

EVALUATING LIPID OXIDATION AND SENSORY CHARACTERISTICS IN BACON
FROM IMMUNOLOGICALLY CASTRATED BARROWS

BY

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THESIS

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Abstract

With increased time after immunological castration, lipid content of bacon increases and unsaturated fatty acid content is reduced. Our objective was to determine the effect of immunological castration (Improvest®, *gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis) management strategies [age of slaughter, and time after second dose (ASD)], on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions. To mimic conditions of a normal production system, all bellies from immunological castrated (IC) barrows were pooled to compare bacon from IC barrows, regardless of management strategy, to physical castrated (PC) barrows and gilts for the same traits. Bellies (N=129) from two slaughter dates were manufactured into bacon under commercial conditions. Immunological castration management strategies included 24 wk old IC pigs 4, 6, 8, or 10 wk ASD, 26 wk old IC pigs 6 wk ASD and 28 wk old IC pigs 8 wk ASD. Gilts and PC barrows were slaughtered at 24, 26, and 28 wk of age and pooled across age. Center-cut bacon was laid-out on parchment paper, packaged in oxygen-permeable poly-vinyl lined boxes, and frozen (-33°C) for 1, 4, 8, or 12 wk to simulate food service conditions. At the end of each storage period, trained sensory panelists evaluated bacon for saltiness, oxidized odor, oxidized flavor, and off flavor on a 15-cm line scale (0 cm indicated none and 15 cm indicated extreme intensity for each characteristic). At similar time points, lipid oxidation (TBARS) and proximate analysis were determined. Both data sets (IC management strategies and IC barrows compared with PC barrows and gilts) were analyzed using the MIXED procedure in SAS with belly as the experimental unit. Model included fixed effects of treatment, wk of storage, their interaction, and blocked by kill date. In both data sets, least square means were separated using the PDIFF option with a Tukey-Kramer adjustment. Overall, as storage time increased, saltiness decreased from 1

to 12 wk ($P < 0.01$), oxidized odor and oxidized flavor increased ($P < 0.01$), and TBARS increased by 0.23-0.30 mg MDA/kg meat from 1 to 12 wk ($P < 0.01$). Moisture and lipid content did not change with storage ($P \geq 0.11$). There was no interaction of treatment and wk within IC treatments or within sex classes ($P \geq 0.11$). Oxidized odor and flavor were unaffected by immunological castration management strategy ($P > 0.21$) or sex class ($P > 0.31$). Among bacon from IC barrows, TBARS were increased in bacon from IC barrows slaughtered at 28 wk of age, 8 wk ASD compared with bacon from IC barrows slaughtered at 24 wk of age, both 4 and 6 wk ASD ($P < 0.01$), with other treatments being similar to both extremes. Lipid content was increased and moisture reduced ($P < 0.01$) in bacon from IC barrows 8 and 10 wk ASD compared with IC pigs at 4 and 6 wk ASD, regardless of age at slaughter. When bacon from IC treatments were pooled, TBARS tended to be increased ($P = 0.06$) by 0.03 mg MDA/kg meat from IC barrows compared to gilts, with bacon from PC barrows not differing from either sex. Bacon from PC barrows contained less ($P < 0.01$) moisture and more ($P < 0.01$) lipid than bacon from IC barrows and gilts. Bacon from IC barrows and gilts had similar lipid and moisture content ($P > 0.05$). Regardless of immunological castration management strategy or sex, bacon became more oxidized with storage. Within IC treatments, lipid oxidation increased as storage time increased, and lipid content was increased in barrows 8 and 10 wk ASD, regardless of age at slaughter. Bacon from IC barrows was more oxidized than bacon from PC barrows, but was similar to bacon from gilts. However, regardless of treatment, there were no differences in sensory attributes of bacon, and after 12 wk of frozen storage bacon did not become rancid. Therefore, using bellies from Improvest® managed pigs for bacon should not result in reductions in shelf life.

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Chapter 1:

Review of the Literature

1.1. Introduction

With the demand for meat continuing to rise due to a growing population (Lutz et al., 2001; Speedy, 2003), the use of technologies designed to increase yields and feed efficiencies of meat animals while continuing to provide affordable, safe, and consistent meat products are needed to a greater extent. One such technology is immunological castration (Improvest[®], *gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis, Kalamazoo, MI). Immunological castration capitalizes upon the ability of intact male pigs to deposit lean muscle more efficiently than physically castrated (PC) barrows, and decreases the incidence of boar taint (Campbell et al., 1989; Dunshea et al., 2001). Along with increased carcass leanness however, immunologically castrated (IC) barrows have increased levels of unsaturated fatty acids compared to PC barrows (Boler et al., 2012). Increased levels of unsaturated fatty acids in products, like bacon and sausage, are more susceptible to lipid oxidation than products that contain increased levels of saturated fatty acids (Rhee et al., 1996). While extensive research has been conducted on the carcass quality and lean cutting yields of IC barrows, no research has been conducted to evaluate the effect immunological castration has on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions. Food service bacon is packaged under oxygen-permeable conditions and stored frozen. Due to the oxygen-permeability of the packaging, meats with increased levels of unsaturated fatty acids will become rancid sooner than product with increased levels of saturated fatty acids, and will have an overall shorter shelf life. This is crucial because bellies from IC barrows are more unsaturated and could

have a compromised shelf life compared with bacon from PC barrows. However, increasing time after second Improvest® dose prior to slaughter or increasing age at slaughter, has resulted in increasing the concentration of saturated fatty acids in belly adipose tissue (Tavárez et al., 2014b). Therefore, it is essential to determine the effect that immunological castration has on lipid oxidation and sensory characteristics of bacon held under simulated food service conditions.

1.2. Immunological Castration

Intact males, when compared with PC barrows, have reduced feed intake, improved feed efficiency, and overall leaner carcasses (Xue et al., 1997). However, meat from intact males possesses a disagreeable odor and flavor referred to as boar taint. Traditionally in the U.S., boar taint has been controlled by physically castrating male pigs prior to 14 d of age (Swine Castration. AVMA, 2014). Immunological castration however, allows producers to take advantage of the improved feed efficiency and leanness associated with intact males, while reducing the risk of boar taint.

1.2.1. Boar Taint

Boar taint is an objectionable flavor and odor that is sometimes detected when pork fat is heated. Boar taint negatively affects the eating experience of boar meat (Babol et al., 1995). Though boars also have less subcutaneous fat when compared to PC barrows (Xue et al., 1997), they develop boar taint through the accumulation of two lipophilic compounds, androstenone and skatole (Dunshea et al., 2001). Androstenone is produced in the testes, with its synthesis linked to testosterone, and is released into circulation to act as a sex pheromone (Claus et al., 1994; Hennessy, 2008). Skatole is synthesized from tryptophan by microbes in the gut, allowing this

compound to be present both in males and females. If not metabolized by the liver, skatole accumulates in adipose tissue (Hennessy, 2008).

Though not all people are able to detect the odors and flavors of boar taint, approximately 25-35% of consumers are sensitive to boar taint (Malmfors and Lundström, 1983; Weiler et al., 2000). Androstenone imparts upon meat an intense sweaty and urine-like odor (Annor-Frempong et al., 1997; Bonneau, 1982), while skatole gives meat an unpleasant musty and fecal odor (Annor-Frempong et al., 1997; Claus et al., 1994). Of the percentage of consumers that can detect boar taint, women make up the majority, and are more sensitive to boar taint than men (Weiler et al., 2000). Androstenone can be detected at levels of 0.2-1.0 μ g/g, while skatole can be detected at a much lower concentration (0.008-0.06 μ g/g) (Annor-Frempong et al., 1997). The concentration threshold for both of these compounds to be deemed unsatisfactory by consumers was reported as being 0.426 μ g/g for androstenone, and 0.026 μ g/g for skatole (Annor-Frempong et al., 1997).

Under normal circumstances, fat samples from PC barrows and gilts have androstenone levels $\leq$ 0.2 μ g/g, while fat samples from intact males have greater than 0.5 μ g/g androstenone. Similarly, PC barrows and gilts normally have skatole levels $\leq$ 0.04 μ g/g skatole while intact males are reported as having skatole levels in excess of 0.2 μ g/g (Prusa et al., 2011). Therefore meat from intact males has been associated with strong odors that are similar to that of feces and urine. This is a more significant issue in the US, since pigs are sent to market at approximately 24 wk of age (Boler et al., 2012), after they have gone through puberty (17.5-20 wk of age) (Phillips and Zeller, 1943) and have begun to produce androstenone. In Europe this is less significant of an issue because pigs are sent to market at a younger age than in the US.

1.2.2. Improvest® Mode of Action

Immunological castration temporarily inhibits testicular function, decreasing androstenone, and allows the IC barrows to eliminate skatole, allowing for a product that is free of boar taint. Improvest® is an immunological product that works like a vaccine to elicit an immune response in intact male pigs. The Improvest® antigen is comprised of a synthetic, incomplete analog of gonadotropin releasing factor (GnRF), bound to a carrier protein, which elicits an immune response within the animal. The result of this immune response is the production of anti-GnRF antibodies by the pig. Under normal conditions, GnRF is released from the hypothalamus and binds to receptors in the anterior pituitary. The pituitary then releases follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones stimulate testicular growth and the production of testosterone by the testicles, leading to sexual maturity, growth, and boar taint.

Castration through use of Improvest® is achieved by a two-dose injection regimen. The first dose (administered no earlier than 9 wk of age) acts as a primer to attenuate the system. The second dose, given at least 4 wk after the first dose, is administered between 3 to 10 wk before slaughter and elicits an anti-GnRF response. The downstream effects of these injections results in a loss of function in the testicles and a regression of the overall reproductive tract of the intact male. This lack of circulating testosterone allows for boar taint compounds to be metabolized by the liver and eliminated in IC barrows (Hennessy, 2008; Janett et al., 2012). Resulting in meat from intact males that no is longer troubled by the unpleasant odors and flavors that is normally associated with boar meat. Furthermore, a decrease in circulating testosterone within the IC barrow allows for increased fat deposition (Oonk et al., 1998). This allows the IC barrows to deposit more backfat and belly fat late in the finishing period prior to slaughter.

1.2.3. Effect of Immunological Castration on Belly Characteristics and Fatty Acid Profile

The major advantage associated with using immunological castration is that IC barrows have improved ADG and feed conversion when compared to PC barrows (Dunshea et al., 2001). Carcasses from IC barrows are also leaner than carcasses from PC barrows, due to IC carcasses having less fat and more muscling (Dunshea et al., 2001; Pauly et al., 2009). Furthermore, when compared to carcasses from PC barrows, carcass cutting yields were increased by 2.5 percentage units in IC barrows. Additionally, carcasses from IC barrows had greater closely trimmed meat yields from the shoulder, loin, and ham when compared to carcasses from PC barrows (Pauly et al., 2009; Boler et al., 2012).

