THE EFFECTS OF DIETARY SOY ISOFLAVONE SUPPLEMENTATION ON CARCASS CUTABILITY AND MEAT QUALITY OF PIGS INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

BY

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THESIS

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ABSTRACT

Porcine reproductive and respiratory virus is (PRRSV) is endemic in the U.S. swine industry. Understanding the viral effects on pig growth and meat quality is essential to characterizing the true impact of infection. Interventions to mitigate the detrimental effects of infection are necessary to successfully overcome these consequences. The objective of this research was to evaluate the effects of porcine respiratory and reproductive syndrome virus (PRRSV) infection and dietary soy isoflavone (ISF) supplementation on carcass cutability and meat quality of commercial pigs. Barrows were randomly allotted to experimental treatments that were maintained throughout the study: non-infected pigs received an isoflavone-devoid control diet (CON, n = 22), and infected pigs received either the control diet (PRRSV-CON, n = 20) or a control diet supplemented with total ISF in excess of 1,600 mg/kg (PRRSV-ISF, n = 25). Following a 7-day adaptation, weanling pigs were inoculated intranasally with either a sham-control (PBS) or live PRRSV (1×10^5 $TCID_{50}$/mL, strain NADC20). Pigs were then raised until 166 days post-inoculation and slaughtered humanely at the University of Illinois Meat Science Laboratory. At 1 d postmortem (192-194 days of age; approximately 120 kg BW), left sides were separated between the 10^{th} and 11^{th} rib for determination of loin eye area (LEA), backfat thickness (BF), and loin quality (ultimate pH, instrumental color, drip loss, visual color, marbling, and firmness). Loin chops were aged 14 d postmortem prior to Warner-Bratzler shear force (WBSF) determination. Belly width, length, thickness, and flop distance were determined. Data were analyzed as a one-way ANOVA in the MIXED procedure of SAS 9.4. Pig was the experimental unit. Least squared means were separated using the probability of difference (PDIFF) option and means were considered significantly different at $P \leq 0.05$. Carcass yield, LEA, BF, and estimated lean percentage did not differ ($P > 0.26$) among treatments. Loins from

CON pigs had increased ultimate pH ($P = 0.01$), reduced L* scores ($P = 0.005$) coupled with darker visual color scores ($P = 0.004$), were firmer ($P < 0.0001$), and had reduced drip loss ($P = 0.01$) compared with PRRSV-CON and PRRSV-ISF pigs. However, WBSF of chops cooked to 71°C did not differ ($P = 0.51$) among treatments after 14 d of aging. Bellies from CON pigs were more firm ($P < 0.01$) compared with bellies from PRRSV-CON and ISF pigs. These data suggest PRRSV infection and soy isoflavone supplementation do not alter carcass characteristics but infection may marginally reduce loin and belly quality. Using the chops mentioned above, a separate cooking methods validation study was completed. The pursuit of novel, more repeatable, and more accurate methods to measure research outcomes is fundamental to the furthering of meat science. The objective of the second study was to determine the ability to detect differences in cook loss and WBSF values between chops aged for differing time periods and cooked to varying degrees of doneness within a sous-vide style cooker. The posterior section of a pork loin was cut into 6 separate 2.54 cm thick chops. The middle four chops were randomly designated for aging of 3 d and cooked to 63°C, aged 7 d and cooked to 63°C, aged 14 d and cooked to 63°C, or aged 14 d and cooked to 71°C. Chops were cooked by placing them in a water bath with an immersion circulator set to the desired end-point temperature for 90 min. Cook loss was calculated and WBSF values were measured. Data were analyzed using a 1-way ANOVA. Least squares means were separated using the probability of difference (PDIFF) option in the MIXED procedure of SAS. Chop served as experimental unit, with loin serving as a block and day as a random effect. Differences in cook loss and tenderness were detected between aging periods. Overall, these data indicate sous-vide is an acceptable cooking method for use in experiments as expected differences in cook loss and WBSF were detected in chops aged to differing time points or cooked to differed degrees of doneness. This, combined with greater

cooking temperature control, convenience, and potentially less degree of doneness variation indicate sous-vide may be more useful in meat science research than grilling.

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If I have seen further, it is by standing upon the shoulders of giants.

- *Sir Isaac Newton*

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CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Pork is the most abundantly consumed protein worldwide, with the United States ranked 8th overall in per capita consumption (Pork Checkoff, 2018). With a global population expected to reach 9 billion people in the near future, raising pork efficiently while maintaining quality is the challenge to the community of producers, packers, and scientists engaged in pork production. Diseases rob herds of their full growth potential and hampers productivity. One of the most endemic pathogens present in the American herd is porcine reproductive and respiratory virus (PRRSV). Postnatally, PRRSV infection is characterized by a loss in efficiency and gain, meaning a reduction in meat produced and thus reduced profitability (Holtkamp et al., 2012). Unfortunately, PRRSV is extremely virulent and spreads quite easily from those infected to the naïve population (Wills et al., 1997). It can also persist for an extended period of time within the environment, making it challenging to eradicate. For these reasons, interventions to mitigate the detrimental effects of infection have been of interest for some time. In particular, nutritional intervention with soy isoflavones has shown promising results in several weaned pig studies, but little is known regarding the long-term effects of supplementation in a wean-to-finish environment. More work is needed to further characterize the benefits, or lack thereof, of dietary isoflavones given to pigs during times of stress. Additionally, the effects of PRRSV infection and isoflavone supplementation on meat yield and quality is unknown.

While maintaining a high health status is ideal, this is not always reality for producers. In cases where nutrient depravation occurs, whether it be through sickness anorexia or producer-

initiated feed depravation, there is still opportunity for correction of the growth curve. Compensatory growth occurs when an animal resumes normal or greater feed intake after a period of reduced voluntary intake. This results in an accelerated rate of gain due to the animal attempting to 'catch up' to its normal cohort. This period of enhanced efficiency can prove beneficial to producers if taken advantage of properly. Compensatory growth has been extensively studied, particularly as a potential feeding regimen for beef cattle. Given that pigs with PRRSV infection are expected to experience feed withdrawal and a period of limited growth, compensatory gain is expected as pigs recover from infection. The effect of this compensatory gain on meat yield and quality, though is unclear. This literature review will background information pertinent to viral infection, pig growth, and pork quality with particular emphasis on PRRSV infection.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

Porcine reproductive and respiratory syndrome virus is a member of the *Arteriviridae* family, part of the genus *Arterivirus.* This family of viruses also includes lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus. All are lipid enclosed single strand RNA viruses (Rossow, 1998). Due to the nature of being an RNA virus, mutations are common within PRRSV viruses, meaning that separate herds infected with PRRSV may have distinct viruses (Rossow, 1998). Currently, there are two distinct PRRSV genotypes recognized: the North American PRRSV isolate and the European isolate. Clinically, this means that pigs vaccinated against one strain may not be protected against the other (Rossow, 1998). Acute PRRSV infection typically lasts approximately 28 days and is largely localized to alveolar macrophages (Chand et al., 2012). Alveolar macrophages exist both in the epithelial lining fluid and ambient air in the lungs (Redente, et al. 2016). In normal function, alveolar macrophages

phagocytize both harmful and harmless particulates that are inhaled during respiration. This leads to degradation of the particulate matter via lysosomes. Alveolar macrophages also play a role in the suppression of inflammation and immune responses, something unique to this class of macrophages (Redente, et al. 2016). When the phagocytic capacity is reached, an immune response may take place as bacterial or viral cells are then free to interact with other types of host cells (Redente, et al. 2016). In summary, a potential host must inhale enough pathogen load to overcome the alveolar macrophage phagocytic capacity in their lungs in order to illicit an immune response.

The current understanding of the immune response to PRRSV is far from complete. The virus induces little expression of interferons, meaning that an adequate immune response is not elicited from the beginning of infection. Additionally, a neutralizing response specific to the infective PRRSV strain may not be mounted for several weeks after exposure (Murtaugh et al., 2002). PRRSV has been reported to increase expression of interleukin (IL)-1β, IL-8, IL-10, IL-12, and tumor necrosis factor (TNF)-α in porcine alveolar macrophages. Two of these cytokines, IL-6 and TNF-α, have been associated with muscle tissue damage (Pyne, 1994) and may also contribute to lung damage, which is why these cytokines play a central role in PRRSV-induced respiratory distress (Chand et al., 2012). Interestingly, PRRSV increases production of IL-10 and IL-12 more so than other porcine respiratory diseases, such as *M. hyopneumoniae* (Thanawongnuwech et al., 2004). The dramatic increase in IL-10 expression early in infection is unusual, as IL-10 is typically produced during the later stages of immune responses due to its immunomodulatory capacity (Redpath, 2001). This early expression of IL-10 may underlie PRRSV persistence within a host. Interleukin-10 has immunosuppressive capabilities through antagonizing IL-12 and other proinflammatory cytokines, as well as suppression of cell-mediated

immune responses and antigen presentation. This hijacking of the immune response by PRRSV induces alveolar macrophages to produce IL-10 in order to utilize its immunomodulatory function to mute the host's defense and allow the infection to progress (Song et al., 2013). The immunosuppressive effects of IL-10 may also explain the high incidence of secondary infections in PRRSV infected herds. These secondary infections can be bacterial, viral, or multifactorial (Murtaugh, et al. 2002). Both IL-10 and further immune system strain due to secondary infections may provide some hints as to the biological mechanism behind PRRSV persistence.

The virus manifests different symptoms depending on the life stage of the pig it has infected. Due to its immunosuppressive qualities (Murtaugh et al., 2002; Redpath et al., 2004; Song et al., 2013), field PRRSV infections are rarely unaccompanied by another opportunistic pathogen (Rossow, 1998). This may confound clinical presentation in infected herds; however, there are some classical symptoms of PRRSV infection.Within the breeding herd, symptoms can vary depending on the trimester the sow is exposed to the virus. Clinically, sow presentation can vary from no symptoms to lethargy, anorexia, and respiratory distress (Rossow, 1998). Unfortunately, the most devastating symptom, abortion and fetus loss, is also the most consistent across infections. Infections transferred in early pregnancy may result in abortions across the herd. Infections transferred in the last trimester are usually characterized by late-term abortion, or litters riddled with stillborn, autolyzed, or mummified piglets (Rossow, 1998). When the economic impact of PRRSV infection within sow herds was analyzed, losses averaged $93.54 per sow in the commercial herd during the infection period. The consequences of PRRSV infection was higher in nucleus herds, with average losses of $380.39 per sow during the infection period (Nieuwenhuis, et al. 2012). These economic losses are largely attributed to the reduction in number of sold pigs, caused by a decrease in number born alive and an increase in

both pre- and post-weaning mortality. These numbers also differ slightly from other economic reports, which estimated losses per commercial production sow to be slightly higher (Holtkamp et al., 2013). Any additional economic losses caused by a reduction in growth of piglets born to PRRSV-infected dams are unknown.

PRRSV can be passed through the placenta and infect fetuses prior to farrowing (Mengeling, Lager and Vorwald, 1998; Rowland, 2010). This means that immune challenge can begin for a neonatal pig immediately at birth. This also means that most non-infected littermates will become infected either via the sow or the sibling piglets (Mengeling, Lager and Vorwald, 1998). Neonatal infection results in an increase in mortality, lethargy, anorexia, (Stevenson et al. 1993; Rossow, 1998) and enlarged lymph nodes with lymph lesions observable on necropsy (Rossow et al., 1994).

