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DISSERTATION

INJECTION-SITE LESIONS IN BEEF MUSCLES AND STUDY OF THE CHEMISTRY RESPONSIBLE FOR GREEN DISCOLORATION

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DEBORAH LYNN ROEBER ENTITLED "INJECTION-SITE LESIONS IN BEEF MUSCLES AND STUDY OF THE CHEMISTRY RESPONSIBLE FOR GREEN DISCOLORATION" BE ACCEPTED AS FULFULLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

INJECTION-SITE LESIONS IN BEEF MUSCLES AND STUDY OF THE CHEMISTRY RESPONSIBLE FOR GREEN DISCOLORATION

Numerous factors affect consumer acceptability of meat products sold via retail outlets. These factors include product quality, color, and packaging systems. Quality defects, such as injection-site lesions, have a negative impact on consumer acceptability. The desirability of the color of muscle can be assured or improved by presence/concentration of antioxidants (such as α-tocopherol) and by the packaging system in which retail products are displayed.

Damaged beef muscle tissue resulting from intramuscular injections of animal-health products represents a "quality control" problem and an economic loss to the beef industry. Audits were conducted in 1998, 1999, and 2000 on 3,190 rounds from cow carcasses in seven different states. Outside round muscles were cut into 1.25-cm slices to identify and characterize lesions. In 1998, 31% and 60% of rounds from carcasses of beef cattle and dairy cattle, respectively, had an injection-site lesion. Frequency of lesions in rounds from carcasses of beef cattle declined 5 percentage points ($P < 0.05$) between 1998 and 1999, and 6 percentage points ($P < 0.05$) between 1999 and 2000. Frequency of lesions in rounds from carcasses of dairy cattle declined 9 percentage points

($P < 0.05$) between 1998 and 1999, and 16 percentage points ($P < 0.05$) between 1999 and 2000. Frequencies of injection-site lesions in muscles of rounds from carcasses of beef cattle were significantly lower than those in muscles of rounds from carcasses of dairy cattle in all three years. Injection-site lesions were most common between the hooks and pins of the hindquarter of beef cattle, and between the pins and hocks of the hindquarter of dairy cattle. Clear lesions and woody calluses exceeded 89%, and occurred more frequently than did other kinds of lesions in muscles of rounds from carcasses of beef and dairy cattle in 1998, 1999, and 2000. Of all injection-site lesions, between 3% and 5% were cystic in muscles of rounds from beef carcasses, and between 2% to 4% were cystic in muscles of rounds from dairy carcasses.

Fifteen individual and sequential national audits of injection-site lesions in fed beef top sirloin butts have been conducted at the steak provisioner/cutting level between November 1995 and July 2000. The national incidence of injection-site lesions in top sirloin butts (n = 240,080) decreased ($P < 0.05$) between November 1995 (11.4%) and July 2000 (2.1%). From November 1995 to July 1997, mean weight per injection-site lesion, across all lesion classes, increased ($P < 0.05$) from 192.5 g to 435.8 g, respectively; mean weight per lesion subsequently decreased ($P < 0.05$) to 249.8 g in July 2000, but was still heavier ($P < 0.05$) than in November 1995.

During the 2000 National Beef Quality Audit, concern was raised by one of the major packers relative to a 'greening' of injection-site lesions located in muscles of chuck steaks and/or roasts packaged in a high-oxygen environment. Supplementation of vitamin E has been shown to enhance retail caselife by maintaining bright, cherry-red color of muscles. Fifty steers were vaccinated with Conquest 5K and were allocated to

one of two groups; control or vitamin E supplementation for 60 days. Following boxed storage and retail display, 80% of control steaks and 96% of vitamin E steaks had a green injection-site lesion present. Of those steaks that exhibited greening, 39.5% of the steaks did not have a visible lesion at the time of packaging. Color attributes; L*, a*, and b*; of muscle tissue did not differ between control and vitamin E steaks prior to packaging or after retail display, nor were there difference in L*, a* or b* in injection-site lesions after retail display. Thiobarbituric acid values were greater ($P < 0.05$) in control steaks at the site of the green injection-site lesion and at a point 6 cm away from the injection-site lesion. Histological evaluation verified the presence of injection-site lesions; the tissue contained fibrous connective tissue/scarring, sheet of macrophages, lymphoid follicles, atrophied muscle fibers, fat cells, and adjuvant. Although results suggested that vitamin E supplementation may delay onset of the chemical reaction causing greening of injection-site lesions when packaged in high oxygen modified atmosphere, and may reduce the degree of oxidative rancidity formation, supplementation does not have the potential to eliminate the 'greening' reaction.

Combinations of myoglobin (Mb), copper sulfate ($CuSO_4$), hydrogen peroxide (H_2O_2), vaccine, and aluminum hydroxide were made and subjected to high partial pressures of oxygen. In phase II, combinations of Mb, copper (Cu), sodium sulfide (NaS), sodium sulfite ($NaSO_3$), sodium sulfate ($NaSO_4$), and H_2O_2 were made at pH 7.2 or 5.5 and subjected to either low or high partial pressures of oxygen. All combinations and extracted lesions were evaluated using spectrophotometry. The pigments from the green lesions from control and Vitamin E supplemented cattle displayed, on average, a 164.5 and 621.3 percent increase at 656 nm as compared to 654 nm, respectively. The

absorbance for the lesions from control and supplemented cattle declined 75 and 109 percent, respectively, from 656 to 658 nm. These changes in absorbance matched the changes in absorbance observed in the sulfmyoglobin and hydroperoxymetmyoglobin pigments made in phase I. Based on the results from phase I, it was concluded that the green color could be a result of $CuSO_4$ or H_2O_2. Combinations, in phase II, which exhibited a positive percent change from 654 to 656 nm and a negative percent change from 656 to 658 nm were Mb+$NaSO_4$, Mb+Cu+H_2O_2, Mb+SO_3, Mb+Cu+NaS, and Mb+Cu+$NaSO_3$, all at pH 7.2 under low partial pressures of oxygen; Mb+$NaSO_4$, Mb+NaS, and Mb+$NaSO_3$, all at pH 7.2 under high partial pressures of oxygen; and Mb+Cu+$NaSO_4$ at pH 5.5 under high partial pressures of oxygen. Results indicated that the 'greening' reaction observed in injection-site lesions of the chucks was a result of the reaction between myoglobin and sulfur, and, as such, suggest that there is an increase of sulfur at the site of the injection-site lesion.

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DEDICATION

I would like to dedicate this dissertation to my immediate and extended family—you all know who you are. This would not have been possible without the love, support and guidance of those around me and those far away. I love you all very much!!

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CHAPTER I

OBJECTIVES OF DISSERTATION

The objectives of this dissertation were:

(1) To characterize the frequency and severity of injection-site lesions in muscles from outside rounds of beef and dairy cow carcasses.

(2) To determine the incidence of injection-site lesions in top sirloin butts from fed steers and heifers using data collected from November 1995 through July 2000.

(3) To determine the cause of the green discoloration of injection-site lesions in muscles of the chuck after high-oxygen, modified atmosphere packaging.

(4) To compare the severity of discoloration of injection-site lesions in chucks from carcasses of cattle supplemented with α-tocopheryl acetate versus discoloration of injection-site lesions in chucks from carcasses of cattle not supplemented with α-tocopheryl acetate.

CHAPTER II

LITERATURE REVIEW

INTRODUCTION

Numerous factors affect consumer acceptability of meat products sold via retail outlets. These factors include product quality, color, and packaging. Quality defects, such as injection-site lesions, have a negative impact on consumer acceptability of beef. Color of meat is, in large part, dependent on the proportional concentrations of reduced myoglobin, oxymyoglobin, and metmyoglobin, which are the three primary forms of myoglobin in muscle. The desirability of the color of muscle can be assured or improved by presence/concentration of antioxidants such as α-tocopherol and by the system, such as modified atmosphere packaging, in which retail products are displayed.

Injection-Site Lesions

Pharmaceuticals and biologicals are commonly administered to cattle at various stages of their lives (Taylor and Field, 1999). If injections are given intramuscularly, in the anatomical region between the hooks and pins, tissue damage occurs (Dexter *et al.*, 1992). The Final Report of the 1991 National Beef Quality Audit (NBQA—1991), raised concerns that producers and/or veterinarians should choose alternative anatomical locations (sites) at which intramuscular injections are administered (Smith *et al.*, 1992).

In the NBQA—1991 Final Report, it was suggested that "Effort must be made to monitor and audit the incidence of injection-site lesions in other anatomical regions of the carcass; particularly important would be determination of occurrence of injection-site lesions in the neck, chuck, bottom round and other sections of carcasses" (Smith *et al.*, 1992). Results of the National Beef Quality Audit–1995 (Smith *et al.*, 1995) revealed that 30-40% of purveyors, retailers, and packers believed that the frequency of injection-site lesions had decreased since NBQA—1991. Even with such improvement, purveyors and retailers still ranked this defect in the top ten quality challenges for fed steer and heifer producers.

Injection-site lesions are seldom detected at packing plants because damage is concealed within the muscles and below subcutaneous fat. Unless top sirloin butts are further processed by packers (including removal of subcutaneous fat and separation of the *biceps femoris* from the *gluteus medius*), injection-site damage will normally be first exposed at retailer or purveyor establishments during portioning of the primal cuts.

Extent of damage at the injection-site is dependent on the calf's age at injection, volume of product injected per site, anatomical site of the injection, route of injection and product injected (Van Donkersgoed *et al.*, 2000). The consequences of injection-site lesions are not limited to lost use of meat product due to the presence of the lesions. As a result of injection-site lesions, visible or not within a steak, tenderness is affected (George *et al.*, 1995b). In top sirloin butt steaks, cores from steaks with injection-site lesions were significantly tougher as far as 7.6 cm from the center of the lesion as compared to cores from control steaks (George *et al.*, 1996). In beef rounds, George *et al.* (1995b) documented that cores taken as far as 7.6 cm from the center of the injection-

site lesion had average shear force values of 5.8 kg, a value that is too high to be considered tender by those in the restaurant or retail trade.

Dexter *et al.* (1994) reported that activities of the National Cattlemen's Association (subsequently, National Cattlemen's Beef Association, NCBA), Quality Assurance Advisory Board led to a reduction in the incidence of injection-site lesions from 21.3% (July 1991) to 10.9% (March 1993). George *et al.* (1996) reported that continuation of these efforts did not result in a reduction in injection-site lesion incidence from July 1993 (10.9%) to July 1995 (10.2%). Van Donkersgoed *et al.* (1997) reported that, in Canada, the incidence of injection-site lesions was 18.8% in top butts, 22.2% in boneless blades, 4.9% in eye of rounds, 1.8% inside rounds, and 7.6% in outside rounds.

In the mid-1990s, concern was registered that, although cattle feeders had received and responded-to the message regarding ways to reduce the incidence of injection-site lesions, the message was not being received or reacted-to by managers in other sectors of the beef production chain, specifically stocker operations and cow-calf producers (Roeber, 2000b). To ascertain the validity of the argument that cow/calf producers, stockers and/or backgrounders might be contributing to the incidence of injection-site lesions—that show up later in the life of the animal—by administering calf-hood intramuscular injections (2 to 6 months of age), a study was conducted by Colorado State University to determine if age of animal at time of injection was influential in the development of injection-site lesions (Roeber, 2000b). The study determined that intramuscular injections: (a) given to two-month-old calves generated lesions (5.3 to 92.7% of the time), and (b) given to six-month-old calves caused lesions (10.0 to 92.3% of the time) in beef cuts from cattle in both groups (a and b), when they were slaughtered

12 to 14 months later (George *et al.*, 1995a). These results provided concrete evidence that the location rather than the timing (when given) of administration of injections that is more important in causing injection-site lesions (George *et al.*, 1995a).

In 1994, it was obvious that initial educational efforts caused some producers to merely change the site of administration of injections—away from the region of the top sirloin butt and to regions like the inside and outside round (areas that are of nearly equal commercial concern as is the top sirloin butt). As a result, an audit was conducted in 1994 to determine the incidence and severity of injection-site lesions in muscles of the round from slaughter steers/heifers. Results of the latter audit in 2000 revealed that 10.37% of rounds from carcasses of fed steers/heifers had an injection-site lesion (Roeber *et al.*, 2000b).

During Face-To-Face interviews of the National Non-Fed Beef Quality Audit—1994 (NNFBQA—1994), one of the cow/bull packers commented that approximately one-in-four (25%) of the rounds fabricated in their facility contained an injection-site lesion that required significant trimming (Smith *et al.*, 1994b).

Recommendations to avoid injection-site lesions provided to cattle producers and veterinarians have included: (1) Administer all clostridial bacterins subcutaneously in the neck regions, preferably using the 'tented' technique, (2) avoid repeat or multiple injections of clostridial bacterins, especially late in the feeding period, (3) avoid intramuscular injections of all injectable products whenever other routes of administration are listed in the label recommendations, (4) use acceptable intramuscular and subcutaneous injection locations, (5) encourage the use of subcutaneous routes whenever possible, (6) inject no more than 10 ml per injection site (less in lighter calves),

(7) change needles every 15 injections (more often, if cattle are dirty), (8) immediately discard bent needles – never straighten or reuse them, (9) do not use products that damage edible tissue at any time during production, (10) choose products that are approved for subcutaneous, intravenous or oral administration whenever possible, and (11) choose products that have low-volume doses whenever possible (George *et al.*, 1997).

While recent data indicates that 50% of cow-calf producers and 35% of veterinarians still administer intramuscular injections in the upper rear leg/hip (NAHMS, 1997), educational programs conducted by the National Cattlemen's Beef Association and state cattlemen's associations have assisted in changing the management practices of numerous producers. The Final Report of the 2000 National Beef Quality Audit (NBQA—2000), it was reported that 11.7% of seedstock producers, 15.4% of cow-calf producers, 15.4% of stocker/backgrounders, and 13.7% of feedlot operators had changed their injection-site location since 1991 (Roeber *et al.*, 2002).

Meat Color

Three properties by which consumers judge meat quality are appearance, texture, and flavor (Faustman and Cassens, 1990). Appearance of beef in the retail case affects acceptability of the product to consumers (Liu *et al.*, 1996); bright-red muscle color suggests freshness to retail customers and is used in beef purchasing decisions (Clydesdale and Francis, 1971). Consumers discriminate against meat cuts that lack fresh appearance (Faustman and Cassens, 1990; Kropf, 1980). The strategy for maximizing the longevity of acceptable fresh muscle color must involve delaying muscle pigment

oxidation and/or enhancing reduction of oxidized muscle pigment (Faustman and Cassens, 1990).

Parameters which influence meat color include, but are not limited to: (1) surface dehydration, (2) temperature, (3) oxygen requirements of the meat, (4) bacterial contamination, (5) pH, and (6) myoglobin concentration (Clydesdale and Francis, 1971). Intensity of color is determined by species, stress, sex, age of animal, rate of postmortem pH decline, and ultimate pH of meat (Lawrie, 1998). Muscles from different meat animal species differ in color intensity because of comparative myoglobin concentrations; beef has the highest and is therefore the darkest, while pork has the lowest and is therefore the lightest (Lawrie, 1998). Factors that affect meat color stability include pH, temperature, relative humidity, light, bacteria, and lipid oxidation (Lawrie, 1998).

Lipid oxidation. Radicals produced during lipid oxidation act directly to promote pigment oxidation, and/or indirectly by damaging pigment reducing systems. The lipid soluble antioxidant, vitamin E, has been included in animal diets to increase lipid stability in meat subsequently obtained from these animals (Lawrie, 1998). Enhancement of vitamin E concentration improved pigment and lipid stability in ground meats (Faustman and Cassens, 1989) and improved muscle color appearance in some steaks (Faustman *et al.*, 1989b).

Oxidation. Many factors regulate meat color stability; however, the process of myoglobin autoxidation (oxidation without assistance from exogenous agents) is paramount (Renerre *et al.*, 1996). Some believe that autoxidation of myoglobin and oxygen consumption rate are the most important factors in color stability, while others believe the activity of the reducing system is the most important (Echevarne *et al.*, 1990).

Oxidative processes affect pigments, lipids, proteins, carbohydrates, vitamins and the overall quality of food (Kanner, 1992). Prooxidants and antioxidants, as well as the state of cells (injured cells are more prone to oxidation), impact overall oxidation (Kanner, 1992). The rate of autoxidation also is dependent on oxygen tension and is highest at half saturation (Watts *et al.*, 1966).

Reduction of oxygen via one electron reduction processes yields several products: superoxide anion (O_2^-) radical, perhydroxyl radical (HO_2^-), hydrogen peroxide (H_2O_2) and a hydroxyl radical (HO^-); all of which participate in the oxidative processes in meat (Kanner, 1992).

Partial oxygen pressure. Faustman and Cassens (1990) cited research that discovered that low, non-zero, oxygen partial pressures enhanced hemoglobin oxidation. Faustman and Cassens (1990) cited research that documented that depth of oxygen penetration in postmortem muscle increased during storage due to the decrease in tissue oxygen consumption. An increase in partial pressure of oxygen can prolong bright red color in muscle during short-term storage (Clark and Lentz, 1973; Bartkowski *et al.*, 1982; Cole, 1986). However, Cole (1986) reported an increase in oxidative rancidity in beef stored in high oxygen environments.

Meat Color Chemistry

Myoglobin. Myoglobin consists of a single polypeptide globin and a prosthetic heme group (Murray *et al.*, 2000). The heme group consists of a centrally located iron atom that has six coordination sites; four sites are in the plane of, and bonded to, the N_2 atoms of four flat porphyrin rings, while the other two sites lie perpendicular in structure

(Murray *et al.*, 2000). One perpendicular site is connected to an N_2 atom of the globin protein molecule and the other site is open and available for binding various ligands (Murray *et al.*, 2000). The heme portion contains a centrally located atom of iron (Fe) within 4 pyrollic rings. The valence state of iron atoms and the ligand bound to the free binding site of heme are the primary factors responsible for the color of hemoglobin and myoglobin and, subsequently, of the muscles in meat (Jeyamkondan *et al.*, 2000).

Myoglobin has a molecular weight of 16,700 Daltons, is equal to 1 of the 4 subunits of hemoglobin, and is primarily found within muscle cells. The ability to combine reversibly with oxygen is the most striking characteristic of myoglobin (Kagen, 1973). Kagen (1973) demonstrated the hyperbolic oxygen binding curve for myoglobin. Quantitative relationships favor more oxygen binding by myoglobin than hemoglobin at low oxygen tensions. The main function of myoglobin is oxygen transport, catalysis, and storage (Murray *et al.*, 2000).

