Meat Quality Assessment of Pork Fed Poultry Fat, Flaxseed Oil, and Supplemented with Vitamin E

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 07, 2017

Keywords: Flaxseed Oil, Poultry Fat, Vitamin E, Dietary Lipids, Meat Quality, Pork

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Abstract

The objective of this research is to understand carcass and meat quality characteristics of pigs fed a combination of poultry fat, flaxseed oil, and supplemented with vitamin E. It is hypothesized that certain combinations of these ingredients may increase intramuscular fat (IMF) percentage while simultaneously decreasing external fat deposition.

Yorkshire pigs (n=96) weighing approximately 50 kg were allocated to pens based on weight and sex, over two trials. Pigs within each trial were born in the same farrowing groups and each pen was allotted two gilts or two barrows. Each pen was randomly assigned to one of 8 dietary treatments in a 4 x 2 factorial arrangement. Corn-soybean meal finisher diets were formulated to contain 0, 2, 4 or 6% lipids and either 11 or 220 IU Vitamin E/kg. For all diets with lipids, 1% flaxseed oil was included and the remaining lipids supplied by poultry fat (0, 1, 3, or 5%).

Pigs were harvested (n=8 groups) when an average pen weight of 110 ± 3 kg was achieved. Following harvest, hot carcass weight (HCW) was recorded. At 24 hours post mortem carcasses were evaluated for last rib fat thickness (LRFT), tenth rib fat thickness (TRFT), loin eye area (LEA), muscle score (MS), percent fat free lean (%FFL), color values (L*, a*, b*), ultimate pH of the ham (pHH) and loin (pHL), and National Pork Producers Council (NPPC) color (NPPCCol) and marbling score (NPPCMar). TRFT, LEA, L*, a*, b*, pHH, NPPCCol, and NPPCMar were determined on the loin eye at the $10^{th}/11^{th}$ rib interface after chilling, prior to carcass fabrication. After carcasses were chilled for 24 h at 4±2°C, 2.54 cm pork chops were

fabricated from the left side of the carcass and individually packaged in vacuum-sealed bags and frozen at -20±2°C for further analysis. Bellies were measured for thickness (BT), and both skin-side up (SSU) and skin-side down (SSD) firmness evaluation were made.

Chops were analyzed for drip loss (DL), vacuum purge loss (VP), marinade uptake (MU), marinade cook loss (MCL), cook loss (CL), Warner-Bratzler Shear Force (WBS), and thiobarbituric acid reactive substances (TBARS). Proximate analysis was performed for the determination of collagen, fat, moisture, protein, and salt content of loin samples. Sensory evaluation by a trained panel was also performed. Statistical analysis was conducted using Proc GLM procedure in SAS (2002). Carcass was the experimental unit and days on feed (DOF) was used as a covariate. Main effects included trial, sex of pig, lipid level, and vitamin E concentration. All interactions were also included in the model.

A 4-way interaction of trial x lipid x sex x vitamin E affected the measurements for SSU (P=0.0430) and CL (P=0.0379). Two 3-way interactions were found in this study. Lipid x vitamin E x sex were different for a* (P=0.0193), pHL (P=0.0007), SSU (P=0.03), belly thickness (P=0.0198), and VP (P=0.0167). A trial x lipid x vitamin E interaction for SSU (P=0.0238), DL (P=0.0471) and CL (P=0.0305) was present. Additionally, a trial x vitamin E interaction was present for TFRT (P=0.03), %FFL (P=0.0350), MS (P=0.0304), SSD (P=0.0042), SSU (P=0.0079), DL (P=0.0490), VP (P=0.0418), and Collagen % (P=0.0225). There was a trial x sex interaction present for LRFT (P=0.0034), VP (P=0.0286), and moisture % (P=0.0390). A lipid x sex interaction was also significant for LRFT (P=0.0031), %FFL (P=0.0164), MS (P=0.0362), and SSU (P=0.0335). A vitamin E x sex interaction was also observed for LRFT (0.0206), SSD (P=0.0003), and SSU (P=0.0018). There was a lipid x vitamin E interaction for TRFT (P=0.0015), %FFL (P=0.0028).

Lipid level, vitamin E concentration, and sex had no effect (P>0.05) on HCW, LEA, %FFL, a*, b*, NPPCCol, pHH, pHL, MS, SSD, SSU, belly thickness, DL, VP, MU, MCL, WBS, % fat, % moisture, % collagen, % protein, % salt, and TBARS. Vitamin E concentration had an effect (P<0.05) on LRFT, TRFT, and NPPCMar. Treatments with inclusion of 220 IU vitamin E produced greater values for LRFT (23.19 vs 21.41 mm), TRFT (21.62 vs 19.26 mm), and NPPCMar (1.87 vs 1.41) than 11 IU vitamin E. In addition, differences were seen across trials for HCW (P=0.0204), MS (P=0.0404), pHH (P<0.0001), pHL (P<0.0001), NPPCCol (P=0.0207), and TBARS (P<0.0001). Trial 1 had greater values for HCW (84.76 vs 81.75 kg), MS (2.57 vs 2.35), pHH (5.85 vs 5.53), pHL (5.67 vs 5.45), and TBARS (0.22 vs 0.15); while NPPCCol was greater in trial 2 (3.24 vs 2.84). Lastly, sex had an effect (P<0.05) on L* and CL. Barrows had greater values (P<0.05) for L* (61.50 vs 58.86) and CL percentage as compared to gilts (17.14 vs 14.89%).

A feeding program utilizing poultry fat in combination with flaxseed oil and vitamin E at these levels will not negatively affect carcass composition or meat quality. While differences are present in this study, all treatments produced pork products which fall within a normal acceptable range for carcass composition and meat quality analysis, all without compromising belly firmness or sensory attributes. Further analysis of fatty acid composition assessment is needed for determining the additional benefits of flaxseed oil inclusion into swine diets.

Acknowledgments

As the end of my time at Auburn University draws near; I have come to the realization that I would not be the woman I am today if it were not for so many individuals who have played a role in this journey, and for the opportunities they have granted me along the way. Not only through graduate school, but through my undergraduate career at Auburn University as well. The good Lord has blessed me beyond measure by giving me the strength and courage to pursue my dreams, and has placed so many influential people in my life to guide me along this path.

First and foremost, I would like to express my sincere gratitude to Dr. Christy Bratcher, who allowed me the opportunity to pursue a degree in Meat Science under her direction. I want to thank her for granting me a chance to be successful and to prove myself when no one else would. I am beyond grateful for her guidance not only as a mentor and professor, but also as a friend throughout my academic career.

I would like to thank my committee members for their patience and support in overcoming numerous obstacles during the completion of this project. Dr. Lisa Kriese-Anderson, Dr. Werner Bergen, and Dr. Lee Chiba have all played vital roles in my success as a graduate student. Additionally, I would like to thank the Department of Animal Sciences faculty and staff for their support, and for the role they have played in shaping me into who I am today. Furthermore, I would like to thank the Meat Lab staff: Barney Wilborn, T. J. Mayfield, Susan Dale, and all the student employees for their assistance in every aspect of my graduate career.

I also want to express my appreciation to my friends and fellow graduate students. I would not have been successful without them. Judson Smith, Katie Sowell, Derek Griffing, Colton Christjohn, Alex Tigue, Dr. Nathan Tapp, Caroline Galvin, Brad Baker, and all other graduate students in the Department of Animal Sciences, I thank you for your invaluable support and friendship. I will cherish our time together, carrying a plethora of memories, laughs, and a few tears with me as we all depart on our life endeavors.

I would like to say a special thank you to my family for their love and unconditional support as this would not have been possible without them. My parents, Pam and David, having instilled in me a hard work ethic, passion, and a drive to achieve anything that I desire. Their emotional support, encouragement, and endless patience has allowed me to realize my potential and granted me the ability to achieve my goals.

Lastly, to my boyfriend Matt, I wish to offer my deepest thanks. His love and support has made every step of this process possible. He has been my rock throughout this journey, as he has kept me grounded and focused. Words cannot express how thankful I am to have him be a part of my life.

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List of Abbreviations

ADG	Average Daily Gain
ALA	Alpha Linolenic Acid
BT	Belly Thickness
CL	Cook Loss
DHA	Docosahexaenoic Acid
DL	Drip Loss
EFA	Essential Fatty Acid(s)
EPA	Eicosapentaenoic Acid
%FFL	Fat-Free Lean Percentage
FA	Fatty Acid(s)
HCW	Hot Carcass Weight
IMF	Intramuscular Fat
LA	Linoleic Acid
LEA	Loin Eye Area
LDL	Low Density Lipoprotein
LRFT	Last Rib Fat Thickness
MCL	Marinade Cook Loss
MT	Metric Ton
MU	Marinade Uptake

MS	Muscle Score
NPPC	National Pork Producer Council
NPPCCol	National Pork Producer Council Subjective Color Score
NPPCMar	National Pork Producer Council Subjective Marbling Score
NAMI	North America Meat Institute
PUFA	Polyunsaturated Fatty Acid(s)
RPCE	Real Per Capita Expenditures
SFA	Saturated Fatty Acid(s)
SDG	Seicoisolariciresinol Diglucoside
SSU	Skin-Side-Up
SSD	Skin-Side-Down
UFA	Unsaturated Fatty Acid(s)
USDA	United States Department of Agriculture
USDA-ERS	United States Department of Agriculture – Economic Research Service
USMEF	United States Meat Export Federation
TBARS	Thiobarbituric Acid Reactive Substances
TRFT	Tenth Rib Fat Thickness
VP	Vacuum Purge
WHC	Water Holding Capacity
WBC	Water Binding Capacity
WBS	Warner-Bratzler Shear Force
ω-FA	Omega Fatty Acid(s)
ω-3 FA	Omega-3 Fatty Acid(s)

ω-6 FA	Omega-6 Fatty Acid(s)

I. Introduction

Meat quality is one of the most important factors to consumers (Font-I-Furnols and Guerrero, 2014). A consumers' intention to purchase a meat product is directly affected by the expected quality of a product (Font-I-Furnols and Guerrero, 2014). Consumer perception of meat quality is derived from the compositional quality or the lean-to-fat ratio, and palatability. Visual appearance, smell, firmness, juiciness, tenderness and flavor contribute to the palpability of a meat product. Consumers have directly affected the swine production system by demanding product standards based upon high quality and organoleptic expectations. Additionally, dietary recommendations suggest a reduction of saturated fatty acids (SFA) and an increased consumption of unsaturated fatty acids (UFA) (Institute of Medicine, 2002), which has resulted in current consumer demands for leaner and healthier pork products. As production practices have shifted to satisfy consumer demands for a leaner pig, there has been an accompanied reduction in IMF content of pork products. IMF content of pork relates to eating quality because IMF directly effects perceived tenderness, juiciness, and contributes to overall palatability. Furthermore, perceived tenderness, juiciness, and palatability are highly correlated with overall quality, intention to purchase, and willingness to pay (Banović et al., 2009; Bello Acebrón and Calvo Dopico, 2000; Lusk et al., 2001). As IMF content increases, tenderness scores increase (Brewer et al., 2001). Thus, the reduction of IMF has caused negative eating experiences for consumers. Supplying the consumer with an overall leaner product with increased IMF would not only increase meat quality attributes, but also consumer acceptance of pork through enhanced

organoleptic characteristics. In addition, the swine industry would benefit from a reduction of subcutaneous carcass fat while simultaneously increasing the IMF content of pork products.

Previous research found through dietary lipid supplementation, it is possible to reduce de novo lipogenesis in pigs to not only satisfy consumer demands for leaner pigs but also increase IMF content to enhance the eating quality of pork (Jakobson and Thorbek, 1993; Bee et al., 2002; Allee et al., 1971a,c; Chillard, 1993; Smith et al., 1996). Increased IMF though dietary lipid supplementation of poultry fat and flaxseed oil, which contains a high concentration of omega-3 fatty acids (ω-3 FA), would also have an additional positive affect on human health due to positive health characteristics of ω-3 FA. Extensive research has been conducted to determine the effects of dietary lipid supplementation on animal performance and the fatty acid (FA) content in pork tissues (Brooks, 1971; Morgan et al., 1992; Wiseman and Agunbial, 1998; Averette Gatlin et al., 2002). Fatty acid composition of dietary fat directly influences FA composition of pork products and the ω-3/ω-6 FA ratio can be altered to favor consumer heath demands (Seerley et al., 1978).

Increasing the concentration of ω-3 FA, a polyunsaturated fatty acid (PUFA), in the diet has beneficial effects on carcass quality and human health, but there could be an accompanying reduction of oxidative stability of pork products. However, inclusion of vitamin E into the swine diet can increase the oxidative stability of the pork. Vitamin E, a natural antioxidant, not only interacts with PUFA at the cellular membrane for stabilization, it also has the potential to increase IMF content and improve meat color (Liu et al., 1995).

The objective of this research was to understand carcass and meat quality characteristics of pigs fed a combination of poultry fat, flaxseed oil, and supplemented with vitamin E.

II. Review of Literature

Swine Production/Pork Production in the United States

U.S. Swine Production

The largest segment of United States (U.S.) agriculture is composed of the meat and poultry industry, which produced 93 billion pounds of meat protein in 2012 (NAMI, 2015). In 2013, 112 million hogs were harvested in the U.S., which produced 23.2 billion pounds of pork (NAMI, 2015). In 2014, the U.S. exported 1.65 billion metric tons (MT, 1 MT= 2204.6 lbs) of pork and pork variety meat, making the annual total pork exports reach a value of $5.6 billion, an increase of 10% from 2013 (NAMI, 2015). The exports in 2013 accounted for 21.5% of U.S. pork production.

Spending less than 6.4% of disposable income, an American male consumes 6.9 oz. of meat per day while a female consumes 4.4 oz (NAMI, 2015). In 2015, the U.S. per capita pork consumption was 64.3 lb., an increase from 59.8 lb. in 2014 (EMI Analytics, 2016). The real per capita expenditures (RPCE) for pork has increased an average of 2.9% annually since 2008 except for one year, for total growth of 20.3%. In 2015, RPCE grew 3% to $198.09 (in 2014 U.S. dollars), which was the highest yearly total since 1991 (Meyer, 2016).

From 2008 to 2013, the U.S. generated approximately 10% of the global pork production, making the U.S. the world's third-largest producer of pork (Giamalva, 2014). The U.S. continued as the third-largest producer through 2016 with China (54,870 in 2015 and 53,500 x 10^3 MT in

2016) and the European Union (23,350 in 2015 and 23,230 x 10^3 MT in 2016) being global pork production leaders (USDA FAS Forecast, 2016).

Economic Impact of the U.S. Pork Production and Exports

Currently, there are over 60,000 pork producers in the U.S., which support over 550,000 jobs (NPPC, 2017). The pork industry generates an estimated $22.3 billion in personal income; adding $39 billion to the GDP (NPPC, 2017). The U.S. swine industry totaled $22.5 billion in 2012. This accounted for 6% of total U.S. agriculture sales (USDA, Ag Census, 2012). A 25% increase since 2007 (USDA, Ag Census, 2012). Currently pork production in the U.S. has an estimated $23.4 billion of gross output; this is linked directly to states' hog slaughtering and processing sectors (NPPC, 2017). The sales from these sectors support additional input purchases, spending and transportation, and other services, as well as consumer-related purchases worth nearly $122 billion (NPPC, 2017). In addition to U.S. consumption of pork products, a large percentage of products are exported to other countries. Since 2008, a large shift in markets has occurred. In 2008, Japan accounted for about one-third of U.S. exports, followed by Mexico, and Canada (USDA ERS, 2017). Japan typically imports equal shares of fresh chilled pork and frozen pork products. In 2008, the U.S. was Japan's number one supplier of fresh pork products, which are typically higher value cuts such as loins. Exported frozen products are mainly boneless bellies and shoulders utilized in processed pork products (USDA ERS, 2017). In 2008, Mexico was the second highest importer of U.S. pork followed by Canada. (USDA ERS, 2017).

A shift occurred in the export/import market since the regression of 2008. According to the U.S. Meat Export Federation (USMEF), total U.S. pork products exported exceeded 2.3 million MT of pork in 2006, including variety meat, with a value of $5.94 billion. Since 2008,

the major importer of U.S. pork was Mexico, exceeding 730,000 MT ($1.35 million), followed by Hong Kong/China with over 540,000 MT ($1.07 million), and Japan importing over 380,000 MT ($1.56 million) (USMEF, 2016). U.S. imports of pork accounts for less than 10% of total global imports with the majority of U.S. pork being imported from Canada and Denmark (USDA ERS, 2017).

U.S. Production Systems

Efficiency is crucial to the success of any livestock operation. Since 2008, the U.S. swine industry has seen an increase in animal feed efficiency as well as breeding efficiency (Giamalva, 2014). Decreasing the input cost of production while increasing the number of piglets per litter resulted in a substantial increase in profitability (Giamalva, 2014). Profitability increases as feed and production efficiency increases (Giamalva, 2014). The demand for food will increase by 70 to 100% by 2050. Thus, continued improvement in management and production practices is vital for sustainability and feeding the world (Godfray, 2010). Improved genetics, management practices, and consolidation within the industry are just a few factors are responsible for these increases in efficiency and profitability.

Pork Supply Chain

In order to maintain and grow a successful industry, several factors need to be considered. Today's farmers have embraced an expanded concept of sustainability that encompasses more aspects of the pork production process. Producers face many challenges including land availability, limited qualified workforce, and rising input costs. With the addition of new technology and sustainable management practices, producers can maximize their business and reduce their environmental footprint.

Integration of new technology has greatly impacted the swine industry and aided in substantial improvements in sow productivity, wean-to-finish growth performance, and carcass composition over the last 35 years (Tokach et al., 2016). Advances in nutrition, genetic selection, and good management practices have led to increased litter size and average daily gain (ADG). Furthermore, increasing market weight and development of leaner carcasses has increased meat quality and carcass yield (National Pork Board, 2016).

The average U.S. swine operation produces more than 4,000 lbs of live weight per sow per year compared with approximately 1,770 lbs in 1980 (Tokach et al., 2016). These improvements are vital to the industry because, without them, it would take an additional 9 million sows to achieve the current level of pork production compared to the current 6 million sows in production (Patience, 2015; Tokach et al., 2016).

Swine Nutrition

The typical commercial swine diet is composed of corn and soybean meal with the addition of vitamins, minerals, and other essential nutrients. A major transition of swine diets has occurred over the last 100 years. In the early 20th century vitamin and minerals were described as unidentified growth factors. Dispensable and indispensable amino acids, with the concept of limiting amino acids was identified in the 1940's (Morrison, 1940). Supplementation of L-lysine HCl in low-crude protein diets achieved similar growth and performance as swine on high protein diets was the next development followed by the determination of other amino acids as a ratio relative to lysine were needed and the concept of ideal protein (Tokach et al., 2016). Utilization of amino acid ratios has led to precisely formulated diets, minimizing crude protein levels while meeting requirements of other amino acids (Tokach et al., 2016). Integration of technology has played a vital role in nutrition as well. Production and utilization of crystalline

amino acids assisted in the reduction of nitrogen excretion in swine waste and has reduced nitrogen requirements by upwards of 40% (Tokach et al., 2016). Advancements in mineral nutrition and development of the enzyme phytase have assisted in minimizing the amount of inorganic phosphorus additives in swine diets. This is vital because only one-third of plant-derived phosphorus is available to the pig for absorption (Tokach et al., 2016). Changes in technology and diet formulation strategies has resulted in substantial improvements in growth rate, feed efficiency, and carcass leanness; all while reducing feed costs per pound of gain and reducing environmental impact (Tokach et al., 2016).

Inclusion of alternative feed ingredients into swine diets has also become a common practice among producers. With the rising cost of corn and soybean meal, other grains, including barley, wheat and oats can be incorporated into the diet (Boggess et al., 2008). Season and geographical location play a large role in alternative ingredient availability and usage. Like the beef industry, swine producers rely on by-products of other industries for feed ingredients: such as grain milling, baking, brewing, distilling, packaging and rendering, fruit and vegetables, vegetable oil, milk, egg and poultry processing. All of the above listed industries produce by-products with various nutritional profiles that can be added into the swine diet for additional benefits (Boggess et al., 2008). These by-products can be a substitution for energy or protein within a complete feed diet. The amount of by-products included into a diet will be dependent upon price, nutrient availability, protein quality, amino acid profile, palatability, presence of anti-nutritional factors, storage life, and the age of the pig for which the feed is intended (Boggess et al., 2008). Some by-products, while serving as a protein or energy source, can also play a role in improving performance traits, efficiency, and carcass composition.

Incorporation of alternative feed ingredients into a diet can also include growth-promoting agents such as beta-agonists. Traditionally incorporated into the diet the last few weeks prior to harvest, beta-agonists convert extra energy animals consume into muscle instead of fat. As an animal ages, it becomes less efficient in turning energy into muscle, therefore incorporating beta-agonists into the diet can help the animal deposit more lean muscle without needing additional feed. Ractopamine hydrochloride (Paylean, Elanco Animal Health, Greenfield, IN) is a phenethanolamine-repartitioning agent that redirects nutrients away from adipose tissue and towards lean tissue deposition (Ricks et al., 1984; Moody et al., 2000). Ractopamine has benefits to improve live animal performance (ADG and improved feed efficiency), N retention, carcass leanness, dressing percentage and water utilization (Storlie, 2012). Performance improvements demonstrated by incorporation of ractopamine can be attributed to increased protein synthesis (Helfrich et al., 1990; Adeola et al., 1992). Several factors affect performance improvements associated with feeding ractopamine to swine including, but not limited to nutrient concentrations of the diet, dietary ractopamine concentration, and duration of feeding (Moody et al., 2000). These benefits are present without compromising meat quality.

Alternative Feed Ingredients and the Impact on Human Health

Dietary Concerns

Dietary recommendations favoring consumption of less saturated fats has led to an increase in demand for foods containing higher levels of UFA (Buckley et al., 1995). The desire to consume foods rich in ω-FA, specifically ω-3 FA, a PUFA, has greatly increased. There is now considerable emphasis on modification of the FA composition of animal tissues, driving the development of new meat products termed designer or functional which contain an increased ω-3

FA content (Buckley et al., 1995). The motivation behind this development is that the human body is unable to produce certain types of fatty acids, specifically ω-3 FA. They must be consumed in the diet. Omega-3 FAs are vital to human health. The Institute of Medicine recommends that adult males and females consume 1.6 g/day and 1.1 g/day of alpha-linolenic acid (ALA) respectively (Institute of Medicine, 2002). These levels of ω-FA are easily obtained in cultures where the diet consists of a large proportion of fatty fish, which are naturally high in ω-FA. The typical diet in western cultures does not meet the required ω-FA levels due to the inadequate supply of fish or consumer dietary preference (Newkirk, 2015).

One characteristic of monogastrics is the capability to directly incorporate dietary FA into body tissues. Fatty acid composition of porcine adipose tissue directly reflects that of their diet (Kouba and Mourot, 1999; Larick et al., 1992). Humans consuming pork products with an altered FA profile can experience a positive effect on their health (Caggiula and Mustad, 1997). In order to satisfy consumers' desire for foods rich in ω-3 FA, swine diets integrate feed additives with high FA content to directly increase the ω-3 FA content in pork products.

Feeding additives such as flaxseed and its derivatives (oil or meal) is one method of altering the FA profile of pork products (Newkirk, 2015). When flaxseed is consumed by swine, there is a strong potential for pigs to deposit greater levels of healthy ω-3 FA into the lean muscle tissue, which can then be consumed in the human diet. Flaxseed has a FA profile containing low levels of saturated fat (9%), moderate levels of monounsaturated fat (18%), and a high concentration of PUFA (73%) (Newkirk, 2015). Flaxseed contains the highest plant-based ω-3 FA concentration with 57% ALA, and 16% ω-6 FA as LA. Linoleic acid and ALA cannot be produced in the human body and must be consumed in the diet, classifying them as essential fatty acids (EFAs). ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA) by the body (Burdge and Wootton, 2002; Harper et al., 2006). EPA and DHA have significant benefits to the management of chronic inflammation, immune disorders, blood pressure management, decreased blood triglyceride levels, and reduction of incidence of coronary heart disease (Newkirk, 2015).