In grower-finisher pigs, PRRSV is much more variable in its presentation. Symptoms can range from respiratory distress, lethargy, fever, and decreased feed intake to no clinical signs at all (Zimmerman et al., 1997). This variation is largely between herds and not among them. Mortality is also lower in PRRSV-infected grower-finisher herds compared with weaned pig populations. It is generally concluded that in practice, the danger from PRRSV comes from its interaction with other pathogens, thus exacerbating the effects of both (Zimmerman et al., 1997). For example, it is common for PRRSV to be a component of the pathogenic conglomerate porcine respiratory disease complex (PRDC). Other likely members of the complex include, but are not limited to, *Mycoplasma hyopneumoniae* and swine influenza virus (Thacker, 2001). It is important to note that due to the number of pathogens that can combine and elicit responses indicative of PRDC, components of isolates will vary by herd and may not always contain PRRSV. Mortality has been found to increase substantially when PRRSV is found in the context

of PRDC (Brockmeier, Halbur, and Thacker, 2002), averaging 4-6% while reaching up to 15% in individual herds (Bochev, 2007). A simple PRRSV infection is known to reduce weight gain and feed intake in grower pigs (Escobar et al., 2004; Schweer et al., 2014; Schweer et al., 2017). This is largely due to self-induced anorexia expressed as a sickness behavior (Johnson, 2002), which is thought to be caused by the increase in circulating inflammatory cytokines including as IL-1, IL-6, IL-8, TNF-α, and IFN-α (Rauw, 2012). This results in production losses such as smaller finished pigs and longer time to market, compounding the costs for treatment and biosecurity measures required once a herd breaks with the virus.

Although estimations are variable, the economic cost associated with PRRSV infection on both individual farm and national levels is astounding (Holtkamp et al., 2013). In the most recent economic analysis of the disease, Holtkamp reported that the majority of the impact was due to loss of revenue rather than additional costs to producers. The reduction in muscle accretion experienced by infected grower-finisher pigs costs the industry an average of $4.67 for every pig slaughtered. Due to the severity of the losses experienced in the breeding herd, the annual cost per infected sow was calculated to be $114.71. Overall, it is estimated that PRRSV infection costs the U.S. pork industry upwards of $663 million annually (Holtkamp, et al. 2012), a huge increase from numbers estimated in 2005 (Neumann et al., 2005).

COMPENSATORY GROWTH

Compensatory growth is a period of accelerated weight gain after a period of slower growth, characterized by an increase in average daily feed intake (ADFI), gain:feed, and average daily gain (ADG). This slow growth period can be induced by feed restriction or sickness anorexia, as would be the case in animals infected with PRRSV. The lack of feed intake during

this time is further taxing on the animal as nutrients are required to form cells needed for an immune response. Despite this, it is expected that during the recovery period, animals that experienced suppressed growth due to sickness will undergo compensatory growth similarly to healthy animals recovering from feed restriction. The growth rate exhibited during this period usually exceeds that of animals that were never restricted (Wilson and Osbourn, 1960; Fox et al., 1972, Ryan, 1990; Hornick et al., 2000; Ballester et al., 2018). This growth can either incompletely or completely compensate for lost muscle accretion during the nutrient restriction period (Wilson and Osbourn, 1960). The extent of recovery can be measured by the compensatory index, which is the ratio between the difference in body weight between a normal and restricted animal at the end of restriction and at the end of the compensatory period. Factors affecting this ratio are expansive, from the severity and length of restriction to the species and age of the animal (Fox et al., 1972; Ryan, 1990; Hornick et al., 2000).

Mechanisms behind compensatory growth

In cattle restricted from feed, blood glucose, IGF-1, insulin, and thyroid hormone were depressed, while growth hormone (GH) was increased (Hayden, Williams and Collier, 1993; Yambayamba, Price, and Foxcroft, 1996). This supports the theory that during nutrient deprivation, an insensitivity to growth hormone is experienced via the suppression of growth hormone receptor synthesis (Hornick et al., 2000). Increased levels of GH stimulated hormones, such as insulin and thyroid hormone, have a negative feedback effect on the production of GH itself. In a feed-restricted state, fewer of these downstream products are being made, resulting in the production of more GH for a longer period of time. During the recovery period, these hormonal levels can be normalized, with no differences observed between restricted cattle and control cattle by day 36 of refeeding (Yambayamba, Price, and Foxcroft, 1996).

Indicating another potential metabolic pathway of importance, it has been reported that the AMPK pathway is downregulated in pigs undergoing compensatory growth when compared to pigs that were never restricted (Ballester et al., 2018). AMPK is a kinase thought to be at the center of numerous metabolic pathways. In short, AMPK promotes catabolic functions in the cell, and inhibits anabolic functions (Ballester et al., 2018). AMPK activation results in increased glycolysis and reduced glycogen synthesis in skeletal muscle, as well as increased fatty acid oxidation (Ballester et al., 2018). Downregulation of AMPK in animals undergoing compensatory growth allows anabolic pathways to become more active, encouraging growth. Animals with downregulation of AMPK would deposit more protein more quickly as refeeding occurs, accelerating their growth curve. This is supported by Therkildsen et al. (2002) who reported that pigs that underwent compensatory growth expressed eEF-2 more quickly after refeeding than μ-calpain. These factors represent factors involved in protein deposition and degradation, respectively, indicating a period where protein is being deposited far faster than it is being degraded. Feed efficiency, measured as the ratio of weight gain to feed intake (gain:feed) increases during refeeding in steers restricted during the growing phase compared to their ad-libitum fed peers, likely due to a reduction in maintenance requirement (Fox et al., 1972; Hayden, Williams, and Collier, 1993; Sainz, de la Torre, and Oltjen, 1995; Yambayamba, Price, and Foxcroft, 1996; Hornick et al., 2000). This indicates a more efficient use of dietary energy during the realimentation period. Restricted and refed steers also have lower plasma urea nitrogen, indicating a more efficient utilization of protein (Fox et al., 1972). Genes associated with cell proliferation and protection from apoptosis were upregulated in pigs restricted from feed (Ballester et al., 2018). All of these changes point to potential metabolic pathways behind the compensatory growth phenomenon.

Body composition and carcass effects of compensatory growth

When an animal is feed-restricted, priority is placed on conserving as much protein as possible while maintaining body function. This leads to increased fat mobilization over protein degradation, resulting in leaner body composition. An exception to this is an already lean animal that does not have the fat reserves to pull energy from, in which weight loss will be primarily comprised of muscle degradation (Hornick et al., 2000). Therefore, during re-alimentation, the composition of gain is largely muscle over adipose tissue (Fox et al., 1972; Sainz, de la Torre, and Oltjen, 1995). Increased fat mobilization during restriction followed by more efficient muscle protein deposition during refeeding results in a leaner animals after compensatory gain.

Steers restricted during the growing phase and refed during the finishing phase had reduced backfat depth and marbling score, but no difference in ribeye area, indicating less relative body fat and increased relative body protein (Sainz, de la Torre, and Oltjen, 1995). Pigs restricted from feed during the grower phase and refed during the finisher phase had similar backfat depths at 110 kg but less intramuscular fat in the longissimus dorsi and biceps femoris (Heyer and Lebret, 2007). Compensatory growth in pigs also decreased dressing percent (Heyer and Lebret, 2007), likely due to increased gastrointestinal tract size resulting from the rapid increase in ADFI during the re-alimentation period. Estimated carcass lean content was increased in pigs that underwent compensatory gain as a result of a greater ham proportion and reduced proportional backfat thickness (Heyer and Lebret, 2007; Therkildsen et al., 2002). Compensatory growth may also increase tenderness in sensory analysis, but these results were not reflected in instrumental tenderness scores (Lametsch et al., 2006) and do not agree with other studies (Therkildsen et al., 2002). During PRRSV infection, pigs have reduced ADG as a consequence of suppressed ADFI (Escobar et al., 2004; Schweer et al., 2014; Schweer et al., 2017). If

infection were cleared during the grower or early finisher phases, it is likely that these animals would undergo refeeding and compensatory gain. Pigs slaughtered after compensatory gain would have less fat deposition and greater proportional lean content.

SOY ISOFLAVONES

Isoflavones that exist naturally in soy plants are phytoestrogenic compounds (Reinli and Block, 1996), meaning that they are structurally similar to estrogen and may activate its receptors in the body (Mazur, 1998). Isoflavones commonly found in soy are daidzein, genistein, and formononetin, along with their various conjugates. Endogenous estrogen levels, along with type and concentration of isoflavones, can affect their potency, which ranges from agonistic to mildly antagonistic. For example, isoflavones may encourage estrogenic activity in men, but be antagonistic in premenopausal women (Reinli and Block, 1996). This antagonistic effect is accomplished by binding to estrogenic receptors and blocking more potent ligands (Kuhn et al., 2004). While they are found in a variety of different foodstuffs, plants of the Leguminosae family have been reported to be the most influential group in regards to isoflavone concentration. Notable members of the family are soybeans, clover, alfalfa, and peanuts. Genistein, considered to be the most potent phytoestrogen, is found in the highest concentration in soybeans and its products (Mazur, 1998). Processing of soy can affect the isoflavone concentration of the end product. Isoflavones are retained during the defatting process, but the majority are lost during the alcohol extraction to generate soy protein concentrate (Kuhn et al., 2004). The unique microbial environment in individual animal's digestive systems can affect the bioavailability of isoflavones (Ren et al., 2001). Isoflavones found in soy are in the glycoside form, which are biologically inactive. In order to be converted to the active, aglycone form, isoflavones must be hydrolyzed in intestinal epithelial cells by brush border enzymes (Andres et al., 2009) or bacterial β-

glucosidases (Cerderroth and Nef, 2009). The ability for microbes to complete this transformation efficiently will alter the bioavailability of isoflavones, leading to variability between individuals that should be considered when analyzing isoflavones' effects.

Estrogens moderate lipogenesis and glucose metabolism (Cerderroth and Nef, 2009). This is accomplished through the activation of AMPK in skeletal muscle and adipose tissue (Cerderroth et al., 2008). General metabolic functions of AMPK have been described above. Specifically, increased intake of phytoestrogens is associated with lower whole body lipid concentration, increased expression of genes important in fatty acid oxidation in both adipose and skeletal muscle, and greater insulin responsiveness in skeletal muscle (Cerderroth et al., 2008). As adipocytes express estrogen receptors, isoflavones are capable of interacting directly with them, increasing their effectiveness of manipulating lipid content and insulin sensitivity. Overall, phytoestrogens help encourage metabolism of energy stores, such as lipid, while encouraging glucose uptake, resulting in leaner whole body composition (Cerderroth et al., 2008).