Despite improvements in yields of lean cuts in carcasses of IC barrows compared with PC barrows, belly quality of IC barrows may be reduced. Bellies from IC barrows are thinner and have narrower belly flop distances than bellies from PC barrows (Boler et al., 2011). The effect of this is that bellies from IC barrows have reduced slicing yield than bellies from PC barrows (Kyle, 2013). These two attributes are likely related as leaner pork carcasses had thinner bellies and increased levels linoleic acid in their adipose tissue (Wood et al., 1989), and increased levels of linoleic acid in lipid decreased belly firmness (Whittington et al., 1986). Bellies from IC barrows also had greater total concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) when compared to PC barrows (Boler et al., 2011; Boler et al., 2012). However, one study reported that IC and PC bellies had similar saturated fatty acid (SFA) and MUFA levels, and only differed with IC having a greater concentration of PUFA than PC barrows (Tavárez et al., 2014a). The difference between these studies is likely due to the second studies use of dried distillers grains with solubles (DDGS)

within the diet. Including DDGS in swine diets has been shown to increase PUFA concentrations within adipose as well as increase iodine values (Xu et al., 2010).

One method that is currently being employed to improve the fatty acid profile of bellies from IC barrows is increasing the time after second Improvest® dose before slaughter. By increasing time after second Improvest® dose before slaughter, from 2 wk to 8 wk, concentrations of PUFA in belly adipose tissue decreased (Tavárez et al., 2014b). However, it should be noted that sending IC barrows to slaughter 2 wk after second dose is off label (Hennessy, 2008), and those animals were not able to enter the food supply. Tavárez et al. (2014b) also reported that increasing time after second dose decreased iodine value from 67.0 to 60.1, but had no effect on total belly lipid content. This reduction in iodine value suggests that as time after second dose increased fat deposited was more saturated and therefore, may be less susceptible to oxidation.

Increasing time after second Improvest® dose before slaughter from 5 to 7 wk reduced the calculated percent lean of IC carcasses (Tavárez et al., 2014a), indicating increased fat deposition as time after second dose increased. Tavárez et al., (2014a) also reported increased back fat thicknesses and increased bacon lipid content as time after second Improvest® dose increased from 5 to 7 wk however, iodine value remained the same. Therefore, increasing time after second dose increased the total amount of belly lipid, which indicates an increase in *de novo* fat synthesis and the potential for decreased levels of PUFA in bacon from IC barrows. Conversely, increasing time after second Improvest® dose prior to slaughter from 4.5 to 7.5 wk, administered in conjunction with ractopamine HCl, did not change the moisture and lipid content of bacon (Lowe, 2013). Ractopamine HCl is a beta-adrenergic agonists that promotes lean muscle growth in swine, by partitioning nutrients away from fat deposition and towards lean

muscle development (Apple et al., 2007). While increasing time after second Improvest® dose before slaughter can increase fat deposition, including ractopamine HCl in the diets negates this effect by continuing to partition the excess nutrients towards lean muscle.

These studies evaluated the effect that Improvest® has on fresh belly and processing characteristics, along with changes fatty acid profiles in adipose tissue as time after second Improvest® dose increased. However, these studies did not report on how the changes in fatty acid profile affected lipid oxidation within the finished product. At this time, there are no data concerning the effect that immunological castration by means of Improvest® has regarding lipid oxidation in bacon.

1.3. Lipid Oxidation

Lipid oxidation, or lipid peroxidation, is the oxidative degradation of lipids. It is a reaction that is auto-catalyzed by oxygen and results in the oxidation of a fatty acid and an accumulation of free radicals, which further propagate the reaction. Meat products with increased levels of PUFA are more prone to oxidizing (Rhee et al., 1996), due to the methylene bridges located adjacent to a double bond causing the hydrogen to become reactive (Herrmann, 1982). This reactive hydrogen is able to disassociate from the fatty acid, resulting in the initiation step of lipid oxidation. Lipid oxidation is a reaction that consists of three stages: initiation, propagation and termination. Initiation occurs when a fatty acid radical is produced through the removal of a hydrogen atom off of a carbon bound to another carbon with a double bond, within an unsaturated fatty acid. The fatty acid radical is an unstable compound and binds with molecular oxygen to create a peroxy-fatty acid radical during the propagation phase (Damodaran and Parkin, 2008). This peroxy-fatty acid radical is also unstable and reacts with

other fatty acids in an attempt to stabilize. This reaction is terminated when a radical reacts with another radical to form a non-radical compound. This mainly occurs when there is an increased concentration of free radicals, thereby increasing the probability of two free radicals interacting with each other (Damodaran and Parkin, 2008). These oxidation end products are mainly reactive aldehydes, an example of which is malondialdehyde (MDA) (Janero, 1990). As a result, the buildup of oxidative end products in meat is responsible for the production of off flavors and off odors that are normally associated with lipid oxidation (Ahn et al., 1993; Fernández et al., 1997).

1.3.1. Thiobarbituric Acid Reactive Substances Assay as a Method to Detect Lipid Oxidation

There are many assays designed to measure lipid oxidation, but one of the most common is the thiobarbituric acid-reactive substances (TBARS) assay (Dawn-Linsley et al., 2005). The TBARS assay measures the end products of lipid oxidation, which are more stable and less susceptible to degradation than the primary products of lipid oxidation (Sørensen and Jørgensen, 1996). There are several different methods to conduct the TBARS assay. These assays include measuring MDA either by extraction, distillation or aqueous acid extraction (Pegg and Shahidi, 2008; Turner et al., 1954). These assays differ in the way MDA is extracted from the meat product, but all conclude by using spectrophotometry to analyze the results. These assays achieve this by quantifying the concentration of the pink (red) chromophore formed by the reaction of 2-Thiobarbituric Acid (TBA) with the end products of lipid oxidation to a known concentration of MDA using spectrophotometry (Sørensen and Jørgensen, 1996).

The inclusion of nitrites into meat inhibits the development of rancidity, and warmed over flavor in cooked meats (Shahidi and Pegg, 1992). However, residual nitrite in cooked meats can interfere with the TBARS assay (Zipser and Watts, 1962). The interference is thought to be

caused by the nitrosation of MDA. This reaction renders the nitrated MDA unavailable to the TBARS assay, resulting in an underestimation of lipid oxidation (Shahidi et al., 1985; Zipser and Watts, 1962). In order to compensate for the interaction of nitrite and MDA, sulfanilamide is added to the assay. Sulfanilamide scavenges the residual nitrite, inhibiting the nitrosation of MDA (Zipser and Watts, 1962), and allowing for more accurate results. This is especially crucial performing the TBARS assay on cured products. Cured products, like bacon, are cured with sodium nitrite with inclusion levels up to 120ppm (FSIS; 9 CFR 424.22). The residual nitrite from this curing process can interfere with the assay and will result in TBARS that are artificially decreased, which could result in one concluding that a sample is acceptable when in fact it is rancid.

1.3.2. Oxidized Odor and Oxidized Flavor

A consequence of lipid oxidation is the formation of off flavors within a product. The buildup of these oxidative end products within meat products is a major contributor to the deterioration of quality of the product over time (Ladikos and Lougovois, 1990). However, this loss of quality in oxidized foods does not make them any less safe to consume (Pearson et al., 1983). This is opposed to microbial degradation, which can also result in a loss of meat quality, but more importantly can impact the safety of the product.

While many undesirable flavors can be lumped into the category of off flavors, the two that are associated with lipid oxidation are oxidized odor and oxidized flavor (also referred to as rancid or warmed-over flavor) (Greene and Cumuze, 1982). These odors and flavors are a result of the end products of lipid oxidation. Included in these oxidative end products are volatile ketones and aldoses can impart an undesirable flavor to the product (Gray, 1978). Untrained consumer panels could detect off flavors associated with lipid oxidation in products that had

TBARS ranging between 0.6 and 2.0 mg MDA/kg of meat. For trained sensory panels, TBARS ranging from 0.5 to 1.0 mg MDA/kg of meat were found to be sufficient for the detection of rancidity (Greene and Cumuze, 1982; Tarladgis et al., 1960). Meaning that a meat product with as little as one part per million of MDA is sufficient for a consumer to consider a product rancid and potentially inedible.

1.3.3. Effect of Immunological Castration on Lipid Oxidation of Other Pork Products

While there are no data concerning the effect immunological castration via Improvest® has on lipid oxidation in bacon, some research has been done on other meat products from IC barrows. In sausage from IC barrows, immunological castration had no effect on TBARS when comparing sausage made from IC barrows to PC barrows (Jones-Hamlow, 2013). Sausage comprised of ground Boston butts (NAMP #407), that was not standardized to a similar fat content, was reported as having similar TBARS throughout 12wk of frozen storage. However, when sausages were formulated to a standard fat content (25% fat); sausage from PC barrows had increased TBARS when compared to sausage from IC barrows. Even though off flavors increased as storage time increased, there was no difference in off flavors between sausage from IC barrows and sausage from PC barrows, for either study (Jones-Hamlow, 2013).

Another experiment from the same author evaluated the effect that immunological castration had on sensory characteristics of enhanced and non-enhanced loins (Jones-Hamlow, 2013). In this study, fresh loins from IC barrows did not differ in off flavor than loins from PC barrows. Once enhanced, loins from IC barrows continued to have similar off flavor sensory scores to loins from PC barrows. All of this data suggests that immunological castration does not affect the sensory attributes of sausage or enhanced loins and pork products from IC barrows should be of similar quality to pork products from PC barrows.

1.4. Bacon

In the United States, pork is the third most consumed meat, behind poultry and beef, with the average American consuming around 23.2 kg of pork in a year (Davis and Lin, 2005). While the per capita pork consumption has remained fairly stable in the past 50 years, the overall volume of pork produced has increased in response to the rising population (Daniel et al., 2011; Davis and Lin, 2005). While individual consumption of pork in the US has remained fairly constant an increased population means more total consumers who are eating 23.2 kg per year. Even with a continued shift towards an increase in poultry consumption, red meat is still the most consumed meat in the US (58% of total meat consumption). Furthermore, of the total meat consumed, 22% is processed (Daniel et al., 2011). Processed meat is defined as meat that has had its fresh meat characteristics altered, by means other than simple grinding or cutting (Pearson and Gillett, 1996). When broken down by type within pork, processed pork dominates fresh pork, with 62% of all pork consumed being processed (Davis and Lin, 2005). Processed pork includes products transformed by grinding, curing, smoking, or seasoning prior to wholesale or retail sale. Included within this category are lunch meats, hot dogs, sausage, bacon, and smoked ham. Of that 62% of pork consumed as processed meats, bacon is the third most consumed processed pork, behind sausage and smoked ham, and makes up 6.3% of all pork consumed (1.5kg/person/year) (Davis and Lin, 2005). According to the United States Bureau of Labor Statistics, the national average price of bacon is \$5.46/sliced lb (U. S. Bureau of Labor Statistics, Average Prices, 2014). The price of bacon has increased 13% since last year (U. S. Bureau of Labor Statistics, Average Prices, 2014) and has grown into a two billion dollar industry (Quick Facts: The pork industry at a glance, 2009).