ALA is first consumed from a dietary source and then is converted to EPA and DHA within the body (Burdge and Wootton, 2002; Harper et al., 2006). EPA undergoes further processing to become eicosanoids, a compound which mimics hormone-like activity. Eicosanoids are involved in the mediation of inflammatory response, pain and fever, blood pressure regulation, clotting factor introduction, maintenance of reproduction function and the regulation of sleep/wake cycle (Newkirk, 2015). ALA demonstrates many additional health benefits as compared to other ω-3 FA; specifically aiding inflammatory response, reduction of blood pressure and incidence of heart disease, and decreased blood triglyceride levels (Newkirk, 2015). On the other hand, eicosanoids produced from ω-6 FA are known to promote inflammation, increase blood pressure and blood clotting. This trait is not seen when eicosanoids are produced from ω-3 FA and especially not from EPA (Newkirk, 2015). DHA produced from ALA is vital to growth and development of fetuses and infants (Newkirk, 2015)

Higher production and consumer demands for leaner pork products have led to an increased rate of growth for pigs. This increased rate of growth has yielded a loss in IMF throughout the carcass. IMF is a major attribute to the eating experience of pork, as it directly affects meat quality and palatability, especially the juiciness and flavor profile. In order to enhance physical and organoleptic characteristics of pork, researchers have shifted their focus to alternative methods to increase IMF while decreasing back fat thickness in pork carcasses to maximize yield and quality of the pork. An increase in IMF can be achieved through dietary lipid

supplementation, specifically ω-3 FA that will ultimately be incorporated into the cellular membranes (Davenel et at., 1999; Simopoulos, 2001; Corino et al., 2002).

Utilizing byproducts generated through the production of animals, such as fat, has a major importance in livestock production. This not only affects livestock producers, but also the meat industry. Fat generated from the poultry industry is not consumed by humans and has few uses. Incorporation of poultry fat into swine diets could lead to many benefits to the entire livestock and meat industry. Poultry fat can be incorporated into feed diets and extensive research has been done in the past on its effects on performance and meat quality when incorporated into poultry and swine diets (Edwards, 1971).

Poultry fat is a readily available fat source for livestock diets, especially in poultry production and processing locations (Seerley et al., 1978). Incorporation of poultry fat has little to no effect on carcass traits, but does alter the FA composition by increasing the ALA concentration (Seerley et al., 1978). In addition, Engel et al. (2001) found that improving the rate and efficiency of gain of swine can be achieved by incorporating poultry fat into the diet. The rate of poultry fat inclusion into swine diets should be limited because research has shown unacceptably soft bellies in swine carcasses (Cannon et al., 1996). Inclusion of dietary lipids containing more than 15% ALA results in meat product with undesirable soft fat, as well as reduced shelf-life, both of which is a result of increased PUFA (Wood et al., 1984). In addition, increased UFA content in poultry fat has been linked to a reduction in pork quality as it can potentially reduce belly firmness, decrease lipid stability, and develop off-flavors (Miller et al., 1993). As the UFA content increases in the diet and tissue, the melting point of the fat in the product decreases. The decline in melting point is a result of increased UFA leading to a reduction in belly firmness and an oilier pork product (Miller et al., 1993).

Dietary Lipid Supplementation

Dietary Lipids and (de novo) Lipogenesis

Excess carbohydrates consumed in the swine diet are converted into lipids for energy storage. This process is known as de novo lipogenesis, or the synthesis of FA endogenously. Lipids are much more energy-dense and are a more efficient form of storage than carbohydrates, providing over twice the amount of energy than carbohydrates (9 vs. 4 kilocalories/g). Fatty acids produced via the metabolic pathway of de novo lipogenesis as well as FA consumed in the diet can be a source for triglyceride synthesis.

Various researchers found reduction of de novo lipogenesis in pigs is possible though dietary lipid supplementation (Allee et al., 1971a, b, c; Chillard, 1993, Smith et al., 1996, Azain, 2001). Supplementation of a swine diet with increased amounts of dietary lipids has the potential to reduce de novo lipogenesis from carbohydrates, resulting in the direct deposition of dietary fat into the body tissue. According to two studies (Jakobsen and Thorbek, 1993; Bee et al., 2002), these results are strictly dependent on the dietary energy status of the animal. When a diet is formulated to provide an adequate amount of energy from sources other than lipid, the dietary fat being consumed would not be utilized as a source of energy, rather the dietary lipids would be directly deposited as body fat both subcutaneous and as IMF.

Dietary Lipids and Omega-3 Fatty Acid Supplementation

Omega-3 FA content in pork has the potential to be increased through direct dietary lipid deposition. Since lipids consumed in the diet can be deposited directly into tissue with minimal alteration, increasing the amount of ω-3 FA in a diet will have a direct effect on the amount of ω-3 FA in pork products. More than 60% of the change in FA composition of porcine adipose

tissue is associated with altering the dietary lipid concentration or source within the first 25 days on feed (Koch et al., 1968; Wood et al., 1994; Wiseman and Agunbiade, 1998).

Intramuscular fat is comprised of FA present in intramuscular adipose tissue and in muscle fibers (Raes et al., 2003). Intramuscular adipose tissue is made-up of isolated or clustered fat cells that lie along the fibers and in the interfascicular area containing mainly triacylglycerolds, while lipids in the fibers are cytosolic droplets of triacyglycerolds, phospholipids, and cholesterol (Raes et al., 2003). The phosopholipid content is relatively constant in muscle tissue and minimally influenced by breed, sex, nutrition, and age. The phospholipid content does depend on the metabolic fiber type of the muscle (Raes et al., 2003). The increased content of mitochondria in more oxidative muscles results in a higher proportion of phospholipids, which are characterized by their high PUFA content (20 to 50% of FA in phospholipids) (Raes et al., 2003). The triacylglycerol content varies in fresh tissue (0.2 to 5 g/100g) and is dependent on the fat level, breed, and muscle location (Sinclair and O'Dea, 1990).

The largest portion of triacylglycerol FA consists of SFA and monounsaturated FA (MUFA) with PUFA (mostly LA, and ALA) making-up between 2 and 30 g/100 g of the total FA content (Raes et al., 2003). Influenced by species, the intramuscular FA composition of monogastrics, specifically the triacylglycerols (7 to 15% PUFA in swine) are a reflection of dietary FA, while the phospholipid composition is less influenced by the diet because they are constituent of cell membranes (Raes et al., 2003).

Membrane properties and other physiological functions would be altered if major changes occurred in the FA profile of cellular membranes (Raes et al., 2003). The PUFA proportion of phospholipids is strictly controlled by a complex enzymatic system which consists of desaturases and elongases. These enzymes function in the conversion of both LA and ALA to

their long chain metabolites; acting on both ω-6 and ω-3 FAs but have preference on ω-3 FA (Brenner, 1989; Raes et al., 2003). Competition is seen for incorporation into phospholipids between the ω-6 and ω-3 FAs leading to some variation in the content of each FA in the phospholipids (Raes et al., 2003). Ultimately, the dietary FA content has control over the ratio of ω-3/ω-6 FA with limited influence from species (Raes et al., 2003).

Meat Quality Overview

Traditionally, nutrition of grower-finishing diet formulations has primarily focused on meeting dietary requirements of the animal for energy and protein to maximize growth performance and carcass lean content, taking caution to avoid any deficiencies (Ellis and McKeith, 2002). Focus has shifted to improving pork quality and improvement of attributes such as muscle color, water-holding capacity (WHC), and pork palatability (Ellis and McKeith, 2002). Palatability, or eating-quality as described by Bonneau and Lebret (2010), is the sensory attributes of pork flavor, tenderness and juiciness, or the main physical and biochemical parameters associated with pH, shear force, WHC, IMF, and oxidative stability. According to Enfalt et al. (1997) consumer acceptability of pork is first based on tenderness, followed by flavor intensity, and level of juiciness. Additionally, meat color is important to consumers. Norman et al. (2003) found consumer acceptability was greater for dark colored pork chops.

Consumers perceive meat quality differently at purchase verse after consumption. This may be related to various physiological product characteristics (Bredahl et al., 1998). The expectation of quality is formed at the point of purchase, and is based on quality ques derived in the store, which includes intrinsic quality ques (physical characteristics of the product) and extrinsic quality ques (brand name, price, distribution outlet, etc.) (Bredahl et al., 1998). Primary contributors to overall quality are oxidative stability, WHC, pH, color, and sensory attributes.

Lipid Oxidation and its Effect on Meat Quality

Inclusion of ω-3 FA, specifically PUFA, into the swine diet results in reduced oxidative stability of pork products. Many factors contribute to the susceptibility of muscle tissue to lipid oxidation. Most importantly is the level of PUFA present in a particular muscle system (Allen and Foegeding, 1981). The process of lipid oxidation occurs when UFA react with molecular oxygen via free radical chain mechanism and form fatty acyl hydroperoxides (peroxides), which are the primary products of oxidation (Gray, 1978). This is followed by secondary reactions that degrade lipids and leads to an increased oxidative rancidity. Lipid oxidation occurs at the membrane level in the intracellular phospholipid fractions of the muscle tissue (Buckley et at. 1995). The subcellular membranes of the mitochondria and microsomes contain phospholipids, which are high in PUFA (Gray and Person, 1987), and the vulnerability of membranes to peroxidation is increased due to the close proximity of a range of prooxidants (Buckley et al. 1995). With any meat product, one of the major causes of quality deterioration during storage is lipid oxidation. The ability to delay lipid oxidation would aid in increasing color stability, shelf life, and positively effecting sensory attributes (Ellis and McKeith, 2002).

Lipid oxidation is initiated after a free lipid radical is formed after a labile hydrogen atom is removed from the fatty acyl chain. The free lipid radical will react with oxygen to form a peroxyradical. The peroxyradical will then obtain another hydrogen from a different hydrocarbon chain and form a hydroperoxide along with a new free radical. The newly produced free radical will initiate the unstoppable chain reaction (Pearson et al. 1977; Enser, 1987). The lipid hydroperoxides produced will undergo homolysis to form hydroxyl and alkoxy radicals. Cleavage via beta-scission of the FA chain adjacent to the alkoxy radical will produce low molecular weight volatile compounds. These compounds are known to have distinct aromas and

can affect flavor profiles of meat products. These include mixtures of aldehydes, ketones, alcohols, hydrocarbons, esters, furans, and lactones (Frankel, 1984). Secondary reactions and further oxidation of the initial peroxides can also lead to flavor deterioration and a negative eating experience. The autoxidation of UFA present in tissue, such as oleic, linoleic, linolenic and arachidonic, will also produce hydroperoxides which undergo a variety of decomposition pathways and produce volatile compounds (Mottram, 1987).

Immediately after slaughter, it is believed autocatalytic peroxidation begins and the changes associated with post-slaughter metabolism and aging provides favorable conditions where the process of lipid oxidation is no longer as tightly controlled. This results in the balance of prooxidative factors/antioxidative capacity favoring oxidation (Buckley et al., 1995). The transition from muscle to meat is achieved by the cessation of blood flow and the start and finish of many metabolic processes (Buckley et al., 1995). Immediately after slaughter, orderly metabolic activities continue but after blood flow has stopped, the products of glycogen break down into lactic acid. Lactic acid settles in the tissue and will gradually lower the pH from neutrality to approximately pH 5.5 (Buckley et al., 1995). It is hypothesized that antioxidant defensive systems (superoxide dismutase, glutathione peroxide, ceruloplasmin, and transferrin) present in the muscle of the live animal stop working due to changes in metabolites and physical properties. The antioxidant defense system may also be weakened by dietary deficiencies in retinol, vitamins C and E, carotenoids, and additional trace elements (Buckley et al., 1995).

Preslaughter effects of stress, events during early postmortem, such as the rate of reduction in pH, ultimate pH, carcass temperature, cold shortening, and additional harvest techniques, like electrical stimulation, may affect the rate and extent of lipid oxidation (Buckley

et al., 1995). This instigates the interactions of prooxidants with UFA and results in the generation of free radicals and propagation of the oxidative reaction (Asghar et al., 1988).

The rate and extent of lipid oxidation has a direct effect on meat quality (Wood and Enser, 1997). Oxidation manifests as a conversion of red muscle pigment myoglobin to brown metmyoglobin. Thus, development of rancid odors and flavors is due to the degradation of PUFA in the tissue membranes (Wood and Enser, 1997). Besides negative effects lipid oxidation has on meat color, lipid oxidation can result in the reduction of fluidity of biomembranes (Dobretsov et al., 1977) as well as the disruption of normal membrane structure and function (Slater et al., 1987). As membranes deteriorate, they can no longer function as a semipermeable barrier, thus contributing to exudative loss from meat (Asghar et al., 1991a; Stanley, 1991). Lipid oxidation causes meat products to experience a decrease in WHC and water binding capacity (WBC), therefore negatively affecting meat quality attributes such as drip loss, purge loss, cook loss, and ability to uptake marinades (Asghar et al., 1991a; Stanley, 1991).

Inhibitions of lipid oxidation

Many things have been shown to inhibit lipid oxidation, including nitrate (Morrissey and Tichivangana, 1985), metal-chelating agents (Sato and Hegarty, 1971) and synthetic antioxidants (Crackel et al., 1988). Consumers have developed a resistance to the use of synthetic antioxidants in food. Interest in using naturally occurring antioxidants, such as vitamin E, ascorbic acid, and glutathione have garnered interest in the food processing industry (Buckley et al., 1995). Vitamin E is an effective way to reduce lipid oxidation. Vitamin E is a lipid-soluble antioxidant that breaks the chain of lipid peroxidation in cell membranes and prevents the formation of lipid hydroperoxides (Halliwell, 1987; Davies et al., 1988). Commonly, vitamin E is incorporated into a diet as α-tocopherol acetate. The antioxidant properties of vitamin E do not

function until it is de-esterified in the gastrointestinal tract (Buckley et al., 1995). Concentration of α-tocopherol in animal tissues has been shown to be directly related to the concentration of vitamin E in the diet (Monahan et al., 1993 a, b). Additionally, the rate and extent of lipid oxidation is directly related to the concentration of α-tocopherol in the tissues (Buckley et al., 1995). Inclusion of up to 200mg/kg of α-tocopherol in swine diets significantly improves lipid stability, and reduces the rate and severity of lipid oxidation in meat products (Monahan et al., 1990a, b).

Meat Color

Visual appearance of a meat product will directly affect consumers' perception of quality and will ultimately determine their decision to purchase a product. Specifically, surface discoloration as a result of lipid oxidation may indicate a lack of product freshness (Smith et al., 1993; Cannon et al., 1995b). Meat color is directly affected by the amount and chemical state of the pigment myoglobin. Oxidation of myoglobin leads to the development of metmyoglobin, and the meat develops an unattractive brown color from the formation of metmyoglobin (Faustman and Cassens, 1990). The rate of discoloration is related to the effectiveness of the oxidation processes and the enzyme reducing system in controlling metmyoglobin levels (Faustman et al., 1989a,b). Incorporation of antioxidants, such as vitamin E, have been shown to effectively control lipid oxidation and the accumulation of metmyoglobin (Liu et al., 1995). According to Monahan et al. (1993a,b), α-tocopherol works as an antioxidant to scavenge free radicals species which are involved in the initiation and propagation of lipid oxidation. The location of Vitamin E within the phospholipid bilayer of cell membranes provides vitamin E with the means of controlling lipid oxidation at a likely initiation site (Hafeman and Hoekstra, 1977). Inclusion of vitamin E at 200 mg/kg in finishing diets has been shown to reduce drip loss, lipid oxidation

(Thiobarbituric acid reactive substances (TBARS) values), increase color stability, and increase final live weight (Onibi et al., 1998; Asghar et al., 1991b; Monahan et al., 1992a,b,c).

Vitamin E improves meat color, even with extended storage times across all species. (Cannon et al., 1995b). Increased color stability, specifically an increase in a* values, or surface redness, was observed in pork chops from pigs fed a supplemental level of α-tocopherol acetate (200 mg/kg of feed) as compared to pigs fed a basal (10 mg/kg) or intermediate concentration (100 mg/kg) after 3 and 6 days of refrigerated storage (Asghar et al., 1991a). In addition, greater a* values in refrigerated pork chops were found as a result of high vitamin E supplementation (200 mg/kg of feed) in pigs as compared to basal supplementation (10 mg/kg) after 2, 4, 6, and 8 days of refrigerated storage (Monahan et al., 1992a). Thiobarbituric acid reactive substances values were significantly influenced by dietary α-tocopherol acetate levels where an increased vitamin E concentration resulted in a reduction in TBARS values, indicating a reduction in lipid oxidation (Monahan et al., 1992a). The increase in color stability of pork products from supplemented pigs was attributed to the reduction in the rate of metmyoglobin formation (Monahan et al., 1992a) while pork color improvements are directly related to an increased concentration of α-tocopherol in the tissue (Asghar et al., 1991a).

Water Holding Capacity and pH

Water holding capacity is defined as the ability of muscle to hold water inherently associated with post-rigor muscle while WBC is the ability of the muscle proteins to hold on to added water from external sources (NPPC, 2000). Both WHC and WBC have an impact on meat quality as they affect many other qualities and economic traits. An inadequate WHC and WBC will result in excess loss of weight of the product during storage, transport, and processing. In addition, water-soluble nutrients will be lost, palatability decreased due to reduced juiciness,

inefficiencies in processing technologies, and excess purge will cause a diminished appearance of packaged products (NPPC, 2000). There are many physical properties partially dependent on WHC including color, texture, and firmness of raw meat, and juiciness and tenderness of cooked meat (Aberle et al., 2001).

A small portion (0.8 to 2.0%) of intramuscular water content is tightly bound to molecules and an additional 4 to 12% of water is bound electrostatically (or immobilized water). The amount of water held electrostatically is dependent on changes within the proteins, and thus on pH (NPPC, 2000). The remaining 60 to 70% of water is considered free water and is not bound to anything. Capillary forces that result from a three-dimensional network of myofilaments and structural proteins hold free water in meat. This is dependent on the space between the filaments (NPPC, 2000). Myofibrillar shrinkage, or a reduction in filament spacing has a direct effect on the volume of water held within the meat. Myofibrillar shrinkage would cause water to be voided from the muscle due to the reduction in space between filaments (NPPC, 2000). Two factors would influence myofibrillar shrinkage, ultimate pH and denaturation of muscle proteins.

A primary aspect in the conversion of muscle to meat is the metabolism of intramuscular glycogen energy stores, which plays a major role in the expression of different quality attributes of fresh pork (NPPC, 2000). Several factors can affect postmortem metabolism: 1) genetic predisposition, 2) elevated metabolism or increased excitability (Grandin, 1994), 3) pre-slaughter stress, and 4) a combination of all of these (NPPC, 2000). Normal pH of a living animal is 7.4, and after harvest in normal conditions, pH will gradually decline over 6 to 8 hours to an ultimate pH. This pH decline is due to accumulation of lactic acid from glycogen breakdown (Aberle et al., 2001). Two extremes can result from postmortem metabolism: a low ultimate pH 5.2 to 5.4

(PSE; pale soft and exudative) or a high ultimate pH greater than 6.0 (DFD; dark firm and dry) (Aberle et al., 2001).

PSE is a condition resulting from rapid breakdown of glycogen into lactic acid early in the postmortem period generally within the 1st hour and while the carcass is still hot and adversely affects meat quality. The low pH causes sarcoplasmic and myofibrillar proteins to denature and shrinking of the myosin filaments occurs, ultimately reducing filament spacing and decreasing WHC (Aberle et al., 2001; NPPC, 2000). The decrease in pH and denaturation of proteins causes the lean tissue to be pale in appearance (Kauffman and Marsh, 1987). In addition to color, ultimate pH also has a direct effect on firmness of meat (NPPC, 2000). The major contractile proteins associated with the formation of the protein lattice are myosin and actin. Myosin binds with actin during contraction resulting in a permanent rigor bond in meat. When myosin is denatured due to low pH, the degree of denaturation will affect both drip loss and softness associated with PSE meat (NPPC, 2000).

An increase in WHC is seen in the DFD condition. DFD occurs when the ultimate pH of the carcass is greater than 6.0. As a result of the negative charge of protein molecules at a pH greater than 5.1, there will be a repulsion between protein molecules, thus increasing the filament space, increasing WHC (NPPC, 2000).

Sensory Attributes

Utilization of a trained sensory panel for the evaluation of meat quality can aid in the determination of consumer acceptability of a product. Typically, trained panelists evaluate a product for tenderness, juiciness, flavor intensity, and the presence of off-flavors. Intramuscular fat content can influence flavor and juiciness perception (Fernandez et al., 1999a,b). Additionally, FA composition of IMF will directly affect the flavor of a pork product. Inclusion

of high levels of dietary lipids, especially high concentrations of PUFA, into the diet will negatively affect meat quality by increasing the susceptibility of the PUFA to lipid oxidation. This increase can contribute to rancidity, off-flavor development, and warmed-over flavor (Gray et al., 1996).

Previous researchers have found varying results on the effects of dietary lipid supplementation and the ability of off-flavor detection using trained sensory panels (Corino et al., 2002; Shackelford et al., 1990; Myer et al., 1992; Miller et al., 1990; Skelly et al., 1975; St John et al., 1987; Van Oeckel et al., 1996). Shackelford et al. (1990) found diets supplemented with 10% rapeseed oil increased the LA concentration of muscle lipid to 3% and increased the incidence of off-flavor detection by panelists. Myer et al. (1992) found similar results in diets supplemented with 12% rapeseed oil. Miller et al. (1990) found overall palatability and flavor scores were reduced in diets supplemented with 10% corn oil. These results vary from Skelley et al. (1975) who reported no differences in sensory characteristics of pork chops from pigs supplemented with soybean meal or roasted soybeans (14 to 30%) were detected. St John et al. (1987) also found no differences in sensory characteristics of meat with 20% canola added to the grower-finishing diet. Intramuscular fat from loins enriched in ALA though dietary supplementation of flaxseed (2.9% ALA and 15% PUFA) had no effect on sensory attributes (Van Oeckel et al., 1996). Differences among studies may be the result of the level of fat supplementation or age and weight of the animal at harvest. An increased harvest weight resulted in increased fat content of the carcass (Pantaleo et al., 2000) which may reduce the influence of dietary fat on muscle lipid composition and effects on sensory characteristics (Corino et al., 2002).

Flaxseed Oil

Overview of Flaxseed

Flax, more commonly known as linseed, is a crop grown in colder regions of the world. Flax, *Linum usitatissimum*, is a member of the genus *Linum* and the family *Linaceae*. Flax is an annual plant that grows to 1.2 m in height and is a tall, slender stemmed plant with slim leaves and blooms blue flowers. The plant produces a fruit in the form of a dry round capsule, 5 to 9 mm in diameter, which contains several glossy yellow or brown seeds. Flax is a food source as well as a fiber crop. It is typically made into textiles and is further processed into linens. The seeds can be harvested for consumption as a whole seed, ground or oil. In addition to dietary supplementation, the oil can be extracted for alternative uses such as a natural supplement as well as its use in an industrial setting (Newkirk, 2015).

Flaxseed in Swine Diets

Flaxseed is incorporated into livestock diets either as whole seed, meal, or flaxseed oil (Newkirk, 2015). Flaxseed contains 42 to 46% fat, 28% dietary fiber, 21% protein, 4% ash, and 6% carbohydrates (Newkirk, 2015). Each form contains a high concentration (20%; DM basis) of ALA (Maddock et al., 2005). Alpha-linolenic acid is an essential ω-3 FA, as well as a precursor for EPA. Eicosapentaenoic acid is also a precursor for the formation of eicosanoids, a hormone-like compound which plays a large role in immune response (Maddock et al. 2005). Additionally, ALA can be further converted into DHA, which assists in controlling cardiovascular disease (Romans et al. 1995a; Goodnight, 1993). DHA also aids in maintaining normal brain growth and development.

Flaxseed has a large dry matter fiber content of 28% (Newkirk, 2015). When consumed as whole flaxseed or as flaxseed meal it contains 1.9 g fiber/T. The risk of heart disease is reduced as a result of the reduction of low-density lipoprotein (LDL) and total cholesterol levels.

Flaxseed contains 85.5 mg/ounce of lignan. Lignan, is a phytochemical, specifically a phytoestrogen which aids in balancing hormones and has a chemical structure similar to that of human estrogen (Newkirk, 2015). Flaxseed contains the highest lignan content as compared to any other plant-based source, proving up to 800 times more (Thompson, 1995). The main lignan in flaxseed is seicoisolariciresinol diglucoside (SDG) which is converted to enterodiol and enterolactone in the colon (Tham et al., 1998). These all have the potential to reduce the risk of heart disease and can reduce the risk of certain types of cancers and osteoporosis (Newkirk, 2015; Tham et al., 1998).

Whole flaxseed or flaxseed meal is more commonly utilized in swine diets rather than flaxseed oil due to its natural antioxidant content. However, to include the whole seed in the diet, it must first undergo treatment to breakdown the protective coating on the seed. Destruction of this coating allows for penetration of the seed by the digestive enzymes for digestion and absorption (Raes et al., 2003). Typically, if fed as a whole seed, an additional treatment process such as crushing, bruising, extrusion or expansion is performed prior to feeding (Raes et al., 2003).