Factors that influence heme pigment functionality and, subsequently, meat color (Price and Schweigert, 1987) include:

1. Iron oxidation state
    a. Ferrous (Fe^{2+}) – red or purple
    b. Ferric (Fe^{3+}) – red or brown
2. Ligand
    a. None (Fe^{2+} only) – purple
    b. Strong (coordinate covalent) – red
    c. Weak (ionic) – red or brown
3. Physical state of protein
    a. Native – red, purple or brown
    b. Denatured – pink or brown
4. Porphyrin Integrity
    a. Intact – red, purple or brown
    b. Substituted – green
    c. Cleaved – green, brown or yellow

Forms of Myoglobin and Common Color Reactions. The three primary reversible forms of the globin pigment in muscle are reduced myoglobin, oxymyoglobin, and metmyoglobin. Reduced myoglobin is purple in color and is the predominant form in the absence of oxygen, oxymyoglobin is red in color and is most desirable in appearance to consumers, and metmyoglobin, which is brown in color, is the predominant color form where low oxygen tensions prevail and is caused by the oxidation of the iron of heme in myoglobin (Cole, 1986; Lawrie, 1998). Additionally, the iron atom in metmyoglobin is oxidized (that is, in the ferric form) and cannot bind oxygen (Lawrie, 1998). Common muscle color reactions are further outlined in Figure 2.1 and Table 2.1.

Heme iron may exist in the reduced ferrous, or oxidized, ferric form. Ferrous heme iron lacks the sixth ligand and is known as deoxymyoglobin (Faustman and Cassens, 1990). When oxygen occupies the sixth binding site of ferrous heme iron, oxymyoglobin results and is responsible for the cherry red color (Faustman and Cassens, 1990). Metmyoglobin is formed when water occupies the sixth coordination position, is incapable of binding oxygen, and is physically inactive (Faustman and Cassens, 1990).

Conditions like low oxygen tension, high temperature, presence of ultraviolet rays, and exposure to atmospheric oxygen for long periods of time cause denaturation of the globin moiety of myoglobin, resulting in a loss of its functional properties (Seideman *et al.*, 1984). Once the globin molecule is denatured, the iron atom is left unprotected which leads to spontaneous oxidation of the iron molecule from the ferrous to ferric state resulting in the formation of brown metmyoglobin (Seideman *et al.*, 1984).

The Green Color Reaction. Greening discoloration in fresh meat is associated with an alteration in heme structure (Lawrie, 1998). Green color is normally attributed to sulfmyoglobin, cholemyoglobin, or verdoheme formation (Lawrie, 1998). Another green discoloration in meat results from interaction between myoglobin and hydrogen peroxide, resulting in the formation of hydroperoxymyoglobin (Lawrie, 1998).

Choleglobin. Green pigmentation in meat has been attributed to an oxidative attack on the pyrole ring of the heme pigments, producing choleglobin (Naughton *et al.*, 1958). Choleglobin can be distinguished from sulfhemoglobin by its conversion to cholehemochrome with an alkali and dithionite (Lemberg and Legge, 1949). Choleglobin is produced from hydrogen peroxide acting on hemoglobin in the presence of ascorbic acid (Lemberg and Legge, 1949).

Hydroperoxymyoglobin. Green pigments with cleaved rings are formed as a result of controlled oxidation by oxygen or peroxides in the presence of reductants (Price and Schweigert, 1987). Metal ions, such as copper and, to a lesser extent, iron, zinc, and aluminum, can catalyze oxidation of oxymyoglobin to metmyoglobin (Price and Schweigert, 1987). Catalase-negative bacteria also can produce green discolorations by causing a build-up of hydrogen peroxide in the tissues; under mildly acid conditions of meat, hydrogen peroxide will react with myoglobin to produce a green pigment known as hydroperoxymetmyoglobin (Price and Schweigert, 1987). The reaction between metmyoglobin and hydrogen peroxide changes with a change in pH (George and Irvine, 1952). At pH 8-9, the reaction is normal and the peak absorbance is at 589 nm; however, at pH 11.1, the peak absorbance is changed to 630 nm (George and Irvine, 1952).

Early studies of reactions between myoglobin and hydrogen peroxide showed that the reactions resulted in production of two derivatives of myoglobin, identifiable from visual inspection by their distinct colors (Reeder *et al.*, 2002). When metmyoglobin reacts with hydrogen peroxide at acidic pH, a stable green specie is formed. The green compound was tentatively identified as a species formed from substitution on the porphyrin ring without ring fission (Reeder *et al.*, 2002).

Sulfmyoglobin. Sulfhemoglobin is a green heme pigment that is physiologically inactive, has unique optical spectra, and reduced oxygen affinities (Chatfield *et al.*, 1987). Hoppe-Seyler first observed the green pigment in 1866; the pigment, called Schwefelmethamoglobin or sulfhemoglobin, is formed from a reaction between oxyhemoglobin and hydrogen sulfide (Lemberg and Legge, 1949; Berzofsky *et al.*, 1971). Sulfmyoglobin occurs when a sulfide group is added to the porphyrin ring (Michel, 1938; Lemberg and Legge, 1949; Price and Schweigert, 1987).

Effects of α-Tocopheryl Acetate on Color Stability

Vitamin E has been used as a dietary supplement for cattle to delay color deterioration of whole muscle and ground beef products because it is an antioxidant that delays the formation of metmyoglobin in muscle tissue (Mitsumoto *et al.*, 1991; Arnold *et al.*, 1992). Because lipid oxidation and muscle pigment oxidation in fresh meat are so closely related and are caused by similar processes (Faustman and Cassens, 1989; Faustman and Cassens, 1990), delaying lipid oxidation with supplemental vitamin E should delay muscle pigment oxidation and meat discoloration (Faustman *et al.*, 1989b; Faustman and Wang, 2000).

Williams *et al.* (1992a) stated that if increased concentrations of α-tocopherol are incorporated into biological membranes within beef muscles, it will delay oxidative processes which result in lipid oxidation and metmyoglobin formation within muscles and thereby delay muscle pigment discoloration. Discoloration is delayed in tissues with increased α-tocopherol concentrations because the increased concentration allows for the natural antioxidant to perform its physiological role of protecting lipid membranes (Williams *et al.*, 1992b). Lipid oxidation has been shown to be significantly lower in steaks from cattle supplemented with vitamin E, as compared to control (from cattle not given supplemental vitamin E), steaks (Faustman and Cassens, 1989; Comstock *et al.*, 1991; Arnold *et al.*, 1993; Stubbs *et al.*, 1999).

The strategy for supplementing feeder cattle with α-tocopheryl acetate is to achieve the ideal concentration (to maximize the antioxidant capacity) without saturating the α-tocopherol level in the muscle above that ideal concentration (Liu *et al.*, 1995). Faustman *et al.* (1989b) proposed 3 μg/g of α-tocopherol as the ideal concentration in the *gluteus medius*, whereas Arnold *et al.* (1993) proposed 3.3 μg/g of α-tocopherol as ideal for the *longissimus lumborum* and 3.8 μg/g of α-tocopherol as ideal for the *gluteus medius*. Other research has shown that caselife of beef retail products can be improved if cattle receive dietary Vitamin E supplementation at levels of 500 to 1,000 IU animal^{-1} d^{-1} for a minimum of 100 d before harvest to attain muscle concentrations of Vitamin E in excess of 3 to 4 μg/g muscle tissue (Smith *et al.*, 1994a, 1996; Westcott *et al.*, 1997). Zerby *et al.* (1999) determined that, among samples of the same retail beef cut, those with muscles containing higher concentrations of α-tocopherol had improved color stability.

The improvement in color stability has a potential to result in a 10.4:1 benefit:cost ratio due to a 3.6% increase in receipts for sale of the meat (Liu *et al.*, 1995). Williams *et al.* (1992a) documented a savings of $0.156/lb that could be realized for beef from cattle supplemented with vitamin E and that use of this technology could save the retail sector of the beef industry $156/1000 lb of meat, resulting in a net return of 26:1. Other researchers have documented that the benefit of feeding vitamin E, with a supplementation cost of $1.43 to $6.00, to the retail sector would be $20.29 to $60.07 per carcass (Smith *et al.*, 1994; Zerby *et al.*, 1999; Westcott *et al.*, 1997).

Effects of Modified Atmosphere Packaging on Color Stability

Modified atmosphere packaging involves the containment of foods under gaseous environments different from that of air so that normal respiration activities of the food and its inherent microbiological populations are retarded (Hintlian and Hotchkiss, 1986; Brody, 1996). When the modified atmosphere packaging operation takes place, the desired combinations/concentrations of one or more gases is in the headspace, but as the contained product continues to respire and its microbial populations multiply and as semi-permeable package structures permit the entry of air, the internal environment is continually being modified (Brody, 1996).

Modified atmosphere packaging allows: (1) greater utilization of resources, (2) better quality control, (3) less waste through utilization of fat, bone, and trimmings, (4) more efficient use of manpower and space, (5) improved profits at the retail and processor levels, (6) use of automated equipment, (7) more uniform products, and (8) better inventory control (Cole, 1986).

Reasons for changing to a central packaging or modified atmosphere packaging system include: (1) meat is no longer the only major consideration for choosing a grocery store, (2) store managers desire control over the meat department, and (3) retailers desire that packers stand behind the quality of products and be responsible for advertising and promotion of their products (Cole, 1986). Central preparation of retail packages of meat offers economic advantages (Farris *et al.*, 1991, as cited by Gill and Jones, 1994). Moving packaging operations of retail cuts from retail stores to the packer level eliminates time-consuming labor for cutting, trimming and overwrapping at retail stores (Jeyamkondan *et al.*, 2000). According to some estimates, modified atmosphere packaging is more cost effective than vacuum packaging (Seideman and Durland, 1984; Hintlian and Hotchkiss, 1986).

Color of the fresh muscle surface depends on the proportional concentrations of three primary derivatives of myoglobin: reduced myoglobin, oxymyoglobin, and metmyoglobin. Reduced myoglobin is the predominant myoglobin form in the absence of oxygen, oxymyoglobin is the oxygenated form of muscle pigment and is responsible for the bright red color, and metmyoglobin creates the brown color which results from the oxidation of the iron heme in the myoglobin molecule of oxymyoglobin or reduced myoglobin (Cole, 1986; Lawrie, 1998).

Maintaining muscle pigment in the oxymyoglobin state with modified atmosphere will provide normal bloomed color for a relatively short distribution period of 8 to 14 days, after which, deleterious oxidative rancidity, microbial, and color changes decrease the consumer acceptability (Cole, 1986).

Modified atmosphere packaging, in combination with optimum temperature control and processing hygiene, is the most effective means of extending storage life of fresh chilled meat and maintaining product quality and safety (Greer *et al.*, 1993). Optimum meat color and bacterial inhibition can be accomplished through use of mixtures of three gases: oxygen, carbon dioxide, and nitrogen (Cole, 1986; Jeyamkondan *et al.*, 2000). Oxygen is commonly used to promote red surface color (Taylor, 1972; Bartkowski *et al.*, 1982; Sebranek, 1986), carbon dioxide inhibits microbial growth (Gill and Tan, 1980; Dixon and Kell, 1989; Farber, 1991), and nitrogen serves as a filler gas to reduce concentrations of other active gases (Seideman and Durland, 1984; Sebranek, 1986; Farber, 1991).

Interaction of oxygen and muscle is dependent on the oxygen tension, amount of myoglobin and surviving activity of the respiratory system in muscle. High oxygen tensions favor prevalence of bright red, oxymyoglobin, whereas low oxygen tensions favor brown, metmyoglobin formation (Figure 2). At high oxygen pressure, the reaction of myoglobin to oxymyoglobin shifts to the left (Cole, 1986). Once red oxymyoglobin is formed, it is stabilized by formation of a highly resonant structure and, as long as the heme remains oxygenated, no further color changes take place (Clydesdale and Francis, 1971). The best way to prevent an initial decrease in redness is to maintain a high partial pressure of oxygen in the atmosphere of the package (Clydesdale and Francis, 1971).

Rickert *et al.* (1958) reported that muscles in fresh meat stored under various partial pressures of oxygen still lost redness, but high concentrations of oxygen temporarily delay discoloration. Bartkowski *et al.* (1982) reported that levels of 40-75% oxygen (more than twice atmospheric oxygen) produced and maintained desirable bright

red beef muscle color for nine days. Taylor (1972) claimed that although an increase of oxygen prolonged bright red muscle color, long-term stability was not feasible. Optimum results in muscle color were obtained when carbon dioxide was used, in combination with high levels of oxygen (85%), at a concentration of 10-15% (Taylor and MacDougall, 1973; Benedict *et al.*, 1975). Meat packaged in high (50-90%) oxygen maintains a desirable oxygenated red muscle color for 8-14 days (Cole, 1986). Muscles in steaks stored under a combination of oxygen and carbon dioxide had lower metmyoglobin fractions and were desirable in appearance after storage for up to eight days, but were unacceptable in appearance when stored for 20 days (Gill and Jones, 1994).

Because metmyoglobin is formed at low oxygen tensions, the oxygen concentration in the gas mixture used for modified atmosphere packaging is usually greater than that of air (Jeyamkondan *et al.*, 2000). The problem with high-oxygen modified atmosphere packaging is that it results in increased lipid oxidation and rancidity (Zhao *et al.*, 1994).

Lipid oxidation is often due to auto-oxidation of unsaturated fatty acids catalyzed by factors that oxidize myoglobin pigments to metmyoglobin (Zhao *et al.*, 1994). Lipid oxidation occurs at a slower rate than discoloration or microorganism growth and is therefore not a major determinant of shelf life in overwrapped, air-permeable packaging systems (Zhao *et al.*, 1994). However, with modified atmosphere packaging, volatile, odor-causing products are retained within the package creating undesirable characteristics in packaged products (Zhao *et al.*, 1994). Jackson *et al.* (1992) reported that beef strip loin steaks packaged in 80% oxygen and 20% carbon dioxide developed strong off-flavors because of the formation of various volatile compounds.

CONCLUSIONS

The incidence of injection-site lesions in top sirloin butts of fed steers/heifers has declined since the initial injection-site lesion audit was conducted in 1991. However, the presence of lesions still negatively impacts product quality. The supplementation of Vitamin E to feedlot cattle enhances retail display life of beef cuts by maintaining a bright, cherry-red muscle color during display. Modified atmosphere packaging, specifically the use of a high-oxygen environment, maintains bright red muscle color in meat for eight to fourteen days after packaging. While numerous studies have documented the impact of each of these defects and/or systems individually, there is limited research on the impact of vitamin E supplementation of feeder cattle on the maintenance of bright red muscle color in meat packaged in modified atmospheres, when injection-site lesions are present.

Table 2.1. Common color reactions: Pigments, their mode of formation, states of heme iron and muscle colors (Source: Price and Schweigert, 1987).

Pigment	Mode of formation	State of Heme iron	Muscle Color
Myoglobin	reduction of metmyoglobin or deoxygenation of oxymyoglobin	Fe^{2+}	purplish red
Oxymyoglobin	oxygenation of myoglobin	Fe^{2+}	bright red
Metmyoglobin	oxidation of myoglobin	Fe^{3+}	brown
Sulfmyoglobin	effect of H_2S on oxymyoglobin	Fe^{3+}	green
Choleglobin	effect of H_2O_2 on oxymyoglobin,	Fe^{2+} or Fe^{3+}	green

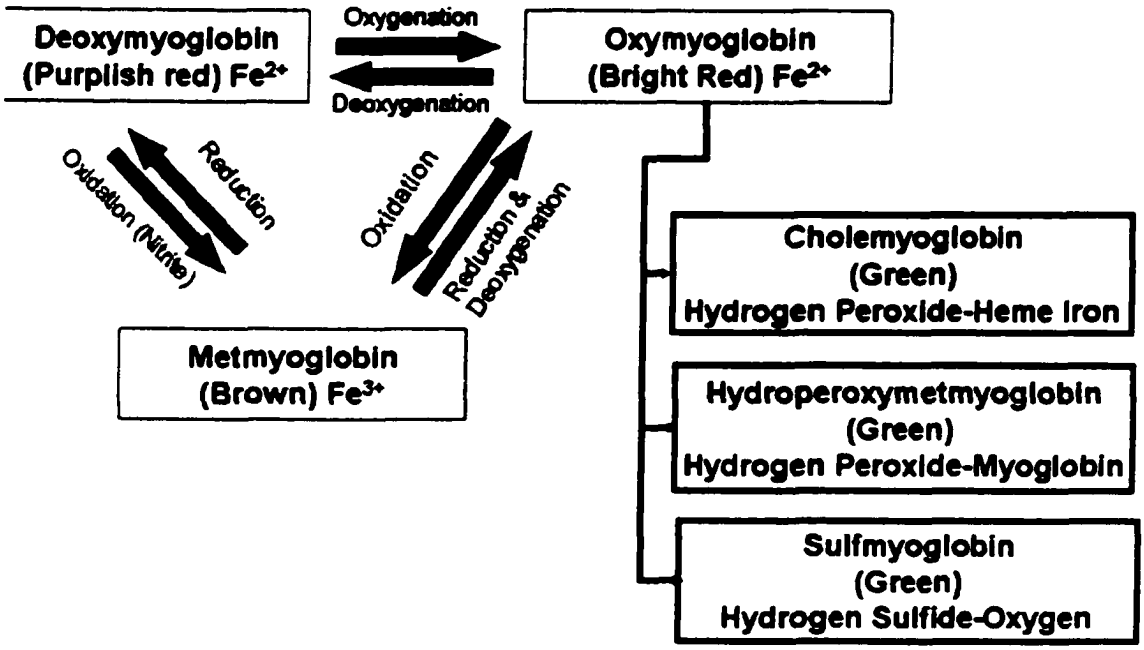


Figure 2.1. Heme pigments of meat. (Source: Faustman and Cassens, 1990; Price and Schweigert, 1987.)