1.4.1. *Techniques Used to Manage Lipid Oxidation in Bacon*

An important factor involved with all food processing is reducing lipid oxidation in order to increase the shelf life of a product. This is of particular concern in a product like bacon that is approximately 30-50% lipid (Brewer et al., 1995) and approximately 15% of that lipid is polyunsaturated (Tavárez et al., 2014b). One way of achieving a longer shelf life in bacon is by the use of sodium nitrite (Donald et al., 1980; Gray et al., 1981). Sodium nitrite, besides imparting the cured color and flavor associated with bacon, works as an antioxidant in meat products (Zubillaga et al., 1984). Sodium nitrite works as an antioxidant by sequestering oxygen and inhibiting it from interacting with fatty acids, while also binding with iron and preventing it from aiding in catalyzing oxidation (Honikel, 2008; Ladikos and Lougovois, 1990). During 5 wk of frozen storage with products including 0, 25 and 50ppm of sodium nitrite, including 25 and 50ppm sodium nitrite reduced TBARS by 25 and 44%, respectively (Shahidi and Pegg, 1992). Increasing sodium nitrite from 0 to 50ppm reduced TBARS in cooked ground pork from 10 μ M to 5 μ M, and increasing nitrite concentrations to 200ppm resulted in a 12 fold reduction in TBARS (Morrissey and Tichivangana, 1985). Therefore, bacon, which has up to 120ppm of sodium nitrite (Pegg and Shahidi, 2008), should have reduced TBARS compared to meat products low in sodium nitrite over similar frozen storage times.

While it is the presence of oxygen with or without the aid of metal cofactors that results in the oxidation of fatty acids, storing products under refrigeration slows lipid oxidation reaction (Labuza and Dugan Jr, 1971). An increase in environmental temperatures allows for greater lipid solubility along with increasing the activity of molecular oxygen, allowing for a more rapid reaction (Holman, 1954). As storage temperature for beef *Longissimus dorsi* steaks increased from 1.6 to 8.3 °C, TBARS increased from 2.8 to 5.4 μ M MDA/kg meat (Jakobsen and Bertelsen,

2000). Freezing meat products can further inhibit lipid oxidation (Dave and Ghaly, 2011; Ladikos and Lougovois, 1990). One study reported that milk frozen for 10 days at -20 °C had decreased levels of MDA compared to milk samples that had been held under refrigeration for 24 hours (Miranda et al., 2004).

Although freezing fat containing products is a better storage mechanism than refrigeration, products held frozen for prolonged periods of time still experience oxidation. As frozen storage time increased from 0 to 8 days, TBARS were increased and were highly correlated with sensory panel evaluations of oxidized flavors and odors in ground pork (Poste et al., 1986). Vacuum packaged bacon that was stored frozen for 84d was reported as having TBARS less than 0.3mg MDA/kg of meat (Leick et al., 2010). In the same study, from 0d of storage to 28d, TBARS increased, but from 28d of storage to 84d TBARS remained the same. Similarly, TBARS increased from 0.1mg MDA/kg of meat to 0.85mg MDA/kg of meat in food service bacon frozen for 90d (Lowe et al., 2014). It is important to note that freezing only slows the rate of oxidation; it does not stop it completely (Ladikos and Lougovois, 1990).

Along with storage temperature, packaging can play a role in limiting lipid oxidation in bacon. Lipid oxidation occurs in the presence of oxygen; removing oxygen or introducing a barrier to prevent oxygen from coming into contact with the product can inhibit lipid oxidation. Bacon stored under a retail setting (vacuum-packaged and stored at 2 °C) had decreased TBARS compared with bacon stored under food service conditions (packaged in oxygen-permeable poly-vinyl lined boxes, frozen at -33 °C) (Lowe et al., 2014). This signifies that under these conditions, while the oxidation reaction was slowed with freezing, removal of oxygen from the environment had a greater impact on inhibiting lipid oxidation than storage temperature. However, this study concluded that, regardless of storage type, TBARS of bacon increased as

storage time increased. Therefore, it was decided that food service style packaging was appropriate for this study. The oxygen-permeability of this style of packaging should provide a harsher environment for the bacon than vacuum packaging. And so, if after 12 wk of frozen storage the food service bacon is still acceptable to a sensory panel then bacon stored in a vacuum container and frozen for the same period of time should still be acceptable as well.

1.5. Objectives

While increasing time after second Improvest® dose prior to slaughter can increase fat deposition and reduce iodine value, no research has been conducted to see how this affects lipid oxidation in a high fat product like bacon. Given that bacon can be 30% lipid, it has a greater potential for oxidation than a lean product like a pork loin chop. It is important to understand the effect that varying immunological castration management strategies (age at slaughter and time after second injection before slaughter) has on lipid oxidation in bacon and its subsequent sensory characteristics. It is also important to investigate the overall effect that castration method and sex has on lipid oxidation and sensory characteristics of bacon in a manner designed to mimic the normal belly supply chain (bellies from IC barrows, PC barrows and gilts of all ages mixed together prior to bacon production) and make the data applicable to a commercial setting.

As time after second dose increases, the fatty acid profile of the belly adipose tissue changes and becomes more saturated. Accordingly, as time after second dose increases, bacon shelf life should be prolonged and oxidized odors and flavors in bacon reduced. It is for that reason why it is important to determine the appropriate immunological castration management strategy (age at slaughter and time after second dose before slaughter) that allows the most favorable bacon to be produced. The objective of this study was twofold, first, was to determine

the effect of immunological castration management strategy, on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions (without an O₂ barrier). Second, was to determine if bacon from IC barrows had increased levels of lipid oxidation and different sensory characteristics than bacon from PC barrows and gilts. Based on these objectives and what is known about IC barrows the hypothesis for the first objective is that as time after second Improvest® dose increases lipid oxidation will decrease. For the second objective, the hypothesis is that when compared to PC barrows and gilts, bacon from IC barrows will have similar lipid oxidation and sensory characteristics, because previous studies found that sausage and enhanced loins from IC barrows were similar to those from PC barrows.

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Chapter 2:

Effect of immunological castration management strategy on lipid oxidation and sensory characteristics of bacon throughout twelve weeks of simulated food service storage

2.1. Abstract

The objectives were to determine the effect of immunological castration management strategy (age at slaughter and time after second dose) and sex, on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions. Bacon was stored frozen for 1, 4, 8 or 12 wk and then evaluated for lipid oxidation (TBARS), moisture and lipid content, and sensory characteristics. As storage time increased, lipid oxidation of bacon increased ($P < 0.01$), regardless of immunological castration management strategy or sex. Within immunological castration management strategies, lipid oxidation increased regardless of treatment, and lipid content increased as time after second dose increased from 4 and 6 wk after second dose to 8 and 10 wk after second dose ($P < 0.05$), regardless of age at slaughter. Lipid oxidation was increased ($P < 0.05$) in immunologically castrated (IC) barrows compared with physically castrated (PC) barrows, but was not different than gilts ($P > 0.05$). However, regardless of treatment, there were no differences in sensory attributes of bacon across treatments. Therefore, using bellies from IC barrows for bacon should not result in reductions in shelf life.

Keywords: Bacon; Immunological castration; Improve; Lipid oxidation

2.2. Introduction

Immunological castration (Improvest[®], *gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis, Kalamazoo, MI) capitalizes upon the ability of intact male pigs to deposit lean muscle more efficiently than physically castrated (PC) barrows, and decreases the incidence of boar taint (Campbell et al., 1989; Dunshea et al., 2001). Along with having leaner carcasses, immunologically castrated (IC) barrows have thinner bellies and narrower flop distances than PC barrows, when averaged over 23 and 25 wk of age at slaughter (Boler et al., 2012). Therefore, bellies from IC barrows may be less desirable for bacon production than bellies from PC barrows. A further consequence of having leaner and thinner bellies is that bellies from IC barrows have increased levels of unsaturated fatty acids compared with PC barrows, when slaughtered at equal age (Boler et al., 2012). This increase in unsaturated fatty acids results in a product that is more susceptible to lipid oxidation (Rhee et al., 1996), and oxidized meats are less desirable to consumers (Campo et al., 2006). Reducing lipid oxidation is important to all foods that contain lipids, but in a product like bacon that is approximately 30-50% lipid (Brewer et al., 1995) and of that lipid approximately 15% polyunsaturated (Tavárez et al., 2014b), it is a necessity.

Limited data concerning IC barrows indicates fresh belly characteristics from IC barrows are more similar to the fresh belly characteristics of gilts, but are inferior to bellies from PC barrows (Kyle, 2013; Lowe, 2013). Increasing age at slaughter in conjunction with increasing time after second dose improved fresh belly characteristics (Boler et al., 2012), and reduced PUFA concentrations in IC adipose tissue (Tavárez et al., 2014b). However, when slaughtered at a constant age, there was no difference in belly green weights or pumped weights of bellies from IC or PC barrows (Boler et al., 2012). This increased saturation of adipose tissue is thought to be

driven by increased feed intake of IC barrows following the second Improvest® dose (Dunshea et al., 2013), leading to an increase in *de novo* fatty acid production and deposition (Kloareg et al., 2007). In other research evaluating the effect of immunological castration on lipid oxidation in high fat products like sausage, sausage from IC barrows was reported as having similar levels of oxidation and similar sensory scores to sausage from PC barrows (Jones-Hamlow, 2013).

While extensive research has been conducted on the carcass quality and cutability of IC barrows, no research has yet been conducted evaluating the effect immunological castration has on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions.

Therefore, the objective of this study was twofold. First, was to determine the effect of immunological castration management strategy [age at slaughter and time after second dose (ASD)] on lipid oxidation and sensory characteristics of bacon packaged under simulated food service conditions. The hypothesis for the first objective was that as time after second Improvest® dose increases the lipid content of the bacon would increase and the rate of lipid oxidation would decrease. The second objective was to determine if bacon from IC barrows had increased levels of lipid oxidation and different sensory characteristics than bacon from PC barrows and gilts. For the second objective, we hypothesized that when compared to PC barrows and gilts, bacon from IC barrows would have similar lipid oxidation and sensory characteristics, because previous studies reported that sausage and enhanced loins from IC barrows were similar to those from PC barrows.

2.3. Materials and Methods

Pigs used during this study were cared for in accordance with University of Illinois Animal Care and Use Committee guidelines.

2.3.1. Raw Materials

Bacon used for this study was selected from a larger experiment, in which approximately 300 pigs of commercial breeding were allotted to treatment in a wean-to-finish building, with IC treatments being allotted at 9 wk of age (Tavárez, 2014). Bellies were sourced from two slaughter dates, two wk apart, with all treatments represented. Treatments were equally represented across sex, castration method and immunological castration management strategy. Management strategies of IC barrows included 24 wk old IC barrows slaughtered at 4, 6, 8, or 10 wk ASD, 26 wk old IC barrows slaughtered at 6 wk ASD and 28 wk old IC barrows slaughtered at 8 wk ASD. Gilts and PC barrows were slaughtered at 24, 26, and 28 wk of age and pooled across age. Treatments were pooled within sex or castration method in order to mimic normal bacon processing conditions, in which bellies from pigs slaughtered at varying ages are sorted based on weight and made into bacon. Pigs were transported to and slaughtered at a USDA Food Safety Inspection Service inspected facility. Bellies were transported under refrigeration to the University of Illinois Meat Science Laboratory, and after processing, weighing and skinning, were boxed, shipped under refrigeration and manufactured into bacon in a commercial facility. Bacon was manufactured and sliced in the manner described by Tavárez (2014), and was transported back to the University of Illinois for sample collection. Population statistics for the subsample of carcasses and bellies used in this study are provided (Table 2.1), but information on the entire population of carcasses for bacon processing characteristics were provided by Tavárez, 2014.