An anti-nutritional factor is present in flaxseed. Anti-nutritional factors are compounds that hamper digestion, absorption, or utilization of nutrients. Anti-nutritional compounds present in flaxseed are linamarin, neolinustatin and linustatin, which are classified as cyanogenic glycosides. These factors reduce the inclusion rate of flaxseed in a diet, especially if not pretreated (Newkirk, 2015; Raes et al., 2003). Anti-nutritional compounds are degraded by ß-

glucosidase in the large intestine but will result in the release of hydrogen cyanide. This is a powerful respiratory inhibitor when absorbed in large quantities (Newkirk, 2015). Negative effects of hydrogen cyanide can be eliminated when the seed undergoes heat treatment during oil extraction. This extraction process will denature the ß-glucosidase, which prevents formation of hydrogen cyanide (Shen et al., 2005; Newkirk, 2015). The concentration of hydrogen cyanide in flaxseed is the highest in immature seed. When immature seeds are fed, they can have a negative effect on animal performance (Newkirk, 2015). A mature flaxseed can be fed without additional treatment and with little or no observed impact of liamarin (Newkirk, 2015).

Flaxseed Oil

Flaxseed oil can be derived through various processes dependent on the final use of the oil. Most of the oil is utilized in industrial products due to the use of solvent extraction (Newkirk, 2015). Oil that is extracted without solvents can be intended for human and livestock consumption. Once oil is extracted from the seed, the residual is considered flaxseed meal and is generally included into livestock diets because of its high protein value (Newkirk, 2015).

There are two main methods for oil extraction: 1) prepress solvent extraction and 2) expeller press extraction. Prepress is the most commonly utilized method in industry, where there is a combination of a mechanical action/pressure and a chemical extraction agent. This proves to be the most effective method to obtain the oil. The steps of prepress oil extractions are as follows as described by Newkirk (2015) in the Flax Feed Industry Guide. First the seed is cleaned and preconditioned, undergoes a flaking process, followed by a cooking process, expelling, solvent extraction, desolventization, cooling and then the discharging of the remaining flaxseed meal. After cleaning, the seeds are warmed to prevent shattering during the flaking process. The flaking process is where the seed is passed between two rollers with a small gap

between them. The applied pressure will rupture the cell wall of the seed, and shapes it into a very thin flake. Formation into a thin flake will increase the surface area of the seed and volume of oil extracted. The seed is then cooked to reduce the viscosity of the oil, allowing the oil to leave the storage bodies of the seed.

The cooking process consists of placing the seeds onto a series of heated plates. Following cooking, the seeds are processed; by placing the heated, flaked seeds into a mechanical expeller press. The mechanical press is made up of a large metal screw system. The seed is passed through, forcing it against the wall of the system pushing the oil out of the seed. The oil is then passed through the wall of the press while the seed and remaining large particles are left behind and collected. The use of a mechanical press will remove about half of the oil from the seeds. The cake or residual seeds and particles are collected at the base of the press for solvent extraction and is transferred to the solvent extractor. Typically, hexane is the solvent of choice, and is flushed through the cake. Hexane solubilizes the oil and removes it from the flaxseed meal cake. Once the oil is removed, the cake undergoes further processing to remove the residual hexane via evaporation. Both the oil and cake are cooled and processed for other purposes including utilization in livestock feed. During the evaporation process, the residual hexane is collected in its vapor form. The vapor and the hexane that was used in the initial oil removal step both contain oil. They both will undergo further treatment to remove the residual hexane. Once the hexane is removed, the flaxseed oil is then sent into further refining.

Prepress solvent extraction is the most efficient way to remove oil from flaxseed but other methods are available. Mechanical expeller extraction is another method that is used, although it is very expensive to build, maintain, and operate an appropriate facility, it is still a very common method of practice. Flaxseed oil that is derived from mechanical expeller

extraction is generally referred to as cold pressed oil, simply stating that the oil never underwent a solvent extraction method. Oil obtained via mechanical expeller extraction is extracted solely by force. Similar to the method for prepress solvent extraction, mechanical extraction utilizes the same high pressure expeller press but utilizes a two-stage press system. This method relies solely on the high pressure and force to remove as much oil as possible. Unfortunately, this method leaves up to 5% of the oil in the residual meal (Newkirk, 2015). The meal from this extraction method is highly desired by livestock producers due to its increased oil content (Newkirk, 2015).

Flaxseed Effects on Carcass Composition and Meat Quality

Studies with flaxseed have reported varying results with regards to animal performance, carcass composition, meat quality, and FA content of pork products (Burdge and Wootton, 2002; Harper et al., 2006; Ellis and McKeith, 2002; Romans et al., 1995 a,b; Matthews et al., 2000). These studies evaluated flaxseed in both swine starter and grower-finishing diets and in addition, examined the effects of feeding duration. Flaxseed meal can be included into swine starter diets up to 3% without adverse effects on growth or feed intake (Newkirk, 2015). Jansman et al. (2007) reported that inclusion of 8.5% expeller meal or 12.5% whole flaxseed into the starter diet of newly weaned pigs caused a depression in weight gain. Newkirk (2015) reported flaxseed oil can be utilized in all diets, including starter diets, without negatively affecting performance. Additionally, supplementation of starter diets with ω-3 FA may improve health status of animals due to ω-3 FA effects on immune system via regulation of eicosanoids (Turek et al., 1996).

According to Newkirk (2015) whole flaxseed and flaxseed meal can be included up to 10% in diets for grower and finishing pigs without negative effects on performance. Early studies indicated whole flaxseed and flaxseed meal could comprise 25% of the diet without affecting animal performance. Matthews et al. (2000) found flaxseed could be included at 5 or

10% in 30 kg grower diets without affecting production performance. Other studies have examined the impact of feeding a 50/50 flaxseed/pea mixture on animal performance. A reduction in animal performance was observed when 50/50 flaxseed/pea was included at 30% of the grower diet but, at 22.5% inclusion, there was no effect on animal performance (Thacker et al., 2004). When evaluated in a finishing diet, Thacker et al. (2004) reported inclusion up to 18% flaxseed/pea resulted in equal performance, but when inclusion reached 24% there was a decrease in weight gain as compared to a traditional diet.

Feeding 0, 5, 10, and 15% ground flaxseed for 25 days prior to harvest had no effect on production or carcass traits (Romans et al., 1995a). Additionally, there were no pork processing problems noted due to lack of muscle and belly firmness (Romans et al., 1995a). Following the initial study, Romans et al. (1995b) found feeding 15% flaxseed for 28 days prior to harvest had no impact on animal performance. Bellies samples from pigs fed a 15% flaxseed diet were able to be identified by trained sensory panelists when compared to untreated control samples during a triangle test. Panelists identified the 15% flaxseed diet bellies based upon the presence of an off-flavor. However, identical loin samples from this study were not identified correctly by the same panelists (Romans et al., 1995a). The ability of panelists to identify the 15% flaxseed treatment was a result of the increased PUFA content in the bellies. Bellies contained a greater lipid content than loins. Additionally, the 15% inclusion of flaxseed did result in a larger ALA and EPA concentration compared to the control samples (Romans et al., 1995a).

Swine, as monogastrics, are able to absorb ALA found in diets more readily since ALA is not biohydrogenated prior to entering the small intestine (Maddock et al., 2005). Fatty acid profile of lean and fat is directly affected by the source of fat in the diet of monogastric species. Thus, feeding flaxseed, meal, or oil can alter the FA profile of the pork product and alter the ratio

of ω-6 to ω-3 FA (Newkirk, 2015). Variation in absorption and response to whole flaxseed and flaxseed oil has been observed (Raes et al., 2003). The main lipid source in flaxseed oil is triacylglycerols, and these are well digested by mammals (Nelson and Ackman, 1988). The complex structure of flaxseed and the location of the oil makes accessibility by digestion enzymes difficult (Raes et al., 2003).

As the concentration of ground flaxseed fed increased (0, 5, 10, and 15%) there was a significant increase in the amount ALA and EPA in both layers of backfat, kidney (leaf) fat, liver, belly, and in longissimus muscle (Romans et al., 1995a). ALA concentration in the inner backfat layer increased from 10 to 23, 37 and 53 mg/g, respectively and EPA increased from 0.09 to 0.20, 0.28 and 0.38 mg/g, respectively. These results indicate feeding flaxseed during the finishing phase can increase the ω-3 content pork products without compromising performance traits (Romans et al., 1995a).

Fontantillas et al. (1998) determined feeding flaxseed oil at 4% for 60 days increased ALA in IMF from 1.14% to 4.94, 7.40, and 7.89% after 0, 17, 31, and 60 days of feeding. Of the maximum ALA enrichment, 70%, was achieved after feeding 30 days while 95% was achieved if fed 60 days (Fontanillas et al., 1998). Nuernberg et al. (2005) reported incorporation of 5% flaxseed oil during the grower-finishing period did not affect carcass composition or meat quality. Feeding flaxseed oil increased the relative content of ALA and long chain ω-3 FA in lipids of muscle, backfat and heart at the expense of arachidonic acid (Nuernberg et al., 2005). Alternatively, D'Arrigo et al. (2002a,b) reported the inclusion of 5% flaxseed oil did negatively influence overall flavor of combined meat and fat samples but did observe an increase in the ω-3 FA and a decrease in ω-6 FA content. In contrast to other studies, Rey et al. (2001) included

0.5% flaxseed oil and 1.5% olive oil or sunflower oil in the diet and fed it for 42 days. This diet increased the ω-3 FA content as well as DHA content within muscle compared to control.

Flaxseed Oil and Meat Quality

In regards to eating experience, flaxseed oil has been included in the diet up 5% without development of off-flavors or a negative impact on sensory attributes (Nuernberg et al., 2005). Other researchers have reported pork eating quality deteriorates after 2.5% inclusion of flaxseed oil (Kratz et al., 2000). Differing results between these studies could be attributed to a difference in duration of feeding, genetic influence, or the use of vacuumed sealed frozen product vs product contained in an oxygen permeable bag (Nuernberg et al., 2005). Increased rancidity due to longer storage time and oxygen exposure may increase the development of off flavor in samples with a greater PUFA content (Nuernberg et al., 2005). In a recent study, flaxseed oil inclusion at 3% in combination with 2% poultry fat demonstrated a slight increase in off-flavor as compared to control samples (Adhikari et al., 2017).

Inclusion of flaxseed oil at 3% (Adhikari et al., 2017; D'Arrigo et al., 2002a, b), and 5% (Nguyen et al., 2004; Nuernberg et al., 2005) successfully increased the PUFA content in backfat and increased the healthy FA profile to a desired level. Supplementation of flaxseed oil at 3 and 5% reduced the firmness of the product, which negatively affected the efficiency of processing and reduced belly quality (Nuernberg et al., 2005; D'Arrigo et al., 2002a,b). Adhikari et al. (2017) also observed the reduction in belly firmness with 3% flaxseed oil in combination with 2% poultry fat.

An increase in lipid oxidation was observed in samples from pigs fed 5% flaxseed oil as compared to control samples from pigs fed 5% olive oil (Nuernberg et al., 2005). Evaluation of 5 dietary treatments containing either flaxseed oil at 30g/kg, a combination of 15g/kg flaxseed oil

plus 15g/kg olive oil, each containing 20 mg/kg or 200mg/kg dietary α-tocopherol acetate, in addition to a control (sunflower oil 30 g/kg plus 20 mg/kg α-tocopherol acetate) was conducted by Hoz et al. (2003). Inclusion of flaxseed oil increased oxidative rancidity as compared to the combination flaxseed/olive oil and control diet. Inclusion of 200 mg/kg α-tocopherol acetate markedly reduced tenderloin fat oxidation as compared to respective diets containing 20 mg/kg α-tocopherol acetate. As dietary vitamin E concentration increased, the vitamin E concentration in lean tissue increased. The increase results in lower induced peroxidation rate of lean and fat (Hoz et al., 2003). Peroxidation rates of diets containing flaxseed and olive oil were one-third lower while flaxseed only diets were one-fifth lower at the increased α-tocopherol acetate level as compared to controls supplemented with a basal concentration (Hoz et al., 2003).

Poultry Fat

Overview of Poultry Fat

Poultry fat, an abundant by-product in certain regions of the U.S., is an inexpensive source of fat for supplementation in swine diets (Engel et al., 2001). Poultry fat has typically been utilized and researched as a component in poultry diets (Edwards, 1971). Specifically, poultry fat is used for its potential to increase ω-3 FA content within tissue (Seerley et al., 1978), increase palatability, and ability to improve ease and efficiency of feed pelleting. Poultry fat is also a highly available dietary energy source. Addition of 5 or 10% poultry fat to swine diets can increase gain/feed and decrease average daily feed intake (ADFI) without affecting carcass characteristics (Williams et al., 1994; Engel et al., 2001). Varying results were reported by Woodworth et al. (1999) who found 6% poultry fat inclusion increased gain/feed ration, and decreased ADFI but negatively affected carcass composition and meat quality.

Poultry Fat and Meat Quality

No differences were observed with the inclusion of 2, 4, or 6% poultry fat in swine diets on ADG, dressing percentage, leaf fat weight, longissimus muscle (LM) pH, back fat, LEA, percentage lean, LM visual evaluation, LM WHC, Warner-Bratzler Shear Force (WBS), sensory evaluation of the LM, and bacon fat color and firmness measurements or bacon processing characteristics (Engel et al., 2001). Addition of poultry fat did improve gain/feed ratio and positively altered the FA profiles of LM and bacon. Inclusion of 6% poultry fat in the diet had little effect on quality of pork LM, belly or bacon (Engel et al., 2001). Alternatively, Woodworth et al. (1999) reported differing results for inclusion of poultry fat at 6%. The Woodworth et al. (1999) study reported an increase in gain/feed ratio but a decreased ADFI, carcass leanness, and a reduction in belly quality. Increased lipid content has the potential to increase carcass fat (Seerley et al., 1978). Lipid inclusion at a 5% level in the diet did produce a significant increase for average backfat thickness, and first rib backfat thickness. Dietary fat at 0, 2.5, and 5% did not influence the majority of carcass trait other than backfat thickness (Seerley et al., 1978).

Vitamin E

Overview of Vitamin E

Inadequate color and WHC are two major concerns of pork marketing. Supplementation of swine diets with vitamin E during the growing and finishing periods may have the potential to improve pork quality overall (Cannon et al., 1995a). Inclusion of increased dietary lipids in swine diets, specifically with high concentrations of UFA, such as flaxseed, may have adverse effects on meat quality (Gatlin et al., 2002). Additional PUFA are more susceptible to oxidation, leading to the development of off-flavors, loss of color, and nutritional values (Pearson et al., 1983). Nutritive value of pork can decline as water-soluble vitamins are purged out of the tissue

as a result of increased lipid oxidation, thus decreasing WHC. The inclusion of antioxidants into the diet, such as vitamin E, can potentially alleviate the possibility of greater lipid oxidation by increasing lipid stability (Pearson et al., 1977).

Vitamin E is an essential nutrient for growth and health in all species of animals (Liu et al., 1995). Vitamin E improves health and plays a diverse role in fetal death and resorption, nutritional myopathy, retinal degeneration, erythrocyte hemolysis, prostaglandin biosynthesis, and T- and B-lymphocytes responsiveness (Machlin, 1984). Vitamin E is a membrane-associated antioxidant that effectively protects vulnerable UFA in cell membranes and plasma lipoproteins from oxidizing agents, both endogenous and exogenous (McCay et al., 1971; Diplock and Lucy, 1973). Additionally, incorporation of α-tocopherol into the lipoprotein matrix of the cell membrane can help maintain cellular integrity and protect UFA from oxidation by free radicals (Tappel, 1962).

The amount and type of UFA in the tissue in addition to the relative amount of pro- and antioxidants, influences the susceptibility of meat to lipid oxidation (Monahan et al., 1993a,b; Gatellier et al., 2000). The source of dietary fat in the diet, (high corn oil, soybean oil, beef tallow, poultry fat, etc.) and the concentration of vitamin E will affect the oxidative stability of muscle and adipose tissue differently dependent on the FA composition of the dietary fat source (Guo et al., 2006).

Including vitamin E into the diet can decrease lipid oxidation, decrease drip loss, and can improve pork color (Asghar et al., 1991a; Monahan et al., 1990a,b, 1992a). In addition to improving meat quality, Asghar et al. (1991b) found supplementation with vitamin E improved ADG and feed efficiency of pigs. Buckley and Morrissey (1992) concluded the rate and extent of lipid oxidation in meat products is dependent on α-tocopherol concentration in the tissue. Dietary

supplementation of α-tocopherol acetate, up to 200 mg/kg of feed, improved the oxidative stability of both raw and cooked pork muscle during storage at 4°C for up to 8 days (Monahan et al. 1990a,b). In addition to pork muscle, oxidative stability of rendered fat was improved (Monahan et al., 1990a). Vitamin E fed at increased levels stabilized the membrane-bound lipids against metmyoglobin/H_2O_2-initiated oxidization (Monahan et al., 1990a). Asghar et al. (1991a) demonstrated in a similar study that high levels of α-tocopherol in subcellular fractions also enhanced membrane stability when exposed to metmyogobin/H_2O_2. Pork chops from pigs receiving a supplemental level of α-tocopherol acetate at 200mg/kg of feed, only had a slight increase in TBARS values when stored at 4°C under fluorescent light for up to 10 days.

Hoz et al. (2003) found the addition of 200 mg kg^{-1} α-tocopherol acetate in a diet containing 30 g kg^{-1} flaxseed oil increased the concentration of α-tocopherol in the tenderloin (close to 3 mg/kg muscle). This increase was greater than the tenderloin (less than 1.0 mg/kg muscle) from a control diet containing 30 g kg^{-1} flaxseed oil and a basal level of α-tocopherol acetate (20 mg kg^{-1}). This was in general agreement with data from other researchers (D'Arrigo et al., 2002a,b; Lopez-Bote and Rey, 2001) for muscle, liver and adipose tissue. Hoz et al. (2003) concluded as vitamin E concentration in the diet increased, the greater the tissue α-tocopherol concentration. Greater tissue α-tocopherol concentration lowers the induced peroxidation rate. Diets supplemented with 200 mg kg^{-1} α-tocopherol acetate were one-fifth lower for peroxidation than basal supplementation. This was also the case in the D'Arrigo et al. (2002a,b) study examining the effect of subcutaneous adipose tissue and liver tissue peroxidation rates. Additionally, flaxseed oil diets containing supplemental and basal concentration of vitamin E reduced ω-6 FA content with a concomitant increase in ω-3 FA concentration; markedly modifying the ω-6/ω-3 ratio with no effect on nutritional composition of the meat (Hoz et al.,

2003). Cheah et al. (1995) reported supplementation with vitamin E prevented PSE and improved tissue WHC of pork. Similar to pork color, Asghar et al. (1991a) concluded that WHC was also directly related to the amount of α-tocopherol incorporated into the cellular membranes of muscles.

Conclusion

Superior meat quality is the desired product of any livestock production system and when it comes to consumer acceptability, meat quality is most important. The successful shift in production methods to meet increased consumer demands while improving IMF content, reducing excess carcass fat, and increasing the ω-3 FA concentration of the products would substantially benefit the pork industry. The inclusion of dietary lipids, especially high PUFA, into swine diets has been shown to positively affect animal growth and performance while increasing the ω-3 FA content to improve human health. In addition, dietary lipid can increase IMF content. Flaxseed oil inclusion also has potential to improve carcass composition and improve human health from increased ω-3 FA content in pork products from supplemented pigs. However, it must be noted that lipid oxidation and presence of off-flavors increase as concentration of flaxseed oil increase. In addition, supplementation with vitamin E can increase color, extend shelf-life, increase oxidative stability, and potentially increase IMF content. Limited research has been done on the complete effects of dietary lipid and vitamin E supplementation on meat quality. Inclusion of high dietary lipids could successfully increase IMF while decreasing excess carcass fat, increase the ω-3 FA content, and work synergistically with vitamin E to improve color and oxidative stability, all while improving meat quality attributes would aid in addressing pork quality issues and contribute to sustainability.

III. Meat Quality Assessment of Pork Fed Poultry Fat, Flaxseed Oil, and Supplemented with Vitamin E

Materials and Methods

Experimental Design

The protocol for animal care, handling, and sampling procedures were approved by the Auburn University Institutional Animal Care and Use Committee. Yorkshire pigs (n=96) weighing approximately 50 kg were obtained from the Auburn University Swine Research and Education Center, Auburn, Alabama. Pigs (n=96) were allocated to pens based on weight and sex over two trials. Pigs within each trial were born in the same farrowing group and each pen was allotted two gilts or two barrows. Each pen was assigned randomly to one of 8 dietary treatments in a 4 x 2 factorial arrangement. There were three gilt pens and three barrow pens per dietary treatment. Pigs in trial 1 were born June 2-7, 2015 and placed on test August 31, 2015. Trial 2 pigs were born August 19-26, 2015 and placed on test November 9, 2015. Corn-soybean meal finisher diets (n=2; 1: 50 to 80kg, 2: 80 to 110kg; Table 1) were formulated to contain 0, 2, 4, or 6% lipids and either 11 (NRC, 2012) or 220 IU vitamin E/kg. For all diets with lipids, 1% flaxseed oil was included and the remaining lipids supplied by poultry fat (0, 1, 3, or 5%). One pig died prior to harvest from trial 2.

Harvest

Pigs were transported to the Lambert-Powell Meat Laboratory, Auburn, Alabama for harvest. Upon arrival, animals were inspected by a United States Department of Agriculture (USDA) inspector, and humanely harvested under the USDA regulations and the Humane Slaughter Act. Pigs were harvested (n=8 groups; Table 2) starting November 4, 2015 to February 10, 2016. Pigs were harvested at an average pen weight of 110 ± 3 kg.

Carcass Evaluation

Following harvest, hot carcass weights (HCW) recorded and carcasses were placed into a 0±2°C cooler. At 24 hours post mortem, the loin eye area (LEA) was exposed between the 10th and 11th thoracic vertebrae only extending 3.81cm past the ventral edge of the longissimus muscle avoiding the belly. Once exposed, the longissimus muscle was allowed to bloom for a minimum of 10 minutes before carcass data were recorded. A trained evaluator performed carcass evaluation for several characteristics to determine carcass quality and yield. Loin eye area was determined at the 10th/11th rib and measured to the nearest tenth of an inch via a plastic measuring grid. Last rib fat thickness (LRFT) and tenth rib fat thickness (TRFT) were measured utilizing a back-fat probe graduated in 1/10 inch increments. Measurements for TRFT were obtained by measuring the fat depth, including the skin, at the ¾ point over the LEA. LRFT was measured perpendicular to the last rib and measurements included the skin.

After appropriate bloom time, the exposed LEA was evaluated for objective color values (L*, a*, b*) using a Hunter Miniscan XE Plus (Model MSXP-4500C; Hunter Laboratories, Reston, VA, USA) using a D_{65} illuminant with a 10^0 observance angle and a 2.54 cm aperture. The colorimeter was calibrated with HunterLab white and black instrument working standard tiles. Color analysis was measured in duplicate on each carcass for accurate representation and

an average value of L*, a*, and b* was recorded. Ultimate pH (24 hours postmortem) of the ham (pHH) and loin (pHL) was assessed via Oakton pH Spear Waterproof Pocket pH Testr™, (OAKTON Instruments, Vernon Hills, IL, USA). Ultimate pHL was measured at the $10^{th}/11^{th}$ rib interface. Ultimate pHH was measured by inserting the probe into a 2.54 cm cut made into lean tissue of the ham. A visual evaluation for muscle score (MS) of the carcass was assigned with a value of 1 (thin), 1.5 to 2.5 (average), or 3 (thick) following the National Pork Producers Council (NPPC, Des Moines, IA, USA) guidelines. Utilizing NPPC visual reference standards, a subjective value for color (NPPCCol), 1 (very pale) to 6 (very dark), was assigned to each carcass based on the color of the lean tissue at the exposed LEA, post blooming. NPPC visual reference standards for the subjective assessment of IMF (marbling) content were used to appropriately assign a marbling score (NPPCMar) based on the amount of IMF interspersed within the lean tissue using a scale of 1 (devoid) to 10 (excessive).

Using carcass measurements, calculations for percent fat-free lean (%FFL) were calculated for each carcass following the equation established by NPPC (2000).

$$\%FFL = \frac{[(8.588 - (21.896 \text{ x TRFT, inches}) + (0.465 \text{ x HCW, lbs}) + (3.005 \text{ x LEA, inches}^2)]}{\text{HCW, lbs}} \, x \, 100$$

Sample Preparation and Packaging

After chilling for 24 hr at 0±2°C and following carcass data collection, carcasses were fabricated into wholesale cuts with loins and bellies removed. Eight 2.54 cm thick chops were fabricated from the left side loin of each carcass, labeled, and individually vacuum-sealed in a 3-layer oxygen barrier bag (Sealed Air, Cryovac, Charlotte, NC, USA) and frozen at -20±2°C for further analysis. Belly quality evaluation was performed after bellies were removed from the carcass. Belly size was evaluated for thickness and firmness; both skin-side up (SSU) and skin-side down (SSD) was measured.

The eight vacuum-sealed chops were held at -20±2°C and removed individually as needed for meat quality evaluation. The first chop was utilized for drip loss (DL), vacuum purge loss (VP), marinade uptake (MU), and marinade cook loss (MCL). The second chop was used for evaluation of cook loss (CL) and Warner-Bratzler Shear Force (WBS). Chops three and four were utilized for proximate analysis (PA), and thiobarbituric acid reactive substance assay (TBARS), respectively. The fifth and sixth chops were used for sensory evaluation. The seventh and eighth chops were designated to be an extra for additional analysis if needed.

