness, redness, yellowness, and vividness between control and ZH-supplemented *longissimus* steaks. ZH steaks had significantly higher hue angles (indicative of greater discoloration and a loss of redness) than control steaks. In conclusion, the limited literature available describes minimal to slightly positive effects regarding display and instrumental color of various cuts from cattle supplemented ZH.

Myoglobin Chemistry

Beef Semimembranosus

Consumers use color as the basis for determining freshness prior to purchasing a cut of meat (Mancini & Hunt, 2005a). Deviations in the color of meat at the retail case may cause consumers to opt for buying other cuts. Large muscles in the beef round typically exhibit differences in protein structure, resulting in an inconsistency in lightness of these muscles (Macdougall, 1982). The beef *semimembranosus* (SM) is an excellent example. The inner portion of the SM near the femur bone tends to chill more slowly than portions located more superficially, resulting in higher degrees of light scattering, more protein denaturation (due to increased temperatures deep within the muscle), and a decreased ability to reduce pigment oxidation (Macdougall, 1982).

In an early study, Follett, Norman, & Ratcliff (1974) analyzed the differences between SM muscles removed from beef carcasses 1 hour or 24 hours postmortem. Data for temperature declines indicated that 1 hr. SM had a more linear, less rapid pH decline ultimately producing a more uniform composition. SM removed at 1 hr. was more tender when cooled between 5 and 15ºC than 24 hr. SM. NAD concentrations remained at levels higher in 1 hr. SM than 24 hr. SM; NAD levels were similar between the muscles after 7 d of storage. The authors noted that NAD concentrations, which influence pigment reducing ability during storage, had a noticeable effect as 1 hr. SM had less discoloration than 24 hr. SM after the 7 d period. Overall, the early removal of the SM from the beef hindquarter resulted in improved color stability and reducing capacity due to more uniform chilling conditions (Follett et al., 1974).

McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell (2005) conducted a large study analyzing the metmyoglobin content, metmyoglobin reductase activity (MRA), oxygen consumption rate (OCR), and instrumental a* values of different beef muscles. The authors classified the SM as intermediate in color stability based upon an average decrease in the $(K/S)_{572}/(K/S)_{525}$ ratio, which indicates the level of metmyoglobin development, across 5 d of retail display. MRA was relatively low for the SM, and OCR increased daily until d 4 of display. A relatively high OCR corresponds to the high a* values (above 24.0) seen for the SM during display.

As of late, research has focused more specifically on differences between the deep (DSM) and superficial (SSM) portions of the SM. Lee, Yancey, Apple, Sawyer, & Baublits (2008), Sammel, Hunt, Kropf, Hachmeister, & Johnson (2002a), and Sammel, Hunt, Kropf, Hachmeister, Kastner, & Johnson (2002b) all found higher pH values for the DSM than the SSM, whereas Seyfert, Mancini, Hunt, Tang, Faustman, & Garcia (2006) and Seyfert, Mancini, Hunt, Tang, & Faustman (2007) reported no difference in pH values between the muscle portions. For SM steaks packaged in high-oxygen (80% O_2, 20% CO_2, HiOx) modified atmosphere packaging (MAP), no significant difference occurred in pH values of fresh muscle; however, steaks enhanced with a 10% solution had significantly higher pH values for the SSM than the DSM (Seyfert, Hunt, Mancini, Hachmeister, Kropf, & Unruh, 2004).

Regarding initial color, the DSM of steaks was a brighter red color than the SSM at the beginning of display (Seyfert et al., 2004; Seyfert et al., 2006; Seyfert et al., 2007). Typically, the bright color of the DSM at the beginning of display darkens faster than the SSM, resulting in a darker DSM in later d of storage (Sammel et al., 2002a; Sammel et al., 2002b; Seyfert et al., 2006). For SM steaks packaged in HiOx, the DSM was brighter on d 0 – 2 of display than the SSM, but not different on d 3 – 7 (Seyfert et al., 2007), or brighter than the SSM on d 0, 2, and 4 (Seyfert et al., 2004). Sawyer, Baublits, Apple, Meullenet, Johnson, & Alpers (2007) reported no difference in display color on d 0 and 6, although the DSM was brighter red than the SSM on d 3.

The percentage of oxymyoglobin (OMb) present on the steak surface was higher (Sammel et al., 2002a; Sammel et al., 2002b) or not different (Seyfert et al., 2004) for the DSM than the SSM on d 0; however, Sammel et al. (2002a), Sammel et al. (2002b), and Seyfert et al. (2004) all reported a higher percentage OMb for the SSM than the DSM on d 2 – 5. The data for display color scores and percentage OMb agree: while the DSM tends to have a brighter color (due to higher OMb amounts) initially, the color usually darkens quickly and the amount of OMb on the steak surface decreases rapidly.

Sawyer et al. (2007) reported similar ($P > 0.05$) discoloration scores between the muscle portions on d 0, 3, and 6 of display. Another study found no difference in discoloration between the DSM and SSM on d 0 and 1, but the DSM was more discolored than the SSM on d 2 – 7 (Seyfert et al., 2006). Percentage metmyoglobin (MMb) on the steak surface was found to be higher for the DSM than the SSM (Sammel et al., 2002a), or not different (Sammel et al., 2002b) on d 0. Both studies reported higher amounts of MMb for the DSM surface in comparison to the SSM on d 1 – 5. Accordingly, Seyfert et al. (2007) and Seyfert et al. (2006) both had an increased metmyoglobin reducing activity (MRA) for the SSM compared to the DSM on d 4 of display, but no difference in MRA between the two muscle portions on d 7. Seyfert et al. (2007) noted that the MRA assay is of less importance when muscles are packaged in HiOx MAP, as the increased oxygen concentration in the atmosphere causes a deeper OMb layer that prevents discoloration via MMb formation during longer shelf life periods.

Regarding instrumental color measurements, the DSM is usually lighter (higher L* values) than the SSM (Lee et al., 2008; Sammel et al., 2002a; Sammel et al., 2002b; Seyfert et al., 2004; Seyfert et al., 2006; Seyfert et al., 2007). Typically the DSM is redder than the SSM (Sammel et al., 2002a; Sammel et al., 2002b) or has no difference in a* values on d 0 (Sawyer et al., 2007; Seyfert et al., 2006; Seyfert et al., 2007); however, the SSM is redder than the DSM during later d of display (Sammel et al., 2002a; Sammel et al., 2002b; Seyfert et al., 2006; Seyfert et al., 2007). Contrastingly, Sawyer et al. (2007) reported higher a* values for the DSM than the SSM on d 6 only of display, and Lee et al. (2008) had a redder DSM compared to the SSM on all d of display.

The same conflicting results have been reported in multiple studies for b* values (yellowness), hue angles (indicative of a loss of redness and increased discoloration), and saturation indices (vividness). Differences between studies may be attributed to the use or lack of electrical stimulation on carcasses, type of chilling regimen, the age of muscles, the use of injection enhancement, location of steaks within the SM, or variation in packaging types.

Muscle from Dairy Breeds

Little information is available regarding the differences in color characteristics of meat from cattle breeds genetically selected for milk production. An early study found the round muscles from Holstein bulls to be paler and more in uniform color than samples from crossbred beef heifers and steers, however, Ziegler, Wilson, & Coble, (1971) cited another study stating that muscle from cattle of dairy genotypes tends to have problems with color stability during retail display in comparison to their beef counterparts. This idea was supported by Faustman & Cassens (1991), who reported higher discoloration scores for *longissimus* and *gluteus medius* steaks from Holstein steers than crossbred beef steers across all 6 d of display. Additionally, the same study found a higher percentage of MMb and increased thiobarbituric acid (TBA) substances (indicative of lipid oxidation) for Holstein muscle samples versus beef samples.

Page, Wulf, & Schwotzer (2001) compared instrumental color values of cattle from different genetic backgrounds. Their results indicated that muscle from traditional beef breeds was significantly lighter, redder, and more yellow than muscle from dairy-type carcasses. Dunne, Keane, O'Mara, Monahan, & Moloney (2004) reported higher L* values for *longissimus* samples from Belgian Blue × Holstein-Friesian (beef) steers in comparison to New Zealand or Irish (dairy) breeds of Friesian steers, but no difference in a*, b*, or chroma values between the three breeds. Belgian Blue × Holstein-Friesian *longissimus* samples had higher hue angles than the Irish Friesian steer samples.

Packaging

The use of packaging materials in the meat industry serves a variety of functions. Some examples include, but are not limited to: preservation, freshness, to provide for consumers' needs, to be a barrier to the external environment, and to extend shelf life for as long as possible (Eilert, 2005; Kropf, 2004). Perhaps the most important function of packaging is to preserve the bright red color (oxymyoglobin) that consumers associate with wholesomeness (Kropf, 2004).

Overwrap Packaging

The basis for overwrap packaging begins with a central processor that fabricates carcasses into subprimals, boxes the cuts, and ships them to a local retailer (Jeyamkondan, Jayas, & Holley, 2000). Upon arrival, large muscles are cut into smaller steaks and roasts, placed on a polystyrene tray, overwrapped (typically with polyvinyl chloride [PVC] film), and placed into the display case (Cole, Jr., 1986). PVC film serves as a good barrier to moisture loss, but allows for the transfer of oxygen to the muscle, and subsequent formation of the OMb pigment (Kropf, 2004). Unfortunately, this desirable bright red color is relatively unstable, lasting approximately 5 d in display before the pigment oxidizes and converts to MMb (Cole, Jr., 1986; Kropf, 2004). Multiple packaging sites, inconsistency in quality, inadequate use of retail display, and excess waste at the retail level are a few other drawbacks to the use of this system (Jeyamkondan et al., 2000).

High-Oxygen MAP

The use of artificial atmospheres in meat packaging serves to fill the role of increased color preservation during display. This type of packaging is characterized by gas blends of 75-80% oxygen and 20-25% carbon dioxide (HiOx); the high atmospheric

O_2 concentration allows a deeper, more oxidative-resistant OMb layer to form while the CO_2 assists in deterring microbial growth (Cole, Jr., 1986; Jeyamkondan, et al., 2000; Kropf, 2004). While shelf life may be up to 2 weeks longer, increased lipid and pigment oxidation from the high oxygen concentrations results in off-odors and flavors (Cole, Jr., 1986; Jeyamkondan et al., 2000; Seyfert et al., 2005). Kropf (2004) suggested using enhancement solutions with antioxidative ingredients to prevent off-odor development.

Stetzer, Wicklund, Paulson, Tucker, Macfarlane, & Brewer (2007) reported a cherry red color for beef strip loin steaks packaged in HiOx and displayed up to 14 d. Top sirloin steaks in HiOx MAP were a more vivid red color with little discoloration up to 14 d of display; however, by d 21 browning and discoloration were evident (John, Cornforth, Carpenter, Sørheim, Pettee, & Whittier, 2005). Sørheim, Nissen, & Nesbakken (1999) found a relatively stable, bright red color in beef loin steaks packaged in HiOx up to approximately 10 d in storage before pigment oxidation and metmyoglobin formation occurred.

Behrends, Mikel, Armstrong, & Newman (2003) evaluated the effects of different packaging methods (PVC or HiOx) on instrumental and visual color variables of the SM. No practical differences occurred in display color or discoloration scores, a* values, or metmyoglobin content. SM steaks packaged in HiOx were lighter (higher L* value) through 10 d of display. The authors suggested that the choice of packaging used is muscle dependent, and that HiOx MAP may even reduce color stability (Behrends et al., 2003). In comparison with PVC overwrap, the use of HiOx MAP for beef cuts results in a brighter, more stable red color and a longer shelf life. However, HiOx MAP is not without its limitations; specifically, the increased off-odors and flavors associated with longer periods of display.

Low-Oxygen, Carbon Monoxide MAP

Using low amounts of carbon monoxide (CO) in combination with a low-oxygen (LoOx) environment for packaging of meat has been approved for use in the United States for less than a decade (Eilert, 2005). Oxygen levels below 1 – 2% are necessary to prevent pigment oxidation and microbial growth; the inclusion of carbon dioxide (usually at 25 – 35%) also aids in controlling spoilage bacteria (Kropf, 2004) while the remainder of the gas blend is typically nitrogen. Cornforth & Hunt (2008) suggested residual oxygen levels be less than 0.15% to prevent metmyoglobin formation. In order to achieve low oxygen levels, oxygen scavengers may be used that bind atmospheric oxygen within the package (Jeyamkondan et al., 2000). One of the defining characteristics of CO MAP is the significant increase in color stability and retail shelf life of meat packaged in this atmosphere (Cornforth & Hunt, 2008; Sebranek & Houser, 2006), even in comparison to HiOx (Kropf, 2004). The main drawbacks to using CO MAP include: a negative perception by consumers, and concern that the extended color shelf life may conceal microbial spoilage (Cornforth & Hunt, 2008).

John et al. (2005), Sørheim et al. (1999), and Stetzer et al. (2007) all reported excellent color stability for beef *longissimus* and *gluteus medius* steaks packaged in CO MAP between a 14 – 21 d shelf life. Exposing beef loin steaks to 5% CO for 24 hours before vacuum packaging results in an acceptable red color for up to 21 d of storage (Jayasingh et al., 2001). Using a barrier bag system, Hunt et al. (2004) exposed PVC overwrapped beef SM steaks to a gas blend containing 0.4% CO for 7, 21, or 35 d before removing steaks from the master bag and placing them into simulated retail display. Results indicated that storage in 0.4% CO for up to 35 d yielded bright red colored steaks at the beginning of display. The DSM benefited slightly from storage in CO from the standpoint of color stability, whereas the SSM had somewhat decreased color stability with longer MAP storage periods (Hunt et al., 2004). One of the primary advantages to packaging beef in CO MAP is the increased color stability during long shelf life periods in comparison to HiOx MAP and PVC overwrap.

Enhancement

The use of injection enhancement has been employed for several decades in the poultry industry, and more recently for pork products (Robbins, Jensen, Ryan, Homco-Ryan, McKeith, & Brewer, 2002; Stetzer, Tucker, McKeith, & Brewer, 2008; Wicklund, Homco-Ryan, Ryan, McKeith, McFarlane, & Brewer, 2005). As companies strive to produce more desirable cuts that consumers are willing to purchase, the use of enhancement has come to the fore-front as a means to improve consistency and eating quality of meat products (Robbins et al., 2002).

Effects on pH

Pietrasik, Dhanda, Shand, & Pegg, (2006), Robbins et al. (2002), Stetzer et al. (2008), and Wicklund et al. (2005) all reported increased pH values for enhanced muscle in comparison to non-injected beef, regardless of percentage pump or which muscle was utilized. All of the previous studies attributed the inclusion of alkaline phosphates in the enhancement solution as the reason for the pH increase.

For beef strip loins injected to a 6% pump, the inclusion of rosemary in the enhancement solution resulted in an increased pH over loins enhanced with lactate or lactate combined with rosemary (Mancini et al., 2005b). Lawrence, Dikeman, Hunt, Kastner, & Johnson (2004) also found a higher pH for beef loins enhanced (11.5% pump) with salt, phosphate, and natural flavoring (containing rosemary) in comparison to a calcium lactate + natural flavoring solution. The difference in pH was attributed to the lower water binding ability of calcium (Lawrence et al., 2004).

Effects on Meat Color

Several studies have evaluated the use of salt and phosphates in enhancement solutions, and their potential effect on meat color development and stability. Wicklund et al. (2005) evaluated instrumental color and visual redness of beef strip loins injected to an 8% pump with a solution containing 0.3% salt and 0.4% phosphate. For beef strips aged up to 28 d prior to enhancement, injected samples were darker, less red and yellow, and less vivid, but a truer red color than their control counterparts. Redness (d 7 only) was not different between injected and control loins; however, panelists rated enhanced samples darker red than controls on d 14, 21, and 28 of ageing.

A similar study using the same percentage pump and ingredient inclusion levels as Wicklund et al. (2005) evaluated the effects of enhancement on instrumental and visual color. Stetzer et al. (2008) reported lower visual brown and red scores, and lower hue angles for enhanced beef muscles than controls, but no difference in yellowness of muscles. For muscles from the round, enhanced *vastus lateralis* was darker than its control counterpart, but had no difference in a*, hue angle, or chroma values. Enhanced *vastus medialis* was a less vivid red with a lower hue angle than the control, but no difference in L* values occurred.

Robbins et al. (2002) found similar instrumental color results for beef *semimembranosus* enhanced with a 10% solution containing 0.4% salt and phosphate. In that study, enhanced SM steaks also had lower display color scores than their control counterparts on d 2 and 4 of display. All three studies concluded that injection enhancement tended to have a negative effect on color attributes of beef during retail display (Robbins et al., 2002; Stetzer et al., 2008; Wicklund et al., 2005). Contrastingly, Pietrasik et al. (2006) reported that enhanced (20% pump with 0.5% salt and 0.3% phosphate) beef *longissimus lumborum* steaks were darker, less red, and less yellow than non-enhanced control steaks, but concluded that enhancement improved the color stability of steaks in that study.

The use of rosemary in enhancement solutions for beef loins resulted in improved L* values throughout 7 d of display, as well as increased a*, b*, and saturation indices initially (Mancini et al., 2005b). By d 7, no difference in redness, yellowness, or vividness occurred between the lactate, rosemary, and lactate + rosemary treatments. Visually, rosemary treated-beef loins were lighter red across all d of display than loins enhanced with the other solutions. Mancini et al. (2005b) concluded that the use of rosemary in enhancement solutions improved color attributes initially, but that those benefits are not carried through long display periods.

Summary

Feeding β-AA to beef cattle has more profound effects on carcass composition and tenderness than meat color. Supplementation of beef or Holstein cattle with any β-AA, including zilpaterol, usually results in minimal to slightly positive effects on meat color. Due to uneven chilling conditions postmortem, the beef *semimembranosus* usually has two different muscle portions, each with diverse color development and color stability properties. Although the type of packaging used for various beef cuts can be muscle dependent, the use of carbon monoxide in an ultra-low oxygen atmosphere yields the most color stability for the longest shelf life period. Multiple studies present conflicting results regarding the effects that injection enhancement has on meat color. The use of rosemary in solution improves initial color attributes, but not necessarily pigment stability, during simulated retail display.

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CHAPTER 3 - The Effects of Zilpaterol Hydrochloride Feeding Duration on Crossbred Beef and Holstein Semimembranosus Steak Color when Packaged in PVC

Abstract

The objective of this research was to determine the effects of zilpaterol hydrochloride (Zilmax®) feeding duration (0, 20, 30, or 40 d) on *semimembranosus* (SM) color development and stability. A 7.62-cm thick portion was removed from 60 crossbred beef (B) and 60 Holstein (H) steer SM subprimals and stored (2ºC) for 21 d. Two 2.54-cm thick steaks were cut, overwrapped with polyvinyl chloride film, and assigned to 0 or 3 d of display. Panelists evaluated the deep (DSM) and superficial (SSM) portions of steaks for initial color, display color, discoloration, pH, L*, a*, b*, hue angle, and saturation indices. No differences ($P > 0.05$) occurred in initial color scores due to Zilmax feeding duration for B or H steaks. The SSM of 20 d B steaks was brighter red ($P < 0.05$) than 40 d B SSM. No differences ($P > 0.05$) in B discoloration scores occurred due to diet. The B DSM had a lower ($P < 0.05$) pH and paler ($P < 0.05$) color than the SSM. Display color scores for B DSM were brighter red ($P < 0.05$) than B SSM initially (d 0 and 1), but B DSM discolored faster ($P < 0.05$) than the SSM on d 1 to 3. H DSM was darker ($P < 0.05$) and more discolored ($P < 0.05$) than the SSM on d 1 to 3 of display. SM steaks from B and H steers fed Zilmax for 20 or 30 d were slightly brighter and less discolored during display than the 40 d Zilmax treatment. Feeding B or H cattle Zilmax for 20 or 30 d will yield steaks equal to or better in color characteristics than steaks from control cattle. Feeding Zilmax for 40 d will likely produce less desirable meat color traits.

Key Words: zilpaterol, beta agonist, display color, overwrap packaging, crossbred beef, Holstein

Introduction

Consumers at retail base purchasing decisions primarily on the color of meat cuts over other factors, as color indicates freshness and age (Jeyamkondan, Jayas & Holley, 2000; John, Cornforth, Carpenter, Sørheim, Pettee & Whittier, 2005; Mancini & Hunt, 2005). Several packaging methods exist that promote color preservation of meat, including the extensively used oxygen-permeable, polyvinyl chloride (PVC) overwrap system. Upon fabrication of carcasses, vacuum packaged subprimal cuts are boxed and shipped to local stores, where subprimals are cut, placed on trays, PVC wrapped, and put into retail display (Cole, Jr., 1986). While PVC overwrap results in a desirable red color expected of fresh beef, it may lead to a shortened retail shelf life (Kropf, 2004).

Beta-adrenergic agonists fed to cattle and swine usually result in increased hot carcass weights and lean tissue development, improved feed efficiencies, and less fat deposition (Beermann, 2004; Dikeman, 2007; Quinn et al., 2008). Ractopamine increased feed efficiencies, hot carcass weights, and longissimus muscle area in Holstein steers (Bass, Beckett & Delmore, 2006). Dikeman (2007) noted that feeding ractopamine hydrochloride to either cattle or pigs resulted in minimal to slightly positive effects on meat color. Quinn et al. (2008) found no significant differences between lightness (L^*), redness (a^*), or yellowness (b^*) values in ractopamine-fed heifers and controls. Zilpaterol fed 30 or 50 d resulted in a longer color display life for beef steaks (Strydom, Buys & Strydom, 2000). Another study (Avendaño-Reyes, Torres-Rodríguez, Meraz-Murillo & Pérez-Linares, 2006) revealed similar L^* values for cattle fed zilpaterol, ractopamine, or no β-agonist on d 1 and 14; however, d 5 control steaks had lower ($P < 0.05$) L^* values (darker color) than steaks from either β-agonist. The same study showed higher ($P < 0.05$) hue angle values (indicative of a loss of redness) for steaks from cattle fed either β-agonist in comparison to controls.

Faustman & Cassens (1991) noted that meat from crossbred cattle tends to discolor more slowly than beef from Holsteins; however, little research has been conducted that analyzes visual color attributes of steaks from cattle of varying phenotypes. Therefore, the objective of this study was to evaluate initial color, color stability, and instrumental color of PVC packaged crossbred beef and Holstein steaks from cattle fed zilpaterol hydrochloride.

Materials and Methods

Animal Selection and Raw Materials

Over 1,000 head of crossbred beef and 2,300 head of Holstein steers were fed at two different commercial feed yards in Texas or California, respectively. Crossbred beef and Holstein steers were allotted randomly to four feeding groups, and fed a typical feedlot finishing diet (see Appendix A) supplemented without or with 7.56 g/ton of Zilmax (100% DM basis, Zilmax®, Intervet, Millsboro, DE). Crossbred beef cattle were implanted on d 0 (arrival at feedlot) and again on d 80 with a Revalor-IS (80 mg trenbolone acetate and 15 mg estradiol). Holstein steers were implanted on d 0 (arrival at feedlot) with Revalor-IS; Holstein steers were implanted with Synovex-S (200 mg progesterone and 20 mg estradiol benzoate) 120 d before the Revalor-IS implant. All cattle were removed from Zilmax supplementation 3 d prior to harvest at two separate commercial facilities in Texas or California. Crossbred beef steers were harvested in late January, whereas the Holstein steers were harvested in early May. Carcasses were electrically stimulated (45 volts) 30 minutes postmortem and chilled at 0 ± 2ºC in a bone-to-bone configuration. Crossbred beef and Holstein carcasses (n = 120) were selected randomly on d 1 postmortem, and 15 inside rounds (*semimembranosus*, SM, NAMP # 168) from each feeding duration (0, 20, 30, or 40 d of Zilmax) were removed from one side of each carcass, vacuum packaged, and commercially shipped to the Kansas State University Meat Laboratory.

Subprimal Processing

On d 9, the vacuum packaged, whole muscle weight of all subprimals was recorded upon arrival. Cuts were unpackaged, drained, and a blotted weight was taken. The percentage purge loss of each muscle was calculated. Subprimals (n = 120 total, 15 from each feeding group for crossbred beef and Holstein carcasses) were trimmed to remove the *adductor* muscle and excess fat, leaving the SM. Muscles were then re-weighed to determine the percentage SM subprimal yield. The anterior portion of each

SM (approximately 7.62-cm thick) was removed, vacuum packaged (Barrier bag 620, Sealed Air Corp., Duncan, SC), and placed into dark storage at 2ºC until d 21 postmortem to simulate retail shelf life patterns.

