Effects of Supplemental Feeding of Plum Juice Concentrate on Fecal Microbial Shedding and Pork Quality

by

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Abstract

Purebred Yorkshire pigs (n=32) were fed at the Auburn University Swine Research and Education Center to determine the influence of supplementation of plum juice concentrate (PJC) on microbial shedding, growth, carcass, and quality characteristics of pork. Pigs were sorted by weight and sex (n=16 barrows, n=16 gilts) and assigned to a pen which housed two pigs per pen. Pens were randomly allotted to one of four treatment groups. Experimental diets contained 0% (Control), 0.5%, 1%, or 2.9% plum juice concentrate (PJC). All basal diets consisted of corn and soybean meal formulated to meet or exceed National Research Council (NRC) recommendations. Pigs were allowed ad libitum access to feed and water, and treatment diets were formulated to be isocaloric. During the feed trial, feed intake and weight gain were measured every 14 d and fecal samples were collected directly from the anus from one pig per pen on days 0, 1, 7, 14, 28, 56, and last day on feed(Group 1=84 d; Group 2=100 d). Pigs were harvested (n=2 groups) at an average pen weight of 114 kg. All pigs were swabbed near the anus to measure microbial load on the hide. Fecal and ham swab samples were plated and enumerated for anaerobic bacteria (AA) and aerobic bacteria (APC). Samples were also enriched to determine prevalence of *Salmonella* spp. and generic *E.coli* (VRBA). Carcass and quality characteristics were recorded according to National Pork Producers Council Guidelines (2000) 24 hr postmortem. Data were analyzed using GLM procedure and mixed model analysis (SAS, 2002). Pen was the experimental unit and independent fixed

effects included: diet, sex, rep, and harvest group for growth, carcass, and meat quality. Diet and day were independent fixed effects for microbial analysis. Results indicated that *Salmonella* spp. was not present in any sample throughout the experiment, therefore no data are reported. Total counts for anaerobic bacteria and *E.coli* were not different among any treatment. Feeding PJC was effective in decreasing fecal bacterial populations for APC (P=0.03) when compared to the control. Additionally, AA (P=0.04) bacteria counts were different for day by treatment effect and showed a decreasing trend for treatments 1.0% PJC and 2.9% PJC. The level of PJC did not affect growth performance or meat quality. Wetness was the only carcass characteristic to be affected by level of PJC supplement (P=0.02), The results of this study demonstrate that supplementing a PJC to growing / finishing pigs does decrease aerobic bacteria as well as decreases anaerobic, aerobic and VRBA counts from d 0 to d 14. However, these results indicate that the supplementation of PJC has no effect on growth, carcass, or quality characteristics of pork.

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I. INTRODUCTION

Microbial food safety is an increasing public health concern worldwide (Zhao et al., 2001). The United States food supply is among the safest in the world (Oliver et al., 2009), however, every year people die, and countless others suffer due to breaches in food safety (Oliver et al., 2009). The most serious meat safety issues, resulting in immediate consumer health problems and recalls from the market place, are from potentially contaminated products associated with bacterial pathogens (Sofos, 2008). Major causes of concern and product recalls stem from the pathogens *Salmonella*, and *Escherichia coli* (*E.coli*) 0157:H7 (Sofos, 2008). *Salmonella* infections are most commonly associated with the consumption of contaminated poultry, beef, and pork (Foley et al., 2008), and while cattle are thought to be the primary reservoir of *E.coli* 0157:H7 (Rice et al., 2003), research has also demonstrated fecal shedding by other domestic livestock such as pigs (Feder et al., 2003).

Animals can become infected by several factors; contact with other infected animals or pests, living in unclean facilities, consuming contaminated feed or water, or harboring pathogens on their hides from feces, soil or general environment (Oliver et al., 2009). Of all of these vectors, fecal shedding is correlated highest with carcass contamination (Elder et al., 2000). Therefore, the most significant intervention strategy in reducing pathogens in meat could be implementing pre-harvest food safety hurdles at the production level, to help prevent fecal shedding

Antibiotics are often included in pig diets to help control gastrointestinal pathogens by disrupting the intestinal microbial ecosystem, consequently improving food safety (Callaway et al., 2003). Antibiotics fed in sub-therapeutic levels however, have become highly controversial due to concern of antibiotic resistance in humans, and antibiotic-residue in animal products (Callaway et al., 2003, Schwarz et al., 2001). Therefore, "natural" methods need to be researched. Fung, (unpublished data) reported that using a "natural" antimicrobial in the form of a plum extract mixed in ground beef suppressed 90% of foodborne pathogens, including *Salmonella* and *E. coli*.

Although food safety is of utmost concern, consumers scrutinize food more today than they did in the past (Troy and Kerry, 2010) and their preference is beginning to change to a more "natural" label (McCarthy et al., 2001; Rhee et al., 2001). Though consumers pay more attention to credence qualities like safety, healthiness, convenience, locality, and ethical factors (Bernués 2003; Issanchou, 1996), consumers still take into consideration meat quality attributes such as; meat color, visible drip, and visible fat (Troy and Kerry, 2010).

The meat industry has typically used synthetic antioxidants such as BHA and BHT to inhibit oxidation and improve meat quality (Chastain et al., 1992; Chen et al., 1984; Sato & Hegarty, 1971), but with consumers becoming more concerned with potential toxicological effects (Decker & Mei, 1996; Rababah et al., 2004) different methods have been studied.

The addition of antioxidants to swine diets during the growing and finishing period has shown to be beneficial in the improvement of pork quality (Cannon et al., 1996). Supplementing the antioxidant, Vitamin E in the form of α – tocopherol has shown to decrease lipid oxidation, drip loss, and improved color of pork when supplemented to growing / finishing pigs (Asghar et al., 1991; Monahan et al., 1990a, b, 1992).

Another method of improving pork quality through the use of antioxidants is post-harvest enhancement of the product. In recent years, research has identified plums as a functional ingredient to not only help reduce foodborne pathogens, but to also improve meat quality by decreasing lipid oxidation and increasing the juiciness of meat without altering the flavor of the product. The indigenous chemical compounds that make up plums serve specific functions when applied to foods. Pectin aids in moisture retention, while malic acid enhances flavor, and sorbitol acts as a natural humectant (Nuñez de Gonzalez et al., 2009). These attributes have been credited for decreasing lipid oxidation, and warmed over flavor (WOF), as well as increasing mouth-feel, juiciness, and nutrients (Nunez de Gonzalez et al., 2008, Yildiz-Turp and Serdaroglu 2010, Leheska et al., 2006, Lee and Ahn 2005).

Therefore, the objective of this study was to test the effect of adding an antioxidant in the form of plum juice concentrate to diets fed to growing / finishing pigs on fecal shedding, growth performance, carcass characteristics, and overall meat quality.

II. REVIEW OF LITERATURE

Consumers scrutinize food more now than ever in the past (Troy and Kerry, 2010). Bernués (2003) and Issanchou (1996) reported that consumers pay more attention to credence qualities like safety, healthiness, convenience, locality, and ethical factors. Becker (1999) goes on to report that these attributes focus primarily on the production and not the product itself. Though consumers are becoming more particular with how the animal was raised, the healthiness of the product, and product safety; consumers still take into consideration meat quality attributes such as: meat color, visible drip, and visible fat (Troy and Kerry, 2010). Challenges to provide a safe, abundant, and nutritious product are complex because all aspects of food production, from farm to fork, must be considered (Oliver et al., 2009). With consumers becoming more educated and preferences changing, researchers and producers must team together in order to meet these demands by decreasing the risk of food-borne pathogens and increasing a high quality, healthy product that consumers will accept.

Pathogens of Concern

Microbial food safety is an increasing public health concern worldwide (Zhao et al., 2001). The United States food supply is among the safest in the world (Oliver et al., 2009), however, every year people die and countless others suffer due to breaches in

food safety (Oliver et al., 2009). The most serious meat safety concerns, resulting in immediate consumer health problems and recalls from the market place, are from potentially contaminated products associated with bacterial pathogens (Sofos, 2008). Major causes of concern and product recalls stem from the pathogens *Salmonella*, and *Escherichia coli* (*E.coli*) 0157:H7 (Sofos, 2008).

Salmonella

In 1885, *Salmonella* species were first isolated from pork by Salmon and Smith (Ruben et al., 1942). Since then, 2,500 serotypes of *Salmonella* have been discovered (Foley and Lynne, 2008). *Salmonella* is a gram-negative rod (Coburn et al., 2007), and due to genetic similarity between serotypes, *Salmonella* has been divided into two species; *Salmonella bongori* and *Salmonella enterica* (Foley and Lynne, 2008), with 99% of all serotypes being grouped in *Salmonella enterica* (Brenner and McWhoter-Murlin, 1998). *Salmonella enterica* is a very common and serious foodborne pathogen, causing 26% of all human foodborne infections, with approximately 1.3 million illnesses and over 500 deaths annually in the United States (Mead et al., 1999). *Salmonella* infections also impact the US economy, costing an estimated 2.3 to 3.6 billion dollars annually due to loss of work, medical care, and loss of life (Buzby et al., 1996; Frenzen et al., 1999).

Salmonella infections are most commonly associated with the consumption of contaminated poultry, beef, and pork (Foley et al., 2008). Many foodborne pathogens, including *Salmonella enterica* typically harbor in pigs' gastrointestinal tracts, farm environment, and on the skin of pigs (McEwen and Fedorka-Cray, 2002). Pigs can become infected with *Salmonella enterica* through breeder herds, contaminated feed and

water, and most commonly, fecal waste (Bailey, 1993). It has also been reported that pigs can become infected with *Salmonella* through their respiratory system and tonsils (deJong and Ekdahl, 1965), allowing other locations of the pig's body to become infected by hematogenous or lymphatogenous spread (Fedorka-Cray et al., 1995). *Salmonella* entering via the fecal-oral route has evolved to be able to withstand and survive a pig's stomach antimicrobial defense of a low pH (Foley and Lynne, 2008). Once in the stomach of the animal, *Salmonella* is able to colonize and spread to the small intestine and colon, attaching to the intestine by pili present on the bacterial cell surface (Foley and Lynne, 2008). Though pigs are known to have *Salmonella* colonies in their gastrointestinal tract (Davies, et al., 1999), they do not show any symptoms of being ill (Callaway, et al., 2007), and are a primary vectors for transmitting *Salmonella* to humans (Borland, 1975).

The primary route for humans to become infected with *Salmonella* is through fecal-oral transmission (Foley and Lynn, 2008). The number of cells needed to make a human ill ranges from as low as 30 cells to as high as 10^9 cells depending on the serotype (Morgan et al., 1994). *Salmonella* can manifest in humans in different forms including; gastroenteritis, bacteremia, typhoid fever, and focal infections (Darwin, and Miller, 1999). Of these forms, gastroenteritis is most common when consuming *Salmonella enterica* contaminated pork, and causes victims to experience diarrhea, abdominal cramps, vomiting, and fever (CDC, 2001). Gastroenteritis symptoms develop within 6 to 72 h after ingestion of bacteria and signs typically last 2 to 7 d, with rare cases causing septicemia and invasive infections of organs and tissues (CDC, 2001; Cohen et al., 1987).

E.coli

Like *Salmonella*, *E. coli* is another harmful pathogen that is commonly found in the food supply. *E. coli* is a gram-negative rod that was first identified as a food-borne pathogen in 1982, after two outbreaks of hemorrhagic colitis were linked to eating contaminated ground beef (Riley et al., 1983). Shiga toxin-producing *E. coli* (STEC) 0157:H7 is the most prominent STEC and causes numerous outbreaks of food-borne diseases worldwide (Meng et al., 2001). *E. coli* 0157:H7 is estimated to cause an average of 500 outbreaks, affecting more than 73,000 people and killing 61 people each year in the United States (Mead et al., 1999). Though *E. coli* 0157:H7 is the most recognized, over 200 other *E. coli* serotypes exist and over 100 strains have been associated with human illnesses (Nataro and Kaper, 1998).

Cattle are thought to be the primary reservoir of *E.coli* 0157:H7 (Rice et al., 2003), but research has also demonstrated fecal shedding by other domestic livestock such as pigs (Feder et al., 2003). Pigs can become infected with *E.coli* 0157:H7 by ingesting bacteria through contaminated feed and water, followed by fecal shedding of the pathogen that, in turn contaminates the farm environment, causing new infections and reinfections of animals both in the gastrointestinal tract and on the hide of the animal (Oliver et al., 2009). Though STEC infects the pig by producing toxins in the gastrointestinal tract, the pig will show no clinical signs of being sick (The Center for Food Security and Public Health, 2006). This allows infected animals to be harvested and equipment or human error in the harvesting process may potentially contaminate carcasses consumers will eat. Another way consumers can be affected by contamination is through the application of manure, as a fertilizer, to a field of produce (McEwen and Fedorka-Cray, 2002). Research has demonstrated the long-term survival of *E.coli*

0157:H7 in manure held under a variety of conditions, so even treated waste could still be a vector of contamination to raw produce (Kuda et al., 1998; Wang et al., 1996).

Consumers become infected with *E. coli* 0157:H7 through eating contaminated product which can lead to serious illness or even death. Humans can become ill with *E. coli* 0157:H7 by ingesting as little as 10 cells (Armstrong et al., 1996). Illness caused by *E.coli* 0157:H7 can be expressed as three different manifestations; hemorrhagic colitis, hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (Doyle and Padhye, 1989; Griffin et al., 1988). Hemorrhagic colitis onset ranges 3 to 9 d after consuming cells and last 2 to 9 d (Doyle, 1991). Symptoms of hemorrhagic colitis consist of sudden and severe abdominal cramping, followed by bloody diarrhea (Doyle, 1991). HUS begins with bloody diarrhea and can also lead to hemolytic anemia, thrombocytopenia (low circulating platelets), or kidney disorder (Doyle, 1991). Individuals with HUS often require dialysis and blood transfusions, and may also suffer from seizures or comas (Doyle, 1991). Some of the symptoms of TTP may exhibit similar to those of HUS, but typically the central nervous system is attacked, leading to blood clots in the brain and usually resulting in death (Doyle, 1991).