2.3.2. Bacon Storage and Sample Preparation

Twenty slices of bacon were collected from the center of each belly (N=129), and used for further analysis. Twelve of the twenty slices were used for sensory analysis, with three of the

twenty slices assigned to each of the four frozen storage times. The remaining eight slices were used for lipid oxidation determination and proximate analysis, with two of the eight slices assigned to each frozen storage time. Bacon was placed in a single layer on 30.48 x 40.64 cm (12x16”) parchment paper and were layered and packaged in oxygen-permeable poly-vinyl lined (Model S-2904, Uline, Pleasant Prairie, WI) corrugated boxes (Model S-4163, Uline, Pleasant Prairie, WI). Boxes of food service bacon were blast frozen (-33°C) for 1, 4, 8, or 12 wk to simulate storage for food service, with bacon for each time point placed in their own designated box. Bacon was analyzed for lipid oxidation and sensory characteristics at the end of each frozen storage time.

2.3.3. Sensory Analysis

Bacon slices were placed on an elevated wire rack on trays and cooked by convection (South Bend Convection Oven, model V-15, South Bend, IN) at 177 °C for 10 min. Halfway through cooking, all trays were rotated in the oven to ensure even cooking. After cooking, slices were cut into bite sized pieces and wrapped in foil prior to sensory evaluation. Approximately two to three bacon pieces were placed in plastic cups with lids and served to panelists. Bacon was evaluated for saltiness, oxidized odor, oxidized flavor, and off flavor. Panelists evaluated bacon using a 15-cm unstructured line scale where 0 = no apparent salt flavor, no oxidized odor, no oxidized flavor, and no off flavor, and 15 = extremely salty, extremely intense oxidized odor, extremely intense oxidized flavor, and extremely intense off flavor.

Upon conclusion of each frozen time point, sensory analysis was conducted using 3 slices of bacon from each belly. Sensory data were collected using trained panels composed of individuals from the University of Illinois Meat Science Laboratory. Sensory characteristics were analyzed by two panels, each consisting of six members who were trained according to the

guidelines of the American Meat Science Association (AMSA, 1995). Six samples were evaluated per panel, with panels conducting evaluations twice a day for six days (12 evaluations per panelist each day), for each time point. Slices were allotted to panels such that each treatment was equally represented in each panel. Panelists were separated by booths and evaluated the bacon under red lighting. Apple juice and unsalted crackers were provided between each sample. Panelists were instructed to remain consistent with either swallowing or expectorating samples.

2.3.4. *Thiobarbituric Acid Reactive Substances (TBARS)*

Upon the completion of each storage time, 2 slices of bacon from each belly were homogenized in a food processor (Cuisinart model DLC 5-Tx, Cuisinart, Stamford, CT), using the pulse function to limit heating the sample prior to analysis. Lipid oxidation was estimated with the thiobarbituric acid reactive substances (TBARS) assay using a modified procedure described by Leick et al. (2010). In order to account for the nitrosation of malondialdehyde (MDA) caused by the presence of residual sodium nitrite, 2 mL of sulfanilamide was added to the bacon homogenate prior to distillation (Shahidi et al., 1985). After incubation, 150 μ L of sample, blank and standard was pipetted in into a 96-well plate and absorbance measured at 530 nm in a plate reader (Synergy HT Multi-Model Microplate Reader, Bio-Tek, Winooski, VT) to determine MDA content. Samples were compared to a standard curve (0-22.5 mg MDA/mL) and TBARS were expressed as mg MDA/kg of meat, and once corrected for lipid content, expressed as μ g MDA/g of fat. Lipid oxidation was corrected for lipid content, expressed as a percentage of weight, using data from proximate analysis. Adjusted TBARS, expressed as μ g MDA/g of fat,

were calculated using the following equation:
$$\frac{mg\ MDA}{kg\ Meat} * \frac{kg\ Meat}{g\ Fat} * \frac{1000mg\ MDA}{mg\ MDA} = \frac{\mu g\ MDA}{g\ Fat}.$$

2.3.5. Proximate Analysis

The homogenate remaining after TBARS analysis was used for moisture and lipid determination. From the homogenized sample, duplicate 5g samples were oven dried at 110 °C for approximately 24h to determine moisture content. Dried samples were then washed in an azeotropic mixture of warm chloroform-methanol, similar to the method described by Novakofski et al. (1989), and weighed to determine total extractable lipid.

2.3.6. Statistical Analysis

Data were analyzed using two different analysis strategies. Analysis strategy 1 evaluated the effect of immunological castration management strategy [age at slaughter and time after second dose (ASD)] (N = 63). This experiment consisted of 6 treatments: IC barrows slaughtered at 24 wk of age 4 wk ASD (n = 11), 6 wk ASD (n = 11), 8 wk ASD (n = 12), or 10 wk ASD (n = 9), IC barrows slaughtered at 26 wk of age, 6 wk ASD (n = 10) and IC barrows slaughtered at 28 wk of age 8 wk ASD (n = 10). Analysis strategy 2 compared bacon from IC barrows with bacon from PC barrows and gilts (N = 129). All 6 IC treatments were pooled (n = 63) in order to compare IC barrows with PC barrows and gilts. Similarly, bacon from PC barrows slaughtered at 24, 26, and 28 wk of age were pooled for the PC treatment (n = 34), and bacon from gilts slaughtered at 24, 26, and 28 wk of age were pooled to for the gilt treatment (n = 32). Bacon was pooled within sex or castration method to mimic normal production systems, where bellies from varying ages and sexes of pigs are mixed to produce bacon.

Both experiments were analyzed using the same model. Data sets were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). Sensory characteristics, proximate analysis and TBARS were analyzed as repeated measures using a first order autoregressive (AR1) covariate structure determined using Akaike's information criteria to minimize variance.

The model included fixed effects of treatment, wk of storage and, their interaction. Block (kill date) was a random variable. Belly served as the experimental unit. Least square means were separated using PDIFF option with a Tukey-Kramer adjustment, to protect against committing a Type I statistical error for multiple comparisons. Differences in means were determined significant at $P \leq 0.05$.

2.4. Results

Analysis strategy 1: Effect of Immunological Castration Management Strategy on Lipid Oxidation and Sensory Characteristics of Food Service Bacon

The objective of analysis strategy 1 was to determine the effect of immunological castration management strategies (age at slaughter, wk ASD), on lipid oxidation and sensory characteristics of bacon packaged for food service. There was no treatment by wk interaction for any trait evaluated in analysis strategy 1 ($P \geq 0.28$) (Figures 2.1 through 2.3).

2.4.1. Proximate Analysis

Moisture and lipid content (Figure 2.1) was affected by immunological castration management strategy. Lipid content was increased by approximately 6 percentage units while moisture was reduced by approximately 4 percentage units in bacon from IC barrows that were 8 and 10 wk ASD compared with bacon from IC barrows that were 4 and 6 wk ASD ($P < 0.01$) regardless of age at slaughter. Moisture and lipid content was similar in bacon from IC barrows that were 4 and 6 wk ASD ($P > 0.05$) regardless of age at slaughter. Moisture tended ($P = 0.10$) to decrease over storage time across all treatments, and lipid content did not change over storage time ($P = 0.33$). Overall, bacon lipid content was greater in IC barrows when slaughtered at 8 or 10 wk ASD when compared with 4 and 6 wk ASD, regardless of age at slaughter.

2.4.2. Lipid Oxidation

As storage time increased, lipid oxidation increased ($P < 0.01$) approximately 0.29mg MDA/kg meat from wk 1 to wk 12 (Figure 2.2A). From wk 1 to wk 4, TBARS increased 0.12mg MDA/kg meat ($P < 0.01$) and from wk 4 to wk 8 lipid oxidation rate did not change ($P = 0.46$). From wk 8 to wk 12, TBARS increased 0.17mg MDA/kg meat ($P < 0.01$).

Overall, lipid oxidation was increased by approximately 0.10mg MDA/kg of meat in bacon from 28 wk old barrows, 8 wk ASD compared with 24 wk old barrows 4 and 6 wk ASD ($P < 0.01$, Figure 2.2B). There was no difference in lipid oxidation among bacon from IC barrows slaughtered at 28 wk of age, 8 wk ASD, IC barrows slaughtered at 26 wk of age, 6 wk ASD, and IC barrows slaughtered at 24 wk of age, 8 or 10 wk ASD ($P > 0.05$). Due to varying lipid contents among IC treatments, TBARS were adjusted for lipid content. Once adjusted for lipid content (Figure 2.2C), there were no differences in lipid oxidation among IC treatments ($P = 0.13$).

2.4.3. Sensory Evaluation

As storage time increased, saltiness decreased ($P < 0.01$, Figure 2.3A). However, there was no difference in saltiness among immunological castration management strategies ($P > 0.05$, Figure 2.3B). Similar to lipid oxidation, oxidized odor increased ($P < 0.01$, Figure 2.3C) and oxidized flavor increased as storage time increased ($P < 0.01$, Figure 2.3D). Oxidized odor and flavor, however, was not affected by immunological castration management strategy ($P \geq 0.21$). Off flavor (data not shown), was not affected by frozen storage time ($P = 0.35$) or immunological castration management strategy ($P = 0.83$).

Analysis strategy 2: Effect of Sex on Lipid oxidation and Sensory Characteristics of Food

Service Bacon

The objective of analysis strategy 2 was to determine if bacon from IC barrows had greater levels of lipid oxidation and different sensory characteristics than bacon from PC barrows and gilts.

There was no sex by wk interaction for any traits evaluated in analysis strategy 2 ($P \geq 0.10$).

2.4.4. Proximate Analysis

Moisture and lipid content did not change over storage time ($P = 0.17$). Lipid content was increased by approximately 6 percentage units and moisture content was decreased by approximately 4 percentage units in bacon from PC barrows compared with bacon from IC barrows and gilts ($P < 0.01$, Figure 2.4). Moisture and lipid content did not differ between IC and gilt bacon ($P = 0.10$, Figure 2.4).

2.4.5. Lipid Oxidation

As storage time increased, overall lipid oxidation increased 0.23mg MDA/kg of meat from wk 1 to wk 12 of storage ($P < 0.01$, Figure 2.5A). From wk 1 to wk 4, TBARS increased 0.08mg MDA/kg meat ($P < 0.01$). From wk 8 to wk 12 TBARS increased 0.16mg MDA/kg meat ($P < 0.01$). Bacon from IC barrows was not more oxidized than gilt bacon or PC bacon ($P = 0.06$, Figure 2.5B). Once TBARS were corrected for lipid content, lipid oxidation, bacon from IC barrows was more oxidized (0.47 μ g MDA/g of fat) than bacon from PC barrows (0.40 μ g MDA/g of fat) ($P = 0.05$), but was not different than bacon from gilts (0.41 μ g MDA/g of fat) ($P = 0.08$, Figure 2.5C and 2.5D). Bacon from PC barrows and gilts had similar levels of oxidation ($P = 0.73$).

2.4.6. Sensory Evaluations

As storage time increased, saltiness decreased ($P < 0.01$, Figure 2.6A). Bacon from PC barrows was saltier than IC bacon ($P < 0.05$), but was not different than bacon from gilts ($P = .90$, Figure 2.6B). Saltiness was similar between bacon from IC barrows and gilts ($P = 0.08$). As storage time increased, oxidized odor increased ($P < 0.01$, Figure 2.6C) and oxidized flavor increased ($P < 0.01$, Figure 2.6D). There was no difference in oxidized odor, oxidized flavor and off flavor across treatments ($P \geq 0.31$). Off flavor was increased as storage time increased ($P < 0.05$, data not shown).

