

Project Title:	Greening Reaction in Beef Injection-site Lesions
Principle Investigator(s):	Keith E. Belk, Ph.D.
Institution(s):	Colorado State University
Completion Date:	May 2004

Background

During the 2000 National Beef Quality Audit, concern was expressed about the green discoloration that occurs in injection-site lesions in muscles of the chuck when packaged in high-oxygen, modified atmosphere packages.¹ Research has shown that meat color is one of the most important factors to consumers when they select beef to purchase at retail.² High-oxygen, modified-atmosphere packaging is used by retailers to increase the color-life of meat products merchandized in retail-ready packaging systems.³ The elevated level of oxygen (40 – 80%) in high-oxygen, modified-atmosphere packages decelerates oxidation of myoglobin in meat and causes beef to exhibit a brighter red color for longer; however, for some unknown reason, oxidation is accelerated in injection-site lesions in a manner causing green discoloration of the lesion. As retailers continue to market ever-increasing amounts of high-oxygen, modified atmosphere packaged steaks and roasts from the chuck, appearance of green injection-site lesions will continue to increase.

Findings and Implications

Injection-site lesions result from damage to muscle tissue when pharmaceuticals are injected with a needle. The most severe damage occurs when pharmaceuticals are delivered by intramuscular injection (IM). Injections administered subcutaneously (SQ), using any non-tented technique, may also cause intramuscular damage.³ The occurrence of injection-site lesions and the severity of lesions vary with the calf's age at injection, product administered, individual animal response to product, contamination and adjuvant used in the vaccine.^{4,5}

Injection-site lesions can vary in appearance and can be classified as: clear, woody callus, nodular, metallic or cystic.⁶ Clear lesions and woody calluses are typical of injections given to animals during the earlier stages of their life, regardless of the product injected. Injection-site lesions grow as the calf grows, similar to how a hide brand enlarges as a calf grows.⁷ Metallic and nodular lesions are typical of pharmaceuticals administered to cattle in mid-to-late feeding phases, while cystic lesions are typical of injections given to cattle late in the finishing phase, regardless of product injected.^{7, 8, 9}

Injection-site lesions were one of the primary quality concerns in the 1991 National Beef Quality Audit and the 1999 National Market Cow and Bull Beef Quality Audit (NMCBBQA) according to packers and end-users.^{10,11} The National Beef Quality Audit – 1991 attributed a nationwide loss of \$54,967,635 per year to injection-site lesions found in the top-sirloin butt in fed steers and heifers.¹⁰ National Cattlemen's Beef Association (NCBA) now recommends that no more than 10 cc be administered per injection-site, and that injection sites should be at least 2 to 3 inches apart, using both sides of the neck when possible. Injections should be administered in the neck region in front of the point of the shoulder. Subcutaneous injections should be administered using the tented technique. If a pharmaceutical can be administered either IM or SQ, always choose SQ. These practices are to prevent the loss of higher valued cuts from the loin and round.



Implementation of these practices reduced the incidence of injection-site lesions in top sirloin butts of fed steers and heifers from 21.3% in July 1991 to 2.1% in July 2000.¹¹

In most cases, detection of injection-site lesions is not possible at the packing plant level of the industry; the lesion is concealed within the muscle and/or beneath the subcutaneous fat layer. Injection-site lesions are predominately detected after the interior of affected muscle is exposed during wholesale/retail fabrication, or by consumers at retail.⁶

Since the 2000 National Beef Quality Audit, a new injection-site challenge has arisen. Distributors and retailers of case-ready chuck steaks and roasts frequently find injection-site lesions. Brian McFarlane, Director of Research and Development at Tyson Fresh Meats, reported that approximately 25% of the chucks fabricated for their case-ready retail line had one or more lesions in the form of an abscess or as damaged tissue, resulting in an approximate loss of \$3 million per year to Tyson Fresh Meats. At fabrication, lesions may not be obvious, woody lesions and clear calluses can be difficult to distinguish from fat, and may not be trimmed off. During the 2000 National Beef Quality Audit, an industry representative expressed concern that green discoloration of some injection-site lesions was occurring in muscles of the chuck when packaged in high-oxygen, modified atmosphere. The first step in addressing this new injection-site challenge was to determine if the green discoloration was really an injection-site. In order to validate that green discoloration was occurring due to injection-site lesions, and not because of something else, histopathological analyses were performed. Analyses of slides made from muscle tissue containing green lesions showed characteristics consistent with those of injection sites. These characteristics included scar tissue, atrophied (shrunken) muscle fibers, adjuvant, and sheets of macrophages and fibrous connective tissue. Results confirmed that green discoloration was an injection-site; not a bruise or other quality defect.