Vacuum Purge Loss

One vacuumed-sealed, 2.54 cm chop was allowed to thaw at 4±2°C for 48 hr. prior to preparing samples for DL, MU, or MCL, VP was measured. Chops were weighed while remaining in vacuum-sealed bags on a Mettler Toledo Classic Plus balance (Mettler Toledo, PB 3002-S/FACT, Columbus, OH, USA) that had been tared to account for weight of vacuum bag and identification tag. Weights were recorded to the nearest 0.01 g. Samples were removed from bag and weighed. VP was determined by the following equation:

$$VP = \frac{[(Weight\ of\ thawed\ sample\ in\ bag,\ \ g) - (\text{Weight of thawed sample removed from bag, } \text{g})]}{(\text{Weight of thawed sample in bag, } \text{g})}\ x\ 100$$

Drip Loss

One vacuumed sealed, 2.54 cm chop was allowed to thaw at 4±2°C for 48 hr. Two 24±2 g samples were obtained from each chop, trimmed to remove any fat and connective tissue. Initial weights were obtained and recorded. Samples were suspended via a fish hook (Model number: 186F-1 Baitholder, Eagle Claw®, Denver, CO, USA), mounted from the lid of a 133 mL presterilized screw cap polypropylene container (25384-144, VWR® International, LLC, Radnor, PA, USA) via a t-pin and hole sealed to avoid any air entry into the container. Samples were stored at 4±2°C for 48 hr. Following the 48 hr incubation period, samples were removed

from hooks and lightly blotted to remove excess surface fluid. Samples were weighed to the nearest 0.01g. Percent DL was calculated by the NPPC (2000) recommended equation and averages determined from the two samples for each carcass.

$$DL = \frac{(Loss\ in\ weight,\ \ g)}{(Initial\ weight,\ \ g)}\ x\ 100$$

Marinade Uptake and Marinade Cook Loss

Using remaining trim from fabricating one chop into two 22 to 26 g samples for DL, fat and connective tissue removed, the sample was ground twice through 6.4 mm plate on the meat grinder attachment for a KitchenAid® stand mixer (Model KSM90, St. Joseph, Michigan, USA). Ground meat was separated into triplicate samples, each weighing 6.00±0.01g, placed into 50 mL presterilized centrifuge tube (89004-364, VWR® International, LLC, Radnor, PA, USA), and weighed to the nearest 0.01 g with cap removed. Ten mL of reagent buffer (3.5% NaCl = 35 g NaCl in 1 liter of water) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) was added to each sample tube and capped tightly. Each tube was vortexed for 15 s and then placed into a 25°C water bath (Thermo-Scientific Precision™ Shallow-Form Reciprocal Shaking Bath, Thermo Fisher Scientific Inc., Waltham, MA, USA) to incubate for 30 minutes. Following incubation, tubes were centrifuged for 20 minutes at 3000 rpm (=800 x g) in an Allegra® X-15R Centrifuge (Beckman Coulter Life Sciences, Indianapolis, IN, USA) at 4°C. Caps were removed from sample tubes and placed upside down to drain excess reagent for 5 minutes. Samples were weighed in tubes, screw cap removed and weights were record to the nearest 0.01 g. MU was calculated by using the NPPC (2000) recommended equation and averages were determined from each set of triplicates for each carcass.

$$MU = \frac{[(\text{Weight of tube and meat after incubation at 25°C},\ \ \text{g}) - (\text{Initial weight of tube and meat},\ \ \text{g})]}{6.00\ g}\ x\ 100$$

Following MU, MCL was determined by utilizing the drained triplicate samples from MU analysis. Drained samples were placed into a preheated, 80°C water bath (Thermo-Scientific Precision™ Shallow-Form Reciprocal Shaking Bath, Thermo Fisher Scientific Inc., Waltham, MA, USA) for 20 minutes. Following the incubation time, samples were removed, water was drained from each sample and cooled to room temperature. Samples were weighed without screw cap and weight recorded to nearest 0.01 g. Marinade cook loss was calculated by using the NPPC (2000) recommended equation and averages determined from each set of triplicate for each pig.

$$MCL = \frac{[(Weight\ of\ tube\ and\ meat\ after\ cooking,\ \ g) - (Initial\ weight\ of\ tube\ and\ meat,\ \ g)]}{6.00\ g}\ x\ 100$$

<u>*Warner-Bratzler Shear Force and Cook Loss*</u>

The *longissimus dorsi* muscle was evaluated for WBS using a 2.54 cm thick chop. Designated vacuum-sealed chops were removed from the freezer and allowed to thaw for 24 hr at 4±2°C. After thawing, chops were removed from the vacuum-sealed bag and raw weight recorded. Chops were cooked on a clam-shell-style grill (Cuisinart® Griddler® GR-4NW, 150 Milford Road, East Windsor, NJ, USA), preheated to approximately 177°C. Temperature was monitored with copper constantan thermocouple wire inserted in the geometric center of each chop and attached to a hand-held Omega data logger HH309A thermometer (OMEGA® Engineering Inc., Stamford, CT, USA) until the internal temperature reached 71°C. Cooked chops were removed from the grill and weighed to determine percent CL. Each chop was labeled, placed on trays, covered with aluminum foil, and allowed to chill at 4±2°C for 24 hr. After 24 hr, six cores (1.27 cm) were removed from each chop with a handheld cork borer (Humboldt H-9672 Cork Borer, Humboldt Mfg. Co., Elgin, IL, USA) ensuring cores were removed parallel to the muscle fiber orientation. WBS was measured using a TA-XT2i Texture Analyser (Texture Technologies Corp. and Stable Micro Systems, Ltd., Hamilton, MA, USA)

following AMSA Research Guidelines (2015). The probe was programmed to be lowered 30.00 mm after detection of resistance. The penetration speed was 3.30 mm/s with a post-test speed of 5.00 mm/s and a pre-test speed of 3.30 mm/s. Each core was sheared once through its center, perpendicular to the muscle fiber orientation. Peak force was measured in kg of force. Average peak force was found from the 6 cores obtained from each chop. CL was determined from the following equation.

$$CL = \frac{[(Weight\ of\ chop\ after\ cooking,\ \ g) - (Initial\ weight\ of\ chop,\ \ g)]}{(Initial\ weight\ of\ chop,\ \ g)}\ x\ 100$$

Thiobarbituric Acid Reactive Substance Assay (TBARS)

A standard was created to produce a regression equation for the prediction of malondialdehyde concentration for the determination of TBARS values. A stock solution was made utilizing 0.2203 g of 1, 1, 3, 3-tetraethoxypropane (TEP) (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in 1 L of deionized water. Six Erlenmeyer flasks were labeled 1 through 6. The TEP stock solution was added to each flask starting with 0 µl in flask 1, followed by 100 µl, 200 µl, 400 µl, 500 µl, and 700 µl to tubes 2 through 6, respectively. Deionized water was added to each flask to achieve a final volume of 10 mL.

In order to determine TBARS values of each sample, two vacuum-sealed 2.54 cm thick chops were allowed to thaw for 24 hr at 4±2°C. After 24 hr, chops were removed from the vacuum-sealed bag and fat and connective tissue removed. Following a modified procedure described by Tarladgis et al. (1960) and performed by Fernando et al. (2003). A 5 g sample was removed from the center of the chop and was completely homologized by blending in a Waring® commercial laboratory blender (Model 57BL30, Waring® Commercial, Torrington, CT, USA) with 30 mL of deionized water. Each sample was blended 60 seconds. The blended sample was then transferred to a 250 mL round bottom flask. An additional 20 mL of deionized water was

added to the blender cup for washing and then transferred to the same 250 mL round bottom flask. A volume of 2.5 mL of 4 N HCl (Thermo Fisher Scientific, Waltham, MA, USA) and 3 to 5 drops of Antifoam B® Silicone Emulsion (Avantor Performance Materials, Inc., Center Valley, PA USA) was added to the mixture, stirred, and distilled at a maximum rate on an Electrothermal™ Heating Mantle (Model CMU0250/CEX1, Cole-Parmer, Stone, Staffordshire, ST15 OSA, UK) until 25 mL of distillate was collected in a 25 mL volumetric flask.

Upon completion of distillation, 5 mL of distillate was pipetted into a 50 mL presterilized centrifuge tube (89004-364, VWR® International, LLC, Radnor, PA, USA) in duplicate. In each tube, 5 mL of 0.02 M 2-thiobarbituric acid (MP Biomedicals, LLC, Solon, OH, USA) in 90% acetic acid (VWR® International, LLC, Radnor, PA, USA) was added, tightly capped, and vortexed (VWR® Analog Vortex Mixer, VWR® International, LLC, Radnor, PA, USA). Tubes were placed into a preheated reciprocal shaking boiling water bath (Thermo Fisher Scientific Inc., Model 2870, Waltham, MA, USA) for 30 minutes. Once removed, tubes were allowed to cool to room temperature. The absorbance was read at 532 nm using a Beckman Coulter® Du® 730 Life Science UV/Vis spectrophotometer (Beckman Coulter®, Brea, CA, USA). TBARS values were determined utilizing a K value of 7.8 obtained from 1, 1, 3, 3,-tetraethoxypropane as the standard.

Proximate Analysis

Following removal of the 5 g sample for TBARS analysis, the remainder of the two thawed chops were completely homogenized using an Ōsterizer 10 speed blender (Sunbeam®-Oster®, Boca Raton, Florida, USA). FOSS FoodScan™ with ISIscan™ software was used to determine moisture, protein, fat, collagen, and salt content of each sample. Once homogenized, a sample cup [D:140 mm, 14 mm height (FOSS Analytical A/S, Foss Allé 1, DK-3400 Hillerød,

Denmark)] was filled completely with sample. Each sample weighed approximately 250 g. Samples were packed completely to ensure no air pockets or gaps existed. The ISIscan™ software was initiated and a check cell procedure was run to calibrate the device prior to evaluating any sample. After evaluation, the samples were individually vacuumed-sealed and frozen at 0°C for further analysis if needed. Data was exported from ISIscan™ software and duplicate runs averaged for each sample for each value.

Trained Sensory Panel Evaluation

The protocol for trained sensory panel evaluation was reviewed and approved by the Auburn University Institutional Review Board for the Protection of Human Subjects (IRB). Prior to beginning sensory evaluation, 15 panelists were selected and trained following the AMSA Research Guidelines (2015). An 8 point hedonic scale was utilized to evaluate six traits of initial juiciness, sustained juiciness, initial tenderness, sustained tenderness, pork flavor intensity and off-flavor intensity (1 = extremely dry, extremely tough, extremely bland, extreme off-flavor to 8 = extremely juicy, extremely tender, extremely intense pork flavor, no off-flavor). Potential off-flavors were identified and with the utilization of various compounds, panelists were trained to be able to distinguish these and identify them correctly. Off-flavor was described by eight descriptors; metallic, salty, livery, grassy, bitter, bloody, rancid, or other-explain.

Panelists evaluated initial juiciness based on the presence and volume of juice excreted by the pork sample with the initial bite. Sustained juiciness was the amount of juice excreted from the sample after 20 chews. Initial tenderness was evaluated based on the firmness of the sample upon initial bite. Sustained tenderness was degree of firmness that the panelists experienced after 20 chews. Pork flavor was simulated using concentrated pork flavor (ProFlavor™ P3306 Pork Flavor, Essentia Protein Solutions, Ankeny, IA, USA) at a high

concentration (3 tsp/cup) (8 on hedonic scale) and a low concentration (1 tsp/cup) (3 on hedonic scale).

Panelists were also trained for potential, project specific, off-flavors including; fish, vitamin E, and flaxseed flavor. 1200 mg fish oil capsules (Nature's Bounty, Inc. Bohemia, NY, USA), 1000 mg flaxseed oil capsules (Spring Valley™, Bentonville, AR), and 1000 IU vitamin E capsules (Spring Valley™, Bentonville, AR) were punctured to recover each oil. The oil was then weighed and mixed with lean ground pork (85:15) and formed into 113.4 g patties using a handheld patty press. For each potential off-flavor, training was performed at three concentrations: 1, 5, and 10%, mimicking a low, moderate, and extreme off-flavor. Patties were cooked to an internal temperature of 71°C following the same procedure as described for WBS.

During sensory evaluation sessions, a trained panel of 8 to 11 panelists was seated in individual, partitioned booths with 250 Lx of red incandescent light. During each session, panelists evaluated 7 to 8 samples, in efforts to minimize any sensory fatigue. Pork loin chop samples were cooked using the same standards as WBS. After cooking, samples were removed from heat and allowed to rest before being cut into 1.27 cm x 1.27 cm cubes with a plastic cutting grid. Each panelist randomly received two cubes from each chop. Each sample was randomly assigned a 3-digit code to ensure no bias, and samples were placed in clear plastic cups with lids. Panelists were given salt-free saltine crackers and diluted apple juice and they were instructed to cleanse their palate by consuming a cracker, followed by a sip of apple juice between each sample. Panelists evaluated samples over a 12-day period, one session per day (n = 95 samples, 7 to 8 samples per session).

Statistical Analysis

Statistical analysis was performed using the general linear model procedure in SAS 9.4 (SAS Inst. Inc., Cary, NC). Carcass was the experimental unit. Lipid level (0, 2, 4, or 6%), vitamin E concentration (11 or 220 IU), sex (F or M), and trial (1 or 2) served as fixed effects and days on feed (DOF) as a covariate. All effects, two-, three-, and four-way interactions were analyzed and considered significant if $P<0.05$. Means were separated using least squares analysis.

III. Meat Quality Assessment of Pork Fed Poultry Fat, Flaxseed Oil, and Supplemented with Vitamin E

Results and Discussion

Results

Three belly quality evaluations were performed for firmness, SSU, SSD and belly thickness (BT). There was a 4-way interaction between trial, lipid content, vitamin E concentration, and sex of pig for SSU belly firmness evaluation (Figure 1). The complexity of this interaction makes the determination of valuable and concise results difficult. There are no obvious trends present within this interaction. Gilts had firmer bellies in trial 2 with a lipid level of 4% and supplemented with 11 IU vitamin E (18.69 cm). Barrows had firmer bellies with a SSU belly measurement of 11.92 cm during trial 1, when fed 2% dietary lipids and supplemented with 11 IU vitamin E. The differences observed in this interaction may possibly be attributed to outliers that are present for SSU belly measurement.

A 4-way interaction between trial, lipid content, vitamin E concentration, and sex of pig for CL is present in this study (Figure 2). Gilts receiving 0 and 2% lipids in trial 1 and all lipid levels in trial 2 (0, 2, 4, and 6%) had greater CL percentages as vitamin E concentration increased from 11 to 220 IU. In trial 1, gilts had a reduction in CL percentage for 4 and 6% lipids as vitamin E concentration increased. Barrows receiving 0, 2, and 6% dietary lipids in trial 1 and 0% during trial 2 had greater CL percentages when fed 220 IU vitamin E. Barrows had lower CL percentages when supplemented with 2, 4, and 6 % dietary lipids and 220 IU vitamins E in trial

2. However, this interaction is very complex and it is believed that the differences observed are also due to the presence of outliers in this study, thus no visible trends are present.

A 3-way interaction between lipid content, vitamin E concentration, and sex of pig was observed for BT (P=0.0198) (Figure 3). Gilts receiving 6% dietary lipids and 11 IU vitamin E had the greatest BT measurements while barrow BT measurements were the greatest when fed 4% dietary lipids and 220 IU vitamin E. In addition, gilt diets containing 4 and 6% lipids had a reduced BT measurement as vitamin E concentration increased. However, barrow BT measurements improved as vitamin E concentration increased within treatments containing 4 and 6% dietary lipids.

Tables 2 and 3 contain the least squares means and p-values for the 3-way interaction between lipid content, vitamin E concentration, and sex of pig present for pHL (P=0.0007) in this study. The range of pHL within this interaction was 5.46 to 5.66 and no trend within this interaction is visible. The pHL values observed are within the normal and desired pH range (5.40 to 6.0) for fresh pork products and are classified as red firm and nonexudative (RFN).

A 3-way interaction for lipid content, vitamin E concentration, and sex of pig for redness (a*) was found in the present study (P=0.0193) (Tables 2 and 3, respectively). Although this interaction is a source of variation for a*, it cannot be completely explained and no visible trend is present in the data. Values for a* range from 7.55 to 9.67, all of which fall into the normal a* colorimeter range for fresh pork products. The ability of a consumer to distinguish any visual differences for redness among any treatment group in this study is highly unlikely due to the small differences in measured a* values.

There was a 3-way interaction between lipid content, vitamin E concentration, and sex of pig for VP (Tables 4 and 5). Gilts had less VP when fed 0, 2, and 4% lipids and 220 IU vitamin

E as compared to 11 IU vitamin E. The inclusion of 6% lipids with 220 IU vitamin E resulted in an increased VP as compared to 11 IU. Vacuum purge was less for barrow treatments containing 0, 4, and 6% lipids with 220 IU vitamin E but barrow VP increased with 2% lipid and 220 IU vitamin E.

The least squares means and p-values for the interaction between trial, lipid content, and vitamin E concentration for DL (P=0.0471) are presented in Tables 6 and 7. Drip loss decreased as vitamin E concentration increased in treatments containing 0 and 4% lipids in trial 1. However, 2 and 6% lipid inclusion produced the greater DL percentage with increased vitamin E supplementation. For trial 2, DL decreased as vitamin E concentration increased with the exception of 2 and 6% dietary lipids during trial 1. Drip Loss was greater in carcasses that received 6% dietary lipids and 220 IU vitamin E.

A trial by sex of pig interaction was observed for moisture content (P=0.0390; Table 8) of the loins. Gilt carcasses contained a greater moisture content in trial 1 than trial 2 (76.38 vs 75.57%) and the moisture content was greater in trial 2 for barrow carcasses (75.83 vs 75.74%). In addition, trial by sex of pig interaction was a source of variation for LRFT (P=0.0034; Table 8). Barrows in trail 2 had less LRFT as compared to all other groups (20.28 mm). Gilts in trial 2 measured the greatest amount of LRFT (24.25 mm). However, the amount of LRFT for barrows in trial 1 (23.11 mm) was equivalent to gilts in trial 2 (24.25 mm). Gilts in trial 1 (21.54 mm) were only different from the barrows in trial 2 (20.28 mm).

A trial by vitamin E concentration interaction was observed for SSD (P=0.0042; Table 9). In trial 1 as vitamin E concentration increased, greater measurements for SSD belly firmness were observed. However, as vitamin E concentration increased in trial 2, belly firmness decreased. This same interaction is present for collagen content (P=0.0225; Table 9). An

increased vitamin E concentration (220 IU) resulted in a greater collagen content in trial 1 loins (1.44 vs 1.49%). In trial 2 pigs supplemented with 11 IU vitamin E had a greater collagen content than pigs supplemented with 220 IU vitamin E (1.59 vs 1.44%).

The trial by vitamin E concentration interaction for TRFT (P=0.0318; Table 9), %FFL (P=0.0350; Table 9), and MS (P=0.0304; Table 9). As vitamin E concentration increased the measured amount of TRFT increased. Specifically, in trial 1 TRFT increased from 18.29 to 22.52 mm as vitamin E concentration increased from 11 IU to 220 IU and in trial 2, TRFT increased from 20.23 to 20.73 mm. In trial 1 as vitamin E concentration increased, %FFL (53.62 vs 51.23%) and MS (2.69 vs 2.44) decreased, while in trial 2 %FFL (51.97 vs 52.35%) and MS (2.25 vs 2.45) was greatest with inclusion of 220 IU vitamin E.

For both SSU (P=0.0018) and SSD (P=0.0003) an interaction between vitamin E concentration and sex of pig was present (Table 10). In gilts, as vitamin E concentration increased belly firmness decreased (SSU; 10.27 vs. 8.28 cm) and (SSD; 13.47 vs 10.80 cm). As for barrows, supplementation with 220 IU vitamin E improved belly firmness in comparison to 11 IU (SSU; 8.87 vs. 6.84 cm) and (SSD; 12.93 vs. 9.08 cm).

A vitamin E concentration by sex of pig interaction for LRFT (P=0.0206; Table 10) demonstrated that supplementation of vitamin E at 11 IU results in gilts with the greatest LRFT (23.05 mm) while barrows had greater LRFT with 220 IU vitamin E (23.63 mm). Barrows supplemented with 220 IU vitamin E were different from barrows receiving 11 IU vitamin E but equivalent to gilts at both concentration of vitamin E. The measured LRFT was equivalent for gilts with 11 and 220 IU vitamin E.

There was a lipid content by vitamin E concentration interaction for TRFT (P=0.0016; Table 11) and %FFL (P=0.0028; Table 11). As lipid content increased from 0 to 6%, TRFT

increased from 16.69 to 21.48 mm, respectively, for treatments with 11 IU vitamin E. This increase was observed across all dietary treatments. There is variation among lipids levels supplemented with 220 IU vitamin E. Lipid inclusion at 4% produced the largest TRFT measurement (23.43 mm) while 6% lipids produced the lowest, 18.12 mm. Lipids at 0 and 4% resulted in a TRFT of 23.07 and 23.43 mm, respectively. Within vitamin E concentration, measurements for TRFT were equivalent for 11 IU vitamin E among all dietary lipid levels except 0%. All treatments containing 220 IU vitamin E were the same except for 6% dietary lipids. The 6% dietary lipid supplementation with 220 IU vitamin E was the ideal combination for the largest reduction of TRFT. In treatments containing 11 IU vitamin E, as the lipid concentration elevated from 0 to 6%, %FFL increased. Although the same trend was not observed for 220 IU vitamin E. Treatment with 6% lipids and 220 IU vitamin E produced the 2nd greatest %FFL among all treatments and was the greatest within 220 IU vitamin E.

A lipid concentration by sex of pig interaction was present for SSU (P=0.0335; Table 12). Gilts performed the best when fed 4% dietary lipids while barrows fed 2% dietary lipids had the greatest SSU belly measurements. Both barrow and gilts had the largest reduction in belly firmness with the inclusion of 6% lipids.

Lipid content by sex of pig interaction was a source of variation for LRFT (P=0.0031; Table 12), TRFT (P=0.0160; Table 12), %FFL (P=0.0164; Table 12), and MS (P=0.0362). The greatest measurement for LRFT in gilts was observed at 0% lipids (25.52 mm) followed by 6, 4, and 2% (22.35, 22.08, and 21.63 mm, respectively). LRFT was greatest for barrows at 4% at 24.10 mm followed by 2, 0, and 6 % lipids (24.02, 20.02, and 18.65 mm, respectively). Carcasses from gilts that received 6% lipids had the greatest amount of TRFT (21.63 mm) followed by 0, 4, and 2% lipids (21.57, 21.36, and 18.88 mm, respectively) but were different

from any other treatment within sex. Carcasses from barrows had the least measured TRFT at 6% lipids (17.97 mm), followed by 0, 2, and 4% lipids with a TRFT of 18.19 mm and 21.96 mm for both 2 and 4% lipids which was equivalent to 0% lipids but different from 2 and 4% within sex. Gilts had the greatest %FFL with 2% dietary lipids while barrows had the greatest %FFL at 6% dietary lipids. Gilts were more heavily muscled and had lower LRFT and TRFT with inclusion of 2% dietary lipid while barrows were heavier muscled with the inclusion of 6% dietary lipids, thus producing the greatest %FFL within each sex at corresponding lipid content.

Table 13 contains the carcass trait least squares means for the main effects evaluated in this study. Trial had an effect on pHH (P<0.0001), pHL (P<0.0001), NPPCCol (P=0.0207), and HCW (P=0.0204). The pH of the ham was greater in trial 1 (5.85) than in trial 2 (5.53). The same trend was present for pHL, 5.67 and 5.45, trial 1 and 2 respectively. Least squares means values was for subjective color were 2.84 (trial 1) and 3.24 (trial 2), demonstrating that trial did affect subjective color (P=0.0207). Trial was a significant source of variation for HCW in this study. Trial 1 carcasses were heavier than carcasses from trial 2 by 3.0 kg (84.8 vs 81.3 kg, trial 1 and 2 respectively). Sex did affect L* values (P=0.0057; Table 13). Loins from barrow carcasses were lighter in white to black ratio than the loins from gilt carcasses (61.50 vs. 58.86). Vitamin E concentration affected NPPCMar (P=0.0066); least squares means values increased as vitamin E concentration increased from 11 IU to 220 IU (1.41 and 1.87) (Table 13). In the present study trial had an effect on DL percentage (P=0.0282; Table 14). In trial 1 DL was 3.34% while in trial 2 DL was 4.12%. There was also an effect on TBARS values (P<0.0001) for trial (Table 14). Trial 1 TBARS value was 0.22 while trial 2 was 0.15. It is also important to note that days on feed affected %FFL (P=0.0392), VP (P=0.0394), LRFT (P=0.0004) and TRFT (P=0.009).