2.5. Discussion

An important factor involved in further processing meat products is retarding the development of lipid oxidation in order to extend shelf life of products. This becomes a greater challenge in products with increased levels of unsaturated fatty acids, as they are more prone to oxidizing (Rhee et al., 1996). As a result of oxidation, oxidized products are less desirable to consumers (Campo et al., 2006). Because bellies from IC barrows have greater total concentration of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) when compared with PC barrows (Boler et al., 2011; Boler et al., 2012), bacon from IC barrows might be more susceptible to lipid oxidation and have reduced shelf life. However, increasing time after second Improvest® dose before slaughter and increasing age at slaughter causes fatty acid profiles of IC barrows to become more similar to PC barrows (Tavárez et al., 2014a;b). Therefore, it was important to understand the effect that immunological castration management strategy has on lipid oxidation and the sensory characteristics of bacon stored under simulated food service conditions.

As storage time increased, lipid oxidation increased, regardless of immunological castration management strategy or sex. Within immunological castration management strategy, IC barrows slaughtered at 24 wk of age, 4 and 6 wk ASD had decreased TBARS when compared with IC barrows slaughtered at 28 wk of age, 8 wk ASD. This likely due to the fact that as time after second Improvest® dose increased from 6 to 8 wk, lipid content of the bacon increased, regardless of age. Other studies have reported an approximately 6 percentage unit increase (71.75% at 4 wk to 77.21% at 8 wk after second dose) in the lipid content of belly adipose tissue as time after second Improvest® dose increased from 4 to 8 wk (Tavárez et al., 2014b). This increase in lipid content is thought to be a result of increased *de novo* fat synthesis due to the increased feed intake of IC barrows after the second Improvest® dose (Dunshea et al., 2001; Lealiifano et al., 2011). The main fatty acids produced from *de novo* synthesis are the saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acid (Mersmann and Smith, 2005). Thus, as pigs continue to consume excess energy, the proportion of saturated fatty acids in adipose tissue is increased and iodine value is ultimately decreased.

While SFA levels increase as a result of *de novo* fat synthesis, an increase in total lipid content can result in increased TBARS (Siu and Draper, 1978). Once TBARS were recalculated based on lipid content, immunological castration did not have an effect on lipid oxidation. Similarly, immunological castration did not affect sensory characteristics. Furthermore, after 12 wk of frozen storage TBARS were still below 0.5mg MDA/kg of meat. This is important because traditionally, TBARS in excess of 0.5mg MDA/kg meat results in sensory data in which trained panelists deem a food to be rancid (Tarladgis et al., 1960). This remained true for this study, after 12 wk of frozen storage; sensory scores indicated that panelists still deemed the bacon from IC barrows to be acceptable. Furthermore, throughout the 12 wk storage time,

sensory scores increased as storage time increased, but at no time did immunological castration management strategy have an impact on oxidized odor and flavor sensory scores relative to PC barrows or gilts.

Within sex class, bacon from PC barrows had an approximately 5 percentage unit increase in lipid content when compared with bacon from IC barrows and gilts. This was expected because PC barrows are reported as having more backfat and thicker bellies than IC barrows (Boler et al., 2012; Tavárez et al., 2014a). However, IC barrows having similar TBARS to PC barrows, was unexpected. Bellies from IC barrows are generally reported as having greater concentrations of MUFA and PUFA in their adipose compared with PC barrows (Boler et al., 2012; Tavárez et al., 2014b). Additionally, in the population from which these bellies were selected, IC barrows slaughtered at 24 wk of age, 4 wk ASD had an increased PUFA percentage compared with PC barrows slaughtered at 24 wk of age (Tavárez, 2014). This increased PUFA percentage should result in bacon from IC barrows with increased TBARS when compared with PC barrows, but as with immunological castration management strategy the differences in lipid content between castration methods affected TBARS. Once TBARS were assessed on a lipid basis, bacon from PC barrows had decreased lipid oxidation compared to IC barrows. However, this difference in TBARS was not reflected in the sensory data.

Overall, bacon from IC barrows had similar oxidized odor and flavor sensory scores when compared with bacon from PC barrows and gilts. In other research evaluating the effect of immunological castration on lipid oxidation in pork sausage and enhanced pork loins, similar results were reported (Jones-Hamlow, 2013). While TBARS and oxidized odor and flavor scores increased with storage time trained pannelists detected no difference among treatments. Given this and the results from Tavárez (2014), increasing time after second Improvest® dose before

slaughter results in a belly with decreased levels of PUFA, and bacon with increased lipid content, that has similar sensory characteristics to bacon from PC barrows and gilts.

2.6. Conclusion

Results from both experiments show immunological castration, within management strategy or when compared with PC barrows and gilts, does affect lipid oxidation but does not affect sensory characteristics of food service type bacon. Regardless of immunological castration management strategy or sex, bacon became more oxidized with storage. Within IC treatments, lipid oxidation increased as storage time increased, and lipid content was increased in barrows 8 and 10 wk ASD, compared with bacon from IC barrows slaughtered 4 and 6 wk ASD, regardless of age at slaughter. Bacon from IC barrows was more oxidized than bacon from PC barrows, but was similar to bacon from gilts. However, regardless of treatment, there were no differences in sensory attributes of bacon, and after 12 wk of frozen storage bacon did not become rancid. Therefore, using bellies from Improvest® managed pigs for bacon should not result in reductions in shelf life.

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2.8. Tables and Figures

Table 2.1: Population statistics on bellies used to make food service bacon

Item	N	Minimum	Maximum	Mean	SEM
Hot Carcass Wt. (kg)	129	77.02	149.78	106.04	0.97
Last Rib Back Fat (mm)	129	5.00	35.00	22.81	0.52
Belly Length (cm)	129	66.04	83.82	74.95	0.26
Belly Width (cm)	129	24.13	34.29	28.90	0.17
Belly Thickness (cm)	129	2.98	5.81	4.17	0.04
Belly Flop (cm)	129	4.32	60.20	26.81	0.91
Green Wt (kg)	129	5.22	12.49	8.37	0.11
Iodine value ¹ (g/100g)	129	57.05	74.01	64.93	0.29

¹Calculated as Iodine value (IV; AOCS, 1998) = C16:1 x (0.95) + C18:1 x (0.86) + C18:2 x (1.732) + C18:3 x (2.616) + C20:1 x (0.785) + C22:1 x (0.723)

Figure 2.1: Effect of immunological castration management strategy on overall moisture and lipid content of food service bacon throughout 12 wk of frozen storage. Immunological castration management treatments include: 24-wk-old IC barrows slaughtered at 4 (IC-24-4), 6 (IC-24-6), 8 (IC-24-8) and 10 (IC-24-10) wk after second dose; and 26 and 28-wk-old IC barrows slaughtered at 6 (IC-26-6) and 8 (IC-28-8) wk after second dose. Data are depicted as least squared means $\pm$ SEM, and means lacking common superscripts (a, b) differ ($P < 0.05$).

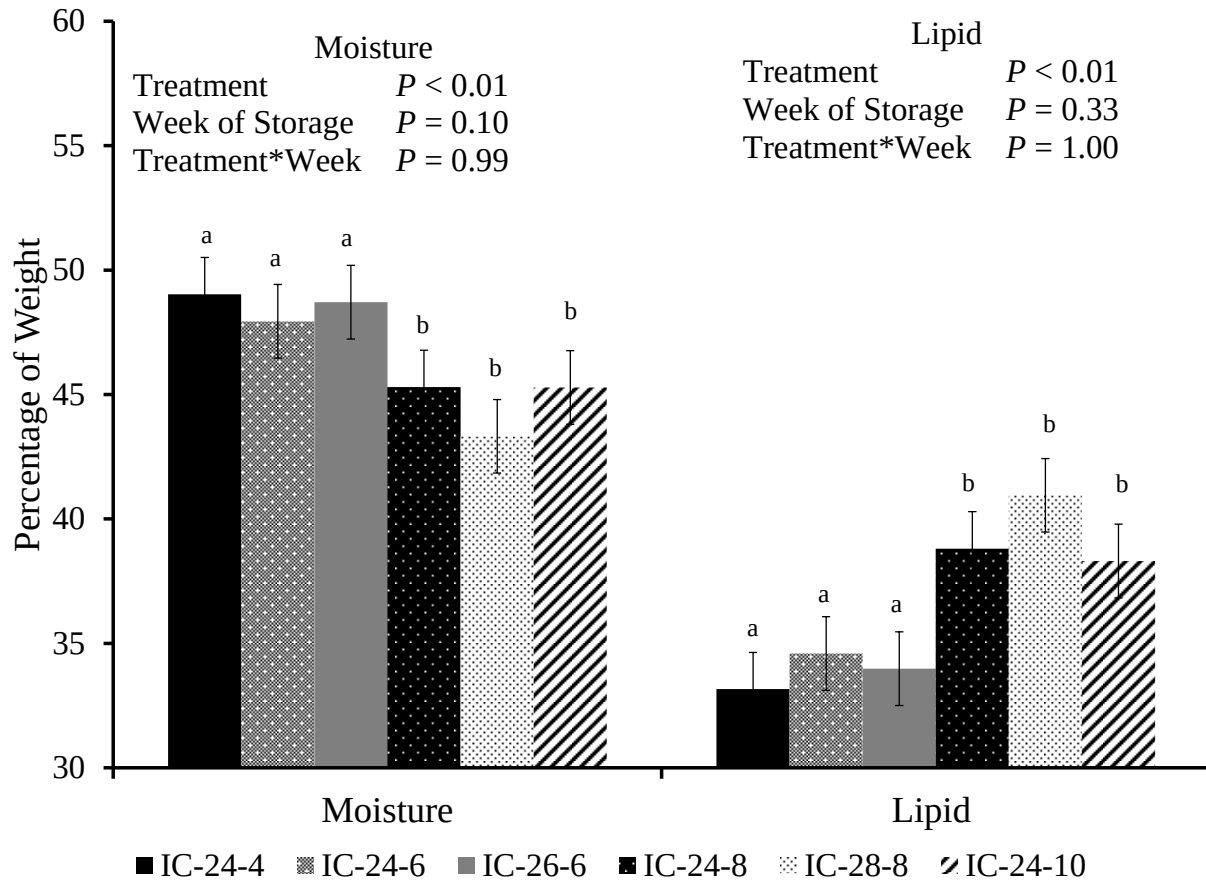


Figure 2.2: Effect of immunological castration management strategy on lipid oxidation of bacon stored for 12 wk in simulated food service conditions. Traits evaluated include TBARS, wk effect (A), TBARS, treatment effect (B), TBARS, corrected for differences in lipid content (C). Immunological castration management treatments include: 24-wk-old IC barrows slaughtered at 4 (IC-24-4), 6 (IC-24-6), 8 (IC-24-8) and 10 (IC-24-10) wk after second dose; and 26 and 28-wk-old IC barrows slaughtered at 6 (IC-26-6) and 8 (IC-28-8) wk after second dose. Data are depicted as least squared means $\pm$ SEM, and means within a panel, lacking common superscripts (a, b, c; indicating differences in overall wk means) (x,y; indicating differences in overall treatment means) differ ($P < 0.05$).