Steak Fabrication

At d 21 postmortem, each SM portion was unpackaged, faced, and two, 2.54-cm thick steaks were cut and placed cut-surface up on either 20.32 cm × 14.61 cm × 1.74 cm or 23.50 cm × 18.42 cm × 1.59 cm foam trays (2S or 4S, Cryovac Sealed Air, Duncan, SC) containing tray diapers. Steaks were overwrapped with a polyvinyl chloride (PVC) oxygen permeable film (MAPAC-M film, 23,250 cc/m^2/24 h, 72 gauge, Resinite Packaging Films, Borden, Inc., North Andover, MA), and assigned to either 0 or 3 d of retail display. All steaks were placed into simulated retail display upon the completion of fabrication and packaging.

pH

Crossbred beef and Holstein steak pH was measured on d 0 and 3 by inserting the tip of a previously calibrated probe (MPI pH probe, glass electrode, Meat Probes Inc., Topeka, KS) twice into the deep SM (DSM) and three times into the superficial SM (SSM). Measurements were averaged within muscle area (DSM or SSM) and a final value was calculated for each portion of a steak.

Retail Display

Steaks were displayed under continual fluorescent lighting (2153 lux, 3000 K, CRI = 85, Bulb model F32T8/ADV830/Alto, Philips, Bloomfield, NJ) at 2 ± 1.3°C in open-topped cases (Unit model DMF8, Tyler Refrigeration Corp., Niles, MI) for 3 d. Display cases were completely filled with one layer of packages that were rotated daily to minimize variation due to package location in the case. Cases automatically defrosted every 12 hours, and case temperature was monitored during display using temperature loggers (RD-TEMP-XT, Omega® Engineering, Inc., Stamford, CT).

Visual Color

Trained color panelists (n = 6 to 8) who passed the Farnsworth-Munsell® 100-hue test (Macbeth, Newsburgh, NY) evaluated each steak region (DSM and SSM) for initial, display color, and discoloration (AMSA, 1991). On d 0 of display, initial color evaluations were made, whereas display color and discoloration scores were recorded daily for steaks on 0 through 3 d of simulated display. The initial color scale used was: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red, 6 = Slightly dark red, 7 = Moderately dark red, 8 = Dark red, and 9 = Very dark red. Panelists scored each muscle region in half-point increments.

The display color scale for evaluating color stability, also rated to the nearest half-point, was: 1 = Very bright red or very bright pinkish red, 2 = Bright red or bright pinkish red, 3 = Dull red or dull pinkish red, 4 = Slightly dark red or slightly dark pinkish red, 5 = Moderately dark red or moderately dark pinkish red, 6 = Dark red to dark reddish tan or dark pinkish red to dark pinkish tan, 7 = Tannish red or tannish pink, and 8 = Tan to brown. According to our scale, panelists were instructed that a score of 5.5 indicated borderline acceptability of steaks.

The discoloration scale indicated, to the nearest whole point, the percentage of surface discoloration due to metmyoglobin formation. The scale used was: 1 = None (0%), 2 = Slight discoloration (1-19%), 3 = Small discoloration (20-39%), 4 = Modest discoloration (40-59%), 5 = Moderate discoloration (60-79%), 6 = Extensive discoloration (80-99%), and 7 = Total discoloration (100%). Panelists were told to ignore edge contamination and cut/muscle irregularities which can lead to premature browning. Daily scores from each panelist for initial color, display color, and discoloration were averaged prior to statistical analysis.

Instrumental Color

Using a calibrated HunterLab MiniScan® XE Plus Spectrophotometer (45/0 LAV, 2.54-cm diameter aperture, 10° standard observer, Illuminant A, Hunter Associates Laboratory, Inc., Reston, VA), steaks were evaluated for instrumental color on d 0 and 3. CIE L*, a*, and b* values were recorded, and used to calculate hue angle ($\tan^{-1} b^*/a^*$)

and saturation index $(a^{*2} + b^{*2})^{1/2}$. Each steak was scanned twice for the DSM and three times for the SSM, and averaged within muscle area for statistical analysis.

Design and Statistical Analysis

The experimental design was a split-plot with the whole plot experimental unit as a crossbred beef or Holstein steer to which feeding treatments were randomly assigned. The subplot experimental units were steaks to which d of retail display was randomly assigned. The repeated measures factor was muscle area (DSM and SSM), which was evaluated daily for visual measurements and on d 0 and 3 for instrumental variables. All crossbred beef and Holstein data were analyzed separately.

Using the MIXED procedure in SAS (SAS Institute, Inc., Cary, NC), subsets of least squares means were subjected to pairwise comparisons using Fisher's LSD procedure at the ($P < 0.05$) level of significance, depending on which main effects and interactions were significant. Diet, Muscle Area, and Day were the main effects tested. Interactions tested were Diet × Display Day, Diet × Muscle Area, Muscle Area × Day, and Diet × Day × Muscle Area.

Results and Discussion

Crossbred Beef Steer Diet Effects

pH

No differences ($P > 0.05$) in pH occurred due to Zilmax feeding duration (Table 3.1). Our pH values were typical of beef muscle and were not likely a factor in any color differences. Avendaño-Reyes et al. (2006) also reported no difference in pH values of *longissimus* steaks from beef steers fed Zilmax, ractopamine, or no β-agonist.

Initial Color

Table 3.1 shows the initial color score means for each dietary regimen for crossbred beef SM steaks packaged in PVC. No main effect differences ($P > 0.05$) occurred for initial color scores due to Zilmax feeding duration. Additionally, there was no significant Diet × Muscle Area interaction for initial color scores (Table 3.2).

Display Color

No Diet × Display Day interaction ($P > 0.05$) occurred for display color values (Table 3.1). The Diet × Muscle Area interaction for display color was significant (Figure 3.1). The DSM portion of steaks had no difference ($P > 0.05$) in display color scores across all Zilmax treatments; however, the SSM of 20 d steaks was brighter red ($P < 0.05$, lower display color scores) than the SSM from 40 d fed steers. For steaks from cattle fed Zilmax 0, 20, or 30 d, no differences ($P > 0.05$) in display color scores occurred between the DSM and SSM portions. Only steaks from the 40 d Zilmax group had a darker ($P < 0.05$) SSM portion in comparison to the DSM. It appears that feeding Zilmax for an extended period of time (40 d) may cause the differences in display color scores that developed between the DSM and SSM.

Discoloration

The feeding duration of Zilmax did not affect discoloration scores, as no significant differences occurred for the Diet × Display Day (Table 3.1) or Diet × Muscle Area (Table 3.2) interactions, or the main effect of diet (Table 3.1).

Instrumental Color

A Diet × Muscle Area × Display Day interaction occurred for L* values of crossbred beef steaks (Table 3.3). Dietary differences only occurred for DSM area on d 0 of display. Steaks from cattle fed for 20 d had a lighter ($P < 0.05$, higher L* value) DSM area on d 0 of retail display than cattle from the 40 d treatment; however, no dietary differences ($P > 0.05$) in L* values were seen on d 0 for the SSM. By d 3 of display, both the DSM and SSM had no differences ($P > 0.05$) in L* values due to diet regimen.

Avendaño-Reyes et al. (2006) displayed *longissimus* steaks from cattle fed Zilmax, ractopamine, or no β-agonist for 14 d and reported no treatment difference ($P > 0.05$) in L* values on d 1 or 14; however, control steaks were darker ($P < 0.05$, lower L* value) on d 5 than steaks from either β-agonist treatment. Regrettably, the Avendaño-Reyes et al. (2006) study did not look at differences due to Zilmax feeding duration.

Within all dietary groups and muscle areas, both the DSM and the SSM lightened ($P < 0.05$) from d 0 to d 3 of display. Within the diet regimens but across muscle areas, the DSM was lighter ($P < 0.05$) than the SSM on d 0 and d 3 of display. These trends agree with Sammel, Hunt, Kropf, Hachmeister, Kastner & Johnson (2002b), who reported higher ($P < 0.05$) L* values for the DSM than the SSM across all 3 d of retail display in PVC overwrap, and increased ($P < 0.05$) L* values for the SSM from d 0 to d 3. In contrast to the present study, Sammel et al. (2002b) saw decreasing ($P < 0.05$) L* values for the DSM from d 0 to 3 of display.

There was no significant Diet × Display Day interaction for redness (a* values) of crossbred beef SM steaks (Table 3.1). However, diet interacted ($P < 0.05$) with muscle area (Table 3.2), as the SSM was redder ($P < 0.05$, higher a* values) than the DSM, regardless of dietary regimen. In several studies, steaks in retail display (from d 1 to 5) also had a redder ($P < 0.05$) SSM than DSM portion (Sammel et al., 2002b; Seyfert, Mancini, Hunt, Tang, Faustman & Garcia 2006). The DSM portion of steaks from crossbred beef steers fed Zilmax for 40 d were redder ($P < 0.05$) than the DSM portion of steaks from all other dietary treatments (Table 3.2). No dietary differences ($P > 0.05$) in a* values occurred for the SSM portion. In contrast, Avendaño-Reyes et al. (2006) saw a significant difference due to diet treatment, where the Zilmax group had lower a* values than the control cattle; although, no differences in feeding duration were tested in their study.

A Diet × Display Day interaction occurred for b*, hue angle, and saturation index values (Table 3.1). On d 0 of display, 40 d steaks were more yellow ($P < 0.05$, higher b* values) and more vivid ($P < 0.05$, higher saturation index values) compared to all other dietary treatments. These dietary differences were not noted ($P > 0.05$) for either variable by d 3 of display. While no diet differences were noted for hue angle values on d 0 of display, steaks from cattle fed 40 d had lower ($P < 0.05$) hue angle values (indicative of less discoloration) on d 3 of display than steaks from 20 or 30 d fed cattle. Overall, steaks on d 0 of display were more yellow, more vivid (higher saturation indices), and had higher hue angle values ($P < 0.05$) than d 3 steaks. Our saturation index values indicated a shift to a less saturated color over time for the crossbred beef SM. Decreased ($P < 0.05$) saturation index values from d 0 to 3 reflect the loss ($P < 0.05$) in redness (lower a* values) and yellowness (lower b* values) of steaks with increasing d of display. The large decrease in saturation index values coincides with the color change from bright to dark red as seen by display color panelist scores.

Seyfert et al. (2006) and Sawyer, Baublits, Apple, Meullenet, Johnson & Alpers (2007) also reported a loss ($P < 0.05$) in yellowness and vividness (saturation index) from d 0 to d 3 of display. Sammel, Hunt, Kropf, Hachmeister, & Johnson (2002a) also had lower ($P < 0.05$) saturation indices on d 3 than d 0 of display, but increased ($P < 0.05$) d 3 hue angle values when compared to d 0. While the difference in hue angle values across d of display was significant, it is not of much practical importance.

Saturation indices and b* values were not significantly different for the Diet × Muscle Area interaction (Table 3.2). Cattle from the 0 and 30 d Zilmax regimens had higher ($P < 0.05$) hue angle values for the DSM than steaks from the 40 d treatment. No differences ($P > 0.05$) were noted between diet groups for the SSM. With exception of the 40 d group, the DSM from all other diet treatments was less red and more discolored ($P < 0.05$, higher hue angle values) than the SSM. This supports our display color results, in which the DSM was more discolored at the end of display according to panelists than the SSM.

Crossbred Beef Steer Muscle Area Effects

pH

DSM pH values were slightly higher ($P < 0.05$) than those from the SSM (Table 3.4). Although significant, the difference in pH value between the DSM and SSM is not large enough to have an impact on muscle functionality. Lee, Yancey, Apple, Sawyer & Baublits (2008) also found higher ($P < 0.05$) pH values for the Cranial-Dorsal quadrant (corresponding to the DSM). In contrast to our study, Seyfert et al. (2006) found no differences ($P > 0.05$) in pH values of crossbred beef DSM and SSM. Confounding results suggest that the postmortem environment the SM is exposed to before the onset of ultimate pH can play an important role in pH differences between the DSM and SSM (Seyfert et al., 2006).

Initial Color

The main effect of muscle area was significant, as the DSM was a lighter cherry red (lower initial color score) than the SSM (Table 3.4). In agreement with our findings, both Sammel et al. (2002b) and Seyfert et al. (2006) reported a lighter red ($P < 0.05$) initial color for the DSM than the SSM. The more pale color typical of the DSM (in comparison to the SSM) can be attributed to high temperatures early on postmortem found within the DSM. Seyfert et al. (2006) noted that early postmortem, the DSM had a low oxygen consumption rate allowing for a bright red initial color to develop upon exposure to oxygen.

Display Color

For both the DSM and SSM, display color scores increased ($P < 0.05$, darker red) daily (Figure 3.2). Both portions of the crossbred beef steaks were borderline acceptability (display color score = 5.5) on d 2, with the DSM having an unacceptable display color score on d 3. On d 0 and 1 of retail display, the DSM was brighter ($P < 0.05$, lower display color score) than the SSM; however, by d 3 of display, the DSM was darker red ($P < 0.05$, higher display color score) than the SSM.

These results suggest that while the DSM is lighter in color at the beginning of simulated retail display, it is less color stable and becomes darker more quickly during display than the SSM. Our data agree with the findings of Sammel et al. (2002b), who also reported significantly lower display color scores for the DSM than the SSM on d 0 of display, but higher DSM display color scores than the SSM on d 2 through 5 of display. Sammel et al. (2002b) described the DSM of beef as similar to the pale, soft, and exudative (PSE) condition in pork. Like PSE pork, the beef DSM experiences high postmortem temperatures that may result in rapid pH declines and excess protein denaturation that negatively impacts meat color stability.

Discoloration

For Muscle Area × Display Day, Figure 3.3 shows that DSM discoloration scores increased ($P < 0.05$) daily through d 3 of display. No differences ($P > 0.05$) occurred in SSM discoloration scores on d 0 and 1, but the SSM portion of steaks discolored ($P < 0.05$) further on both d 2 and 3 of display. Overall, the DSM of crossbred beef steaks discolored at a faster rate as evidenced by higher (*P* < 0.05) discoloration scores than the SSM on d 1, 2, and 3 of simulated retail display. By d 3 of display, the DSM was discolored close to 40% in comparison to almost 20% for the SSM portion.

McKenna, Mies, Baird, Pfeiffer, Ellebracht & Savell (2005) grouped the SM into a category of intermediate color stability based upon a $(K/S)_{572}/(K/S)_{525}$ value (indicative of a metmyoglobin increase) less than 1.20 after 5 d of retail display. This agrees with our results, as both muscle areas of steaks were only about 40% discolored by d 3 of display. Sammel et al. (2002a) found a higher percentage of metmyoglobin (as well as less oxymyoglobin) for the DSM portion on d 2 through 5 of retail display in comparison to the SSM. Our visual discoloration scores agree with their findings, as the DSM was more discolored (*P* < 0.05) than the SSM on d 2 and 3 of retail display.

Instrumental Color

Both areas (DSM and SSM) were less red (*P* < 0.05, lower a* value) on d 3 than d 0 of retail display (Table 3.4). The DSM was also consistently less red (*P* < 0.05) than the SSM on both d of display. Sawyer et al. (2007) reported no difference (*P* > 0.05) in a* values for the DSM and SSM portions on d 0 or d 3, although an overall loss of redness (*P* < 0.05) from d 0 to d 3 occurred for both portions. Similarly, Seyfert et al. (2006) had no difference (*P* > 0.05) in d 0 DSM and SSM a* values. In contrast with our results, Sammel et al. (2002b) reported a redder (*P* < 0.05) DSM than the SSM on d 0. This difference was reversed by d 3, where the SSM was redder (*P* < 0.05) than the DSM in their study. Confounding results from multiple studies support the idea that differences in early postmortem environments can alter SM muscle color and color stability in both portions.

No Muscle Area × Display Day interaction occurred for yellowness (b* values) or vividness (saturation indices) (Table 3.4). Muscle area main effect differences were significant, as the SSM was more (*P* < 0.05) yellow and more (*P* < 0.05) vivid in color than its DSM counterpart (data not shown).

Both muscle areas had lower (*P* < 0.05) hue angle values on d 3 than d 0 of retail display (Table 3.4). This agrees with our Diet × Display Day hue angle values for all the Zilmax treatments. On d 0 the DSM had a lower (*P* < 0.05) mean hue angle; however, by d 3 the SSM had a lower ($P < 0.05$) hue angle. Sammel et al. (2002b) also reported a lower ($P < 0.05$) DSM hue angle value than the SSM on d 0, but contrasting to our results, the DSM also had a lower hue angle than the SSM on d 3 of their study. Based upon our hue angle results, it appears that the color change involved equal shifts in both a* and b* values that did not reflect a large movement toward brown, just an overall color deterioration.

Holstein Steer Diet Effects

pH

A significant difference in pH due to the main effect of diet occurred (Table 3.5). Steaks from Holsteins fed Zilmax for 40 d had higher ($P < 0.05$) pH values than steaks from the 20 and 30 d dietary treatments. Our pH values are comparable to those of Moloney, Allen, Joseph, Tarrant & Convey (1994) who reported pH values between 5.47 and 5.51 for Friesian steers fed various levels of the β-agonist L-644,969. No differences ($P > 0.05$) occurred in pH values of loin (5.41) and sirloin (5.43) from Holstein and beef steers (Faustman & Cassens, 1991). Beef steers fed zilpaterol had an average pH of 5.43 compared to 5.44 for cattle fed no β-agonist (Avendaño-Reyes et al., 2006). Nonetheless, pH value differences in the present study were small and not of practical consequence.

Initial Color

Zilmax feeding duration had no significant effect on initial color scores of Holstein SM steaks (Table 3.5). Diet regimen did not interact ($P > 0.05$) with muscle area to affect initial color scores (Table 3.6). These results agree with our findings for crossbred beef steer initial color scores due to diet.

Display Color

As expected, display color scores increased ($P < 0.05$) daily through the end of display (Figure 3.4). The only difference in Holstein display color scores due to Zilmax diet occurred on d 1 of display. Holsteins fed Zilmax for 40 d produced moderately darker red (*P* < 0.05, higher display scores) steaks on d 1 than their 30 d counterparts. Through the end of display, no differences (*P* > 0.05) in display color scores among all dietary regimens occurred. There was no significant Diet × Muscle Area interaction for any of the feeding durations (Table 3.5).

Discoloration

Discoloration scores were similar (*P* > 0.05) across dietary regimen, with the exception of d 2 (Figure 3.5). Steaks from 20 and 30 d fed cattle had less (*P* < 0.05) discoloration on d 2 of display than 40 d fed Holsteins. In contrast to our visual findings, Strydom et al. (2000) reported that *longissimus lumborum* and *gluteus medius* steaks from crossbred beef steers fed Zilmax 50 d tended to have less metmyoglobin formation than 0 or 30 d supplemented cattle. In the present study, steaks from the 40 d Zilmax treatment tended (*P* > 0.05) to have higher display color and discoloration scores on d 1 and 3 of display. By the end of display, all steaks reached modest (40-59%) to moderate (60-79%) discoloration levels.

Instrumental Color

No Diet × Muscle Area interaction occurred for any of the instrumental variables measured (Table 3.6). While no significant interaction occurred due to Zilmax feeding duration and d of display for lightness (Table 3.5), a trend (*P* = 0.0505) was seen for L* values as steaks from all dietary regimens tended to become darker from d 0 to 3 of display. Strydom, Frylinck, & Marais (2007) also reported no difference (*P* > 0.05) in lightness (L* values) of *longissimus lumborum* steaks from bulls fed zilpaterol or no β-agonist.

Steaks from all dietary regimens were a more vivid red (*P* < 0.05, greater a* and saturation indices) color on d 0 than d 3 of retail display (Table 3.5). No differences (*P* > 0.05) in redness or saturation index values due to Zilmax feeding duration were noted for steaks on d 0 or 3. Contrastingly, Strydom et al. (2007) noted higher (*P* < 0.05) chroma (saturation index) values for *longissimus* steaks from zilpaterol supplemented cattle when compared to control steaks.

On d 0 of display, steaks from the 40 d Zilmax group were more yellow (*P* < 0.05, higher b* value) than steaks from 0 or 20 d fed Holsteins. There were no differences (*P* > 0.05) in b* values due to dietary treatment by d 3 of display. Steaks from all diet regimens were less yellow (*P* < 0.05, lower b* values) on d 3 than at the beginning of display (d 0).

Moloney et al. (1994) had increased (*P* < 0.05) hue angle values after 2 d of display for longissimus steaks from Friesian steers fed L-644,969. Although the Diet × Display Day interaction was not significant for hue angle values in our study, we also had numerically higher hue angle values for all diet regimens after d 3 of display (Table 3.5).

Holstein Steer Muscle Area Effects

pH

No differences (*P* > 0.05) in pH were noted for the DSM and SSM portions (Table 3.7). In beef cattle, Seyfert et al. (2006) reported no differences (*P* > 0.05) in pH values between the DSM and SSM; however, Lee et al. (2008) had higher (*P* < 0.05) pH values for the DSM than the SSM.

Initial Color

Muscle area main effect differences occurred (Table 3.7), as the DSM portion was a lighter cherry red color (*P* < 0.05, lower initial color score) than the SSM. Sammel et al. (2002b) also noted lower (*P* < 0.05) DSM initial color scores in comparison to the SSM.

Display Color

While the DSM was brighter red (*P* < 0.05, lower display scores) than the SSM on d 0, the DSM was darker red (*P* < 0.05, higher display scores) than the SSM on all other d of display (Figure 3.6). For Holstein steaks, both muscle areas (DSM and SSM) reached unacceptable visual color scores (greater than 5.5) by d 2 of simulated retail display. The same results occurred for both muscle areas of crossbred beef steer steaks.

Seyfert et al. (2006) also reported lower (*P* < 0.05) DSM display color scores than the SSM on d 1, but after d 3, the DSM was a darker red (*P* < 0.05, higher display scores) than the SSM through the end of display. The accelerated darkening of the DSM of is consistent with other available literature.

Discoloration

The rate at which DSM display color scores increased may be due in part to the faster rate at which metmyoglobin accumulated for that muscle portion (Figure 3.7). Although no differences ($P > 0.05$) occurred in DSM and SSM discoloration scores on d 0 of display, the DSM was more ($P < 0.05$) discolored than the SSM on d 1 through 3 of retail display. Both muscle areas had higher ($P < 0.05$) discoloration scores daily through the end of display. By d 3, the DSM was over 60 to 79% discolored, whereas the SSM was only modestly discolored (40 – 59%).

In comparison to our crossbred beef steak discoloration scores (between 2.0 and 4.0 on d 3), our Holstein steaks tended to have higher discoloration scores (between 4.0 and 6.0 on d 3) at the end of display. Faustman & Cassens (1991) found a higher ($P < 0.05$) percentage of metmyoglobin formation for Holstein *longissimus* and *gluteus medius* steaks than crossbred beef steer steaks daily through 6 d of display.

Instrumental Color

Table 3.7 shows the mean values for Muscle Area × Day interactions for all instrumental variables. Overall, both portions (DSM and SSM) of Holstein steaks became darker, less red and yellow, more discolored (higher hue angles), and less vivid (lower saturation indices) in color from d 0 to d 3 of display ($P < 0.05$). Moloney et al. (1994) reported a trend ($P = 0.06$) toward darker (lower L* values) *longissimus* steaks after 3 d of display. The DSM was lighter ($P < 0.05$, higher L* value) than the SSM on d 0 and 3 of display (Table 3.7). Lee et al. (2008) similarly had higher ($P < 0.05$) L* values for the CrD quadrant (corresponding to the DSM) than the SSM of crossbred beef steaks. No differences ($P > 0.05$) were observed in a* or b* values between the DSM and SSM on d 0; however, by d 3 the SSM was redder ($P < 0.05$, higher a* value) and more yellow ($P < 0.05$, higher b* value) than the DSM.