Lipid content, vitamin E concentration, and sex of pig had no effect (P>0.05) on HCW, LEA, %FFL, a*, b*, NPPCCol, pHH, pHL, MS, SSD, SSU, BT, DL, VP, MU, MCL, WBS, % fat, % moisture, % collagen, % protein, % salt, and TBARS. Additionally, sensory attributes were not affected by any dietary treatment in this study (Table 15).

<u>Discussion</u>

Carcass composition

The hypothesized result of the present study was that carcass composition would be positively altered as a result of reduced de novo lipogenesis from increased dietary lipid supplementation. Reducing de novo lipogenesis through dietary lipid supplementation, as described in a review by Moser (1977), can be achieved with inclusion of 5% dietary lipids into a typical corn-soybean finisher diet. The inclusion of up to 5% lipids has the potential to reduce body fat and increase leanness in the carcasses. The objective of reducing of de novo lipogenesis would be the reduction of carcass fat while simultaneously increasing IMF. Decreasing carcass fat would directly increase %FFL and MS of the carcasses and improve yield and increase profitability. The increased %FFL accompanying increased MS, is desired by producers and the meat industry.

The trial by vitamin E concentration interaction for TRFT demonstrated that as vitamin E concentration increased the measured amount of TRFT increased. Muscle score and %FFL are also directly affected by vitamin E concentration within trial. As %FFL increased a direct increase was observed for MS. In trial 1, the reduction in %FFL for pigs supplemented with 220 IU vitamin E can be attributed to the greater TRFT measurements as a result of increased dietary vitamin E. The %FFL is directly affected by TRFT. A substantial increase in TRFT measurement was observed in trial 1 (18.29 to 22.52 mm) as vitamin E concentration increased from 11 to 220

IU. The increase in TRFT resulted in a reduced %FFL. The greater increase in TRFT during trial 1 can be attributed to the increased days on feed for harvest period 4 during trial 1, in addition to the increased age at harvest at final harvest during trial 1. Furthermore, of the 47 pigs receiving vitamin E supplementation at 220 IU, 28 of them reached market weight during the 3rd and 4th harvest periods within their trial, while of the 48 pigs receiving 11 IU vitamin E, 26 finished during the 1st and 2nd harvest days within each trial. This may suggest that more time is required for pigs receiving increased vitamin E supplementation to reach market weight, thus allowing more time for back fat deposition.

In other research, the majority of researchers found no differences in TRFT, LRFT, and %FFL with the inclusion of vitamin E in the diet, at any concentration (Cannon et al., 1995a,b; Onibi et al.,1998; Guo et al., 2006; Engel et al., 2001). The present study found conflicting results as compared to Engel et al. (2001) who reported no differences in dressing percent with the inclusion of poultry fat into swine diets. Additionally, it has been reported that flaxseed (4 to 10 g/kg) in the diet has no effect on back fat thickness or lean meat percentage in barrows or gilts (Van Oeckel et al., 1997). Flaxseed oil at 4 or 5% has also been included in swine diets with no differences measured for carcass traits including TRFT and LRFT. (Fontanillas et al., 1998; Nuernberg et al., 2005). The present study follows similar results found by Guo et al. (2006) and a summary by Pettigrew and Moser (1991) who found that TRFT tended (P=0.09) to increase with lipid supplementation. An increase in TRFT was observed as lipid content increased with inclusion of 11 IU vitamin E in the present study and variation was also present for 220 IU vitamin E inclusion.

Overall, gilts had similar measurements for TRFT and LRFT as compared to barrows (Gilts: 20.86 and 22.90 mm; Barrows: 20.02 and 21.70 mm, TRFT and LRFT respectively.

Twenty-four of the 47 total gilts in this study were harvested during the last harvest periods within each trial, the increased days on feed and age at harvest for gilts could explain the increased amount of fat deposition observed. Typically, barrows are expected produce heavier and fatter carcasses, while gilts will have a greater %FFL (Ellis et al., 1996; Langlois and Minvielle, 1989). Thus, the greater %FFL and MS observed in the present study for gilts is to be expected. Although the present study contradicts (Ellis et al., 1996; Langlois and Minvielle, 1989) who found that gilts had greater values for TRFT and LRFT.

The 6% dietary lipid supplementation with 220 IU vitamin E produced the ideal combination for the largest reduction of TRFT and greatest %FFL when sex is disregarded. One explanation of the decreased TRFT in the interaction between lipid content and vitamin E concentration could be the reduction of de novo lipogenesis. Additionally, pigs receiving this dietary treatment finished during the first 3 harvest periods during trial 1. In trial two, 3 of the 5 pigs receiving this treatment finished during the first harvest period, while two finished during the last harvest period in trial 2 (Table 16). It is important to note that the 3rd harvest period in trial 1 and the last harvest period in trail 2 were at comparable in age (168 and 164 days respectively) (Table 17). The 6% lipid treatment supplemented with 220 IU vitamin E treatment was not represented during the final harvest period in trial 1, so not having additional days on feed or benefit from increased age, could have contributed to the reduction in TRFT for this particular treatment. Overall, it can be concluded that feeding low levels of dietary lipids with low levels of vitamin E increases %FFL by decreasing TRFT. Pigs that received 6% lipids and 220 IU vitamin E are the exception; producing the second largest %FFL (54.13%) within all treatments and greatest within 220 IU vitamin E treatments. Again, directly relating to the effect of TRFT on %FFL, as the 6% and 220 IU vitamin E treatment performed best for TRFT.

The results found in the present study for MS agrees with previous research which states that the inclusion of 4 and 5% flaxseed oil had no effect on carcass composition (Fontanillas et al., 1998; Nuernberg et al., 2005). Including poultry fat at 2.5 and 5% into finishing diets only produced small changes in carcass characteristics, none of which were different (Seerley et al., 1978). In agreement with Seerley et al. (1978), a study by Engel et al. (2001) reported no differences were found in carcass composition with inclusion of 6% poultry fat into finishing diets. Though differences were observed for MS in the lipid content by sex of pig interaction, this demonstrates that gilts required less lipid inclusion (2%) to produce the greatest MS. Muscle score for gilts at 2% was different from 0 and 4% lipids, while barrows produced the greatest MS with 6% dietary lipid inclusion, but was equivalent to 0% lipids.

Most literature reports no differences in HCW due to inclusion of vitamin E in the diet (Asghar et al., 1991a,b; Onibi et al., 1998) except if vitamin E is added at 500 mg/kg (Gou et al., 2006, Cannon et al., 1995a,b; Hoving-Bolink et al., 1998; Asghar et al., 1991a,b). Since the highest level of vitamin E inclusion in this study was 220 IU, it was not expected that HCW would be different. There were no differences for lipid content, vitamin E concentration of sex of pig for HCW in the present study. Flaxseed oil was included at 1% in all diets for this study and no differences in HCW were detected. These results are similar to a study conducted by Nuernberg et al. (2005) where flaxseed was included at 4% in swine diets and no differences were observed for HCW. Days on feed tended (P=0.0713) to influence HCW. As expected, the longer pigs were on feed, an increased HCW was observed. This directly relates to the variation observed for HCW across trials. Trial 1 pigs experienced a greater age at harvest for the final harvest period than trial 2 pigs. Thus, an increased HCW was observed during trial 1.

Consumers measure perceived freshness of a meat product primarily by color (Monahan et al., 1994). Research studies suggest that the inclusion of α-tocopherol acetate in animal diets has the potential to positively affect surface color characteristics, in addition increase color stability (Asghar et al., 1988, 1991a,b). An increase in color stability was reported as a direct result of an increased concentration of dietary vitamin E (Asghar et al., 1991a). Results from previous research vary from the results obtained in this study. Greater a* values were reported in frozen chops from pigs that received vitamin E supplementation as compared to control samples not receiving vitamin E. The increase in color stability for supplemented chops was attributed to vitamin E reducing the formation of metmyoglobin (Monahan et al. 1992a). A reduction in a* values could be attributed to greater lipid oxidation in samples due to the larger lipid and PUFA content which would be expected as dietary lipid concentration increased. Additionally, as the vitamin E concentration within the loin tissue increases, an increased a* value should be observed. As lipid content, specifically PUFA content, increases more vitamin E is needed to stabilize biological membranes in order to avoid oxidation (Onibi et al., 2000). A reduction in lipid stability will directly reduce a* values (Onibi et al., 2000). Although the present study did not produce an improvement in a* value, results were similar to results presented by Guo et al. (2006). Inclusion of DL-α-tocopherol acetate at 40 and 200 IU and dietary lipid supplementation had no effect ($P>0.05$) on L*, a*, or b* (Guo et al., 2006). It is believed that there was no increase in a* values during the present study due to flaxseed oil's low inclusion. Thus, the increase in PUFA was not significant enough to increase the rate of lipid oxidation to require increased vitamin E for stabilization.

The influence of sex on color in the present study disagrees with results reported by Bereskin et al. (1978) and Hiner et al. (1965) which suggest that barrows have reduced color

values than gilts. The greater L* value for barrows can be attributed to the greater marbling score in barrow loins. As subjective marbling scores improve, objective color values for L* become lighter (more white). The greater NPPCMar score as seen with an increase in vitamin E concentration contradicts previous research, which revealed little differences among treatment groups with high dietary lipid supplementation with the addition of 40 or 200 IU vitamin E (Guo et al., 2006). While these values are different, the likelihood of consumers being able to determine any differences due to the slight increase in marbling is unlikely.

The present study produced conflicting results for the effectiveness of vitamin E and its ability to positively improve and stabilize pork color while it has proven to effectively stabilize and improve beef color (Liu et al., 1995). The color increase in trial 2 can be attributed to a decrease in lipid oxidation as a result of increased color stability from vitamin E supplementation but this increase is only a slight increase. There is no biological difference between trial 1 and trial 2 is observed. Overall the least squares means for NPPCCol observed in this study are in agreement with the greater objective a* values (8.52 and 8.94, trial 1 and 2 respectively). The increase in color stability is directly related to the α-tocopherol concentration of the muscle tissue (Cannon et al., 1995a,b). Specifically, 3.0 to 3.7 μg/g of tissue is needed to stabilize beef color and extend shelf life (Faustman et al. 1989a). In regards to pork, α-tocopherol concentration is also directly related to increase color stability and shelf-life but the exact concentration is yet to be identified (Asghar et al., 1991a).

Many factors contribute to the development of ultimate pH ranging from genetics, transportation, temperature, lairage, and preharvest stress (NPPC, 2000). The increase in ultimate pHH and pHL during trial 1 can be attributed to the unusual increase in ambient temperature during trial 1 harvest periods. Ultimate pH of meat has an effect on many meat quality attributes.

An increase or decrease in pH has the potential to either positively or negatively affect factors including but not limited to WHC, WBC, DL, and CL. In regards to the dietary components of this study, previous research found that vitamin E incorporation at 10, 100, and 200 IU produced no differences for pH among treatment groups (Monahan et al., 1990a,b). Dietary lipid supplementation combined with vitamin E at 40 and 200 IU, also produced no differences in pH based on dietary treatments (Guo et al., 2006). Additionally, result in the present study in regards to sex and ultimate pH are supported by previous research that determined that sex has been shown to have no impact on pH (Cisneros et al., 1996; Leach et al., 1996).

Meat Quality

Based on prior research conducted with the inclusion of dietary lipids and vitamin E in swine diets it was hypothesized as vitamin E concentration increased within the diet, a reduction in DL would be observed. The potential for vitamin E to increase lipid stability will result in a lower DL. Oxidative stability is a major concern when flaxseed oil is included in the swine diet because of the potential to increase lipid oxidation rates. Research has shown inclusion of flaxseed oil at 5% decreases ($P<0.05$) the oxidative stability of pork products (Nuernberg et al., 2005). The inclusion of vitamin E at 200 mg/kg has been shown to improve the oxidative stability of pork when supplemented with flaxseed oil at 3% or increased poultry fat in the ration (D'Arrigo et al., 2002a,b; Guo et al., 2006). Fresh pork samples from pigs treated with 200 mg/kg of vitamin E had a lower rate of DL than pigs not supplemented with vitamin E (Onibi et al., 1998). Frozen chops from pigs that received vitamin E supplementation at 200 mg/kg had the lowest and most desired DL values ($P<0.05$), when compared to treatments containing 10 and 100 mg/kg vitamin E (Asghar et al., 1991a, b). Vitamin E supplementation at 200 and 500 IU also reduced DL in fresh and frozen samples allowing researchers to conclude that these results

could be attributed to the reduction in lipid oxidation in biological membranes (Onibi et al., 2000). The reduction in rate of DL, or increased WHC, was found to be directly related to the α-tocopherol content in the cellular membranes of the muscle (Asghar et al., 1991a). Cheah et al. (1995) found dietary vitamin E supplementation prevented PSE and improved tissue WHC. In regards to lipid supplementation, poultry fat inclusion at 2, 4, and 6% found no difference in DL at 24 or 48 hours, WBC, or CL percentage (Engel et al., 2001). Results found in the present study are not supported by results from other researchers where VP was determined after 28 and 56 days of vacuum-storage and did not differ ($P>0.05$) from the control as compared to samples supplemented with 100 mg vitamin E (Cannon et al., 1995a,b). The larger inclusion rate of vitamin E in the present study may be an explanation for the differing result. Supplementation at 220 IU may elevate the α-tocopherol concentration in the tissue to adequately reduce oxidation to increase WHC. The increase in VP in chops from pigs receiving 11 IU of vitamin E correlates with the reduction in DL with 11 IU vitamin E inclusion. The increased moisture loss due to VP, reduced the amount of available moisture within the sample prior to DL determination. As VP increased, a reduction in DL would be expected. In addition, the increased in moisture content can be attributed to the decreased VP within the same treatment. As VP increased, moisture content decreased.

While there is a difference ($P<0.05$) for the trial by lipid concentration interaction for DL, values observed during both trials for all lipid levels fall within the normal, desired range of 2 to 6% (NPPC, 2000). The greater DL in trial 2 is related to the decreased pH during trial 2 as compared to trial 1. The pH in trial 1, is closer to the pH of DFD meat but is still within a normal and acceptable range. One characteristic of a greater pH, as well as DFD, is increased WHC.

The results of this study are not similar to Latorre et al. (2004) who determined that sex influenced CL. In this study, barrows had less CL percentages than gilts. However, other researchers have found that sex has no effect on CL percentages (Cisneros et al., 1996; Ellis et al., 1996).

As hypothesized from prior research, increasing dietary lipid content, specifically PUFA, may reduce the oxidative stability of pork products thus elevating TBARS values (Monahan et al., 1992a,b,c). The reduction in oxidative stability leads to a reduced storage life, quality, and can negatively affect other meat characteristics (Buckley et al., 1995). Overall, in the present study the inclusion of poultry fat and flaxseed oil (1%) was at a concentration so low that the negative effects of increased lipid oxidation as a result of increased PUFA were not observed. Thus, extreme differences were not seen for TBARS values.

Proximate analysis of loins was evaluated for protein, moisture, fat, collagen, and salt content for all treatments. The hypothesized result of increased dietary lipid supplementation was a greater fat content within loins due to the reduction of de novo lipogenesis which results in greater IMF. No differences were observed ($P>0.05$) for protein, fat, or salt content in this study. It was observed that as collagen content increased, greater values were observed for SSD, within each trial. This relationship suggests that greater collagen content increased the rigidity of the belly, thus increasing SSD measurements.

Belly Quality

The initially hypothesis for this study was that as dietary lipid content increased, belly quality and firmness would diminish. Previous studies indicated increased dietary lipid content, inclusion of poultry fat, or other fat source within a swine diet can adversely affect belly quality by reducing belly firmness leading to processing difficulties (Cannon et al., 1996; Miller et al.,

1993). It is important to maintain integrity of the belly as it is the most valuable cut obtained from the pork carcass. Researchers have found that inclusion of more than 6% dietary lipids has resulted in a reduction in belly firmness (Woodworth et al., 1999; Engel et al., 2001). Although results vary among treatment groups in regards to belly quality and firmness in the present study, it can be concluded that supplementing the swine diet with increased dietary lipids, 1% flaxseed oil, and vitamin E does not negatively affect belly quality. Additionally, belly firmness measurements for all dietary treatments in this study were not different as compared to control diets.

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Conclusion

In conclusion, a feeding program utilizing poultry fat in combination with flaxseed oil and vitamin E at these levels will not negatively affect carcass composition or meat quality assessed in the project. Overall, for this study vitamin E fed at 220IU in combination with 6% dietary lipids for all pigs is most acceptable when evaluated for the most important meat quality traits. It is important to note that animals are commercially finished in same-sex groups, therefore determination of the best treatment in regards to sex is valuable. Males performed best when fed 6% dietary lipids while females performed best at 2%. While differences are present in this study, all treatments produced pork products which fall within the normal acceptable range for carcass composition and meat quality analysis all without compromising belly firmness or sensory attributes. Further analysis of FA composition assessment is needed for determining the additional benefits of flaxseed inclusion into swine diet.

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Implications

Increased dietary lipid supplementation with the inclusion of vitamin E into swine diets can be performed without negatively affecting carcass composition and meat quality. Although variation was seen throughout treatments, no treatment resulted in a reduction in carcass composition or meat quality. Maintaining the integrity of bellies is of particular interest because bellies have the greatest value from a pork carcass. It is crucial to ensure that diet manipulation and supplementation does not negatively affect belly firmness. A result of decreased belly firmness would be the decrease in profitability, processing efficacy, and overall reduction in consumer acceptance.

Dietary lipid supplementations have been researched over the years, with varying results. Incorporation of high levels of poultry fat into the diet not only benefits the swine producers but it also benefits the poultry industry. Geographically, the south-east region of the U.S. produces a large volume of poultry. The incorporation of poultry fat as a dietary fat and energy source without adverse effects on meat quality and yield would only positively affect both poultry and swine producers.

Producers could adopt a feeding program utilizing poultry fat in combination with flaxseed oil, and Vitamin E at these levels and will not negatively affect the variables for carcass composition or meat quality assessed in the project. Further analysis of fatty acid composition

assessment is needed for determining any additional benefits of flaxseed inclusion into swine diet.

Additional research as it pertains to meat quality that would be of benefit in this study is color evaluation of the frozen loins as they were removed from the freezer and thawed. Some research suggests that vitamin E can have the potential to increase oxidative stability and improve the color of pork products throughout the duration of long term freezing. Adding an evaluation of color stability of frozen pork could be beneficial for better understanding vitamin E and its long-term effects as it pertains to frozen storage. In addition, an evaluation of fresh pork product storage methods and a shelf-life evaluation for varying storage methods would also be beneficial in determination of the overall benefit that vitamin E provides.

Table 1. Composition of Experimental Finisher 1 Diets (as fed)[1]

	Lipid Content							
	0		2		4		6	
	Vitamin E Concentration							
Ingredient, g/kg	11	220	11	220	11	220	11	220
Corn	700.2	700.2	667.6	667.7	635.1	635.1	602.6	602.6
SBM (47.5% CP)	273.5	273.5	285.3	285.3	297.1	297.1	308.9	308.9
Animal Fat	0.00	0.00	10.00	10.00	30.00	30.00	50.00	50.00
Flaxseed Oil	0.00	0.00	10.00	10.00	10.00	10.00	10.00	10.00
Dicalcium Phosphate	11.9	11.9	12.76	12.76	13.72	13.72	14.63	14.63
Limestone	8.39	8.39	8.29	8.29	8.12	8.12	7.96	7.96
Salt	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin-mineral[2]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Calculated Composition[3]								
DE, Mcal/kg	3.40	3.40	3.50	3.50	3.60	3.60	3.70	3.70
CP, g/kg	188.2	188.2	191.2	191.2	194.1	194.1	197.1	197.1
Ca, g/kg	7.00	7.00	7.21	7.21	7.42	7.42	7.62	7.62
P, g/kg	6.00	6.00	6.16	6.16	6.34	6.34	6.51	6.51
Ca:P	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
SID Lys, g/kg	8.50	8.50	8.75	8.75	9.00	9.00	9.25	9.25
SID Lys:DE, g/Mcal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Ca:DE, g/Mcal	2.06	2.06	2.06	2.06	2.06	2.06	2.06	2.06
P:DE, g/Mcal	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76

[1] Finisher 2 diets were similar but contained 7.30g standardized ideal digestible (SID) Lys/kg in the diet with 0% lipids. Vitamin E premix (220 IU/kg) was included in place of corn. SBM = Soybean Meal, CP = Crude Protein, and DE = Digestible Energy.
[2] Provided the following (unit/kg diet): Fe (ferrous sulfate), 150mg; Zn (zinc oxide), 150mg, Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; I (ethylenediamin dihydroiodide), 5 ppm; Se (sodiu, selenite), 3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; vitamin E, 11 IU; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline (choline chloride), 110 mg.
[3] To maintain a constant ratio (Chiba et al., 1991 a,b), Lys, Ca, or P content was adjusted for DE accordingly.

Table 2. Interaction between Lipid Content, Vitamin E Concentration, and Sex of Pig Least Squares Means for Carcass Traits

	Lipid Content																
	0				2				4				6				
	F		M		F		M		F		M		F		M		
Trait[1]	11	220	11	220	11	220	11	220	11	220	11	220	11	220	11	220	SEM
HCW, kg	80.9	82.6	83.2	82.9	82.5	79.8	83.7	84.2	84.8	82.6	86.2	84.1	83.3	80.4	85.3	85.7	2.79
LRFT, mm	24.92	28.12	18.43	21.61	23.93	19.33	22.81	25.24	21.24	22.93	20.78	27.43	22.10	22.59	17.07	20.23	1.98
TRFT, mm	18.05	25.10	15.33	21.05	17.13	20.62	20.80	23.13	20.62	22.10	19.18	24.75	24.68	18.58	18.28	17.66	1.90
LEA, cm^2	42.45	40.49	45.34	40.18	46.00	45.20	42.02	41.19	39.71	42.71	41.88	39.55	35.38	44.14	44.32	45.97	3.31
FFL, %	53.61	49.60	55.55	51.39	54.86	53.24	51.99	50.74	51.42	51.62	52.69	49.50	48.54	53.91	53.71	54.35	1.44
L*	58.84	59.80	60.39	60.69	61.77	58.75	62.49	62.09	58.15	60.13	63.52	60.21	56.22	57.26	61.01	61.62	2.01
a*	8.86^{abc}	8.63^{abc}	8.44^{abc}	9.51^{b}	8.04^{ac}	9.18^{b}	9.55^{b}	7.55^{a}	9.06^{abc}	8.29^{abc}	8.14^{abc}	8.52^{abc}	9.27^{abc}	9.67^{b}	8.85^{abc}	8.13^{abc}	0.63
b*	16.09	16.38	15.97	16.99	16.06	16.40	17.22	15.93	16.50	16.00	16.16	16.02	15.88	15.95	16.35	15.59	0.65
pHH	5.65	5.73	5.77	5.71	5.76	5.68	5.62	5.75	5.61	5.70	5.74	5.71	5.65	5.70	5.65	5.66	0.06
pHL	5.56^{bcde}	5.53^{abcd}	5.66^{e}	5.54^{abcd}	5.61^{cde}	5.52^{abcd}	5.46^{a}	5.63^{cde}	5.44^{a}	5.53^{abcd}	5.62^{cde}	5.50^{ab}	5.58^{bcde}	5.64^{de}	5.58^{bcde}	5.57^{bcde}	0.04
MS	2.74	2.36	2.38	2.13	2.75	2.62	2.13	2.01	2.37	2.99	2.77	2.25	2.25	2.42	2.38	2.76	0.23
NPPC Color[2]	2.91	3.01	3.17	2.92	2.98	3.36	3.02	2.71	3.48	3.05	2.75	2.73	3.38	3.19	2.94	2.98	0.37
NPPC Marbling[2]	1.70	1.85	1.21	1.96	1.01	2.26	1.73	1.55	1.76	2.19	1.15	1.89	1.50	1.56	1.20	1.66	0.37
SSD[3], cm	13.35	14.50	9.05	13.02	9.51	9.27	11.56	12.68	16.97	10.21	8.54	15.15	14.07	9.23	7.18	10.90	1.90
SSU[3], cm	10.92^{cd}	9.70^{bc}	6.97^{ab}	9.35^{bc}	7.37^{abc}	8.16^{abc}	9.17^{bc}	8.69^{bc}	13.88^{d}	8.72^{abc}	5.17^{a}	9.43^{bc}	8.91^{bc}	6.52^{ab}	6.05^{ab}	8.01^{abc}	1.40
Belly Thickness, mm	30.80^{a}	39.83^{abc}	37.43^{abc}	32.67^{a}	35.95^{ab}	35.95^{ab}	39.32^{abc}	32.87^{a}	36.75^{abc}	35.26^{ab}	37.03^{abc}	43.89^{bc}	45.97^{c}	35.62^{ab}	33.96^{ab}	38.12^{abc}	3.90