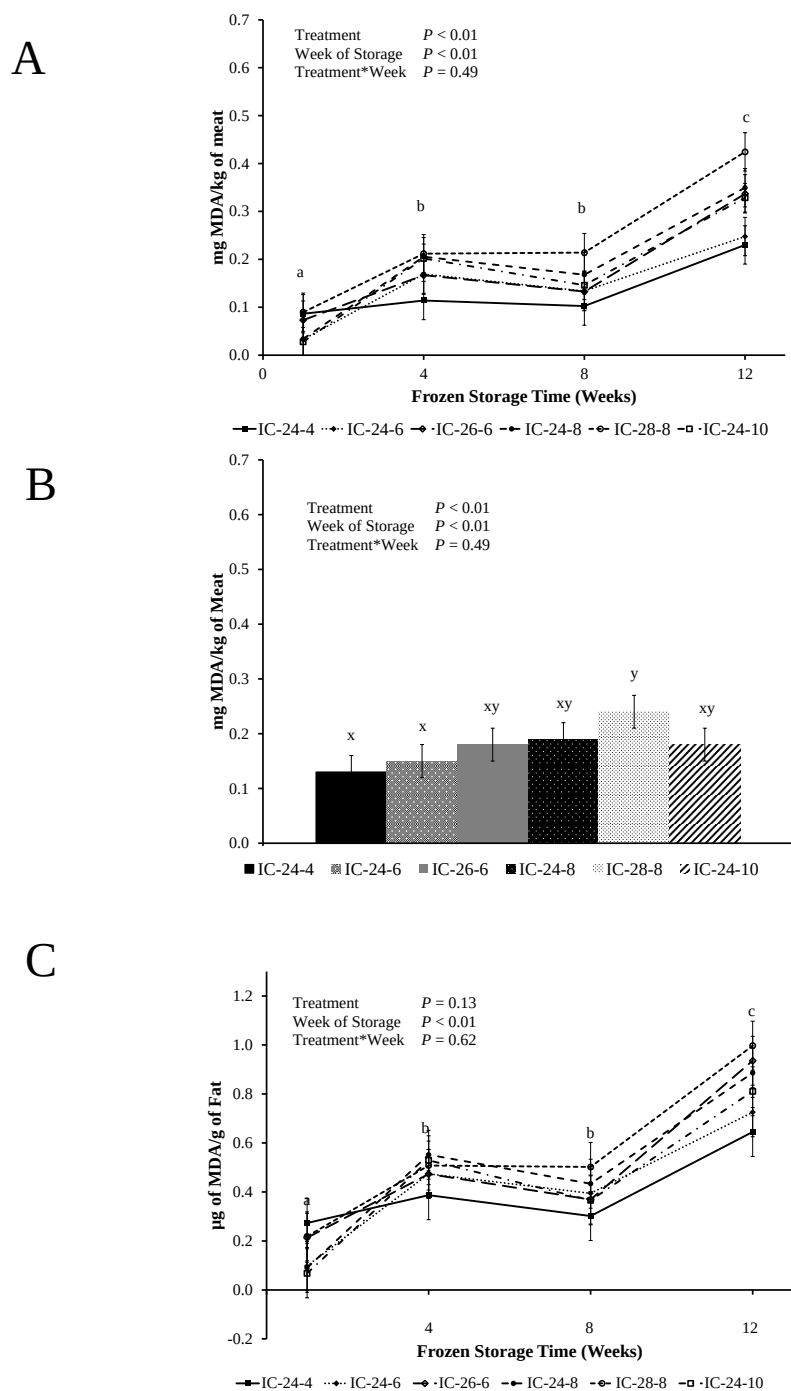


Figure 2.3: Effect of immunological castration management strategy on sensory characteristics of bacon stored for 12 wk in simulated food service conditions. Traits evaluated include saltiness (A, B), oxidized odor (C), and oxidized flavor (D). Off flavor (not depicted) was unaffected ($P > 0.05$) by immunological castration management strategy. Immunological castration management treatments include: 24-wk-old IC barrows slaughtered at 4 (IC-24-4), 6 (IC-24-6), 8 (IC-24-8) and 10 (IC-24-10) wk after second dose; and 26 and 28-wk-old IC barrows slaughtered at 6 (IC-26-6) and 8 (IC-28-8) wk after second dose. Data are depicted as least squared means $\pm$ SEM, and means within a panel, lacking common superscripts (a, b, c; indicating differences in overall wk means) differ ($P < 0.05$).

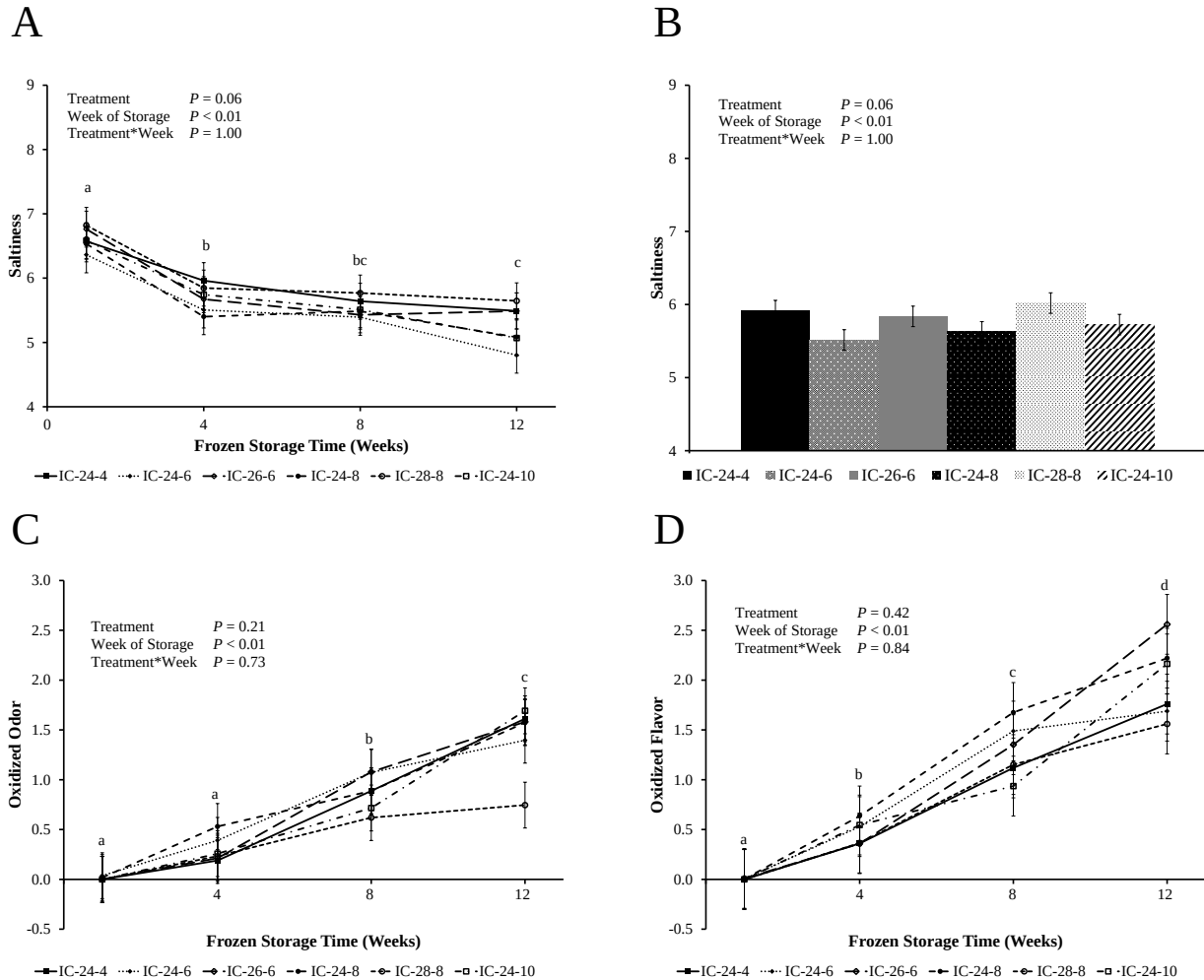


Figure 2.4: Effect of sex on overall moisture and lipid content of food service bacon from immunologically castrated (IC) barrows, physically castrated (PC) barrows and gilts throughout 12 wk of frozen storage. Data are depicted as least squared means $\pm$ SEM, and means within a panel, lacking common superscripts (a, b) differ ($P < 0.05$).

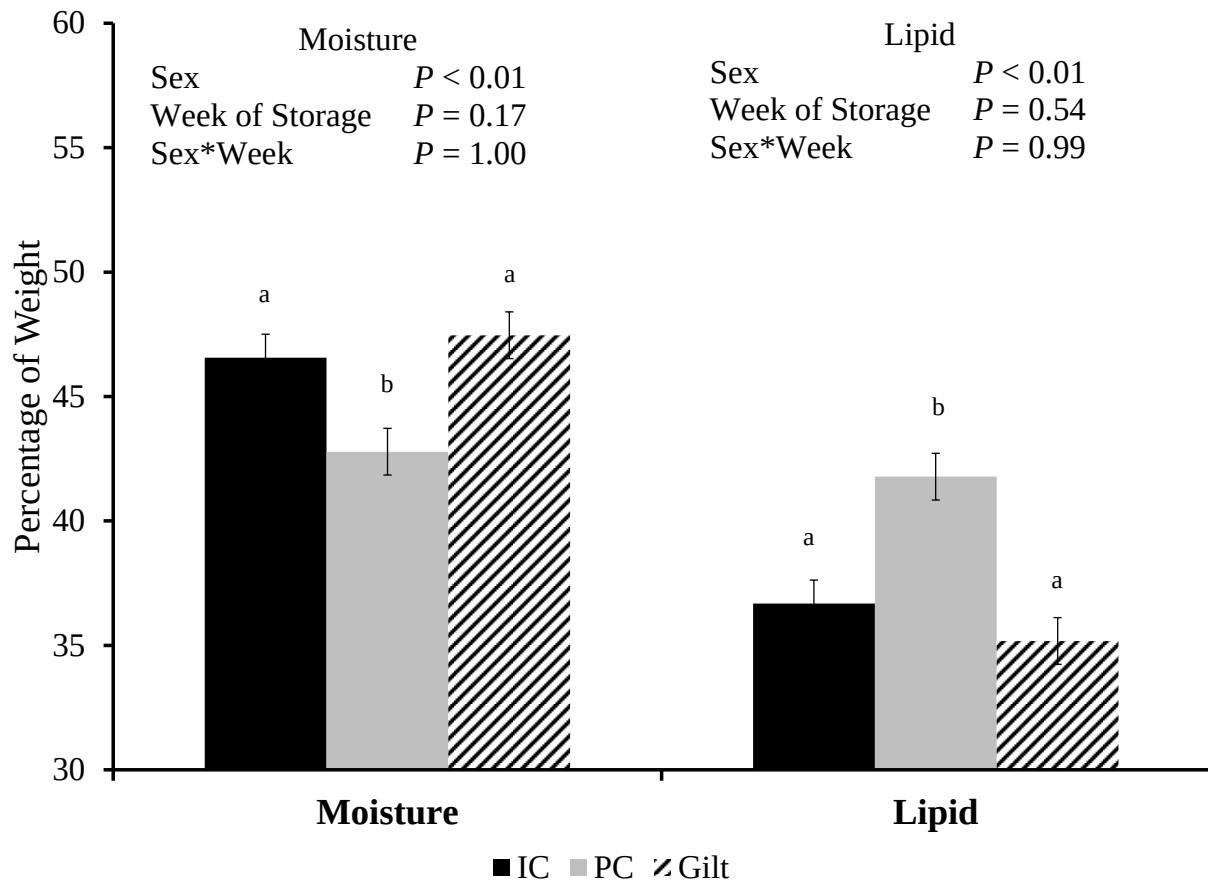


Figure 2.5: Effect of sex on lipid oxidation of food service bacon from immunologically castrated (IC) barrows, physically castrated (PC) barrows and gilts throughout 12 wk of frozen storage. Traits evaluated include TBARS, wk effect (A), TBARS, treatment effect (B), TBARS, corrected for differences in lipid content, wk effect (C), TBARS, corrected for differences in lipid content, treatment effect (D). Data are depicted as least squared means $\pm$ SEM, and means within a panel, lacking common superscripts (a, b, c; indicating differences in overall wk means) (x, y; indicating differences in overall treatment means) differ ($P < 0.05$).