Our results agree with those of Sammel et al. (2002b), who reported lower ($P < 0.05$) a* and b* values for the DSM than the SSM on d 3 of display. While we had no differences ($P > 0.05$) in a* or b* values between both portions on d 0, they reported greater ($P < 0.05$) redness and yellowness for the DSM than the SSM on the first d of display.

The DSM and SSM had similar ($P > 0.05$) hue angle values on d 0 of display. The DSM had higher ($P < 0.05$) hue angle values than the SSM on d 3 of retail display, confirming the loss in redness (lower a* value) of the DSM throughout display. Saturation indices, while similar ($P > 0.05$) initially during display, were lower ($P < 0.05$, loss of vividness) for the DSM than the SSM on d 3 of display.

Summary

There were no practical differences in pH values of crossbred beef or Holstein SM steaks due to Zilmax feeding duration or muscle area. Only a few significant differences occurred in display and instrumental color of steaks from crossbred beef and Holstein steers fed Zilmax. Steaks from crossbred beef and Holstein steers fed Zilmax for 40 d were redder (higher a* values) and more yellow (higher b* values) initially, but color panelists evaluated 40 d steaks as darker and more discolored in later d of display. Our data indicate that feeding Zilmax to crossbred beef or Holstein steers for 20 or 30 d will have an equal or slight advantage in display color stability of steaks packaged in a traditional PVC overwrap system in comparison to steaks from non-supplemented cattle. Feeding Zilmax for 40 d causes minor detrimental effects on display color and discoloration scores.

More notable than Zilmax diet differences was the variation in color development and stability of the SM muscle areas. While the DSM had better initial and display color scores at the beginning of simulated display, it became dark and discolored rapidly after only 1 to 2 d.

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Table 3.1 Diet, Day, and Diet × Display Day means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in PVC

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.46	5.46	5.44	5.46	**------**
Initial color [b]	4.8	4.4	4.6	4.7	**------**
Display color [c]	**4.5**	**4.3**	**4.3**	**4.5**	
d 0	2.8	2.6	2.6	2.8	**2.7 [h]**
d 1	3.9	3.5	3.7	3.9	**3.7 [g]**
d 2	5.5	5.3	5.4	5.7	**5.5 [f]**
d 3	5.9	5.6	5.7	5.9	**5.8 [e]**
Discoloration [d]	**2.0**	**1.9**	**1.9**	**1.9**	
d 0	1.0	1.0	1.0	1.0	**1.0 [h]**
d 1	1.3	1.2	1.3	1.2	**1.2 [g]**
d 2	2.1	2.2	2.2	2.2	**2.2 [f]**
d 3	3.4	3.3	3.0	3.2	**3.2 [e]**
a*	**32.2 [y]**	**32.3 [y]**	**32.2 [y]**	**33.5 [z]**	
d 0	40.6	40.5	40.4	42.3	**41.0 [e]**
d 3	23.9	24.1	24.0	24.6	**24.2 [f]**
b*	**------**	**------**	**------**	**------**	
d 0	40.0 [ey]	39.7 [ey]	40.2 [ey]	42.1 [ez]	**------**
d 3	21.7 [fz]	22.0 [fz]	22.1 [fz]	21.9 [fz]	**------**
Hue angle	**------**	**------**	**------**	**-------**	
d 0	44.5 [ez]	44.4 [ez]	44.8 [ez]	44.8 [ez]	**------**
d 3	42.4 [fyz]	42.5 [fz]	42.7 [fz]	41.7 [fy]	**------**
Saturation index	**------**	**------**	**------**	**------**	
d 0	56.9 [ey]	56.7 [ey]	57.0 [ey]	59.7 [ez]	**------**
d 3	32.3 [fz]	32.6 [fz]	32.7 [fz]	33.0 [fz]	**------**

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[a] SE: Initial = 0.14, Display = 0.16, Discoloration = 0.11, a* = 0.43, b* = 0.42, Hue angle = 0.25, Saturation index = 0.58.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[c] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.
[d] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.
[efgh] Means across day within diet (within variable) with no letter do not differ ($P > 0.05$); means across day within diet (within variable) without a common letter differ ($P < 0.05$).
[yz] Means across diet within day (within variable) with no letter do not differ ($P > 0.05$); means across diet within day (within variable) without a common letter differ ($P < 0.05$).

Table 3.2 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in PVC

	Diet [1], day			
Variable	0	20	30	40
Initial color [b]				
DSM	4.5	4.1	4.2	4.4
SSM	5.1	4.7	4.9	5.0
Discoloration [c]				
DSM	2.2	2.2	2.1	2.1
SSM	1.7	1.7	1.6	1.7
a*				
DSM	30.0 [ey]	30.7 [ey]	30.9 [ey]	32.4 [ez]
SSM	34.5 [fz]	33.9 [fz]	33.6 [fz]	34.6 [fz]
b*				
DSM	29.3	29.6	30.1	31.0
SSM	32.3	32.0	32.2	33.0
Hue angle				
DSM	44.4 [ez]	43.9 [eyz]	44.2 [ez]	43.5 [ey]
SSM	42.6 [fz]	43.0 [fz]	43.3 [fz]	43.1 [ez]
Saturation index				
DSM	41.9	42.7	43.1	44.8
SSM	47.3	46.6	46.5	47.8

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: Initial = 0.15, Discoloration = 0.10, L* = 0.89, a* = 0.43, b* = 0.43, Hue angle = 0.25, Saturation index = 0.58.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red, 6 = Slightly dark red.

[c] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[ef] Means across muscle area within diet (within variable) with no letter do not differ ($P > 0.05$); means across muscle area within diet (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across diet within muscle area (within variable) with no letter do not differ ($P > 0.05$); means across diet within muscle area (within variable) without a common letter differ ($P < 0.05$).

Table 3.3 Diet × Muscle Area × Display Day means[a] for L* values of Zilmax[b] fed-crossbred beef steer steaks packaged in PVC

	Muscle Area [c]			
	DSM		SSM	
Diet, day	d 0 [d]	d 3	d 0	d 3
0	40.6 [epyz]	43.0 [fpz]	35.3 [eqz]	40.1 [fqz]
20	41.5 [epz]	42.8 [fqz]	36.5 [eqz]	41.3 [fqz]
30	40.1 [epyz]	44.3 [fpz]	36.4 [eqz]	40.9 [fqz]
40	38.7 [epy]	42.3 [fpz]	34.8 [eqz]	39.8 [fqz]

[a] SE: DSM = 0.89, SSM = 0.89.
[b] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[c] DSM = deep SM, SSM = superficial SM.
[d] Day of simulated retail display.
[ef] Means within diet across day (within muscle area) without a common letter differ (*P* < 0.05).
[pq] Means within diet across muscle area (within day) without a common letter differ (*P* < 0.05).
[yz] Means within day across diet (within muscle area) without a common letter differ (*P* < 0.05).

Table 3.4 Muscle Area and Muscle Area × Display Day means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in PVC

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.47 [y]	5.45 [z]
Initial color [b]	------	------	4.3 [y]	4.9 [z]
a*			------	------
d 0	40.0 [ey]	42.0 [ez]		
d 3	22.0 [fy]	26.3 [fz]		
b*			30.0 [y]	32.4 [z]
d 0	39.2	41.8		
d 3	20.9	22.9		
Hue angle			------	------
d 0	44.4 [ey]	44.9 [ez]		
d 3	43.6 [fy]	41.1 [fz]		
Saturation index			43.2 [y]	47.1 [z]
d 0	55.9	59.2		
d 3	30.4	34.9		

[1] DSM = deep SM, SSM = superficial SM.
[a] SE: Initial = 0.15, a* = 0.43, b* = 0.43, Hue angle = 0.25, Saturation index = 0.58.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[ef] Means across day within muscle area (within variable) with no letter do not differ (*P* > 0.05); means across day within muscle area (within variable) without a common letter differ (*P* < 0.05).
[yz] Means across muscle area within day (within variable) with no letter do not differ (*P* > 0.05); means across muscle area within day (within variable) without a common letter differ (*P* < 0.05).

Table 3.5 Diet, Day, and Diet × Display Day means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in PVC

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.46 [yz]	5.44 [y]	5.43 [y]	5.48 [z]	------
Initial color [b]	4.6	4.5	4.8	4.6	------
L*	**42.1**	**42.6**	**41.5**	**40.9**	
d 0	42.9	43.0	42.1	41.7	**42.4 [e]**
d 3	41.2	42.2	41.0	40.2	**41.2 [f]**
a*	------	------	------	------	
d 0	29.5 [ez]	30.0 [ez]	29.8 [ez]	30.2 [ez]	------
d 3	18.3 [fz]	17.4 [fz]	18.5 [fz]	17.2 [fz]	------
b*	------	------	------	------	
d 0	23.8 [ey]	24.0 [ey]	24.5 [eyz]	25.3 [ez]	------
d 3	18.1 [fz]	17.2 [fz]	17.8 [fz]	18.3 [fz]	------
Hue angle	**41.9**	**42.0**	**41.9**	**43.7**	
d 0	38.8	38.7	39.3	39.9	**39.2 [f]**
d 3	45.0	45.3	44.6	47.4	**45.6 [e]**
Saturation index	------	------	------	-----	
d 0	37.9 [ez]	38.5 [ez]	38.6 [ez]	39.3 [ez]	------
d 3	25.8 [fz]	24.5 [fz]	25.8 [fz]	25.2 [fz]	------

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[a] SE: pH = 0.01, Initial = 0.15, L* = 0.62, a* = 0.73, b* = 0.46, Hue angle = 0.68, Saturation index = 0.81.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[ef] Means across day within diet (within variable) without a letter do not differ (*P* > 0.05); means across day within diet (within variable) without a common letter differ (*P* < 0.05).
[yz] Means across diet within day (within variable) without a letter do not differ (*P* > 0.05); means across diet within day (within variable) without a common letter differ (*P* < 0.05).

Table 3.6 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in PVC

	Diet [1], day			
Variable	0	20	30	40
Initial color [b]				
DSM	4.5	4.3	4.5	4.4
SSM	4.7	4.6	4.8	4.7
Display color [c]				
DSM	5.2	5.0	5.0	5.3
SSM	4.9	4.7	4.7	5.0
Discoloration [d]				
DSM	3.2	3.1	3.0	3.3
SSM	2.6	2.4	2.6	2.9
L*				
DSM	43.5	44.3	42.8	42.6
SSM	40.6	41.0	40.3	39.3
a*				
DSM	22.7	22.6	23.1	22.4
SSM	25.2	24.8	25.2	24.9
b*				
DSM	20.4	20.5	20.9	21.4
SSM	21.5	20.7	21.4	22.2
Hue angle				
DSM	42.8	43.6	43.1	45.2
SSM	41.0	40.4	40.8	42.1
Saturation index				
DSM	30.6	30.7	31.2	31.2
SSM	33.1	32.3	33.1	33.4

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[a] SE: Initial = 0.16, Display = 0.17, Discoloration = 0.15, L* = 0.67, a* = 0.73, b* = 0.48, Hue angle = 0.68, Saturation index = 0.81.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[c] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.
[d] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

Table 3.7 Muscle Area and Muscle Area × Display Day means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in PVC

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.45	5.45
Initial color [b]	------	------	4.4 [y]	4.7 [z]
L*				
d 0	44.3 [ey]	40.6 [ez]	------	------
d 3	42.3 [fy]	40.0 [fz]	------	------
a*				
d 0	29.7 [ez]	30.1 [ez]	------	------
d 3	15.8 [fy]	20.0 [fz]	------	------
b*				
d 0	24.3 [ez]	24.5 [ez]	------	------
d 3	17.3 [fy]	18.4 [fz]	------	------
Hue angle				
d 0	39.2 [ez]	39.2 [ez]	------	------
d 3	48.2 [fy]	43.0 [fz]	------	------
Saturation index				
d 0	38.4 [ez]	38.8 [ez]	------	------
d 3	23.5 [fy]	27.2 [fz]	------	------

[1] DSM = deep SM, SSM = superficial SM.

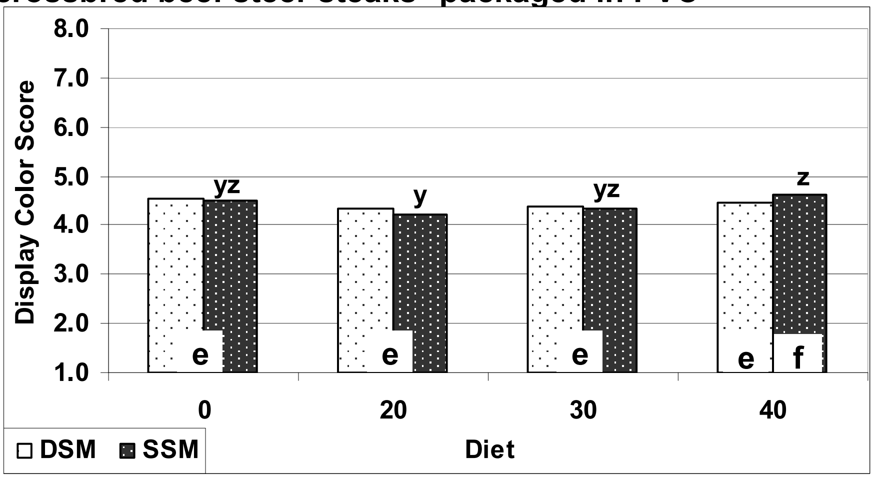
[a] SE: pH = 0.01, Initial = 0.07, L* = 0.32, a* = 0.38, b* = 0.24, Hue angle = 0.39, Saturation index = 0.42.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[ef] Means across day within muscle area (within variable) without a letter do not differ ($P > 0.05$); means within muscle area across day (within variable) without a common letter differ ($P < 0.05$).

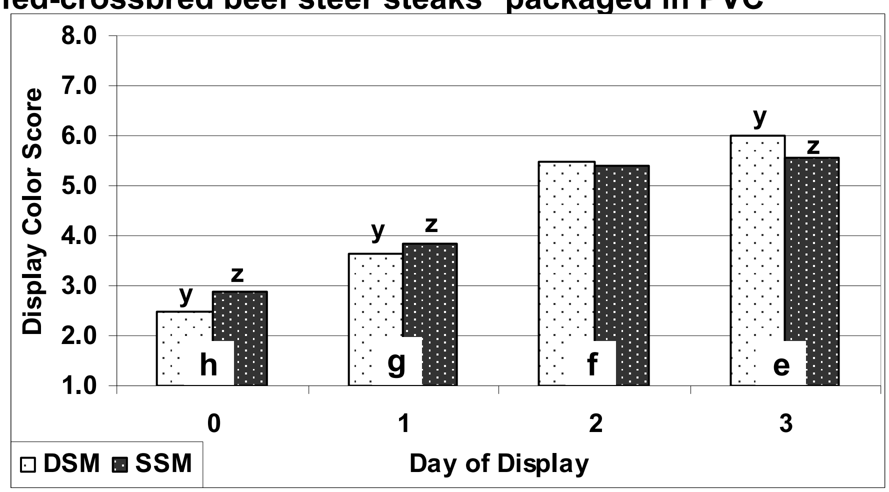
[yz] Means across muscle area within day (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within day (within variable) without a common letter differ ($P < 0.05$).

Figure 3.1 Diet × Muscle Area means[a] for display color scores[b] of Zilmax[c] fed-crossbred beef steer steaks[d] packaged in PVC



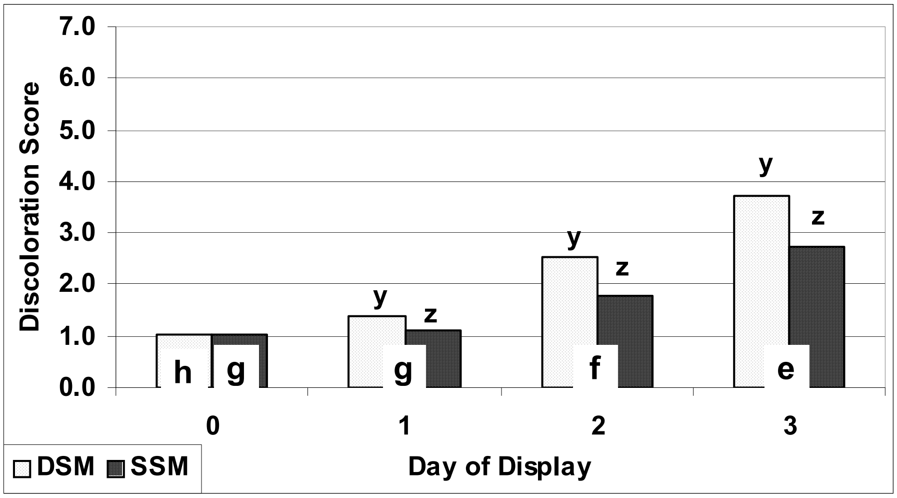
[a] SE: DSM = 0.15, SSM = 0.15.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[ef] Bars across muscle area within diet without a common letter differ ($P < 0.05$).
[yz] Bars across diet within muscle area with no letter do not differ ($P > 0.05$); bars across diet within muscle area without a common letter differ ($P < 0.05$).

Figure 3.2 Muscle Area × Display Day means[a] for display color scores[b] of Zilmax[c] fed-crossbred beef steer steaks[d] packaged in PVC



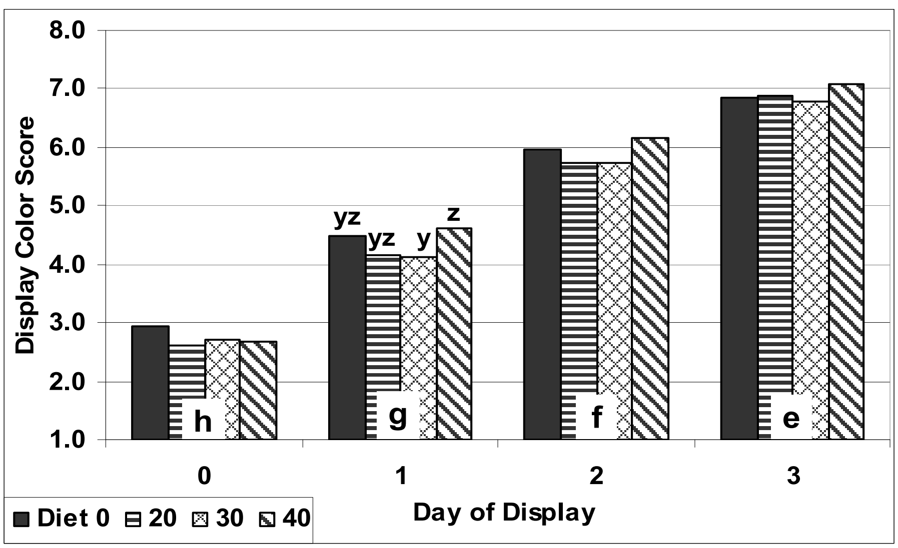
[a] SE: DSM = 0.09, SSM = 0.09.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red, 7 = Tannish red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[efgh] Bars across day within muscle area without a common letter differ ($P < 0.05$).
[yz] Bars across muscle area within day with no letter do not differ ($P > 0.05$); bars across muscle area within day without a common letter differ ($P < 0.05$).

Figure 3.3 Muscle Area × Display Day means[a] for discoloration scores[b] of Zilmax fed-crossbred beef steer steaks[c] packaged in PVC



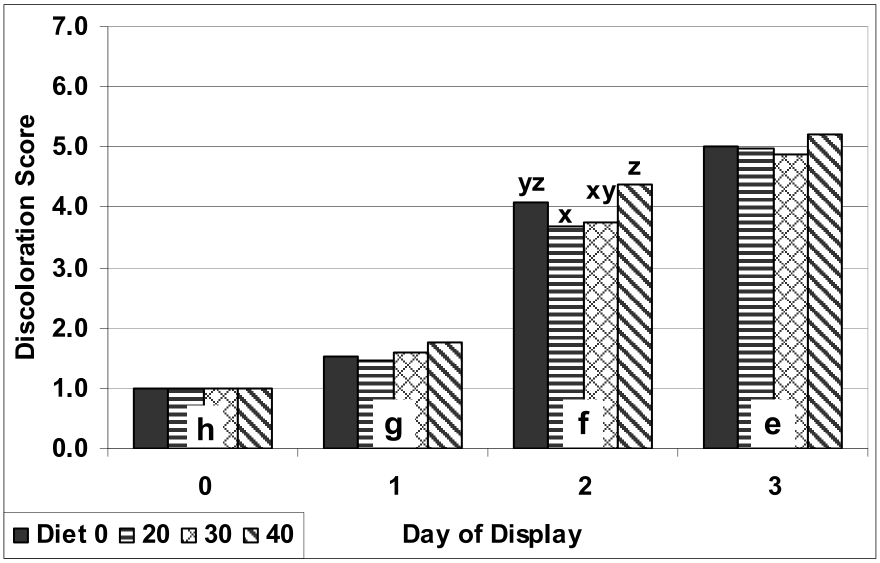
[a] SE: DSM = 0.06, SSM = 0.06.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.
[c] DSM = deep SM, SSM = superficial SM.
[efgh] Bars across day within muscle area without a common letter differ ($P < 0.05$).
[yz] Bars across muscle area within day with no letter do not differ ($P > 0.05$); bars across muscle area within day without a common letter differ ($P < 0.05$).

Figure 3.4 Diet × Display Day means[a] for display color scores[b] of Zilmax[c] fed-Holstein steer steaks packaged in PVC



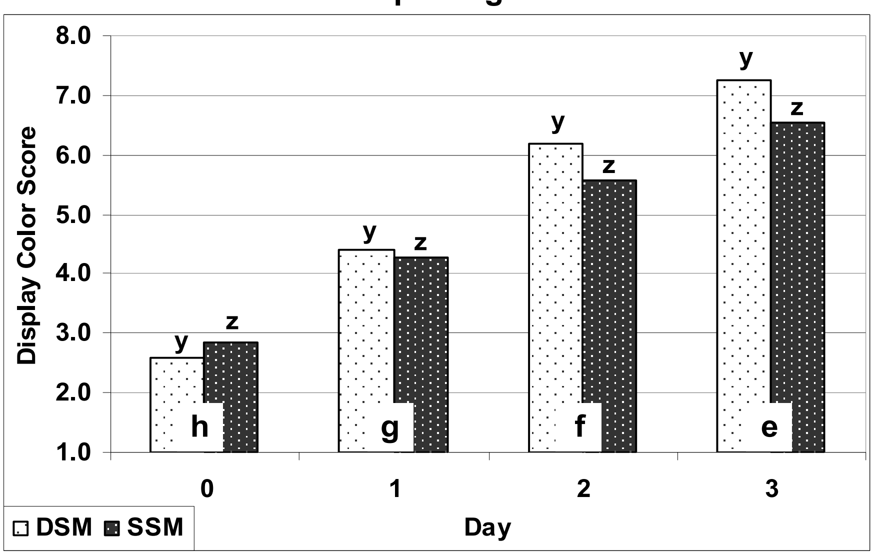
[a] SE: Diet = 0.18.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red, 7 = Tannish red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efgh] Bars across display day within diet without a common letter differ ($P < 0.05$).
[yz] Bars across diet within day with no letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 3.5 Diet × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-Holstein steer steaks packaged in PVC



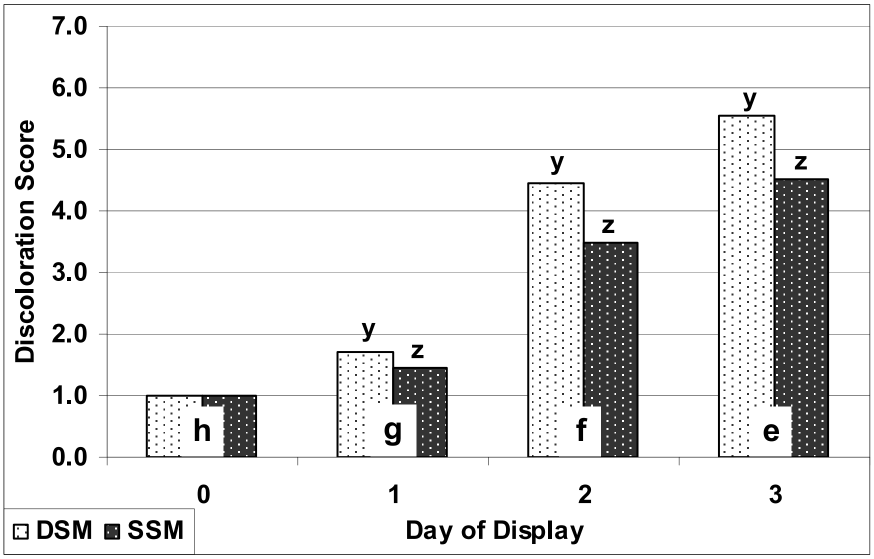
[a] SE: Diet = 0.16.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%, 5 = 60-79%, 6 = 80-99%.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efgh] Bars across display day within diet without a common letter differ ($P < 0.05$).
[xyz] Bars across diet within day with no letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 3.6 Muscle Area × Display Day means[a] for display color scores[b] of Zilmax fed-Holstein steer steaks[c] packaged in PVC



[a] SE: DSM = 0.09, SSM = 0.09.
[b] Display color scale: 1 = Very bright red, 3=Dull red, 5 = Moderately dark red, 7=Tannish red.
[c] DSM = deep SM, SSM = superficial SM.
[efgh] Bars across display day within muscle area without a common letter differ ($P < 0.05$).
[yz] Bars across muscle area within display day without a common letter differ ($P < 0.05$).