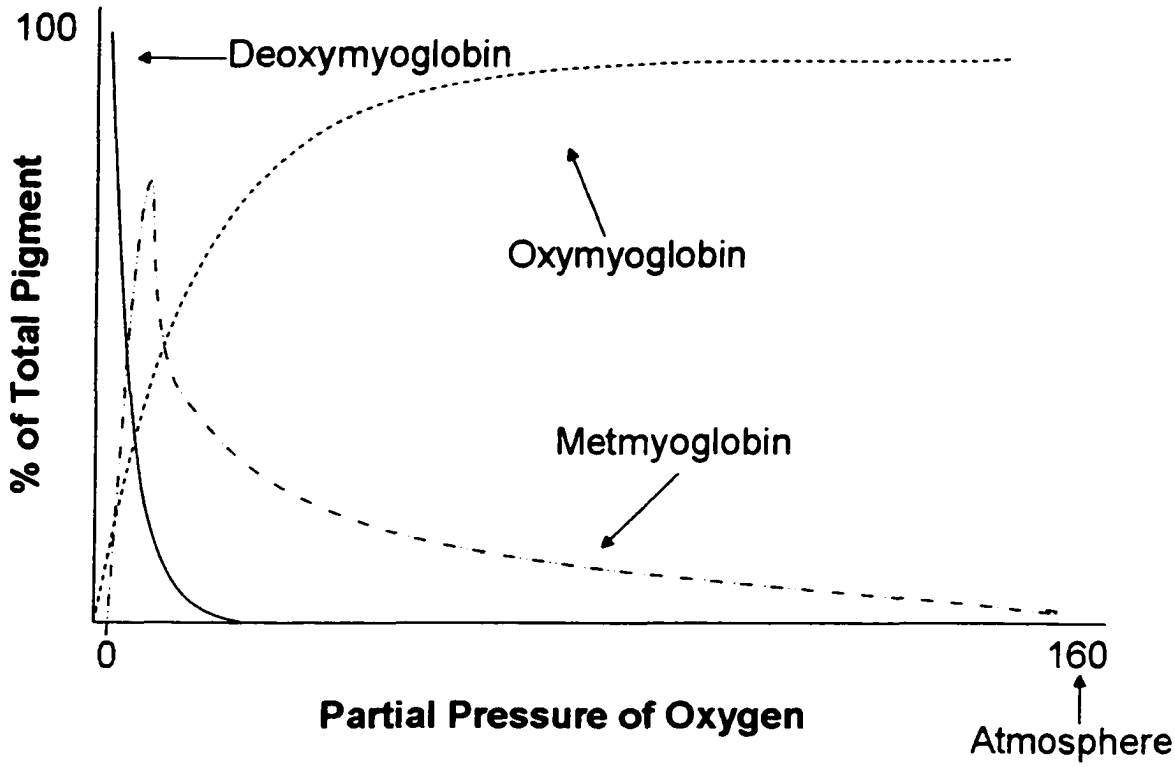


Figure 2.2. The relationship between partial pressure of oxygen and the concentrations of muscle pigments (Redeveloped from Aberle *et al.*, 2001).

CHAPTER III

FREQUENCIES OF INJECTION-SITE LESIONS IN MUSCLES FROM ROUNDS OF DAIRY AND BEEF COW CARCASSES

ABSTRACT

Frequency of injection-site lesions in muscles from top sirloins and rounds in fed cattle carcasses is well documented; this study characterized frequency and severity of lesions in muscles from rounds of beef and dairy cow carcasses. Audits were conducted in 1998, 1999, and 2000 on 3,190 rounds from cow carcasses. Outside round muscles were cut into 1.25-cm slices to characterize lesions. In 1998, 31% of rounds from carcasses of beef cattle and 60% of rounds from carcasses of dairy cattle had an injection-site lesion. Frequency of lesions in rounds from carcasses of beef cattle declined 5 percentage points ($P < 0.05$) between 1998 and 1999, and 6 percentage points ($P < 0.05$) between 1999 and 2000. Frequency of lesions in rounds from carcasses of dairy cattle declined 9 percentage points ($P < 0.05$) between 1998 and 1999, and 16 percentage points ($P < 0.05$) between 1999 and 2000. Frequencies of injection-site lesions in muscles of rounds from carcasses of beef cattle were lower ($P < 0.05$) than those in muscles of rounds from carcasses of dairy cattle in all three years. Injection-site lesions were most common between the hooks and pins of the hindquarter of beef cattle and between the pins and hocks of the hindquarter of dairy cattle. Clear lesions and woody calluses

exceeded 89%, and occurred more frequently than did other kinds of lesions in muscles of rounds from carcasses of beef and dairy cattle in 1998, 1999, and 2000. Of all injection-site lesions, between 3% and 5% were cystic in muscles of rounds from carcasses of beef cattle, similar to the 2% to 4% of cystic lesions found in muscles of rounds from carcasses of dairy cattle. Although yearly data indicated that frequencies of injection-site lesions declined, there remains a need for educational programs and continued improvement in beef quality assurance practices among both beef and dairy producers.

INTRODUCTION

Results of the National Market Cow and Bull Beef Quality Audit—1999 (NMCBBQA—99) revealed (Roeber *et al.*, 2000a) that approximately 44% of product from carcasses of market cows and bulls is sold as whole muscle cuts (as 100% visual lean pieces or as primal cuts). The presence of injection-site lesions in whole muscle cuts, such as the top sirloin and outside round, limits their use and value. Roeber *et al.* (2001a) reported that, in NMCBBQA—99, "occurrence of injection-site lesions in muscles" was included among the top five quality challenges for both beef and dairy market cows and bulls. The NMCBBQA—99 and the National Non-Fed Beef Quality Audit—1994 (NNFBQA—94), respectively, attributed losses of $1.46 and $0.66 for each market cow or bull harvested in those years to the occurrence of injection-site lesions (Roeber *et al.*, 2000a). Because injection-site lesions are concealed in muscles and/or are under subcutaneous fat, they are seldom found during fabrication at the packing plant and appear, instead, during wholesale/retail fabrication or at the consumer level.

The frequency of injection-site lesions in muscles from top sirloins and outside rounds in carcasses from fed cattle is well documented (Dexter *et al.*, 1994; George *et al.*, 1995b; Roeber *et al.*, 2001b; Van Donkersgoed *et al.*, 1999). The objective of the current study was to characterize the frequency and severity of lesions in muscles from outside rounds of beef and dairy cow carcasses. Monitoring of the frequency of injection-site lesions in muscles from outside rounds of beef cow and dairy cow carcasses allows educational efforts of state and national beef quality assurance (BQA) programs to target, more definitively, management practices of producers that can be changed to minimize occurrence of such defects.

MATERIALS AND METHODS

To obtain estimates of the national frequency and severity of injection-site lesions in muscles of outside rounds from beef and dairy cow carcasses, data were collected in each of four packing plants in 1998 and in each of seven packing plants in both 1999 and 2000. Packing plants in which rounds were sampled were selected by geographic location and daily harvest/fabrication capacity. Among packing plants, it was believed that carcasses represented cows from small, medium, and large beef and dairy enterprises.

At each packing plant, 200 outside rounds (*biceps femoris* plus *semitendinosus* muscles) were selected randomly except that selection ensured that outside rounds were included from both dairy cow and beef cow carcasses during each collection period. Each muscle from each outside round was cut into 1.25-cm slices from the cranial to caudal ends and evaluated for lesions by a trained researcher. Lesions were documented

by location, depth from the outside of the muscle, and diameter of the lesion. Lesion location was recorded by the muscle (*biceps femoris* or *semitendinosus*) and the region or quadrant (Figure 3.1) in which it was present. The quadrants were identified as Q1 through Q4. Quadrant Q4 was located at the cranial (or proximal) end of the primal cut (outside round) and contained only the *biceps femoris* muscle. The remaining three quadrants Q1, Q2, and Q3 were defined as even thirds of the remaining primal cut (containing both the *biceps femoris* and *semitendinosus* muscles) with Q3 adjacent to Q4, and with Q1 being the most caudal (or distal) third, at the shank end. Lesion depth was measured from the outside surface of the muscle (fat cover excluded) to the center of the lesion. Lesion diameter was equivalent to the length of the lesion through the primal cut; similar to documenting the number of steaks the lesion affected. The damaged tissue was classified (gross evaluation) into one of five lesion types using the 5-point system originally described by Dexter *et al.* (1994).

Statistical Analysis. The frequency of injection-site lesions, as percentages, was analyzed using the Frequency Procedure of SAS (SAS, 1999). Differences between frequency percentages for years (1998, 1999, 2000) and for muscles from beef cow vs. dairy cow carcasses were determined by calculating the chi-square statistic. Analyses of variance for number of lesions per round, lesion depth, and lesion diameter for audit period and breed type (beef or dairy) were conducted using the general linear models procedures of SAS (1999). Least significant differences were used to identify statistical differences among mean values for number of lesions per round, lesion depth, and lesion diameter when analysis of variance demonstrated effects ($\alpha = 0.05$) for audit period or the origin (beef vs. dairy) of muscle.

RESULTS AND DISCUSSION

For all three years of these audits, frequencies of injection-site lesions and average number of lesions per round that had a lesion were higher ($P < 0.05$) in outside round muscles from dairy carcasses than for beef carcasses (Table 3.1). This result was not unexpected because at the National Market Cow and Bull Beef Quality Audit Strategy Workshop in 1999, it was estimated that dairy cows receive several more injections per year than do beef cows (Roeber *et al.*, 2000a).

Cumulatively, across the three years of audits in the present study, frequencies of injection-site lesions in outside round muscles from beef cow carcasses and dairy cow carcasses were 26% and 49%, respectively. An estimate of 33 to 35% frequency of injection-site lesions in muscles from outside rounds from cow and bull carcasses in Canada reported by Van Donkersgoed *et al.* (1998b, 1999) was within the range (26 to 49%) of the current study.

The frequency of injection-site lesions in muscles from rounds from carcasses of beef cattle declined 5 percentage points (from 31% to 26%) from 1998 to 1999, and an additional 6 percentage points (from 26% to 20%) from 1999 to 2000 ($P < 0.05$, Table 3.1). The frequency of injection-site lesions in muscles from rounds from carcasses of dairy cattle declined 9 percentage points (from 60%, to 51%) from 1998 to 1999, and an additional 16 percentage points (from 51%, to 35%) from 1999 to 2000 ($P < 0.05$, Table 3.1). In 1998, 1999, and 2000, the frequency of injection-site lesions was greater ($P < 0.05$) in muscles from rounds from carcasses of dairy cattle than in those from rounds from carcasses of beef cattle (Table 3.1). While there was no difference in the average number of lesions in those muscles that had a lesion present among years studied, in

muscles of rounds from beef or dairy type cattle, muscles from rounds from carcasses of beef cattle had fewer ($P < 0.05$) lesions per round than did muscles from rounds from carcasses of dairy cattle in all three years (Table 3.1).

Identification of locations within the outside round muscles at which injection-site lesions occurred should be useful to educators as they design programs to assist cattle producers in reducing the occurrence of this defect in market cow and bull carcasses. Injection-site lesions occurred more frequently ($P < 0.05$) in the *semimembranosus* muscles and in caudal quadrants (Q1 and Q2) in rounds from dairy cow carcasses than in those from beef cow carcasses, suggesting that more dairy cattle than beef cattle are given injections from behind, and those injections may be administered while the animals are restrained in self-locking head restraints in the feeding area or perhaps in the milking parlor (Table 3.2). The location at which most ($P < 0.05$) of the injections are administered to beef cattle appears to be between the hooks and the pins (Q3 and Q4), likely from above while cattle are handled through a chute (Table 3.2).

The kind of lesion is useful in characterizing the length of time between administration of the injection and the time of harvest (George *et al.*, 1995b; George *et al.*, 1995a; Dexter *et al.*, 1994). Clear lesions and woody calluses are typical of injections given to animals in earlier stages of their life (as calves or at times before weaning), metallic and nodular lesions are typical of pharmaceuticals administered to cattle mid-to-late feeding phases, and cystic lesions are typical of injections given to cattle late in the finishing phase (George *et al.*, 1995b; George *et al.*, 1995a; Dexter *et al.*, 1994). In 1998, 1999, and 2000, in muscles of rounds from both beef cow and dairy cow carcasses, the frequencies of both clear lesions and woody lesions were greater ($P < 0.05$) than the

frequencies of nodular, metallic, and cystic lesions (Table 3.3). In muscles of rounds from beef and dairy cow carcasses, the frequencies of cystic lesions were not statistically different from 1998 to 1999, or from 1998 to 2000, indicating that two to five percent of cows likely received intramuscular injections near the time of harvest. In the most recent audit of fed steer and heifer carcasses, frequencies of cystic lesions in top sirloin butts and rounds were only 0.24% and 0.0% of total lesions, respectively (Roeber *et al.*, 2000b).

Depth and diameter of injection-site lesions were recorded and used for prediction of relative amounts of saleable product lost as a result of the lesion (Table 3.4). Diameters of lesions varied among the kind of lesions in muscles of beef and dairy cow rounds. Cystic lesions, in muscles of rounds from carcasses of dairy cattle, damaged more saleable product ($P < 0.05$) per lesion than did clear, metallic, nodular, and woody lesions. In rounds from carcasses of beef cattle, the diameter of cystic lesions was similar to that of nodular lesions, but larger than that of the other kinds of lesions (Table 3.4). Lesions were closer ($P < 0.05$) to the outside surface of the muscles of the round in beef and dairy carcasses in 1999 as compared to 1998 and closer ($P < 0.05$) to the surface in 2000 as compared to 1999 (Table 3.4). There was no apparent difference ($P > 0.05$) in the diameter of the lesions in the muscles of beef cow rounds in 1998, 1999, and 2000 (Table 3.4).

IMPLICATIONS

The frequency of injection-site lesions in muscles of the round from beef and dairy cow carcasses declined by 11 and 25 percentage points, respectively, from 1998 to

2000. These reductions in the frequency of injection-site lesions are substantial, but not sufficient, because one of five beef cattle rounds and more than one of three of dairy cattle rounds still have injection-site lesions. Injection-site lesions in beef and rounds from carcasses of dairy cattle cost beef and dairy industries, in total, over $9 million annually (Roeber *et al.*, 2000a). Additionally, the most recent National Animal Health Monitoring System report indicated that 47% of producers and 37% of veterinarians administer intramuscular injections in the upper or lower rear leg of cows (USDA, 1998) so the need for further educational effort is apparent. Continuous monitoring of the frequency of injection-site lesions in muscles of the round from beef and dairy cow carcasses allows educational efforts of state and national quality assurance programs to target, more definitively, management practices of producers that can minimize occurrence of such defects in end-products.

Table 3.1. Frequency of injection-site lesions in 1998, 1999, and 2000.

	Beef type			Dairy type		
	1998	1999	2000	1998	1999	2000
Total pieces audited	135	824	736	243	586	666
Pieces with lesion(s)	42	212	147	146	299	230
Percent of rounds with lesion(s)	$31^{a,y}$	$26^{b,y}$	$20^{c,y}$	$60^{a,x}$	$51^{b,x}$	$35^{c,x}$
Number of lesions per piece that had a lesion (Mean ± S.E.)	$1.2^{y} \pm 0.5$	$1.2^{y} \pm 0.5$	$1.4^{y} \pm 0.8$	$1.6^{x} \pm 1.3$	$1.6^{x} \pm 1.2$	$1.7^{x} \pm 1.2$
Maximum lesions in one round	3	4	6	11	12	8

[a,b,c] Subcolumn percentages, within each breed type and comparing years, with differing superscript letters differ ($P < 0.05$).

[x,y] Subcolumn percentages, within each year and comparing breed type, with differing superscript letters differ ($P < 0.05$).

Table 3.2. Injection-site lesion frequency data, by location within the outside round muscles, for all plants in 1998, 1999, and 2000, as a percentage of total lesions.

Location[1] in outside round muscles	Beef type			Dairy type		
	1998 %	1999 %	2000 %	1998 %	1999 %	2000 %
Semitendinosus Q1(shank end)	$6^{a,x}$	$0^{b,y}$	$9^{a,x}$	$11^{ab,x}$	$15^{a,x}$	$10^{b,x}$
Semitendinosus Q2	$7^{ab,x}$	$5^{b,y}$	$14^{a,y}$	$15^{b,x}$	$24^{a,x}$	$17^{b,x}$
Semitendinosus Q3	$14^{a,x}$	$10^{a,x}$	$3^{b,y}$	$14^{a,x}$	$11^{ab,x}$	$7^{b,x}$
Semitendinosus all quadrants	$27^{a,x}$	$15^{b,y}$	$27^{a,x}$	$40^{b,x}$	$50^{a,x}$	$34^{b,x}$
Biceps femoris Q1 (shank end)	$1^{b,x}$	$2^{b,y}$	$6^{a,x}$	$8^{b,x}$	$13^{a,x}$	$10^{ab,x}$
Biceps femoris Q2	$12^{ab,x}$	$8^{b,x}$	$18^{a,y}$	$18^{b,x}$	$16^{b,x}$	$31^{a,x}$
Biceps femoris Q3	$22^{a,x}$	$21^{a,x}$	$10^{b,y}$	$22^{a,x}$	$13^{b,y}$	$17^{ab,x}$
Biceps femoris Q4 (sirloin end)	$38^{b,x}$	$53^{a,x}$	$38^{b,x}$	$13^{a,y}$	$8^{a,y}$	$9^{a,y}$
Biceps femoris all quadrants	$73^{b,x}$	$85^{a,x}$	$73^{b,x}$	$60^{a,x}$	$51^{b,y}$	$66^{a,x}$

[1] Locations included two muscles and four quadrants (Q1 to Q4, Figure 1) in outside rounds of carcasses.

[a,b,c] Subcolumn percentages, within each breed type and comparing years, with differing superscript letters differ ($P < 0.05$).

[x,y] Subcolumn percentages, within each year and comparing breed types, with differing superscript letters differ ($P < 0.05$).

Table 3.3. Injection-site lesion frequency data, by kind of lesion, for all plants in 1998, 1999, and 2000, as a percentage of total lesions.

	Beef type			Dairy type		
Kind of Lesion	**1998**	**1999**	**2000**	**1998**	**1999**	**2000**
Clear[a]	$48^{g,xy}$	$39^{g,y}$	$59^{g,x}$	$50^{g,y}$	$45^{g,y}$	$67^{g,x}$
Woody[b]	$48^{g,x}$	$54^{g,x}$	$34^{g,y}$	$39^{g,x}$	$46^{g,x}$	$29^{g,y}$
Nodular[c]	NA^{f}	$3^{h,x}$	$1^{h,x}$	$6^{h,x}$	$7^{h,x}$	$2^{h,y}$
Metallic[d]	NA^{f}	1^{h}	NA^{f}	$1^{h,x}$	NA^{f}	NA^{f}
Cystic[e]	$5^{h,x}$	$3^{h,x}$	$4^{h,x}$	$4^{h,x}$	$2^{h,x}$	$2^{h,x}$

[a] Clear: Lesion containing primarily clear connective tissue.