Pork consumption has increased 20% in the last century (Buzby and Farah, 2006), and with increase in consumption comes potential increase of *Salmonella* and *E.coli* foodborne illness for consumers (Foley and Lynne, 2008). Because *Salmonella* and *E.coli* can live undetected in pigs (Callaway et al., 2008), be present prior to entering a processing facility, and are known to be found on pork carcasses through cross contamination in processing plants (Foley et al., 2008), food safety hurdles must be implemented and tailored to specific livestock species while still being applicable to large

numbers of animals (Callaway et al., 2008). Food producers have a moral and ethical responsibility to produce the safest food for consumers (Rollin, 2006). One area that could contribute to the reduction of *Salmonella* and *E.coli* contaminated meat is on-farm pre-harvest food safety (Foley et al., 2008).

Pre-Harvest Food Safety

Traditionally, food safety research has targeted improvements in post-harvest sanitation and safety in meat (Elder et al., 2000). Recently researchers have begun to stress the importance of intervention strategies in live animal production, by developing new technologies for pre-harvest food safety in meat (Callaway et al., 2003). Some pre-harvest pathogen control techniques that have been explored include; diet manipulation, use of feed additives, antibiotics, bacteriophage therapy, immunizations, competitive exclusion, prebiotics, probiotics, and proper animal management such as pen management, clean feed, chlorinated water, and little to no stress during transportation (Sofos, 2004a, 2004b, 2005; Stopforth & Sofos, 2006). Animals can become infected by several factors; contact with other infected animals or pests, living in unclean facilities, consuming contaminated feed or water, or harboring pathogens on their hides from feces, soil or general environment (Oliver et al., 2009). Of all of these vectors, fecal shedding is correlated highest with carcass contamination (Elder et al., 2000). Therefore, the most significant intervention strategy in reducing pathogens in meat could be implementing pre-harvest food safety hurdles at the production level, such as supplementation of antibiotics and antimicrobials in the feed, to help reduce foodborne pathogenic bacterial populations in the gut and on the hide of pigs prior to slaughter (Hynes and Wachsmuth, 2000).

Antibiotics

Antibiotics are often included in pig diets to help control gastrointestinal pathogens by disrupting the intestinal microbial ecosystem, consequently improving food safety (Callaway et al., 2003). Antibiotics have also been supplemented in sub-therapeutic levels to improve growth performance and feed efficiency since the 1950's (Kiser, 1976). Antibiotics fed in sub-therapeutic levels however, have become highly controversial due to concern of antibiotic resistance in humans, and antibiotic-residue in animal products (Callaway et al., 2003, Schwarz et al., 2001). Specific concerns of antibiotic resistance include: the potential for higher risk of infection in humans during treatment with antibiotics, the potential for failure of human antibiotics in treating of infections, increased severity of illness caused by antibiotic resistant pathogens, and potential for co-selection of higher virulence genes (Institute of Food Technologist, 2006). Due to these concerns, the European Commission (EC) has prohibited the use of antibiotic feed additives as growth promoters in pig production since January 2006 in the European Union (European Commission, 2003). As a consequence of the ban from the EC the US cannot export pork that has been supplemented with antibiotics for consumption in the European Union. With regulatory pressure and rising consumer concern, alternative strategies must be researched and implemented to continue to create a safe and efficient product for both consumers, and producers (Bae et al., 1999).

Antimicrobials

One alternative to supplementing antibiotics in swine diets is by supplementing different forms of antimicrobials into the diet. By adding antimicrobials to feed or water,

pigs still receive the benefit of antibiotics by inhibiting of the growth of pathogens, but not the negative publicity of antibiotics. Whitney et al. (2006) supplemented an antimicrobial in the form of bacitracin methylene disalicylate, to growing pigs to reduce or inhibit intestinal lesions caused by the pathogen *Lawsonia intracellularis*. Another example of supplementing antimicrobials into swine diets is the use of a potato (Solanum tuberosum L. cv. Gogu Valley) protein to reduce bacterial populations in feces of weanling pigs (Jin et al., 2008).

The study conducted by Whitney et al. (2006) tested the effect of including 10% of distiller dried grains with solubles (DDGS), and also tested the inclusion of an antimicrobial, bacitracin methylene disalicylate, in the diet of growing pigs to determine the reduction or inhibition of growth of intestinal lesions when inoculated with *Lawsonia intracellularis*. One hundred 17 d old pigs were randomly allotted to one of five treatments; negative control, corn-soy diet, not inoculated (NC); positive control, corn-soy diet, inoculated (PC); 10% DDGS diet, inoculated (D); positive control, with antimicrobial supplementation, inoculated (PC + A); and 10% DDGS diet, with antimicrobial supplementation, inoculated (D + A). DDGS have shown to reduce the spread of pathogenic organisms in the intestine (Hampson et al., 1999) due to fiber influence. Fiber can stimulate secretion of the epithelium, impairing bacterial adhesion (Smith and Halls, 1968). Results were that pigs fed diets containing antimicrobial supplementation had a lower occurrence of lesions in the jejunum and tended to have reduced total tract lesion length. Likewise, pigs fed D + A had reduced fecal shedding of *Lawsonia intracellularis*, determining supplementation of DDGS and antimicrobial is beneficial in reducing pathogens in the gastrointestinal tract and feces of pigs.

The study conducted by Jin et al. (2008) used 280 weaned pigs (Landrace X Yorkshire X Duroc) in a 28 d feeding period to examine the effect of supplementing an antimicrobial peptide in the form of potato protein (PP) as an antibiotic replacer to decrease the level of bacteria in the feces. Pigs were randomly assigned to one of five treatments: a positive control, a basal diet with antibiotics (PC) and 4 potato protein diets, which consisted of a basal diet supplemented with 0%, .25%, .50%, or .75% PP. Diets were fed for 28 d, and body weight and feed disappearance were measured every two weeks. Fecal samples were collected on days 0, 7, 14, 21, and 28 to determine levels of bacteria present in feces. The conclusion was that supplementing swine diets with PP, reduced bacterial counts in the feces and improved ADG, ADFI, and G: F, which demonstrated that PP was a useful alternative to antibiotic supplementation.

Although, supplementing natural antimicrobials in swine diets does reduce pathogens and overall bacteria in feces, it does not completely eliminate all microbes that could potentially harm the food supply. Ayres (1955) stated the origin of microflora are numerous and can be found on hide, hoofs, tools, air, water and workers within the processing facility. Dockerty et al. (1970) found that scalding and singeing were helpful methods in decreasing microbes on the carcass of pigs. However, following the process of scalding and singeing, researchers found heading, eviscerating, and splitting of the carcass, increased microbial loads (Dockerty et al., 1970). With the risk of animals, equipment, and workers increasing the microbial loads in meat, an impact at the pre-harvest level will not solve all food safety issues. However, a reduction at one stage of production should logically produce an impact further down the production chain (Oliver et al., 2009). Therefore, supplementing antimicrobials to pigs prior to harvest could

potentially lead to cleaner pigs (both in their gastrointestinal tract and on their hide) entering the processing facility.

Pre-Harvest Meat Quality

Food safety is always of utmost concern, and begins at the farm level. However, another continuous concern is meat quality, which can also be influneced at the farm level. Meat quality has become progressively more important to the pork industry as it faces the challenge of increased competition with other red meat species (Cannon et al., 1996), and with improving its presence in a global market (Hasty et al., 2002). As Cannon et al. (1995) demonstrated in a pork quality audit, pork struggles with two main quality concerns: inadequate color and poor water-holding capacity. Lipid oxidation is another concern in the pork industry, and has also shown to affect the quality of color, water-holding capacity, flavor, texture, nutritive value, and safety (Buckley et al., 1995). Though there are many pre- and post- harvest methods that have been shown to improve pork quality, supplementing antioxidants in swine diets is one way that has been shown to increase multiple quality attributes in pork.

Antioxidants

The supplementation of antioxidants in swine diets during the growing and finishing period has been shown to be beneficial in the improvement of pork quality (Cannon et al., 1996). Many researchers have determined that varying levels of vitamin E in the form of α-tocopheryl used as a diet additive in pigs can help improve meat quality. Vitamin E is a potent antioxidant and has shown to decrease lipid oxidation, drip loss, and improves color of pork (Asghar et al., 1991; Monahan et al., 1990a,b, 1992). Vitamin

E improves pork quality through its antioxidant properties. (Buckley et. al., 1995). Antioxidants retard or inhibit lipid oxidation (Kim et al., 2003) by inhibiting radical forming enzymes (Nagao et al., 1999).

Cannon et al. (1996) determined that supplementation of vitamin E fed in the form of α-tocopheryl at levels of 100mg/kg to growing pigs is successful in reducing lipid oxidation in pork. The reduction of lipid oxidation in pork has also been credited for prolonging shelf life in both fresh and frozen pork (Guo et al., 2006). Vitamin E increases color stability (Asghar et al., 1991), and increases a* values for refrigerated chops from pigs supplemented vitamin E, compared with chops from pigs receiving no vitamin E supplementation (Monahan et al., 1992). Lauridsen et al. (1999) attributed improvements of pork quality to improved water-holding capacity due to supplementing antioxidants in the form of vitamin E. By utilizing antioxidants in swine diets, pork producers can increase pork quality (Lauridsen et al., 1999), without altering taste or visual appearance (Cannon et al., 1995).

Phenolics

Plums

Over the last two decades, research has revealed that plums have a high phenolic content (Donovan et al., 1998; Gil et al., 2002; Kim et al., 2003; Nakatani et al., 2000; Wang et al., 1996) which could potentially be useful in both pre-harvest food safety and pre-harvest meat quality, due to the phenolic properties found in plums. Phenolics are composed of an aromatic benzene ring substituted with a hydroxl group (Kim et al., 2003). Some forms of phenolics are free radical scavenger antioxidants (Di Carlo et al.,

1999) that protect cells from oxidative damage (Anderson et al., 1994). Plums have the strongest activity of all fruit and vegetables in the human diet (McBride, 1999). Other forms of phenolics are effective natural antimicrobials (Bowles and Juneja, 1998). Antimicrobial activity in phenolics is unclear, but theories of the reaction include; a reaction with the cell membrane, or inactivation of cellular enzymes, or possibly a combination of the two (Davidson & Branen, 1981). Other research has led to the theory that it may be related to the free hydroxyl group (Prindle & Wright, 1977). Phenolics can also serve as a natural colorant stability source (Francis, 1989). All forms of these phenolics are naturally found in plums, allowing plums to be a multi-functional phenolic source. It has been reported that the most effective antioxidant in plums is also one of the most effective antimicrobials. The antioxidant and antimicrobial, chlorgenic acid is typically found in high concentrations in plums (Davidson and Breanen, 1981). Another multi phenolic found in plums is anthocyanin, which has antioxidant (Igarashi et al., 1989), antimicrobial (Beuchat and Golden, 1989), and color stability properties (Francis, 1989).

Plum Products

Plums are a versatile fruit, and can be consumed in a variety of way including in fresh, dried or juiced. Plum juice concentrate is obtained by extracting the juice from the fruit and elevating the concentration to 70° brix (70g sugar/100g juice) (Sunsweet Growers, 2008). Prunes are acquired by dehydrating fresh plums at 85-90°C for 18 hours (Piga et al., 2003). Plums have often been classified as a functional food, because of their unique chemical composition (Piga et al., 2003). The same benefits that are found in plums are also found in prunes and plum juice. Cevallos-Casals et al. (2006) determined

that levels of anthocyanin content, overall phenolic content and levels of antioxidant activity in prunes were higher over that of levels found in fresh plums. It is because plums and prunes possess such high levels of phenols that they are now being used to improve the meat industry.

Functional Ingredient

The meat industry has typically used synthetic antioxidants such as BHA and BHT to inhibit oxidation and warmed over flavor (WOF) in meat products (Chastain et al., 1982; Chen et al., 1984; Sato & Hegarty, 1971), but with consumers becoming more concerned with potential toxicological effects (Decker & Mei, 1996; Rababah et al., 2004), their preference is beginning to change to a more "natural" label (McCarthy et al., 2001; Rhee et al., 2001). In recent years, plums and plum based products such as prune juice concentrate, plum puree, and plum juice concentrate have been researched as being used as a functional ingredient to help reduce foodborne pathogens, decrease lipid oxidation just as well as synthetic antioxidants, and increase the juiciness of meat, without altering the flavor of the product. The indigenous chemical compounds that make up plums serve specific functions when applied to foods, pectin aids in moisture retention, while malic acid enhances flavor, and sorbitol acts as a natural humecant (Nuñez de Gonzalez et al., 2009). A study conduct by Fung (unpublished data), reported that a 3% prune extract solution when added to ground beef could suppressed 90% of foodborne pathogens, including *Salmonella* and *E. coli*. The antioxidant properties of prunes also work as a functional ingredient by reducing lipid oxidation and WOF in roast beef by adding either 2.5% fresh prune juice or 2.5% dried plum concentrate to a brine (Nunez de Gonzalez et al., 2008). Yildiz-Turp and Serdaroglu (2010) found that plum

puree could be incorporated to beef patties at levels of 5 or 10% without causing any detrimental effects to the product. When added at 5% patties resulted in highest cook yield and moisture retention, while 10% plum puree was most accepted during sensory analysis, and both levels were successful in inhibiting lipid oxidation. Lee and Ahn (2005) reported that when 3% of plum extract when added to low fat content meats, can improve mouth-feel due the sorbitol in the plums which helps to increase moisture in the product. Meat quality can also be improved by increasing juiciness, even when reheated when supplementing prune extract (Fung, unpublished data). Nutrient levels can also be increased by adding plum extracts to products due to the phenolic content, which is appealing to consumers (Leheska et al., 2006). Although different levels and forms of plums have shown to improve meat quality it has also shown that products darken color and become unacceptable to consumers (Nunez de Gonzalez et al., 2009).

Conclusion

Though, plum products are effective when supplemented as a functional ingredient in meat, not all consumers prefer an enhanced product. Previous research has focused on adding plum products to a meat product as a processing ingredient. Therefore, the objective of this study is to improve pre-harvest food safety and pre-harvest meat quality in pork by supplementing plum juice concentrate to growing / finishing pigs. Supplementing these natural antimicrobials and antioxidants in the form of plum juice concentrate, could potentially decrease microbial loads in feces of the pigs and furthermore, reduce the amount of microbes found on the hide of the animals at harvest. Another potential benefit of supplementing the antioxidants to pigs is the possibility of the plum juice concentrate in the pork meat to act as a functional ingredient to reduce

oxidation, warmed-over flavor, increase color stability, and increased overall shelf-life of the product.