Supplementation in healthy pigs

Supplementation with soy isoflavones increased ADG in newly weaned gilts grown to 30 kg, with the majority of this gain being lean tissue (Cook, 1998). When pigs were supplemented with isoflavones from the beginning of the grower phase (30 kg BW) to the end of the finisher phase (113 kg BW), there were no differences in growth until late finishing, where pigs supplemented with isoflavones had reduced daily gain and reduced feed intake. This did not result in any effect in overall ADG between groups (Payne et al., 2001). These results are echoed by Kuhn et al. (2004), who report no differences in growth outcomes across the weaning,

grower, and finisher phases in pigs fed diets with elevated isoflavone levels. Pigs devoid of isoflavones did have a lower percentage lean tissue and greater percentage of fat at slaughter (Payne et al., 2001). This disagrees with Kuhn et al. (2004), who observed no differences in carcass leanness. In a separate experiment by Payne et al. (2001), increasing levels of supplemental isoflavones did not improve growth beyond that exhibited by pigs fed soybean meal, which contained naturally occurring levels of isoflavones. In this same study, there were no differences in meat quality in pigs either supplemented or deprived of isoflavones similar to the results of Kuhn et al. (2004). Overall, these agree that in a healthy pig model, increased feeding of isoflavones does not affect growth outcomes or meat quality, but may result in leaner carcasses.

Potential mechanisms for antiviral properties

Isoflavones have also been reported to have antiviral properties (Andres et al., 2009), although the mechanism of action for this ability has not been fully illuminated. Isoflavones somehow alter the host cell or the virus such that viral binding, entry, protein expression, or replication are no longer efficient (Andres et al., 2009), and there is *in vivo* evidence suggesting that isoflavones may enhance the adaptive immune response by increasing the proportion of helper T cells (Smith et al., 2017). One proposed pathway is the inactivation of NF-κB by genistein (Andres et al., 2009). NF-κB is a transcription factor for several proinflammatory genes, such as inflammatory cytokines and adhesion proteins. It has also been associated with delayed apoptosis in macrophages, offering another explanation for the enhanced inflammatory responses exhibited with high NF-κB levels (Lawrence, 2009). Inhibition of this transcription factor may mitigate the inflammatory response associated with many viral infections, reducing the severity of the effect exacted on the body. An anti-inflammatory effect would be extremely

beneficial in a PRRSV infection. Another proposed mechanism is that isoflavones may act as inhibiters on protein tyrosine kinases (PTKs), which may be the mechanism of genistein's NF-κB modulation (Andres et al., 2009). These enzymes are a class of kinases that phosphorylate tyrosine residues on substrates within the cell and exist in two subclasses: those that are transmembrane receptors and those that are not receptors. Both are critical members of their specific signaling pathways, the outcomes of which span a wide range of functions (Hubbard and Till, 2000). One function of the Src family of PTKs, a subclass of PTKs, is to help induce inflammatory responses in tissues. Inhibition of these kinases has improved animal responses to acute inflammatory stress (Okutani et al., 2006). Modulation of Src PTKs by isoflavones may reduce the inflammatory effects of PRRSV infection. More pertinent to PRRSV, the PTK, Syk, has been pointed to as a modulator of inflammation in the lung (Ulanova et al., 2005). If isoflavones effectively inhibit Syk, or other PTKs, PRRSV may not be able to induce as vicious an inflammatory response or hijack alveolar macrophages, potentially reducing its virility. It is important to note that the antiviral effect of isoflavones is dose dependent, and more work is needed to establish the effective concentrations. *In vitro* studies do suggest that frequent, low doses of isoflavones may be more effective than a single high dose (Evers et al., 2005).

Supplementation in virally challenged pigs

In newly weaned pigs, increasing supplementation of genistein resulted in reduced serum viral load and serum interferon levels through day post-inoculation (DPI) 24 (Greiner et al., 2001), suggesting a mitigation of virility. This is likely accomplished before DPI 4, as the rate of viral clearance after that was not different between groups, indicating that supplementation prior to infection may aid in combatting the virus. This differs from others, who report a lessening of serum viral load and TNF-α levels only at DPI 14 (Rochell et al., 2015); however, the source of

isoflavones differs between these two experiments. Rochell et al. (2015) fed differing inclusion levels of soybean meal rather than supplementing only genistein, and each treatment used included higher levels of total isoflavones than any of the genistein inclusions used by Greiner et al. (2001). This could be the cause of the discrepancy, indicating differing effects between individual isoflavones compared to a whole meal. In Greiner et al. (2001), supplementation improved growth performance at lower levels of supplementation. Prior to inoculation, weight gain tended to increase in pigs supplemented with 200 and 400 ppm genistein when compared to pigs not given any supplementation. Efficiency was increased with supplementation, with pigs fed at the 400 ppm level being the most efficient. Conversely, pigs supplemented with 800 ppm of genistein had substantially reduced weight gain and feed intake when compared to all other groups (Greiner et al., 2001), suggesting a reduction in palatability. This data provides some direction when considering an effective dose of isoflavones if they are to be fed as a preventative measure. Once infected, supplementation at 200 and 400 ppm did improve feed intake, but ending body weight and efficiency were not different among groups (Greiner et al., 2001). This differs from others, where pigs fed higher amounts of soybean meal had greater body weight at DPI 14, likely due to increased ADG (Rochell et al., 2015; Smith et al., 2019). Similar to data gathered for the pre-infection period, further supplementation of isoflavones combined with a naturally rich source such as soybean meal does not further enhance their growth or immune benefits (Smith et al., 2019).

In summary, isoflavone supplementation during PRRSV infection may reduce viral effects resulting in increased ADG compared to non-supplemented pigs. This would minimize any compensatory growth response because feed restriction during infection would be minimal. Therefore, pigs supplemented with isoflavones during infection would be more similar to their

healthy counterparts in terms of carcass composition, while those allowed to complete full compensatory gain would be leaner. The effect of isoflavone supplementation during an immune challenge on meat quality is unknown.

PORK LOIN QUALITY

Color

Meat color is the most influential trait consumers evaluate when selecting product in the fresh case (Mancini and Hunt, 2005). While it is subjective, consumers correlate color with freshness and wholesomeness. The molecule largely responsible for the final color of meat is myoglobin, and color can differ based on myoglobin form. Myoglobin is a sarcoplasmic hemeprotein, similar to hemoglobin in the blood. The iron in the center of myoglobin has the ability to form six bonds, one of which is used to bind ligands (Mancini, 2013). These ligands, along with the state of the iron molecule, determine meat color. Different combinations of these two properties result in four possible states of myoglobin. Deoxymyoglobin results from a ferrous iron and no ligand in the sixth bonding site, and results in the meat appearing a dark purple. Metmyoglobin contains a ferric iron and water as the ligand, and results in a brown color (Govindarajan and Snyder, 1973; Mancini, 2013). These are the least desirable states of myoglobin in regards to consumer preference. Carboxymyoglobin also has a ferrous iron, but carbon monoxide is bound at the sixth site. Oxymyoglobin also has a ferrous iron with oxygen bound. Both of these myoglobin states result in bright red meat, and are the most desirable (Govindarajan and Snyder, 1973; Mancini, 2013). Color intensity is caused by the concentration of myoglobin in the muscle (Seideman et al., 1984). As a sarcoplasmic protein, myoglobin is

bound to free water in the muscle. When purge occurs, water leaking out will take myoglobin with it, thus diminishing the intensity of the color and reducing the value of the product.

The interaction between temperature and pH decline postmortem is what determines the water holding capacity of meat (Scheffler and Gerrard, 2007). In life, muscle utilizes both oxidative and anaerobic metabolism to meet energy demands under differing physiological conditions. Once the animal is terminated via exsanguination, oxygen can no longer be delivered to muscle cells, forcing them to switch to anaerobic energy sources. The main anaerobic pathway in which muscle will obtain energy for contraction is glycolysis. The end product of glycolysis is pyruvate, which is then converted to lactate in order to regenerate the NAD+ required for glycolysis to continue to occur. Lactate is an acidic waste molecule that under normal circumstances would be removed by the blood. The lack of blood means that lactate builds up in the muscle as metabolism occurs, resulting in a lowering of the muscle pH (Scheffler and Gerrard, 2007). The amount of glycogen available for breakdown in the muscle helps determine how much lactate is generated, driving the ultimate pH. Typically, muscle will reach a pH of 5.3 to 5.7 at 24 h postmortem, but an accelerated decline would mean dipping below a pH of 6.0 within the first 60 minutes after exsanguination. An accelerated decline also means a depletion of energy substrates more quickly, meaning that rigor mortis would be reached at a higher temperature, thus increasing degradation of structural proteins and increasing purge (Wismer-Pendersen and Briskey, 1961; Scheffler and Gerrard, 2007).

There are several antemortem factors that influence the pH decline. Stress results in decreased glycolytic potential at 135 min postmortem, indicating a quicker pH drop resulting in lower water holding capacity (Hambrecht et al., 2005). Acute infection may increase antemortem physiological stress, potentially having an impact on the pH and temperature decline, thereby

influencing meat quality. While data regarding the impact of a PRRSV infection on meat quality is minimal, others have reported no effect on meat quality when utilizing alternative pathogens. In a *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* joint infection, there were no differences between the infective and control groups (Outhouse, 2017). *M. hyopneumoniae* is a respiratory pathogen commonly responsible for pneumonia and is one of the most frequent pathogens to complex with PRRSV. Similar to PRRSV, it also occurs within the epithelial lining of the lungs (Thacker et al., 2000). *L. intracellularis* is a bacteria that propagates within enterocytes causing reduced digestive and absorptive capacity resulting in stunted growth (Stege et al., 2004). *M. hyopneumoniae* also an inflammatory disease, increasing production of TNF-α, IL-1, and IL-6 in experimentally infected pigs (Thacker et al., 2000; Choi et al., 2006). While PRRSV also increases circulating levels of these cytokines, as well as IL-10 and IL-12 (Murtaugh et al., 2002), PRRSV increases IL-10 and IL-12 to levels beyond those induced by *M. hyopneumoniae* and for a longer period of time (Thanawongnuwech et al., 2004). The prolonged elevated levels of circulating IL-10 and IL-12 observed in PRRSV may indicate an extended active infection period compared to simple *M. hyopneumoniae* infections. A longer active infection period would indicated more time muscle would be exposed to cytokines such as TNF-α and IL-6, both of which have been associated with muscle damage (Pyne, 1994). Longer exposure to these damaging inflammatory cytokines may mean more opportunity for reduced meat quality in PRRSV-infected pigs. In particular, pre-damaged proteins may be more prone to further postmortem degradation, resulting in greater opportunity for water leakage and reduced fresh meat color.

Marbling

Marbling has long been associated with higher quality meat, particularly in beef cattle. Even within pork, increasing marbling content increased the likelihood of purchase due to a perceived improved eating experience (Moeller et al., 2010a). There are conflicting data regarding whether or not increased marbling truly does impact tenderness and juiciness. Some report that higher marbled pork chops were correlated with improved tenderness (Lonergan et al., 2007; Cannata et al., 2010), while others have found that marbling in pork does not affect tenderness or juiciness (Rincker et al., 2008; Wilson et al., 2016). It is important to note that the average proportion of marbling (2-3%) exhibited in commercial pigs corresponds to the proportion required to grade Select and Standard in beef cattle (Savel, 1986). This may mean that modern pork simply does not deposit enough marbling to result in tenderness and juiciness improvements observed in beef cattle with increasing quality grade (Wheeler et al., 1994).