Figure 3.7 Muscle Area × Display Day means[a] for discoloration scores[b] Zilmax[c] fed-Holstein steer steaks[d] packaged in PVC



[a] SE: DSM = 0.09, SSM = 0.09.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%, 5 = 60-79%, 6 = 80-99%.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[efgh] Bars across display day within muscle area without a common letter differ ($P < 0.05$).
[yz] Bars across muscle area within display day with no letter do not differ ($P > 0.05$); bars across muscle area within day without a common letter differ ($P < 0.05$).

CHAPTER 4 - The Effects of Zilpaterol Hydrochloride Feeding Duration on Crossbred Beef and Holstein Steer Enhanced Semimembranosus Steak Color when Packaged in High or Low-Oxygen MAP

Abstract

The objective of this research was to determine the effects of zilpaterol hydrochloride (Zilmax®) feeding duration (7.56 g/ton for 0, 20, 30, or 40 d) on color development and stability of *semimembranosus* (SM) steaks from 60 crossbred beef (B) and 60 Holstein (H) carcasses. SM subprimals (n = 120 total, 30 from each Zilmax duration) were enhanced, cut into five, 2.54-cm thick steaks, packaged in 80% O_2/20% CO_2 (HiOx) or 69.6% N_2/30% CO_2/0.4% CO (LoOx) MAP, and assigned to 0, 3, or 5 d (HiOx) or 0 or 9 d (LoOx) of display. Panelists evaluated the deep (DSM) and superficial (SSM) portions of steaks for initial color, display color, and discoloration; pH, L*, a*, b*, hue angle, and saturation indices were measured. For steaks in HiOx, the DSM of 20 and 30 d B steaks on d 4 and the DSM of 20 d B steaks on d 5 was brighter ($P < 0.05$) red than 40 d Zilmax B DSM. On d 1 and 5, the SSM of 20 d HiOx B steaks was brighter ($P < 0.05$) red than 40 d B SSM. The SSM of 40 d HiOx B steaks was darker ($P < 0.05$) red on d 3 than the SSM from all other dietary regimens. On d 5, HiOx 20 d Zilmax H steaks were darker ($P < 0.05$) than H steaks from all other feeding durations. HiOx 20 d H steaks were more discolored ($P < 0.05$) on d 3 – 5 than all other diet treatments. For steaks in LoOx, 30 d B steaks were brighter ($P < 0.05$) red than 0 or 40 d B steaks on d 0 and 9. LoOx 20 d B steaks were brighter ($P < 0.05$) red than 40 d steaks on d 3. DSM and SSM from 40 d LoOx H steaks tended ($P > 0.05$) to have improved display color compared to all other dietary regimens. B and H steer steaks in LoOx MAP from all feeding durations were less than 20% discolored through d 9. The DSM was lighter ($P < 0.05$) than the SSM on d 0 and 9 for B steaks packaged in HiOx and LoOx and H steaks in LoOx, respectively. In summary, feeding Zilmax to B steers for 20 or 30 d yields brighter red, less discolored steaks than other treatments during display in HiOx or

LoOx. H steaks in LoOx MAP have improved color qualities over control steaks when fed Zilmax for any duration, whereas H steaks in HiOx have less color stability when fed 20 d of Zilmax in comparison to all other treatments.

Key Words: zilpaterol, beta agonist, display color, modified atmosphere packaging, crossbred beef, Holstein

Introduction

With the change in location of meat fabrication from the retail sector to more centralized processing, the use of modified atmosphere packaging (MAP) has become more prevalent (Kropf, 2004). One of the main added benefits of MAP is the extended color life seen for case-ready packages (Eilert, 2005; Jayasingh, Cornforth, Carpenter, & Whittier, 2001; Seyfert, Hunt, Mancini, Hachmeister, Kropf, & Unruh, 2004; Sørheim, Nissen, & Nesbakken, 1999).

Zilpaterol fed 30 or 50 d resulted in a longer display color life for beef steaks (Strydom, Buys & Strydom, 2000). Another study (Avendaño-Reyes, Torres-Rodríguez, Meraz-Murillo & Pérez-Linares, 2006) revealed similar L* values for cattle fed zilpaterol or no β-agonist on d 1 and 14; however, d 5 control steaks were ($P < 0.05$) darker than steaks from the zilpaterol group. Few studies exist analyzing the effects of feeding zilpaterol on color development and stability of muscles from supplemented cattle.

High oxygen (HiOx) MAP has an oxygen concentration four times higher than the atmosphere which results in the delayed formation of metmyoglobin in comparison to atmospheric concentrations (Jeyamkondan, Jayas, & Holley, 2000). By delaying metmyoglobin development, the extended color life typical of steaks in atmospheric systems can be achieved. Inclusion of carbon dioxide in the gas blend aids in retarding microbial growth while extending shelf life (Kropf, 2004). One drawback to HiOx MAP can be the hastened oxidative rancidity of lipids (Jeyamkondan et al., 2000; John, Cornforth, Carpenter, Sørheim, Pettee, & Whittier, 2005; Kropf, 2004); however, the inclusion of antioxidants (such as rosemary) may slow the oxidative process (Mancini et al., 2005). The HiOx MAP system has fewer critical factors to control than the use of ultra-low oxygen MAP systems, resulting in more wide-spread use.

The use of carbon monoxide (CO) in a low oxygen (LoOx) MAP system was approved in 2004 (Cornforth & Hunt, 2008). Benefits to using CO in MAP include a more stable red color resulting in a longer shelf life and improved meat flavor due to less lipid oxidation in comparison to HiOx MAP (Cornforth & Hunt, 2008; Eilert, 2005; Jayasingh et al., 2001; Kropf, 2004). CO MAP is not without its critics as consumers are concerned with the inability to detect potential spoilage because of extended color life

(Cornforth & Hunt, 2008). Sørheim et al. (1999) found that meat packaged in low levels of CO with carbon dioxide formed an acceptable red color complimented by low microbial levels. The same red color was formed in HiOx MAP, but the color was unstable over longer shelf life periods.

Both gas blends (HiOx and LoOx) are associated with benefits and challenges to their use in commerce. This study focused on the effects of these two packaging types on display color development, color stability, and instrumental color of crossbred beef and Holstein *semimembranosus* steaks from cattle fed zilpaterol hydrochloride.

Materials and Methods

Animal Selection

Over 1,000 head of crossbred beef and 2,300 head of Holstein steers were fed at two different commercial feed yards in Texas or California, respectively. Cattle were allotted randomly to four feeding groups (0, 20, 30, or 40 d) and fed a typical feedlot finishing diet (Appendix A) with or without zilpaterol hydrochloride (7.56 g/ton of Zilmax®, Intervet, Millsboro, DE). Crossbred beef cattle were implanted on d 0 (arrival at feedlot) and again on d 80 with a Revalor-IS (80 mg trenbolone acetate and 15 mg estradiol). Holstein steers were implanted on d 0 (arrival at feedlot) with Revalor-IS; Holstein steers were implanted with Synovex-S (200 mg progesterone and 20 mg estradiol benzoate) 120 d before the Revalor-IS implant. All cattle were removed from Zilmax supplementation 3 d prior to harvest (d 0) at two separate commercial facilities in Texas or California. Crossbred beef steers were harvested in late January, whereas the Holstein steers were harvested in early May.

Raw Materials

Carcasses were electrically stimulated (45 volts) 30 minutes postmortem and chilled at 0 ± 2ºC in a bone-to-bone configuration. Crossbred beef (324 to 439 kg, A-maturity) and Holstein carcasses (360 to 484 kg, A-maturity) were selected randomly on d 1 postmortem, and 15 inside rounds (s*emimembranosus*, SM, NAMP # 168) from each feeding group were removed from one side of each carcass, vacuum packaged, and commercially shipped to the Kansas State University Meat Laboratory.

Subprimal Processing

Upon arrival, the weight of the vacuum packaged subprimal was recorded (d 9). Cuts were unpackaged and drained; a blotted weight was collected and percentage subprimal purge loss of each muscle was calculated. Subprimals (n = 120 total, 15 from each feeding group for crossbred beef and Holsteins) were trimmed to remove the *adductor* muscle and any excess fat, leaving the SM. Each SM muscle was re-weighed to determine percentage SM muscle yield. A 7.62 cm-thick anterior portion of each SM was removed, re-vacuum packaged (Barrier bag 620, Sealed Air Corp., Duncan, SC), and put into dark storage until d 21 postmortem for overwrap packaging. The remaining SM portion was vacuum packaged and placed into dark storage at 2ºC until d 10 postmortem.

Enhancement and Steak Fabrication

On d 10, the larger SM portion was removed from the package and weighed. Sets of 4 randomly selected muscles (1 from each feeding group) were passed once through a multiple-needle injector (Model N30, Wolftec, Inc., Werther, Germany). Each SM was injected to a 10% pump with a solution containing 0.3% sodium chloride, 0.35% phosphate (BRIFISOL 85 Instant, BK Giulini Corp., Simi Valley, CA), and 0.05% rosemary extract (NatureGuard™ Rosemary Extract, Newly Weds Foods Co./NORAC, Edmonton, Alberta, Canada). Following a 10-minute post-pump drain period, each SM was re-weighed to determine the percentage pump. The SM was faced and fabricated into five, 2.54-cm thick steaks. Three steaks were assigned to a high-oxygen (HiOx) modified atmosphere packaging (MAP) system (80% O_2, 20% CO_2) for 0, 3, or 5 d of retail display. The remaining two steaks were allotted to a low-oxygen, carbon monoxide (LoOx) MAP system (69.6% N_2, 30% CO_2, and 0.4% CO) for either 0 or 9 d of retail display. All MAP steaks were placed with the fresh cut surface up in 24.5 cm × 14.3 cm × 5.0 cm rigid polypropylene trays (CS1178, Cryovac Sealed Air Corp., Duncan, SC) containing tray diapers (Dri-Loc Soaker Pads, AC-50, Sealed Air Corp., Duncan, SC), covered with oxygen-barrier film (Lid 550, 1.0 mils; less than 20.0 oxygen transmission cc/24 h/m^2 at 4.4°C with 100% relative humidity (RH), and moisture vapor transmission less than 0.1 g/24 h/645.2 cm^2 at 4.4°C and 100% RH, Cryovac Sealed Air Corp., Duncan, SC), and packaged (Ross Jr. S-3180, Ross, Midland, VA). HiOx and LoOx packages were boxed and placed into dark storage for 4 and 11 d, respectively, before being put into simulated retail display. For LoOx packaged steaks, two activated oxygen scavengers (ActiveTech™, Pactiv, Chicago, IL) were included in each package to eliminate residual O_2 during storage and display.

pH

The pH was measured on d 0, 3, and 5 or d 0 and 9 for HiOx or LoOx packaged steaks, respectively, by inserting the tip of a previously calibrated probe (MPI pH probe, glass electrode, Meat Probes Inc., Topeka, KS) twice into the deep SM (DSM) and three times into the superficial SM (SSM). Measurements were averaged and a final value was calculated for the DSM and SSM portions of each steak.

Retail Display

All steaks were displayed under constant fluorescent lighting (2153 lux, 3000 K, CRI = 85, Bulb model F32T8/ADV830/Alto, Philips, Bloomfield, NJ) at 2 ± 1.3°C in open-topped cases (Unit model DMF8, Tyler Refrigeration Corp., Niles, MI). Packages were rotated daily in order to minimize variation due to package location in the case. Cases defrosted every 12 h, and temperature was monitored throughout display using temperature loggers (RD-TEMP-XT; Omega® Engineering, Inc., Stamford, CT).

Visual Color

Trained panelists (n = 6 to 8) who had passed the Farnsworth-Munsell® 100-hue test (Macbeth, Newsburgh, NY) conducted initial, display color, and discoloration evaluations (AMSA,1991) on each steak region (DSM and SSM). Steaks packaged in HiOx and LoOx were in simulated retail display for 5 d and 9 d, respectively. Initial color evaluations were made on d 0 of retail display, whereas display color and discoloration scores were recorded daily. The initial color scale used across all packaging treatments was: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red, 6 = Slightly dark red, 7 = Moderately dark red, 8 = Dark red, and 9 = Very dark red; panelists scored each region to half-point increments.

The display color scale, also rated to the nearest half-point, was: 1 = Very bright red or very bright pinkish red, 2 = Bright red or bright pinkish red, 3 = Dull red or dull pinkish red, 4 = Slightly dark red or slightly dark pinkish red, 5 = Moderately dark red or moderately dark pinkish red, 6 = Dark red to dark reddish tan or dark pinkish red to dark pinkish tan, 7 = Tannish red or tannish pink, and 8 = Tan to brown. Panelists used 5.5 on the scale as a point of reference indicating borderline acceptability of steaks.

The discoloration scale used to indicate, to the nearest whole point, the percentage of surface discoloration due to metmyoglobin formation was: 1 = None (0%), 2 = Slight discoloration (1-19%), 3 = Small discoloration (20-39%), 4 = Modest discoloration (40-59%), 5 = Moderate discoloration (60-79%), 6 = Extensive discoloration (80-99%), and 7 = Total discoloration (100%). Panelists were instructed to ignore browning due to "edge" contamination or muscle/cut irregularities.

Instrumental Color

Steaks were evaluated for instrumental color at 0, 3, and 5 d (HiOx) and 0 and 9 d (LoOx) using a calibrated HunterLab MiniScan® XE Plus Spectrophotometer (45/0 LAV, 2.54-cm-diameter aperture, 10° standard observer, Illuminant A, Hunter Associates Laboratory, Inc., Reston, VA). CIE L* (0 = black, 100 = white), a* (negative = green, positive = red), and b* (negative = blue, positive = yellow) values were obtained and used to calculate hue angle ($\tan^{-1} b^*/a^*$) and saturation index $(a^{*2} + b^{*2})^{1/2}$. Scans from each steak or muscle region (n = 2 for DSM, n = 3 for SSM) were averaged for statistical analysis.

Odor and Gas Concentration

MAP packages were evaluated for carbon dioxide (CO_2), oxygen (O_2), and carbon monoxide (CO) head space gas concentrations (Tri-Gas MAP Headspace Analyzer, Model 900121, sampling rate = 5 ml/sec, resolution = CO: 0.001%, CO_2: 0.01%, O_2: 0.01%, Bridge Analyzers, Inc., Alameda, CA) at 0, 3, and 5 d of visual display for HiOx or 0 and 9 d for LoOx.

Odor scores were subjectively measured on all d 9 LoOx steaks immediately after the packages were opened by two individuals familiar with typical off-odors of meat products. The following scale was used: 1 = No off odor, 2 = Slight off odor, 3 = Small off odor, 4 = Moderate off odor and 5 = Extreme off odor, with values greater than 3.5 considered unacceptable.

Subprimal and Steak Yields

SM whole muscle yield was determined from weights taken upon arrival of muscles, and again after being removed from the package, drained, and blotted. Subprimal cut loss was calculated using the following formula: % weight loss = [(Wt. of packaged subprimal – Wt. of empty vacuum bag) – Wt. of drained subprimal ÷ (Wt. of packaged subprimal – Wt. of empty vacuum bag)] × 100.

Pump yields were determined from SM weights taken prior to enhancement and after the 10-minute post-pump drain period. Percent pump = [(Unpumped cut wt. – Pumped and drained cut wt.) ÷ Unpumped cut wt.] × 100 was the formula used for calculation.

Percent weight loss of steaks during display was calculated on HiOx and LoOx steaks using direct weights of ingoing and outcoming steaks. The formula used for calculation was: % weight loss = [(Wt. of ingoing steak – Blotted weight of outcoming steak) ÷ Ingoing steak wt.] × 100. Data are presented in Appendix C.

Design and Statistical Analysis

The experimental design was a split-plot design with the whole plot experimental unit as a crossbred beef or Holstein steer to which feeding treatments were randomly assigned. Individual steaks were the subplot experimental units assigned randomly to d of retail display. Visual and instrumental color traits were repeat measures taken on each muscle area (DSM and SSM). Using the MIXED procedure in SAS (SAS Institute, Inc., Cary, NC), least squares means were subjected to pairwise comparisons using Fisher's LSD procedure at the ($P < 0.05$) level of significance, depending on which main effects and interactions were significant. Diet, Muscle Area, and Day were the main effects tested. Interactions tested were Diet × Day, Diet × Muscle Area, Muscle Area × Display Day, and Diet × Day × Muscle Area.

Results and Discussion

HiOx Crossbred Beef Steer Diet Effects

pH

No differences ($P > 0.05$) in pH values occurred for the main effect of diet for crossbred beef steer steaks packaged in HiOx (Table 4.1). Avendaño-Reyes et al. (2006) also found no difference ($P > 0.05$) in pH values of *longissimus* steaks from cattle supplemented with or without (control) zilpaterol hydrochloride.

Gas Concentrations

There were no Diet × Display Day interactions or differences ($P > 0.05$) in gas concentrations due to Zilmax feeding duration (Table 4.1). Oxygen concentrations decreased ($P < 0.05$) during 5 d of display, whereas carbon dioxide levels increased ($P < 0.05$).

Initial Color

Initial color scores were similar ($P > 0.05$) due to Zilmax feeding duration (Table 4.1). Initial color scores for the DSM and SSM according to diet regimen were not different ($P > 0.05$, Table 4.2). Although the interaction was not significant, the DSM had numerically lower initial color scores (brighter red) than the SSM for all diet groups.

Display Color

A significant Diet × Muscle Area × Display Day interaction occurred for display color scores (Table 4.3). For the DSM portion, steaks from cattle fed 0, 20, and 40 d had no differences ($P > 0.05$) in display color scores on d 0 and 1, increased ($P < 0.05$) scores on d 2, 3, and 4, but no differences ($P > 0.05$) in display color between d 4 and 5. The DSM of 30 d fed cattle had no differences ($P > 0.05$) in display color scores on d 0 and 1 or d 1 and 2, but darkened ($P < 0.05$) on d 3 and 4. Display color scores for the 30 d DSM were not significantly different on d 4 and 5 of display. No differences ($P > 0.05$) in display color scores occurred due to dietary regimen for the DSM portion for d 0 through 3. On d 4, the DSM of steaks from the 20 and 30 d treatments was brighter red

(*P* < 0.05, lower display scores) than steaks from cattle fed 40 d Zilmax. On d 5, only the DSM portion of 20 d steaks was brighter (*P* < 0.05) than the DSM from 40 d fed crossbred beef steers. No literature was found looking at the effects of β-agonist supplementation on color development and stability of the SM muscle portions.

The SSM portion of steaks from cattle fed Zilmax for 0, 30, or 40 d was the brightest red (*P* < 0.05) on d 0, had increased (*P* < 0.05) display color scores on d 1 through 4, but no differences (*P* > 0.05) in display color on d 4 and 5. 20 d SSM was the brightest red (*P* < 0.05) on d 0 and 1, darkened (*P* < 0.05) on d 2 – 4 of display, but had no differences (*P* > 0.05) in display scores on d 4 and 5. No differences (*P* > 0.05) occurred in display color scores for the SSM portion due to dietary regimen on d 0 and 2 of simulated display. On d 1 and 5, the SSM portion of 20 d crossbred beef steer steaks was brighter red (*P* < 0.05) than their 40 d counterparts. The SSM of 40 d steaks was significantly darker on d 3 of display than the SSM from all other dietary regimens. On d 4, the SSM of 30 d Zilmax treated steaks was brighter red (*P* < 0.05) than the 40 d SSM.

Overall, DSM and SSM display color scores increased (*P* < 0.05) from d 0 to 5 of simulated retail display. Behrends, Mikel, Armstrong, & Newman (2003), John et al., (2005), and Seyfert, Hunt, Mancini, Hachmeister, Kropf, & Unruh (2004) all reported increased display color scores (darker color) for the end of display in comparison to d 0 of display. Within all d of display and diet treatments, the DSM portion had lower (*P* < 0.05) display color scores than the SSM. In agreement with our findings, Seyfert et al., (2004) noted lower display color scores for the DSM than the SSM. Sammel, Hunt, Kropf, Hachmeister, Kastner, & Johnson (2002b) reported a lower (*P* < 0.05) DSM display color score in comparison to the SSM on d 0, but the DSM was darker (*P* < 0.05, higher display scores) than the SSM on d 2 through 5 of display. Differences in chilling of the DSM and SSM may explain the inconsistency in display color scores seen in various studies. In the present study, the DSM from all dietary regimens was still considered acceptable (display color score less than 5.5) by panelists on d 5 of display whereas the SSM from cattle supplemented with Zilmax for 0, 30, and 40 d was borderline unacceptable by the end of display.

Discoloration

Figure 4.1 shows the Diet × Display Day interaction for discoloration scores. No differences ($P > 0.05$) in steak discoloration occurred due to Zilmax feeding duration on d 0, 1, or 2 of display. Steaks from the 40 d diet regimen had increased ($P < 0.05$) discoloration scores in comparison to the other dietary regimens on d 3 through 5 of display. By the end of display, steaks from all diet groups were less than 40% discolored. No Diet × Muscle Area interaction was noted for discoloration scores of crossbred beef steer steaks (Table 4.2).

Instrumental Color

No Diet × Display Day or Diet × Muscle Area interaction occurred ($P > 0.05$) for any of the instrumental color variables (Table 4.1 and 4.2). The diet main effect was significant only for hue angle values where steaks from control cattle were overall less discolored ($P < 0.05$, 39.6, lower hue angle values) than steaks from the 20 or 30 d treatments (Table 4.1). Avendaño-Reyes et al. (2006) also reported lower ($P < 0.05$) hue angle values for *longissimus* steaks from control cattle versus those fed zilpaterol.

As expected, all diet regimens had a numerical decrease ($P > 0.05$) in a* and b* values from d 0 through 5 of display (Table 4.1). Saturation indices decreased ($P < 0.05$) daily through d 5 of display (Table 4.1).

HiOx Crossbred Beef Steer Muscle Area Effects

pH

The SSM had a typical, but lower ($P < 0.05$) pH than the DSM portion (Table 4.4). Contrastingly, Seyfert et al. (2004) reported similar pH values for the DSM and SSM of enhanced steaks.

Initial Color

Muscle area was significant, as the DSM was a lighter cherry red ($P < 0.05$, lower initial color score) than the SSM (Table 4.4). Our initial color scores were similar to Seyfert et al. (2004), who reported lower initial color scores for the DSM (3.2) than the SSM (4.8) for HiOx packaged steaks. In another study, the DSM portion of steaks packaged in PVC overwrap had numerically (P > 0.05) lower initial color scores than the SSM (Sammel et al., 2002b).

Discoloration

Both the DSM and SSM had no differences (P > 0.05) in discoloration scores on d 0 and 1. However, both muscle portions discolored (P < 0.05) on d 2 through 5, and the DSM portion was more discolored (P < 0.05) than the SSM (Figure 4.2).

Follett, Norman, & Ratcliff (1974) noted high biochemical activity of the DSM only a few hours postmortem at high temperatures, resulting in a poor ability to reduce pigment, which could explain the increased rate of DSM discoloration seen in the present study. Macdougall (1982) and Sammel et al. (2002b) also noted that slow chilling of the DSM at higher temperatures than the SSM would denature DSM proteins resulting in poor reducing capacity.