[b] Woody: Lesion characterized by infiltration with organized connective tissue and fat.

[c] Nodular: Lesion with nodules, the central foci of necrosis, surrounded by granulomatous inflammation.

[d] Metallic: Lesion containing mineralized remnants of muscle cells.

[e] Cystic: Encapsulated lesion containing fluid.

[f] NA: Frequency of lesions in this category for a given year accounted for less than 1 percent of all lesions identified.

[g,h] Percentages in the same column, comparing kind of lesion in a breed type for a given year, with differing superscript letters differ ($P < 0.05$).

[x,y] Kind of lesion percentages, within each breed type and comparing years, with differing superscript letters differ ($P < 0.05$).

Table 3.4. Depth and diameter of injection-site lesions arrayed by kind of lesion, year, and breed type.

	Depth (cm) ± SE		Diameter (cm) ± SE	
Trait	**Beef Type**	**Dairy Type**	**Beef Type**	**Dairy Type**
Kind of Lesion[d]				
Clear	$2.06^{y} \pm 0.08$	$2.51^{b,x} \pm 0.05$	$4.75^{b} \pm 0.20$	$5.46^{c} \pm 0.33$
Woody	$2.18^{y} \pm 0.10$	$2.69^{a,x} \pm 0.08$	$5.61^{b,y} \pm 0.20$	$6.38^{b,x} \pm 0.18$
Nodular	1.68 ± 0.23	$2.21^{b} \pm 0.20$	$5.79^{ab} \pm 1.22$	$5.82^{bc} \pm 0.41$
Metallic	0.64 ± 0.00	$1.75^{b} \pm 0.38$	$3.73^{b} \pm 0.03$	$5.41^{c} \pm 1.30$
Cystic	2.34 ± 0.33	$3.00^{a} \pm 0.36$	$8.20^{a} \pm 0.89$	$11.35^{a} \pm 1.30$
Year[e]				
1998	$2.29^{a} \pm 0.23$	$2.97^{a} \pm 0.10$	5.23 ± 0.41	$6.02^{b} \pm 0.20$
1999	$1.85^{b,y} \pm 0.10$	$2.41^{b,x} \pm 0.05$	$5.44^{y} \pm 0.20$	$7.26^{a,x} \pm 0.18$
2000	$1.47^{c,y} \pm 0.05$	$1.93^{c,x} \pm 0.05$	$5.08^{y} \pm 0.23$	$7.37^{a,x} \pm 0.46$

[a,b,c] Means in the same column, for either kind of lesion or year, with differing superscript letters differ ($P < 0.05$).

[d] Data for combined years.

[e] Data for combined kinds of lesions.

[x,y] Means in the same row and for the same trait (depth or diameter) with differing superscript letters differ ($P < 0.05$).

Figure 3.1. Quadrants of the hindquarter in which injection-site lesions were identified. Quadrant Q4 is located at the cranial (or proximal) end of the primal cut (outside round) and contains only the *biceps femoris* muscle. The remaining three quadrants Q1, Q2, and Q3 were defined as even thirds of the remaining primal cut (containing both the *biceps femoris* and *semitendinosus* muscles) with Q3 adjacent to Q4, and with Q1 being the most caudal (or distal) third, at the shank end.

CHAPTER IV

INCIDENCE OF INJECTION-SITE LESIONS IN TOP SIRLOIN BUTTS FROM CARCASSES OF FED CATTLE

ABSTRACT

Damaged beef muscle tissue resulting from intramuscular injections of animal-health products represents a "quality control" problem and an economic loss to the beef industry. Fifteen individual and sequential national audits of injection-site lesions in beef top sirloin butts were conducted at the steak provisioner/cutting level between November 1995 and July 2000. The national incidence of injection-site lesions in top sirloin butts (n = 240,080) decreased ($P < 0.05$) between November 1995 (11.4%) and July 2000 (2.1%). From November 1995 to July 1997, mean weight per injection-site lesion, across all lesion classes, increased ($P < 0.05$) from 192.5 g to 435.8 g, respectively; mean weight per lesion subsequently decreased ($P < 0.05$) to 249.8 g in July 2000, but was still heavier ($P < 0.05$) than in November 1995. Results of these audits suggest that producers have changed injection practices. These changes have likely been in response to educational efforts such as the National Cattlemen's Beef Association and state beef quality assurance programs. Analyses of results for lesion classes, partitioning lesions according to chronological stages of the healing process, suggested that the majority of lesions were

induced at times which coincide with cow-calf, stocker, or early finishing-period stages of cattle production.

INTRODUCTION

Results of the National Beef Quality Audit–1995 (Smith *et al.*, 1995) revealed that 30-40% of purveyors, retailers, and packers believed that the frequency of injection-site lesions decreased since a similar audit was conducted in 1991. Even with such improvement, purveyors and retailers still ranked this defect in the top ten challenges for fed steer and heifer quality. Pharmaceuticals are commonly administered to cattle at various stages of their lives (Taylor and Field, 1999). If injections are administered intramuscularly, in the anatomical region between the hooks and pins, tissue damage occurs (Dexter *et al.*, 1992). Injection-site lesions are seldom detected at packing plants because damage is concealed within the muscles and subcutaneous fat. Unless top sirloin butts are further processed by packers (including removal of subcutaneous fat and separation of the *biceps femoris* from the *gluteus medius*), injection-site damage will normally be exposed at retailer or purveyor establishments during portioning of the primal cuts.

Dexter *et al.* (1994) reported that activities of the National Cattlemen's Association (subsequently National Cattlemen's Beef Association, NCBA) Quality Assurance Advisory Board led to a reduction in the incidence of injection-site lesions from 21.3% (July 1991) to 10.9% (March 1993). George *et al.* (1996) reported that continuation of these efforts did not result in a reduction in injection-site lesion incidence from July 1993 (10.9%) to July 1995 (10.2%). Since July 1995, 15 audits were

conducted to determine the impact of beef quality assurance efforts of cattlemen's organizations on the incidence of injection-site lesions in the top sirloin butts of fed steers and heifers. This analysis is a sequel to those by Dexter *et al.* (1994) and George *et al.* (1996), and continues the reporting of results of national audits of incidence of injection-site lesions in top sirloin butts from fed steers and heifers using data collected from November 1995 through July 2000.

MATERIALS AND METHODS

General Protocol. In order to obtain ongoing assessments of the incidence/severity of injection-site lesions in top sirloin butts on a national scale, data were collected from individual steak-cutting plants located nationwide. Audits in each of four plants were conducted in November 1995; in each of March, July, and November of 1996, 1997, 1998, and 1999; and in March and July of 2000. Facilities audited were selected according to (1) U.S. geographic location, and (2) quantities of top sirloin butts processed at that location. In order to ensure that adequate quantities of top sirloin butts were evaluated, two shifts (8-9 h) were audited at each plant visited during each audit period. Audit procedures were identical to those described by Dexter *et al.* (1994).

At each of the audited facilities, all steak cutters were provided verbal instructions concerning the audit process and were shown how the affected tissue (injection-site lesion) appeared in top sirloin butts/steaks. Instructions also were provided regarding actions to take when questionable tissue was discovered; an appropriate course of action was to hold the product for evaluation by the investigator before excision of the tissue. As each individual top sirloin butt was portioned into individual steaks, injection-site

damage that was exposed was excised from all affected steaks. The excised damaged tissue was subsequently classified using a 5-point classification system as described by Dexter *et al.* (1994) and weighed (to the nearest g).

Statistical Analysis. Data representing percentage incidence of injection-site lesions were analyzed using the Frequency Procedure of SAS (SAS, 1999). Differences between incidence values associated with the 15 audit time periods were determined by calculating the chi-square statistic. Means for lesion weight were computed and analysis of variance was conducted using the GLM procedures of SAS (1999). Least significant differences were used to identify statistical differences among mean lesion weights when AOV demonstrated an effect of the audit period and/or lesion type ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Average incidence of injection-site lesions during the audit period of November 1995 was 11.40%, which was numerically higher than the 10.19% incidence reported by George *et al.* (1996) for the audit period of July 1995. The average weight of injection-site lesions excised from affected top sirloin butts during the November 1995 audit was 192.5 g, which also was numerically heavier than the 152.8 g lesion weight found in July 1995 (George *et al.*, 1995).

Over the entire 15 audit periods included in this report, incidence of injection site lesions decreased (Table 4.1) from a high of 11.40% in the first audit period (November 1995) to a low of 2.06% in the last audit period (July 2000). The decline in numerical injection-site lesion incidence was continuous, with each subsequent incidence lower ($P < 0.05$) than the preceding audit incidence, over the 15 audit periods with the exception

of: (a) the July 1996 to November 1996 audit periods, the July 1997 to November 1997 audit periods, the November 1998 to March 1999 audit periods, and the November 1999 to March 2000 audit periods, where the incidence did not change, and (b) the March 1998 to July 1998 to November 1998 audit periods, where incidence of injection site lesions peaked ($P < 0.05$) in July 1998 and then returned to previous levels in November 1998. The incidence of injection-site lesions in fed steer and heifer top sirloin butts was lower than incidences reported by Van Donkersgoed *et al.* (1997 and 1998a); in those two studies, the incidences of lesions in Canadian fed beef top sirloin butts were 18.8% and 13.3% in the fall of 1996 and in the spring of 1997, respectively. Reduced incidence of injection-site lesions from November 1995 to July 2000 corresponded with the downward trend reported by Dexter *et al.* (1994); in that study, lesion incidence declined over the six audit periods between July 1991 to March 1993. During the period covered by the report of George *et al.* (1996), no decrease in injection-site lesion incidence occurred over seven audit periods from July 1993 to July 1995. Across the entirety of the period covered by successive reports of Dexter *et al.* (1994), then George *et al.* (1996), and now this study, the incidence of injection site lesions has declined 19.2 percentage points, suggesting that producers have changed injection administration practices. These changes were likely a response to educational efforts such as the National Cattlemen's Beef Association and state beef quality assurance programs. Even with such decline of incidence in top sirloin butts in past years, the beef industry must remain cautious and the education must continue to develop as the incidence of injection-site lesions in fed steer and heifer rounds was 11.3% in the 2000 audit (n = 7,436).

Average weight of trim per lesion (Table 4.1) resulting from the presence of injection-site lesions generally increased from 192.5 g in November 1995 to a peak in July 1997 of 435.8 g; mean trim loss has sporadically declined since July 1997 and was 249.8 g in July 2000. The increase in weight of injection-site lesion trim between November 1995 and July 2000 was not consistent with the findings of Dexter *et al.* (1994), who found that mean weight of trim loss per lesion declined from July 1991 to March 1993, but was consistent with the findings of George *et al.* (1996) who found that mean lesion trim weight increased from July 1993 to July 1995. The spike in mean lesion excision weights in 1997 coincide with the report of George *et al.* (1996) demonstrating toughening of muscle up to 7.62 cm away from the core of injection-site lesions and suggests that a short-term change in excision procedures for lesions may have been initiated by purveyors. Possible reasons for the recent increase in the weight of an injection-site lesion include changes in steak-cutting (hand cutting vs automation) and the changes in products (types of products, dosage of products, and adjuvant changes)(D. Griffin, personal communication).

Over the entire 15 audit period (November 1995 to July 2000), incidence of lesions classified as "cystic" (encapsulated lesion containing fluid) and "woody callus" (older lesion that is characterized by infiltration with organized connective tissue and fat) did not change ($P > 0.05$; Table 4.2). Incidence of lesions classified as "nodular" (lesion with nodules, the central foci of necrosis, surrounded by granulomatous inflammation) and "mineralized" (lesion containing mineralized remnants of muscle cells) decreased ($P < 0.05$), while the incidence of lesions classified as "clear" (older lesion that primarily contains clear connective tissue) increased ($P < 0.05$). Overall, 84% of the lesions

examined between November 1995 and July 2000 were classified as "older" lesions (either "woody callus" or "clear"). Possible reasons for changes in lesion classification include more critical selection of vaccine types, lower dose vaccines, adjuvant changes, and the introduction of new products (D. Griffin, personal communication).

Mean lesion weight by type and audit period are presented in Table 4.3. The mass of tissue surrounding injection-site lesions that was excised by purveyors during portioning increased ($P < 0.05$) from November 1995 through July 2000 for "clear" and "woody callus" lesions, but not for other classes of lesions. Mean weights of "cystic", "nodular", and "mineralized" lesions did not increase when comparing audits of November 1995 vs. July 2000, but excised weights of "nodular" lesions increased ($P < 0.05$) to a peak value of 470.5 g in July 1997 and weights of excised "mineralized" lesions increased ($P < 0.05$) to a peak value of 482.3 g in November 1998.

IMPLICATIONS

Injection-site lesions have caused enormous economic loss to the U.S. beef industry and have been a serious quality assurance problem. Reductions in lesion incidence in top sirloin butts from U.S. fed steer and heifers for the period of November 1995 through July 2000 -- from 11.4% to 2.1%, respectively -- generated an approximate net savings of $2.15 per steer or heifer slaughtered, which equates to an industry-wide savings of $76,078,100, based on the projected 30.31 million steers and heifers to be harvested in 2000 (USDA, 2000).

Table 4.1. Summary of injection-site damage (incidence and weight of lesions) in beef top sirloin butts for fifteen audits.

Audit Period	Packer Locations[a]	Steak Cutter Locations[b]	Number of Subprimals Evaluated	Incidence of Lesions[c] (%)	Average weight[d] of trim per lesion ± SE (g)
November 1995	IA, IL, KS, NE, TX, UT	AR, CO, IL, TN, WA	19,814	11.40[e]	192.5[k] ± 3.1
March 1996	CO, IL, KS, NE, TX, UT	AR, CA, CO, IL, TN	19,935	10.29[f]	211.1[j] ± 3.7
July 1996	AZ, CO, KS, NE, TX, WI	AR, CA, IL, TN	19,197	8.51[g]	212.2[j] ± 3.7
November 1996	KS, NE, TX, UT	AR, CA, IL, TN	21,617	9.03[g]	231.2[i] ± 4.2
March 1997	CO, KS, NE, TX,	AR, CA, IL, TN	19,065	7.48[h]	227.7[i] ± 4.6
July 1997	KS, NE	CA, IL, TN	11,088	5.61[i]	435.8[e] ± 12.9
November 1997	AZ, CO, IL, KS, NE, TX, UT, WI	CA, IL, TN, TX	14,644	5.59[i]	284.4[f] ± 7.2
March 1998	CO, KS, NE, UT	CA, IL, TX	12,927	4.75[j]	161.1[l] ± 6.1
July 1998	AZ, KS, NE, PA, TX	CA, TN, TX	8,693	6.07[i]	229.3[i] ± 6.2
November 1998	KS, NE, TX	CA, IL, TN, TX	8,044	4.43[j]	201.0[jk] ± 6.8
March 1999	CA, ID, KS, NE	CA, IL, TN, TX	16,237	4.64[j]	278.2[fg] ± 6.6
July 1999	KS, NE, TX	CA, IL, TN, TX	16,466	3.40[k]	210.7[jk] ± 4.7
November 1999	KS, NE, NY, TX	IL, TN, TX	10,772	2.67[l]	261.7[gh] ± 7.5
March 2000	KS, NE, TX	CA, IL, TN, TX	21,126	3.02[l]	229.4[i] ± 7.9
July 2000	CO, KS, NE, TX	CA, IL, TN, TX	20,455	2.06[m]	249.8[h] ± 9.3

[a] Packer-location origin of top sirloin butts.
[b] Steak-cutting facilities at which top sirloin butts were evaluated.
[c] Percentage of top sirloin butts that had an injection-site lesion.
[d] Average weight per lesion after excision.
[e,f,g,h,i,j,k,lm] Values, within a column, lacking a common superscript letter differ ($P < 0.05$).

Table 4.2. Percentage incidence (of lesions excised) of injection-site lesions stratified by five types of lesion classification.

Audit Period	Lesion Classification				
	Cystic[a]	Nodular[b]	Mineralized[c]	Clear[d]	Woody Callus[e]
November 1995	0.75^{ghi}	28.34^{f}	0.13^{gh}	46.06^{kl}	24.71^{l}
March 1996	0.59^{ghi}	17.02^{h}	0.00^{h}	49.39^{jk}	33.01^{hi}
July 1996	0.31^{i}	25.95^{f}	0.00^{h}	43.64^{l}	30.11^{ij}
November 1996	0.56^{ghi}	25.87^{f}	0.51^{f}	45.44^{kl}	27.61^{jk}
March 1997	0.35^{hi}	19.20^{g}	0.49^{f}	53.33^{i}	26.63^{kl}
July 1997	1.13^{fg}	21.86^{g}	0.48^{fg}	49.84^{ijk}	26.69^{jkl}
November 1997	0.73^{ghi}	16.85^{h}	0.85^{f}	45.79^{kl}	35.78^{gh}
March 1998	2.61^{f}	12.87^{hi}	0.65^{f}	52.77^{ij}	31.11^{hij}
July 1998	0.57^{ghi}	11.17^{ij}	0.76^{f}	49.62^{ijk}	37.88^{fg}
November 1998	1.40^{fg}	14.89^{hi}	0.84^{f}	57.58^{g}	25.28^{kl}
March 1999	0.93^{gh}	9.15^{jk}	0.00^{h}	49.34^{jk}	40.58^{fg}
July 1999	0.36^{ghi}	7.14^{kl}	0.00^{h}	48.93^{jk}	43.57^{f}
November 1999	0.69^{ghi}	4.51^{lm}	0.00^{h}	53.82^{h}	40.97^{fg}
March 2000	0.47^{ghi}	5.64^{lm}	0.31^{fg}	54.08^{g}	39.50^{fg}
July 2000	0.24^{i}	4.03^{m}	0.00^{h}	72.75^{f}	22.99^{l}

[a] Cystic = Encapsulated lesion containing fluid.

[b] Nodular = Lesion with nodules, the central foci of necrosis, surrounded by granulomatous inflammation.

[c] Mineralized = Lesion that contains mineralized remnants of muscle cells.