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

^{abcde} =Means within the same row with common superscripts do not differ. ($P>0.05$)

Table 3. Carcass Trait P-Values

Trait[1]	P>F						
	Lipid	Vitamin E	Sex	L x S	L x V	V x S	L x S x V
HCW, kg							
LRFT, mm		0.0481		0.0031		0.0206	
TRFT, mm		0.0068		0.0160	0.0016		
LEA, cm^2							
FFL, %				0.0164	0.0028		
L*			0.0265				
a*							0.0193
b*							
pHH							
pHL							0.0007
MS				0.0362			
NPPC Color[2]							
NPPC Marbling[2]		0.0066					
SSD[3], cm						0.0003	
SSU[3], cm				0.0335		0.0018	0.0364
Belly Thickness, mm							0.0198

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

Table 4. Interaction between Lipid Content, Vitamin E Concentration, and Sex of Pig Least Squares Means for Meat Quality Traits

	Lipid Content																
	0				2				4				6				
	F		M		F		M		F		M		F		M		
Trait[1]	11	220	11	220	11	220	11	220	11	220	11	220	11	220	11	220	SEM
DL, %	4.21	2.98	4.97	4.01	4.16	2.85	5.67	3.98	2.50	2.78	3.86	2.58	3.86	3.68	2.99	4.55	0.72
VP, %	9.31^{bc}	8.58^{bc}	11.11^{acd}	7.64^{b}	12.66^{a}	9.74^{bc}	8.07^{bd}	11.20^{acd}	10.24^{ac}	9.22^{bc}	13.23^{a}	9.29^{bc}	7.94^{bd}	9.54^{bc}	11.41^{ac}	10.58^{acd}	1.28
MU, %	12.25	9.24	11.10	13.57	12.00	9.86	9.30	9.09	12.66	15.76	6.75	13.77	12.54	20.22	10.03	10.76	3.75
MCL, %	19.19	23.04	21.57	19.72	20.74	21.01	23.27	24.46	20.84	18.23	23.79	18.97	18.57	11.62	21.25	21.69	2.72
WBS, kg	4.79	5.07	4.53	4.39	4.31	4.96	4.07	3.29	4.32	3.90	4.01	4.24	4.76	3.79	4.64	3.44	0.58
CL, %	15.78	17.78	15.63	16.92	14.48	16.02	17.43	17.00	14.97	16.23	21.46	16.67	14.31	9.51	16.90	15.11	1.66
TBARS	0.15	0.16	0.20	0.17	0.22	0.17	0.21	0.18	0.15	0.20	0.21	0.17	0.19	0.18	0.18	0.22	0.02
Collagen, %	1.53	1.47	1.52	1.44	1.42	1.42	1.48	1.46	1.59	1.50	1.54	1.48	1.51	1.47	1.53	1.47	0.10
Fat, %	3.93	4.15	3.96	4.49	4.73	3.39	4.72	4.88	4.13	3.99	4.38	4.45	3.81	4.37	4.02	3.66	0.53
Moisture, %	76.41	75.83	76.45	75.64	75.69	76.74	75.56	75.27	75.45	75.66	75.79	75.33	76.28	75.74	75.58	76.63	0.47
Protein, %	26.11	26.27	26.44	25.84	26.36	26.65	26.37	26.24	26.36	26.59	26.63	26.33	26.11	25.87	26.44	26.86	0.36
Salt, %	0.78	0.75	0.79	0.73	0.64	0.75	0.78	0.72	0.70	0.75	0.67	0.68	0.71	0.75	0.73	0.69	0.07

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

^{abcde} =Means within the same row with common superscripts do not differ. (P>0.05)

Table 5. Meat Quality Trait P-Values

Trait[1]	P>F						
	Lipid	Vitamin E	Sex	L x S	L x V	V x S	L x S x V
DL, %							
VP, %							0.0167
MU, %							
MCL, %							
WBS, kg							
CL, %	0.0228		0.0220				
TBARS							
Collagen, %							
Fat, %							
Moisture, %							
Protein, %							
Salt, %							

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

Table 6. Interaction between Trial, Lipid Content, and Vitamin E Concentration Least Squares Means for Meat Quality Traits

		Lipid Content								
		0		2		4		6		
Trait[1]	Trial	11	220	11	220	11	220	11	220	SEM
DL, %	1	3.75^{abe}	3.37^{abcd}	3.74^{abce}	4.53^{ce}	3.11^{abc}	2.53^{ab}	2.58^{ab}	3.10^{abc}	0.74
	2	5.43^{ef}	3.61^{abce}	6.09^{f}	2.30^{a}	3.25^{abc}	2.84^{abc}	4.27^{bce}	5.14^{def}	
VP, %	1	10.45	8.13	10.44	10.55	13.84	8.24	10.93	9.89	1.23
	2	9.97	8.09	10.29	10.38	9.62	10.28	8.43	10.23	
MU, %	1	12.70	11.00	11.16	7.55	7.96	15.15	11.23	19.21	3.62
	2	10.65	11.81	10.14	11.40	11.45	14.38	11.34	11.78	
MCL	1	20.79	21.29	22.48	25.05	23.91	17.62	19.94	14.10	2.62
	2	19.97	21.47	21.53	20.43	20.73	19.58	19.89	19.22	
WBS, kg	1	5.32	4.63	4.06	4.32	4.77	4.56	4.63	3.38	0.56
	2	4.00	4.84	4.31	3.94	3.55	3.58	4.76	3.84	
CL, %	1	16.34^{bc}	17.53^{bcd}	14.40^{abc}	18.04^{cd}	21.58^{d}	16.56^{bc}	15.16^{abc}	11.05^{a}	1.61
	2	15.08^{abc}	17.17^{bc}	17.52^{bcd}	14.98^{abc}	14.85^{abc}	16.34^{bc}	16.06^{bc}	13.56^{ab}	
TBARS	1	0.24	0.20	0.27	0.18	0.22	0.24	0.20	0.22	0.02
	2	0.11	0.14	0.16	0.17	0.15	0.12	0.17	0.19	
Collagen, %	1	1.52	1.48	1.27	1.49	1.53	1.50	1.44	1.50	0.09
	2	1.54	1.44	1.63	1.40	1.59	1.48	1.61	1.44	
Fat, %	1	3.89	3.87	4.44	4.12	4.64	4.42	3.94	4.18	0.51
	2	4.00	4.77	5.00	4.15	3.86	4.02	3.88	3.85	
Moisture, %	1	76.69	76.40	75.83	76.22	75.85	75.67	75.89	75.92	0.45
	2	76.18	75.07	75.42	75.79	75.39	75.32	75.97	76.45	
Protein, %	1	26.20	26.11	26.87	26.31	26.32	26.53	26.60	26.49	0.35
	2	26.35	26.00	25.87	26.58	26.66	26.39	25.95	26.24	
Salt, %	1	0.81	0.81	0.68	0.74	0.66	0.72	0.69	0.70	0.06
	2	0.76	0.67	0.74	0.73	0.70	0.71	0.75	0.74	

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

^{abcde} = Means within the same rows for trials 1 and 2 within trait with common superscripts do not differ. ($P>0.05$)

Table 7. Meat Quality Trait P-Values

	P>F						
Trait[1]	Trial	Lipid	Vitamin E	L x V	T x L	T x V	T x L x V
DL, %	0.0282					0.0490	0.0471
VP, %						0.0418	
MU, %							
MCL, %							
WBS, kg							
CL, %		0.0228					0.0305
TBARS	<0.0001						
Collagen, %						0.0225	
Fat, %							
Moisture, %							
Protein, %							
Salt, %							

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

Table 8. Interaction between Trial and Sex of Pig Least Squares Means for Carcass and Meat Quality Traits

	Trial					P>F
	1		2			
Trait[1]	F	M	F	M	SEM	T x S
HCW, kg	83.7	85.9	80.6	82.9	1.55	
LRFT, mm	21.54[ac]	23.11[bc]	24.25[b]	20.28[a]	1.10	0.0034
TRFT, mm	20.63	20.17	21.09	19.87	1.06	
LEA, cm^2	42.93	43.15	41.09	41.96	1.85	
FFL, %	52.33	52.53	51.87	52.45	0.80	
L*	59.68	62.28	58.04	60.72	1.12	
a*	8.80	8.25	8.96	8.92	0.35	
b*	16.29	16.34	16.02	16.22	0.36	
pHH	5.83	5.87	5.53	5.53	0.03	
pHL	5.65	5.69	5.45	5.45	0.02	
MS	2.68	2.45	2.44	2.25	0.13	
NPPC Color[3]	2.96	2.71	3.38	3.09	0.21	
NPPC Marbling[3]	2.00	1.58	1.46	1.51	0.20	
SSD[4], cm	10.70	10.99	13.58	11.03	1.06	
SSU[4], cm	8.79	8.14	9.76	7.57	0.78	
Belly Thickness, mm	36.72	37.35	37.31	36.47	2.17	
Trait[2]						
DL, %	3.22	3.46	3.54	4.70	0.43	
VP, %	9.32[ab]	11.30[b]	9.99[ab]	9.33[a]	0.71	0.0286
MU, %	14.94	9.05	11.19	12.05	2.08	
MCL, %	18.30	23.00	20.02	20.68	1.51	
WBS, kg	4.76	4.16	4.21	3.99	0.32	
CL, %	14.32[a]	18.34[b]	15.45[a]	15.94[a]	0.93	0.0248
TBARS	0.21	0.23	0.15	0.16	0.01	
Collagen, %	1.44	1.49	1.54	1.50	0.05	
Fat, %	3.80	4.58	4.33	4.06	0.30	
Moisture, %	76.38[b]	75.74[ab]	75.57[a]	75.83[ab]	0.26	0.0390
Protein, %	26.27	26.58	26.31	26.20	0.20	
Salt, %	0.74	0.71	0.72	0.73	0.04	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

[3] National Pork Producers Council standards (2000)

[4] Pork belly firmness assessment

[abc] = Means within the same row with common superscripts do not differ. (P>0.05)

Table 9. Interaction between Trial and Vitamin E Concentration Least Squares Means for Carcass and Meat Quality Traits

	Trial					P>F
	1		2			
Trait[1]	11	220	11	220	SEM	T x V
HCW, kg	85.8	83.8	81.7	81.8	1.30	
LRFT, mm	20.99	23.67	21.83	22.70	0.92	
TRFT, mm	18.29[a]	22.52[b]	20.23[a]	20.73[ab]	0.88	0.0318
LEA, cm^2	43.91	42.18	40.37	42.68	1.54	
FFL, %	53.62[b]	51.23[a]	51.97[ab]	52.35[ab]	0.67	0.0350
L*	61.65	60.32	58.95	59.82	0.94	
a*	8.48	8.56	9.07	8.81	0.29	
b*	16.47	16.16	16.09	16.15	0.30	
pHH	5.82	5.89	5.55	5.52	0.03	
pHL	5.67	5.67	5.46	5.44	0.02	
MS	2.70[b]	2.44[ab]	2.25[a]	2.45[ab]	0.11	0.0304
NPPC Color[3]	2.82	2.85	3.34	3.13	0.17	
NPPC Marbling[3]	1.56	2.02	1.25	1.71	0.17	
SSD[4], cm	9.28[a]	12.41[b]	13.28[b]	11.33[ab]	0.89	0.0042
SSU[4], cm	7.78	9.15	9.33	8.00	0.65	
Belly Thickness, mm	36.12	37.96	38.18	35.60	1.81	
Trait[2]						
DL, %	3.29[a]	3.38[a]	4.76[b]	3.47[a]	0.36	0.0490
VP, %	11.42[b]	9.20[a]	9.58[a]	9.75[a]	0.60	0.0418
MU, %	10.76	13.23	10.89	12.34	1.74	
MCL, %	21.78	19.51	20.53	20.17	1.27	
WBS, kg	4.70	4.22	4.16	4.05	0.27	
CL, %	16.87	15.79	15.88	15.51	0.77	
TBARS	0.23	0.21	0.15	0.15	0.01	
Collagen, %	1.44[a]	1.49[ab]	1.59[b]	1.44[a]	0.04	0.0225
Fat, %	4.23	4.14	4.19	4.20	0.25	
Moisture, %	76.06	76.05	75.74	75.66	0.22	
Protein, %	26.50	26.36	26.21	26.30	0.17	
Salt, %	0.71	0.74	0.74	0.71	0.03	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

[3] National Pork Producers Council standards (2000)

[4] Pork belly firmness assessment

[ab] = Means within the same row with common superscripts do not differ. (P>0.05)

Table 10. Interaction between Vitamin E Concentration and Sex of Pig Least Squares Means for Carcass Traits

	Vitamin E					
	11		220			P>F
Trait[1]	F	M	F	M	SEM	V x S
HCW, kg	82.9	84.6	81.3	84.2	1.40	
LRFT, mm	23.05[bc]	19.77[a]	22.74[ac]	23.63[bc]	1.00	0.0206
TRFT, mm	20.12	18.40	21.60	21.65	0.96	
LEA, cm^2	40.89	43.39	43.14	41.72	1.67	
FFL, %	52.11	53.48	52.10	51.49	0.72	
L*	58.75	61.85	58.99	61.15	1.01	
a*	8.81	8.74	8.94	8.42	0.32	
b*	16.13	16.43	16.18	16.13	0.33	
pHH	5.67	5.70	5.70	5.71	0.03	
pHL	5.55	5.58	5.56	5.56	0.02	
MS	2.53	2.42	2.60	2.29	0.12	
NPPC Color[2]	3.19	2.97	3.15	2.83	0.19	
NPPC Marbling[2]	1.49	1.32	1.97	1.77	0.18	
SSD[3], cm	13.47[b]	9.08[a]	10.80[ac]	12.93[bc]	0.96	0.0003
SSU[3], cm	10.27[b]	6.84[a]	8.28[ac]	8.87[bc]	0.70	0.0018
Belly Thickness, mm	37.27	36.94	36.67	36.89	1.96	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

[abc] = Means within the same row with common superscripts do not differ. (P>0.05)

Table 11. Interaction between Lipid Content and Vitamin E Concentration Least Squares Means for Carcass Traits

	Lipid									
	0		2		4		6			P>F
Trait[1]	11	220	11	220	11	220	11	220	SEM	L x V
HCW, kg	82.1	82.7	83.1	82.00	85.5	83.3	84.3	83.1	1.91	
LRFT, mm	21.68	23.86	23.37	22.29	21.01	25.18	19.59	21.41	1.36	
TRFT, mm	16.69^{a}	23.07^{b}	18.96^{ab}	21.87^{b}	19.90^{ab}	23.43^{b}	21.48^{bc}	18.12^{ac}	1.30	0.0016
LEA, cm^2	43.90	40.34	44.01	43.20	40.80	41.13	39.85	45.06	2.28	
FFL, %	54.58^{b}	50.49^{a}	53.43^{bc}	51.99^{ab}	52.05^{ab}	50.56^{a}	51.12^{ac}	54.13^{b}	0.98	0.0028
L*	59.62	60.24	62.13	60.42	60.83	60.17	58.61	59.44	1.38	
a*	8.65	9.07	8.79	8.37	8.60	8.40	9.06	8.90	0.43	
b*	16.03	16.68	16.64	16.17	16.33	16.01	16.12	15.77	0.44	
pHH	5.71	5.72	5.69	5.72	5.67	5.70	5.65	5.68	0.04	
pHL	5.61	5.54	5.54	5.58	5.53	5.51	5.58	5.61	0.03	
MS	2.56	2.25	2.44	2.32	2.57	2.62	2.32	2.59	0.16	
NPPC Color[2]	3.04	2.97	3.00	3.03	3.12	2.89	3.16	3.08	0.25	
NPPC Marbling[2]	1.46	1.90	1.37	1.91	1.45	2.04	1.35	1.61	0.25	
SSD[3], cm	11.20	13.76	10.53	10.98	12.76	12.68	10.62	10.06	1.31	
SSU[3], cm	8.94	9.53	8.27	8.43	9.53	9.08	7.48	7.26	0.96	
Belly Thickness, mm	34.12	36.25	37.64	34.41	36.89	39.58	39.97	36.87	2.67	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

[abc] = Means within the same row with common superscripts do not differ. (P>0.05)

Table 12. Interaction between Lipid Content and Sex of Pig Least Squares Means for Carcass Traits

	Lipid									
	0		2		4		6			P>F
Trait[1]	F	M	F	M	F	M	F	M	SEM	L x S
HCW, kg	81.8	83.1	81.2	83.9	83.7	85.2	81.9	85.5	2.06	
LRFT, mm	25.52^{bd}	20.02^{ac}	21.63^{ac}	24.02^{bd}	22.08^{acd}	24.10^{bd}	22.35^{bc}	18.65^{a}	1.47	0.0031
TRFT, mm	21.57^{ac}	18.19^{a}	18.88^{ac}	21.96^{bc}	21.36^{ac}	21.96^{bc}	21.63^{bc}	17.97^{a}	1.41	0.0160
LEA, cm^2	41.47	42.76	45.60	41.60	41.21	40.72	39.76	45.14	2.46	
FFL, %	51.60^{ab}	53.47^{ab}	54.05^{b}	51.37^{a}	51.52^{ab}	51.10^{a}	51.23^{a}	54.03^{b}	1.06	0.0164
L*	59.32	60.54	60.26	62.29	59.14	61.86	56.74	61.31	1.49	
a*	8.75	8.97	8.61	8.55	8.67	8.33	9.47	8.49	0.46	
b*	16.23	16.48	16.23	16.58	16.25	16.09	15.91	15.97	0.48	
pHH	5.69	5.74	5.72	5.68	5.65	5.72	5.67	5.66	0.04	
pHL	5.54	5.60	5.57	5.55	5.48	5.56	5.61	5.57	0.03	
MS	2.55^{ab}	2.26^{ab}	2.69^{b}	2.07^{a}	2.68^{ab}	2.51^{a}	2.34^{a}	2.57^{b}	0.17	0.0362
NPPC Color[2]	2.96	3.04	3.17	2.87	3.27	2.74	3.28	2.96	0.27	
NPPC Marbling[2]	1.78	1.59	1.64	1.64	1.98	1.52	1.53	1.43	0.27	
SSD[3], cm	13.93	11.03	9.39	12.12	13.59	11.84	11.65	9.03	1.41	
SSU[3], cm	10.31^{bc}	8.16^{ac}	7.77^{ac}	8.93^{abc}	11.30^{b}	7.30^{a}	7.71^{ac}	7.03^{a}	1.03	0.0335
Belly Thickness, mm	35.32	35.05	35.95	36.09	36.01	40.46	40.79	36.04	2.89	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

[abcd] = Means within the same row with common superscripts do not differ. (P>0.05)

Table 13. Carcass Traits Least Squares Means for Main Effects

Trait[1]	Trial			Sex			Vitamin E			Lipid				
	1	2	SEM	F	M	SEM	11	220	SEM	0	2	4	6	SEM
HCW, kg	84.8[a]	81.8[b]	0.91	82.1	84.4	1.07	83.7	82.8	0.89	82.4	82.5	84.4	83.7	1.35
LRFT, mm	22.33	22.27	0.64	22.90	21.70	0.76	21.41[a]	23.19[b]	0.63	22.77	22.83	23.09	20.50	0.96
TRFT, mm	20.40	20.48	0.62	20.86	20.02	0.73	19.26[a]	21.62[b]	0.60	19.88	20.42	21.66	19.80	0.92
LEA, cm^2	43.04	41.53	1.08	42.01	42.56	1.28	42.14	42.43	1.06	42.12	43.60	40.97	42.45	1.61
FFL, %	52.43	52.16	0.47	52.10	52.49	0.55	52.80	51.79	0.46	52.54	52.71	51.31	52.63	0.70
L*	60.98	59.38	0.65	58.86[a]	61.50[b]	0.77	60.30	60.07	0.64	59.93	61.27	60.50	59.03	0.98
a*	8.52	8.94	0.20	8.88	8.58	0.24	8.78	8.68	0.20	8.86	8.58	8.50	8.98	0.30
b*	16.32	16.12	0.21	16.16	16.28	0.25	16.28	16.16	0.21	16.36	16.41	16.17	15.94	0.31
pHH	5.85[a]	5.53[b]	0.02	5.68	5.70	0.02	5.68	5.71	0.02	5.71	5.70	5.69	5.67	0.03
pHL	5.67[a]	5.45[b]	0.01	5.55	5.57	0.02	5.56	5.56	0.01	5.57	5.56	5.52	5.59	0.02
MS	2.57[a]	2.35[b]	0.07	2.56	2.35	0.09	2.47	2.44	0.07	2.40	2.38	2.59	2.45	0.11
NPPC Color[2]	2.84[a]	3.24[b]	0.12	3.17	2.90	0.14	3.08	2.99	0.12	3.00	3.02	3.00	3.12	0.18
NPPC Marbling[2]	1.79	1.48	0.12	1.73	1.54	0.14	1.41[a]	1.87[b]	0.12	1.68	1.64	1.75	1.48	0.18
SSD[3], cm	10.84	12.30	0.62	12.14	11.01	0.73	11.28	11.87	0.61	12.48	10.76	12.72	10.34	0.92
SSU[3], cm	8.47	8.66	0.45	9.27	7.86	0.54	8.55	8.57	0.44	9.24	8.35	9.30	7.37	0.68
Belly Thickness, mm	37.04	36.89	1.27	37.02	36.91	1.50	37.15	36.78	1.24	35.19	36.02	38.23	38.42	1.89

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

[ab] = Means within the same row and main effect with common superscripts do not differ. ($P>0.05$)

Table 14. Meat Quality Traits Least Squares Means for Main Effects

Trait[1]	Trial			Sex			Vitamin E			Lipid				
	1	2	SEM	F	M	SEM	11	220	SEM	0	2	4	6	SEM
DL, %	3.34[a]	4.12[b]	0.25	3.38	4.08	0.29	4.03	3.43	0.24	4.04	4.17	2.93	3.77	0.37
VP, %	10.32	9.66	0.42	9.65	10.32	0.49	10.50	9.47	0.41	9.16	10.42	10.50	9.87	0.62
MU, %	11.99	11.62	1.22	13.07	10.55	1.44	10.83	12.78	1.19	11.54	10.06	12.23	13.39	1.82
MCL, %	20.65	20.35	0.88	19.16	21.84	1.05	21.15	19.84	0.87	20.88	22.37	20.46	18.28	1.32
WBS, kg	4.46	4.10	0.19	4.49	4.08	0.22	4.43	4.14	0.18	4.70	4.16	4.12	4.15	0.28
CL, %	16.33	15.69	0.54	14.89[a]	17.14[b]	0.64	16.37	15.65	0.53	16.53[a]	16.23[a]	17.33[a]	13.96[b]	0.81
TBARS	0.22[a]	0.15[b]	0.01	0.18	0.19	0.01	0.19	0.18	0.01	0.17	0.19	0.18	0.19	0.01
Collagen, %	1.46	1.52	0.03	1.49	1.49	0.04	1.52	1.47	0.03	1.49	1.45	1.53	1.50	0.05
Fat, %	4.19	4.19	0.17	4.06	4.32	0.20	4.21	4.17	0.17	4.13	4.43	4.24	3.96	0.26
Moisture, %	76.06	75.70	0.15	75.97	75.78	0.18	75.90	75.86	0.15	76.08	75.82	75.56	76.06	0.23
Protein, %	26.43	26.26	0.12	26.29	26.39	0.14	26.35	26.33	0.12	26.17	26.41	26.48	26.32	0.18
Salt, %	0.73	0.73	0.02	0.73	0.72	0.03	0.72	0.73	0.02	0.76	0.72	0.70	0.72	0.03

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

[ab] = Means within the same row and main effect, with common superscripts do not differ. (P>0.05)

Table 15. Least Squares Means for Sensory Panel Evaluation

Sensory Attribute*	Trial			Sex			Vitamin E			Lipid				
	1	2	SEM	F	M	SEM	11	220	SEM	0	2	4	6	SEM
Initial Juiciness	5.84	5.44	0.19	5.59	5.69	0.09	5.64	5.64	0.08	5.75	5.44	5.65	5.71	0.12
Sustained Juiciness	5.63	5.33	0.19	5.45	5.51	0.09	5.46	5.50	0.08	5.57	5.32	5.53	5.51	0.12
Initial Tenderness	5.30	5.32	0.24	5.18	5.44	0.12	5.22	5.40	0.10	5.40	5.18	5.50	5.16	0.15
Sustained Tenderness	5.24	5.16	0.25	5.04	5.36	0.12	5.08	5.32	0.10	5.26	5.07	5.39	5.08	0.16
Pork Flavor Intensity	5.83	5.91	0.14	5.84	5.90	0.07	5.85	5.89	0.06	5.92	5.75	5.86	5.96	0.09
Off-Flavor Intensity	7.78	7.68	0.11	7.70	7.74	0.06	7.67	7.78	0.05	7.76	7.66	7.67	7.80	0.07

*No significant differences were observed for any sensory attribute within any dietary treatment.