Instrumental Color

For L* and hue angle variables, the Muscle Area × Display Day interaction was significant (Table 4.4), while b* values showed a strong trend (P = 0.0564). Muscle area main effect was significant for a* and b* values where the DSM was less red (25.3 vs. 26.4) and more yellow (22.4 vs. 21.5) than the SSM. Saturation indices were not different (P > 0.05) between muscle areas. Similar a* values and saturation indices were reported by Seyfert, Mancini, Hunt, Tang, & Faustman (2007) and Sawyer et al. (2007), respectively.

The DSM portion of crossbred beef steaks was lighter (P < 0.05, higher L* values) than the SSM on all d of display. Seyfert et al. (2004) and Seyfert et al. (2007) also noted that the SSM was darker (P < 0.05, lower L* values) than the DSM across all d of display. In our study, DSM L* values were not significantly different at the beginning (d 0) and end (d 5) of display, but the DSM was lighter (P < 0.05) on d 3 than d 0 and 5. The SSM lightened (P < 0.05, higher L* values) daily through the end of display. In contrast with our results, Seyfert et al. (2004) reported decreased (P < 0.05) SSM L* values daily through the end of display.

Overall, the DSM was more discolored (P < 0.05, higher hue angles) than the SSM across all d of display. Sammel, Hunt, Kropf, Hachmeister, & Johnson (2002a) also reported increased ($P < 0.05$) hue angles for the DSM of PVC-packaged steaks from 0 to 5 d of display. In the present study, no differences ($P > 0.05$) in SSM hue angle values occurred on d 0 and 5 of display; however, the SSM on d 3 was less discolored ($P < 0.05$, lower hue angle) than either d 0 or d 5.

HiOx Holstein Steer Diet Effects

Gas Concentrations

Although a significant Diet × Display Day interaction occurred for CO_2 gas concentrations (Table 4.5), the percentage of CO_2 in the packages was within 2%, and all concentrations were near the 20% target. No large differences occurred in CO_2 concentrations according to diet regimen. Oxygen levels were 81.0, 77.4, and 78.1% on d 0, 3, and 5, respectively. Overall, the MAP gas blend was close to the desired 80% O_2/20% CO_2 level.

pH

No differences ($P > 0.05$) due to Zilmax feeding duration occurred for pH values which ranged from 5.76 to 5.79 (Table 4.5). While the Diet × Muscle Area interaction was not significant, pH values for the DSM were numerically higher ($P > 0.05$) than the SSM across all diet treatments (Table 4.6). Our pH values for Holstein steaks were higher than those reported by Dunne, Keane, O'Mara, Monahan, & Moloney (2004) and Moloney, Allen, Joseph, Tarrant, & Convey (1994), probably due to phosphate in the enhancement solution (Seyfert et al., 2005).

Initial Color

Steaks across all diet regimens were moderately light cherry red on d 0 of display (Table 4.5). The DSM of steaks for all diet treatments had lower ($P > 0.05$) initial color scores than the SSM (Table 4.6).

Display Color

No differences ($P > 0.05$) occurred in display color scores due to Zilmax treatment until the end of display (Figure 4.3). On d 5, steaks from the 20 d feeding duration were darker (P < 0.05) than steaks from all other feeding durations. Steaks from all dietary regimens were an unacceptable tannish red color at the end of display. Although no interaction between diet regimen and muscle area occurred, the DSM of steaks from all diets had lower (P > 0.05) display color scores than their SSM counterparts (Table 4.6).

Discoloration

Discoloration scores for all feeding durations were stable through d 2 of display, but all steaks accumulated more (P < 0.05) metmyoglobin from d 3 through the end of display (Figure 4.4). These data agree with the d 5 display color scores, as steaks from the 20 d diet regimen were more discolored (P < 0.05) on d 3, 4, and 5 than steaks from the 0, 30, and 40 d feeding durations. By the end of display, 20 d steaks approached 40% metmyoglobin on the steak surface, whereas all other dietary treatments had only 30% metmyoglobin. Although not significant, the DSM had numerically higher discoloration scores than the SSM across all feeding durations (Table 4.6).

Instrumental Color

There were no differences (P > 0.05) in a* values and hue angles among diet regimens on d 0 of display (Table 4.5). On d 3, both a* and hue angle values indicated that steaks from the 20 and 30 d treatments were generally less red (P < 0.05) than control steaks. By d 5, steaks from the 20 d feeding duration had the lowest (P < 0.05) a* and hue angle values among all dietary treatments. The loss of redness in 20 d steaks by the end of display may have contributed to greater accumulation of metmyoglobin (higher discoloration scores) also noted for the 20 d treatment on d 3 to 5 of display. In a study comparing zilpaterol, ractopamine, and no β-agonist treatments, no differences (P > 0.05) in a* values of beef *longissimus* steaks occurred among the three dietary treatments (Avendaño-Reyes et al., 2006); however, the study did not look at the effects of various feeding durations. Behrends, Mikel, Armstrong, & Newman (2003), John et al. (2005), and Seyfert et al. (2005) all reported decreasing a* values for MAP beef steaks during simulated display. No Diet × Display Day interactions occurred for L*, b*, and saturation indices (Table 4.5).

Diet × Muscle Area interactions were significant for a*, b*, and saturation indices (Table 4.6). The SSM of control steaks was redder (*P* < 0.05) than the DSM; steaks from the 20, 30, and 40 d treatments had no differences (*P* > 0.05) in redness of the DSM and SSM. Control steaks had a redder (*P* < 0.05) SSM portion than the SSM of steaks from all other diet regimens. No differences (*P* > 0.05) in DSM a* values occurred across Zilmax feeding durations.

The DSM of Holstein steaks from all Zilmax treatments was more yellow (*P* < 0.05) than their SSM counterparts. No differences (*P* > 0.05) were noted in DSM b* values across diet treatments; however, the SSM of control steaks was more yellow (*P* < 0.05) than the SSM of 20, 30, or 40 d steaks.

While saturation indices were not different (*P* > 0.05) for the DSM and SSM of control and 20 d steaks, the DSM was more vivid (*P* < 0.05) than the SSM of both 30 d and 40 d Holstein steaks. The DSM of control and 40 d steaks was more vivid (*P* < 0.05) than the DSM of 20 d steaks. The SSM of steaks from control cattle was more vivid (*P* < 0.05) than the SSM of steaks from all other Zilmax treatments. Moloney, Allen, Joseph, Tarrant, & Convey (1994) found a significant linear effect for saturation indices of *longissimus* steaks from Friesian steers fed the β-agonist L-644,969; on d 2, 3, and 6 of display, steaks from dairy cattle fed no β-agonist were more vivid (*P* < 0.05, higher saturation indices) than steaks from cattle supplemented with L-644,969. Diet × Muscle Area interactions were not significant for L* and hue angle values (Table 4.6). Main effect means indicated that steaks from all dietary regimens became darker as d of display increased. These data agree with display panelist’s scores.

HiOx Holstein Steer Muscle Area Effects

The main effect and interaction means for d of display and the DSM and SSM muscle areas are presented in Table 4.7. These two muscle areas of the SM represent slow chilled (DSM) and faster chilled (SSM) areas of this large, hind limb muscle. Numerous reports indicate significant differences for these two areas.

pH

The pH of the DSM was slightly higher than the SSM (Table 4.7). Both of these values are higher than those for non-enhanced beef muscle Sammel et al. (2002b) and *longissimus* steaks from Friesian steers (Moloney et al., 1994). The inclusion of alkaline phosphate (used to raise pH) in the enhancement solution is most likely responsible for the higher pH values (Seyfert et al., 2004).

Initial Color

The DSM portion of Holstein steaks was a lighter ($P < 0.05$, lower initial color score) cherry red than the SSM (Table 4.7). Sammel et al. (2002b), Seyfert et al. (2004), and Seyfert et al. (2007) all reported lower ($P < 0.05$) initial color scores for the DSM than the SSM, primarily due to the decreased oxygen consumption rate of the DSM which causes the development of a bright color at the beginning of display (Seyfert, Mancini, Hunt, Tang, Faustman, & Garcia, 2006).

Display Color

The DSM of Holstein steaks darkened ($P < 0.05$) daily, whereas the SSM darkened ($P < 0.05$) on d 0 and 1, had no difference ($P > 0.05$) on d 2 and 3, but had increased ($P < 0.05$) display color scores on d 4 and 5 of display (Figure 4.5). The DSM was lighter ($P < 0.05$, lower display scores) than the SSM on d 0 through 4; however, there were no differences ($P > 0.05$) in display color scores between the DSM and SSM on d 5. Seyfert et al. (2007) reported lower ($P < 0.05$) DSM display color scores than the SSM for d 0 to 2 of display before no significant difference in display color occurred between the muscle portions for d 3 to 7 of display in HiOx MAP. In the present study, both muscle portions were unacceptable and almost dark red (5.9) in color according to panelists by the end of display, whereas Seyfert et al. (2007) reported acceptable display color scores according to panelists for all 7 d of simulated display.

Discoloration

Both the DSM and SSM were 20-39% discolored by d 5 of display (Table 4.7). Muscle area main effects were significant, as the DSM of Holstein steaks had a higher ($P < 0.05$) discoloration score than the SSM. Sawyer et al. (2007) analyzed the effects of steak location within the SM muscle (dorsal, medial, or ventral) on differences in color variables. Their study found no differences ($P > 0.05$) in discoloration scores between the DSM and SSM of steaks from the dorsal and medial muscle portions; however, the

DSM had a higher (*P* < 0.05) discoloration score than the SSM of steaks cut from the ventral portion of the SM (Sawyer et al., 2007). Increased discoloration scores of the DSM may be due to the lower (*P* < 0.05) metmyoglobin reducing activity of the DSM in comparison to the SSM of steaks packaged in HiOx on d 0 and 4 of display (Seyfert et al., 2007).

Instrumental Color

The muscle area main effect was significant for L*, as the DSM was lighter (*P* < 0.05) than the SSM (Table 4.7). Seyfert et al. (2007) also reported higher (*P* < 0.05) L* values for the DSM of beef steaks in comparison to the SSM on d 0, 4, and 7 of display in a HiOx MAP atmosphere.

The DSM of Holstein steaks was redder (*P* < 0.05) than the SSM on d 0 (Table 4.7). By d 3, there were no differences (*P* > 0.05) in redness between the muscle portions. On d 5, the SSM was redder (*P* < 0.05) than the DSM. Sammel, Hunt, Kropf, Hachmeister, & Johnson (2002a) also had higher (*P* < 0.05) a* values for the DSM than the SSM of PVC-packaged steaks on d 0 of display; however, the SSM was redder (*P* < 0.05) than the DSM on d 1 to 5. Contrastingly, Seyfert et al. (2004) reported higher (*P* < 0.05) a* values for the SSM than the DSM of HiOx packaged steaks on d 0 through 4 of display. In the present study, both muscle portions had a loss (*P* < 0.05) in redness from d 0 until the end of display.

On all d of display, the DSM was more yellow (*P* < 0.05, higher b* values) than the SSM (Table 4.7). The DSM was the most yellow (*P* < 0.05) on d 0 of display; no differences (*P* < 0.05) in b* values occurred between d 3 and 5. For the SSM, b* values were highest (*P* < 0.05) on d 0 and decreased (*P* < 0.05) on d 3 before increasing (*P* < 0.05) at the end of display. Sammel et al. (2002a) reported higher (*P* < 0.05) b* values for the DSM in comparison to the SSM of PVC-packaged steaks on d 0 to 5 of display, whereas Seyfert et al. (2004) had higher (*P* < 0.05) b* values for the SSM than the DSM on d 2 and 4 of HiOx retail display. Hue angles increased (*P* < 0.05) for the both muscle portions from the beginning to the end of display (Table 4.7). On all d of display, the DSM was more discolored (*P* < 0.05, higher hue angle) than the SSM.

As expected, saturation indices decreased (*P* < 0.05) daily for the DSM and from d 0 to 3 for the SSM (Table 4.7). The DSM was more vivid (*P* < 0.05) than the SSM on d 0; however, this difference was not noted on d 3 or 5 of display. Seyfert et al. (2007) reported no differences (*P* > 0.05) in chroma values between muscle portions on d 0 or 7 of simulated display in HiOx packaging; however, in their study the SSM was more (*P* < 0.05) vivid than the DSM on d 4.

LoOx Crossbred Beef Steer Diet Effects

pH

Steaks from crossbred beef cattle fed Zilmax for 30 d had a lower (*P* < 0.05) pH than steaks from all other diet regimens (Table 4.8); however, pH differences were small and would not compromise muscle functionality. No differences (*P* > 0.05) in pH values occurred due to diet regimen and muscle area (Table 4.9).

Gas Concentrations

Carbon monoxide concentrations were not different (*P* > 0.05) across diet regimens on d 0 (Table 4.8), but were lower than the 0.4% target. The main effect of day was significant for CO_2, as d 0 had a lower (*P* < 0.05) carbon dioxide concentration than d 9.

Odor Scores

Odor scores at the end of display were not significantly different for Zilmax feeding duration (Table 4.8). All steaks had a small to moderate off-odor.

Initial Color

The DSM of steaks from all diet treatments was a lighter (*P* < 0.05) cherry red than their SSM counterparts (Figure 4.6). Within muscle areas, the 20 and 30 d DSM and SSM of crossbred beef steaks had lower (*P* < 0.05) initial color scores than the DSM of control steaks and the SSM of 40 d steaks, respectively. In agreement with our findings, Hunt et al. (2004) reported numerically lower initial color scores for the DSM in comparison with the SSM of steaks exposed to a LoOx MAP system.

Display Color

On d 0 and 9 of display, crossbred beef steaks from cattle fed Zilmax for 30 d were brighter ($P < 0.05$) red than control or 40 d steaks (Figure 4.7). The 20 d treatment had lower ($P < 0.05$) display color scores than 40 d steaks on d 3 of display. On d 4 and 5, 30 d steaks were brighter ($P < 0.05$) red than steaks from crossbred beef cattle fed Zilmax for 40 d. No literature was found looking at the effects of Zilmax feeding duration on display color scores of SM steaks in MAP packaging.

Control steaks had no differences ($P > 0.05$) in display color scores on d 0 and 1, but darkened ($P < 0.05$) on d 2 through the end of display. Steaks from the 20 and 40 d treatments were not different ($P > 0.05$) in display scores on d 0, 1, 7, and 8, but 20 and 40 d steaks darkened ($P < 0.05$) on d 2 through 7 and again on d 9. Steaks from the 30 d feeding duration darkened ($P < 0.05$) from d 0 to 2 and from d 5 through the end of display; no differences ($P > 0.05$) in display color scores occurred between d 3 and 4 for the 30 d treatment. At the end of display, all steaks were still considered an acceptable slightly dark red. Sørheim et al. (1999) reported a red to bright red color for up to 14 d of display for beef loin steaks packaged in LoOx MAP at 4ºC. Sirloin steaks packaged in LoOx were bright red to reddish tan for 21 d of storage (John et al., 2005).

Of the three packaging types, steaks packaged in LoOx had the lowest numerical display color scores at the end of retail display in the current study. Although not significant, John et al. (2005) reported numerically lower display scores (brighter red color) for LoOx sirloin steaks in comparison to HiOx MAP. Moreover, Stetzer, Wicklund, Paulson, Tucker, Macfarlane, & Brewer (2007) and Sørheim et al. (1999) noted that *longissimus* steaks in HiOx were not as red on d 14 of display as LoOx steaks. Stetzer et al. (2007) also stated that the capability of LoOx MAP to preserve the bright red color in meat during display is a benefit of its use over HiOx MAP. The Diet × Muscle Area interaction was not significant in our study, although the DSM of all diet regimens had numerically lower display color scores than the SSM (Table 4.9).

Discoloration

No differences (*P* > 0.05) in discoloration scores due to diet regimen and muscle area occurred (Table 4.9). Discoloration scores were not different (*P* > 0.05) until d 8 and 9 of display (Figure 4.8). On d 8, steaks from the 30 d feeding duration were less discolored (*P* < 0.05) than control steaks. By d 9, both 30 and 40 d Zilmax treatments had lower (*P* < 0.05) discoloration scores than control and 20 d steaks. Overall, LoOx steaks from all diet regimens had almost no discoloration until d 9, when all diet treatments were slightly more discolored (*P* < 0.05) than on d 0 through 8 of display.

In our study, Diet × Display Day discoloration scores of SM steaks packaged in LoOx were below 2.0 for all feeding durations and 9 d of display, whereas SM steaks in HiOx MAP had Diet × Display Day discoloration scores close to 3.0 after only 5 d. Sørheim et al. (1999) reported higher visual color scores (indicative of more discoloration) for HiOx-packaged beef loin steaks and pork chops after 10 and 14 d, respectively, when compared to steaks and chops in LoOx MAP.

Instrumental Color

No interactions (*P* > 0.05) for L* values due to diet regimen and d of display or muscle area were observed (Tables 4.8 and 4.9). However, the main effect of diet was significant, as steaks from the 20 and 30 d feeding regimens were lighter (*P* < 0.05) than control steaks. The DSM had numerically higher (*P* > 0.05) L* values than the SSM for all Zilmax feeding durations.

There were no significant interactions or main effect dietary differences for a*, b* and saturation index values (Tables 4.8 and 4.9). Although not statistically significant, steaks on d 0 for all diet regimens were more (*P* > 0.05) vivid red and yellow than steaks at the end of display. Numerical differences in redness, yellowness, and vividness were negligible between the DSM and SSM of steaks from all feeding durations. Hunt et al. (2004) suggested that LoOx MAP may compensate for muscle portion differences of the SM by improving color stability of the DSM 1 to 2 d over other packaging systems.

Hue angle values were not different (*P* > 0.05) for crossbred beef steaks due to Zilmax feeding duration and d of display (Table 4.8). The Diet × Muscle Area interaction was significant (Table 4.9), as the DSM of steaks from all dietary treatments was more discolored ($P < 0.05$, higher hue angles) than the SSM. Within muscle area, no differences ($P > 0.05$) in hue angles occurred due to diet regimen for the SSM; however, the DSM of 20 d steaks was more ($P < 0.05$) discolored than the DSM of steaks from all other feeding durations. Hue angle values in our study were comparable to those of John et al. (2005) who reported hue angles for sirloin steaks of 34.1 and 34.0 after 7 and 14 d of display in LoOx MAP.

LoOx Crossbred Beef Steer Muscle Area Effects

pH

The DSM of crossbred beef steer steaks in LoOx had a lower ($P < 0.05$) pH than the SSM portion (Table 4.10), although differences in pH were not of practical significance. In HiOx packaging, the DSM and SSM portions of crossbred beef steaks had an increased difference in pH values of 0.28 and 0.23 units, respectively, in comparison to their LoOx counterparts (Table 4.4).

Display Color

The DSM portion of steaks darkened ($P < 0.05$) daily through the end of display (Figure 4.9). The SSM had increased ($P < 0.05$) display scores daily with the exception of d 7 and 8, when no change ($P > 0.05$) in SSM display color occurred. In comparison to the SSM, the DSM had lower ($P < 0.05$) display color scores daily during display. Contrastingly, Hunt et al. (2004) reported higher visual color scores for the DSM in comparison with the SSM across 35 d of shelf life for steaks exposed to CO via a barrier bag system. According to panelists, both muscle portions were still an acceptable moderately dark red color on d 9. Sørheim et al. (1999) reported acceptable visual color scores through 14 d of display for beef loin steaks packaged in LoOx.

Discoloration

Discoloration scores between muscle portions or across d of display were similar ($P > 0.05$, Table 4.10). The DSM and SSM were less than 20% discolored after 9 d of simulated retail display.

Instrumental Color

Both the DSM and SSM were darker (*P* < 0.05, lower L* values) on d 9 than d 0 of display (Table 4.10), which agrees with the increased display color scores observed for both muscle portions on d 9 in comparison to the beginning of display (Figure 4.9). The DSM was also lighter (*P* < 0.05, higher L* values) than the SSM at the beginning and end of display. Seyfert et al. (2007) also reported higher L* values for the DSM than the SSM of steaks packaged in MAP at 0, 4, and 7 d of display.

The Muscle Area × Display Day interaction was not significant for a*, b*, hue angle, or saturation index values (Table 4.10); however, the main effects of muscle area were different (*P* < 0.05) for b*, hue angle, and saturation indices. Yellowness, hue angles, and saturation indices for the DSM were higher (*P* < 0.05) than the SSM, respectively. In another study, Seyfert et al. (2007) had no differences (*P* > 0.05) in a* values or saturation indices between the DSM and SSM of MAP steaks on d 0.

LoOx Holstein Steer Diet Effects

pH

No differences (*P* > 0.05) in pH values of Holstein steaks occurred due to diet regimen (Table 4.11), or for the interaction of Diet × Muscle Area (Table 4.12). Moloney et al. (1994) reported no differences in ultimate pH values (5.47 and 5.51) of the SM muscle from Friesian steers fed increasing doses of L-644,969. Our higher pH values may be due to the use of an alkaline phosphate in the enhancement solution (Seyfert et al., 2005).

Gas Concentrations

No Diet x Display Day interaction occurred for CO_2 or CO concentrations (Table 4.11). Diet main effect was significant, as control packages had the highest (*P* < 0.05) CO_2 levels in comparison to 20, 30, and 40 d. CO and CO_2 concentrations were less than their 0.4% and 30% target levels, respectively.

Odor Scores

Off-odor scores were not different ($P > 0.05$) across diet regimens (Table 4.11), and all steaks had an acceptable odor after d 9 of display.

Initial Color

Steaks from all diet regimens could be characterized as moderately light cherry red in color (Table 4.11). The DSM had numerically lower ($P > 0.05$) initial color scores than the SSM for all diet treatments (Table 4.12). The tendency for a lighter red color in the DSM is likely due to differences in carcass chilling Sammel et al. (2002b) and not to Zilmax feeding duration.

Display Color

Table 4.13 contains the display color means for the Diet × Muscle Area × Display Day interaction. For the DSM, no differences ($P > 0.05$) occurred in display color scores due to Zilmax feeding duration on d 1 to 5, d 7, and d 9 of display. At the beginning of display, the DSM portion of 20 and 40 d steaks was lighter ($P < 0.05$, lower display color scores) red than the DSM of control steaks. The DSM of 20 and 30 d steaks (d 6) and the 20 d DSM (d 8) were lighter ($P < 0.05$) red than the DSM of control steaks.

No differences ($P > 0.05$) in SSM display color scores across dietary treatments occurred on d 2, 3, 6, and 7. On d 0 and 1, the SSM of control steaks was darker ($P < 0.05$, higher display scores) than 40 d and 20 d SSM, respectively. The SSM of control steaks was darker ($P < 0.05$) red than 30 and 40 d SSM portions on d 4 of display. On d 5 and 9, the SSM of 40 d fed steaks was lighter ($P < 0.05$) red than its 30 d counterpart. The SSM from the 40 d diet regimen had lower ($P < 0.05$) display color scores than the SSM of control or 30 d steaks on d 8 of display. Overall, both muscle portions from steaks of Holsteins supplemented Zilmax for 40 d tended ($P > 0.05$) to have equal or improved display color scores compared to the other dietary regimens.

Display color scores for the DSM across all diet regimens and 20, 30, and 40 d SSM increased ($P < 0.05$) from d 0 to d 3 of display before decreasing ($P < 0.05$) on d 4, and increasing ($P < 0.05$) again to the end of display (d 9). The SSM portion of steaks from control Holsteins increased ($P < 0.05$) from d 0 to d 9 of display.

The DSM portion of all steaks was acceptable for the entire display period, whereas the SSM of steaks from all feeding durations was borderline unacceptable by d 9. For crossbred beef steaks packaged in LoOx, the DSM was also lighter (*P* < 0.05) than the SSM on d 0 to 4. Improved color stability of the DSM may be due to the inclusion of CO in LoOx MAP that increases shelf life of that muscle portion (Hunt et al., 2004).