[d] Clear = Older lesion that contains primarily clear connective tissue.

[e] Woody Callus = Older lesion characterized by infiltration with organized connective tissue and fat.

[f, g, h, i, j, k, l, m] Percentages, within a column, lacking a common superscript letter differ ($P < 0.05$).

Table 4.3. Mean (± SE) weight (g) per injection-site lesion stratified by five types of lesion classification.

Audit Period	Lesion Classification				
	Cystic[a]	Nodular[b]	Mineralized[c]	Clear[d]	Woody Callus[e]
November 1995	$346.4^{fg} \pm 55.6$	$146.4^{jk} \pm 3.8$	$94.7^{hi} \pm 25.0$	$116.2^{o} \pm 3.7$	$223.7^{jk} \pm 8.3$
March 1996	$358.7^{fg} \pm 76.6$	$177.0^{j} \pm 5.6$	-	$143.6^{mn} \pm 3.2$	$216.8^{k} \pm 9.5$
July 1996	$266.6^{g} \pm 106.6$	$168.6^{jk} \pm 5.5$	-	$147.7^{lmn} \pm 4.4$	$221.8^{jk} \pm 8.9$
November 1996	$291.2^{g} \pm 77.5$	$189.6^{ij} \pm 6.6$	$81.3^{i} \pm 24.5$	$150.5^{klmn} \pm 5.0$	267.6^{hi} 10.3
March 1997	$218.2^{g} \pm 48.0$	$186.6^{ij} \pm 8.0$	$188.4^{ghi} \pm 27.3$	$159.3^{jklm} \pm 4.6$	$240.9^{ij} \pm 13.1$
July 1997	$291.9^{g} \pm 65.8$	$470.5^{f} \pm 28.6$	$311.3^{fgh} \pm 105.8$	$299.1^{f} \pm 15.8$	$525.0^{f} \pm 26.1$
November 1997	$310.5^{fg} \pm 84.2$	$216.6^{hi} \pm 15.2$	$356.9^{ab} \pm 91.8$	$240.4^{g} \pm 11.1$	$277.8^{gh} \pm 11.9$
March 1998	$293.6^{g} \pm 83.3$	$94.2^{k} \pm 13.9$	$352.8^{fg} \pm 90.3$	$108.4^{o} \pm 6.8$	$149.0^{l} \pm 12.1$
July 1998	$256.7^{g} \pm 91.6$	$180.1^{ij} \pm 16.6$	$316.8^{fgh} \pm 82.8$	$175.3^{hij} \pm 7.3$	$224.1^{jk} \pm 12.1$
November 1998	$439.0^{f} \pm 166.8$	$158.2^{jk} \pm 14.7$	$482.3^{f} \pm 246.3$	$127.2^{no} \pm 6.1$	$206.7^{k} \pm 14.7$
March 1999	$482.4^{f} \pm 245.9$	$235.1^{gh} \pm 18.6$	-	$197.7^{h} \pm 6.8$	$303.0^{g} \pm 11.3$
July 1999	$194.0^{g} \pm 52.0$	$169.8^{jk} \pm 16.4$	-	$151.2^{jklmn} \pm 5.5$	$213.9^{k} \pm 7.8$
November 1999	$512.0^{f} \pm 318.7$	$208.5^{hij} \pm 23.0$	-	$196.2^{hi} \pm 7.5$	$267.8^{hi} \pm 13.0$
March 2000	$374.4^{fg} \pm 92.0$	$298.1^{g} \pm 30.9$	$132.0^{ghi} \pm 18.0$	$168.6^{ijkl} \pm 10.4$	$215.1^{k} \pm 12.8$
July 2000	$93.0^{g} \pm 0.0$	$173.1^{jk} \pm 30.2$	-	$171.4^{hijk} \pm 8.3$	$296.7^{gh} \pm 28.1$

[a] Cystic = Encapsulated lesion containing fluid.

[b] Nodular = Lesion with nodules, the central foci of necrosis, surrounded by granulomatous inflammation.

[c] Mineralized = Lesion containing mineralized remnants of muscle cells.

[d] Clear = Older lesion that contains primarily clear connective tissue.

[e] Woody Callus = Older lesion characterized by infiltration with organized connective tissue and fat.

[f, g, h, i, j, k, l, m, n, o] Means, within a column, lacking a common superscript letter differ ($P < 0.05$).

CHAPTER V

THE IMPACT OF α-TOCOPHERYL ACETATE SUPPLEMENTATION ON DISCOLORATION OF INJECTION-SITE LESIONS IN RETAIL CUTS FROM THE CHUCK DURING STORAGE

ABSTRACT

During the 2000 National Beef Quality Audit, concern was raised by one of the major packers about a 'greening' discoloration condition of injection-site lesions in muscles of chuck steaks and/or roasts that were packaged in a high-oxygen environment. The objective of this study was to compare the severity of discoloration of injection-site lesions in chucks from carcasses of cattle supplemented with dietary α-tocopheryl acetate vs. that in chucks from carcasses of control, non-supplemented cattle. Fifty steers were vaccinated with a product shown to cause lesions that exhibit a 'greening' effect when packaged in a modified atmosphere environment. Steers were allocated to one of two treatment groups; control or vitamin E supplementation (1,000 IU/hd/d) for 60 d. After slicing, chuck steaks were packaged using a high-oxygen, modified atmosphere packaging system and were evaluated for the greening effect during boxed storage and retail display, histopathologic evaluation, or TBA and collagen determination. Following boxed storage and retail display, 80% of steaks from control, non-supplemented cattle

and 96% of steaks from cattle supplemented with vitamin E had a green injection-site lesion present. The injection-site lesions in steaks from control, non-supplemented cattle turned green, on average, at 78.6 hr after high-oxygen packaging and the injection-site lesions in steaks from cattle supplemented with vitamin E turned green, on average, at 87.3 hr after high-oxygen packaging. Across both treatment groups, of those steaks that exhibited greening, 39.5% of steaks did not have a visible lesion at the time of packaging. Color attributes (L*, a*, and b*) of muscle tissue did not differ between steaks from the control group and steaks from cattle supplemented with vitamin E before packaging or after retail display, nor were there differences in L*, a* or b* in injection-site lesions after retail display. Thiobarbituric acid values were greater ($P < 0.05$) in steaks from control, non-supplemented cattle at the site of the green injection-site lesion, and at a point 6 cm away from the injection-site lesion, than in steaks from Vitamin E supplemented cattle. Histopathologic evaluation verified the presence of injection-site lesions; the tissue contained fibrous connective tissue/scarring, sheet of macrophages, lymphoid follicles, atrophied muscle fibers, fat cells, and adjuvant. Although results suggested that vitamin E may delay formation of green discoloration, results indicated that vitamin E did not have the potential to reduce the incidence of green injection-site lesions observed in chuck muscles packaged in high-oxygen modified atmosphere.

INTRODUCTION

Appearance of beef in the retail case affects acceptability of the product to consumers (Liu *et al.*, 1996); bright-red muscle color suggests freshness to retail

consumers and is used as beef purchasing criteria by consumers (Clydesdale and Francis, 1971). Consumers discriminate against meat cuts that lack fresh appearance and, therefore, a strategy for maximizing acceptable fresh muscle color must involve delaying muscle pigment oxidation and/or enhancing reduction of oxidized muscle pigment (Faustman, 1990). During the 2000 National Beef Quality Audit, concern was raised by one major beef packer about a 'greening' discoloration condition of injection-site lesions in muscles of chuck steaks and/or roasts that were packaged in a high-oxygen environment.

Vitamin E has been used as a dietary supplement for cattle to delay color deterioration of whole-muscle and ground beef products because it is an antioxidant that delays the formation of metmyoglobin in muscle tissue (Mitsumoto *et al.*, 1991; Arnold *et al.*, 1992), thereby delaying meat discoloration (Faustman *et al.*, 1989b; Faustman and Wang, 2000). Studies have used high levels of vitamin E supplementation with conventional feeding times (2,000 to 3,000 IU/day for 30 to 100 d), while others have explored the use of lower supplementation levels (300 to 500 IU/day) with extended d (210 to 310 d) on feed (Arnold *et al.*, 1992; Faustman *et al.*, 1989a; Smith *et al.*, 1996). Delmore *et al.* (1998) documented that supplementation of cows with 50,400 IU of α-tocopheryl acetate within as few as fourteen d (900 IU/day for fourteen d) resulted in an improvement in retail caselife of beef from those cows. The objectives of this study were (1) to determine what pharmaceutical product could be used to recreate the green discoloration in injection-site lesions in muscles of the chuck after being packaged in a high-oxygen, modified atmosphere environment, and (2) to compare the severity of discoloration of injection-site lesions in chucks from carcasses of cattle

provided 1,000 IU/hd/d of supplemental α-tocopheryl acetate versus discoloration of injection-site lesions in chucks from carcasses of cattle not supplemented with α-tocopheryl acetate when packaged in a high-oxygen environment.

MATERIALS AND METHODS

Determination of the Pharmaceutical Product. Pharmaceutical companies and veterinarians were interviewed to determine, from their perspective, the market share of various products in the feedlot industry. Once interviews were completed, twelve (n = 12) pharmaceutical products were chosen for evaluation based on their market share in the industry, the class of product, and the type of adjuvant used in the product. Products chosen for evaluation included: (1) Ivermectin (Ivomec), Aspen Resources (Kansas City, MO); (2) Conquest 5K (Vira Shield), Aspen Resources (Kansas City, MO); (3) ScourBos 9, Grand Labs (Larchwood, IA); (4) Vision 7 with Spur, Intervet (Millsboro, DE); (5) CattleMaster 4, Pfizer Animal Health (Exton, PA); (6) Micotil 300, Elanco Animal Health (Indianapolis, IN); (7) Liquamycin (LA200), Pfizer Animal Health (Exton, PA); (8) One Shot Ultra 7, Pfizer Animal Health (Exton, PA); (9) Nuflor, Schering Plough Animal Health (Union, NJ); (10) BoviShield 4+L5, Pfizer Animal Health (Exton, PA); (11) Pyramid 4, Fort Dodge Animal Health (Fort Dodge, IA); and (12) Alyhydrogel 1.3% (aluminum hydroxide gel adjuvant), Accurate Chemical and Scientific Corp. (Westbury, NJ).

Twenty-four (n = 23) cattle were vaccinated 37 d before harvest with one of the twelve products (two animals/product with the exception of one animal being vaccinated with Alyhydrogel) to determine what product could be used to recreate the green

discoloration in muscles of the chuck after being packaged in a high-oxygen, modified atmosphere environment. Product administration followed label directions with the following exceptions: only 5 cc of a 9cc dosage of Ivermectin was administered, Conquest 5K did not clear withdrawal prior to harvest, ScourBos 9 did not clear withdrawal prior to harvest and was administered to feedlot steers, only 5 cc of a 13 cc dosage of Micotil was administered, only 5 cc of a 27 cc dosage of Nuflor was administered, and, lastly, all subcutaneous injections were administered using the non-tented technique. Cattle were harvested at the Colorado State University Meat Laboratory and chilled for 48 hours.

After chilling and removal of chucks, carcasses were condemned from use in the food chain. Chucks were sliced into 2.5 cm steaks and inspected/evaluated for the presence of an injection-site lesion corresponding to the area at which an injection was administered. When an injection-site lesion was present, two steaks were placed on a styrofoam tray, overwrapped and placed in a high-oxygen master pack at King Soopers (Denver, CO). When a lesion was not visible in the chuck, one steak was removed from the chuck ~15 cm posterior to the neck end and placed in a high-oxygen master pack. Following packaging, master packs were placed into boxed storage for five d and then placed on tables in a cooler maintained at 1 to 3°C under lighting conditions (Phillips Delux Warm White Florescent lamps, 24 h/d, the surface of the meat was exposed to 900 to 1365 lux) recommended by AMSA (1991) appropriate for simulation of retail display for three d.

Cattle Selection and Administration of Injections. Fifty yearling steers (n = 50) with known treatment history (no cattle previously receiving injections in the chuck

and/or all previous injections administered on only one, and on the same side of animals) were selected at a cooperating feedyard. Each steer received an intramuscular injection, administered by a cooperating veterinarian, on the right side in the *serratus ventralis* immediately anterior to the shoulder blade and as close to the spinal column as was practical. The intramuscular vaccination was administered in a manner previously shown to cause lesions that exhibit the 'greening' effect when packaged in a high-oxygen modified atmosphere environment, as demonstrated in the pharmaceutical determination phase of the project. Cattle were followed through the feeding phase to assure that all other feedlot processing vaccinations were administered to the animal in the neck region on the left side, the side opposite to that in which the injections of interest were administered for analysis in the present study.

The 25 cattle from each group (control and vitamin E supplemented) were shipped to harvest and tag transfer was completed by CSU personnel on the harvest floor in order to maintain the identity of cattle as they were converted into carcasses.

Vitamin E Supplementation. Cattle (n = 25) were supplemented with 1,000 IU/hd/d of α-tocopheryl acetate, top dressed on daily rations, for 60 d (total of 60,000 IU/hd during finishing).

Chuck Selection. Chucks were tagged, identified with a number which corresponded to that of the carcass, and tracked through fabrication. Chucks (NAMP 113, NAMP, 1997) were boxed and shipped to the Colorado State University Meat Laboratory. Upon arrival at Colorado State University, chucks were stored in vacuum packages at refrigerated temperatures (2°C) until four d postmortem, as might be the practice for product that is to be case-ready portioned and packaged. At four d

postmortem, chucks were sliced into 2.5 cm steaks and inspected/evaluated for the presence of an injection-site lesion corresponding to the area at which an injection was administered. Two steaks from each of the chucks in which lesions were visually identified were placed in high-oxygen, modified atmosphere packages. One steak was shipped to Food Safety Net Services (San Antonio, TX) for TBA analysis and the other steak was placed into boxed storage. When an injection-site lesion was not visible in the chuck, one steak was removed from the chuck ~15 cm posterior to the neck end and placed in a high-oxygen modified atmosphere package for boxed storage.

Boxed Storage and Retail Case Display. Packaged chuck steaks were placed in boxed storage for three d to simulate the typical amount of time product is in transit from packaging until being placed in a retail display case. After three d of boxed storage, each chuck steak was placed on tables in a cooler maintained at 1 to 3°C under lighting conditions (Phillips Delux Warm White Florescent lamps, 24 h/d, the surface of the meat was exposed to 900 to 1365 lux) recommended by AMSA (1991) to simulate retail display conditions.

Color Evaluation. Color of each steak prior to packaging was measured using a HunterLab MiniScan XE hand-held spectrophotometer equipped with a 6 mm aperture (HunterLab Associates, Inc., Reston, VA) to determine values for CIE L* (brightness; 0 = black, 100 = white), a* (redness/greenness; positive values = red, negative values = green), and b* (yellowness/blueness; positive values = yellow, negative values = blue) following procedures of the Commission Internationale de l'Eclairage (CIE, 1976). Values for "lesion" L*, a*, and b* were determined by computing the average of three readings obtained from the injection-site lesion location, if present, or an area

representative of the location at which most lesions were occurring if a lesion was not visible, and was collected before packaging and after retail display when the modified atmosphere could be sacrificed. After retail display, objective measures of "muscle" L*, a*, and b* also were collected from normal muscle tissue that did not exhibit the 'greening' reaction.

In addition to the objective evaluations of color obtained from lesions before packaging, and following retail display, a subjective color evaluation was conducted to monitor the presence of 'greening.' The subjective color score (0 if no discoloration, 1 if green discoloration was present) was recorded at the time of packaging; at 6, 12, 24, and 48 h after packaging during boxed storage, and at 72 h, which represented the completion of boxed storage and the beginning of simulated retail display. Steaks also were evaluated every 12 h during simulated retail display.

TBA and Collagen Analysis. One steak from each chuck with a visible lesion (n = 27; n = 15 from the vitamin E group, n = 12 from the control group) was packaged in a high-oxygen, modified atmosphere package and shipped to Food Safety Net Services (San Antonio, TX) for TBA analysis (Tarladgis *et al.*, 1960). Thiobarbituric acid (TBA) analysis was conducted using samples from the location of the 'greening,' as well as 6 cm away from the greening effect, to determine if differences in TBA values were associated with discoloration or due to treatment. Additionally, 9 of the 27 steaks (n = 5 from the vitamin E group, n = 4 from the control group) were selected for hydroxyproline determination (Procedure AOAC #990.26; AOAC, 2000). Steaks for hydroxyproline determination were selected based on the location of the lesions in order to compare lesions that were in the same muscle and in similar locations. Hydroxyproline analysis

was conducted using samples obtained at two locations on each of the nine steaks; locations were at a point that was at the center of the lesion and at a point approximately 6 cm away from the center of the lesion in the same muscle.

Histopathologic Examination. Following retail display, five chucks per treatment group were evaluated for histopathologic characteristics; each chuck selected for histopathologic analysis was characterized at the site of the injection-site lesion. Histopathologic examination was performed by the Department of Pathology, Colorado State University. Tissue samples were placed in 10% formalin for fixation, microtone slicing, and coded for submission. Microscopic examination was used to confirm the presence or absence of injection-site lesion characteristics.

Data Analysis. Chi-square was used to determine if differences existed in the frequency of green discoloration between cattle that were and were not supplemented α-tocopheryl acetate. Analysis of variance was used to determine if differences existed the time at which green discoloration of injection-site lesions was observed in the *serratus ventralis* muscle between the cattle that were vs. were not provided supplemental α-tocopheryl acetate. The analysis of variance model included dietary vitamin E supplementation level (0 or 1,000) as a fixed main effect, and retail display time as a repeated measure. Data were analyzed using the general linear models procedure of SAS (1999); a pairwise t-test was used to separate means when the model demonstrated a treatment effect ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Determination of the Pharmaceutical Product. Visible injection-site lesions were present in 87% of the chucks. Both steaks from each of the two chucks obtained from the steers vaccinated with Conquest 5K and one pair of steaks from a chuck obtained from a steer vaccinated with ScourBos 9 exhibited the green discoloration at 48 h of boxed storage. After an additional 3 d of boxed storage and 3 d of simulated retail display, no additional steaks revealed the green discoloration in the muscles of the chuck. Both products resulting in the green discoloration included Xtend III as a mineral oil-based adjuvant in the product.

Carcass Characteristics. Carcass characteristics, by group, of the fifty cattle utilized in this study are presented in Table 5.1. There were no differences in carcass characteristics between the control and vitamin E groups.