Table 16. Treatment Representation per Harvest Day

	Trial								
	1				2				
	DOH*								
% Lipid	1	2	3	4	5	6	7	8	N
0	0	4	4	4	2	2	2	6	24
2	2	2	2	6	4	2	2	4	24
4	2	0	6	4	2	6	4	0	24
6	2	6	2	2	3	6	0	2	23
Vitamin E									
11	2	8	6	8	6	10	0	8	48
220	4	4	8	8	5	6	8	4	47
Sex									
F	0	6	4	14	3	6	4	10	47
M	6	6	10	2	8	10	4	2	48
N	6	12	14	16	11	16	8	12	

*DOH = Harvest period n=8

Table 17. Simple Means of Days on Feed, Age at Harvest, and Hot Carcass Weight Across Slaughter Groups

	Trial							
	1				2			
	Harvest Group							
DOH*	1	2	3	4	5	6	7	8
Age at Harvest	154	161	168	182	153	160	167	174
Days on Feed	65	72	79	93	72	79	86	93
HCW	83.7	81.8	84.1	86.8	80.9	82.4	82.6	81.8

*DOH = Harvest period n=8

Figure 1. Interaction between Trial, Lipid Content, Vitamin E Concentration, and Sex of Pig for Skin-Side-Up Belly Measurement

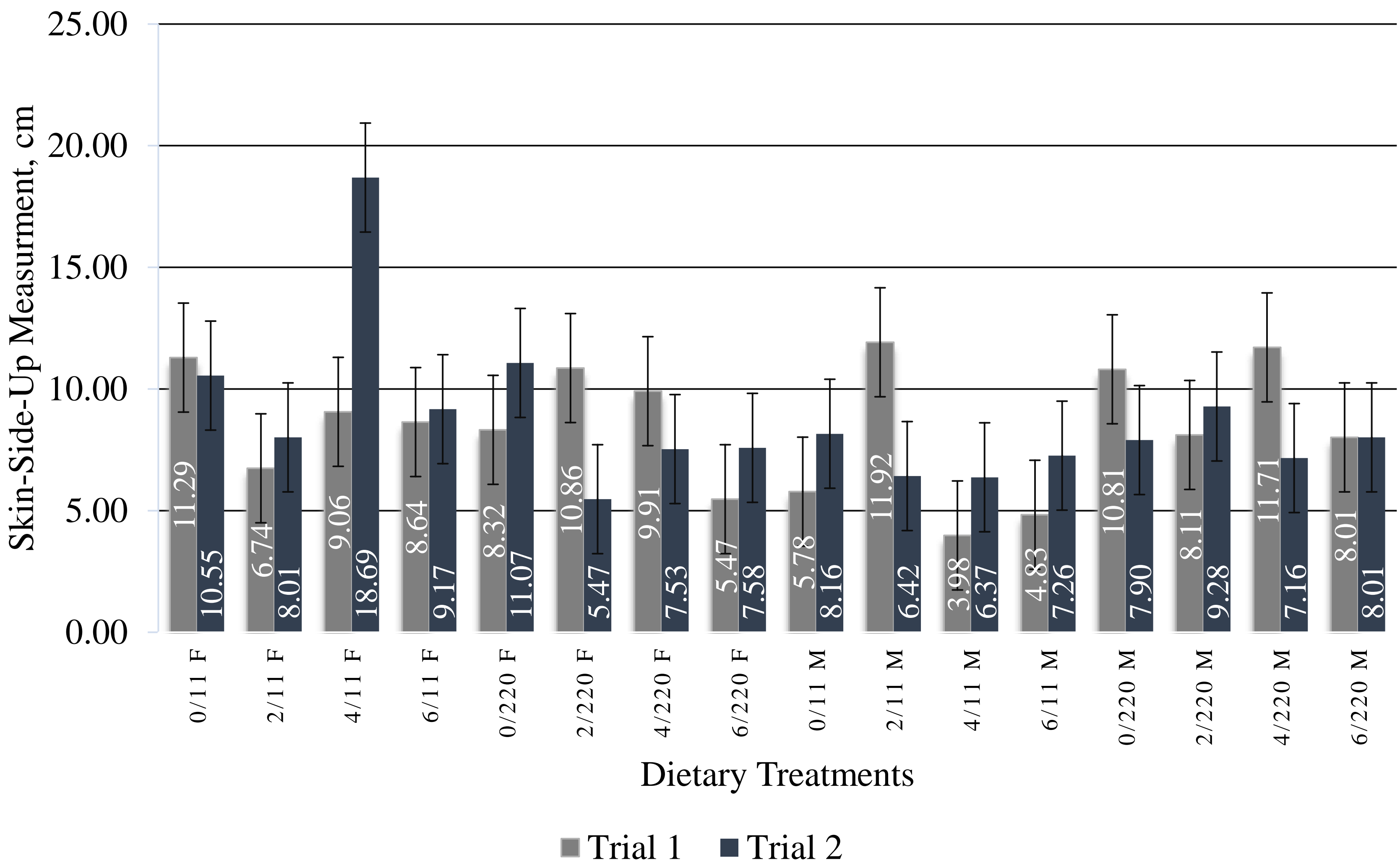


Figure 2. Interaction between Trial, Lipid Content, Vitamin E Concentration, and Sex of Pig for Cook Loss

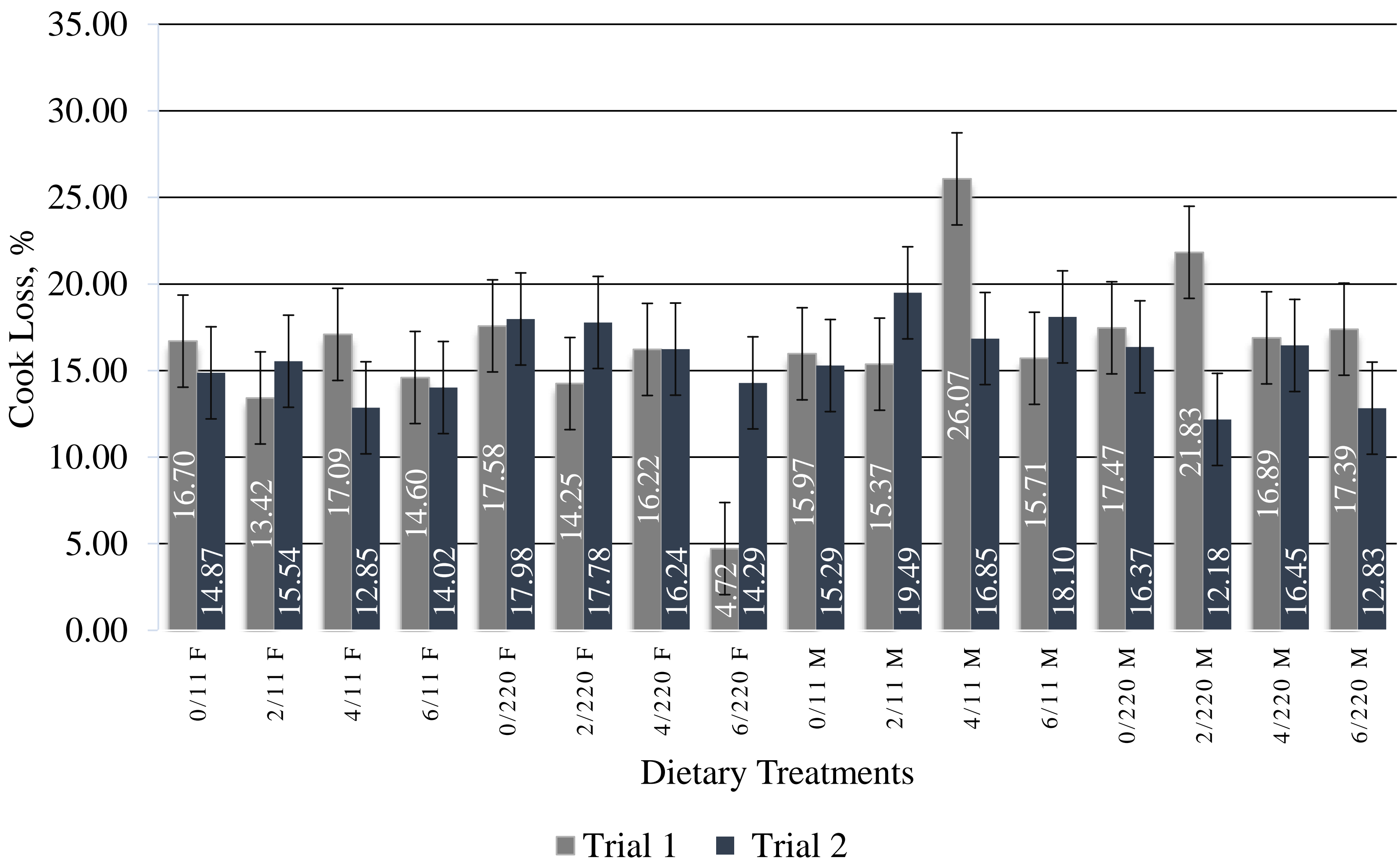
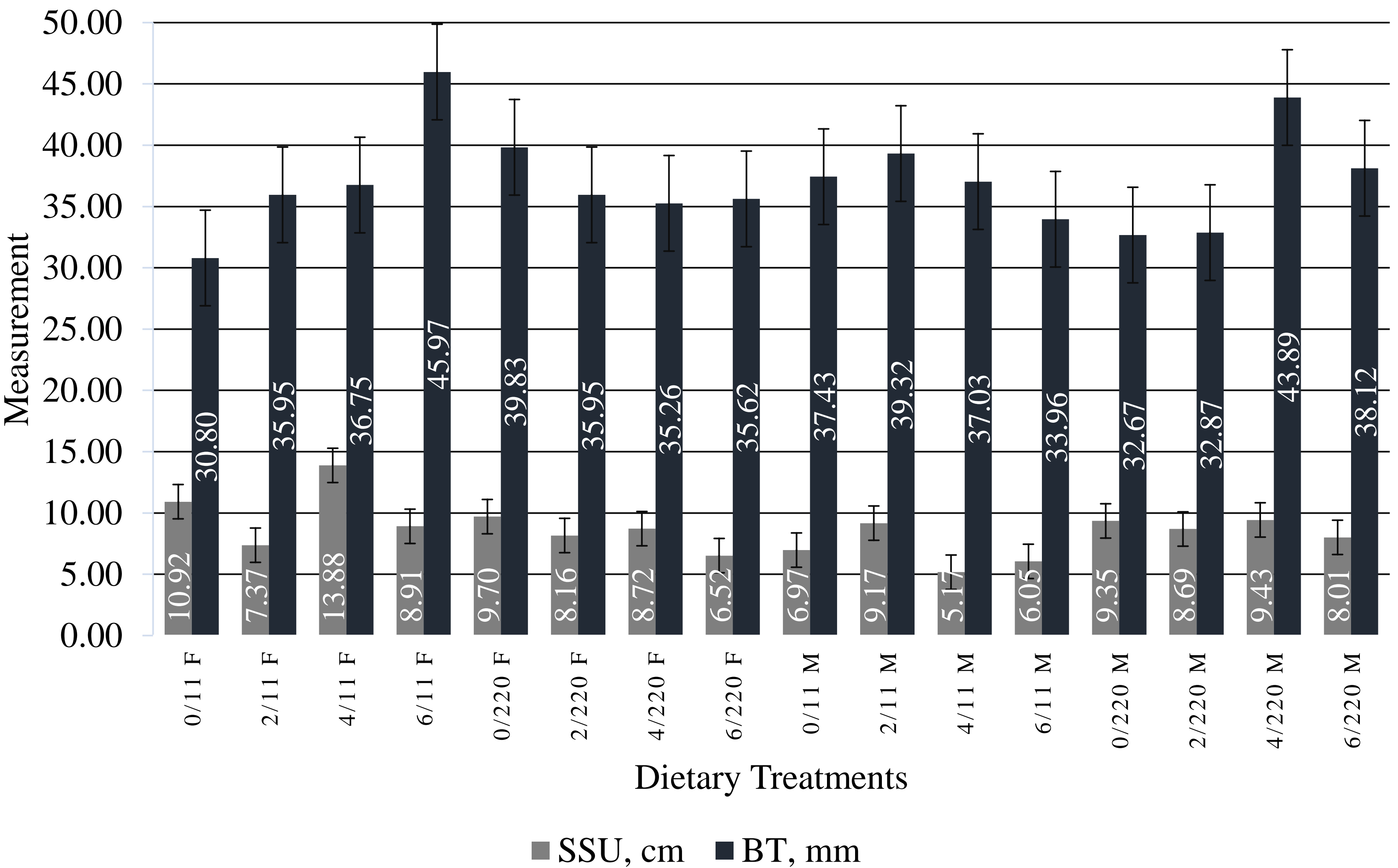


Figure 3. Interaction between Lipid Content, Vitamin E Concentration, and Sex of Pig for Skin-Side-Up and Belly Thickness Measurements



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Appendices

Appendix A

48 Hour Drip Loss

National Pork Producers Council Publication. 2000. Pork Composition and Quality Assessment Procedures. Des Moines, IA

1. Post-rigor, fresh muscle of choice (usually the *longissimus*) is sectioned perpendicular to fiber orientation. Each section should be 4 cm think and taken from a standards location. Duplicate analysis from adjacent section is recommended.
2. Using a stainless-steel coring device, 4 cm in diameter, cut sample from center of the section. The sample should weigh at least 40 g to 50 g (24±2 g in present study). It is very important that the weight be standardized within a reasonable range (10g) to maintain a constant surface area to volume ratio. Small samples, (20 g) as compared to large ones (75 g), will have larger surface area/volume ratio and, thus, will lose proportionally more fluids.
3. Weigh cored sample to neatest 0.1 g (0.01 g in our study) on a balance.
4. Suspend sample on a hook (S-hook or fish hook works well) and contain it in a plastic bag, freezer container, or wax-coated box. Insure that container does NOT touch the sample and that the humidity and airflow remain constant. The temperature needs to be constant (usually 2-4°C). It is important that the samples are fresh and NOT frozen.

5. Keep sample at 4°C for 48 hr. Other times such as 24 or 72 hr are appropriate to insure differences in water holding capacity for a given test. However, the 48 hr is recommended and should be used when comparing results with other laboratories.
6. After 48 hr, remove sample from hook, blot (do not squeeze) remaining surface fluids twice with paper toweling, and weigh to nearest 0.1 g (0.01 g in our study).
7. Percentage drip is calculated by dividing loss in weight (due to drip) by initial weight x 100. Duplicate values should agree to < 10%.
8. For longissimus sample taken at 24 hr postmortem suspend for hr at 4°C the following % drip loss values serve as guidelines for establishing quality category*:
    a. RSE and PSE: > 6%
    b. RFN and DFD: < 6% (DFD mostly < 2%).

*DFD = Dark, Firm, and Dry; RFN = Reddish-pink, Firm, and Nonexudative; RSE = Reddish-pink, Soft, and Exudative; PSE = Pale, Soft and Exudative.

$$DL = \frac{(Loss\ in\ weight,\ \ g)}{(Initial\ weight,\ \ g)}\ x\ 100$$

Appendix B

Marinade Uptake

National Pork Producers Council Publication. 2000. Pork Composition and Quality Assessment Procedures. Des Moines, IA

1. Remove external fat from muscle. Subsequently, grind meat through 6.4-mm (1/4inch) plate. Conduct all measurements in triplicate.
2. Weigh and number 50 mL centrifuge tubes (without cap). Record the weight of tubes to second decimal place (0.01 g).
3. Weigh 6.00 ± 0.01g of ground meat into each centrifuge tube.
4. Add 10 mL of reagent buffer (3.5% NaCl = 35 g NaCl in 1 liter of water).
5. Place screwcap on tightly and shake gently until samples break apart.
6. Vigorously shake an additional 15 seconds.
7. Place tubes in water bath for 30 minutes 'incubation' at 25°C.
8. After incubation, centrifuge for 20 minutes at 3000 rpm (= 800 x g).
9. Remove cap and put tube upside down to drain water for 5 minutes.
10. Weigh samples and tubes (without screw cap), and record to the second decimal place (0.01 g).

$$MU = \frac{[(\text{Weight of tube and meat after incubation at 25°C, g}) - (\text{Initial weight of tube and meat, g})]}{6.00\ g}\ x\ 100$$

Appendix C

Marinade Cook Loss

National Pork Producers Council Publication. 2000. Pork Composition and Quality Assessment Procedures. Des Moines, IA

1. Loosely cap drained tubes and place rack of tubes into 80°C (preheated) water bath for 20-minutes (time sharply).
2. Remove and drain cook-out water and completely cool samples to 20-22°C.
3. Weigh the tube and meat (without screw cap) and record weight to 0.01 g.
4. Discard tubes and sample.

$$MCL = \frac{[(Weight\ of\ tube\ and\ meat\ after\ cooking,\ g) - (Initial\ weight\ of\ tube\ and\ meat,\ g)]}{6.00\ g}\ x\ 100$$

Appendix D

Vacuum Purge Loss

1. Remove vacuum-sealed samples from the freezer and place in 4°C for 48 hours to thaw.
2. Tare scale to account for weight of vacuum bag and I.D. tag.
3. Weigh thawed sample while sealed in vacuum bag, record weight to the nearest 0.01 g.
4. Remove samples from vacuum bag, weigh, and record weight to the nearest 0.01 g.

$$VP = \frac{[(Weight\ of\ thawed\ sample\ in\ bag,\ \ g) - (\text{Weight of thawed sample removed from bag, g})]}{(\text{Weight of thawed sample in bag, g})}\ x\ 100$$

Appendix E

Thiobarbituric Acid Reactive Substance Assay

1. Remove external fat from muscle. Obtain a 5.00 g ± 0.01g sample of meat.
2. Blend sample in Waring blender with 30 mL of deionized water for 1 minute.
3. Transfer blended sample to a 250 mL round bottom flask.
4. Rinse blender cup with additional 20mL of deionized water and transfer to the same 250 mL round bottom flask.
5. Add 2.5 mL of 4N HCL and 3-5 drops of Antifoam B® Silicone Emulsion (Avantor Performance Materials, Inc., Center Valley, PA USA) to the sample.
6. Distilled the sample at maximum rate and collect 25 mL of distillate in a 25 mL volumetric flask.
7. Pipette 5 mL of distillate into 50 mL presterilized centrifuge tube with screw cap. Perform in duplicate.
8. Add 5 mL of 0.02 M 2-thiobarbituric acid in 90% acetic acid to distillate.
9. Tighten caps completely and mix using a vortex mixer for 5 seconds.
10. Place the tubes, sealed tightly, into boiling water bath for 30 minutes.
11. Remove from water bath and allow sample to cool to room temperature.
12. Measure the absorbance at 532 nm using a spectrophotometer.

13. Calculate K values using 1, 1, 3, 3,-tetraethoxypropane as the standard (7.8) and calculate TBARS values.

$$TBARS\ Value = (Absorbance\ value) x\ (K\ value\ or\ 7.8)$$

Distillation Set-Up:

TEP Solution:

TEP FW: 220.3

Standard Solution: 0.2203 g → 1000 mL is $1 * 10^{-3}$ mol/mL

100 µl = $1 * 10^{-7}$ mol/mL

200 µl = $2 * 10^{-7}$ mol/mL

400 µl = $4 * 10^{-7}$ mol/mL

500 µl = $5 * 10^{-7}$ mol/mL

700 µl = $7 * 10^{-7}$ mol/mL

1. Prepare stock $1 * 10^{-3}$ solution (refrigerate up to a week, if needed).
2. Create dilutions using the amounts provided above by adding the solution and bringing to volume at 100 mL. Distill 50 mL, add 5 mL TBA and 5 mL of distilled solution to test tube. Heat in water bath for 35 minutes. After heating, place in ice bath for 10 minutes. Read at 532 absorbance.

Appendix F

Warner-Bratzler Shear Force and Cook Loss

American Meat Science Association (AMSA) Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat
AMSA, Champaign, Illinois, USA

Standard Equipment:

Warner-Bratzler Shear Force can be performed using the original Warner-Bratzler shear machine or an automated testing machine (Instron, United, Texture Technologies, etc.) with a Warner-Bratzler shear blade and crosshead speed of 200 or 250 mm/minute.

Warner-Bratzler shear blade specifications include:

1. Blade thickness of 1.1684 mm (0.046 inches)

2. V-notched (60°angle) cutting blade,

3. Cutting edge beveled to a half-round

4. Corner of the V rounded to a quarter-round of a 2.363 mm diameter circle

5. Spacers providing the gap for the cutting blade to slide through of 2.0828 mm thickness.

    1. After cooking and recording final cooked temperature and weight, steaks should be chilled overnight at 2 to 5°C before coring. Chilling firms the steak making it easier to obtain uniform diameter cores. If chilling is not used, some protocol to obtain consistent steak temperature before coring should be followed, such as allowing steaks to reach room temperature (23°C).

2. Round cores should be uniformly 1.27 cm (0.5 inch) in diameter and removed parallel to the longitudinal orientation of the muscle fibers so that the shearing action is perpendicular to the longitudinal orientation of the muscle fibers.
3. Cores can be obtained using a hand-held coring device (cork borer) or an automated coring device (drill press with cork borer attached).
4. Coring devices must be in good condition and sharp or the core diameters will not be consistent and will result in spurious increased variation in shear values.
5. A minimum of six cores should be obtained from each sample (this may require 1 or more steaks or chops depending on the muscle and species). Cores that are not uniform in diameter, have obvious connective tissue defects or otherwise would not be representative of the sample should be discarded.
6. If steaks/chops were chilled, cores should be kept refrigerated until sheared to maintain consistent temperature. All values obtained should be used for mean calculation, unless visual observation indicates some reason a value should be discarded (e.g., a piece of connective tissue).
7. Each core should be sheared once in the center to avoid the hardening that occurs toward the outside cooked edge of the sample.
8. Shear tests that do not follow these equipment or sample specifications should not be referred to as "Warner Bratzler" shear force (such as square holes in the shear blade, square meat samples, straight edged shear blade, or blade not properly beveled, etc.).

Cook loss percentage was determined by the following equation:

$$CL = \frac{[(Weight\ of\ chop\ after\ cooking,\ \ g) - (Initial\ weight\ of\ chop,\ \ g)]}{(Initial\ weight\ of\ chop,\ \ g)}\ x\ 100$$

Appendix G

Proximate Analysis

FoodScan™ with ISIscan™
FOSS Analytical A/S, Foss Allé 1, DK-3400 Hillerød, Denmark

1. Thaw samples at 4°C for 24 hours.
2. Remove external fat from muscle. Subsequently, grin meat through 3.0-mm (1/8inch) plate or a food homogenizer. Conduct all measurements in duplicate.
3. Obtain enough sample to fill D: 140 mm (14 mm height) sample cup completely.
    a. About 250 g
4. Each sample should be packed the same to insure uniform analysis. Pack sample into cup to avoid any air pockets or gaps. Check the sample cup bottom to ensure this.
5. Open the FoodScan door; place the sample cup in the sample cup holder, making sure that the small pin in the cup holder is securing the cup.
6. Close the door and make sure the door handle is pushed all the way to its upright position.
7. A sample scan is initiated from the product tree. Double click the appropriate sample type.
8. Enter sample registration details, including User Defined Fields.
9. Click “Collect”. Allow the cycle to complete.
10. Once finished run the analysis a second time.