III. EFFECTS OF FEEDING PLUM JUICE CONCENTRATE ON FECAL SHEDDING IN GROWING / FINISHING PIGS

Abstract

Yorkshire pigs (n=32) were fed at the Auburn University Swine Research and Education Center to determine the effects of adding plum juice concentrate (PJC) to growing / finishing pigs' diets on microbial shedding. Pigs were sorted by weight and sex (n=16 barrows, n=16 gilts) and assigned to a pen which housed two pigs per pen. Pens were randomly allotted to one of four treatment groups. Experimental diets contained 0% (Control), 0.5%, 1%, or 2.9% plum juice concentrate (PJC). All basal diets consisted of corn and soybean meal formulated to meet or exceed National Research Council (NRC) recommendations. Pigs were allowed ad libitum access to feed and water, and treatment diets were formulated to be isocaloric. During the feed trial, fecal samples were collected from one pig per pen on days 0, 1, 7, 14, 28, 56, and the last day on feed (Group 1 =84 d; Group 2 =100 d). At an average pen weight of 114 kg pigs were humanely harvested and the ham of all pigs were swabbed following stunning, and exsanguination to determine if PJC could decrease microbes on the hide by decreasing microbial flora in the gastrointestinal tract. Fecal and ham swab samples were plated and enumerated for anaerobic (AA) and aerobic bacteria (APC). Samples were also enriched to determine prevalence of *Salmonella* spp. and generic *E.coli* (VRBA). Microbial analysis data were

analyzed using the GLM procedure. Results indicated *Salmonella* spp. was not present in any sample throughout the experiment and total counts for anaerobic bacteria, and *E.coli* were not different among any treatment. However, feeding PJC was effective in decreasing fecal bacterial populations for APC (P=0.03) when compared to the control. The fecal populations of bacteria among days did decrease for APC (P=<.0001), and VRBA (P=<.0001). Additionally, APC (P=0.52) and VRBA (P=0.34) counts were not different for day by treatment interaction. However, there was a day by treatment interaction for AA (P=0.04) bacteria and showed a trend of decreasing bacterial populations for treatments 1.0% PJC as well as 2.9% PJC. In conclusion, the results of this study demonstrate that adding PJC to growing / finishing pigs' diets decreases aerobic bacteria and also decreases anaerobic, aerobic and VRBA counts from d 0 to d 14.

Introduction

Microbial food safety is an increasing public health concern worldwide (Zhao et al., 2001). The United States food supply is among the safest in the world (Oliver et al., 2009), however, every year people die, and countless others suffer due to breaches in food safety (Oliver et al., 2009). The most serious meat safety concerns, resulting in immediate consumer health problems and recalls from the market place, are from potentially contaminated products associated with bacterial pathogens (Sofos, 2008). Major causes of concern and product recalls stem from *Salmonella* and *Escherichia coli* (*E.coli*) 0157:H7 (Sofos, 2008). *Salmonella* infections are most commonly associated with the consumption of contaminated poultry, beef, and pork (Foley et al., 2008), while cattle are thought to be the primary reservoir of *E.coli* 0157:H7 (Rice et al., 2003),

research has also demonstrated fecal shedding by other domestic livestock such as pigs (Feder et al., 2003).

Animals can become infected by several means; contact with other infected animals or pests, living in unclean facilities, consuming contaminated feed or water, or harboring pathogens on their hides from feces, soil or general environment (Oliver et al., 2009). Of all these vectors, fecal shedding is correlated highest with carcass contamination (Elder et al., 2000). Therefore, the most significant intervention strategy in reducing pathogens in meat could be implementing pre-harvest food safety hurdles at the production level, to help prevent fecal shedding.

Antibiotics are often included in pig diets to help control gastrointestinal pathogens by disrupting the intestinal microbial ecosystem, consequently improving food safety (Callaway et al., 2003). Antibiotics fed in sub-therapeutic levels however, have become highly controversial due to concern of antibiotic resistance in humans, and antibiotic-residue in animal products (Callaway et al., 2003, Schwarz et al., 2001). Therefore, "natural" methods need to be researched. Fung, (unpublished data) reported that using a "natural" antimicrobial in the form of a plum extract mixed in ground beef suppressed 90% of foodborne pathogens, including *Salmonella* and *E. coli*.

Therefore, the objective of this study was to determine the effects of adding a "natural" antimicrobial to growing / finishing pigs' diets in the form of plum juice concentrate (PJC) on microbial loads and fecal shedding.

Materials and Methods

Animals and Diets

Yorkshire pigs (n=32) were acquired at a BW of 22.68 kg + 4.54 kg and raised during the growing / finishing phases in an open barn with half concrete and half slatted floors at Auburn University Swine Research and Education Center (IACUC PRN: 2011-1890). Pigs were sorted by weight and sex (n=16 barrows, n=16 gilts) and assigned to a pen which housed two pigs per pen. Pens were randomly allotted to one of four treatment groups. Experimental diets contained 0% (Control), 0.5%, 1%, or 2.9% plum juice concentrate (PJC). All basal diets consisted of corn and soybean meal formulated to meet or exceed National Research Council (NRC) recommendations. Pigs were allowed ad libitum access to feed and water, and treatment diets were formulated to be isocaloric. During the feed trial fecal samples were collected directly from the anus of one pig per pen on days 0, 1, 7, 14, 28, 56, and the last day on feed (Group 1 =84 d; Group 2 =100 d). At an average pen weight of 114 kg pigs were transported (n=2 groups) to Auburn University Lambert - Powell Meats Laboratory, and held in lairage over-night. Pigs were humanely harvested under USDA – FSIS inspection. The ham of all pigs were swabbed following stunning, and exsanguination to determine if PJC could decrease microbes on the hide by decreasing microbial flora in the gastrointestinal tract.

Microbial Analysis

Fecal samples were weighed, and buffered peptone water (BPW) (Neogen, Lansing, MI) was added in a 1:1 ratio. The sample was then stomached for 1 min at 240 rpm (Seward, UK). Dilutions were made in 0.1% peptone water (PW) (Neogen, Lansing,

MI) and plated on Anaerobic Agar (AA) (Becton Dickinson, Sparks, MD) to determine total anaerobic counts, Standard Methods Agar (APC) (Neogen, Lansing, MI) to determine aerobic plate counts, Xylose-Lysine-Tergitol 4 (XLT4) (Neogen Lansing, MI) to determine *Salmonella spp.* counts and Violet Red Bile Agar (VRBA) (Neogen, Lansing, MI) to determine coliform and generic *Esherichia coli* (E. coli) counts. Anaerobic agar was incubated in 7.0 L anaerobic chambers (Mitshubishi, Japan) with anaerogen sachets (Oxoid, Hampshire, UK) at 37°C for 48 h. PCA, XLT4 and VRBA were incubated aerobically at 37°C for 24 h. Results were reported as CFU/g.

Prevalence of *Salmonella spp.* was determined by enriching the original fecal and BPW solution and incubating for 24 h at 37°C. From this, 0.1 mL was placed into 10 mL of Rappaport-Vassiliadis broth (RV) (Neogen, Lansing, MI) and incubated at 37°C for 24 h. Enriched samples were streak plated on XLT4 Agar and incubated at 37°C for 24 h. Results were reported as positive/negative.

On the day of harvest, samples from the hams of individual pigs were obtained. Swabs containing neutralizing buffer (Swab Environtrans Hardy Diagnostics Santa Maria, CA) were used to sample an area 7.6 cm to the right of anus. Samples were spread plated on AA, APC, XLT4 and VRBA and incubated as described above; results were reported as CFU/ cm^2. To determine prevalence of *Salmonella*, 1 mL of neutralizing buffer was placed into 9 mL BPW and incubated at 37°C for 24 h. From this, 0.1 mL was added to 10 mL RV broth and incubated at 37°C for 24 h. Enriched RV samples were streak plated on XLT4 and incubated for 24 h at 37°C. Results were reported as positive/negative.

Statistical Analysis

Microbial data was analyzed using the GLM procedure (SAS, 2002). Pen was the experimental unit and independent fixed effects included diet and day.

Results and Discussion

Microbial Analysis

The effect of PJC on bacterial populations in feces of growing / finishing pigs is presented in Table 1. *Salmonella* spp. was not present in any sample throughout the experiment and no data is reported. Total anaerobic bacteria (P=0.63), and *E.coli* (P=0.12) were not different. Feeding PJC was effective in decreasing fecal bacterial populations for APC (P=0.03). Jin et al. (2008) conducted an experiment to compare supplementing antibiotics (positive control) to supplementing a natural antimicrobial to weanling pigs. The natural antimicrobial fed was potato protein at levels of 0.00% 0.25% 0.50% and 0.75%. The results of that experiment compare to that of the present study. Data suggest that any level of antimicrobial fed to pigs does numerically decreased total bacteria. Fung, (unpublished) reported that by using plums as a functional ingredient could decrease pathogen growth in meat, however, that does not agree with the results of the present study.

The effect of day on bacterial populations in feces of growing / finishing pigs is presented in Table 2. The fecal populations of bacteria among days was different for AA (P=<.0001), APC (P=<.0001), and VRBA (P=<.0001). Anaerobic bacteria significantly decreased between d 1 and 7 (P=.0008), 14 and 28 (P<.0001), 56 and pre-harvest (P<.0001), and pre-harvest and post-harvest swab (P=<.0001). Aerobic bacteria

decreased between d 1 and 7 (P<.0001), and d 14 (P<.0001). Additionally, there was a difference between d 1 and post-harvest swab (P=<.0001). *E.coli* decreased between d 1 and 7 (P=.0002), 1 and 14(P<.0001), 1 and 28 (P=.0002), and post-harvest swab (P=<.0001). This data compares to Jin et al. (2008) who reported a decrease for fecal populations of total bacteria, coliform bacteria, and *Staphylococcus* spp. on d 28. Staphylococcus also decreased at d 21 when fed the potato protein antimicrobial.

Day by treatment interaction results are reported in Figures 1, 2, and 3. There was a diet by day interaction for anaerobic (P=0.04) bacteria counts, and showed a decreasing trend for control, 1.0%, and 2.9% treatments from d 0 to d 7, d 14 to 28, and d 56 to post-harvest swab. Supplementing 0.5% PJC also showed a decrease in count between d 56 and post-harvest swab. The reasons for these results are unclear. However, from d 0 to d 14 there is a clear trend of decreasing counts for AA. This could indicate that as the pig increases in body weight levels of PJC need to be increased to meet microbial inhibiting levels.

Implications

The results of this study demonstrate that supplementing a PJC to growing / finishing pigs does decrease aerobic bacteria. This study also reveals that PJC does help to decrease anaerobic, aerobic and VRBA counts from d 0 to d 14. Further research is needed to identify if feeding PJC 14 d prior to harvest could result in lowest bacteria counts, or if continuing to increase levels of PJC as the pigs grow could help to reduce fecal bacteria populations.

IV. GROWTH AND CARCASS CHARACTERISTICS OF PIGS FED PLUM JUICE CONCENTRATE AS A FEED ADDITIVE

Abstract

Yorkshire pigs (n=32) were fed at the Auburn University Swine Research and Education Center to determine the influence of supplementation of plum juice concentrate (PJC) on growth, carcass, and quality characteristics of pork. Pigs were sorted by weight and sex (n=16 barrows, n=16 gilts) and assigned to a pen which housed two pigs per pen. Pens were randomly allotted to one of four treatment groups. Experimental diets contained 0% (Control), 0.5%, 1%, or 2.9% plum juice concentrate (PJC). All basal diets consisted of corn and soybean meal formulated to meet or exceed National Research Council (NRC) recommendations. Pigs were allowed ad libitum access to feed and water, and treatment diets were formulated to be isocaloric. During the feed trial (Group 1=84 d; Group 2=100 d), feed intake and weight gain were measured every 14 d. Pigs were harvested (n=2 groups) at an average pen weight of 114 kg, and carcass and quality characteristics were recorded according to National Pork Producers Council Guidelines (2000). Growth and carcass data were analyzed using mixed model analysis (SAS, 2002). While, the level of PJC did not affect ADG, G: F or ADFI, there was a date of harvest effect on ADG (P=0.001) and G: F (P=0.009). Wetness was the only carcass characteristic to be affected by level of PJC supplement (P=0.02), however, muscle scores were higher (P=0.05) for pigs harvested on the first harvest day. Dressing

percentage was greater (P<.0001) for pigs harvested on the second harvest day. Fat thickness measured along the midline of the 10th rib was thicker (P=0.02) for barrows and barrows had a greater (P=.0005) loin pH. Feeding of PJC as a feed additive had no effect on quality characteristics; however, pigs from the second harvest date had lower Warner-Bratzler shear force values (P=0.02). There were no differences in initial or sustained tenderness or juiciness, pork flavor intensity or off flavor (P>0.05). Finally, gilts had more fat free lean (P=0.05), percentage fat free lean (P=0.006), and drip loss (P=0.03), but barrows had a higher percentage (P=0.04) cook loss. Independent of harvest group or sex, these results indicate that the supplementation of PJC has no effect on growth, carcass, or quality characteristics of pork.

Introduction

Consumers scrutinize food more today than they did in the past (Troy and Kerry, 2010) and their preference is beginning to change to a more "natural" label (McCarthy et al., 2001; Rhee et al., 2001). Though consumers pay more attention to credence qualities like safety, healthiness, convenience, locality, and ethical factors (Bernués 2003; Issanchou, 1996), consumers still take into consideration meat quality attributes such as; meat color, visible drip, and visible fat (Troy and Kerry, 2010).

The meat industry has typically used synthetic antioxidants such as butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) to inhibit oxidation and improve meat quality (Chastain et al., 1992; Chen et al., 1984; Sato & Hegarty, 1971), but with consumers becoming more concerned with potential toxicological effects (Decker & Mei, 1996; Rababah et al., 2004) different methods have been studied.

The supplementation of antioxidants in swine diets during the growing and finishing period has shown to be beneficial in the improvement of pork quality (Cannon et al., 1996). Supplementing the antioxidant, Vitamin E in the form of α – tocopherol has shown to decrease lipid oxidation, drip loss, and improve color of pork when supplemented to growing / finishing pigs (Asghar et al., 1991; Monahan et al., 1990a, b, 1992).