Discoloration

While the Diet × Muscle Area interaction for discoloration scores of Holstein steaks was not significant (Table 4.12), discoloration scores were different (*P* < 0.05) by diet regimen and display day (Figure 4.10). Steaks from cattle fed Zilmax for 0, 20, or 30 d discolored (*P* < 0.05) on d 2 and 3 from the beginning of display before decreasing (*P* < 0.05) discoloration scores occurred on d 4. Steaks from the 0, 20, and 30 d diet groups were slightly more discolored (*P* < 0.05) by the end of display than on d 4. Steaks from the 40 d feeding duration discolored (*P* < 0.05) on d 2 and 3 from d 0 of display before discoloration scores decreased (*P* < 0.05) on d 4 of display. Discoloration scores for the 40 d feeding group were not different (*P* > 0.05) on d 4 from d 9 of display. Steaks from all feeding durations were less than 20% discolored during the entire 9 d display, thus the slight increase in metmyoglobin would not likely be of practical importance. The inclusion of CO in the gas blend possibly served to inhibit myoglobin oxidation during longer shelf life periods (Cornforth & Hunt, 2008).

Differences (*P* < 0.05) in discoloration scores across Zilmax feeding durations occurred on d 2 to 3 and d 7 to 9 of display (Figure 4.10). On d 2 and 9, 30 d Holstein steaks were the most (*P* < 0.05) discolored in comparison to all other diet treatments. On d 3, control steaks were less (*P* < 0.05) discolored than 20 and 30 d steaks; by d 7 control and 20 d steaks had lower (*P* < 0.05) discoloration scores than steaks from the 30 d feeding duration. On d 8 of simulated display, control steaks were less (*P* < 0.05) discolored than their 30 d counterparts. However, discoloration scores of Holstein steaks in LoOx across all feeding durations and d of display were low.

Instrumental Color

No significant interaction (Tables 4.11 and 4.12) or diet main effect differences occurred for L* or hue angle values. Steaks were darker ($P < 0.05$) on d 9 than d 0 of display (Table 4.11).

The DSM of steaks from 0, 30, and 40 d feeding durations were a more ($P < 0.05$) vivid red color than the SSM portion (Table 4.12). No differences ($P > 0.05$) in a* values or saturation indices occurred between the DSM and SSM of the 20 d treatment. Within the SSM, 30 and 40 d steaks were a less ($P < 0.05$) vivid red than the SSM of control and 20 d steaks. The DSM of control steaks was redder ($P < 0.05$) than the DSM of 20 and 40 d treatments. The DSM of steaks from all feeding regimens was more yellow ($P < 0.05$) than the SSM, with the DSM of control steaks being more yellow ($P < 0.05$) than its 20 d counterpart. For the SSM, control steaks had higher ($P < 0.05$) b* values than 30 d steaks. Saturation indices were also higher ($P < 0.05$) for the DSM portion of control steaks in comparison to the same muscle area from the 20 and 30 d feeding groups. These instrumental values are indicative of a bright red color and thus support panelist visual scores for display color and discoloration.

LoOx Holstein Steer Muscle Area Effects

pH

The DSM had a slightly higher ($P < 0.05$) pH than the SSM (Table 4.14). These findings agree with DSM pH values of crossbred beef and Holstein steaks packaged in HiOx, when compared to the SSM portion.

Initial Color

The DSM was a lighter ($P < 0.05$) cherry red than the SSM portion of steaks (Table 4.14). These results are also consistent with our findings for initial color scores of crossbred beef and Holstein steaks packaged in HiOx, as well as Sammel et al. (2002b) and Seyfert et al. (2004). A lighter color early postmortem for the DSM has been attributed to excess light scattering from protein denaturation at high temperatures (Macdougall, 1982).

Discoloration

Discoloration scores for the DSM and SSM increased ($P < 0.05$) on d 2 and 3 from the beginning of display before decreasing ($P < 0.05$) on d 4 (Figure 4.11). Both muscle portions were more ($P < 0.05$) discolored at the end of display than on d 4 through 8. While statistically significant, increased discoloration scores on d 2 and 3 were minor.

Discoloration scores between the DSM and SSM were similar ($P > 0.05$) during display except on d 1, 4, and 7. On d 1 and 7, the DSM had higher ($P < 0.05$) discoloration scores than the SSM, whereas the SSM was more discolored ($P < 0.05$) than the DSM on d 4 of display. Differences in discoloration between the DSM and SSM had little practical significance, as both muscle portions were less than 20% discolored during the 9 d of simulated display. Carbon monoxide in the gas blend may act as an antioxidant to prevent the oxidation of myoglobin (Cornforth & Hunt, 2008). The added color stability would allow for an increased shelf life of cuts packaged in LoOx MAP (Eilert, 2005).

Instrumental Color

No significant interactions occurred for a*, b*, hue angles, or saturation indices of LoOx packaged Holstein steaks due to muscle area and d of display (Table 4.14). The muscle area main effect was significant (Table 4.14), as the DSM was redder, more yellow, and more vivid than the SSM of Holstein steaks ($P < 0.05$). Also, the DSM had a higher ($P < 0.05$) hue angle than the SSM, which is indicative of more discoloration, probably due to the low reducing capacity of the DSM (Follett, Norman, & Ratcliff, 1974).

The DSM was lighter ($P < 0.05$, higher L* values) than the SSM on d 0 and 9 of display. L* values were also higher ($P < 0.05$) on d 0 than d 9 of display for both muscle portions. Differences in instrumental values between muscle portions were minimized by packaging the SM muscle in LoOx MAP. Hunt et al. (2004) reported numerically lower a* values on d 0 and 6 of display for the DSM in comparison to the SSM of steaks packaged in a LoOx MAP barrier bag system for 7 d prior to being overwrapped in PVC.

Summary

All pH values for crossbred beef and Holstein steaks packaged in HiOx and LoOx MAP were within an acceptable range and would not negatively impact SM color. Few significant differences in instrumental color variables for crossbred beef or Holstein SM steaks occurred for either packaging treatment due to Zilmax feeding duration. Steaks from crossbred beef steers fed Zilmax for intermediate durations (20 or 30 d) and packaged in HiOx or LoOx were slightly brighter red and less discolored during simulated display than control or 40 d steaks. Holstein steaks in HiOx MAP from the 20 d Zilmax treatment were the darkest and most discolored at the end of display in comparison to all other feeding regimens. All Zilmax feeding durations for Holstein steaks packaged in LoOx resulted in improved display color over control steaks.

In comparison to traditional packaging methods, HiOx and LoOx MAP improved display and instrumental color differences between the DSM and SSM. Both muscle portions of crossbred beef and Holstein steaks were the brightest red and least discolored at the end of display in LoOx MAP. The use of LoOx MAP for SM steaks will minimize differences in color between the DSM and SSM.

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Table 4.1 Diet, Day, and Diet × Display Day means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in HiOx

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.71	5.71	5.71	5.71	**------**
Initial color [b]	4.2	4.0	4.0	4.2	**------**
O_2 gas, %	**79.7**	**80.4**	**78.2**	**81.0**	
d 0	82.8	82.0	76.5	81.9	**80.8** [e]
d 3	82.1	81.4	83.5	83.1	**82.5** [e]
d 5	74.1	77.9	74.7	78.0	**76.2** [f]
CO_2 gas, %	**20.0**	**20.1**	**19.2**	**20.2**	
d 0	18.8	18.8	17.2	18.9	**18.4** [f]
d 3	20.6	20.3	20.4	20.6	**20.5** [e]
d 5	20.6	21.3	20.1	21.0	**20.8** [e]
L*	**48.1**	**49.5**	**49.7**	**48.7**	
d 0	47.4	48.3	48.6	47.4	**47.9** [f]
d 3	48.5	50.2	50.1	49.3	**49.5** [e]
d 5	48.4	49.9	50.5	49.3	**49.5** [e]
a*	**26.4**	**25.6**	**25.7**	**25.6**	
d 0	33.0	32.5	32.3	32.6	**32.6** [e]
d 3	25.4	24.5	24.6	24.7	**24.8** [f]
d 5	20.9	19.9	20.2	19.4	**20.1** [g]
b*	**21.9**	**22.1**	**22.1**	**21.6**	
d 0	27.7	27.7	27.4	27.5	**27.5** [e]
d 3	20.6	21.0	21.1	20.5	**20.8** [f]
d 5	17.5	17.6	17.9	16.9	**17.5** [g]
Hue angle	**39.6** [y]	**40.9** [z]	**40.8** [z]	**40.4** [yz]	
d 0	40.0	40.4	40.2	40.0	**40.1** [f]
d 3	39.0	40.7	40.6	39.7	**40.0** [f]
d 5	40.0	41.8	41.7	41.5	**43.2** [e]
Saturation index	**34.4**	**33.9**	**33.9**	**33.5**	
d 0	43.1	42.7	42.4	42.7	**42.7** [e]
d 3	32.7	32.3	32.4	32.1	**32.4** [f]
d 5	27.3	26.6	27.0	25.9	**26.7** [g]

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.01, Initial = 0.13, O_2 = 2.1, CO_2 = 0.54, L* = 0.66, a* = 0.44, b* = 0.36, Hue angle = 0.39, Saturation index = 0.53.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[efg] Means across day within diet (within variable) without a letter do not differ ($P > 0.05$); means across day within diet (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across diet within day (within variable) without a letter do not differ ($P > 0.05$); means across diet within day (within variable) without a common letter differ ($P < 0.05$).

Table 4.2 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in HiOx

	Diet [1], day			
Variable	0	20	30	40
Initial color [b]				
DSM	3.4	3.1	3.2	3.4
SSM	5.0	4.9	4.8	5.0
Discoloration [c]				
DSM	1.9	1.8	1.8	2.1
SSM	1.6	1.6	1.7	1.9
L*				
DSM	50.7	52.7	52.8	51.7
SSM	45.5	46.2	46.7	45.6
a*				
DSM	25.6	24.9	25.4	25.2
SSM	27.3	26.3	26.0	26.0
b*				
DSM	22.2	22.4	22.7	22.2
SSM	21.6	21.9	21.6	21.1
Hue angle				
DSM	41.1	42.2	41.9	41.9
SSM	38.2	39.7	39.8	39.0
Saturation index				
DSM	33.9	33.5	34.1	33.6
SSM	34.9	34.2	33.8	33.5

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[a] SE: Initial = 0.14, Discoloration = 0.10, L* = 0.63, a* = 0.39, b* = 0.32, Hue angle = 0.35, Saturation index = 0.47.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[c] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

Table 4.3 Diet × Muscle Area × Display Day means[a] for display color scores[b] of Zilmax fed-crossbred beef steer steaks packaged in HiOx

	Day of display					
	0	1	2	3	4	5
	DSM [c]					
Diet [d]						
0	2.4 ^{hpz}	2.5 ^{hpz}	2.8 ^{gpz}	3.7 ^{fpz}	5.0 ^{epyz}	5.1 ^{epyz}
20	2.3 ^{hpz}	2.3 ^{hpz}	2.8 ^{gpz}	3.4 ^{fpz}	4.8 ^{epy}	4.8 ^{epy}
30	2.3 ^{hpz}	2.5 ^{ghpz}	2.6 ^{gpz}	3.5 ^{fpz}	4.9 ^{epy}	5.0 ^{epyz}
40	2.4 ^{hpz}	2.5 ^{hpz}	2.6 ^{gpz}	3.7 ^{fpz}	5.3 ^{epz}	5.3 ^{epz}
	SSM					
Diet						
0	3.1 ^{iqz}	3.3 ^{hqyz}	3.7 ^{gqz}	4.2 ^{fqy}	5.3 ^{eqyz}	5.5 ^{eqyz}
20	3.0 ^{hqz}	3.1 ^{hqy}	3.4 ^{gqz}	4.1 ^{fqy}	5.3 ^{eqyz}	5.4 ^{eqy}
30	2.9 ^{iqz}	3.4 ^{hqyz}	3.5 ^{hqz}	4.2 ^{gqy}	5.2 ^{fqy}	5.5 ^{eqyz}
40	2.9 ^{iqz}	3.6 ^{hqz}	3.8 ^{gqz}	4.7 ^{fqz}	5.7 ^{eqz}	5.8 ^{eqz}

[a] SE: DSM = 0.14, SSM = 0.14.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red, 7 = Tannish red.
[c] DSM = deep SM, SSM = superficial SM.
[d] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efghi] Means across diet within day (within muscle area) without a common letter differ ($P < 0.05$).
[pq] Means across muscle area within diet (within day) without a common letter differ ($P < 0.05$).
[yz] Means across diet within day (within muscle area) without a common letter differ ($P < 0.05$).

Table 4.4 Muscle Area and Muscle Area × Display Day means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in HiOx

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.75 [y]	5.67 [z]
Initial color [b]	------	------	3.2 [z]	4.9 [y]
L*			------	------
d 0	51.8 [fy]	44.1 [gz]		
d 3	52.7 [ey]	46.3 [fz]		
d 5	51.5 [fy]	47.5 [ez]		
a*			25.3 [z]	26.4 [y]
d 0	32.3	33.0		
d 3	24.2	25.4		
d 5	19.4	20.8		
b*			22.4 [y]	21.5 [z]
d 0	27.7	27.4		
d 3	21.4	20.2		
d 5	18.0	17.0		
Hue angle			------	------
d 0	40.6 [gy]	39.7 [ez]		
d 3	41.5 [fy]	38.5 [fz]		
d 5	43.2 [ey]	39.3 [ez]		
Saturation index			33.8	34.1
d 0	42.5	42.9		
d 3	32.3	32.5		
d 5	26.5	26.9		

[1] DSM = deep SM, SSM = superficial SM.

[a] SE: pH = 0.01, Initial = 0.07, L* = 0.38, a* = 0.28, b* = 0.23, Hue angle = 0.24, Saturation index = 0.34.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[efg] Means across day within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across day within muscle area (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across muscle area within day (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within day (within variable) without a common letter differ ($P < 0.05$).

Table 4.5 Diet, Day, and Diet × Display Day means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in HiOx

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.79	5.77	5.76	5.76	**------**
Initial color [b]	4.6	4.2	4.3	4.3	**------**
O_2 gas, %	**77.6**	**81.2**	**78.5**	**78.0**	
d 0	81.5	80.2	81.3	81.0	**81.0 [e]**
d 3	77.7	78.7	77.4	75.8	**77.4 [f]**
d 5	73.7	84.5	76.9	77.0	**78.0 [f]**
CO_2 gas, %	**------**	**------**	**------**	**------**	
d 0	19.3 [ez]	18.8 [ez]	19.2 [ez]	18.9 [efz]	**------**
d 3	19.6 [ez]	19.5 [ez]	19.3 [ez]	19.1 [ez]	**------**
d 5	17.1 [fx]	19.6 [ez]	19.1 [eyz]	17.8 [fxy]	**------**
L*	**42.9**	**43.7**	**43.4**	**44.2**	
d 0	43.7	44.1	43.9	44.4	**44.0 [e]**
d 3	42.8	43.7	43.5	44.5	**43.6 [f]**
d 5	42.3	43.4	42.8	43.7	**43.0 [g]**
a*	**------**	**------**	**------**	**------**	
d 0	32.4 [ez]	31.7 [ez]	31.7 [ez]	31.7 [ez]	**------**
d 3	26.6 [fz]	24.8 [fy]	25.5 [fy]	25.7 [fyz]	**------**
d 5	25.1 [gz]	22.5 [gy]	24.4 [gz]	24.6 [gz]	**------**
b*	**23.9**	**23.2**	**23.3**	**23.4**	
d 0	26.7	26.3	26.1	26.3	**26.4 [e]**
d 3	22.5	21.5	21.6	22.1	**21.9 [f]**
d 5	22.4	21.7	22.1	21.9	**22.0 [f]**
Hue angle	**------**	**------**	**------**	**------**	
d 0	39.6 [ez]	39.7 [ez]	39.4 [ez]	39.7 [ez]	**------**
d 3	40.2 [fz]	41.0 [fz]	40.3 [fz]	40.7 [fz]	**------**
d 5	41.9 [gy]	44.2 [gz]	42.2 [gy]	41.7 [gy]	**------**
Saturation index	**36.8 [z]**	**35.1 [y]**	**35.8 [y]**	**36.0 [yz]**	
d 0	42.0	41.2	41.1	41.2	**41.4 [e]**
d 3	34.8	32.9	33.4	33.9	**33.7 [f]**
d 5	33.7	31.3	33.0	33.0	**32.7 [g]**

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.01, Initial = 0.14, O_2 = 2.3, CO_2 = 0.50, L* = 0.64, a* = 0.44, b* = 0.31, Hue angle = 0.38, Saturation index = 0.50.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[efg] Means across day within diet (within variable) without a letter do not differ ($P > 0.05$); means across day within diet (within variable) without a common letter differ ($P < 0.05$).

[xyz] Means across diet within day (within variable) without a letter do not differ ($P > 0.05$); means across diet within day (within variable) without a common letter differ ($P < 0.05$).

Table 4.6 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in HiOx

	Diet [1], day			
Variable	0	20	30	40
pH				
DSM	5.86	5.83	5.82	5.83
SSM	5.73	5.71	5.70	5.68
Initial color [b]				
DSM	4.0	3.5	3.6	3.5
SSM	5.2	4.9	5.1	5.0
Display color [c]				
DSM	4.0	4.0	3.9	3.8
SSM	4.7	4.7	4.7	4.6
Discoloration [d]				
DSM	1.8	1.9	1.8	1.7
SSM	1.5	1.8	1.7	1.6
L*				
DSM	45.5	46.7	46.5	47.2
SSM	40.4	40.7	40.2	41.2
a*				
DSM	27.6 [ez]	26.2 [ey]	27.3 [ez]	27.6 [ez]
SSM	28.4 [fz]	26.4 [ey]	27.1 [ey]	27.0 [ey]
b*				
DSM	24.1 [ez]	23.8 [ez]	23.9 [ez]	24.3 [ez]
SSM	23.7 [ez]	22.6 [fy]	22.6 [fy]	22.5 [fy]
Hue angle				
DSM	41.2	42.5	41.4	41.5
SSM	39.9	40.8	39.9	39.9
Saturation index				
DSM	36.7 [ez]	35.4 [ey]	36.4 [eyz]	36.8 [ez]
SSM	37.0 [ez]	34.8 [ey]	35.3 [fy]	35.2 [fy]

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.01, Initial = 0.15, Display = 0.13, Discoloration = 0.11, L* = 0.65, a* = 0.40, b* = 0.29, Hue angle = 0.35, Saturation index = 0.45.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[c] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.

[d] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[ef] Means across muscle area within diet (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within diet (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across diet within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across diet within muscle area (within variable) without a common letter differ ($P < 0.05$).

Table 4.7 Muscle Area and Muscle Area × Display Day means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in HiOx

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.84 [z]	5.71 [y]
Initial color [b]	------	------	3.6 [y]	5.0 [z]
Discoloration [c]			1.8 [z]	1.6 [y]
d 0	1.0	1.0		
d 1	1.1	1.0		
d 2	1.2	1.1		
d 3	1.7	1.5		
d 4	2.3	2.2		
d 5	3.3	3.1		
L*			46.5 [z]	40.6 [y]
d 0	46.8	41.2		
d 3	46.6	41.0		
d 5	46.0	40.1		
a*			------	------
d 0	32.3 [ey]	31.4 [ez]		
d 3	25.5 [fz]	25.7 [fz]		
d 5	23.8 [gy]	24.5 [gz]		
b*			------	------
d 0	27.2 [ey]	25.5 [ez]		
d 3	22.5 [fy]	21.3 [gz]		
d 5	22.4 [fy]	21.7 [fz]		
Hue angle			------	------
d 0	40.1 [gy]	39.1 [gz]		
d 3	41.5 [fy]	39.6 [fz]		
d 5	43.3 [ey]	41.7 [ez]		
Saturation index			------	------
d 0	42.2 [ey]	40.5 [ez]		
d 3	34.1 [fz]	33.4 [fz]		
d 5	32.7 [gz]	32.8 [fz]		

[1] DSM = deep SM, SSM = superficial SM.
[a] SE: pH = 0.01, Initial = 0.07, Discoloration = 0.07, L* = 0.32, a* = 0.25, b* = 0.17, Hue angle = 0.20, Saturation index = 0.28.
[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.
[c] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.
[efg] Means across day within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across day within muscle area (within variable) without a common letter differ ($P < 0.05$).
[yz] Means across muscle area within day (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within day (within variable) without a common letter differ ($P < 0.05$).

Table 4.8 Diet, Day, and Diet × Display Day means[a] for instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in LoOx

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.46 [z]	5.48 [z]	5.42 [y]	5.47 [z]	**------**
CO_2 gas, %	**26.5**	**25.6**	**26.2**	**26.5**	
d 0	25.4	24.7	25.4	25.9	**25.3 [f]**
d 9	27.5	26.6	27.0	27.2	**27.1 [e]**
CO gas, %	**------**	**------**	**------**	**------**	
d 0	0.28 [ez]	0.28 [ez]	0.29 [ez]	0.28 [ez]	**------**
d 9	0.21 [fy]	0.23 [fz]	0.24 [fz]	0.24 [fz]	**------**
Odor score [b]	3.9	3.9	3.7	3.5	**------**
L*	**48.6 [y]**	**51.0 [z]**	**50.9 [z]**	**49.1 [yz]**	
d 0	50.4	52.4	52.5	50.2	**51.4 [e]**
d 9	46.7	49.5	49.3	47.9	**48.4 [f]**
a*	**36.3**	**35.8**	**36.6**	**36.9**	
d 0	37.0	36.5	37.1	37.4	**37.0 [e]**
d 9	35.5	35.1	36.2	36.3	**35.8 [f]**
b*	**25.0**	**25.2**	**25.2**	**25.5**	
d 0	26.0	26.2	26.3	26.4	**26.2 [e]**
d 9	24.0	24.3	24.2	24.5	**24.2 [f]**
Hue angle	**34.6**	**35.1**	**34.5**	**34.6**	
d 0	35.1	35.5	35.3	35.1	**35.2 [e]**
d 9	34.0	34.7	33.7	34.1	**34.1 [f]**
Saturation index	**44.1**	**43.8**	**44.5**	**44.8**	
d 0	45.3	45.0	45.5	45.8	**45.4 [e]**
d 9	42.9	42.7	43.5	43.8	**43.2 [f]**

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[a] SE: pH = 0.01, CO_2 = 0.42, CO = 0.01, Odor = 0.20, L* = 0.72, a* = 0.40, b* = 0.36, Hue angle = 0.23, Saturation index = 0.51.
[b] Odor scale: 1 = No off odor, 2 = Slight off odor, 3 = Small off odor, 4 = Moderate off odor.
[ef] Means across day within diet (within variable) without a letter do not differ ($P > 0.05$); means across day within diet without a common letter differ ($P < 0.05$).
[yz] Means across diet within day (within variable) without a letter do not differ ($P > 0.05$); means across diet within day without a common letter differ ($P < 0.05$).

Table 4.9 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks[b] packaged in LoOx

	Diet [1], day			
Variable	0	20	30	40
pH				
DSM	5.47	5.49	5.44	5.50
SSM	5.44	5.46	5.39	5.44
Display color [c]				
DSM	3.1	2.9	2.8	3.2
SSM	4.5	4.2	4.1	4.4
Discoloration [d]				
DSM	1.1	1.2	1.1	1.1
SSM	1.1	1.1	1.1	1.1
L*				
DSM	53.0	55.1	54.7	52.8
SSM	44.1	46.8	47.1	45.3
a*				
DSM	36.6	35.7	36.5	37.0
SSM	36.0	35.9	36.7	36.7
b*				
DSM	25.8	25.9	25.5	25.9
SSM	24.3	24.5	24.9	25.0
Hue angle				
DSM	35.2 [ey]	36.0 [ez]	34.9 [ey]	35.0 [ey]
SSM	33.9 [fz]	34.2 [fz]	34.1 [fz]	34.2 [fz]
Saturation index				
DSM	44.7	44.2	44.6	45.2
SSM	43.4	43.5	44.4	44.5

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.02, Display = 0.11, Discoloration = 0.03, L* = 0.76, a* = 0.40, b* = 0.36, Hue angle = 0.23, Saturation index = 0.51.

[b] DSM = deep SM, SSM = superficial SM.

[c] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.

[d] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[ef] Means across muscle area within diet (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within diet (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across diet within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across diet within muscle area (within variable) without a common letter differ ($P < 0.05$).