11. After both cycles have finished, remove sample cup from the device and remove sample.

12. Data then can be exported from ISIscan™ software

Appendix H

Trained Sensory Panel Evaluation Form

ID:			Date		Project:		
Sample #	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Flavor Intensity	Off Flavor Intensity	Off Flavor Descriptor
1							
2							
3							
4							
5							
6							
7							
8							

Juiciness	Tenderness	Flavor Intensity	Off Flavor	Off Flavor Descriptors
8= Extremely Juicy 7= Very Juicy 6=Moderately Juicy 5= Slightly Juicy 4= Slightly Dry 3= Moderately Dry 2= Very Dry 1= Extremely Dry	8= Extremely Tender 7= Very Tender 6=Moderately Tender 5= Slightly Tender 4= Slightly Tough 3= Moderately Tough 2= Very Tough 1= Extremely Tough	8= Extremely Intense 7= Very Intense 6=Moderately Intense 5= Slightly Intense 4= Slightly Bland 3= Moderately Bland 2= Very Bland 1= Extremely Bland	8= No Off Flavor 7= Slight Off Flavor 6= Small Off Flavor 5= Modest Off Flavor 4= Moderate Off Flavor 3= Very Off Flavor 2= Intense Off Flavor 1= Extreme Off Flavor	8= Metallic 7= Salty 6= Livery 5= Grassy 4= Bitter 3= Bloody 2= Rancid 1= Other-Explain

Appendix I

The Pork Flavor Lexicon adapted from Chu (2015)

Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat; American Meat Science Association Copyright © 2015
201 W Springfield Ave, Suite 1202 Champaign, Illinois USA 61820 800-517-2672

ATTRIBUTE	DEFINITION	REFERENCE
Astringent	The chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as a puckering/dry and associated with tannins or alum	Lipton Tea, 1 bag = 6.0 (F) Lipton Tea, 3 bags = 12.0 (F)
Boar taint	Aromatic associated with boar taint; hormone-like; sweat, animal urine	0.1g 3-methylindole = 13.0 (A) Androstenone = 15.0 (A)
Bitter	The fundamental taste factor associated with a caffeine solution	0.05% caffeine in water = 2.0 (F) 0.08% caffeine in water = 5.0 (F)
Bloody/ Serumy	An aromatic associated with blood on cooked meat products; closely related to metallic aromatic	Boneless Pork Chop, 135°F = 2.0 (F & A)
Brown/ Roasted	A round, full aromatic generally associated with pork suet that has been broiled	Pork Fat, cooked = 3.0 (F), 4.0 (A)
Burnt	The sharp/acrid flavor note associated with over roasted pork muscle, something over baked or excessively browned in oils	Arrowhead Puffed Barley Cereal® = 5.0 (A & F)
Cardboardy	Aromatic associated with slightly oxidized fats and oils, reminiscent of wet cardboard packaging	Dry cardboard = 5.0 (F), 3.0 (A) Wet cardboard = 7.0 (F), 6.0 (A)
Chemical	Aromatic associated with garden hose, hot Teflon pan, plastic packaging and petroleum-based products such as charcoal lighter fluid	1 drop Clorox in 200 mL water = 6.5 (F) Ziploc Bag = 13.0 (aroma)
Fat-like	Aromatics associated with cooked animal fat	Pork fat, cooked = 10.0(F); 7.0(A)
Floral	Sweet, light, slightly perfume impression associated with flowers	0.12 oz Clorox Wipe Liquid in 4 oz Water= 8.0 (A) Geraniol = 7.5 (A) 1:1 White Grape Juice to Water = 5.0 (F & A)

Heated oil	The aromatics associated with oil heated to a high temperature	Wesson Oil, microwaved 3 min = 7.0 (F& A) Lay's Potato Chips = 4.0 (A)
Liver-like	Aromatics associated with cooked organ meat/liver	Pork Liver, cooked = 15.0 (F); 12.0(A)
Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons	Dole Pineapple Juice = 6.0 (A & F) 0.10% KCl solution= 1.5 (A & F)
Nutty	Nutty characteristics are: sweet, oily, light brown, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc.	Diamond Shelled Walnut, ground for 1 min= 6.5 (F)
Pork identity	Amount of pork flavor identity in the sample	Boneless Pork Chop, 175°F = 7.0 (F), 5.0 (A) 80/20 Ground Pork, cooked = 6.0 (F); 5.0 (A)
Refrigerator stale	Aromatics associated with products left in the refrigerator for an extended period time and absorbing a combination of odors (lack of freshness/flat)	80/20 Ground Pork, cooked, left chilled overnight = 6.0 (F), 8.0 (A)
Salty	The fundamental taste factor of which sodium chloride is typical	0.2% Salt in Water = 2.5 (F) 0.35% Salt in Water = 5.0 (F)
Soapy	An aromatic commonly found in unscented hand soap	0.12 oz Clorox Wipe Liquid in 4 oz Water= 3.0 (A) 0.5g Ivory Bar Soap in 100mL water = 6.5 (A)
Sour	The fundamental taste factor associated with citric acid solution	0.05% citric acid in Water = 2.0 (F) 0.08% Citric Acid in Water = 5.0 (F)
Spoiled/Putrid	The presence of inappropriate aromatics and flavors that is commonly associated products. It is a foul taste and/or smell that indicates product is starting to decay and putrefy	Boneless Pork Chop, 175°F, left out for 24 hours then refrigerate for 6 days = 3.0 (A) 80/20 Ground Pork, cooked, same as above = 5.0 (A)
Sweet	The fundamental taste factor associated with a sucrose solution	0.05% Sugar in Water = 2.0 (F) 0.08% Sugar in Water = 5.0 (F)
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides	0.035% Accent flavor = 7.5 (F)
Vinegary	Aroma notes associated with vinegar	1.1g Vinegar in 200g water = 6.0 (F); 4.0 (A)
Warmed-over	Perception of a product that has been previously cooked and reheated	80/20 Ground Pork, cooked, left chilled overnight and reheated = 5.0 (A & F)

Appendix Table 1. Interaction between Trial, Lipid Content, Vitamin E Concentration, and Sex of Pig Least Squares Means for Carcass Traits

		0				2				4				6				
	Trial	F		M		F		M		F		M		F		M		
Trait[1]		11	220	11	220	11	220	11	220	11	220	11	220	11	220	11	220	SEM
HCW, kg	1	82.2	84.9	84.8	82.2	85.9	79.9	86.4	87.0	86.8	82.9	87.7	84.7	85.0	81.6	87.3	86.9	4.46
	2	79.7	80.4	81.6	83.5	79.1	79.6	81.0	81.3	82.8	82.3	84.6	83.5	81.7	79.2	83.3	84.6	
LRFT, mm	1	21.89	24.15	16.10	26.12	19.91	18.75	24.43	26.55	22.84	22.88	23.37	29.16	22.03	19.91	17.37	21.82	3.17
	2	27.96	28.10	20.76	17.10	27.96	19.91	21.18	23.93	19.63	22.98	18.18	25.70	22.17	25.28	16.77	18.64	
TRFT, mm	1	16.95	26.76	13.21	21.61	15.75	21.68	22.03	23.09	18.86	23.56	19.28	26.83	23.16	18.29	17.02	18.29	3.04
	2	19.14	23.44	17.45	20.48	18.51	19.56	19.56	23.16	22.39	20.62	19.07	22.67	26.20	18.86	19.53	17.02	
LEA, cm^2	1	44.11	43.38	46.86	40.69	47.18	42.25	41.69	42.43	43.62	41.76	41.14	40.48	36.89	44.28	49.76	42.18	5.31
	2	40.79	37.61	43.83	39.68	44.83	48.15	42.34	39.96	35.81	43.67	42.62	38.62	33.87	44.00	38.87	49.76	
FFL, %	1	54.62	49.39	56.96	51.36	55.59	51.90	51.22	50.96	53.07	50.62	52.43	48.69	49.47	53.96	55.61	52.98	2.20
	2	52.60	49.81	54.14	51.42	54.13	54.58	52.77	50.51	49.76	52.61	52.95	50.32	47.61	53.87	51.80	55.72	
L*	1	58.40	60.56	59.87	58.26	63.32	58.98	64.90	65.05	61.96	59.62	64.67	60.95	58.16	56.49	61.92	62.65	3.22
	2	59.29	59.04	60.91	63.12	60.22	58.51	60.08	59.13	54.34	60.64	62.36	59.46	54.28	58.04	60.10	60.59	
a*	1	9.20	9.13	8.45	9.07	8.34	8.52	9.34	6.57	8.86	7.78	6.83	9.16	8.16	10.38	8.67	7.87	1.00
	2	8.53	8.14	8.42	9.94	7.74	9.85	9.76	8.52	9.26	8.79	9.45	7.87	10.38	8.97	9.02	8.38	
b*	1	16.40	16.94	16.06	16.26	16.53	15.84	18.04	15.65	16.86	15.51	15.27	17.14	16.05	16.18	16.56	15.76	1.03
	2	15.77	15.82	15.88	17.71	15.60	16.96	16.41	16.22	16.13	16.49	17.05	14.90	15.70	15.71	16.14	15.41	
pHH	1	5.74	5.85	5.90	5.87	5.87	5.89	5.70	6.02	5.75	5.93	5.97	5.88	5.80	5.86	5.79	5.82	0.09
	2	5.55	5.61	5.65	5.54	5.65	5.48	5.54	5.48	5.47	5.47	5.51	5.54	5.50	5.55	5.51	5.51	
pHL	1	5.63	5.58	5.82	5.63	5.70	5.64	5.49	5.77	5.54	5.66	5.80	5.57	5.68	5.79	5.71	5.74	0.07
	2	5.48	5.47	5.51	5.45	5.52	5.41	5.44	5.48	5.34	5.39	5.44	5.43	5.48	5.49	5.44	5.41	
MS	1	2.99	2.48	2.51	2.26	3.01	2.23	2.24	2.03	2.74	2.98	3.03	2.50	2.50	2.51	2.51	2.51	0.37
	2	2.48	2.24	2.25	2.00	2.48	3.01	2.01	2.00	2.00	3.00	2.51	2.00	2.00	2.33	2.25	3.01	
NPPC Color[2]	1	3.45	2.87	3.01	2.81	2.60	2.62	2.45	2.68	2.95	3.37	2.68	3.02	2.73	3.10	2.60	2.35	0.59
	2	2.37	3.16	3.23	3.02	3.37	4.10	3.60	2.73	4.02	2.73	2.81	2.45	4.02	3.28	3.27	3.60	
NPPC Marbling[2]	1	2.30	2.11	0.91	2.45	0.91	2.11	2.05	1.35	2.05	3.11	0.85	2.23	2.01	1.41	1.41	1.41	0.59
	2	1.11	1.58	1.51	1.48	1.11	2.41	1.41	1.76	1.48	1.26	1.45	1.55	0.98	1.71	0.98	1.91	
SSD[3], cm	1	10.80	12.10	6.45	14.95	8.67	11.46	13.18	12.29	10.80	10.19	6.89	15.84	12.61	8.99	4.86	13.43	2.91
	2	15.91	16.92	11.65	11.08	10.35	7.01	9.94	13.08	23.15	10.22	10.19	14.45	15.53	9.47	9.49	8.35	
SSU[3], cm	1	11.29[be]	8.32[abe]	5.78[acd]	10.81[be]	6.74[abe]	10.86[bce]	11.92[be]	8.11[abe]	9.06[abe]	9.91[abe]	3.98[a]	11.71[e]	8.64[abe]	5.47[ac]	4.83[a]	8.01[abe]	2.24
	2	10.55[abe]	11.07[bde]	8.16[abe]	7.90[abe]	8.01[abe]	5.47[ac]	6.42[ab]	9.28[abe]	18.69[f]	7.53[ab]	6.37[ac]	7.16[abe]	9.17[abe]	7.58[abe]	7.26[ab]	8.01[abe]	
Belly Thickness, mm	1	31.00	40.13	39.71	33.96	36.53	35.37	38.93	28.98	38.93	36.96	38.51	52.02	38.33	36.53	27.01	39.71	6.24
	2	30.61	39.53	35.16	31.38	35.37	36.53	39.71	36.75	34.56	33.57	35.55	35.76	53.61	34.70	40.91	36.53	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down) [2] National Pork Producers Council standards (2000) [3] Pork belly firmness assessment [abcdef]=Means within the same rows for trials 1 and 2 within trait with common superscripts do not differ (P>0.05)

Appendix Table 2. Carcass Trait P-Values for Main Effects

	P>F				
Trait[1]	Trial	Lipid	Vitamin E	Sex	DOF
HCW, kg	0.0204				
LRFT, mm			0.0481		0.0004
TRFT, mm			0.0068		0.0091
LEA, cm^2					
FFL, %					0.0392
L*				0.0265	
a*					
b*					
pHH	<0.0001				
pHL	<0.0001				
MS	0.0404				
NPPC Color[2]	0.0207				
NPPC Marbling[2]			0.0066		
SSD[3], cm					
SSU[3], cm					0.0079
Belly Thickness, mm					

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)
[2] National Pork Producers Council standards (2000)
[3] Pork belly firmness assessment

Appendix Table 3. Carcass Trait P-Values for All Interactions

	P>F										
Trait[1]	T x L	T x S	T x V	L x S	L x V	S x V	T x L x S	T x L x V	T x S x V	L x S x V	T x L x S x V
HCW, kg											
LRFT, mm		0.0034		0.0031		0.0206					
TRFT, mm			0.0318	0.0160	0.0016						
LEA, cm^2											
FFL, %			0.0350	0.0164	0.0028						
L*											
a*										0.0193	
b*											
pHH											
pHL										0.0007	
MS			0.0304	0.0362							
NPPC Color[2]											
NPPC Marbling[2]											
SSD[3], cm			0.0042			0.0003					
SSU[3], cm			0.0346	0.0335		0.0018		0.0238		0.0364	0.0430
Belly Thickness, mm										0.0198	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

Appendix Table 4. Interaction between Trial, Lipid Content, Vitamin E Concentration, and Sex of Pig Least Squares Means for Meat Quality Traits

		0				2				4				6				
	Trial	F		M		F		M		F		M		F		M		
Trait[1]		11	220	11	220	11	220	11	220	11	220	11	220	11	220	11	220	SEM
DL, %	1	3.34	3.47	4.16	3.27	3.85	4.21	3.63	4.84	3.26	2.26	2.95	2.79	2.94	2.40	2.22	3.78	1.22
	2	5.08	2.48	5.78	4.74	4.47	1.49	7.71	3.12	1.73	3.30	4.77	2.37	4.79	4.96	3.75	5.32	
VP, %	1	8.30	7.95	12.61	8.31	12.93	7.91	7.95	13.19	11.27	8.43	16.42	8.05	8.70	9.07	13.16	10.72	2.05
	2	10.33	9.20	9.61	6.98	12.39	11.56	8.19	9.20	9.20	10.02	10.04	10.53	7.18	10.20	9.67	10.45	
MU, %	1	15.84	7.93	9.56	14.07	12.14	10.18	10.18	4.91	12.83	17.57	3.08	12.73	13.48	29.53	8.98	8.88	6.00
	2	8.66	10.55	12.64	13.07	11.85	9.53	8.42	13.28	12.49	13.94	10.41	14.81	11.60	10.91	11.09	12.64	
MCL, %	1	17.34	22.87	24.24	19.71	21.51	21.07	23.45	29.02	21.22	18.75	26.60	16.49	18.98	4.63	20.90	23.56	4.35
	2	21.04	23.22	18.90	19.73	19.97	20.95	23.09	19.90	20.47	17.71	20.99	21.45	18.18	18.61	21.59	19.83	
WBS, kg	1	5.39	5.12	5.25	4.14	4.49	4.97	3.64	3.67	5.34	4.68	4.20	4.44	4.69	3.40	4.58	3.37	0.93
	2	4.19	5.03	3.81	4.64	4.12	4.95	4.50	2.92	3.29	3.12	3.82	4.02	4.82	4.17	4.70	3.51	
CL, %	1	16.70^{bcde}	17.58^{bcde}	15.97^{bcde}	17.47^{eg}	13.42^{bce}	14.25^{bce}	15.37^{bcde}	21.83^{dgh}	17.09^{cde}	16.22^{bcde}	26.07^{fh}	16.89^{bcde}	14.60^{bce}	4.72^{a}	15.71^{bcde}	17.39^{eg}	2.66
	2	14.87^{bcde}	17.98^{eg}	15.29^{bce}	16.37^{bcde}	15.54^{bcde}	17.78^{bcde}	19.49^{defg}	12.18^{bc}	12.85^{bce}	16.24^{bcde}	16.85^{bcde}	16.45^{bcde}	14.02^{bce}	14.29^{bce}	18.10^{eg}	12.83^{bce}	
TBARS	1	0.20	0.19	0.27	0.21	0.29	0.17	0.25	0.20	0.21	0.25	0.24	0.23	0.18	0.21	0.21	0.23	0.04
	2	0.09	0.14	0.13	0.13	0.15	0.18	0.17	0.15	0.10	0.14	0.19	0.10	0.20	0.16	0.14	0.21	
Collagen, %	1	1.47	1.52	1.56	1.44	1.15	1.54	1.40	1.44	1.54	1.49	1.52	1.52	1.36	1.50	1.52	1.49	0.15
	2	1.60	1.42	1.48	1.45	1.69	1.31	1.57	1.49	1.63	1.52	1.56	1.44	1.67	1.43	1.54	1.46	
Fat, %	1	3.75	3.23	4.03	4.52	4.17	3.35	4.72	4.88	4.25	3.90	5.04	4.95	3.81	3.94	4.08	4.43	0.85
	2	4.11	5.08	3.88	4.46	5.29	3.44	4.71	4.87	4.01	4.08	3.72	3.95	3.81	4.80	3.96	2.89	
Moisture, %	1	76.67	76.84	76.70	75.96	76.38	76.81	75.27	75.64	76.05	76.00	75.65	75.34	76.40	75.90	75.38	75.95	0.75
	2	76.15	74.82	76.20	75.32	75.00	76.68	75.86	74.91	74.85	75.32	75.93	75.32	76.15	75.58	75.78	77.32	
Protein, %	1	25.92	26.26	26.48	25.96	26.72	26.08	27.02	26.54	26.27	26.62	26.37	26.43	26.48	25.84	26.71	27.14	0.58
	2	26.31	26.28	26.40	25.72	26.01	27.22	25.72	25.94	26.45	26.55	26.88	26.23	25.74	25.90	26.17	26.58	
Salt, %	1	0.81	0.84	0.82	0.77	0.63	0.78	0.74	0.70	0.68	0.76	0.64	0.68	0.62	0.79	0.76	0.60	0.11
	2	0.76	0.66	0.76	0.69	0.65	0.72	0.82	0.74	0.71	0.74	0.69	0.68	0.80	0.71	0.71	0.78	

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances) ^{abcdefg} = Means within the same rows for trials 1 and 2 within trait with common superscripts do not differ

Appendix Table 5. Meat Quality Trait P-Values for Main Effects

Trait[1]	P>F Trial	Lipid	Vitamin E	Sex	DOF
DL, %	0.0282				
VP, %					0.0394
MU, %					
MCL, %					
WBS, kg					
CL, %		0.0228		0.0220	
TBARS	<0.0001				
Collagen, %					
Fat, %					
Moisture, %					
Protein, %					
Salt, %					

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

Appendix Table 6. Meat Quality Trait P-Values for All Interactions

	P>F										
Trait[1]	T x L	T x S	T x V	L x S	L x V	S x V	T x L x S	T x L x V	T x S x V	L x S x V	T x L x S x V
DL, %			0.049					0.0471			
VP, %		0.0286	0.0418							0.0167	
MU, %											
MCL, %											
WBS, kg											
CL, %		0.0248						0.0305	0.0167		0.0379
TBARS	0.0789							0.0964		0.0997	
Collagen, %			0.0225						0.0904		
Fat, %		0.0348									
Moisture, %		0.0390									
Protein, %											
Salt, %											

[1] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)

Appendix Table 7. Interaction between Trial, Lipid Content, and Vitamin E Concentration Least Squares Means for Carcass Traits

		0		2		4		6		
Trait[1]	Trial	11	220	11	220	11	220	11	220	SEM
HCW, kg	1	83.5	83.6	86.1	83.5	87.3	83.8	86.14	84.2	2.69
	2	80.6	81.9	80.0	80.5	83.7	82.9	82.5	81.9	
LRFT, mm	1	19.00	25.13	22.17	22.65	23.11	26.02	19.70	20.87	1.91
	2	24.36	22.59	24.56	21.92	18.91	24.34	19.47	21.96	
TRFT, mm	1	15.08	24.19	18.89	22.39	19.07	25.21	20.09	18.29	1.84
	2	18.29	21.96	19.03	21.36	20.73	21.65	22.86	17.94	
LEA, cm^2	1	45.48	42.03	44.43	42.34	42.38	41.12	43.33	43.23	3.21
	2	42.31	38.64	43.59	44.05	39.21	41.14	36.37	46.88	
FFL, %	1	55.79	50.37	53.40	51.41	52.75	49.66	52.54	53.47	1.39
	2	53.37	50.61	53.45	52.55	51.36	51.46	49.71	54.79	
L*	1	59.13	59.41	64.11	62.02	63.31	60.29	60.04	59.57	1.94
	2	60.10	61.08	60.15	58.82	58.35	60.05	57.19	59.32	
a*	1	8.83	9.10	8.84	7.54	7.84	8.47	8.42	9.13	0.61
	2	8.47	9.04	8.75	9.19	9.36	8.33	9.70	8.68	
b*	1	16.23	16.60	17.28	15.74	16.06	16.32	16.31	15.97	0.62
	2	15.83	16.77	16.00	16.59	16.59	15.69	15.92	15.56	
pHH	1	5.82	5.86	5.79	5.95	5.86	5.90	5.79	5.84	0.06
	2	5.60	5.58	5.59	5.48	5.49	5.50	5.50	5.53	
pHL	1	5.73	5.61	5.59	5.71	5.67	5.62	5.70	5.76	0.04
	2	5.49	5.446	5.48	5.44	5.39	5.41	5.46	5.45	
MS	1	2.75	2.37	2.63	2.13	2.88	2.74	2.51	2.51	0.21
	2	2.36	2.12	2.25	2.51	2.26	2.50	2.13	2.67	
NPPC Color[2]	1	3.27	2.84	2.52	2.65	2.81	3.20	2.67	2.73	0.36
	2	2.80	3.09	3.48	3.42	3.42	2.59	3.65	3.44	
NPPC Marbling[2]	1	1.60	2.28	1.48	1.73	1.45	2.67	1.71	1.41	0.35
	2	1.31	1.53	1.26	2.09	1.46	1.40	0.98	1.81	
SSD[3], cm	1	8.62	13.53	10.92	11.87	8.84	13.02	8.73	11.21	1.84
	2	13.78	14.00	10.14	10.08	16.67	12.34	12.51	8.91	
SSU[3], cm	1	8.53	9.57	9.33	9.49	6.52	10.81	6.74	6.74	1.35
	2	9.35	9.49	7.21	7.37	12.53	7.34	8.22	7.79	
Belly Thickness, mm	1	35.35	37.05	37.73	32.18	38.72	44.49	32.67	38.12	3.77
	2	32.88	35.46	37.54	36.64	35.05	34.66	47.26	35.62	

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)
[2] National Pork Producers Council standards (2000)
[3] Pork belly firmness assessment

Appendix Table 8. Carcass Trait P-Values

Trait[1]	P>F						
	Trial	Lipid	Vitamin E	L x V	T x L	T x V	T x L x V
HCW, kg	0.0204						
LRFT, mm			0.0481				
TRFT, mm			0.0068	0.0016		0.0318	
LEA, cm^2							
FFL, %				0.0028		0.0350	
L*							
a*							
b*							
pHH	<0.0001						
pHL	<0.0001						
MS	0.0404					0.0304	
NPPC Color[2]	0.0207						
NPPC Marbling[2]			0.0066				
SSD[3], cm						0.0042	
SSU[3], cm						0.0346	0.0238
Belly Thickness, mm							

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

Appendix Table 9. Simple Means by Trial for Carcass and Meat Quality Traits

Trait[1]	Trial 1	Std Dev	Trial 2	Std Dev
HCW, kg	84.38	5.39	81.93	4.81
LRFT, mm	22.99	6.07	22.13	3.85
TRFT, mm	20.88	4.77	20.16	4.19
LEA, cm^2	42.38	6.17	41.76	6.76
FFL, %	52.38	3.28	52.36	3.21
L*	60.84	4.45	59.63	3.64
a*	8.59	1.45	8.85	1.13
b*	16.39	1.44	16.12	1.05
pHH	5.84	0.16	5.54	0.08
pHL	5.66	0.14	5.45	0.06
MS	2.54	0.50	2.36	0.49
NPPC Color[3]	2.81	0.73	3.19	0.74
NPPC Marbling[3]	1.90	0.86	1.45	0.62
SSD[4], cm	11.68	4.57	11.90	4.46
SSU[4], cm	9.23	3.33	8.35	3.28
Belly Thickness, mm	37.70	8.63	36.61	8.18
Trait[2]				
DL, %	3.36	1.21	4.09	2.07
VP, %	9.73	3.08	9.89	2.48
MU, %	12.16	8.78	11.64	5.19
MCL, %	20.43	6.99	20.38	3.85
WBS, kg	4.49	1.32	4.04	0.96
CL, %	16.33	4.71	15.72	3.26
TBARS	0.22	0.04	0.15	0.05
Collagen, %	1.46	0.22	1.52	0.15
Fat, %	4.19	1.04	4.28	1.08
Moisture, %	76.06	0.92	75.61	1.04
Protein, %	26.40	0.80	26.29	0.62
Salt, %	0.72	0.16	0.72	0.08

[1] Carcass Trait Abbreviations: LRFT (Last Rib Fat Thickness), TRFT (Tenth Rib Fat Thickness), LEA (Loineye Area), FFL (Percent Fat Free Lean), pHH (pH Ham), pHL (pH Loin), MS (Muscle Score), SSD (Belly skin-side-down), SSU (Belly skin-side-down)

[2] National Pork Producers Council standards (2000)

[3] Pork belly firmness assessment

[4] Meat Quality Trait Abbreviations: MU (Marinade Uptake), MCL (Marinade Cook Loss), DL (Drip Loss), VP (Vacuum Purge), WBS (Warner-Bratzler Shear Force), CL (Cook Loss), TBARS (Thiobarbituric Acid Reactive Substances)