Another method of improving pork quality through the use of antioxidants is post-harvest enhancement of the product. In recent years, research has identified plums as a functional ingredient to help reduce foodborne pathogens, decrease lipid oxidation, and increase the juiciness of meat without altering the flavor of the product. The indigenous chemical compounds that make up plums serve specific functions when applied to foods. Pectin aids in moisture retention, while malic acid enhances flavor, and sorbitol acts as a natural humectant (Nuñez de Gonzalez et al., 2009). These attributes have been credited for decreasing lipid oxidation, and warmed over flavor (WOF), as well as increasing mouth-feel, juiciness, and nutrients (Nunez de Gonzalez et al., 2008, Yildiz-Turp and Serdaroglu 2010, Leheska et al., 2006, Lee and Ahn 2005).

The objective of this study was to determine the effect of supplementing growing / finishing pigs' diets with an antioxidant in the form of plum juice concentrate on growth performance, carcass characteristics, and overall meat quality.

Materials and Methods

Animals and Diets

Yorkshire pigs (n=32) were acquired at a BW of 22.68 kg $\pm$ 4.54 kg and were raised during the growing finishing phases in an open barn with half concrete and half

slatted floors at Auburn University Swine Research and Education Center (IACUC PRN: 2011-1890). Pigs were sorted by weight and sex (n=16 barrows, n=16 gilts) and assigned to a pen which housed two pigs per pen. Pens were randomly allotted to one of four treatment groups. Experimental diets contained 0% (Control), 0.5%, 1%, or 2.9% plum juice concentrate (PJC). All basal diets consisted of corn and soybean meal formulated to meet or exceed National Research Council (NRC) recommendations. Pigs were allowed ad libitum access to feed and water, and treatment diets were formulated to be isocaloric. During the feed trial (Group 1 =84 d; Group 2 =100 d) feed intake and weight gain were measured every 14 d. At an average pen weight of 114 kg pigs were transported to Auburn University Lambert - Powell Meats Laboratory.

Harvesting and Carcass Data

Pigs were humanely harvested at Auburn University Lambert – Powell Meats Laboratory under USDA – FSIS inspection. Carcasses were chilled for 24 h at 2 ± 1°C. The pH was monitored in both the right loin and ham at 30 min, 45 min, 3 h, 6 h, and 24 h after the time the animal was stunned. The pH was measured by using a portable pH spear probe meter (pH Spear. Eutech Instruments Oakton). Both live weight and hot carcass weight were recorded to determine dressing percentage. Following the 24 h chill period, carcasses were ribbed between the 10^{th} and 11^{th} rib. Backfat was measured at the 10^{th} and last rib, and loin eye area (LEA) was also measured. Backfat and LEA were adjusted based on the recommended equation of NPPC (2000).

Adjusted Backfat to 250 lb. =

Actual Backfat + [(250-actual wt.) x actual backfat/ (actual wt.-b)]

Adjusted LEA to 250 lb. =

Actual LEA + [(250-actual wt.) x actual LEA/ (actual wt. + 155)]

Evaluation of subjective scores for marbling, wetness, firmness, and muscle score, were determined by a trained observer using published visual standards (NPPC 2000). Additionally, the longissimus muscle at the 10^{th} rib was evaluated for objective color measurements with a Hunter Miniscan XE Plus (Hunter Lab, Reston, VA) to determine Hunter L*, a*, and b* values. The Miniscan was calibrated according to the manufacturer's recommendations and utilized a D65 light source, a 10° viewing angle, and a 35mm viewing area. Following carcass evaluation, a section of the longissimus muscle was removed from the 11^{th} rib to the last lumbar vertebrae from each carcass to use for meat quality analysis. Starting at the 11^{th} rib end, one 5.1cm thick chop was cut for drip loss, moisture uptake (MU), and marinade cook loss (MCL). One 2.54cm thick chop was removed for fatty acid analysis, two for Warner-Bratzler Shear Force (WBS) determination, one for Thiobarbituric Acid Reactive Substances (TBARS), and two for sensory evaluation. Chop samples for drip loss, MU, and MCL were analyzed fresh, while chops for FA, WBS, TBARS, and sensory were vacuum packaged and frozen for later analysis.

Meat Quality Analysis

Drip Loss, Marinade Uptake, and Marinade Cook Loss

One 5.1cm fresh chop was fabricated from the longissimus muscle and cut to two 4cm thick cubes weighing between 40 to 50 g. Samples were then trimmed free of fat and connective tissue. Cubes were weighed, then suspended by a fish hook (Model

number:121 – 2/0, Eagle Claw®) in a plastic sample cup (500 mL Polypropylene Straight –Sided Jar, Nalgene®, VWR International, LLC., West Chester, PA, USA) for 48 h at a temperature of 4°C. Following the 48 h time period samples were removed from hooks and blotted to remove excess surface fluid. Samples were then weighed to the nearest 0.1g. Percent drip was calculated by NPPC (2000) recommended equation, and averaged across the two samples for each pig.

Percent Drip Loss =

(Loss in Weight / Initial Weight) x 100

Using excess trim from fabricating one 5.1cm into two 4cm cubes for drip loss, the meat was ground using a coffee bean grinder, (Mr. Coffee Inc., Cleveland, Ohio) and separated into triplicate samples weighing 6.00 $\pm$ 0.01, and placed in 50 ml centrifuge tube that were weighed to the nearest 0.01 g without the cap on. 10 ml of reagent buffer (3.5% NaCl = 35 g in 1 liter of water) was added to each sample and vortexed for 15 s before being placed in a water bath to incubate for 30 min at 25°C. Following incubation, samples were centrifuged using a Beckman Coulter Allegra X-15 R (Beckman Coulter, Inc., Brea, CA, USA) swinging bucket rotor for 20 m at 3000 rpm and 13°C. Samples were then left upside down to drain for 5 min, and weights were recorded with the cap off to the nearest 0.01 g. Moisture uptake was then calculated by using the NPPC (2000) equation and an average was determine across each set of triplicates.

Moisture Uptake =

[(weight tube and meat, after incubation at 25°C) – (initial weight of tube and meat)/6.00g x 100]

Additionally, marinade cook loss was analyzed by using the drained triplicate samples from MU. Samples were placed into an 80°C water bath and left for 20 m. Once samples were removed from the water bath they were left to cool to an average temperature of 21°C, and weight were recorded of tubes with cap off. The NPPC (2000) equation was used to calculate MCL before calculating averages for each pig.

Marinade Cook Loss =

[(weight of tube and meat, after cooking) – (initial weight of tube and meat)/6.00g] x 100

Fatty Acid Analysis

All fatty acid standards were purchased through Nu-Chek Prep Inc., Elysian, Minnesota, except Hexanes (J.T. Baker, Phillipsburg, New Jersey), MeOH (Fisher Scientific, Fair Lawn, New Jersey), KOH (Sigma-Aldrich, St. Louis, Missouri), and Supelco standard FAME mixture (47885-U) (Supelco, Bellefonte, Pennsylvania).

Frozen samples were thawed to room temperature. Samples were uniformly distributed by grinding for 10 to 15 s in a room-temperature coffee bean grinder (Mr. Coffee Inc., Cleveland, Ohio). 1.0 g of meat was placed into a 16 × 125 mm screw-cap Pyrex culture tube (Corning Laboratory Science Company, New York) to which 1.0 mL of the C13:0 internal standard (0.5 mg of C13:0/mL of MeOH), 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added. The tube was incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking every 20 min to properly permeate, dissolve, and hydrolyze the sample. After cooling below room temperature in a cold tap water bath, 0.58 mL of 24 N H_2SO_4 in water was added. The tube was mixed by inversion and with precipitated K_2SO_4 present was incubated again in a 55°C water bath for 1.5 h with hand-

shaking every 20 min.

After FAME synthesis, the tube was cooled in a cold tap water bath. Three milliliters of hexane was added, and the tube was mixed for 5 min on a vortex. The tube was then centrifuged for 5 min in a Beckman-Coulter Allegra X-15R centrifuge, and the hexane layer, containing the FAME, was removed and placed into a 2ml GC vial. The vial was capped and placed at -20°C until GC analysis.

The fatty acid composition of the FAME was determined by capillary GC on a SP-2560, 100m × 0.25 mm × 0.20 µm capillary column (Supelco) installed on a Shimadzu 2014 gas chromatograph equipped with a Shimadzu AOC-20i auto injector, a flame ionization detector, and split injection. The initial oven temperature was 140°C, held for 5 minutes, subsequently increased to 240°C at a rate of 4°C min-1, and then held for 20 minutes. Helium was used as the carrier gas at a flow rate of 0.5 mL_min-1, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260°C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times with FAME standards purchased from Supelco.

Warner-Bratzler Shear Force and Cook Loss

Warner Bratzler shear force (WBS) evaluation was performed using pork loin chops cut to 2.54cm thickness were thawed in a vacuum package bag at approximately 4°C for 24 h. Chops were weighed and placed on a Calphalon Removable Plate Grill (Caphalon, Perrysburg, OH) clamshell style contact grill pre-heated to 177°C. Temperature was monitored with copper constantan thermocouple wire inserted into the geometric center of the chop and attached to a hand-held Omega data logger HH309A

(Omega, Stamford, CT) temperature recorder. Chops were cooked for 7 min which resulted in a final temperature of 71°C. Cooked chops were removed and reweighed before being placed on non-absorbent wax-coated paper to cool to room temperature then chops were wrapped in aluminum foil and placed in refrigerator at 4°C for approximately 24 h. Six 1.27cm-diameter cores were removed from each chop with a brass cork borer (Model 1601A Series Brass Cork Borer, Boekel Scientific, Feasterville, PA) parallel to the longitudinal orientation of the muscle fibers. Each core was sheared once at its center using a TA-XT2i Texture Analyser (Texture Technologies Corp., Scarsdale, NY). The peak force measurements were averaged from the six cores of each sample and were used for analysis. The probe was programmed to be lowered 30 mm after detection of resistance. The penetration speed was 3.3 mm/s with a post-test speed of 10 mm/s and a pre-test speed of 2.0 mm/s. Cook loss was measured as the percent of pre-cooked weight lost during cooking.

Cook Loss =

(Initial weight – Cooked weight) x 100

Thiobarbituric Acid Reactive Substances

TBARS were determined using a modified method of Wang et al. (2003). Briefly, five grams of meat free of fat and connective tissue were homogenized in 15 mL of 7.5% trichloroacetic acid, 0.1% propyl gallate, and 0.1% EDTA using a bullet style blender (Bella Cucini Rocket Blender, Bella Cucina Artful Food, Inc., Montreal Canada) for approximately 30 s. Samples were then centrifuged at 1,500 x *g* using a Beckman Coulter Allegra X-15 R (Beckman Coulter, Inc., Brea, CA, USA) swinging bucket rotor. After

centrifugation, samples were filtered through no. 4 Whatman paper (Whatman, plc, Kent, UK). Samples were then loaded into a 96-well microplate (Greneir Bio-one, Frickenhausen, Germany) 32 samples in triplicate wells and incubated at 40°C for 130 min in a VWR Incubating Microplate Shaker (VWR International, LLC., West Chester, PA, USA). Microplates were then read using a Multiskan EX (Thermo Fisher Scientific, Waltham, MA, USA) absorbance reader at 540 nm.

Standards were made using a stock solution of tetraethoxypropane (TEP) at 1 mM/L. The stock solution was diluted to 0, 2, 4, 6, 8, 10, 20, and 30 μM/mL. A standard curve was then generated for each plate and used for the samples on each respective plate, and results are expressed as mg malondialdehyde / kg meat.

Sensory Evaluation

A trained panel of nine members conducted a sensory evaluation of boneless top loin chops according to guidelines by AMSA (1995). Chops were cooked as previously described for WBS evaluation. Chops were allowed to rest before being cut into 1cm x 1cm x cooked chop thickness pieces, and placed in a plastic cup with lids. Samples were held in a warming oven for a minimal amount of time prior to sensory evaluation. Two pieces of each sample were given to panelist to evaluate for initial and sustained juiciness, initial and sustained tenderness, pork flavor, off flavor intensity, and off flavor descriptor on a 8 point scale, where 1 = extremely dry, extremely tough, extremely bland, and no off flavor, and 8 = extremely juicy, extremely tender, extremely intense pork, and extremely off flavor respectively. Panelists were instructed to cleanse their palate with a salt-free saltine cracker and sip apple juice before each sample. Panel members evaluated

samples over a four day period (n= 32 total samples, 8 samples per d) in secluded partitioned booths with red incandescent light. The form given to sensory panelist to evaluate samples can be found in Appendix F.

Statistical Analysis

Data were analyzed using mixed model analysis (SAS, 2002). Pen was the experimental unit and independent fixed effects included: diet, sex, rep, and harvest group.

Results and Discussion

Animal Diets

Treatment effect of supplementing PJC to growing / finishing pigs is presented in Table 4. There were no differences in ADG, G: F, ADFI, or finishing live weight among treatments. However, in comparison with harvest group 2, group 1 had improved ADG (P=0.01), G: F (P=0.07), and finishing live weight (P=0.005) (Table 5). Blair and English (1965) demonstrated that barrows are more efficient and grow faster than gilts. Therefore, improved growth performance among harvest group 1 may be associated with the pigs of that group being majority barrows. Although supplementing varying levels of PJC did not differ from the control group, these results agree with similar studies which supplemented an antioxidant (Vitamin E), in which no difference in growth performance or feed efficiency was observed (Amer and Elliot 1973, Roth and Kirschgessner, 1975, Monahan et al., 1990, and Cannon et al., 1996).

Carcass Data

The effect of treatment, sex and harvest group of ham and loin pH is presented in figures 4, 5, 6, and 7. Pigs across all treatments showed a gradual decline in ham pH, resulting in quadratic (P=0.005) effect. Loin pH concluded that gilts had a lower pH (P=0.0005) than barrows, and also tended to have a lower ultimate loin pH (P=0.06). The results of the present study disagree with results of Eikelenboom and Hoving-Bolink (1993) who suggest gilts maintain a higher pH than barrows. This discrepancy may be caused by a difference of temperature on day of harvest, in which predominantly gilts were harvested on the cooler of the two days. Lefaucheur et al. (1991) demonstrated that temperature does have an effect on pH, reporting that pigs reared in cooler temperatures resulted in a faster postmortem pH decline and lower ultimate pH in the longissimus dorsi muscle.