Chucks from carcasses of 45 steers that were administered one of 8 different vaccines were evaluated for injection-site lesions and green discoloration once packaged in a high oxygen, modified-atmosphere.³ Twenty-eight of the forty-five chucks (62%) had visible injection-site lesions at the time of fabrication. After three days of boxed storage in high-oxygen, modified atmosphere packages, 15 of the 28 chucks with visible lesions exhibited green discoloration. Two more chucks exhibited green discoloration after being placed in simulated retail display, including a chuck that did not have a visible injection-site lesion initially.

The extent of greening varies with pharmaceuticals and oxidation of tissue. Differences found among differing pharmaceutical products may be a result of an immune response and/or product formulation, specifically for the adjuvant.^{3,12} The greening reaction observed in injection site lesions of the chuck may be the result of a reaction between oxygenated myoglobin, copper, and/or sulfate.¹²

Myoglobin is the pigment protein found in muscle that gives it its red color. It is thought that copper and sulfate may be present in the injection-site due to an immune response or because these minerals can be present in the adjuvant of some vaccines. Adjuvants are insoluble and are used in vaccines to exaggerate an immune response in order to deliver antigens in vaccines to parts of the body that aid in disease resistance. Pharmaceutical companies incorporate differing adjuvants into different vaccines to achieve specific objectives; these adjuvants and their ingredients are proprietary. Therefore, it is difficult to determine if adjuvants include sulfate or copper, or if the adjuvant causes the greening reaction in injection-site lesions. Some scientists think copper is the culprit. Copper easily oxidizes when it is exposed to oxygen; an example is the obvious discoloration of copper piping when it is exposed to oxygen and moisture. This

reaction may be similar to what occurs in injection-site lesions when they are exposed to high levels of oxygen in modified atmosphere packaging.

When cuts from the chuck containing an injection-site lesion are packaged in a high oxygen, modified-atmosphere environment, and green discoloration of the injection-site lesion occurs, retailers reject the product. If the product is not rejected and happens to be placed in a retail display case, consumers reject it, viewing it as spoiled (or worse). Francois Bere of Cargill says “It is hard to detect injection sites at the time of packaging, but green discoloration stands out just at the time of sale. These packages are not only a 100% loss to the retailer, but may cause the loss of a customer. A rough estimate would be [that] slightly less than 0.1% of trays [with green injection-site lesions] may make it to the retailer's display case. This would vary seasonally. In my opinion, the impact is significant not only from rate of occurrence, but also from visual impact to the customer.”

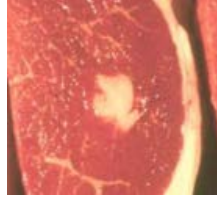
Green discoloration of injection-sites is not just a problem in whole-muscle cuts; green spots have been known to appear in ground chuck when packaged in a high-oxygen modified-atmosphere as well. Currently, two of the five largest grocery chains in the United States package beef cuts in high oxygen, modified-atmosphere packages. In one situation, the retailer packages their own beef products; the other has their distributors' package product for retail distribution. Distributors and retailers, regardless of the company responsible for packaging the product, are experiencing significant losses due to the green discoloration of injection-site lesions.