Fat Quality

Fatty acid profiles of pork can have an astounding impact on quality, especially of higher fat products like bellies, bacon and sausages. Unsaturated fatty acids have a higher propensity to oxidize, increasing the likelihood of rancidity in the meat (Wood et al., 2004). Oxidized fats may introduce unwelcome flavor volatiles that are off-putting to consumers. Unsaturated fats are also less firm, and as such, may be detrimental to belly and bacon quality. In bellies with higher proportions of unsaturated fatty acids, bacon was oilier, less firm, had less desirable fat coloration, and had a lower slicing yield. Bacon high in unsaturated fatty acids also scored lower in consumer panels, meaning a greater dislike for the product (Shackelford et al., 1990).

Fat deposition is affected by where an animal is on its growth curve. Energy utilization in the body can be thought of as a series of steps, with the first requirement needing to be fulfilled before the animal can move on to the next one. The first requirement is energy put towards maintenance of bodily functions. The second is protein accretion, witnessed externally as muscle growth. Once these systems are saturated with the energy they need to be the most efficient, then excess energy ingested is deposited as lipid (Bastianelli and Sauvant, 1997). As the animal matures, less energy is required for muscle growth and so more lipid is deposited. It is commonly accepted that fat deposition occurs in a defined order regardless of species: abdominal, intermuscular, subcutaneous and then intramuscular (Pethick and Dunshea, 1996; Pethick et al., 2006). These depositions are not exclusive, and increased intramuscular fat is typically accompanied by increased subcutaneous, or even intermuscular, fat content (Pethick et al., 2006).

The fatty acid composition of various depots also changes throughout growth (Kloareg et al., 2007). Pigs slaughtered at a lighter ending live weight had a reduced concentration of the essential fatty acid, alpha-linolenic, which is a desirable nutrient. These pigs also had increasing amounts of unsaturated fatty acids, namely 14:1, 16:1, and 20:2, coupled with a lower overall carcass lipid content (Kloareg et al., 2007). Diet and ADG were not different between slaughter weight groups, indicating that earlier in the growth curve, pigs are depositing a higher proportion of unsaturated fatty acids. Other studies report similar results, with slower-growing pigs depositing a higher proportion of polyunsaturated fatty acids in the belly (Correa et al., 2008). The development of depots also has an impact on the fat firmness. The more underdeveloped the depot is, the higher the water content is, resulting in softer fat (Wood et al., 2004).

These data have interesting implications regarding the effect of compensatory growth on fat quality. Due to the delay in their growth curve, animals slaughtered while undergoing compensatory growth may have a higher proportion of unsaturated fatty acids, detrimentally impacting fat quality. However, the fast growth rate exhibited during compensatory growth may escalate lipid deposition rate. There is a potential for increased deposition of intramuscular fat if re-alimentation is completed prior to slaughter (Lebret, 2008). Pigs that undergo compensatory growth but are slaughtered at the same weight as controls have slightly less backfat, illustrating the delay in lipid deposition within this group (Duckworth, 1965). This is confirmed in more recent pig (Heyer and Lebret, 2007; Therkildsen et al., 2002) and beef cattle studies (Sainz, de la Torre, and Oltjen, 1995). However, this is not maintained in other studies that report no differences in fat deposition or lean content between controls and those pigs that underwent compensatory growth (Outhouse, 2017). This study observed compensatory growth as a result from a pathogenic challenge, and these results may be confounded by the effects of illness.

Firmness

Firmness in fresh meat is a desirable trait to consumers, who demonstrate an aversion to soft, light meat (Brewer and McKeith, 2006). Water holding capacity is correlated to firmness, as a lower water holding capacity is usually associated with a loss of integrity in the perimysium and endomysium (Joo et al., 2013). As described above, this loss of structure originates from a combination of rapid pH decline and high internal temperature. Careful control of these through best processing practices is the most consistent method to improving firmness.

EATING EXPERIENCE

Tenderness

Tenderness is one of the most important components of a positive eating experience (Norman et al., 2003; Cannata et al., 2010; Dilger et al., 2010; O'Quinn et al., 2018). The driving force behind meat tenderness is the postmortem enzymatic proteolysis, affected by the interaction between the pH and temperature declines (Kemp et al., 2010). The first wave of proteolysis is largely attributed to the activity of calpains, which are proteases located in the sarcoplasm. Three isoforms have been identified in skeletal muscle: μ-calpain, m-calpain, and p94 (Kemp et al., 2010). Although p94 is in the calpain family, there is little evidence to indicate it is important in postmortem protein degradation, unlike the other two isoforms. Both μ-calpain and m-calpain are calcium-activated, inhibited by calpistatin, and their main substrate is myofibrillar structural proteins (Koohmaraie, 1992; Kemp et al., 2010). The second wave of proteolysis is defined by the activity of proteasomes. In life, proteasomes in skeletal muscle consist of two subunits and are ATP and ubiquitin dependent. When the muscle undergoes postmortem metabolism and ATP stores are depleted, the proteasome cleaves into its subunits, of which the 20S unit does not require ATP (Kemp et al., 2010). This system hydrolyzes structural proteins already damaged by other proteolysis systems, enhancing their effect. Proteasome activity can persist at ultimate pH levels down to 5.6 and a full week postmortem (Lamare et al., 2002). Increased sarcomere length and decreased collagen content are correlated with increased sensory tenderness (Dilger et al., 2010), but the interaction between these and post-mortem proteolysis is the best predictor (Wheeler, Shackelford, and Koohmaraie, 2000). Tenderness increases with post-mortem aging (Ellis et al., 1998; van Laack et al., 2001; Channon et al., 2003; Dilger et al., 2010; Clark et al., 2014; Jones-Hamlow et al., 2015). Longer aging periods

allow the enzymatic systems described above to continue to denature structural proteins, dissolving muscle foundation (Wagner, 2007). Although myosin and actin are the two most abundant proteins in muscle, recent studies have reported that these two only undergo minor changes mainly due to oxidation (Huff-Lonergan et al., 2010). The critical phenomenon is the degradation of structural proteins such as titin, troponin T, desmin, nebulin, and filamin (Kołczak et al., 2003; Huff-Lonergan et al., 2010). Tenderization begins with a steep decline in WBSF values over days 1-7. The next time period (d 7-14) is characterized by a continued, but less dramatic, reduction in peak values. It is generally accepted that this decline ceases at approximately 14 d post-mortem (Dransfield et al., 1981; van Laack et al. 2001; Rees et al., 2002; Dilger et al., 2010), but some have reported continued tenderization until day 21 (Clark et al., 2014). As the aging phenomenon occurs across species and muscles, it can be used to manufacture differences in tenderness for other research purposes. Those seeking to validate new cooking or packaging methods benefit from its consistency.

Negative correlations between marbling content and instrumental tenderness have been reported (van Laack et al., 2001; Lonergan et al., 2007; Rincker et al., 2008; Wilson et al., 2016), implying that as intramuscular fat increases instrumental tenderness also increases. It is important to note that these correlations account for little of the variation present, and are likely not the only factor contributing to differences in tenderness. Some report that increasing marbling results in greater sensory tenderness scores, but no differences in instrumental tenderness scores (Cannata et al., 2010). Conversely, others report no differences in sensory panel scores among marbling groups (Rincker et al., 2008; Wilson et al., 2016). Based on these studies, marbling content in pork is likely one of many variables affecting inherent tenderness rather than being the sole contributor.

End point cooking temperature has a substantial effect on tenderness of chops. As degree of doneness drops from the historic target of 71°C to the more modern 63°C, both sensory and instrumental tenderness increases (Rincker et al., 2008; Moeller et al., 2010a; Moeller et al., 2010b; Klehm et al., 2018). The most consistent and impactful method for manipulating pork chop tenderness is the lowering of the end point cooking temperature.

Juiciness

Juiciness is resultant from an increased water holding capacity such that more water is retained through processing, storage, and cooking. Excluding the dry, firm, and dark (DFD) meat phenomenon, some water is inherently lost through these processes. Increased marbling content is associated with increased sensory juiciness scores in some studies (Rincker et al., 2008; Cannata et al., 2010; Moeller et al., 2010a; Moeller et al., 2010b); however, the increases in juiciness are so small that their practicality is questionable. In other studies, no effect is detectable (Lonergan et al., 2007; Wilson et al., 2016; Klehm et al., 2018). Elevated ultimate pH results in greater juiciness (Cameron et al., 1990; Huff-Lonergan et al., 2002; Lonergan et al., 2007). As described above, this is likely due to less protein degradation, meaning a greater water holding capacity. Improved water holding capacity combined with reduced drip and cook loss would logically result in juicier chops, though studies examining this relationship are not conspicuous in the literature. One well-documented relationship is that between end point temperature and juiciness. Lower end point temperature results in improved juiciness scores (Wood et al., 1995; Rincker et al., 2007; Moeller et al., 2010a; Moeller et al., 2010b; Klehm et al., 2018), again demonstrating the impact of lowered degree of doneness in pork.

Flavor

Many natural compounds found in raw meat come together to form the flavor profile unique to the species of origin. The main constituents of these flavor precursors are proteins, acids, sugars, and fat, along with volatile components such as products of oxidation (Imafidon and Spanier, 1994). Flavor can be affected by age and sex of the animal, diet, carcass handling, and further processing. Any trial investigating a treatment effect on meat quality should measure flavor to ensure that the product stays within the boundaries of consumer acceptability.

SOUS-VIDE COOKING

Sous-vide is the process of cooking food in a heat stable, sealed package using a water bath. It has been a topic of interest in the meat and food science arenas since the 1990s (Schellekens, 1996; Baldwin, 2012). It began as a method for increasing shelf stability in processed foods, but has quickly grown in popularity within restaurants, home kitchens, and now meat science research. Due to the nature of the water bath, the meat being cooked can never exceed the set temperature, regardless of the time it is submerged. In a logistical manner, when using a sous-vide apparatus for research purposes, the cooking of the chops would not have to be monitored to ensure they do not exceed the desired degree of doneness. Even heating of the water also eliminates hot and cold spots that are commonly found when grilling. Total submergence also allows completely even heating throughout the meat, barring any overcrowding. There are many potential benefits of using the sous-vide method in a research context, including greater reproducibility (Keller, 2008; Baldwin, 2012) and further control over doneness (Baldwin, 2012), although some of these claims are anecdotal and have yet to be substantiated by objective scientific measure (Creed, 1998). If proven, these benefits would

mean a reduction in the variability attributed to cooking, thus allowing greater insight into the effects of treatments on inherent qualities of the meat.

OBJECTIVES

As reviewed, pigs undergoing a viral challenge will experience reduced growth. Dietary soy isoflavones may help mitigate the efficiency loss within an infected herd. During recovery, pigs may undergo compensatory growth, potentially affecting their body composition and meat quality. Therefore, the objective of this study was to characterize how PRRSV affects carcass characteristics and meat quality, and whether or not soy isoflavones were beneficial to the growth or quality of infected pigs. Furthermore, establishment of new methods to be used in research examining meat quality is pertinent to the furthering of meat science. Sous-vide has become a method of renewed interest due to its potential benefits in reducing variability and increasing repeatability. Thus, the objective of the second study was to validate sous-vide as a method to be used for cooking pork chops in a research setting.