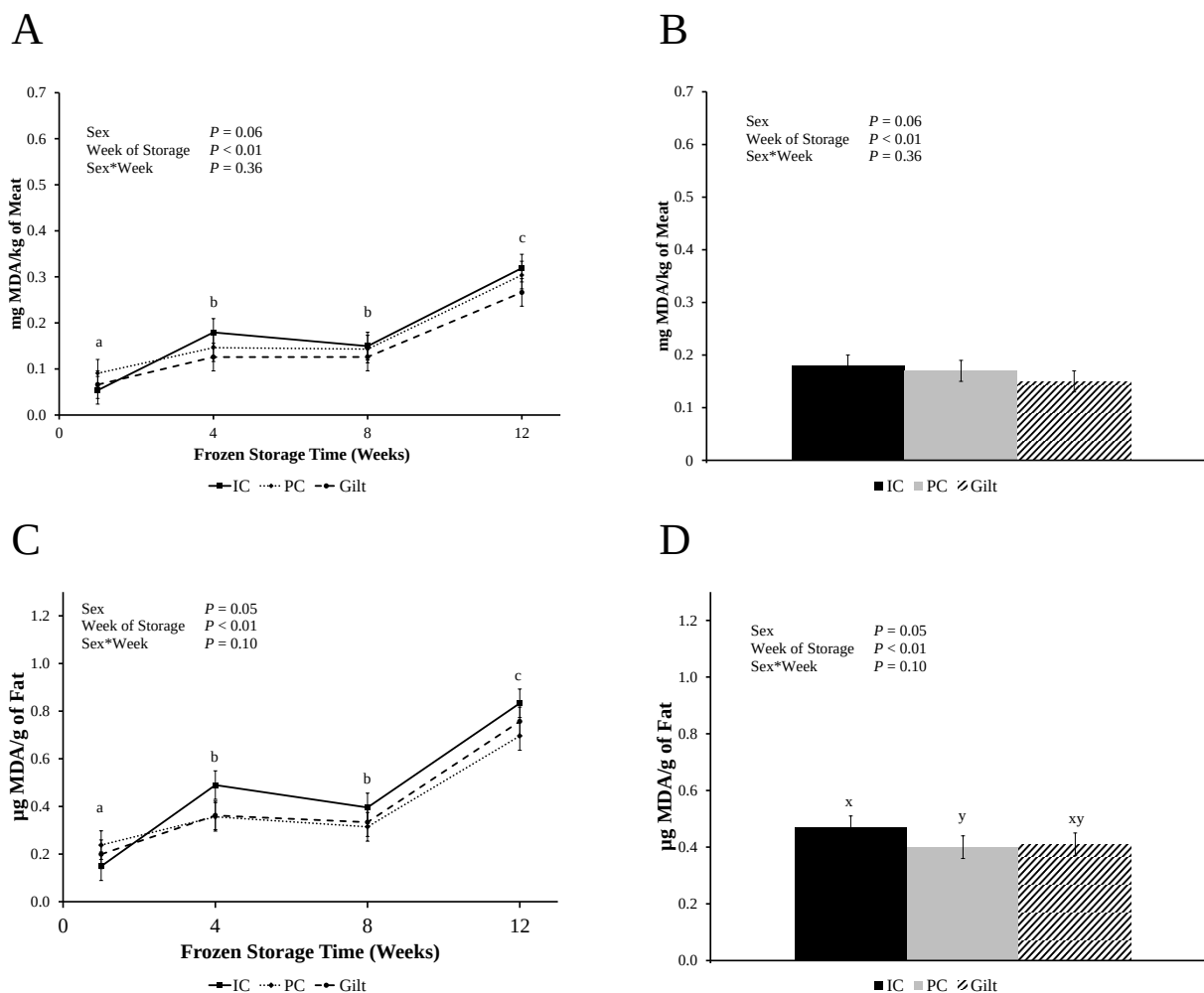
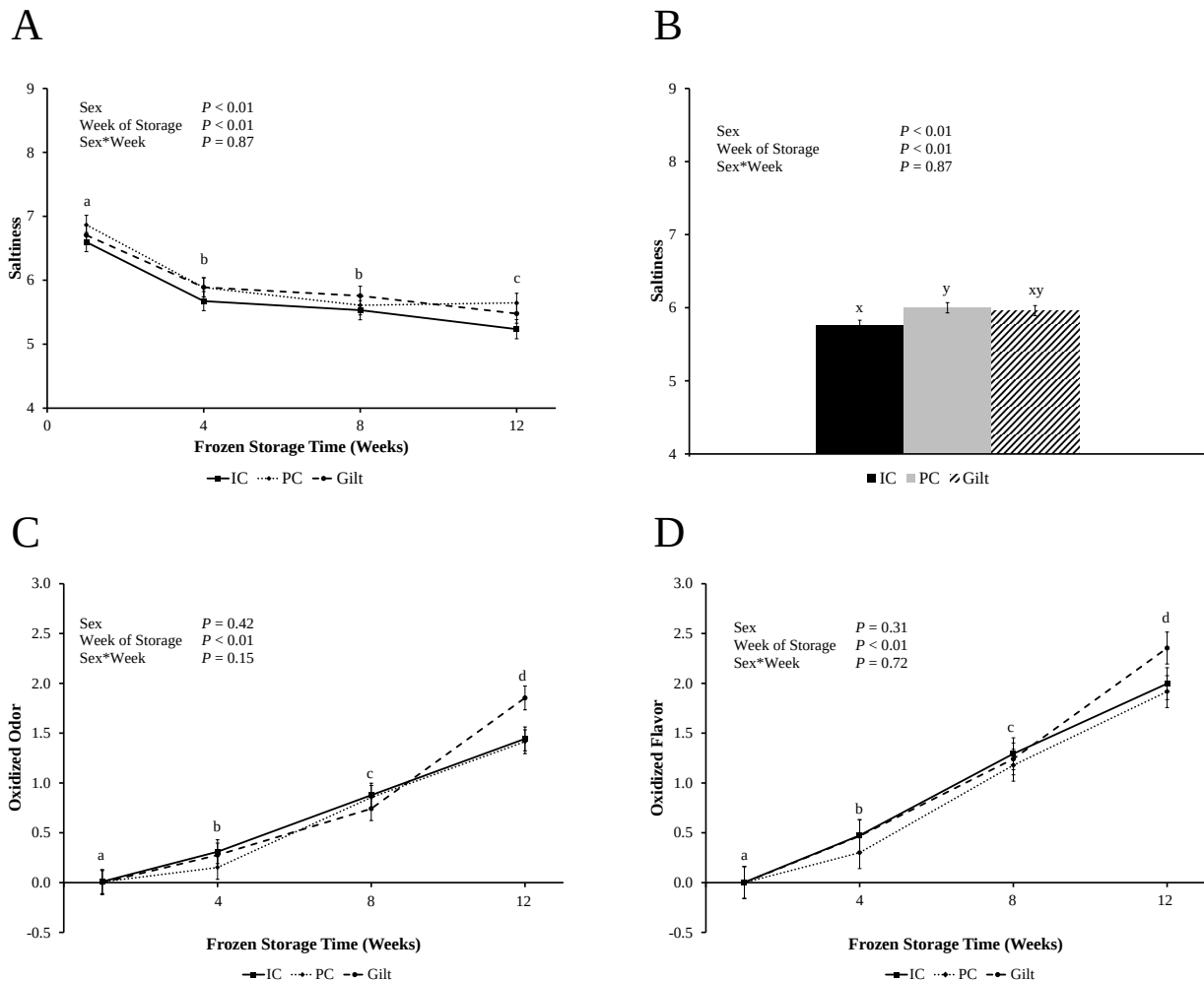


Figure 2.6: Effect of sex on sensory characteristics of food service bacon from immunologically castrated (IC) barrows, physically castrated (PC) barrows and gilts throughout 12 wk of frozen storage. Traits evaluated include saltiness (A, B), oxidized odor (C), and oxidized flavor (D). Off flavor (not depicted) was unaffected ($P > 0.05$) (A), saltiness. (B), oxidized odor. (C), oxidized odor. (D), off flavor (not shown) was unaffected ($P > 0.05$) by sex. Data are depicted as least squared means $\pm$ SEM, and means within a panel lacking common superscripts (a, b, c, d; indicating differences in overall wk means) (x, y; indicating differences in overall treatment means) differ ($P < 0.05$).



Chapter 3:

Summary of the effect of immunological castration on fresh belly quality and bacon processing characteristics, shelf life, and sensory characteristics

3.1. Introduction

Immunological castration (Improvest[®], *gonadotropin releasing factor analog-diphtheria toxoid conjugate*, Zoetis, Kalamazoo, MI) is a means of controlling boar taint in intact males. Improvest[®] is a two injection regime and is administered subcutaneously at the base of the ear, at approximately 16 and 20 wk of age. While the first dose primes the immune system, the second dose triggers an immune response that results in a loss of function of the testis and a clearing of boar taint compounds (androstenone and skatole) from the pig. Managing pigs with Improvest[®] allows for producers to take advantage of the increased carcass leanness and improved feed efficiency of boars compared to physically castrated (PC) barrows (Xue et al., 1997). However, one disadvantage of leaner pigs is increased content of unsaturated fatty acids in adipose (Wood et al., 2008). Therefore, the likelihood of lean pigs having fat quality issues (soft, oily, and yellow fat) are increased compared with fatter pigs. This can ultimately have a negative impact on fat quality in fresh bellies, can reduce bacon slicing yields, and can impact shelf life of the finished product.

3.2. Effect of Immunological Castration on Fresh Belly Quality

Bellies from Improvest[®] managed pigs are on average 10.3 cm firmer (as measured by belly flop distance) and 0.5 cm thicker than bellies from boars (Boler et al., 2011; Kyle et al., 2014). However, bellies from immunologically castrated (IC) barrows are thinner (26.4 cm) and less firm (3.8 cm) than bellies from PC barrows (35.9 cm, 4.1 cm, respectively), but are thicker

(22.9 cm) and firmer (3.6 cm)bellies from gilts (Kyle et al., 2014; Lowe et al., 2014). In IC pigs, one potential strategy to alter belly quality is to increase the time interval between the second Improvest® dose and slaughter.