Table 4.10 Muscle Area and Muscle Area × Display Day means[a] for display and instrumental color variables of Zilmax fed-crossbred beef steer steaks packaged in LoOx

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.47 [z]	5.44 [y]
Discoloration [b]			1.1 [z]	1.1 [y]
d 0	1.0	1.0		
d 1	1.0	1.0		
d 2	1.0	1.1		
d 3	1.1	1.1		
d 4	1.1	1.0		
d 5	1.0	1.0		
d 6	1.1	1.0		
d 7	1.1	1.1		
d 8	1.2	1.1		
d 9	1.5	1.4		
L*			------	------
d 0	55.2 [ey]	47.6 [ez]		
d 9	52.6 [fy]	44.1 [fz]		
a*			36.4	36.3
d 0	37.3	36.8		
d 9	35.6	35.9		
b*			25.8 [z]	24.7 [y]
d 0	26.9	25.5		
d 9	24.7	23.8		
Hue angle			35.3 [z]	34.1 [y]
d 0	35.8	34.7		
d 9	34.7	33.5		
Saturation index			44.7 [z]	43.9 [y]
d 0	46.0	44.8		
d 9	43.3	43.1		

[1] DSM = deep SM, SSM = superficial SM.

[a] SE: pH = 0.01, Discoloration = 0.03, L* = 0.40, a* = 0.26, b* = 0.24, Hue angle = 0.14, Saturation index = 0.33.

[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[ef] Means across day within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across day within muscle area (within variable) without a common letter differ ($P < 0.05$).

[yz] Means across muscle area within day (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within day (within variable) without a common letter differ ($P < 0.05$).

Table 4.11 Diet, Day, and Diet × Display Day means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in LoOx

	Diet [1], day				Main effect means
Variable	0	20	30	40	Day
pH	5.71	5.71	5.71	5.70	------
CO_2 gas conc.	**27.1 [z]**	**26.2 [y]**	**25.7 [y]**	**26.3 [y]**	
d 0	25.5	24.8	24.6	24.7	**24.9 [f]**
d 9	28.7	27.6	26.9	27.8	**27.8 [e]**
CO gas conc.	**0.23**	**0.24**	**0.24**	**0.25**	
d 0	0.27	0.27	0.27	0.28	**0.27 [e]**
d 9	0.20	0.21	0.22	0.21	**0.21 [f]**
Odor score [b]	3.2	3.2	3.2	3.3	------
Initial color [c]	4.5	4.4	4.5	4.3	------
L*	**45.7**	**47.1**	**45.8**	**46.5**	
d 0	46.2	47.6	46.4	46.9	**46.8 [e]**
d 9	45.1	46.5	45.2	46.1	**40.6 [f]**
a*	**36.6 [z]**	**35.6 [y]**	**35.3 [y]**	**35.4 [y]**	
d 0	36.7	35.4	34.9	35.2	**35.6 [f]**
d 9	36.5	35.9	35.6	35.6	**35.9 [e]**
b*	**24.1 [z]**	**23.5 [y]**	**22.9 [y]**	**23.2 [y]**	
d 0	24.3	23.3	22.8	23.2	**23.4**
d 9	23.9	23.6	23.0	23.3	**23.5**
Hue angle	**33.4**	**33.4**	**33.0**	**33.3**	
d 0	33.5	33.4	33.2	33.3	**33.3 [e]**
d 9	33.2	33.4	32.8	33.2	**33.2 [f]**
Saturation index	**43.8 [z]**	**42.7 [y]**	**42.1 [y]**	**42.4 [y]**	
d 0	44.0	42.4	41.7	42.1	**42.6 [f]**
d 9	43.7	43.0	42.4	42.6	**43.0 [e]**

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.01, CO_2 = 0.32, CO = 0.005, Odor = 0.22, Initial = 0.14, L* = 0.67, a* = 0.31, b* = 0.28, Hue angle = 0.16, Saturation index = 0.41.

[b] Odor scale: 1 = No off odor, 2 = Slight off odor, 3 = Small off odor, 4 = Moderate off odor.

[c] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[ef] Means across day within diet (within variable) without a letter do not differ (*P* > 0.05); means across day within diet (within variable) without a common letter differ (*P* < 0.05).

[yz] Means across diet within day (within variable) without a letter do not differ (*P* > 0.05); means across diet within day (within variable) without a common letter differ (*P* < 0.05).

Table 4.12 Diet × Muscle Area means[a] for display and instrumental color variables of Zilmax fed-Holstein steer steaks packaged in LoOx

	Diet [1], day			
Variable	0	20	30	40
pH				
DSM	5.73	5.73	5.73	5.72
SSM	5.69	5.69	5.69	5.68
Initial color [b]				
DSM	3.6	3.5	3.6	3.5
SSM	5.4	5.2	5.4	5.1
Discoloration [c]				
DSM	1.1	1.1	1.2	1.1
SSM	1.1	1.1	1.1	1.1
L*				
DSM	49.6	51.1	50.2	50.6
SSM	41.8	43.1	41.4	42.4
a*				
DSM	37.2 [ez]	35.8 [ey]	36.3 [eyz]	36.3 [ey]
SSM	36.0 [fz]	35.5 [ez]	34.4 [fy]	34.6 [fy]
b*				
DSM	24.5 [ez]	23.8 [ey]	23.8 [eyz]	24.0 [eyz]
SSM	23.7 [fz]	23.2 [fyz]	22.0 [fx]	22.5 [fxy]
Hue angle				
DSM	33.4	33.6	33.2	33.5
SSM	33.3	33.1	32.7	33.0
Saturation index				
DSM	44.5 [ez]	43.0 [ey]	43.4 [ey]	43.5 [eyz]
SSM	43.1 [fz]	42.4 [ez]	40.7 [fy]	41.2 [fy]

[1] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[a] SE: pH = 0.01, Initial = 0.15, Discoloration = 0.01, L* = 0.72, a* = 0.34, b* = 0.29, Hue angle = 0.17, Saturation index = 0.43.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[c] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[ef] Means across muscle area within diet (within variable) without a letter do not differ (*P* > 0.05); means across muscle area within diet (within variable) without a common letter differ (*P* < 0.05).

[xyz] Means across diet within muscle area (within variable) without a letter do not differ (*P* > 0.05); means across diet within muscle area (within variable) without a common letter differ (*P* < 0.05).

Table 4.13 Diet × Muscle Area × Display Day means[a] for display color scores[b] of Zilmax fed-Holstein steer steaks packaged in LoOx

	Day of display									
	0	1	2	3	4	5	6	7	8	9
Diet [c]	DSM [d]									
0	2.8 [jpz]	2.8 [jpz]	3.2 [ipz]	3.9 [fpz]	3.5 [hpz]	3.3 [ipz]	3.7 [gpz]	3.7 [gpz]	4.2 [epz]	4.0 [epz]
20	2.4 [kpy]	2.6 [jpz]	3.0 [ipz]	3.7 [epz]	3.3 [ghpz]	3.2 [hpz]	3.3 [gpy]	3.5 [fpz]	3.8 [epy]	3.8 [epz]
30	2.5 [ipyz]	2.7 [ipz]	3.2 [hpz]	3.9 [epz]	3.2 [ghpz]	3.2 [hpz]	3.3 [gpy]	3.7 [fpz]	4.0 [epyz]	4.0 [epz]
40	2.4 [kpy]	2.7 [jpz]	2.9 [ipz]	3.7 [fpz]	3.2 [hpz]	3.2 [hpz]	3.4 [gpyz]	3.5 [gpz]	3.9 [epyz]	4.0 [epz]
	SSM									
0	4.4 [jqz]	4.5 [iqz]	4.7 [hqz]	5.1 [gqz]	5.1 [gqz]	5.1 [gqyz]	5.3 [fqz]	5.3 [fqz]	5.7 [eqz]	5.6 [eqyz]
20	4.1 [jqyz]	4.1 [jqy]	4.5 [iqz]	4.9 [gqz]	4.8 [hqyz]	5.1 [fgqyz]	5.2 [fqz]	5.1 [fqz]	5.4 [eqyz]	5.4 [eqyz]
30	4.3 [iqyz]	4.3 [iqyz]	4.7 [hqz]	5.2 [gqz]	4.7 [hqy]	5.3 [fgqz]	5.3 [fgqz]	5.4 [fqz]	5.7 [eqz]	5.7 [eqz]
40	3.9 [kqy]	4.2 [jqyz]	4.6 [iqz]	4.8 [hqz]	4.6 [iqy]	4.9 [ghqy]	5.1 [fqz]	5.0 [fgqz]	5.3 [eqy]	5.4 [eqy]

[a] SE: 0.14.
[b] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[efghijk] Means across day within diet (within muscle area) without a common letter differ (*P* < 0.05).
[pq] Means across muscle area within diet (within day) without a common letter differ (*P* < 0.05).
[yz] Means across diet within day (within muscle area) without a common letter differ (*P* < 0.05).

Table 4.14 Muscle Area and Muscle Area × Display Day means[a] for instrumental color variables of Zilmax fed-Holstein steer steaks packaged in LoOx

	Muscle Area [1]		Main effect means	
Variable	DSM	SSM	DSM	SSM
pH	------	------	5.72 [y]	5.69 [z]
Initial color [b]	------	------	3.6 [y]	5.3 [z]
L*			-------	-------
d 0	51.2 [ey]	42.4 [ez]		
d 9	49.6 [fy]	42.0 [fz]		
a*			36.4 [y]	35.1 [z]
d 0	36.1	35.0		
d 9	36.7	35.1		
b*			24.0 [y]	22.8 [z]
d 0	23.9	22.9		
d 9	24.2	22.8		
Hue angle			33.4 [y]	33.0 [z]
d 0	33.5	33.2		
d 9	33.4	32.9		
Saturation index			43.6 [y]	41.9 [z]
d 0	43.3	41.8		
d 9	43.9	41.9		

[1] DSM = deep SM, SSM = superficial SM.

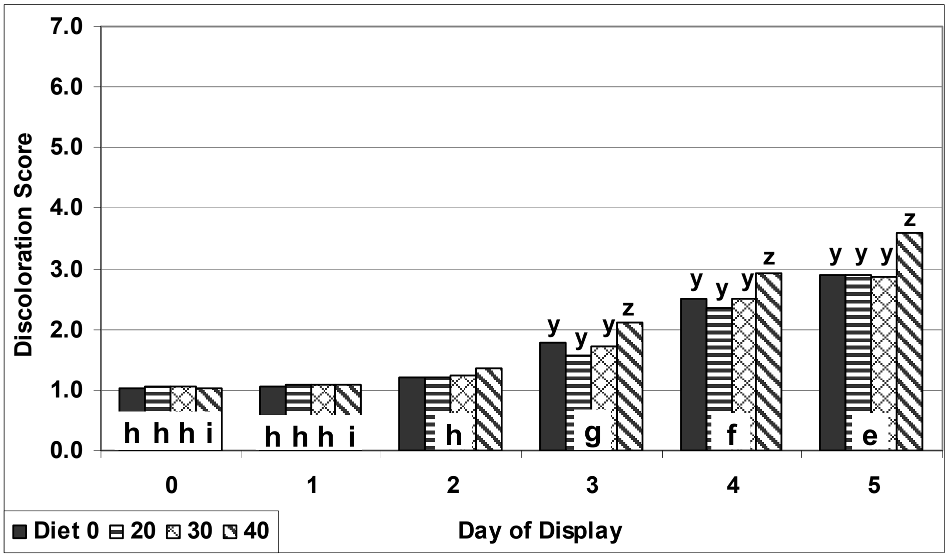
[a] SE: pH = 0.01, Initial = 0.07, L* = 0.34, a* = 0.18, b* = 0.15, Hue angle = 0.09, Saturation index = 0.22.

[b] Initial color scale: 1 = Purplish pink or red or reddish tan of vacuum packages, 2 = Bleached, pale red, 3 = Slightly cherry red, 4 = Moderately light cherry red, 5 = Cherry red.

[ef] Means across day within muscle area (within variable) without a letter do not differ ($P > 0.05$); means across day within muscle area (within variable) without a common letter differ ($P < 0.05$).

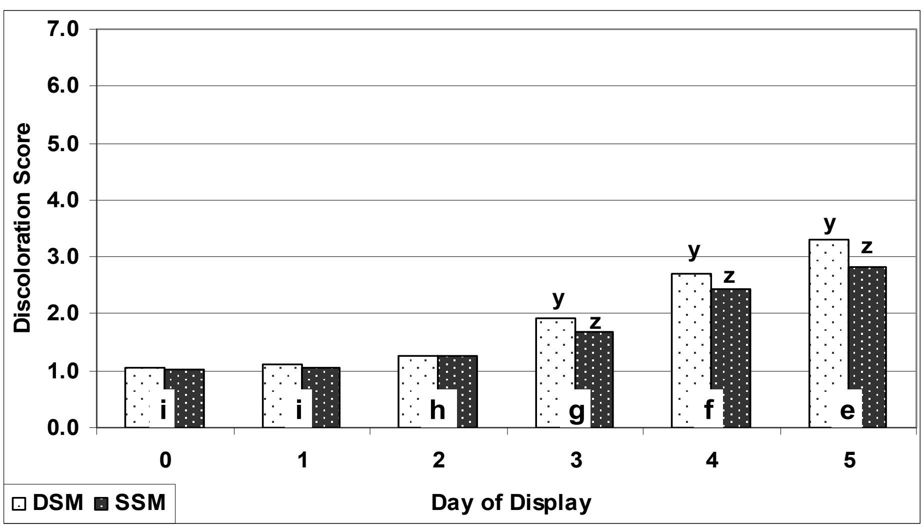
[yz] Means across muscle area within day (within variable) without a letter do not differ ($P > 0.05$); means across muscle area within day (within variable) without a common letter differ ($P < 0.05$).

Figure 4.1 Diet × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-crossbred beef steer steaks packaged in HiOx



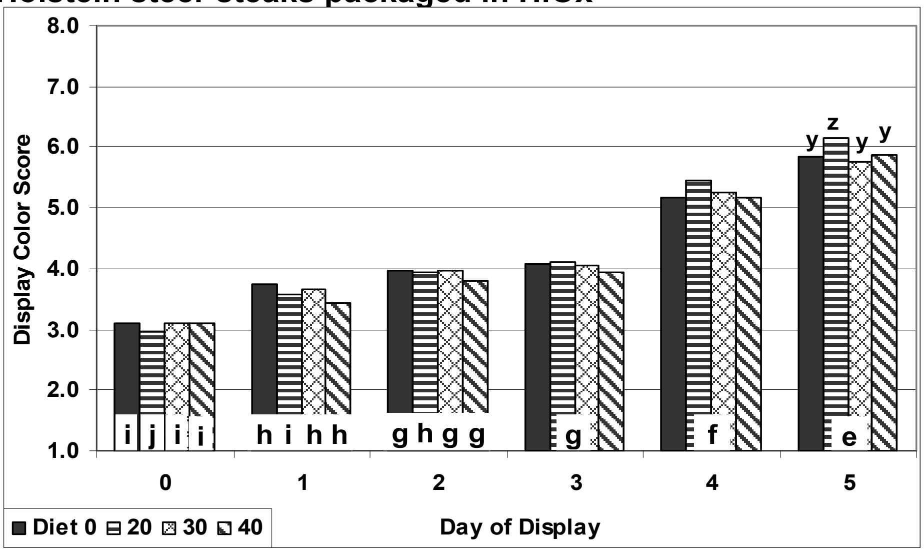
[a] SE: 0.11.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%, 5 = 60-79%.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efghi] Bars within diet across day without a common letter differ ($P < 0.05$).
[yz] Bars within day across diet without a letter do not differ ($P > 0.05$); bars within day across diet without a common letter differ ($P < 0.05$).

Figure 4.2 Muscle Area × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-crossbred beef steer steaks[d] packaged in HiOx



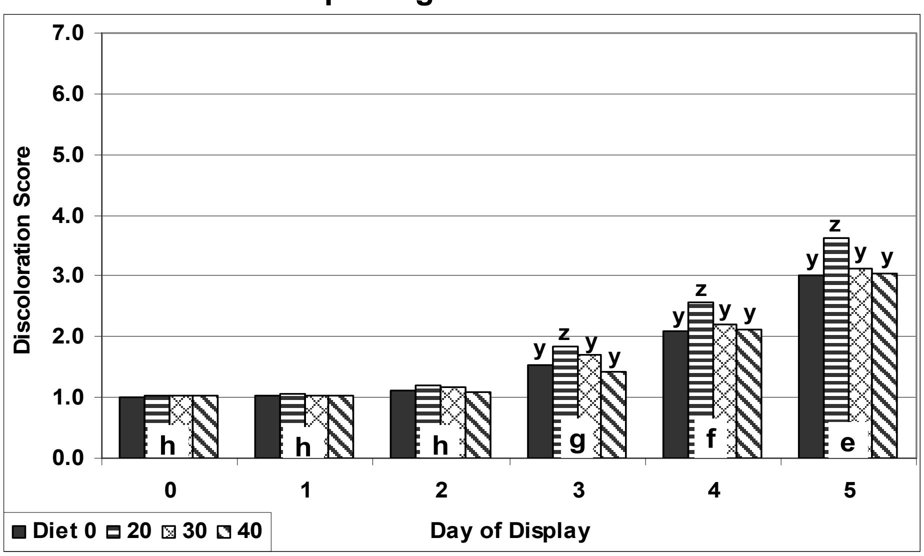
[a] SE: 0.07.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%, 5 = 60-79%.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[efghi] Bars within muscle area across day without a common letter differ ($P < 0.05$).
[yz] Bars within day across muscle area without a letter do not differ ($P > 0.05$); bars within day across muscle area without a common letter differ ($P < 0.05$).

Figure 4.3 Diet × Display Day means[a] for display color scores[b] of Zilmax[c] fed-Holstein steer steaks packaged in HiOx



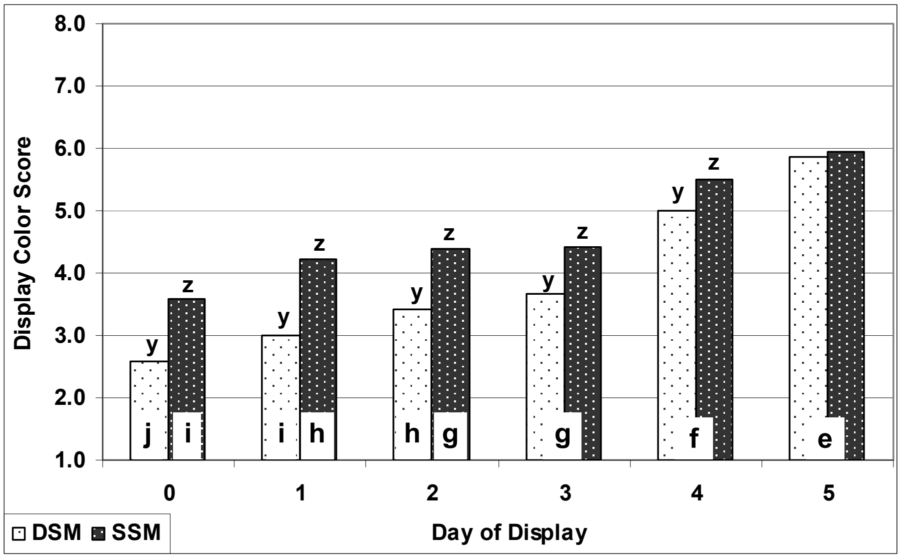
[a] SE: 0.14.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red, 7 = Tannish red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efghij] Bars across day within diet without a common letter differ ($P < 0.05$).
[yz] Bars across diet within day without a letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 4.4 Diet × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-Holstein steer steaks packaged in HiOx



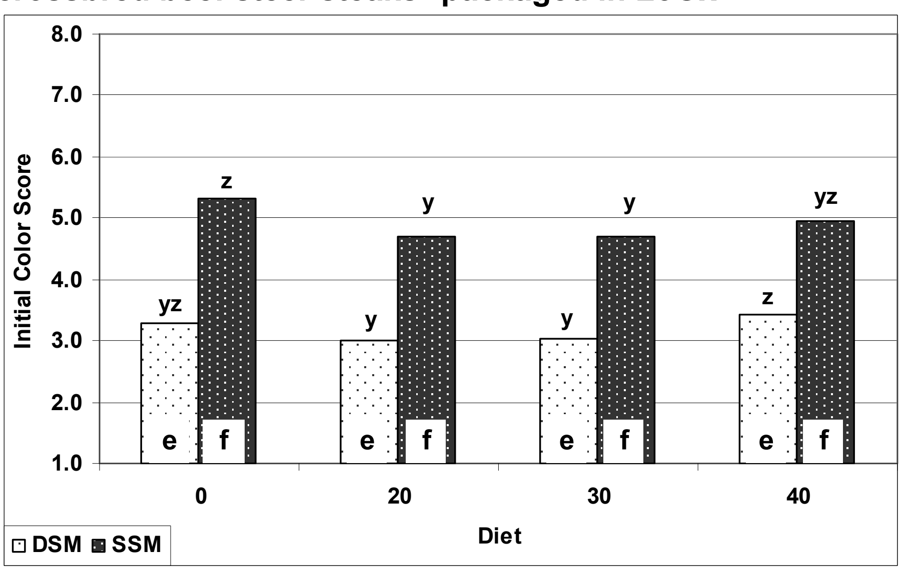
[a] SE: 0.14.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[efgh] Bars across day within diet without a common letter differ ($P < 0.05$).
[yz] Bars across diet within day without a letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 4.5 Muscle Area × Display Day means[a] for display color scores[b] of Zilmax fed-Holstein steer steaks[c] packaged in HiOx



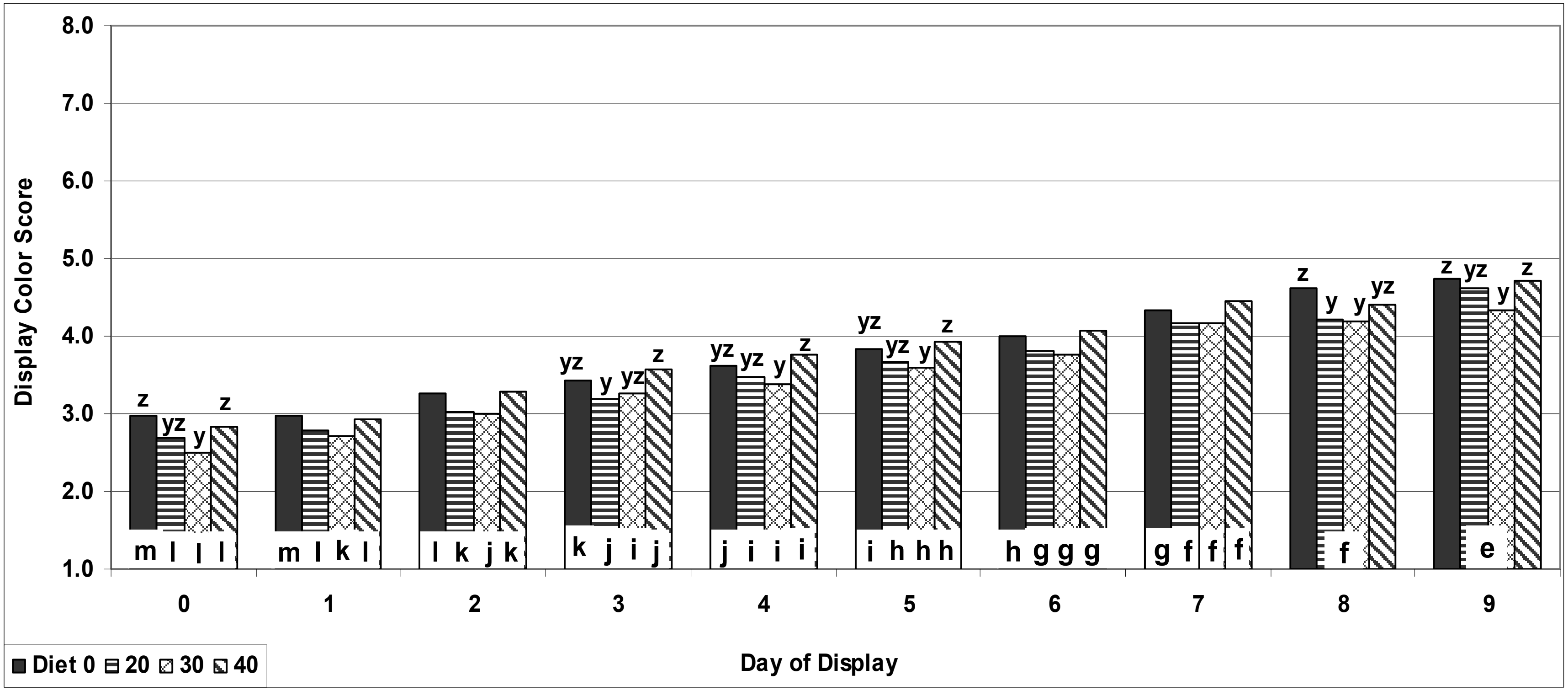
[a] SE: 0.07.
[b] Display color scale: 1 = Very bright red, 3 = Dull red, 5 = Moderately dark red, 7 = Tannish red.
[c] DSM = deep SM, SSM = superficial SM.
[efghij] Bars across day within muscle area without a common letter differ (*P* < 0.05).
[yz] Bars across muscle area within day without a letter do not differ (*P* > 0.05); bars across muscle area within day without a common letter differ (*P* < 0.05).