The effect of treatment, sex, and harvest group on carcass characteristics of pig supplemented PJC are presented in tables 6, 7, 8 and 9. Pigs in harvest group 2 had a higher dressing percentage (P= <.0001) than that of harvest group 1. Pigs in group 2 were majority gilts, in which our results would agree with previous research that states females tend to have a higher dressing percentage (Langlois and Minivelle, 1989). There were no differences in hot carcass weight (HCW) among sex or harvest group which could be attributed to group 1 having higher live weights, while group 2 had higher dressing percentages and therefore may have balanced overall HCW between harvest groups.

There was no difference in last rib fat, however gilts were leaner when measured at the 10^{th} rib (P=0.02). Gilts also had lower adjusted back fat (P=0.02), higher fat free lean (P=0.05), higher percent fat free lean (P=0.006) larger loin eye area (LEA) and

larger adjusted LEA (P=0.02) than barrows. These results agree with Friend and MacIntyre (1970) who reported gilts being leaner and having larger LEA. Barrows had increased marbling (P=0.04) and pigs that were harvested first (group 1) tended to have a higher muscle score (P=0.05). This is likely because the majority of group 1 were barrows, which are more efficient and grow faster (Blair and English, 1965).

There were no differences for Hunter L* and a*, yet gilts tended to have lower b* value (P=0.07). These results agree with others (Cannon et al., 1996; Houben et al., 1998; Zanardi et al., 1999) who observed no effects on Hunter L* a* b* when supplementing an antioxidant of vitamin E on color of fresh pork. Firmness of LEA showed no difference, however, wetness did vary among treatments (P=0.02), with treatment of 0.5% PJC producing the most ideal wetness of a 2. The present study carcass data results agree with Cannon et al. (1996) who supplemented varying levels of the antioxidant, Vitamin E and reported no differences among treatments. However, the treatment effect of wetness was not expected, the more antioxidants supplement tended to be the most exudative. This contradicts previous research which suggests antioxidants can help to increase water-holding capacity (Lauridsen et al., 1999).

Meat Quality

Drip Loss, Marinade Uptake, and Marinade Cook Loss

The effect of treatment, sex, and harvest group on drip loss, marinade uptake, and marinade cook loss are presented in Tables 10 and 11. There were no differences among treatments for MU, MCL, or drip loss. This is in agreement with Cannon et al. (1996), who reported no effect of adding antioxidants (vitamin E) to growing / finishing pigs' diets on drip loss in fresh loin chops. In contrast, Lauridsen et al. (1999) and Cheah et al.

(1995) observed a reduction in drip loss in longissimus samples when measured following slaughter. Yildiz – Turp and Serdaroglu, (2010) reported beef patties formulated with plum puree had lower cooking yields and Nuñez de Gonzalez et al. (2008) stated that increasing plum contents to roast beef also increased cooking loss. Yildiz – Turp and Serdaroglu, (2010) attribute low cook yields to plums blocking the effect on water binding ability of meat proteins. In contrast University of Arkansas (Unplubished data), reports water holding capacity analysis showed that plum fiber marinade retained moisture as well as the phosphate control, however plum fiber marinade chicken did have a higher cook loss.

Although there was no treatment effect on drip loss, there was a sex effect (P=0.03) in which gilts had a higher percentage drip loss. This suggest that females had more free water present, which could be due to the high pH that the males maintain causing the water to become more immobilized.

Fatty Acid Analysis

The effect of treatment and sex for fatty acid levels in pigs supplemented PJC are presented in Tables 12 and 13. Barrows had an increase percentage of C16:0 (P=0.01), C17:1 (P=0.01) and total saturated fatty acids (SFA) (P=0.01). Females had an increase percentage of C22:6n3 (P=0.01), ratio (omega-3 to omega-6 ratio) (P=0.04), and omega-3 (P=0.02) fatty acids . The current data agrees with Zhang et al, (2007) who reported barrows had higher concentrations of SFA and mono unsaturated fatty acids. Nürnberg et al, (2005), also reported that barrows had higher levels of SFA than gilts. Although pigs fed 0.5% PJC showed to have highest levels of C 18:2 N6T (P=0.03) there is no

circumstantial evidence to suggest treatment should play a role in FA levels, because there are no increased levels of FA in PJC.

Warner-Bratzler Shear Force and Cook Loss

The effect for treatment, sex, and harvest group on WBS and cook loss of pigs supplemented PJC is presented in Tables 10 and 11. There were no differences among treatments for WBS or percent cook loss. However, results showed that harvest group 1 was less tender than harvest group 2 (P=0.02) when analyzed by WBS method (1.43 kg vs. 1.15 kg, respectively). Results also showed that cook loss was greater for males (P=0.04) majority of which were harvested in group 1. These results are likely due to pigs being harvested under extreme heat conditions of that particular day, which could have implicated in the resulting PSE carcasses. PSE meat develops a looser muscle structure and is associated with a lower water-holding capacity, resulting in a tougher product (Romans et al. 2001). These results agree with van der Wal et al. (1988), who reported a difference in toughness and cook loss in PSE pork loins than in DFD pork loins.

Thiobarbituric Acid Reactive Substances

There were no differences among treatments or sex for TBARS. The results support a previous study by Cannon et al. (1996), who determined that thiobarbituric acid values were unaffected after vacuum packaging and allowing one day of display. In contrast, it has been reported TBARS values decrease when plum puree is added to beef patties at levels of 5 and 10 percent (Yildiz – Turp and Serdaroglu, 2010). Therefore, the results of the presents may not have been supplemented at high enough concentration to warrant uptake into the muscle tissue.

Sensory Evaluation

The effect of treatment, sex, and harvest group on sensory attributes of pig supplemented PJC is presented in Tables 14 and 15. PJC did not influence sensory characteristics of tenderness, juiciness, pork flavor intensity, or off flavor intensity. Although, PJC did not increase sensory attributes, it also did not decrease sensory attributes, therefore maintaining consumers' expectations. These results agree with Nuñez de Gonzalez et al. (2008) who reported small changes among treatments of plum added to boneless beef roast. Therefore, the results of the present study may not have been supplemented at high enough concentration to warrant uptake into the muscle tissue to have results similar to Yildiz – Turp and Serdaoglu (2010), who reported enhancing beef patties at levels of 5% and 10% with plum puree increased juiciness, texture, and sweetness.

Implications

Growth performance, carcass characteristics and meat quality were unaffected by supplementation of plum juice concentrate in growing / finishing pig diets. Therefore, adopting this method appears to be ineffective. However, there is a potential that the levels fed in this study were not high enough to warrant any meat quality differences. Additionally, plums are known antioxidants and could potentially help to improve growth, carcass and meat quality if fed in different forms, such as the fruit itself, or by adding PJC in growing / finishing pigs' diets at the increased and successful percentages that have been achieved by previous post-harvest enhancement research.

Table 1. LS Means ± SEM for Treatment Effects of Feeding PJC to Growing / Finishing Pigs on Anaerobic, Aerobic, and VRBA Plate Counts

	Treatment				
Plate	Control	0.5%	1.0%	2.9%	P > F
Anaerobic	5.96 ± 0.14	5.88 ± 0.14	5.72 ± 0.14	5.93 ± 0.14	0.63
Aerobic	6.14 ± 0.014[a]	5.60 ± 0.14[b]	5.80 ± 0.14[abc]	5.68 ± 0.14[bc]	0.03
VRBA	5.65 ± 0.19	5.06 ± 0.19	5.55 ± 0.19	5.29 ± 0.19	0.12

[abc] Values within same row with same superscript are not different (P>0.05)

Table 2. LS Means ± SEM for Day Effects of Feeding PJC to Growing / Finishing Pigs on Anaerobic, Aerobic, and VRBA Plate Counts

	Day								
Plate	0	1	7	14	28	56	Pre-Harvest	Post-Harvest Swab	P > F
Anaerobic	7.00 ± 0.20[a]	6.50 ± 0.20[ab]	5.51 ± 0.20[cde]	5.99 ± 0.21[bc]	5.06 ± 0.20[df]	7.59 ± 0.20	5.27 ± 0.20[ef]	4.05 ± 0.20	<.0001
Aerobic	7.11 ± 0.20	6.00 ± 0.20[a]	5.26 ± 0.20[bc]	4.89 ± 0.20[bd]	5.46 ± 0.20[c]	6.14 ± 0.20[ae]	6.53 ± 0.20[e]	5.07 ± 0.20[cd]	<.0001
VRBA	6.39 ± 0.27[a]	6.40 ± 0.27[a]	5.17 ± 0.27[b]	4.48 ± 0.27[b]	5.18 ± 0.27[b]	6.02 ± 0.27[a]	6.25 ± 0.27[a]	3.19 ± 0.27	<.0001

[abcdef] Values within same row with same superscript are not different (P>0.05)

Table 3. Composition (as-fed basis) of Experimental Basal Diets for Growing / Finishing Phases

Item	Growing Phase	Finishing Phase
% Protein	13.7	12.7
Metabolized Energy, Kcal/kg	3531	3509
% Fat	8.5	8
% Calcium	0.51	0.46
% Phosphorus	0.39	0.36

Table 4. LS Means ± SEM for Treatment Effects of Feeding PJC to Growing / Finishing Pigs on ADFI, G:F, ADG, Finishing Live Wt, and Adj. Days 250 lbs.

	Treatment				
	Control	0.5% PJC	1.0% PJC	2.9% PJC	P>F
ADFI, kg/d	2.34 ± 0.20	2.37 ± 0.20	2.26 ± 0.18	2.21 ± 0.18	0.57
G:F, kg/kg	0.40 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.41
ADG, kg/d	0.93 ± 0.06	0.97 ± 0.06	0.95 ± 0.05	0.89 ± 0.06	0.25
Finishing Live Wt., kg	104.55 ± 5.31	109.49 ± 5.31	106.27 ± 4.71	102.32 ± 4.92	0.22
Adj. Days 250 lbs.	169.55 ± 3.10	165.36 ± 3.10	166.29 ± 2.85	173.23 ± 2.96	0.27

Table 5. LS Means ± SEM for Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on ADFI, G:F, ADG, Finishing Live Wt., and Adj. Days 250 lbs.

	Sex			Harvest Group		
	Barrow	Gilt	P>F	1	2	P>F
ADFI, kg/kg	2.40 ± 0.18	2.20 ± 0.18	0.19	2.37 ± 0.18	2.22 ± 0.18	0.30
G:F, kg/kg	0.39 ± 0.01	0.42 ± 0.19	0.15	0.43 ± 0.01	0.39 ± 0.01	0.07
ADG, kg/d	0.94 ± 0.05	0.93 ± 0.05	0.70	1.01 ± 0.05	0.87 ± 0.05	0.01
Finishing Live Wt., kg	106.41 ± 5.46	104.91 ± 5.46	0.74	112.68 ± 5.46	98.64 ± 5.46	0.005
Adj. Days 250 lbs.	166.03 ± 2.84	171.19 ± 2.84	0.31	153.87 ± 2.84	183.35 ± 2.84	<.0001

Table 6. LS Means ± SEM for Treatment Effects of Feeding PJC to Growing / Finishing Pigs on HCW, Dressing Percentage, LEA, Adj. LEA, Last Rib Fat, Adj. Back Fat, and Muscle Score

	Treatment				
	Control	0.5% PJC	1.0% PJC	2.9% PJC	P>F
HCW, kg	77.88 ± 4.53	82.50 ± 4.53	82.16 ± 4.02	78.43 ± 4.19	0.27
Dressing Percentage	74.88 ±0.86	75.82 ± 0.86	77.49 ± 0.76	76.91 ± 0.79	0.12
LEA, cm^2	42.90 ± 0.21	48.00 ± 0.21	46.83 ± 0.18	45.55 ± 0.19	0.09
Adj. LEA, cm^2	45.29 ± 0.22	49.48 ± 0.22	48.90 ± 0.20	48.65 ± 0.20	0.21
10^{th} Rib Fat, cm	2.2 ± 0.07	2.1 ± 0.07	2.1 ± 0.06	2.0 ± 0.06	0.81
Last Rib Fat, cm	2.4 ± 0.07	2.7 ± 0.07	2.7 ± 0.06	2.7 ± 0.06	0.68
Adj. Back Fat, cm	2.3 ± 0.07	2.1 ± 0.07	2.3 ± 0.06	2.2 ± 0.06	0.76
Muscle Score	1.97 ± 0.07	2.19 ± 0.07	2.13 ± 0.06	2.03 ± 0.06	0.17

Table 7. LS Means ± SEM for Treatment Effects of Feeding PJC to Growing / Finishing Pigs on Marbling, Wetness, Firmness, L*, a*, b*, PFFL, and FFL

	Treatment				
	Control	0.5% PJC	1.0% PJC	2.9% PJC	P>F
Marbling	1.57 ± 0.19	1.38 ± 0.19	1.50 ± 0.17	1.18 ± 0.18	0.47
Wetness	1.81^{ab} ± 0.16	2.00^{a} ± 0.16	1.50^{bc} ± 0.14	1.31^{c} ± 0.15	0.02
Firmness	1.46 ± 0.20	1.83 ± 0.20	1.25 ± 0.18	1.40 ± 0.19	0.22
L*	59.76 ± 1.33	58.06 ± 1.33	59.70 ± 1.18	61.83 ± 1.23	0.25
a*	10.61 ± 0.59	10.64 ± 0.59	10.61 ± 0.53	11.55 ± 0.55	0.56
b*	17.22 ± 0.88	16.37 ± 0.88	17.53 ± 0.78	18.20 ± 0.81	0.51
% FFL	52.29 ± 1.01	53.95 ± 1.01	53.10 ± 0.90	53.99 ± 0.94	0.63
FFL, kg	40.67 ± 2.30	44.37 ± 2.30	43.62 ± 2.04	42.13 ± 2.13	0.12

[abc] Values within same row with same superscript are not different (P>0.05)

Table 8. LS Means ± SEM for Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on HCW, Dressing Percentage, LEA, Adj. LEA, Last Rib Fat, Adj. Back Fat, and Muscle Score