Boxed Storage and Retail Display. The percentages of chuck steaks with a green injection-site lesion at each boxed storage and retail display time, by treatment, are presented in Table 5.2. No steaks exhibited the greening reaction until 48 h of boxed storage time. At the end of boxed storage, 48% of the steaks from control, non-supplemented cattle and 44% of the steaks from cattle supplemented with vitamin E had a green injection-site lesion (Table 5.2). Following 96 h of retail display, 80% of steaks from control, non-supplemented cattle and 72% of steaks from cattle supplemented with vitamin E had a green injection-site lesion (Table 5.2). Of steaks that eventually exhibited greening of the injection-site, 60% of the control group and 61% of the vitamin E supplementation group turned green during boxed storage (Table 5.3). The injection-site lesions in steaks from control, non-supplemented cattle turned green, on average,

between the 72 hr and 84 hr evaluations (between the end of boxed storage and before 12 hours in the retail display case) and the injection-site lesions in steaks from cattle supplemented with vitamin E turned green, on average, between the 84 hr and 96 hr evaluations (between 12 hr and 24 hr in simulated retail display). The difference between chuck steaks from carcasses of fed cattle in the control and vitamin E supplementation groups did not differ statistically (P = 0.10). The trend may have economic and consumer satisfaction consequences (Table 5.3) because discoloration appeared to be slightly delayed in steaks from cattle supplemented with vitamin E, however, there is potential, with the delay, for the lesions to discolor after being sold to a consumer.

Of those steaks that eventually exhibited greening, 39.5% (n = 8 steaks from the control group, n = 7 steaks from the vitamin E group) of the steaks did not have a visible lesion at the time of packaging (data not presented in tabular form). Sixty-five percent of the steaks that did not have a visible lesion at the time of packaging turned green, on average, 92.8 ± 39.2 hr after packaging (at 20 hr of retail display) (data not presented in tabular form).

Color attributes (L*, a*, and b*) of injection-site lesions did not differ before packaging or after retail display between steaks from the control group and steaks from cattle supplemented with vitamin E (Table 5.4). However, the difference in a* between muscle tissue vs. injection-site lesion tissue after retail display was greater ($P < 0.05$) in steaks from control, non-supplemented cattle than in steaks from cattle supplemented with vitamin E (Table 5.4). Injection-site lesion a* values decreased from 8.04 and 8.11, at the time of cutting, to 3.90 and 4.29 following retail display in steaks from the control group and steaks from cattle supplemented with vitamin E, respectively (Table 5.4).

These values indicate that, from an objective measurement, the discoloration of lesions was more visible in steaks from the control group vs. steaks from the vitamin E supplementation group.

Thiobarbituric acid values were greater ($P < 0.05$) in steaks from control, non-supplemented cattle, at the site of the green injection-site lesion and at a point 6 cm away from the injection-site lesion, than in steaks from cattle supplemented with vitamin E. Steaks from control, non-supplemented cattle had TBA values of 1.576 and 0.947 at the injection-site lesion and at a point 6 cm away from the lesion, respectively, while TBA values in steaks from cattle supplemented with vitamin E were 0.400 and 0.239 at the site of the lesion and 6 cm away, respectively. Lower TBA values in steaks from cattle supplemented with vitamin E indicated that there was less lipid oxidation in the steaks as compared to the steaks from control, non-supplemented cattle. Hydroxyproline levels were elevated ($P < 0.05$) at the injection-site lesion vs. a point 6 cm away from the lesion site in steaks from the control group and the vitamin E supplemented group.

Histopathologic Evaluation. Figure 5.1 illustrates a microscopic view of the cross-section of normal muscle tissue. The tissue contained very distinct muscle fibers and very little fibrous connective tissue. Figures 5.2 through 5.4 illustrate cross-sections of green injection-site lesions. The lesions contained fibrous connective tissue/scarring, sheet of macrophages, lymphoid follicles, atrophied muscle fibers, fat cells, and adjuvant. George *et al.* (1995a, 1995b) reported similar histopathologic findings in injection-site lesions administered at branding and weaning, as well as at sites 2.54 and 5.08 cm away from the center of injection-site lesions indicating that tissue damage is not limited to the visible injection-site lesion, but is common around the lesion site.

IMPLICATIONS

Although results suggested that vitamin E may delay formation of green discoloration, results indicated that vitamin E did not have the potential to reduce the incidence of green injection-site lesions observed in chuck muscles packaged in high-oxygen modified atmosphere. While elevated vitamin E concentration has been demonstrated to enhance retail caselife by maintaining color stability of muscle pigments, this study did not support the hypothesis that supplementation of vitamin E to cattle might eliminate the greening discoloration of misplaced injection-site lesions. Data also indicated that incomplete visibility of lesions upon cutting, and before packaging, poses difficulties in identifying these defects at the packer/processor level. Additional research should be conducted to develop methods of detection of injection-site lesions before chuck steaks are packaged in high-oxygen, modified atmosphere environments.

Table 5.1 Means and standard deviations for carcass characteristics stratified by control vs. treatment groups.

Carcass trait	Control ± SD (n = 25)	Vitamin E Treatment ± SD (n = 25)
Hot carcass weight (kg)	366.40 ± 24.97	372.04 ± 26.55
Longissimus muscle area (cm^2)	87.03 ± 6.52	84.84 ± 7.74
Kidney, pelvic and heart fat (%)	1.62 ± 0.33	1.56 ± 0.30
Fat thickness (cm)	1.27 ± 0.30	1.40 ± 0.28
Yield grade	2.83 ± 0.46	3.09 ± 0.49
Marbling score[a]	354.80 ± 41.64	380.40 ± 48.69

[a] Marbling score is coded as 300=slight 00, 350=slight 50, 400=small 00.

Table 5.2. Percentages of chuck steaks exhibiting the 'greening' reaction at each color evaluation stratified by control vs. treatment groups.

Evaluation Time[a]	Control (n = 25)	Vitamin E (n = 25)
Boxed Storage-time 0	0	0
Boxed Storage-time 6 hr	0	0
Boxed Storage-time 12 hr	0	0
Boxed Storage-time 24 hr	0	0
Boxed Storage-time 48 hr	36	24
Boxed Storage-time 72 hr	48	44
Retail Display-time 12 hr	60	48
Retail Display-time 24 hr	60	52
Retail Display-time 36 hr	68	52
Retail Display-time 48 hr	68	56
Retail Display-time 60 hr	68	56
Retail Display-time 72 hr	72	56
Retail Display-time 84 hr	80	72
Retail Display-time 96 hr	80	72

[a] No differences existed between control vs. treatment groups at any time period.

Table 5.3. Overall percentages of chuck steaks exhibiting the 'greening' reaction, percentage of those exhibiting 'greening' that turned green during boxed storage, percentage of those exhibiting 'greening' that turned green during retail display, and the least squares mean hr until 'greening' stratified by control vs. treatment groups.

Trait[a]	Control (n = 25)	Vitamin E Treatment (n = 25)	SEM
Percentage exhibiting 'greening'	80.0 (n=20)	72.0 (n = 18)	0.434
Percentage of those exhibiting 'greening' that turned green during boxed storage	60.0 (n = 12)	61.1 (n = 11)	0.502
Percentage of those exhibiting 'greening' that turned green during retail display	40.0 (n = 8)	38.9 (n = 7)	0.502
Least squares mean time until 'greening' (hr)[b]	78.6	87.3	39.8

[a] No differences ($P > 0.05$) existed between control vs. treatment groups for any of the traits.

[b] $P = 0.10$.

Table 5.4. Least square mean values for CIE L*, a*, and b* color attributes and mean differences between muscle color and lesion color after retail display by control vs. treatment groups.

Color Attribute	Control (n = 25)	Vitamin E Treatment (n = 25)	SEM
Lesion L* (before packaging)	42.76	43.17	5.86
Lesion a* (before packaging)	8.04	8.11	2.15
Lesion b* (before packaging)	10.30	10.55	1.52
Lesion L* (after display)	49.77	48.80	8.96
Lesion a* (after display)	3.90	4.29	1.46
Lesion b* (after display)	9.84	10.21	1.47
Difference in L* between normal muscle tissue after display and lesion after display	-9.69	-7.91	4.30
Difference in a* between normal muscle tissue after display and lesion after display	6.55[a]	5.71[b]	1.29
Difference in b* between normal muscle tissue after display and lesion after display	2.32	1.64	1.48

[a,b] Means within a row bearing a common superscript do not differ ($P > 0.05$).

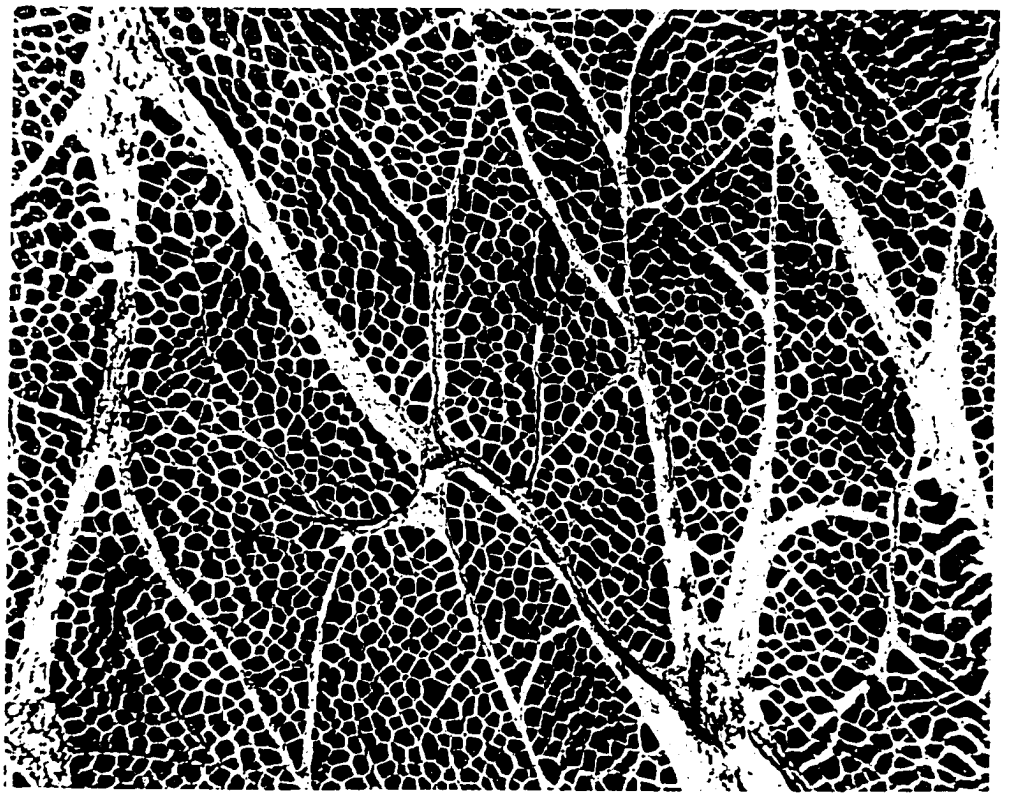


Figure 5.1. Histopathologic cross-section of normal muscle. The image contains clearly identifiable muscle fibers and contains very little connective tissue.

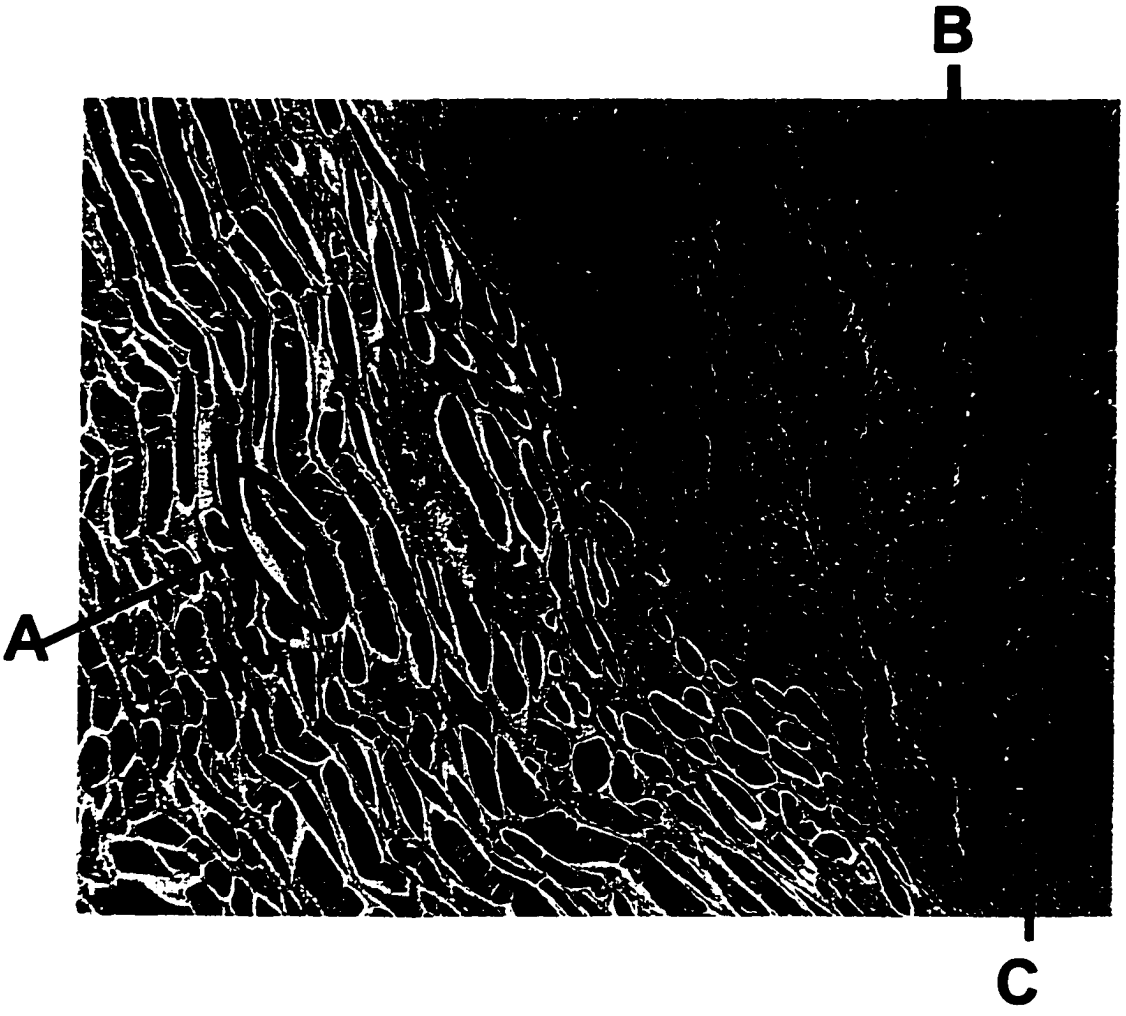


Figure 5.2. Histopathologic cross-section of a green injection-site lesion. Tissue consists of fibrous connective tissue or scarring (A), sheets of macrophages and fibrous connective tissue walling-off adjuvant (B), and lymphoid follicles (C).

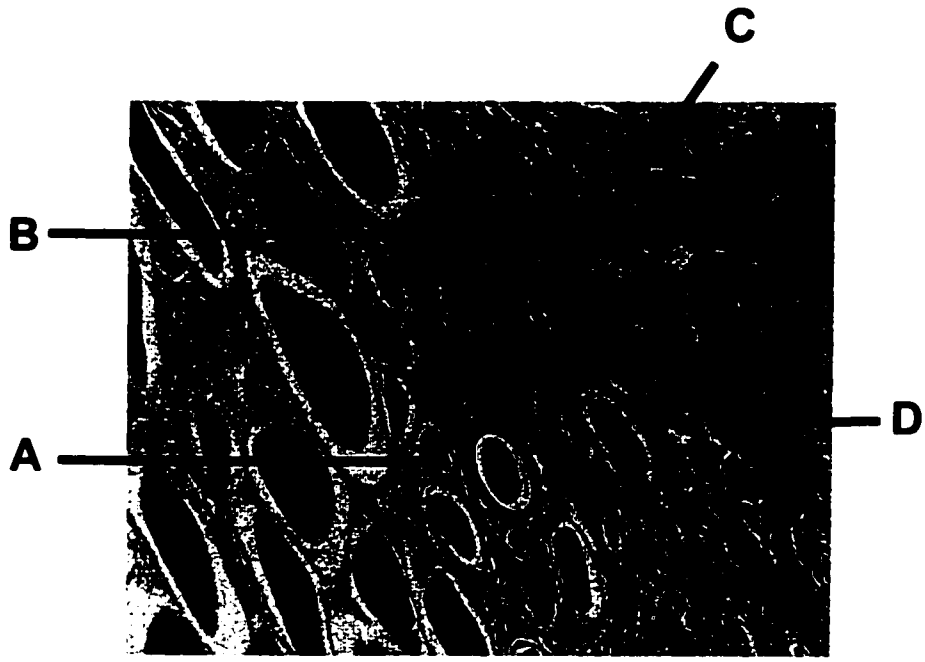


Figure 5.3. Histopathologic cross-section of an injection-site lesion after onset of the green discoloration. Tissue consists of atrophied muscle fibers (A), scar tissue or connective tissue (B), adjuvant (C), and nuclei of muscle fibers (D).