Increasing time after second dose in IC barrows from 4 to 6 wk increased belly flop distance by 3.4 cm (11.9 to 15.3 cm), but did not alter belly thickness (Boler et al., 2012). Similarly, increasing time after second dose from 5 to 7 wk in IC barrows increased belly thickness by 0.6 cm (from 3.4 to 4 cm), and belly flop distance by 1.4 cm (from 14.7 to 16.1 cm) (Table 3.1, adapted from Tavárez et al., 2014a). Furthermore, at 5 wk after second dose, bellies from IC barrows were 0.3 cm thinner than bellies from PC barrows, but at 7 wk after second dose, belly thickness only differed by 0.1 cm (Tavárez et al., 2014a). Within IC barrows slaughtered at 24 wk of age, belly thickness, belly flop distance increased as time after second dose increased from 4 to 10 wk after second dose (Tavárez, 2014). While bellies from IC barrows had similar thickness measurements to bellies from PC barrows as time after second dose increased, belly flops remained different. Increasing age at slaughter in PC barrows increased flop distances by 2.27 cm (from 16.7 to 18.97 cm) and increasing age at slaughter in IC barrows increased flop distances by 1.4 cm (14.7 to 16.1 cm) (Tavárez et al., 2014a). Therefore, the difference in flop distance remained unchanged over time between the two castration methods.

The cause of these improvements in fresh belly characteristics is thought to be linked to increased *de novo* fat synthesis as a result of increased feed intake in IC barrows after second dose (Dunshea et al., 2001; Lealiifano et al., 2011). Excess carbohydrates consumed as a result of increased feed intake are used to synthesize fat. Accordingly, *de novo* synthesis is reflected with an approximately 6 percentage unit increase in lipid content (71.75% at 4 wk to 77.21% at 8

wk after second dose) in belly adipose tissue of IC barrows, compared with an approximately 3 percentage unit increase in lipid content (81.67% at 4 wk to 84.88% at 8 wk) in belly adipose tissue of PC barrows (Tavárez et al., 2014b). As age at slaughter increased from 24 to 28 wk of age, lipid content of bacon was increased, and iodide value was reduced in IC barrows but not in PC barrows and gilts combined (Tavárez, 2014). Increasing lipid content of belly adipose tissue leads to bellies that are firmer (Wood et al., 2008) and have fewer problems during bacon slicing (Enser et al., 1984).

The main fatty acids produced from *de novo* synthesis are the saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acid (Mersmann and Smith, 2005). Thus, as pigs continue to consume excess energy, the proportion of saturated fatty acids in adipose tissue is increased. Within IC barrows slaughtered at 24 wk of age, bacon lipid content increased as time after second dose increased, and iodine value tended to decrease, from 4 to 10 wk after second dose (Tavárez, 2014). Furthermore, in IC barrows as time after second dose increased, the iodine value of belly adipose was reduced by approximately 3 percentage units (63.98 at 4 wk to 60.85 at 8 wk after second dose). However, in PC barrows iodine value of belly adipose tissue was unchanged (61.53 at 4 wk to 61.64 at 8 wk) (Tavárez et al., 2014b).

Overall, IC barrows are 3.7 kg heavier and have 2.1mm less backfat than PC barrows. Belly adipose lipid content is reduced by 8.9 percentage units and iodine values are 0.5 units (66.2 vs 65.7) greater in IC barrows compared to PC barrows. Furthermore, belly flop distance (7.1 cm) and thickness (0.3 cm) are reduced in IC barrows compared with PC barrows. It is also important to note that when dried distillers grains with solubles (DDGS) is included in the diet, belly flop distances and iodine values are worse than those from pigs fed a corn-soy diet (Boler

et al., 2012; Tavárez et al., 2014a), regardless of castration method. This means that diet and castration method both contribute to fresh belly characteristics.

Based on available data it is optimal to slaughter IC barrows at least 8 wk after second dose to maximize fresh belly quality. However, increasing time after second dose to improve fresh belly quality is not without its limitations. Increasing time after second dose from 4 to 6 wk decreased the overall feed efficiency of IC barrows (Puls, 2014). Furthermore, as time after second dose prior to slaughter nears 10 wk, the risk of boar taint in the meat is increased, due to a decrease in circulating antibodies (Hennessy, 2008).

3.3. Effect of Immunological Castration on Bacon Processing

Along with reducing belly flop distances and thickness measurements, immunological castration can alter bacon slicing yields. Immunologically castrated barrows slaughtered between 4.5 and 6.5 wk after second dose (at approximately 130 kg ending live weight) had similar backfat to PC barrows (28.5 vs 28.9 mm, respectively) but had 4.8 percentage units less lipid in their bacon compared to PC barrows (Kyle et al., 2014). Furthermore, slicing yields (expressed as a percentage of green weight) were approximately 4.7 percentage units less in IC barrows compared to PC barrows and gilts. Feeding ractopamine HCl to IC barrows improved slicing yields by 2.76 percentage units (93.6 vs 96.4%) (Kyle et al., 2014). One possible explanation of this effect is an increased secondary lean area of the slice, which might help reduce shatters. However, Lowe (2013) reported no differences in slicing yields of IC barrows fed ractopamine HCl.

Bacon slicing yields also increased as time after second dose increased, but was similar between sex classes (Tavárez, 2014). Furthermore, slicing yields were improved with increasing

age at slaughter. In IC barrows slaughtered at 5 and 7 wk after second dose, increasing time after second dose prior to slaughter improved slicing yields by approximately 2 percentage units when compared with PC barrows (6.1 percentage units less at 5 wk, 4.6 percentage units less at 7 wk) (Tavárez et al., 2014a). This improvement in slicing yields is likely due to the increased rate of lipid deposition in bacon in IC barrows compared to PC barrows. From 5 to 7 wk, IC barrows had a 7.6 percentage unit increase in bacon lipid content, while PC barrows only had a 4.3 percentage unit increase. While, slicing yields in IC barrows did improve as time after second dose increased, slicing yields of IC barrows were 5 percentage units lower than PC barrows at 7 wk after second dose (Tavárez et al., 2014a). Overall, slicing yields, calculated as a percentage of green weight or cooked weight were on average 5.3 and 4.0 percentage units greater in PC barrows compared to IC barrows and lipid content of PC bacon was approximately 5 percentage units greater than bacon from IC barrows. Increasing age at slaughter and time after second dose improved both fresh and cured belly characteristics, but age at slaughter had a greater marginal impact on fresh and cured belly characteristics.

3.4. Effect of immunological castration on lipid oxidation and sensory characteristics of bacon

While increasing time after second Improvest® dose prior to slaughter can increase fat deposition and reduce iodine value, it is also important to understand the effect that these changes have on lipid oxidation and sensory characteristics of bacon. As storage time increased, lipid oxidation (TBARS) and oxidized odor and flavor increased, regardless of IC treatment or sex class (Herrick, 2014). Among IC barrows, lipid oxidation was increased in bacon from IC barrows slaughtered at 28 wk of age, 8 wk after second dose compared with bacon from IC

barrows slaughtered at 24 wk of age, both 4 and 6 wk after second dose. However, in IC barrows, lipid content of bacon was increased by approximately 5 percentage units in barrows slaughtered at 8 and 10 wk after second dose compared to barrows slaughtered 4 and 6 wk after second dose, regardless of age at slaughter. Once TBARS were corrected for lipid content, there were no differences in lipid oxidation among Improvest® managed barrows.

When compared to bacon from PC barrows and gilts, bacon from IC barrows had similar levels of lipid oxidation. However, bacon from PC barrows had an approximately 5 percentage unit increase in bacon lipid content than bacon from IC barrows and gilts, which were similar. Once TBARS were corrected for lipid content, bacon from IC barrows had increased levels of lipid oxidation compared with PC barrows, but was not different than bacon from gilts. While bacon from IC barrows was more oxidized than bacon from PC barrows, this difference was not detected by a trained sensory panel.

When comparing sensory characteristics from intact males (IM) to IC barrows and PC barrows, IM had a detectable boar aroma and boar flavor, while both IC and PC barrows were devoid of boar aroma and flavor (Little et al., 2014). Intact males had the least desirable cured bacon aroma and flavor; however, there were no differences in bacon from IC barrows and PC barrows. Furthermore, there was no difference in off-flavor sensory scores between IC and PC barrows, slaughtered at 5 or 7 wk after second dose (Little et al., 2014). Within IC barrows there was no difference in oxidized odor and oxidized flavor sensory scores among treatments (Herrick, 2014). Additionally, when compared with PC barrows and gilts, bacon from IC barrows had similar sensory attributes. Therefore, using bellies from IC barrows results in bacon that has similar to bacon from IC barrows and should not have decreased shelf life.

3.5. Conclusion

Carcasses from IC barrows have improved feed efficiency and are leaner than carcasses from PC barrows. However, increased carcass leanness results in bellies from IC barrows that had decreased slicing yields and increased iodine values compared to bellies from PC barrows. Furthermore, bacon from IC barrows has increased levels of lipid oxidation compared to PC barrows, but this increase in oxidation was not detectable by a trained sensory panel. Increasing time after second dose (> 6wk) and age at slaughter can help curb these differences and results in bellies from IC barrows that are more similar to PC barrows. Additionally within IC barrows, increasing time after second dose before slaughter results in an increase in lipid content in bacon and overall improvements in bacon slicing yields. Data from all of these studies suggest that at heavier (>135kg) finishing weights, bellies from IC barrows become more similar to bellies from PC barrows and become more superior to gilts.

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3.7. Tables

Table 3.1 Effect of immunological castration on fresh belly characteristics of barrows slaughtered at 5 and 7 wk after second Improvest® dose

Item	5 wk after second Improvest® dose		SEM	P - value
	Physical Castrate	Immunological Castrate		Cast.
Length, cm	67.79	67.84	0.48	0.43
Width, cm	25.08	25.78	0.47	0.05
Thickness ¹ , cm	3.77	3.44	0.10	<0.01
Flop distance, cm	16.71	14.72	1.71	0.07
Iodine value ² , g/100g	66.86	67.17	0.76	0.63
Item	7 wk after second Improvest® dose		SEM	P - value
	Physical Castrate	Immunological Castrate		Cast.
Length, cm	70.05	69.86	0.81	0.77
Width, cm	26.21	26.42	0.54	0.63
Thickness ¹ , cm	4.09	3.92	0.10	0.07
Flop distance, cm	18.97	16.14	1.80	0.44
Iodine value ² , g/100g	66.76	65.74	0.99	0.21

¹Thickness is the average of 8 measurements collected on along the belly, where locations 1 to 4 is from the anterior to posterior position of the dorsal edge of the belly and locations 5 to 8 are from the anterior to posterior position of the ventral edge of the belly

²Calculated as Iodine value (IV; AOCS, 1998) = C16:1 x (0.95) + C18:1 x (0.86) + C18:2 x (1.732) + C18:3 x (2.616) + C20:1 x (0.785) + C22:1 x (0.723)

Data adapted from Tavárez et al., 2014a