	Sex			Harvest Group		
	Barrows	Gilts	P>F	1	2	P>F
HCW, kg	80.76 ± 4.65	79.72 ± 4.65	0.79	80.25 ± 4.65	80.23 ± 4.65	0.99
Dressing Percentage	76.08 ± 0.88	76.47 ± 0.88	0.81	71.36 ± 0.88	81.19 ± 0.88	<.0001
LEA, cm^2	42.90 ± 0.21	48.77 ± 0.21	0.03	45.42 ± 0.21	46.26 ± 0.21	0.73
Adj. LEA, cm^2	44.77 ± 0.23	51.42 ± 0.23	0.02	45.74 ± 0.23	50.39 ± 0.23	0.10
10^{th} Rib Fat, cm	2.5 ± 0.07	1.7 ± 0.07	0.02	2.1 ± 0.07	2.1 ± 0.07	0.94
Last Rib Fat, cm	2.7 ± 0.07	2.6 ± 0.07	0.56	2.9 ± 0.07	2.4 ± 0.07	0.12
Adj. Backfat, cm	2.6 ± 0.07	1.8 ± 0.07	0.02	2.1 ± 0.07	2.3 ± 0.07	0.42
Muscle Score	2.02 ± 0.07	2.13 ± 0.07	0.38	2.21 ± 0.07	1.95 ± 0.07	0.05

Table 9. LS Means ± SEM for Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on Marbling, Wetness, Firmness, L*, a*, b*, PFFL, and FFL

	Sex			Harvest Group		
	Barrow	Gilt	P>F	1	2	P>F
Marbling	1.79 ± 0.20	1.02 ± 0.20	0.04	1.17 ± 0.20	1.65 ± 0.20	0.19
Wetness	1.53 ± 0.16	1.78 ± 0.16	0.40	1.66 ± 0.16	1.66 ± 0.16	1.00
Firmness	1.40 ± 0.21	1.57 ± 0.21	0.66	1.52 ± 0.21	1.44 ± 0.21	0.83
L*	59.88 ± 1.36	59.79 ± 1.36	0.97	59.64 ± 1.36	60.04 ± 1.36	0.87
a*	10.82 ± 0.61	10.88 ± 0.61	0.96	10.99 ± 0.61	10.71 ± 0.61	0.79
b*	18.88 ± 0.91	15.78 ± 0.91	0.07	16.21 ± 0.91	18.45 ± 0.91	0.07
% FFL	50.52 ± 1.04	56.14 ± 1.04	0.006	53.14 ± 1.04	53.52 ± 1.04	0.006
FFL, kg	40.77 ± 2.36	44.72 ± 2.36	0.05	42.61 ± 2.36	42.87 ± 2.36	0.89

Table 10. LS Means ± SEM for Treatment Effects of Feeding PJC to Growing / Finishing Pigs on Drip Loss, Marinade Uptake, Marinade Cook Loss, WBS, and Cook Loss

	Treatment				
	Control	0.5% PJC	1.0% PJC	2.9% PJC	P>F
% Drip Loss	0.79 ± 0.25	1.17 ± 0.25	0.92 ± 0.22	1.50 ± 0.23	0.19
% Marinade Uptake	30.43 ± 8.90	37.25 ± 8.90	31.08 ± 7.89	22.02 ± 8.24	0.67
% Marinade Cook Loss	39.14 ± 2.97	40.25 ± 2.97	37.22 ± 2.63	33.87 ± 2.75	0.41
WBS, kg	1.53 ± 0.24	1.33 ± 0.24	1.21 ± 0.21	1.51 ± 0.22	0.10
% Cook Loss	16.88 ± 1.56	13.87 ± 1.56	13.27 ± 1.38	15.64 ± 1.44	0.34

Table 11. LS Means ± SEM for Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on Drip Loss, Marinade Uptake, Marinade Cook Loss, WBS, and Cook Loss

	Sex			Harvest Group		
	Barrow	Gilt	P>F	1	2	P>F
% Drip Loss	0.54 ± 0.26	1.64 ± 0.26	0.03	0.87 ± 0.26	1.33 ± 0.26	0.32
% Marinade Uptake	34.40 ± 9.13	25.99 ± 9.13	0.61	22.05 ± 9.13	38.34 ± 9.13	0.33
% Marinade Cook Loss	39.30 ± 3.04	35.94 ± 3.04	0.55	33.19 ± 3.04	42.04 ± 3.04	0.12
WBS, kg	1.27 ± 0.24	1.51 ± 0.24	0.22	1.43 ± 0.24	1.15 ± 0.24	0.02
% Cook Loss	18.08 ± 1.60	11.75 ± 1.60	0.04	12.06 ± 1.60	17.77 ± 1.60	0.06

Table 12. LS Means ± SEM for Effects of feeding PJC to Growing / Finishing Pigs on Fatty Acids

	Control	0.5% PJC	1.0% PJC	2.9% PJC	P > F
C 8:0	0.13 ± 0.04	0.08 ± .04	0.16 ± 0.04	0.05 ± 0.04	0.31
C 10:0	0.16 ± 0.07	0.20 ± 0.06	0.14 ± 0.06	0.08 ± 0.07	0.58
C 11:0	0.27 ± 0.06	0.23 ± 0.06	0.14 ± 0.06	0.16 ± 0.06	0.43
C 12:0	0.30 ± 0.07	0.11 ± 0.07	0.22 ± 0.07	0.30 ± 0.07	0.13
C 13:0	1.17 ± 0.10	1.31 ± 0.97	1.25 ± 0.09	1.24 ± 0.10	0.82
C 14:0	0.16 ±0.07	0.15 ±0.06	0.14 ± 0.06	0.14 ± 0.06	0.99
C 14:1	1.46 ± 0.19	1.77 ± 0.18	1.54 ± 0.18	1.52 ± 0.18	0.65
C 15:0	0.11 ± 0.05	0.15 ± 0.05	0.19 ± 0.05	0.10 ± 0.05	0.58
C 16:0	22.15 ± 0.55	21.7 ± 0.53	21.60 ± 0.53	22.17 ± .55	0.81
C 16:1	4.21 ± 0.18	4.78 ± 0.17	4.27 ± 0.17	4.53 ± 0.18	0.1
C 17:0	1.48 ± 0.21	1.52 ± 0.20	1.41 ± 0.20	1.68 ± 0.21	0.81
C 17:1	9.61 ± 0.19	9.00 ± 0.18	9.52 ± 0.18	9.18 ± 0.18	0.09
C 18:0	0.15 ± 0.05	0.14 ± 0.05	0.19 ± 0.05	0.08 ± 0.05	0.53
C 18:1 N9T	0.64 ± 0.05	0.67 ± 0.04	0.59 ± 0.04	0.65 ± 0.05	0.59
C 18:1 N9C	38.94 ± 1.22	37.27 ± 1.18	38.13 ± 1.18	37.79 ± 1.22	0.8
C 18:2 N6T	0.05 ± 0.05	0.19 ± 0.04	0.01 ± 0.04	0.11 ± 0.05	0.03
C 18:2 N6C	11.80 ± 0.86	12.48 ± 0.83	12.89 ± 0.83	12.23 ± 0.86	0.83
C 20:0	0.47 ± 0.07	0.53 ± 0.07	0.51 ± 0.07	0.62 ± 0.07	0.55
C 18:3 N6	0.19 ± 0.06	0.19 ± 0.05	0.27 ± 0.06	0.29 ± 0.06	0.56
C 20:1	0.98 ± 0.07	0.88 ± 0.06	0.97 ± 0.06	0.92 ± 0.07	0.66
C 18:3 N3	0.10 ± 0.03	0.07 ± 0.03	0.15 ± 0.03	0.05 ± 0.03	0.14
C 21:0	0.11 ± 0.06	0.29 ± 0.06	0.21 ± 0.06	0.21 ± 0.06	0.22
C 20:2	0.34 ± 0.06	0.47 ± 0.06	0.38 ± 0.06	0.43 ± 0.6	0.45
C 22:0	0.60 ± 0.11	0.44 ± 0.10	0.61 ± 0.10	0.67 ± 0.11	0.5
C 20:3 N6	0.16 ± 0.06	0.24 ± 0.06	0.13 ± 0.06	0.19 ± 0.06	0.63
C 20:4 N6	2.86 ± 0.33	2.99 ± 0.32	2.83 ± 0.32	2.92 ± 0.33	0.99
C 20:3 N3	0.09 ± 0.08	0.23 ± 0.07	0.10 ± 0.07	0.14 ± 0.08	0.5
C 20:4 N6	2.86 ± 0.33	2.99 ± 0.32	2.83 ± 0.32	2.92 ± 0.33	0.99
C 23:0	0.05 ± 0.03	0.11 ± 0.03	0.05 ± 0.03	0.02 ± 0.03	0.31
C 22:2	0.03 ± 0.01	0.03 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.15
C 20:5 N3	0.04 ± 0.02	0.09 ± 0.02	0.00 ± 0.02	0.02 ± 0.02	0.06
C 24:1	0.24 ± 0.10	0.44 ± 0.09	0.16 ± 0.09	0.21 ± 0.10	0.17
C 22:6 N3	0.93 ± 0.16	1.25 ± 0.15	1.27 ± 0.15	1.30 ± 0.16	0.35
Total	99.99 ± 0.01	1.00.01 ± 0.01	100.00 ± 0.01	100.01 ± 0.01	0.39
SFA	27.31 ± 0.36	26.96 ± 0.35	26.83 ± 0.35	27.52 ± 0.36	0.5
MUFA	56.09 ± 1.09	54.81 ± 1.06	55.16 ± 1.06	54.79 ± 1.09	0.83
PUFA	16.60 ± 1.33	18.23 ± 1.28	18.01 ± 1.29	17.68 ± 1.33	0.82
Ratio	0.61 ± 0.06	0.68 ± 0.05	0.67 ± 0.05	0.65 ± 0.06	0.79
N6	15.07 ± 1.21	16.09 ± 1.17	16.12 ± 1.17	15.74 ± 1.21	0.92
N3	1.16 ± 0.18	1.64 ± 0.17	1.51 ± 0.17	1.50 ± 0.18	0.28
N6N3	13.64 ± 1.43	11.33 ± 1.38	11.83 ± 1.38	11.11 ± 1.43	0.61

Table 13. LS Means ± SEM for Sex Effects of feeding PJC to Growing / Finishing Pigs on FA

	Barrows	Gilts	P > F
C 8:0	0.14 ± 0.03	0.07 ± 0.03	0.14
C 10:0	0.13 ± 0.05	0.16 ± 0.05	0.57
C 11:0	0.19 ± 0.04	0.21 ± 0.04	0.75
C 12:0	0.26 ± 0.05	0.20 ± 0.05	0.39
C 13:0	1.22 ± 0.07	1.27 ± 0.07	0.63
C 14:0	0.13 ± 0.05	0.17 ± 0.05	0.61
C 14:1	1.43 ± 0.13	1.72 ± 0.13	0.15
C 16:0	22.77 ± 0.39	21.04 ± 0.39	0.01
C 16:1	4.47 ± 0.13	41.42 ± 0.13	0.77
C 17:0	1.37 ± 0.15	1.67 ± 0.15	0.18
C 17:1	9.60 ± 0.13	9.05 ± 0.13	0.01
C 18:0	0.17 ± 0.04	0.12 ± 0.04	0.36
C 18:1 N9T	0.59 ± 0.03	0.68 ± 0.03	0.06
C 18:1 N9C	38.65 ± 0.86	37.41 ± 0.86	0.33
C 18:2 N6T	0.07 ± 0.03	0.12 ± 0.03	0.34
C 18:2 N6C	11.75 ± 0.61	12.95 ± 0.61	0.19
C 20:0	0.51 ± 0.05	0.56 ± 0.05	0.45
C 18:3 N6	0.22 ± 0.04	0.25 ± 0.04	0.57
C 20:1	0.94 ± 0.05	0.94 ± 0.05	0.95
C 18:3 N3	0.08 ± 0.02	0.10 ± 0.02	0.58
C 21:0	0.17 ± 0.04	0.24 ± 0.04	0.26
C 20:2	0.35 ± 0.41	0.46 ± 0.04	0.07
C 22:0	0.52 ± 0.08	0.64 ± 0.08	0.29
C 20:3 N6	0.16 ± 0.04	0.20 ± 0.04	0.53
C 20:4 N6	2.29 ± 0.23	3.21 ± 0.23	0.08
C 20:3 N3	0.14 ± 0.05	0.14 ± 0.05	0.98
C 20:4 N6	2.59 ± 0.23	3.21 ± 0.23	0.08
C 23:0	0.05 ± 0.02	0.05 ± 0.02	0.93
C 22:2	0.01 ± 0.01	0.02 ± 0.01	0.39
C 20:5 N3	0.03 ± 0.02	0.04 ± 0.02	0.88
C 24:1	0.21 ± 0.07	0.32 ± 0.07	0.29
C 22:6 N3	0.97 ± 0.11	1.41 ± 0.11	0.01
Total	99.99 ± 0.004	100.00 ± 0.004	0.29
SFA	27.74 ± 0.26	26.58 ± 0.26	0.01
MUFA	55.89 ± 0.77	54.54 ± 0.77	0.24
PUFA	16.37 ± 0.94	18.89 ± 0.94	0.78
Ratio	0.59 ± 0.04	0.72 ± 0.04	0.04
N6	14.79 ± 0.86	16.73 ± 0.86	0.13
N3	1.22 ± 0.13	1.68 ± 0.13	0.02
N6N3	13.02 ± 1.01	10.93 ± 1.01	0.17

Table 14. LS Means ± SEM for Effects of Feeding PJC to Growing / Finishing Pigs on Initial Juiciness, Sustained Juiciness, Initial Tenderness, Sustained Tenderness, Pork Flavor, and Off Flavor

Variable	Control	0.5% PJC	1.0% PJC	2.9% PJC	P > F
Initial Juiciness[a]	5.47 ± 0.32	5.62 ± 0.32	5.32 ± 0.28	4.82 ± 0.30	0.3
Sustained Juiciness[a]	5.20 ± 0.34	5.42 ± 0.34	5.06 ± 0.30	4.66 ± 0.31	0.42
Initial Tenderness[b]	5.06 ± 0.35	5.67 ± 0.35	4.97 ± 0.31	4.91 ± 0.32	0.37
Sustained Tenderness[b]	4.87 ± 0.41	5.40 ± 0.41	4.86 ± 0.36	4.59 ± 0.38	0.56
Pork Flavor[c]	4.99 ± 0.15	4.98 ± 0.15	4.89 ± 0.13	4.88 ± 0.14	0.91
Off Flavor[d]	1.18 ± 0.09	1.19 ± 0.09	1.34 ± 0.08	1.16 ± 0.08	0.38