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CHAPTER 2

EFFECT OF DIETARY SOY ISOFLAVONE SUPPLEMENTATION ON CARCASS CUTABILITY AND MEAT QUALITY OF PIGS INFECTED WITH PORCINE REPRODUCTIVE AND RESPIRATORY VIRUS

ABSTRACT

Porcine reproductive and respiratory virus is (PRRSV) is endemic in the U.S. swine industry. Understanding the viral effects on pig growth and meat quality is essential to characterizing the true impact of infection. The objective was to evaluate the effects of porcine respiratory and reproductive syndrome virus (PRRSV) infection and dietary soy isoflavone supplementation on carcass cutability and meat quality of commercial pigs. Barrows were randomly allotted to experimental treatments that were maintained throughout the study: non-infected pigs received an isoflavone-devoid control diet (CON, n = 22), and infected pigs received either the control diet (PRRSV-CON, n = 20) or that supplemented with total ISF in excess of 1,500 mg/kg (PRRSV-ISF, n = 25). Following a 7-day adaptation, weanling pigs were inoculated intranasally with either a sham-control (PBS) or live PRRSV (1×10^5 $TCID_{50}$/mL, strain NADC20). Pigs were then raised until 166 days post-inoculation and slaughtered humanely at the University of Illinois Meat Science Laboratory. At 1 d post-mortem (192-194 days of age; approximately 120 kg BW), left sides were separated between the 10^{th} and 11^{th} rib for determination of loin eye area (LEA), backfat thickness (BF), and loin quality (ultimate pH, instrumental color, drip loss, visual color, marbling, and firmness). Loin chops were aged 14 d post-mortem prior to Warner-Bratzler shear force (WBSF) determination. Belly width, length, thickness, and flop distance were determined. Data were analyzed as a one-way ANOVA in the

MIXED procedure of SAS 9.4 with pig as the experimental unit. Least squared means were separated using the probability of difference (PDIFF) option. Means were considered significantly different at $P \leq 0.05$. Carcass yield, LEA, BF, and estimated lean percentage did not differ ($P > 0.26$) among treatments. Loins from CON pigs had increased ultimate pH ($P = 0.01$), reduced L* scores ($P = 0.005$) coupled with darker visual color scores ($P = 0.004$), were firmer ($P < 0.0001$), and exhibited reduced drip loss ($P = 0.01$) compared with PRRSV-CON and PRRSV-ISF pigs. However, WBSF did not differ ($P = 0.51$) among treatments after 14 d of aging. Bellies from CON pigs were more firm compared with bellies from PRRSV-CON and ISF pigs ($P < 0.01$). These data suggest PRRSV infection and soy isoflavone supplementation did not alter carcass characteristics but may have marginally reduced loin and belly quality.

Key words: PRRSV, pork, cutability, meat quality

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRSV) is arguably one of the most concerning health challenges in the United States swine herd, with an estimated economic loss of $664 million annually (Holtkamp et al., 2013). While PRRSV can affect all pigs regardless of life stage, approximately 55% of this economic loss is attributed solely to reductions in performance within the grow-finish herd (Holtkamp et al., 2013). In a 21 d study, infection with PRRSV lowered the ADG of growing pigs by 30% (Schweer et al., 2016). A prolonged infection that is accompanied by this depression in growth may have a significant effect on a herd's productivity, meaning a delay in rapid lean growth. Depending on the timing of slaughter relative to PRRSV infection, this can potentially affect carcass composition. Alterations of lean growth may be affected by compensatory gain during recovery from PRRSV.

Compensatory growth resulted in increased calculated lean (Heyer and Lebret, 2007); however, the extent and effect of compensatory growth on carcass composition of pigs infected with PRRSV has not been determined. A dietary intervention of interest for use in PRRSV-infected pigs is supplemental soy isoflavones. As phytoestrogenic compounds (Reinli and Block, 1996) commonly found in soybeans, isoflavones have antiviral activity (Andres et al., 2009). Feeding 29% soybean meal improved ADG and lowered serum viral load in weanling pigs infected with PRRSV compared to pigs fed 17.5% soybean meal (Greiner et al., 2001; Rochell et al., 2015). Additionally, supplementation with dietary soy isoflavones in pigs infected with PRRSV at 28 days of age reduced mortality, but did not affect growth during or after infection (Smith et al., 2019).

It is not known if any modulation by isoflavones during the infective period affects the carcass characteristics and meat quality of grow-finish pigs. Therefore, the objective of this study was to establish how PRRSV infection affects carcass composition and meat quality of pigs, and to determine the potential of supplementation with isoflavones to mitigate effects. It is expected that infection will impact both carcass composition and meat quality, while isoflavone supplementation will mitigate these effects by modulating infection severity.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Dietary treatments and experimental design

A total of 96 barrows at 21 d of age were randomly allotted to 1 of 3 treatment groups. The first group was treated with a sham PBS inoculation and fed a basal corn-soy based diet that was practically devoid of isoflavones (CON; n = 24). The second group was infected with 1 ×

10^5 $TCID_{50}$/mL of the NADC20 strain of PRRSV and fed the same basal diet (PRRSV-CON; *n* = 36). The third group was infected with the same dosage of PRRSV, and fed the basal diet supplemented with > 1,500 mg/kg of isoflavones (PRRSV-ISF; *n* = 36). Infection, diet preparation, and design of this trial has been described previously (Smith et al., 2019). Over the course of the 145 d trial, 29 pigs were removed from the trial due to mobility issues or complications resultant of the PRRSV infection.

Carcass characteristics

At 166 days post-inoculation, pigs (n = 67) were transported to the University of Illinois Meat Science Laboratory (Urbana, IL) and held in lairage for a minimum of 13 hours. Feed was withheld but water was provided free-choice. Pigs were weighed immediately before slaughter (ending live weight; ELW). All pigs were slaughtered under the inspection of the Food Safety and Inspection Service of the United States Department of Agriculture. Pigs were immobilized using head-to-heart electrical stunning and terminated via exsanguination. Carcasses were weighed approximately 45 min post-mortem to obtain a hot carcass weight (HCW). Carcass yield was determined by dividing HCW by ELW and expressed as a percentage.

Carcasses were chilled at 4°C for a minimum of 20 hours. Left sides were separated between the 10^{th} and 11^{th} rib to expose the longissimus dorsi muscle (LD). Acetate paper and a permanent marker were used to trace the LD. Tracings were measured in duplicate on a digitizer tablet (Wacom, Vancouver, WA). The average of two measurements was reported as the loin eye area (LEA). Backfat thickness was measured 75% of the way around the LD from the dorsal processes of the vertebral column. Estimated fat-free lean, expressed as a percentage, was calculated using the following equation (Burson and Berg, 2001):

$$\textit{Estimated fat-free lean (\%)} = (8.588 + (0.465 \times \textit{HCW, lbs.}) - (21.896 \times \textit{fat thickness, in}) + (3.005 \times \textit{loin muscle area, in}^2)) / \textit{HCW, lbs.}) \times 100$$

Carcass fabrication and cutability

Fabrication of pork carcasses followed the same procedure as outlined by Boler et al. (2011). At 1 d post-mortem, sides were weighed to obtain a chilled side weight. Primals, subprimals, and retail cuts were weighed to determine percentage of chilled side weight. Leaf fat and standardized trim were removed and weighed independently. The front foot and back foot were removed at the upper knee and hock joints (radiocarpal joint and tibiotarsal joint, respectively) and weighed together. Sides were cut to yield a pork leg (NAMP #401), skin-on whole loin (NAMP #410), whole belly (NAMP #408), and whole shoulder (NAMP #403). Pork legs were skinned to produce a trimmed ham (NAMP #402), then fabricated to an inside, outside, knuckle, lite butt (a portion of the gluteus medius) and inner shank. Whole loins were trimmed to meet the specifications of a NAMP #410 trimmed loin, then fabricated to a NAMP #413 sirloin, NAMP #414A tenderloin, NAMP #422 backribs, and backbones. Due to ribbing at the 10th rib, loins were separated into anterior and posterior portions. Spareribs (NAMP #416) were removed from the belly. Bellies were then weighed. Whole shoulders were weighed with jowl and neckbones on, then fabricated to neckbones (NAMP #421), jowl (NAMP #419), boneless picnic (NAMP #405A) and boneless Boston butt (NAMP #406A).

Carcass cutability of primals and subprimals was calculated by dividing the cut weight by the chilled side weight, expressed as a percentage. These weights were then used to calculate lean bone-in yield, lean boneless yield, carcass bone-in yield, and carcass boneless yield according to Lowell et al. (2019).

Loin and Belly Quality

After fabrication, posterior portions of the LD (posterior to the $10^{th}/11^{th}$ rib junction) were used for quality measurements. Once all loins were collected (approximately 30 hours post-mortem), the most anterior end of the portion was refaced and allowed to oxygenate for approximately 20 minutes. Ultimate pH was measured in the chop face using a pH meter (Hanna Instruments, Smithfield, RI, USA). Beginning at the anterior face, a total of five chops (2.54 cm thick) were collected for analysis. The most anterior 2.54-cm chop was collected for proximate analysis and further quality measurements. Instrumental color was measured on the cut face of these chops using a chromameter (model CR-400; Minolta Camera Co., Ltd., Osaka, Japan) using a D65 light source, 2° observer angle, 8 mm aperture, and calibrated using a white tile. Subjective firmness (NPPC, 1991), visual color and marbling scores (NPPC 1999) were recorded by a single trained technician. These chops were then individually vacuum packaged and frozen at -20°C until proximate analysis. The next four anatomically sequential chops were randomly assigned to either 3, 7, or 14 d aging periods for Warner-Bratzler shear force (WBSF) and cook loss. These chops were all cooked to 63°C. Two chops were chosen for 14 d aging, then each was randomly assigned either 63°C or 72°C end point cooking temperatures. Chops were then individually vacuum packaged and placed in refrigeration until the assigned aging time, when they were frozen at -20°C until further analysis. The 14 d aged chops cooked to 72°C were used for another study (Bryan et al., 2019) and those results are not reported here. A sixth chop (1.27 cm thick) was then collected for drip loss.

Bellies were laid flat on tables before analysis. Fresh belly quality including length, width, thickness, and flop distance was determined as described by Lowell et al. (2018).

Proximate Analysis

Chops were allowed to partially thaw, and were then homogenized using a food processor (Cuisinart, Inc., Stamford, CN, USA). Five-gram samples were taken in duplicate from each chop and were placed in labelled aluminum tins. Moisture content was determined after drying in a 110°C oven for at least 24 h. Lipid content was determined after extraction using a chloroform:methanol solution for at least 8 h, as described by Novokofski et al. (1989). All proximate analysis results were expressed as a percentage of the original sample weight.