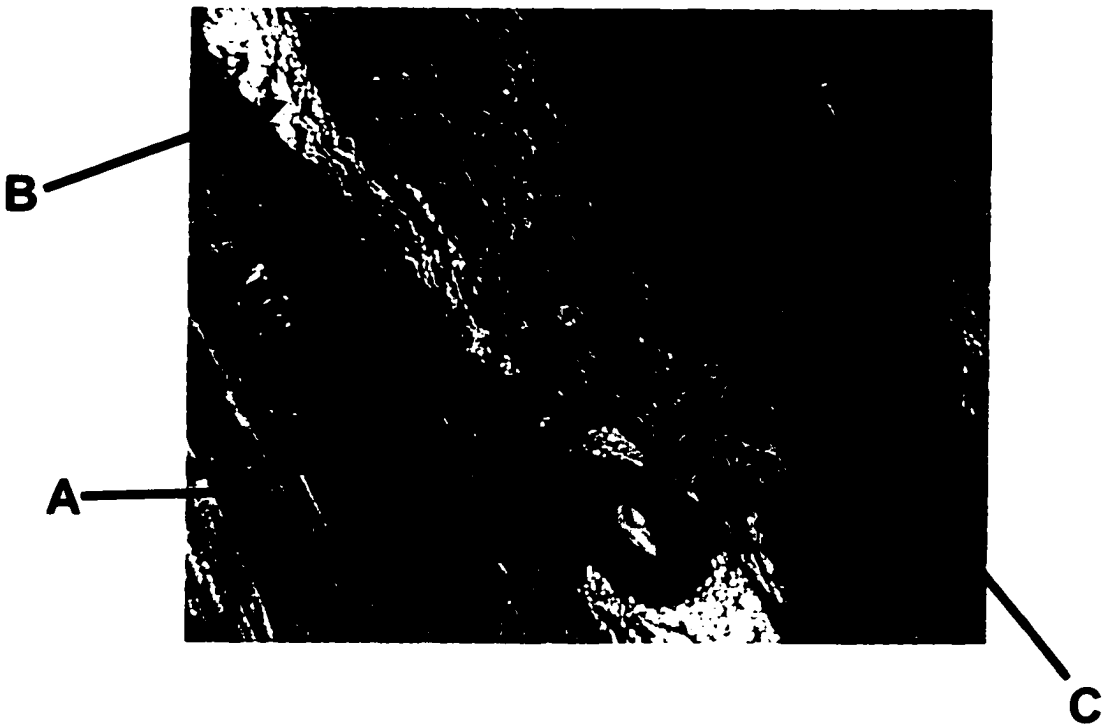


Figure 5.4. Histopathologic cross-section of a green injection-site lesion stained with Masson's trichrome stain. Tissue consists of muscle fibers (A), fat cells (B), and fibrous connective tissue (C).

CHAPTER VI

THE GREENING REACTION OBSERVED IN INJECTION-SITE LESIONS IN MUSCLES OF THE CHUCK

ABSTRACT

During the 2000 National Beef Quality Audit, concern was raised by one of the major packers about a 'greening' discoloration condition of injection-site lesions in muscles of chuck steaks and/or roasts that were packaged in a high-oxygen environment. The objective of this study was to determine the pigment responsible for the green discoloration that occurs in injection-site lesions in muscles of the chuck when such cuts are packaged in high-oxygen, modified atmosphere packages by studying color reactions *in vitro*. Green injection-site lesions were extracted from muscles of the chuck. In phase I, solutions of myoglobin (Mb), copper sulfate ($CuSO_4$), hydrogen peroxide (H_2O_2), vaccine, and aluminum hydroxide (AlOH), as well as combinations of two or more of the solutions, were subjected to high partial pressures of oxygen. In phase II, solutions of two or more of Mb, copper (Cu), sodium sulfide (NaS), sodium sulfite ($NaSO_3$), sodium sulfate ($NaSO_4$), and/or H_2O_2 were made at pH 7.2 or 5.5 and subjected to either low or high partial pressures of oxygen. Solutions from phase I and phase II and extracted lesions from chucks from cattle supplemented vitamin E and from cattle not

supplemented vitamin E were evaluated using spectrophotometry. The pigments from the green lesions from steaks from control cattle and from steaks from vitamin E supplemented cattle displayed, on average, a 164.5 and 621.3 percent increase in absorbance/μg protein at 656 nm as compared to 654 nm, respectively. The absorbance for the lesions from control and supplemented cattle declined 75 and 109 percent, respectively, from 656 to 658 nm. These changes in absorbance matched the changes in absorbance observed in the sulfmyoglobin and hydroperoxymetmyoglobin pigments made in phase I. Based on the results from phase I, it was likely that the green color resulted from a reaction between myoglobin and either $CuSO_4$ or H_2O_2. Solutions, in phase II, which exhibited a positive percent change from 654 to 656 and a negative percent change from 656 to 658 were Mb+$NaSO_4$, Mb+Cu+H_2O_2, Mb+SO_3, Mb+Cu+NaS, and Mb+Cu+$NaSO_3$, all at pH 7.2 under low partial pressures of oxygen; Mb+$NaSO_4$, Mb+NaS, and Mb+$NaSO_3$, all at pH 7.2 under high partial pressures of oxygen; and Mb+Cu+$NaSO_4$ at pH 5.5 under high partial pressures of oxygen. Results indicated that the 'greening' reaction observed in injection-site lesions of the chucks maybe a result of the reaction between myoglobin, copper, and sulfur, and, given the increased copper concentration at the injection-site lesion, suggests that there is an increased concentration of sulfur either at the lesion site or in pharmaceutical products. Further research investigating the causative agents in the chemical reaction causing the green discoloration of injection-site lesions in chuck steaks packaged in high-oxygen need to be conducted with emphasis on sulfur and copper compounds.

INTRODUCTION

Beef muscle tissue damaged as a result of administration of animal health products intramuscularly is a quality challenge facing the beef industry. Injection-site lesions may be caused by contamination, animal sensitivity to the vaccine, vaccination injury, or the adjuvant used to enhance the immune response (Troxel *et al.*, 2001). Vaccines containing an oil adjuvant produce larger, more persistent lesions than vaccines containing aluminum hydroxide (Straw et al., 1990). Incidence of injection-site lesions in top sirloin butts of fed steers and heifers has declined from a high of 21.3% in July of 1991 (Dexter *et al.*, 1994) to 2.1% in July of 2000 (Roeber *et al.*, 2001b). The decline in the incidence of lesions has been one of the major success stories for the beef industry since 1991 (Smith *et al.*, 2001). However, during the 2000 National Beef Quality Audit, concern was raised by one major packer about a 'greening' discoloration that was apparently occurring in injection-site lesions manifested in muscles of chuck steaks and/or roasts packaged in a high-oxygen environment (Smith *et al.*, 2001).

Most green discoloration in muscles of fresh meat is associated with an alteration (saturation of a double bond) in heme structure (Lawrie, 1998). Green color in muscle is normally attributed to hydroperoxymetmyoglobin, sulfmyoglobin, or choleglobin (Price and Schweigert, 1987).

Hydroperoxymetmyoglobin forms from the reaction of hydrogen peroxide and myoglobin under mildly acidic conditions in meat. The pigment is green and is mildly oxidized; the distal histidine is the site of the oxidation in the reaction (Price and Schweigert, 1987). Sulfhemoglobin is a green heme pigment that is physiologically inactive and has reduced oxygen affinities (Chatfield *et al.*, 1987). Sulfmyoglobin results

from adding a sulfide group to the porphyrin ring (Michel, 1938; Lemberg and Legge, 1949; Price and Schweigert, 1987). Choleglobin is another green pigment associated with excessive meat pigment oxidation sufficient to cause porphyrin-ring opening (Price and Schweigert, 1987). Choleglobin is produced from hydrogen peroxide acting on hemoglobin in the presence of ascorbic acid (Lemberg and Legge, 1949).

This study was conducted to determine the pigment responsible for the green discoloration of injection-site lesions that occurs in muscles of the chuck when packaged in modified atmosphere, high-oxygen environments by studying color reactions *in vitro*.

MATERIALS AND METHODS

Materials. A single vaccine, identified in previous endeavors as causing a 'greening' discoloration of injection-site lesions in meat stored in modified atmosphere, high-oxygen packages, was chosen for use during the *in vitro* determination of the cause of the chemical reaction resulting in the discoloration. All chemicals used were reagent grade or better and were purchased from Fisher Scientific (Houston, TX). Deionized water was used throughout the study. Myoglobin was from horse skeletal muscle (ICN Biomedical, Aurora, OH) and was used without further purification. Fresh 1% and 3% H_2O_2 was prepared for each phase of the experiment by diluting stock solution with water. All experiments were conducted at room temperature.

Phase I.

Preparation of 'Green' Injection-Site Lesions. Twenty (N = 20; n = 10 lesions from chuck steaks from cattle not supplemented with Vitamin E, n = 10 lesions from

chuck steaks from cattle supplemented with Vitamin E) green injection-site lesions from a companion study, as well as eighteen (N = 18) green lesions supplied by a cooperating packing plant processing facility, were extracted from affected steaks using a sterile knife. Myoglobin pigments were extracted from the muscle as described by Smith and Carpenter (1970).

In vitro Reactions. *In vitro* solutions of muscle (MSC) or myoglobin (Mb), 3% hydrogen peroxide (H_2O_2), aluminum hydroxide (AlOH), 1 ppm copper sulfate ($CuSO_4$), vaccine (VAC), and combinations of two or more of the solutions were made. Solutions generated included: Mb, AlOH, $CuSO_4$, H_2O_2, VAC, MSC, Mb+$CuSO_4$, Mb+VAC, MSC+$CuSO_4$, MSC+VAC, MSC+H_2O_2, MSC+$CuSO_4$+VAC, Mb+$CuSO_4$+VAC, Mb+AlOH, and MSC+AlOH. Three ml of solubilized myoglobin, prepared according to Morey *et al.* (1973), were added to each of the solutions that were to contain myoglobin. Each reaction was replicated eight times. Reactions were subjected to high partial pressures of oxygen for 24 h before further evaluation. Sulfmyoglobin and hydroperoxymetmyoglobin were generated according to the protocol outlined by Michel (1938) and Morey *et al.* (1973), respectively.

Spectrophotometric Analysis. Following storage in the high oxygen environment, 1 ml of each sample replicate was analyzed using the Hewlett Packard Spectrophotometer (HP89532R UV-Visible Spectrophotometer; Wilmington, DE) to obtain absorbance values from 430 to 670 nm. Protein concentration was determined according to Bradford (1976). Protein concentrations were used to calculate absorbance:protein concentration ratios for statistical analysis.

Statistical Analysis. Ratios of absorbance:protein concentration and changes in absorbance:protein ratios from two sequential wavelengths were analyzed using the General Linear Models procedure of SAS (1999). The model included the presence of myoglobin/muscle, vaccine, copper sulfate, hydrogen peroxide, and aluminum hydroxide as fixed main effects. A pairwise t-test was used to separate means when the model demonstrated a significant main effect (α = 0.05).

Phase II.

In vitro Reactions. *In vitro* solutions of two or more of myoglobin (Mb), 3% hydrogen peroxide (H_2O_2), 1 ppm copper (Cu), 0.3 ppm sodium sulfide (Na_2S), 0.3 ppm sodium sulfite (Na_2SO_3), and/or 0.3 ppm sodium sulfate (Na_2SO_4) were made. Solutions generated included: Mb+Cu, Mb+Na_2S, Mb+Na_2SO_3, Mb+Na_2SO_4, Mb+Cu+Na_2S, Mb+Cu+Na_2SO_3, Mb+Cu+Na_2SO_4, Mb+Cu+H_2O_2, H_2O_2+Na_2S, H_2O_2+Na_2SO_3, H_2O_2+Na_2SO_4, Mb+H_2O_2+Na_2S, Mb+H_2O_2+Na_2SO_3, Mb+H_2O_2+Na_2SO_4, Mb+H_2O_2, and Cu+H_2O_2. For each of the reactions, myoglobin was solubilized as described for phase I, and 3 ml of the myoglobin solution were added to 1 ml of the appropriate solution(s); reactions without myoglobin contained equal proportions of each solution in a total of 4 ml. Reactions were mixed at a pH of 5.5 or 7.2 and were subjected to either high partial pressures of oxygen or to environmental pressures of oxygen for 24 h before further evaluation. Each reaction was replicated three times in a 2 pH x2 partial pressures of oxygen blocked factorial design.

Spectrophotometric Analysis. Following storage in the appropriate environment, 1 ml of the each sample replicate was analyzed using the Hewlett Packard

Spectrophotometer (HP89532R UV-Visible Spectrophotometer; Wilmington, DE) to obtain absorbance values from 640 to 670 nm. Protein concentration was determined as previously described. Protein concentrations were used to calculate absorbance:protein concentration ratios for statistical analysis.

Statistical Analysis. Ratios of absorbance:protein concentration and changes in absorbance:protein ratios from two sequential wavelengths were analyzed using the General Linear Models procedure of SAS (SAS, 1999). The model included the presence of myoglobin, copper, sulfide, sulfite, sulfate, and hydrogen peroxide as fixed main effects. A pairwise t-test was used to separate means when the model demonstrated a significant main effect (α = 0.05). If main effects were significant, an additional model was used to analyze interactions between main effects.

RESULTS

Characteristics of 'Green' Injection-Site Lesions. Figure 6.1 displays the absorbance characteristics of green injection-site lesions of steaks from control and vitamin E supplemented cattle. The absorbance/μg protein of the green pigments from injection-site lesions from control and Vitamin E supplemented cattle displayed, on average, a 164.5 and 621.3 percent increase ($P < 0.05$) from 654 to 656 nm, respectively. The absorbance for the lesions from control and supplemented cattle declined ($P < 0.05$) 75 and 109 percent, respectively, from 656 to 658 nm. These changes in absorbance matched the changes in absorbance observed in the sulfmyoglobin and hydroperoxymetmyoglobin pigments generated in phase I. The absorbance•(μg/ml protein)$^{-1}$ of sulfmyoglobin was 0.00065, 0.00099, and -0.00025 at 654, 656, and 658 nm,

respectively, representing an increase of 52.8% from 654 to 656 nm, followed by a decline of 125.3% from 656 to 658 nm. A similar trend was observed for hydroperoxymetmyoglobin where the absorbance•(μg/ml protein)$^{-1}$ was 0.00032, 0.00037, and 0.00023 at 654, 656, and 658 nm, respectively, representing an increase of 15.4% from 654 to 656 nm, followed by a decline of 38.8% from 656 to 658 nm.

Phase I. The ratio of absorbance to μg protein for phase I products demonstrated that, in comparison to the characteristics of the lesions, the green color was not a result of AlOH. The green characteristics also were not typical of solutions which included muscle tissue (MSC, MSC+$CuSO_4$, MSC+VAC, MSC+H_2O_2, MSC+$CuSO_4$+VAC, and MSC+AlOH), but were typical of some solutions generated with myoglobin, $CuSO_4$, H_2O_2, and Mb+$CuSO_4$ (Figure 6.2). The percent change from 654 nm to 656, as well as the percent change from 656 nm to 658 nm, for each of the solutions exhibiting similar characteristics of extracted green injection-site lesions is presented in Table 6.1. Based on the results from Phase I, the green color denoted by an increase in absorbance at 656 nm appeared to result from reactions of Mb with either $CuSO_4$ or H_2O_2. Therefore, the second phase was designed to determine the effects of elemental Cu, sulfate, sulfite, sulfide, and H_2O_2 on the greening reaction.

Phase II. The ratio of absorbance to μg/ml protein for phase II products demonstrated that, in comparison to the characteristics of the lesions, myoglobin had to be present in the solution for the green reaction to occur. Further analysis indicated that the solutions that exhibited a positive percent change in the absorbance per μg/ml protein from 654 nm to 656 nm and a negative percent change from 656 nm to 658 nm were Mb+$NaSO_4$, Mb+Cu+H_2O_2, Mb+SO_3, Mb+Cu+NaS, and Mb+Cu+$NaSO_3$, all at pH 7.2

under atmospheric partial pressures of oxygen; Mb+$NaSO_4$, Mb+NaS, and Mb+$NaSO_3$, all at pH 7.2 under high partial pressures of oxygen; and Mb+Cu+$NaSO_4$ at pH 5.5 under high partial pressures of oxygen (Figure 6.3). The percent change from 654 nm to 656 nm, as well as from 656 nm to 658 nm, is presented in Table 6.2.

DISCUSSION

Absorbance per μg/ml protein of sulfmyoglobin and hydroperoxymetmyoglobin peaked at 617 nm and 589 nm, respectively, as previously documented by Nicol *et al.* (1970) and George and Irvine (1952), respectively. In addition, absorbance ratios of sulfmyoglobin and hydroperoxymetmyoglobin peaked at 656 nm.

Lesions evaluated during the study identified a dramatic change in absorbance to protein concentration ratios from 654 nm to 656 nm and from 656 nm to 658 nm. In fact, absorbance ratios from lesions were more characteristic of changes seen in absorbance to protein concentration ratios of sulfmyoglobin and hydroperoxymyoglobin in Phase I of the study. Lesions from steaks from cattle supplemented vitamin E resulted in a larger ($P < 0.05$) percent increase in absorbance to protein concentration ratios than control lesions. The difference between the absorbance to protein concentration ratios may be attributed, in part, to the production of oxidized glutathione, which could serve as another source of sulfur for the development of sulfmyoglobin (Figure 6.4).

Phase I *in vitro* color reactions revealed that greening discoloration could not be created with solutions including muscle, but could occur in a solution of Mb+$CuSO_4$. The Mb+$CuSO_4$ solution generated a green product that resembled sulfmyoglobin and/or hydroperoxymetmyoglobin. It also was apparent from data obtained in Phase I that the

'greening' reaction observed in lesions under a high-oxygen, modified atmosphere environment caused an increase in the absorbance to protein concentration ratio from 654 nm to 656 nm, followed by a decline in the ratio from 656 nm to 658 nm. Based on the results of Phase I, Phase II was conducted to evaluate the effects of hydrogen peroxide, a pure, elemental copper source, and three different sources of sulfur (sulfide, sulfite, or sulfate) on the change in absorbance from 654 nm to 656 nm and from 656 nm to 658 nm in order to determine if the 'greening' characteristic was a result of copper, sulfur, or hydrogen peroxide reactions.

Phase II data indicated that the 'greening' reaction observed in lesions in modified atmosphere packaging is related more so to the presence of sulfur, in any of the three forms (SO_4, SO_3, or S), than to the presence of copper or hydrogen peroxide. The addition of sodium sulfate, sodium sulfite, or sodium sulfide, individually, to myoglobin under neutral pH conditions resulted in absorbance characteristics typical of the green pigments produced in Phase I and of the green pigments extracted from the lesions in the study. The presence of copper with sodium sulfide or sodium sulfite and myoglobin at neutral pH and under atmospheric environment, with sodium sulfate and myoglobin at a low pH under high partial pressures of oxygen, as well as with hydrogen peroxide and myoglobin at neutral pH under low partial pressures of oxygen also resulted in absorbance to protein concentration ratios similar to the green pigments produced in Phase I of the study. Given the conditions under which the discoloration occurs, the combination of copper and sodium sulfate with myoglobin is likely the cause of the green pigment. Since copper is present at an injection-site lesion as a result of the immune

response, it would seem likely that sulfur is either in the pharmaceutical product and/or that sulfur is a by-product of vitamin E metabolism in the muscle tissue.