[a]Scored on an 8-point scale (1 = extremely dry, 8 = extremely juicy)

[b]Scored on an 8-point scale (1 = extremely tough, 8 = extremely tender)

[c]Scored on an 8-point scale (1 = extremely bland, 8 = extremely intense)

[d]Scored on an 8-point scale (1 = no off flavor, 8 = extreme off flavor)

Table 15. LS Means ± SEM for Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on Initial Juiciness, Sustained Juiciness, Initial Tenderness, Sustained Tenderness, Pork Flavor, and Off Flavor

	Sex			Harvest Group		
Variable	Barrow	Gilt	P > F	1	2	P > F
Initial Juiciness[a]	5.17 ± 0.33	5.44 ± 0.33	0.65	5.46 ± 0.33	5.16 ± 0.33	0.62
Sustained Juiciness[a]	5.20 ± 0.34	4.97 ± 0.34	0.71	5.07 ± 0.34	5.10 ± 0.34	0.96
Initial Tenderness[b]	4.97 ± 0.35	5.34 ± 0.35	0.57	5.20 ± 0.35	5.11 ± 0.35	0.89
Sustained Tenderness[b]	4.92 ± 0.42	4.94 ± 0.42	0.97	4.92 ± 0.42	4.94 ± 0.42	0.97
Pork Flavor[c]	5.03 ± 0.15	4.85 ± 0.15	0.52	5.00 ± 0.15	4.87 ± 0.15	0.63
Off Flavor[d]	1.22 ± 0.09	1.21 ± 0.09	0.94	1.26 ± 0.09	1.18 ± 0.09	0.62

[a]Scored on an 8-point scale (1 = extremely dry, 8 = extremely juicy)

[b]Scored on an 8-point scale (1 = extremely tough, 8 = extremely tender)

[c]Scored on an 8-point scale (1 = extremely bland, 8 = extremely intense)

[d]Scored on an 8-point scale (1 = no off flavor, 8 = extreme off flavor)

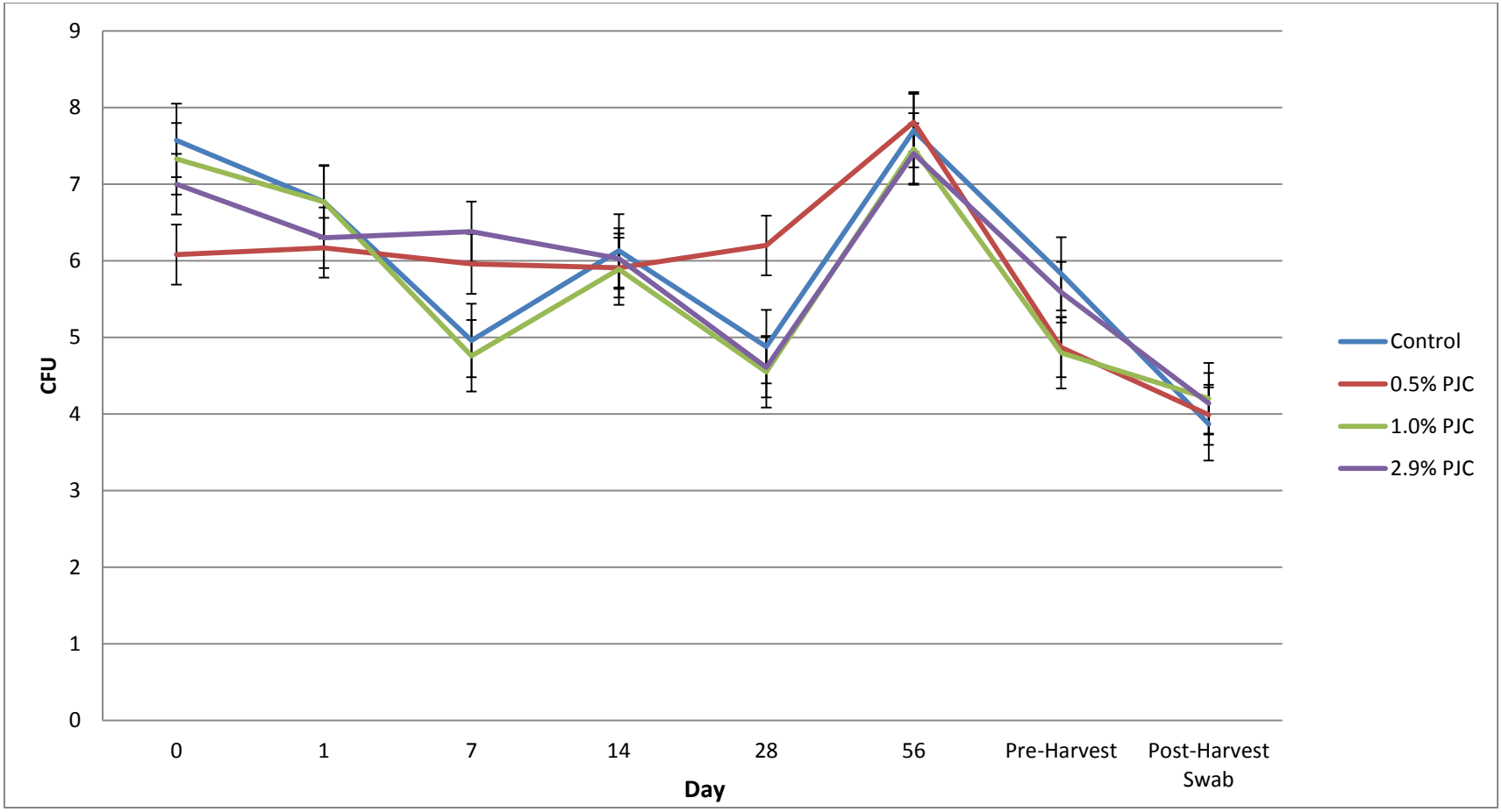


Figure 1. Day x Treatment Interaction of Feeding PJC to Growing / Finishing Pigs on Anaerobic Plate Count

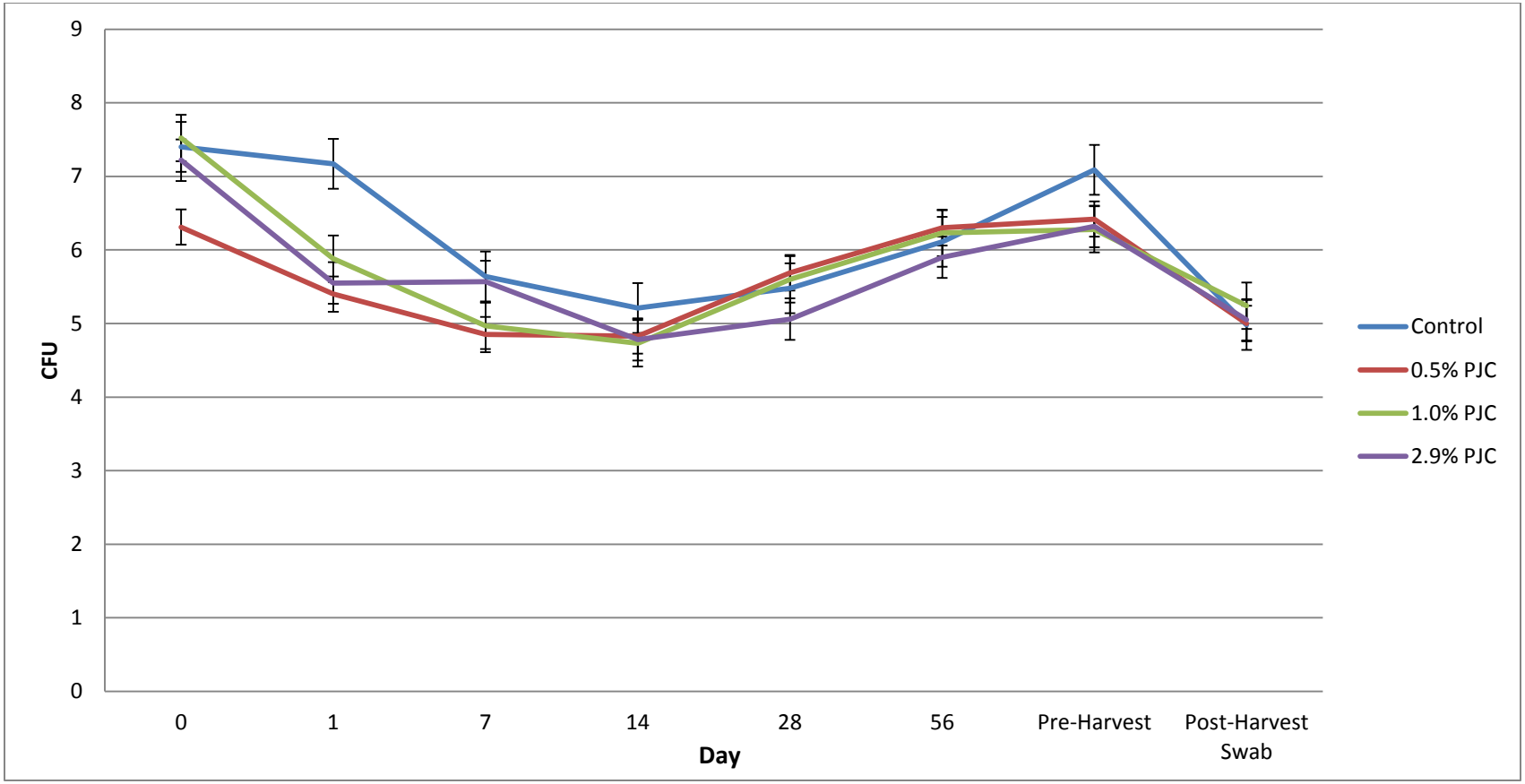


Figure 2. Day x Treatment Interaction of Feeding PJC to Growing / Finishing Pigs on Aerobic Plate Count

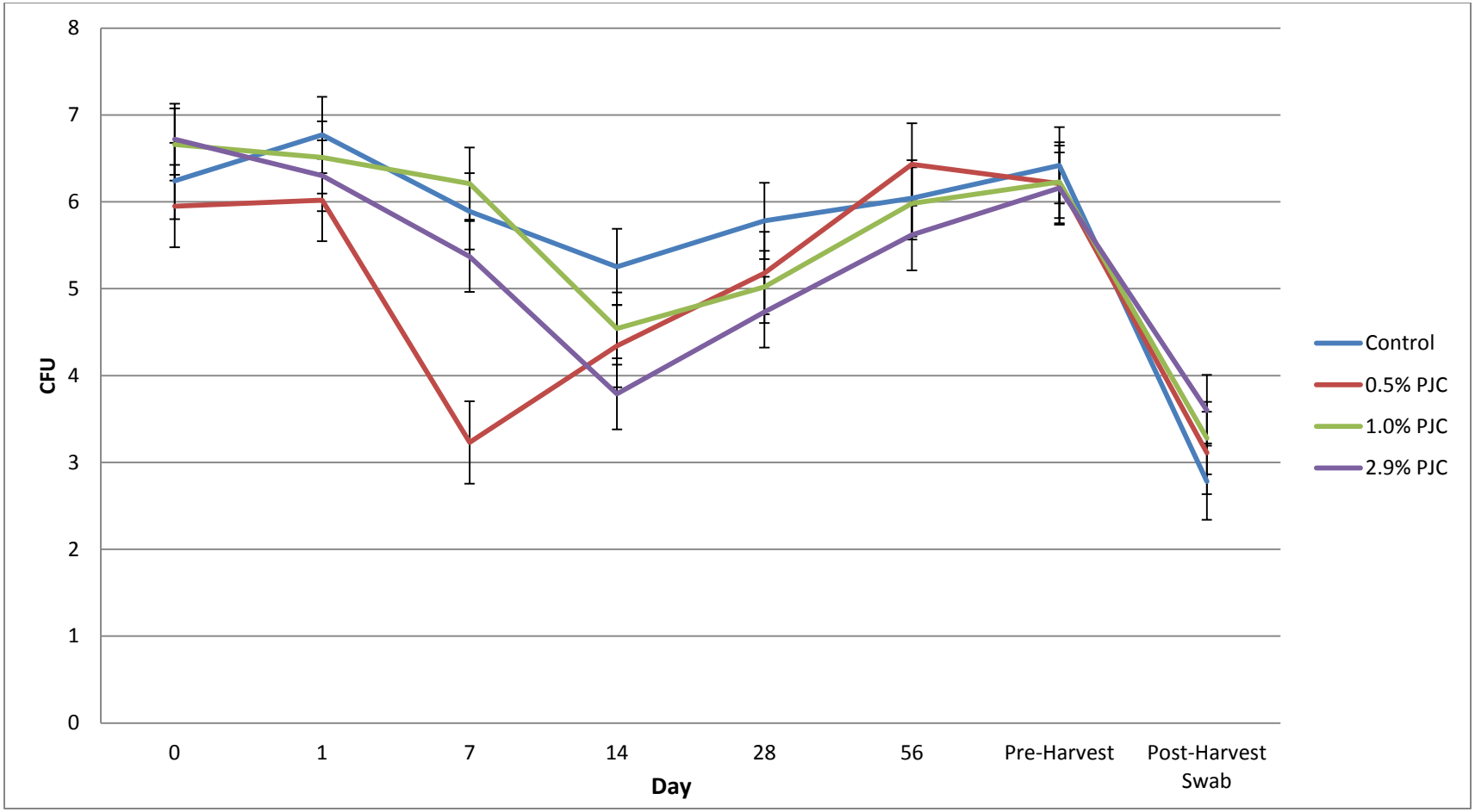


Figure 3. Day x Treatment Interaction of Feeding PJC to Growing / Finishing Pigs on VRBA Count