Warner-Bratzler Shear Force and Cook Loss

Chops obtained from these pigs were used in a separate study exploring cooking methods (Bryan et al., 2019). Only chops aged 3, 7, or 14 d and cooked to 63°C were used for the purposes of this study. Chops were allowed to thaw in packaging at 4°C for at least 24 h prior to analysis. Thawed chops were weighed in packaging for an initial weight. A precision immersion cooker (ANOVA Applied Electronics, Inc., San Francisco, CA, USA) was tightened to the rim of a 37 L tub. The tub was then filled with approximately 25 L of water, and the cooker was set to 71°C. Once the water was heated, no more than 13 chops were placed into the water bath and allowed to cook for 90 min. A validation study was completed prior to analysis to confirm this cooking time was appropriate for chops to reach the desired internal temperature (data not shown). After 90 min, chops were removed from packages and internal temperature was measured using a handheld meat thermometer. If a chop had not reached the desired degree of doneness (63°C), it was repackaged and placed back into the water bath. Chops were allowed to cool to approximately 22°C before a final weight was measured. For each group of chops cooked

together, packages were removed, dried, and then weighed together to determine an average packaging weight. Cook loss was determined by the following equation:

$$Cook\ loss\ (\%) = \{[(Initial\ wt\ (g) - packaging\ wt\ (g))\text{-}Cooked\ wt\ (g)] / Initial\ wt\ (g)\} \times 100$$

A portion of each cooled chop was cut to determine the direction of muscle fibers. A total of 4 cores measuring 1.25 cm in diameter were then removed parallel to the muscle fibers. Cores were sheared using a texture analyzer (model TA.HD Plus; Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, TK) with a blade speed of 3.33 mm/s and a load cell capacity of 100 kg. Peak values of each core were averaged to yield a single shear force value for each chop.

Drip Loss

Immediately after collection, chops were weighed. A fish hook with twine attached was placed between the subcutaneous fat and longissimus muscle. The chop was then placed in a plastic bag, suspended by the twine from a pole and allowed to hang for 24 hours at 4°C. After 24 h, chops were weighed again. Drip loss was calculated as:

$$Drip\ Loss\ (\%) = ((Initial\ wt,\ g.) / (Final\ wt.,\ g)) \times 100$$

Statistical Analysis

Data were analyzed as a one-way ANOVA in the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Pig was used as the experimental unit. Least squared means were separated using the probability of difference (PDIFF) option. Normality of residuals was tested using the UNIVARIATE procedure. Homogeneity of variances was tested using Levene's option in the GLM procedure. Means were considered significantly different at $P \leq 0.05$. Cook loss and

WBSF data were analyzed as a two-way ANOVA in the MIXED procedure of SAS 9.4. Aging period was included as a repeated measure in an unstructured covariance structure. Least squared means were separated using a PDIFF statement.

RESULTS

Carcass Characteristics and Cutability

Ending live weight, HCW, and carcass yield did not differ ($P \geq 0.23$) among treatments. Loin eye area, backfat thickness, and estimated fat free lean were not different ($P \geq 0.32$) between treatments (Table 2.1). However, numerically ELW, HCW and LEA were reduced approximately 4% in pigs infected with PRRSV compared with non-infected pigs. Spareribs from CON pigs were both heavier ($P = 0.01$) and a greater percentage of chilled side weight ($P = 0.01$) compared with PRRSV pigs (Table 2.2). Shoulder cutability was not different among treatments (Table 2.3). CON pigs had approximately 0.17 kg heavier ($P =0.05$) backribs than both PRRSV-CON and –ISF pigs (Table 2.4). CON pigs also had approximately 0.10 kg heavier ($P =0.03$) outsides than PRRSV-CON and –ISF pigs (Table 2.5). Boneless ham, shoulder, loin, and natural fall belly yields were not different ($P > 0.09$; Table 2.6). Bone-in and boneless carcass and lean yields were also not affected by treatment ($P > 0.32$; Table 2.6).

Loin quality

Infection with PRRSV reduced loin quality (Table 2.7). Drip loss was increased by approximately 1.27% units ($P = 0.04$) in chops from infected pigs compared to CON pigs. Both PRRSV-CON and PRRSV-ISF pigs had an ultimate pH 0.07 units lower ($P = 0.04$) than CON pigs. PRRSV-CON and PRRSV-ISF loins were lighter ($P \leq 0.02$) than CON pigs by approximately 0.34 and 3.28 units in visual and instrumental color, respectively. Firmness was

reduced ($P < 0.001$) by approximately 0.79 units in PRRSV-CON and –ISF pigs compared with CON pigs. Moisture and fat content were not different between treatments. After a 3 d aging period, chops from CON pigs required 0.39 kg less ($P < 0.05$) to shear than chops from PRRSV-CON pigs. Chops from PRRSV-ISF pigs were intermediate but not different to either of the other treatments. After a 7 d aging period, chops from PRRSV-ISF pigs required 0.39 kg less ($P < 0.05$) to shear than chops from PRRSV-CON pigs. Chops from CON pigs were intermediate but not different from to the other two treatments. After a 14 d aging period, chops from PRRSV-ISF pigs required approximately 0.36 kg less ($P <0.05$) to shear than chops from both PRRSV-CON and CON pigs. There were no differences in cook loss until the 14 d aging period, in which chops from CON pigs had approximately 1.35% units less ($P <0.05$) cook loss than chops from both PRRSV-CON and –ISF pigs.

Belly quality

Belly quality was altered by treatment (Table 2.8). Belly width tended ($P = 0.08$) to be affected by treatment with bellies from CON pigs being 1.42 cm wider ($P < 0.05$) than bellies from PRRSV-ISF. Bellies from PRRSV-CON pigs were intermediate to the other two treatments. Belly flop was reduced ($P < 0.05$) approximately 3.5 cm in bellies from PRRSV-CON and –ISF pigs compared with bellies from CON pigs. However, length and thickness were not different between treatments.

DISCUSSION

The goal of this study was to characterize the carcass composition and cutability and meat quality resulting from pigs infected with PRRSV. Given that PRRSV infection was expected to transiently reduce growth rate and potentially then result in compensatory gain, the

hypothesis was that pigs infected with PPRSV would have leaner carcass composition with lower intramuscular fat content. Supplementation with isoflavones was expected to mitigate the effects of PRRSV, meaning that less compensatory growth would occur. The hypothesis was that pigs supplemented with isoflavones would more closely resemble their non-infected counterparts in both carcass composition and meat quality.

Reductions in growth during infections is usually attributed to anorexia during illness. This anorexia is pertinent to survival of the animal during the infection (Johnson, 2002; Murray & Murray, 1979). During recovery, refeeding triggers compensatory growth, characterized by an increase in average daily gain and feed efficiency above that of an animal that never fasted (Heyer and Lebret, 2007; Therkildsen et al., 2004). While a period of anorexia was observed in this study, compensatory growth was never observed. Although pigs did return to feeding during the recovery period, they did not maintain an intake level conducive to realimentation and compensatory growth levels were not achieved through the final phase of the feeding trial. This could be caused by a few extenuating circumstances. For example, a secondary bacterial infection was prevalent in PRRSV-infected groups; this may have suppressed feed intake for longer than a PRRSV infection alone. This combination of PRRSV and the bacterial infection may also explain the high mortality observed in these groups. Secondly, the end of the feeding trial took place at the end of summer, meaning the daily high temperatures were approximately 32.2°C. Increased ambient temperature decreases feed intake (Collin et al., 2001; Pearce et al., 2013), and thereby reduces growth (St-Pierre, Cobanov, & Schnitkey, 2003). Given the absence of compensatory gain, the lack of differences in estimated lean, carcass cutability, and fat depth were expected. While not statistically significant, weights were depressed in PRRSV-infected pigs but weight reductions were proportional in the body resulting in no alterations in cutability.

Pigs infected with PRRSV had reduced loin quality and belly quality. Loins from pigs infected with PRRSV, regardless of diet, had reduced ultimate pH, increased drip loss, lighter visual and instrumental color, and were less firm than loins from uninfected pigs. Belly quality, namely belly flop distance, was reduced in bellies from pigs infected with PRRSV. Despite the positive effect of dietary soy isoflavones on mortality in this (Smith et al., 2019) and other studies (Andres et al., 2009; Rochell et al.,2015), supplementation of isoflavones in this trial did not mitigate the detrimental effects of PRRSV on loin or belly quality.

The impact of infection on pork quality is relatively unknown. Outhouse (2017) utilized a *Mycoplasma hyopneumoniae* plus *Lawsonia intracellularis* coinfection as opposed to a PRRSV infection. In that study, loin quality was not different between infected and non-infected pigs, but one must also consider that the immune response to *M. hyopneumoniae* plus *L. intracelluaris* differs from that of PRRSV. PRRSV elevates inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor-α, IL-10, and IL-12 for a longer period of time compared to *M. hyopneumoniae* and *L. intracelluaris* (Thanawongnuwech, 2004; Senthikumar et al., 2019). Prolonged exposure to this inflammation may damage muscle tissue (Pyne, 1994), which may help to explain why meat quality outcomes were affected in our PRRSV study. Although visual and instrumental color, pH, and firmness were reduced in PRRSV pigs, the magnitude to which they are different is not necessarily of practical value. Ultimate pH only differed by 0.5 units, not likely to affect other quality attributes in any substantial nature. Visual color differed by less than a half score on the NPPC scale, also not likely to affect purchase intent. This is reflected in L* scores, where only a 3.4 unit difference was observed.

Expected differences in fat deposition and quality due to compensatory growth were partially observed, namely in the belly. Pigs infected with PRRSV had a lower belly flop than

pigs not infected with PRRSV, indicating reduced fat quality. Softer bellies have a higher proportion of unsaturated fatty acids (Shackelford et al., 1990), so it is likely PRRSV infected pigs deposited a greater proportion of unsaturated fatty acids in their belly fat. Unsaturated fatty acids also have a greater propensity for oxidation and rancidity (Wood et al., 2004), clearly undesirable in bacon. In consumer panels, bellies higher in unsaturated fatty acids scored lower than those with a greater proportion of saturated fatty acids (Shackelford et al., 1990).

In conclusion, cutability was not influenced by infection status, while pigs infected with PRRSV had reduced loin and belly quality. Supplementation with dietary isoflavones did not alter the response to PRRSV infection in terms of loin or belly quality. The severity of the infection in this study, however, prevented full realimentation of pigs and therefore, precluded compensatory gain. Future studies with more mild depressions in growth may result in more impacts on carcass composition and cutability.

TABLES AND FIGURES

Figure 2.1. Effect of PRRSV infection and dietary treatment on cook loss of loin chops aged 3, 7, and 14 days. Data within a time-point with differing letters are different ($P \leq 0.05$).

Figure 2.1.