The direct cause of green discoloration in injection-site lesions when packaged in a high-oxygen, modified-atmosphere is not known. More information regarding the ingredients of adjuvants is required to draw any conclusions regarding biochemical reactions that may occur within muscle and adipose tissue that results in greening of injection-site lesions. The most efficient way, at present, to address this beef quality challenge is to reduce the prevalence of injection-site lesions in marketable portions of the chuck. This goal can be achieved by administering intramuscular injections in a tightened injection-site zone, at least a hand's width ahead of the shoulder. Subcutaneous injections should be administered using the tented technique in front of the shoulder or in the dewlap, where whole muscle cuts are not derived.¹³

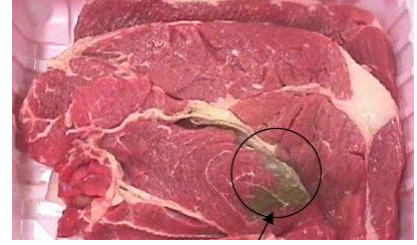
Chute use and design also is important because it can affect access to the tightened injection-site zone during vaccination. Often, when an animal is caught by the head gate, the head gate and or neck-extender is covering the injection-site zone. Dr. Temple Grandin, a well-known animal behaviorist, industry consultant, and designer of livestock handling facilities acknowledges that some companies which design/manufacture cattle chutes are aware of this problem and are designing chutes to make the injection-site zone more accessible. Dr. Grandin strongly suggests utilizing proper animal handling techniques during cattle processing to insure that animals entering a chute move at a walk/trot; thereby allowing the animal to be caught at the proper location and facilitating access to the proper injection-site zone. In some situations, it is possible to remove neck-extenders from a chute head-gate, making the injection-site zone accessible from the front of the chute. Some hydraulic chutes have hydraulic head-benders that can be used to keep the animals neck turned to the side, making the neck more accessible. When using hydraulic head benders, the bar must be closed behind the jaw bone, not on the jaw bone, in order to keep the calf calm and relaxed. By working together, producers, pharmaceutical companies, and researchers can conquer this quality challenge together.



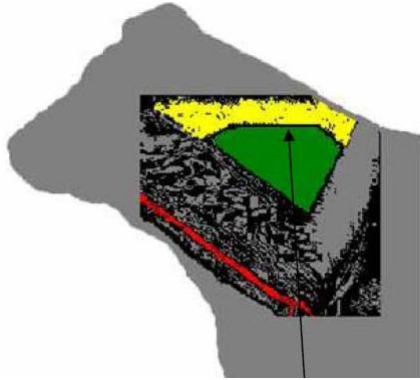
Nodular injection-site lesion



Woody Callus injection



Green discoloration of an Injection-site lesion



Injection-site zone

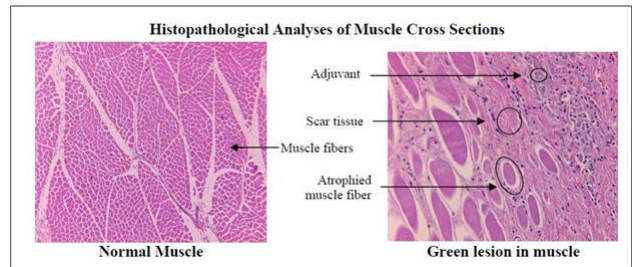


Table 1. Incidence of visible injection-site lesions in beef chucks at the time of fabrication resulting from administration of eight differing vaccines and the incidence of green discoloration of injection-site lesions when packaged in high-oxygen modified-atmosphere.

Pharmaceutical	Route of Administration	n	Visible Lesions at Fabrication		Green Discoloration Following Boxed Storage		Green Discoloration Following Retail Display	
			Total	%	#	%	#	%
Control	N/A	5	0	0	0	0	0	0
Presponse® HM	IM ^a	5	4	80	3	60	3	60
PYRAMID® 4 + Presponse®	IM	5	0	0	0	0	0	0
Triangle® 9 + Type II BVD	IM	5	5	100	1	20	1	20
Prism® 4	IM	5	0	0	0	0	0	0
Vira Shield® 5	SQ ^b	5	5	100	4	80	4	80
BarVac® Alpha-7	SQ	5	5	100	2	40	3	60
BarVac® Express 5 PHM	SQ	5	3	60	3	60	4	80
Poly-Bac B® 3R	SQ	5	5	100	2	40	2	40
Totals		45	27	60	15	33	17	38

^aIntramuscular
^bSubcutaneous