Figure 4. Effects of Feeding PJC to Growing / Finishing Pigs on Ham pH

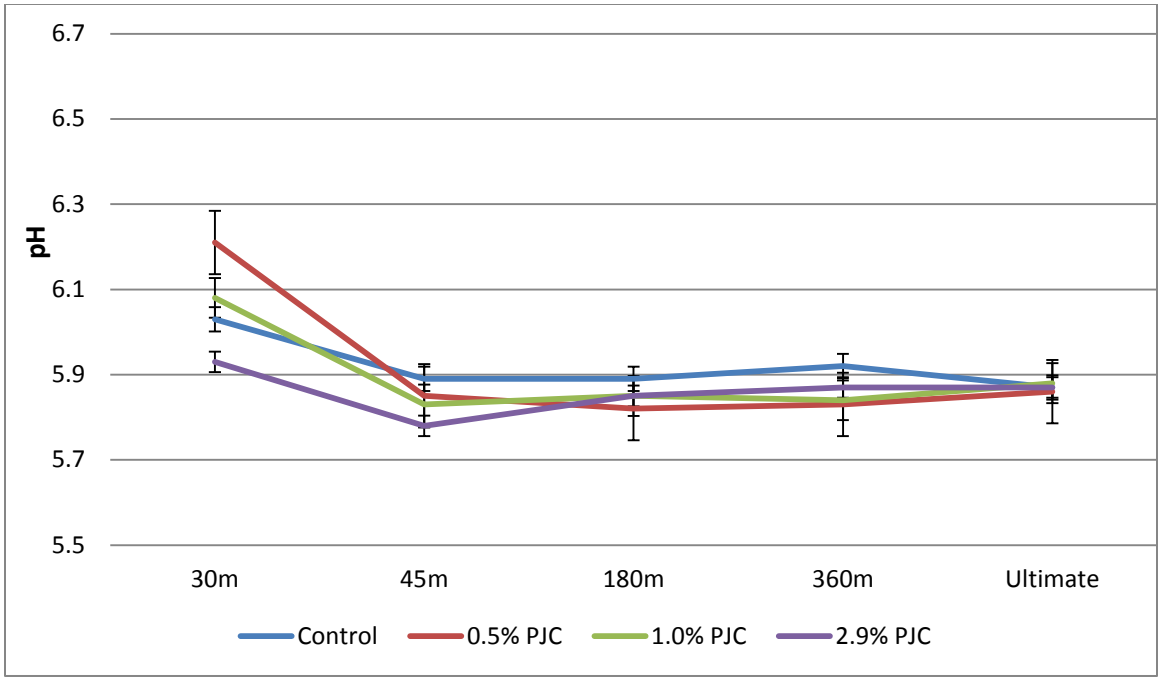


Figure 5. Effects of Feeding PJC to Growing / Finishing Pigs on Loin pH

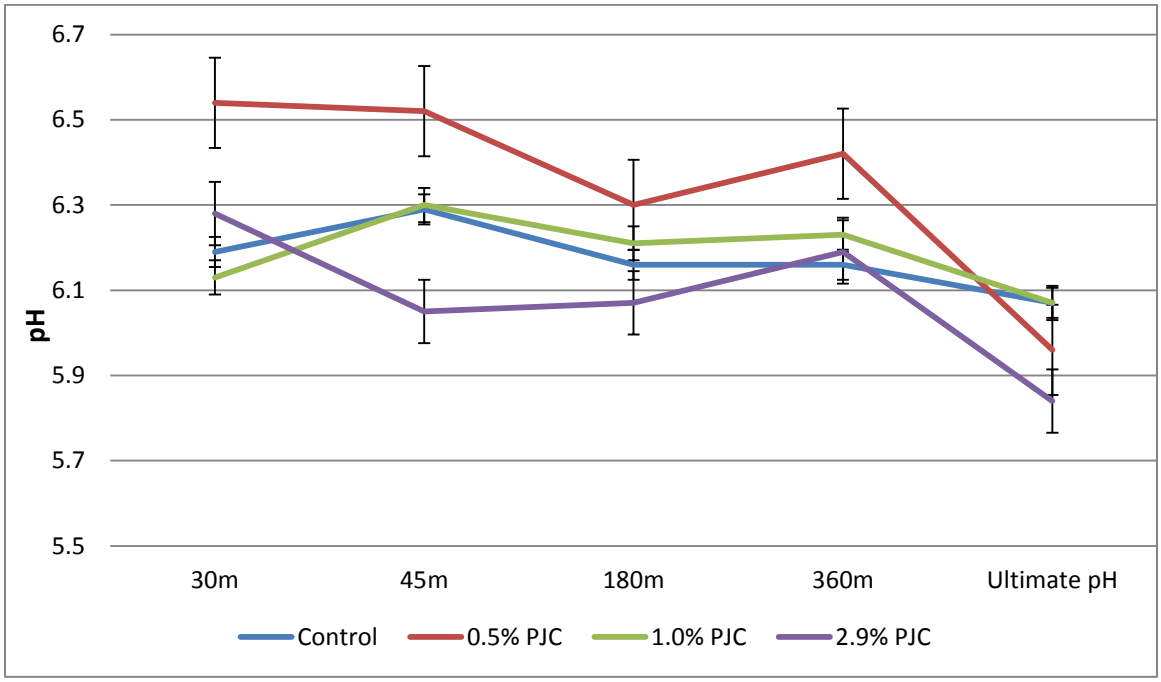


Figure 6. Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on Ham pH

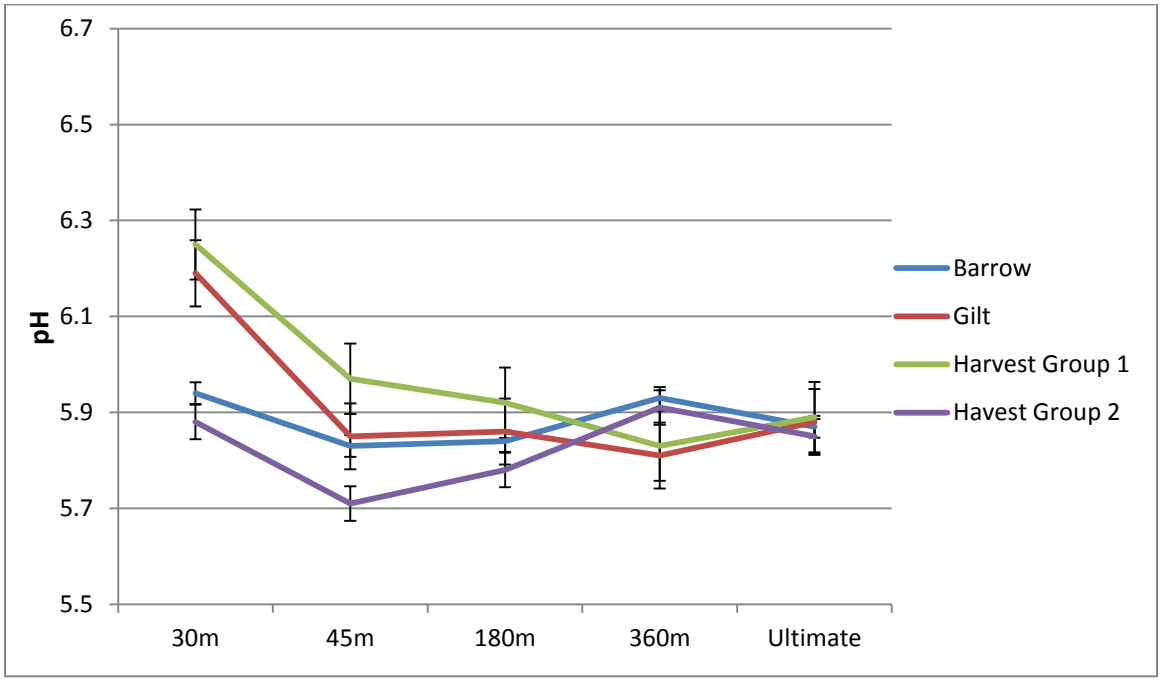
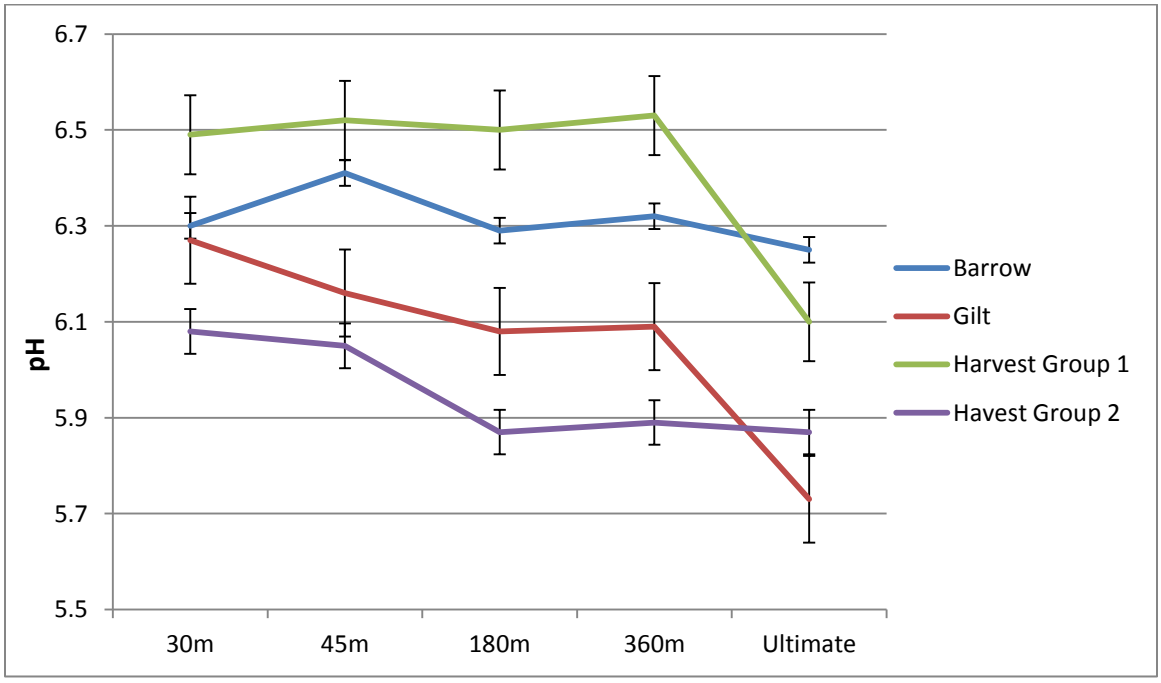


Figure 7. Sex and Harvest Group Effects of Feeding PJC to Growing / Finishing Pigs on Loin pH



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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 show 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. 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 0.01 g 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.

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.

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, grin meat through 6.4-mm (1/4-inch) 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 bugger (3.5% NaCl = NaCl in 1 liter of water).
5. Place scewcap on tightly and shake gently until samples break apart.
6. Vigorously shake an additional 15 seconds.
7. Put 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).

Marinade Uptake =

(weight tube and meat, after incubation at 25°) – (initial weight of tube and meat) / 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 tube an 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

Marinade Cook Loss =

(weight tube and meat, after cooking) – (initial weight of tube and meat) / 6.00 g x 100

Appendix D

Thiobarbituric Reactive Substances Assay

Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A., Phillips, B. 2002. Modified extraction method for determining 2-thiobarbituric acid values in meat with increase specificity and simplicity. J. Food Sci. 67:2833-2836.

A. Solutions

a. TCA Extraction solution

7.5% (w/v) trichloroacetic acid
0.1% (w/v) EDTA
0.1% (w/v) Propyl Gallate

b. 80 mM TBA solution

1.15 g Thiobarbituric acid into 100 mL ddH2O

c. Standard Solution

Make a 1 mM solution by adding 240 µL of tetraethoxypropane to 1L

B. Standards

a. Dilute 1mM stock solution to 80 nM /L

b. Then make standards following the table below in individual tubes

mg / kg TEP	TEP (µL)	TCA (µL)	Pipette Setting
0	0	2000	1000 x 2
2	50	1950	975 x 2
4	100	1900	950 x 2
6	150	1850	925 x 2
8	200	1800	900 x 2
10	250	1750	875 x 2
20	500	1500	750 x 2
30	750	1250	625 x 2

C. Sample preparation and extraction procedure

a. Mince meat sample and weigh out 5 g

b. Place meat into a 50 mL centrifuge tube and add 15 mL TCA Extraction solution

c. Homogenize meat for 20-30 sec using a blender

d. Place lid back on centrifuge tube

e. Centrifuge at 1,500 x *g* for 15 min

f. Remove from centrifuge and filter through No. 4 Whatman paper

D. Incubation and Reading

a. Load 96-well microplate

b. Each sample should be loaded in triplicate with 125 µL / well (See diagram below for details)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	A	A	A	I	I	I	Q	Q	Q
B	2	2	2	B	B	B	J	J	J	R	R	R
C	4	4	4	C	C	C	K	K	K	S	S	S
D	6	6	6	D	D	D	L	L	L	T	T	T
E	8	8	8	E	E	E	M	M	M	U	U	U
F	10	10	10	F	F	F	N	N	N	V	V	V
G	20	20	20	G	G	G	O	O	O	W	W	W
H	30	30	30	H	H	H	P	P	P	X	X	X

c. After sample are loaded, pipette 125 µL of TBA Solution into each well

d. Incubate for 130 min at 40o C

e. Remove plates from incubator and read at 540 nm on plate reader

Appendix E

Fatty Acid Methyl Esters

O'Fallon, J. V., Busboom, J. R., Nelson, M. L., Gaskins, C. T. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511-1521.

A. Solutions and Chemicals
- a. Hexane
- b. Methanol (MeOH)
- c. 10 N KOH
- d. 24 N H_2SO_4

B. Direct Fatty Acid Methylation

a. Mince meat sample and weigh out 1 g

b. Place 1 g meat into a 16 mL screwtop tube

c. Add 5.3 mL of MeOH, 0.7 mL of KOH and standard to meat sample in tube. Place cap on tube and place in water bath at 55^{o} C. If waterbath has a shaker attachment, turn shaker attachment on to desired setting. If there is no shaker attachment, vortex samples for 5 s every 20 min. Incubate for 90 min.

d. After incubation, place samples in cold tap water and allow to cool to below room temperature.

e. After cooling, add 0.58 mL of 24 N H_2SO_4. Mix tube by inversion and make sure K_2SO_4 precipitate is present. Place tube back in water bath and incubate for 90 min at 55^{o} C. If there is no shaker attachment, vortex samples for 5 s every 20 min.

f. Repeat step d.

g. After cooling, add 3 mL of hexane and vortex for 5 min.

h. Centrifuge tubes for 5 min at 1,500 x *g*.

i. Remove hexane layer and place in fatty acid vial.

j. Place fatty acid vials in freezer until time of analysis

Appendix F

Trained Sensory Evaluation Form

Name Date Project

Sample Number	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Pork Flavor Intensity	Off Flavor Intensity	Off Flavor Descriptor

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