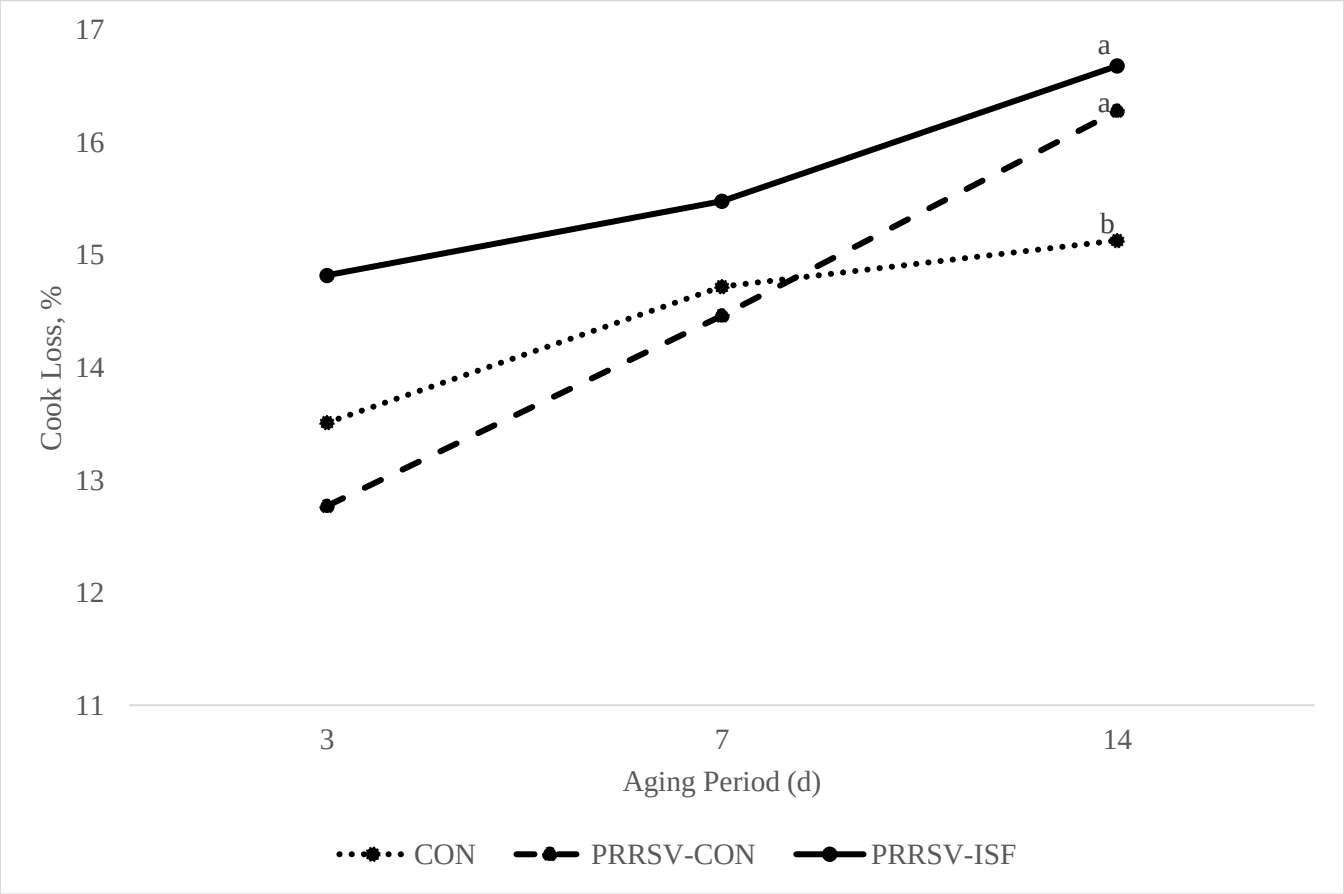

Figure 2.2. Effect of PRRSV infection and dietary treatment on Warner-Bratzler shear force (WBSF) values of loin chops aged 3, 7, and 14 days. Data within a time-point with differing letters are different ($P \leq 0.05$).

Figure 2.2.

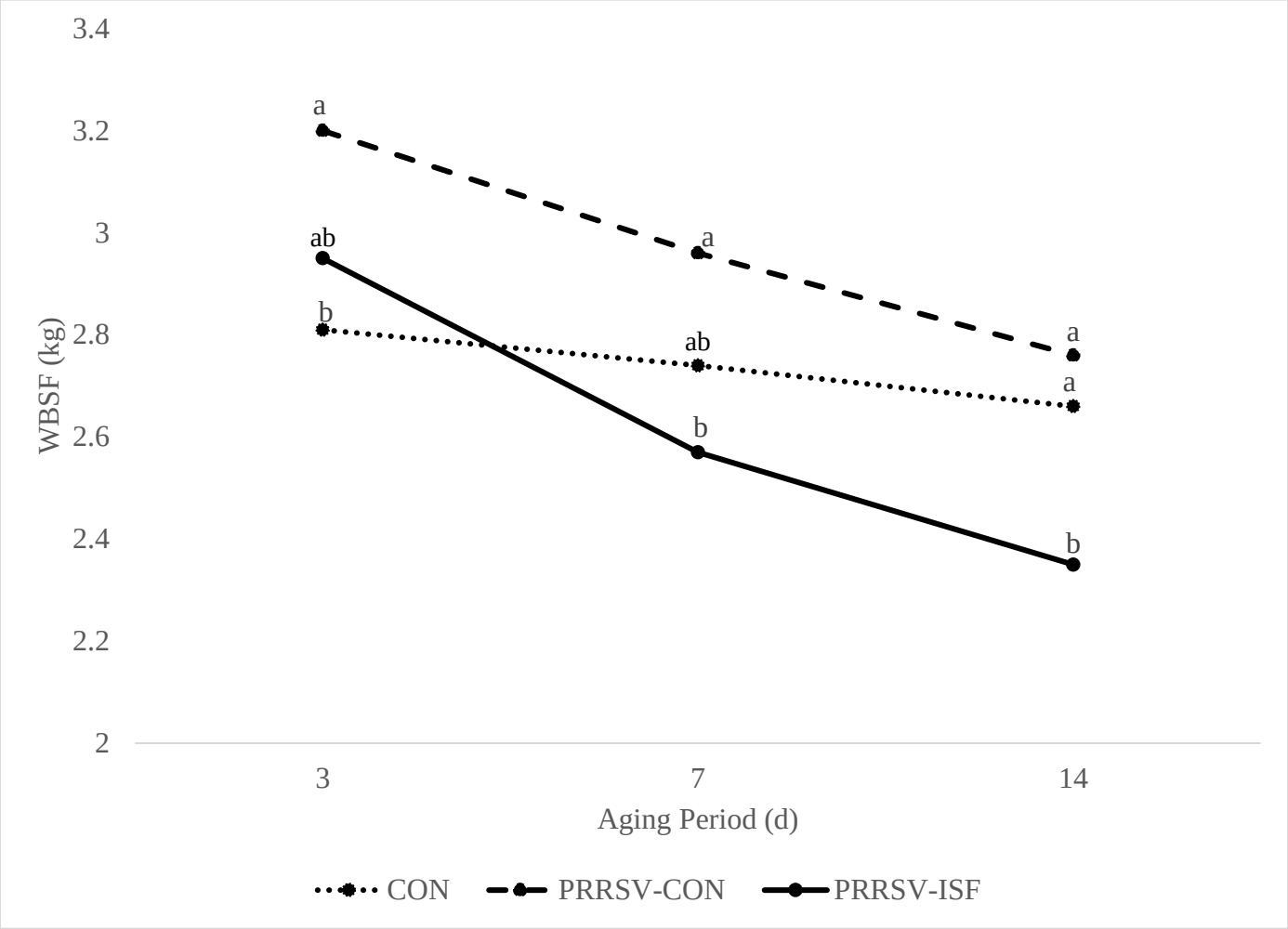

Table 2.1. Effects of PRRSV infection and isoflavone supplementation on carcass characteristics.

	Treatment[1]				
Item	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Pigs, n	22	20	25		
Ending live weight, kg	125.52	121.58	121.14	2.37	0.32
HCW[2], kg	98.59	95.30	94.17	2.01	0.23
Carcass yield, %	78.51	78.38	78.00	0.28	0.36
Loin eye area, cm^2	45.80	44.27	43.46	1.19	0.32
Backfat thickness, cm	2.21	2.20	2.23	0.11	0.98
Estimated lean[3], %	51.65	51.44	51.21	0.52	0.81

[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones
[2]HCW = hot carcass weight
[3]Estimated lean, % = (8.588 + (0.465 × HCW, lbs.) – (21.896 × fat thickness, in) + (3.005 × loin muscle area, in^2)) / HCW, lbs.) × 100

Table 2.2. Effects of PRRSV infection and isoflavone supplementation on primal cutability.

	Treatment[1]				
Item[2]	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Sides, n	20	22	25		
Chilled side wt, kg	48.86	47.25	46.78	0.99	0.26
Whole shoulder, kg	13.15	12.85	12.85	0.29	0.66
% chilled side wt	26.97	27.19	27.46	0.26	0.36
Bone-in Boston, kg	4.01	3.98	3.97	0.10	0.96
% chilled side wt	8.21	8.42	8.48	0.12	0.22
Bone-in picnic, kg	5.69	5.58	5.47	0.13	0.43
% chilled side wt	11.67	11.81	11.69	0.15	0.77
Whole loin, kg	13.11	12.61	12.43	0.30	0.21
% chilled side wt	26.64	26.70	26.57	0.23	0.92
Whole ham, kg	11.19	10.87	10.72	0.21	0.22
% chilled side wt	22.91	23.03	22.96	0.18	0.89
Whole belly, kg	7.30	7.00	6.85	0.20	0.23
% chilled side wt	14.92	14.79	14.61	0.19	0.45
Spareribs, kg	1.93[a]	1.75[b]	1.78[b]	0.06	0.01
% chilled side wt	3.97[a]	3.71[b]	3.81[a]	0.07	0.02

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$)
[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones
[2]Whole shoulder = NAMP #403, Bone-in Boston = NAMP #406, Bone-in picnic = NAMP #405, Whole loin = NAMP #410, Whole ham = NAMP #401, Spareribs = NAMP # 416

Table 2.3. Effects of PRRSV infection and isoflavone supplementation on shoulder cutability.

	Treatment[1]				
Item[2]	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Shoulders, n	22	20	25		
Jowl, kg	1.61	1.49	1.5	0.05	0.12
% chilled side wt	3.31	3.16	3.19	0.07	0.27
Neckbones, kg	1.11	1.13	1.16	0.04	0.60
% chilled side wt	2.29	2.39	2.49	0.08	0.19
Clear plate, kg	0.72	0.74	0.74	0.03	0.89
% chilled side wt	1.48	1.55	1.58	0.04	0.20
Bone-in Boston, kg	4.01	3.98	3.97	0.10	0.96
% chilled side wt	8.21	8.42	8.48	0.12	0.22
Boneless Boston, kg	3.76	3.74	3.74	0.09	0.99
% chilled side wt	7.70	7.92	7.98	0.12	0.17
Bone-in picnic, kg	5.69	5.58	5.47	0.13	0.43
% chilled side wt	11.67	11.81	11.69	0.15	0.77
Boneless picnic, kg	4.12	4.01	3.95	0.11	0.50
% chilled side wt	8.43	8.48	8.44	0.13	0.97

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$).

[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones

[2]Jowl = NAMP # 419, Neckbones = NAMP #412, Bone-in Boston = NAMP # 406, Boneless Boston = NAMP #406A, Bone-in picnic = NAMP #405, boneless picnic = NAMP # 405A

Table 2.4. Effects of PRRSV infection and isoflavone supplementation on loin cutability.