Figure 4.6 Diet × Muscle Area means[a] for initial color scores[b] of Zilmax[c] fed-crossbred beef steer steaks[d] packaged in LoOx



[a] SE: 0.14.
[b] Initial color scale: 1 = Purplish pink or red, 3 = Slightly cherry red, 5 = Cherry red.
[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[d] DSM = deep SM, SSM = superficial SM.
[ef] Bars across muscle area within diet without a common letter differ (*P* < 0.05).
[yz] Bars across diet within muscle area without a common letter differ (*P* < 0.05).

Figure 4.7 Diet × Display Day means[a] for display color scores[b] of Zilmax[c] fed-crossbred beef steer steaks packaged in LoOx



[a] SE: 0.11.

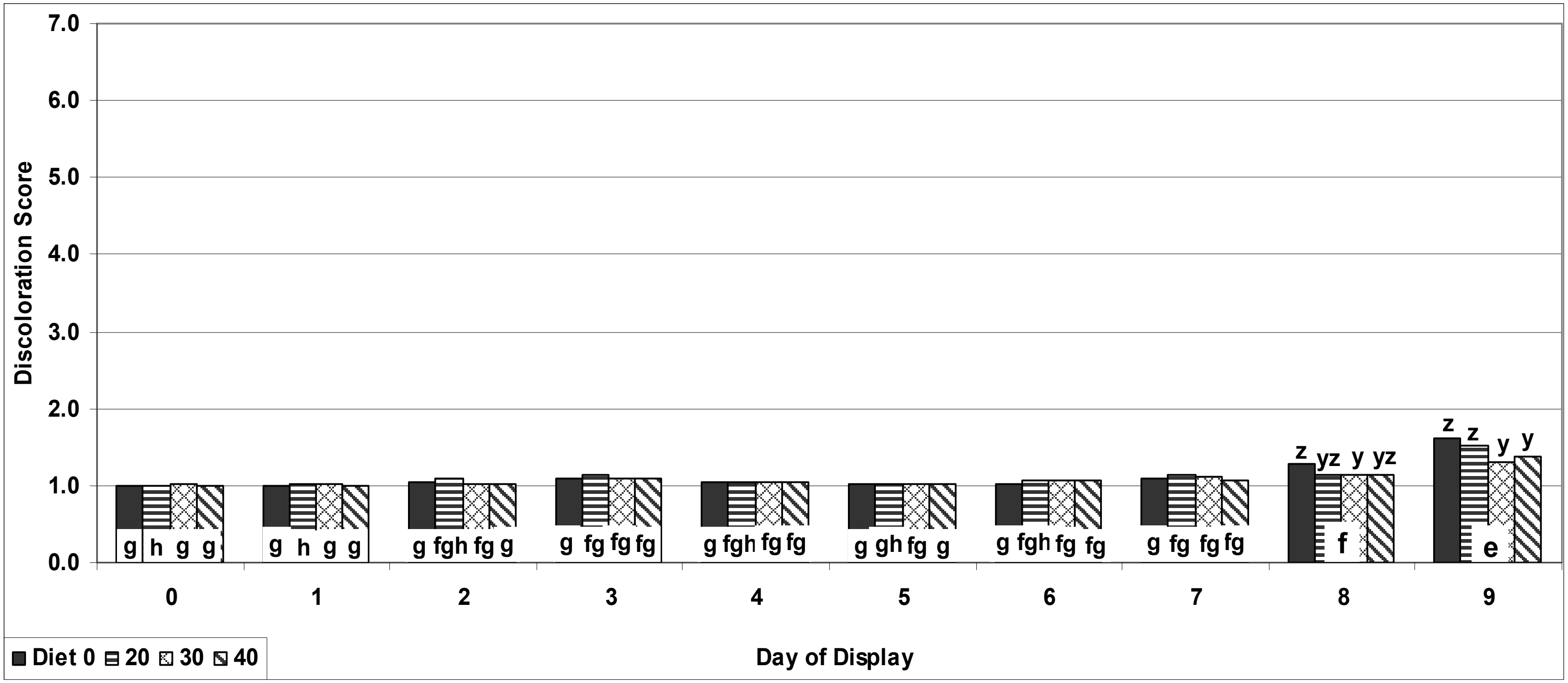
[b] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.

[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[egfhijklm] Bars across day within diet without a common letter differ ($P < 0.05$).

[yz] Bars across diet within day without a letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 4.8 Diet × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-crossbred beef steer steaks packaged in LoOx



[a] SE: 0.05.

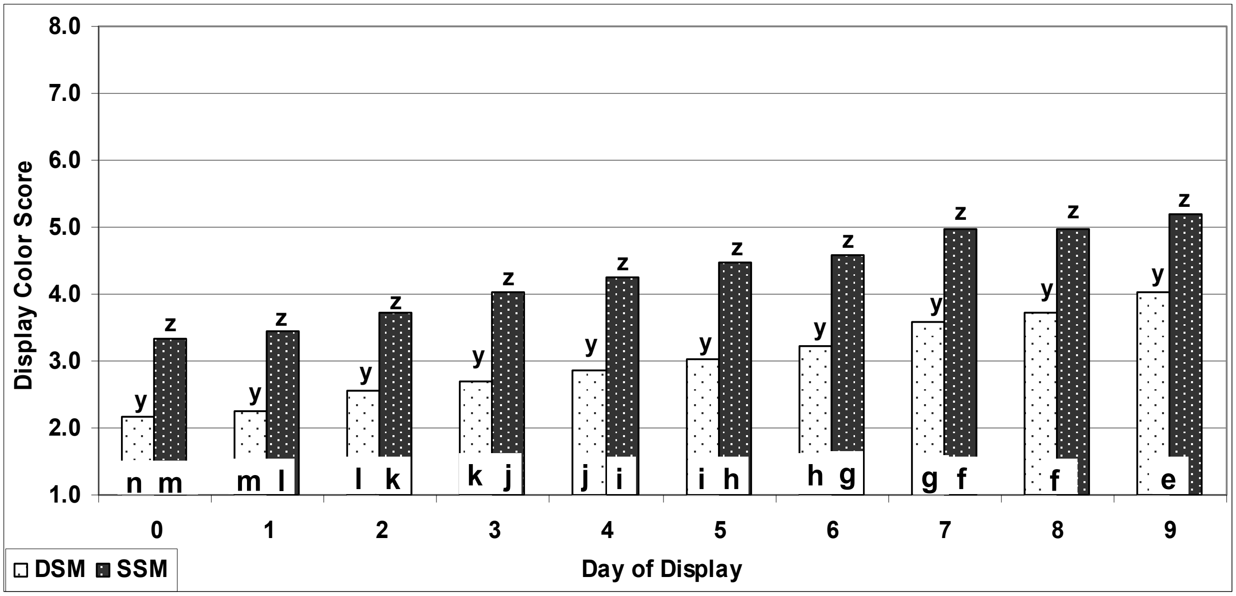
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[efgh] Bars across day within diet without a common letter differ (*P* < 0.05).

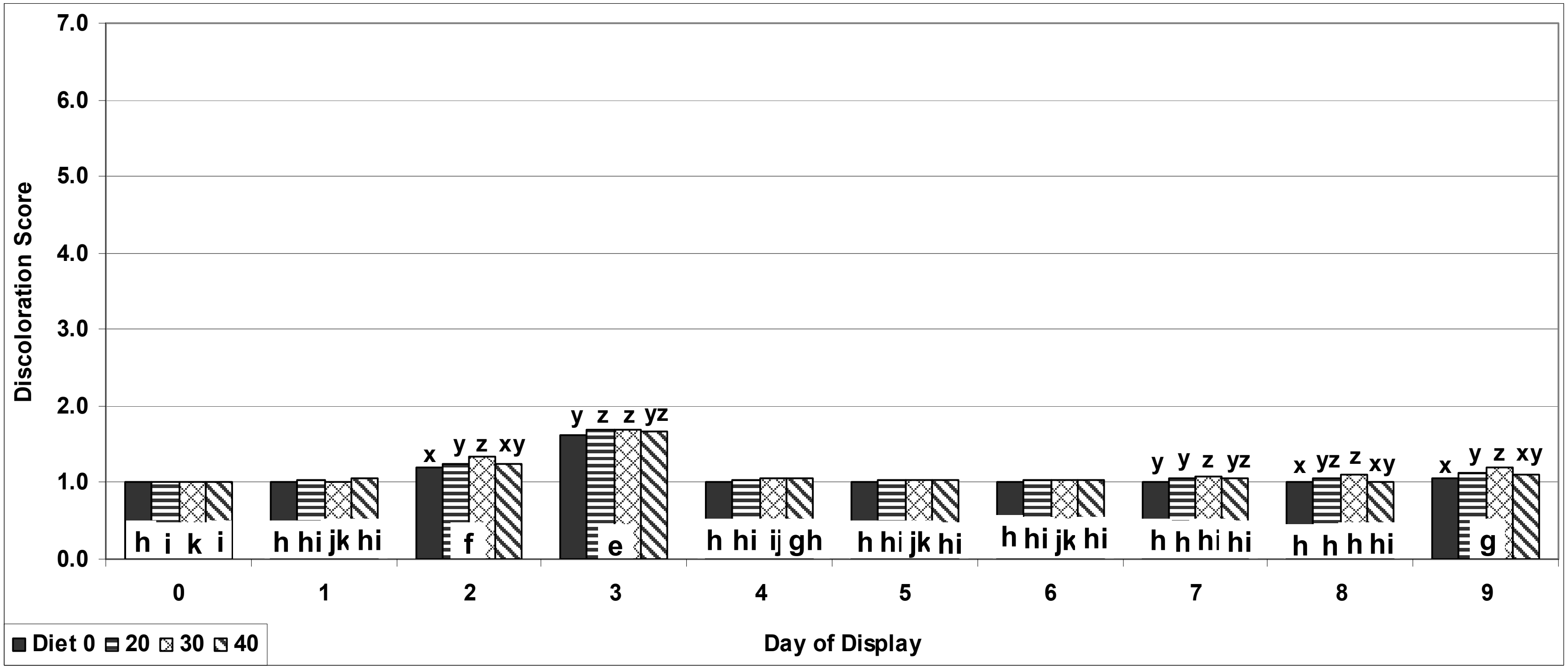
[yz] Bars across diet within day without a letter do not differ (*P* > 0.05); bars across diet within day without a common letter differ (*P* < 0.05).

Figure 4.9 Muscle Area × Display Day means[a] of display color scores[b] of Zilmax-fed crossbred beef steer steaks[c] packaged in LoOx



[a] SE: 0.06.
[b] Display color scale: 1 = Very bright red, 2 = Bright red, 3 = Dull red, 4 = Slightly dark red, 5 = Moderately dark red, 6 = Dark red.
[c] DSM = deep SM, SSM = superficial SM.
[efghijklmn] Bars across day within muscle area without a common letter differ (*P* < 0.05).
[yz] Bars across muscle area within day without a common letter differ (*P* < 0.05).

Figure 4.10 Diet × Display Day means[a] for discoloration scores[b] of Zilmax[c] fed-Holstein steer steaks packaged in LoOx



[a] SE: 0.02.

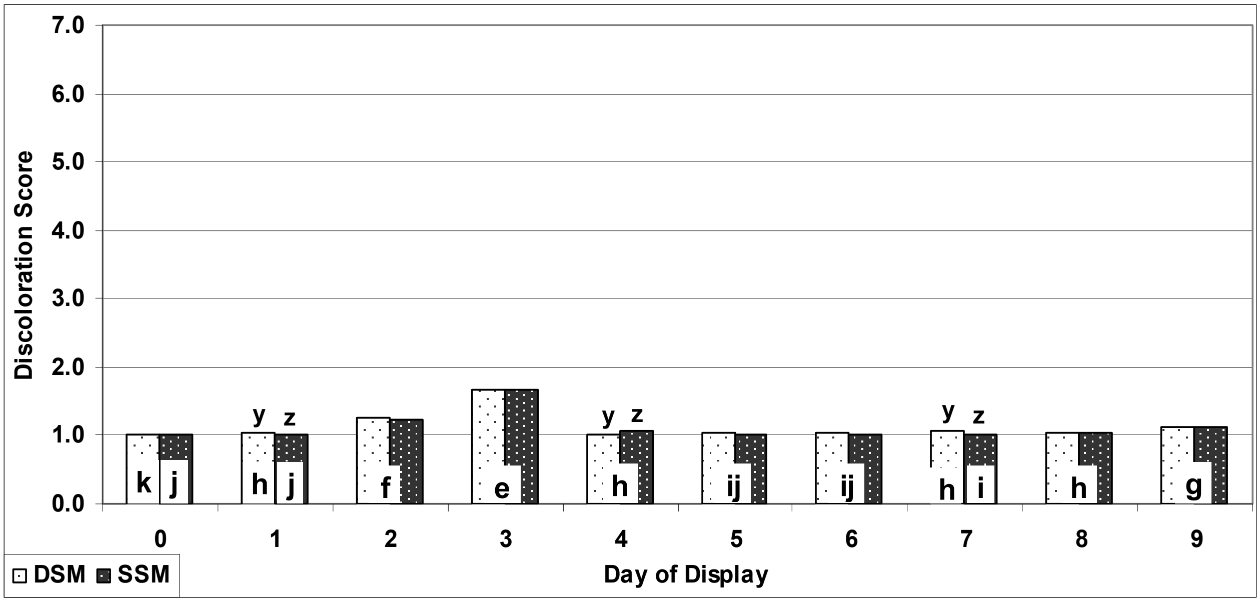
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.

[c] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.

[efghijk] Bars across day within diet without a common letter differ ($P < 0.05$).

[xyz] Bars across diet within day without a letter do not differ ($P > 0.05$); bars across diet within day without a common letter differ ($P < 0.05$).

Figure 4.11 Muscle Area × Display Day means[a] for discoloration scores[b] of Zilmax-fed Holstein steer steaks[c] packaged in LoOx



[a] SE: 0.01.
[b] Discoloration scale: 1 = 0%, 2 = 1-19%, 3 = 20-39%, 4 = 40-59%.
[c] DSM = deep SM, SSM = superficial SM.
[efghijk] Bars across day within muscle area without a common letter differ ($P < 0.05$).
[yz] Bars across muscle area within day without a letter do not differ ($P > 0.05$); bars across muscle area within day without a common letter differ ($P < 0.05$).

CHAPTER 5 - Conclusions from this Thesis

1. The supplementation of Zilmax to crossbred beef steers will not have any practical effects on *semimembranosus* steak color.

2. Feeding Zilmax for 20 or 30 days to crossbred beef steers will result in steak color characteristics equal to or better than steaks from control (no Zilmax) cattle.

3. The 40 day Zilmax feeding duration will tend to darken crossbred beef steak color in comparison to the 20 and 30 day feeding durations.

4. Holstein *semimembranosus* steaks from cattle with or without supplemented Zilmax will have more variability in color stability than crossbred beef steer steaks.

5. Regardless of Zilmax supplementation, color differences due to *semimembranosus* muscle areas (deep versus superficial) will still occur.

6. Packaging type will affect color shelf life with steaks in low-oxygen/carbon monoxide atmospheres having a longer shelf life than high-oxygen atmospheres which will be greater than steaks overwrapped with polyvinyl chloride.

7. Color characteristics of steaks regardless of dietary regimen or muscle will worsen as day of display increases.

Appendix A - Crossbred Beef and Holstein Steer Diet Composition

Table A.1 Diet (DM basis) fed to crossbred beef steers[a] supplemented with or without Zilmax[b] for 0, 20, 30, or 40 d

Ingredient [c]	Percent Included
Steamflaked Corn	74.3
Dried Distillers Grain, Corn	7.4
Chopped Alfalfa Hay	5.5
Corn Silage	5.8
Fat, animal	3.2
Finisher Supplement	3.8
Microingredient	0.03

[a] Days on Feed = 161, cattle were implanted on d 0 (Revalor-IS: 80 mg TBA and 15 mg estradiol) and d 80 (Revalor-IS).
[b] Zilmax was fed at a concentration of 7.56 g/ton on a 100% DM basis; cattle were withdrawn from Zilmax supplementation 3 d prior to harvest.
[c] Cattle were fed Rumensin and Tylan while not being supplemented with Zilmax; control steers were fed Rumensin and Tylan during the entire finishing phase.

Table A.2 Diet (DM basis) fed to Holstein steers[a] supplemented with or without Zilmax[b] for 0, 20, 30, or 40 d

Ingredient [c]	Percent Included
Steamflaked Corn	69.5
Alfalfa Hay	10.4
Sudangrass Hay	4.6
Yellow Grease	5.1
Corn Dried Distillers Grain	7.8
Urea	0.5
Calcium Carbonate	1.2
Ultraferm	0.8
Mineral Premix	0.2

[a] Cattle were implanted on d 0 (Revalor-IS: 80 mg TBA and 15 mg estradiol); steers were implanted (Synovex-S: 200 mg progesterone and 20 mg estradiol benzoate) 120 d before the Revalor-IS implant.
[b] Zilmax was added into a liquid supplement containing Ultraferm and Urea at 0.8% (100% DM basis) of ration; cattle were withdrawn from Zilmax supplementation 3 d prior to harvest.
[c] Cattle were fed Rumensin (30 g/ton) while not being supplemented with Zilmax; control steers were fed Rumensin during the entire finishing phase.

Appendix B - Carcass Data for Crossbred Beef and Holstein Steers

Table B.1 Carcass Data Summary for Crossbred Beef and Holstein steers fed Zilmax for 0, 20, 30, or 40 d

Type	Diet [a] (d Zilmax fed)	HCW [b] (kg)	PYG [c]	Adj. PYG	Fat Thickness (cm)	REA [d] (cm^2)	KPH [e] (%)	Final YG	Marbling Score
Crossbred Beef Steers	0	379	3.2	3.3	1.3	84.5	2.0	3.2	Slight 50
	20	378	3.1	3.2	1.2	94.2	1.9	2.6	Slight 70
	30	378	3.2	3.3	1.3	96.1	2.0	2.7	Slight 60
	40	367	3.4	3.5	1.5	99.4	2.0	2.6	Slight 50
Holsteins	0	404	2.6	2.6	0.6	88.4	2.8	2.6	Small 20
	20	420	2.8	2.8	0.8	91.0	3.0	2.9	Slight 80
	30	414	2.6	2.6	0.6	100.0	2.7	2.1	Slight 60
	40	427	2.6	2.6	0.6	93.5	2.2	2.5	Slight 90

[a] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[b] HCW = Hot carcass weight.
[c] PYG = Preliminary yield grade.
[d] REA = Ribeye area.
[e] KPH = Kidney, pelvic, and heart fat.

Appendix C - Means for Steak Weight Loss during Display

Table C.1 Zilmax-fed crossbred beef steer steaks packaged in HiOx[a]

Diet [b]	Pre-display wt. (g)	Post-display wt. (g)	% Wt. loss [c]
0	612.3	562.5	8.1
20	567.0	530.7	6.4
30	598.7	567.0	5.3
40	589.7	557.9	5.4

[a] SE: 0.04.
[b] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[c] % wt. loss = [(wt. of ingoing steak – blotted wt. of outcoming steak) ÷ ingoing steak wt.] × 100.

Table C.2 Zilmax-fed Holstein steer steaks packaged in HiOx[a]

Diet [b]	Pre-display wt. (g)	Post-display wt. (g)	% wt. loss [c]
0	539.8 [x]	476.3 [x]	11.8
20	589.7 [y]	526.2 [y]	10.8
30	621.4 [z]	557.9 [z]	10.2
40	616.9 [yz]	553.4 [yz]	10.3

[a] SE: 0.02.
[b] Cattle were fed Zilmax for 0, 20, 30, or 40 d prior to harvest.
[c] % wt. loss = [(wt. of ingoing steak – blotted wt. of outcoming steak) ÷ ingoing steak wt.] × 100.
[xyz] Means across diet within variable without a common letter differ ($P < 0.05$).

Table C.3 Zilmax-fed crossbred beef steer steaks packaged in LoOx[a]

Diet [b]	Pre-display wt. (g)	Post-display wt. (g)	% wt. loss [c]
0	580.6	517.1	10.9
20	553.4	503.5	9.0
30	562.5	512.6	8.9
40	557.9	512.6	8.1

[a] SE: 0.04.
[b] Cattle were fed Zilmax for 0, 20, 30 or 40 d prior to harvest.
[c] % wt. loss = [(wt. of ingoing steak – blotted wt. of outcoming steak) ÷ ingoing steak wt.] × 100.

Table C.4 Zilmax-fed Holstein steer steaks packaged in LoOx[a]

Diet [b]	Pre-display wt. (g)	Post-display wt. (g)	% wt. loss [c]
0	1.11 [x]	0.93 [x]	16.2
20	1.18 [y]	0.98 [y]	16.9
30	1.24 [z]	1.05 [z]	15.3
40	1.25 [z]	1.07 [z]	14.4

[a] SE: 0.02.
[b] Cattle were fed Zilmax for 0, 20, 30 or 40 d prior to harvest.
[c] % wt. loss = [(wt. of ingoing steak – blotted wt. of outcoming steak) ÷ ingoing steak wt.] × 100.
[xyz] Means across diet within variable without a common letter differ ($P < 0.05$).

Appendix D - Initial Color Sheet for All Packaging Types

COLOR SCORING SCALES FOR INTERVET ZILMAX RESEARCH PROJECT
Multi-university Study 2007 – Use 6 to 8 trained panelists

NAME: ______________________________ DATE: ____________

Initial Color Score Scale: To characterize on the initial appearance

1. Purplish pink or red or reddish tan of vacuum packages
2. Bleached, pale red
3. Slightly cherry red
4. Moderately light cherry red
5. Cherry red
6. Slightly dark red
7. Moderately dark red
8. Dark red
9. Very dark red

*****Score to half-point increments*****

Sample ID	Color Score (Deep)	Color Score (Superficial)	Sample ID	Color Score (Deep)	Color Score (Superficial)
280			290		
281			291		
282			292		
283			293		
284			294		
285			295		
286			296		
287			297		
288			298		
289			299		

Appendix E - Display Color Sheet for All Packaging Types

COLOR SCORING SCALES FOR INTERVET ZILMAX RESEARCH PROJECT
Multi-university Study 2007 – Use 6 to 8 trained panelists

NAME: ______________________________ DATE: ___________

HiOx MAP / LoOx-CO MAP
Muscle Color Score Scale

1. Very bright red or pinkish red
2. Bright red or pinkish red
3. Dull red or pinkish red
4. Slightly dark red or pinkish red
5. Moderately dark red or pinkish red

5.5. Borderline acceptability to panelist

6. Dark red or dark reddish tan or Dark pinkish red or dark pinkish tan
7. Tannish red or tannish pink
8. Tan to brown

****Score to half-point increments****

Discoloration Scale
Surface % MetMb

1. None (0%)
2. Slight discoloration (1-19%)
3. Small discoloration (20-39%)
4. Modest discoloration (40-59%)
5. Moderate discoloration (60-79%)
6. Extensive discoloration (80-99%)
7. Total discoloration (100%)

****Whole point increments only****

Sample ID	Color Score (Deep)	Discoloration Score (Deep)	Color Score (Superficial)	Discoloration Score (Superficial)
500				
501				
502				
503				
504				
505				
506				
507				
508				
509				
510				