IMPLICATIONS

The solution of myoglobin and vaccine did not result in green color characteristics, which indicated that the immune response is potentially involved in the development of such discoloration. Additionally, in Phase I, the solution of myoglobin and copper sulfate did result in the green discoloration. Further results indicated that the 'greening' reaction observed in injection-site lesions of the chucks maybe a result of the reaction between myoglobin, copper, and sulfur, and, given the increased copper concentration at the injection-site lesion, suggests that there is an increased concentration of sulfur either at the lesion site, sulfur in pharmaceutical products, and/or a by-product of vitamin E metabolism in the muscle tissue. Further research investigating the causative agents in the chemical reaction causing the green discoloration of injection-site lesions in chuck steaks packaged in high-oxygen need to be conducted with emphasis on sulfur and copper compounds.

Table 6.1. Comparison of the percent change in spectrophotometric absorbance per µg/ml protein from 654 nm to 656 nm and from 656 nm to 658 nm in Phase I *in vitro* solutions and 'green' lesions.

	Percent Change	
Sample	**654 to 656 nm**	**656 to 658 nm**
Lesions (Phase I)	7[b]	-18[b]
Control Lesions	165[b]	-75[b]
Vitamin E Lesions	621[a]	-109[a]
Copper Sulfate	11[b]	-54[b]
Hydrogen Peroxide	214[b]	-71[b]
Myoglobin	6[b]	-52[b]
Myoglobin+Copper Sulfate	44[b]	-89[a]
Sulfmyoglobin	53[b]	-125[a]
Hydroperoxymetmyoglobin	15[b]	-39[b]

[a,b] Percentages, within a column, bearing a common superscript letter are not different ($P > 0.05$).

Table 6.2. Comparison of the percent change in spectrophotometric absorbance per µg/ml protein from 654 nm to 656 nm and from 656 nm to 658 nm in Phase II *in vitro* solutions and 'green' lesions.

	Percent Change	
Sample[a]	**654 to 656 nm**	**656 to 658 nm**
Control Lesions	165^{cd}	-75^{d}
Vitamin E Lesions	621^{b}	-109^{cd}
Mb+$NaSO_4$, pH 7.2, high ppO	317^{bc}	-181^{b}
Mb+ $NaSO_4$, pH 7.2, low ppO	10^{d}	-91^{cd}
Mb+Cu+$NaSO_4$, pH 5.5, high ppO	2^{d}	-130^{bcd}
Mb+Cu+H_2O_2, pH 7.2, low ppO	41^{d}	-286^{a}
Mb+Cu+NaS, pH 7.2, low ppO	4^{d}	-171^{bc}
Mb+Cu+$NaSO_3$, pH 7.2, low ppO	8^{d}	-119^{bcd}
Mb+NaS, pH 7.2, high ppO	11^{d}	-164^{bc}
Mb+$NaSO_3$, pH 7.2, low ppO	1^{d}	-107^{cd}
Mb+$NaSO_3$, pH 7.2, high ppO	5^{d}	-179^{b}

[a] Sample abbreviations: Mb = myoglobin, $NaSO_4$ = sodium sulfate, high ppO = high partial pressures of oxygen (high-oxygen environment), low ppO = low partial pressures of oxygen (atmospheric environment), Cu = elemental copper, H_2O_2 = hydrogen peroxide, NaS = sodium sulfide, $NaSO_3$ = sodium sulfite.

[b,c,d] Percentages, within a column, bearing a common superscript letter are not different ($P > 0.05$).

Figure 6.1. Ratios of absorbance to µg/ml protein for 'green' injection-site lesions from chucks from control cattle and from Vitamin E supplemented cattle. C.Les = lesions from chucks from control cattle; E.Les = lesions from chucks from cattle supplemented with Vitamin E.

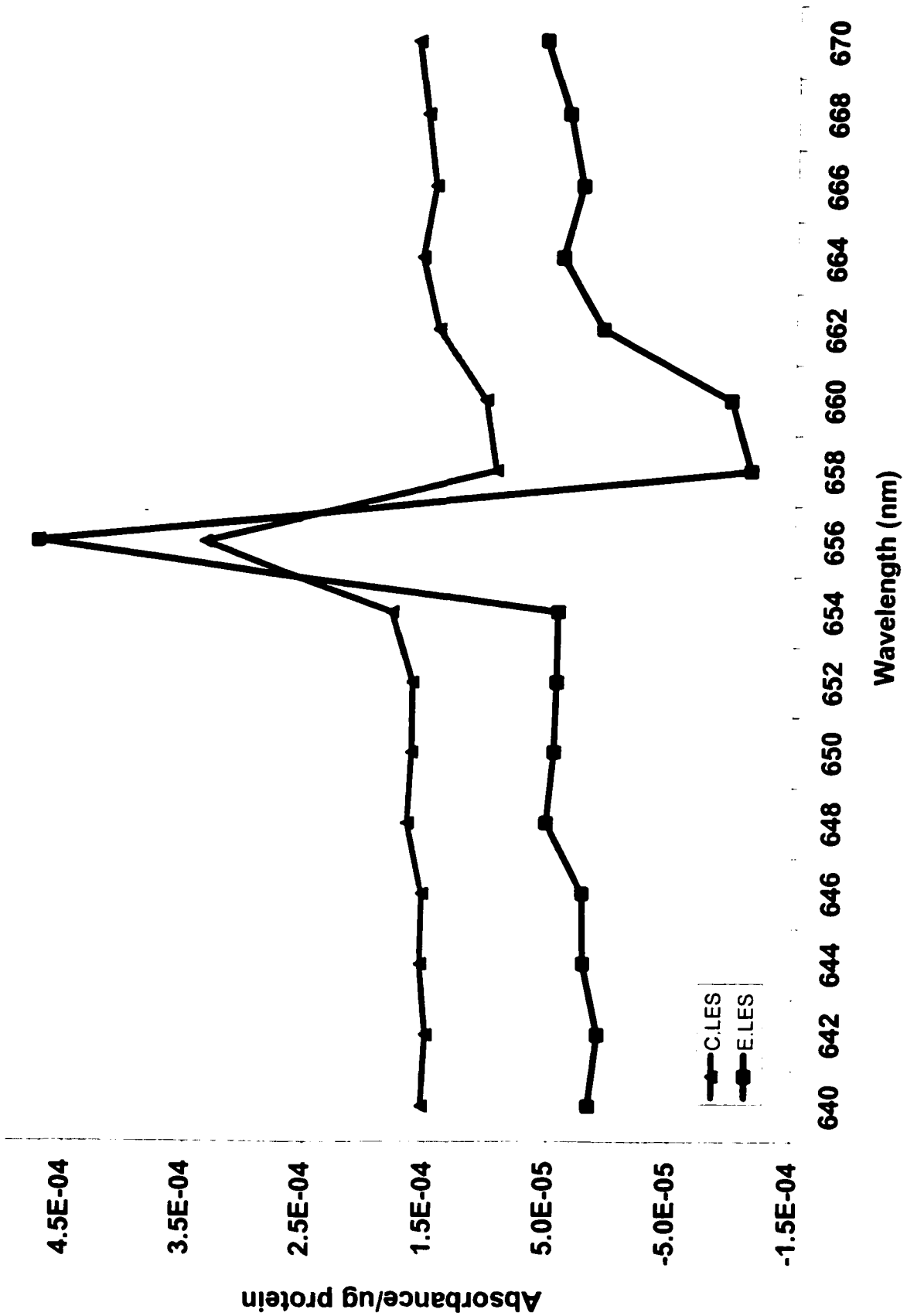


Figure 6.2. Ratios of absorbance to µg/ml protein for 'green' injection-site lesions and *in vitro* color reactions produced under high partial pressures of oxygen. H_2O_2 = hydrogen peroxide, HPMb = hydroperoxymetmyoglobin, Mb = myoglobin, MbCu = myoglobin plus copper sulfate, SMb = Sulfmyoglobin, Lesion = green lesions supplied by a cooperating packer.

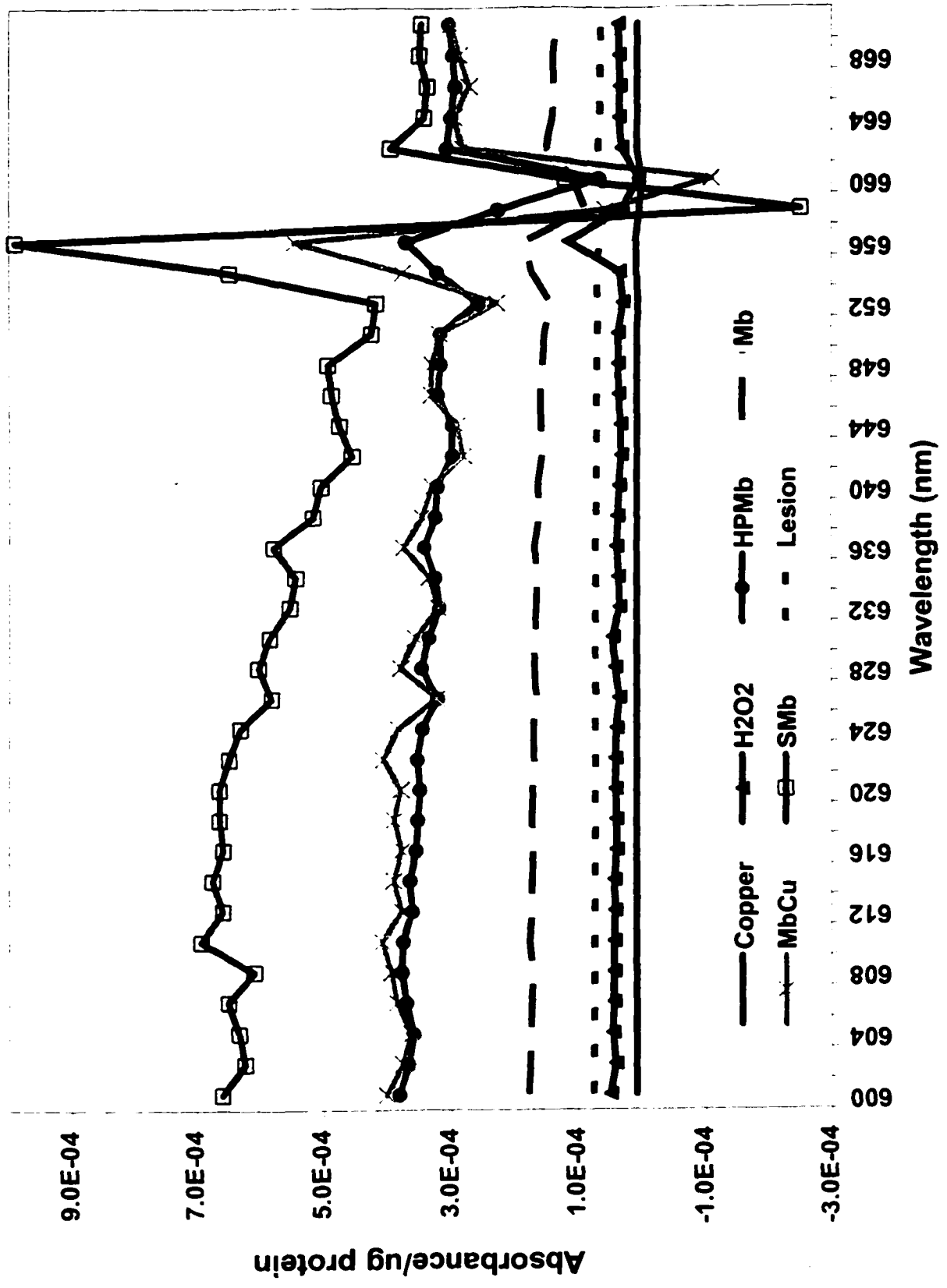
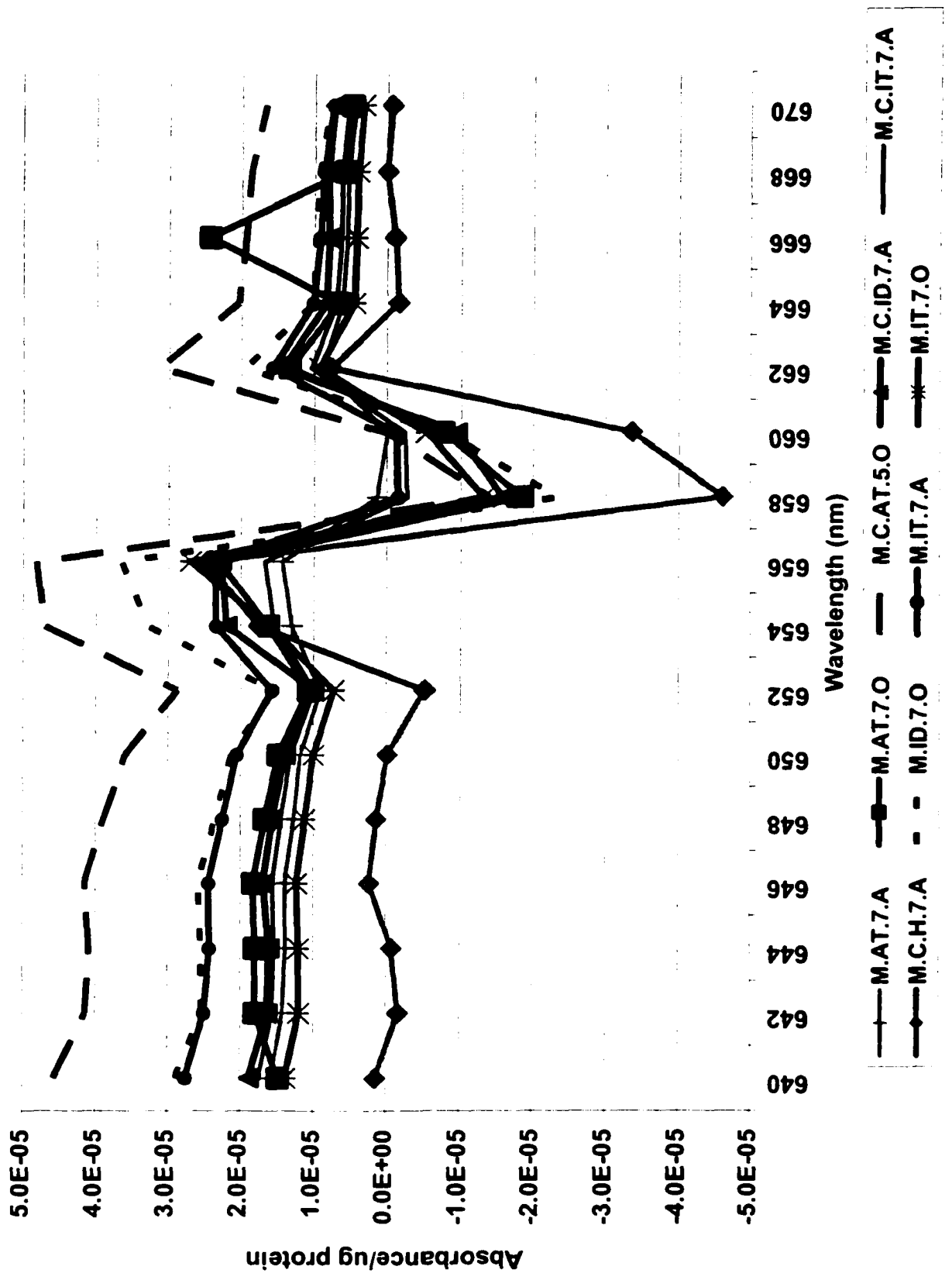


Figure 6.3. Ratios of absorbance to µg/ml protein for Phase II *in vitro* color reactions produced under high or low partial pressures of oxygen and mixed at pH 5.5 or 7.2. M = myoglobin, AT = sodium sulfate, 7 = pH 7.2, A=atmospheric environment/low partial pressures of oxygen, O = high oxygen environment/high partial pressures of oxygen, C = copper, 5 = pH 5.5, ID = sodium sulfide, IT = sodium sulfite, H = hydrogen peroxide.



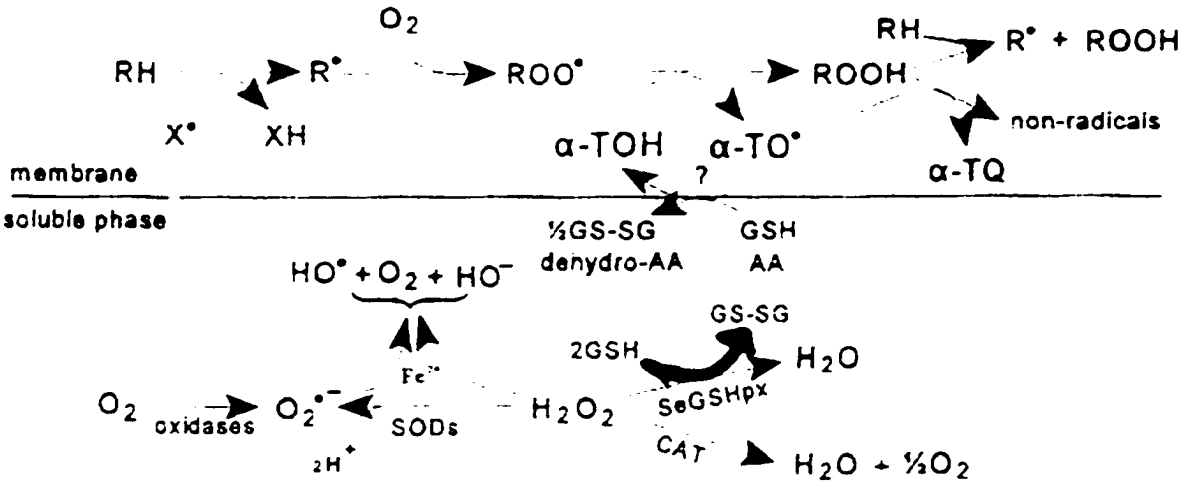


Figure 6.4. Cellular antioxidant defense system diagram. CAT, catalase; GSH, reduced glutathione; GSSG, oxidized glutathione; α-TQ, α-tocopherylquinone; α-TO, α-tocophenoxyl radical; α=TOH, α-tocopherol; SeGSHpx, selenium-dependent glutathione peroxidase; SOD, superoxide dismutase (Combs, 1992; reprinted with permission from Academic Press, Orlando, FL).

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