	Treatment[1]				
Item[2]	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Loins, n	22	20	25		
Trimmed loin, kg	10.64	10.34	10.20	0.20	0.26
% chilled side wt	21.79	21.93	21.83	0.18	0.86
Canadian back, kg	3.31	3.21	3.22	0.07	0.51
% chilled side wt	6.79	6.82	6.90	0.11	0.77
Tenderloin, kg	0.45	0.45	0.45	0.01	0.97
% chilled side wt	0.93	0.96	0.97	0.03	0.36
Sirloin, kg	0.85	0.81	0.81	0.02	0.26
% chilled side wt	1.75	1.71	1.73	0.04	0.74
Backribs, kg	1.04[a]	0.88[b]	0.87[b]	0.06	0.05
% chilled side wt	2.14	1.86	1.87	0.12	0.14
Backbone, kg	2.03	2.05	2.09	0.08	0.81
% chilled side wt	4.15	4.35	4.48	0.14	0.21

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$).
[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones
[2]Trimmed loin = NAMP #410, Canadian back = NAMP #414, Tenderloin = NAMP # 414A, Sirloin = NAMP #413D, Backribs = NAMP # 422

Table 2.5. Effects on PRRSV infection and isoflavone supplementation on ham cutability.

	Treatment[1]				
Item[2]	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Hams, n	20	22	25		
Trimmed ham, kg	9.53	9.23	9.13	0.17	0.19
% chilled side wt	19.54	19.56	19.55	0.19	0.99
Inside, kg	2.61	2.46	2.47	0.06	0.14
% chilled side wt	5.34	5.21	5.28	0.08	0.50
Outside, kg	1.74[a]	1.66[ab]	1.62[b]	0.04	0.03
% chilled side wt	3.56	3.52	3.48	0.07	0.66
Lite butt, kg	0.27	0.26	0.26	0.01	0.91
% chilled side wt	0.55	0.56	0.57	0.03	0.84
Knuckle, kg	1.35	1.33	1.36	0.03	0.83
% chilled side wt	2.77	2.83	2.92	0.06	0.18
Inner shank, kg	0.65	0.63	0.65	0.01	0.47
% chilled side wt	1.32[a]	1.33[ab]	1.39[b]	0.02	0.05

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$).
[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones
[2]Trimmed ham = NAMP #402, Inside = NAMP #402F, Outside = NAMP #402E, Knuckle = NAMP # 402H

Table 2.6. Effect of PRRSV infection and isoflavone supplementation on carcass and lean yield.

	Treatment[1]				
Item	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Pigs, n	22	20	25		
Boneless ham[2], kg	6.61	6.33	6.36	0.13	0.24
Boneless shoulder[3], kg	7.87	7.75	7.69	0.18	0.75
Natural fall belly, kg	7.30	7.00	6.85	0.20	0.23
Boneless loin[4], kg	4.62	4.48	4.48	0.10	0.47
Bone-in carcass yield[5], %	76.13	76.51	76.17	0.29	0.59
Boneless carcass yield[6], %	52.19	52.25	52.30	0.33	0.97
Bone-in lean yield[7], %	61.21	61.72	61.56	0.36	0.58
Boneless lean yield[8], %	37.27	37.46	37.70	0.34	0.60

[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones

[2]Boneless ham, kg = (inside, kg (NAMP #402F) + outside, kg (NAMP #402E) + knuckle, kg (NAMP #402H) + lite butt, kg + inner shank, kg)

[3]Boneless shoulder, kg = (boneless Boston, kg (NAMP # 406A) + boneless picnic, kg (NAMP #405A)

[4]Boneless loin, kg = (Canadian back, kg (NAMP #410) + tenderloin, kg (NAMP #414A) + sirloin, kg (NAMP #413D)

[5]Bone-in carcass yield, % = ((trimmed ham, kg (NAMP #402) + bone-in Boston, kg (NAMP #406) + bone-in picnic, kg (NAMP #405) + trimmed loin, kg (NAMP #410) + whole belly) / chilled side, kg)*100

[6]Boneless carcass yield, % = ((inside, kg (NAMP #402F) + outside, kg (NAMP #402E) + knuckle, kg (NAMP #402H) + Canadian back, kg (NAMP # 410) + tenderloin, kg (NAMP # 414A) + sirloin, kg (NAMP #413D) + boneless Boston, kg (NAMP #406A) + boneless picnic, kg (NAMP #405A) + whole belly, kg) / chilled side, kg)*100

[7]Bone-in lean yield, % = ((trimmed ham, kg (NAMP #402) + bone-in Boston, kg (NAMP #406) + bone-in picnic, kg (NAMP #405) + trimmed loin, kg (NAMP #410) / chilled side, kg)*100

[8]Boneless lean yield, % = ((inside, kg (NAMP #402F) + outside, kg (NAMP #402E) + knuckle, kg (NAMP #402H) + Canadian back, kg (NAMP #410), + tenderloin, kg (NAMP #414A) + sirloin, kg (NAMP #413D), boneless Boston, kg (NAMP #406A) + boneless picnic, kg (NAMP #405A))/chilled side, kg)*100

Table 2.7. Effects of PRRSV infection and isoflavone supplementation on loin quality.

	Treatment[1]				
Item	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Loins, n	22	20	25		
Drip loss, %	3.31[a]	4.53[b]	4.65[b]	0.43	0.04
Ultimate pH	5.74[a]	5.67[b]	5.67[b]	0.02	0.04
Visual color[2]	3.55[a]	3.23[b]	3.18[b]	0.10	0.01
Visual marbling[3]	2.25	2.13	2.14	0.13	0.72
Subjective firmness[4]	3.82[a]	2.90[b]	3.16[b]	0.14	<0.0001
Moisture, %	74.14	74.28	74.09	0.19	0.74
Lipid, %	3.13	2.89	3.16	0.17	0.45
Lightness, L*[5]	53.81[a]	56.96[b]	57.21[b]	0.96	0.02
Redness, a*[6]	9.76[a]	11.13[b]	10.10[ab]	0.44	0.08
Yellowness, b*[7]	7.35[a]	8.70[b]	7.87[ab]	0.40	0.06

[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones

[2]NPPC color based on the 1999 standards measured in half point increments where 1=palest, 6=darkest

[3]NPPC marbling based on the 1999 standards measured in half point increments where 1 = least amount of marbling, 6 = greatest amount of marbling

[4]NPPC firmness based on the 1991 scale measured in half point increments where 1 = softest, 5 = firmest

[5]L* measures darkness (0) to lightness (100; greater L* indicates a lighter color)

[6]a* measures redness (greater a* indicates a redder color)

[7]b* measures yellowness (greater b* indicates a more yellow color)

[a-b]LS means within a row having different superscripts are statistically different ($P < 0.05$)

Table 2.8. Effects of PRRSV infection and isoflavone supplementation on belly quality

	Treatment[1]				
Item	CON	PRRSV-CON	PRRSV-ISF	SEM	*P*-values
Bellies, n	22	20	25		
Length, cm	70.05	71.34	70.33	0.79	0.47
Width, cm	28.30[a]	27.10[ab]	26.88[b]	0.50	0.08
Flop, cm	15.30[a]	11.96[b]	11.66[b]	0.93	<0.01
Thickness[2], cm	3.74	3.66	3.58	0.09	0.35

[1]Treatments: CON = non-infected fed basal diet, PRRSV-CON = infected fed basal diet, PRRSV-ISF = infected fed basal diet + isoflavones

[2]Thickness was an average of 8 measurements, 4 anterior to posterior on the dorsal half and 4 anterior to posterior on the ventral half

[a-b]LS means within a row having different superscripts are statistically different ($P \leq 0.05$)

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CHAPTER 3

TECHNICAL NOTE: A METHOD FOR DETECTION OF DIFFERENCES IN COOK LOSS AND TENDERNESS OF AGED PORK CHOPS COOKED TO DIFFERING DEGREES OF DONENESS USING SOUS VIDE

ABSTRACT

Validation of new methods to improve accuracy and repeatability of analyses is vital to the furthering of meat science. The objective was to determine the ability to detect differences in cook loss and Warner-Bratzler shear force (WBSF) values between chops aged for differing time periods and cooked to varying degrees of doneness with in a sous-vide style cooker. Loins from pigs (HCW = 96 kg) humanely slaughtered at the University of Illinois Meat Science Laboratory were separated between the 10^{th} and 11^{th} rib into anterior and posterior sections. The posterior section was cut into 6 separate 2.54 cm thick chops. The middle four chops were randomly designated for aging of 3 d and cooked to 63°C, aged 7 d and cooked to 63°C, aged 14 d and cooked to 63°C, or aged 14 d and cooked to 71°C. Chops were cooked by placing them in a water bath with an immersion circulator set to the desired end-point temperature for 90 min. Cook loss was calculated for each chop by measuring initial and final weight, and accounting for packaging weight. Four cores measuring 1.25 cm in diameter were cut parallel to the muscle fibers from each chop and analyzed for WBSF. Data were analyzed using a 1-way ANOVA. Least squares means were separated using the probability of difference (PDIFF) option in the MIXED procedure of SAS. Among chops cooked to 63°C, chops aged 3 d has less ($P < 0.01$) cook loss than those aged 7 d, and chops aged 7 d had less ($P < 0.01$) cook loss than those aged 14 d. Among chops aged for 14 d, chops cooked to 71°C had greater ($P < 0.001$) cook loss than

chops cooked to 63°C. Differences in tenderness were also detected between aging periods. Among chops cooked to 63°C, chops aged 3 d required more ($P = 0.02$) force to shear than those aged 7 d, but chops aged 7 d did not differ ($P = 0.15$) from those aged 14 d. Chops aged 14 d and cooked to 71°C required ($P < 0.0001$) more force than those aged 14 d and cooked to 63°C. Overall, these data indicate sous-vide is an acceptable cooking method for use in experiments as expected differences in cook loss and WBSF were detected in chops aged to differing time points or cooked to differed degrees of doneness.

Key words: aging, pork, sous-vide, tenderness

INTRODUCTION

Sous-vide cooking is a method of the cooking of food products by immersion in a heated water bath. The water bath temperature is maintained by a submerged heating element and water is circulated to maintain a constant temperature. Sous-vide became a research topic of interest in the 1990s, primarily as a method of extending shelf life of minimally processed foods (Baldwin, 2012). The commercial application of this consisted of vacuum packaging and pre-cooking products in water at lower temperatures for a longer period of time than used with traditional cooking methods (Schellekens, 1996). Currently, sous-vide is becoming more popular with chefs and private households due to the precision temperature control and reproducibility (Baldwin, 2012). Because the water bath temperature is equal to that of the desired endpoint cooking temperature of the food itself, foods can be cooked and held at that temperature. Therefore, even with extended cooking times, the desired endpoint temperature cannot be exceeded. These advantages may be of importance to meat science researchers, particularly in the context of sensory and tenderness assays. Greater control of the degree of doneness has the potential to

reduce variability among samples. Reproducibility reduces the variation between studies due to cooking. However, very little work is available to validate the use of sous-vide cooking in meat quality research.

Postmortem aging increases tenderness of pork products (Ellis et al., 1998; van Laack, et al., 2001; Channon et al., 2003; Dilger et al., 2010; Clark et al., 2014; Jones-Hamlow et al., 2015). Sarcoplasmic and myofibrillar proteins are denatured, leading to a weakening of the muscle structure and subsequently an increase in tenderness (Wagner, 2007). The differences in tenderness can be detected using Warner-Bratzler shear force (WBSF) in pork chops cooked to 70°C, with a decline in peak force values from chops aged 1 to 20 d (Ha et al., 2017). This tenderization occurs via a rapid decline in peak force values during early aging (d 1-7) followed by a less steep decline in peak force values (d 7-14) until finally a plateau is reached (Dransfield et al., 1981; van Laack et al. 2001; Rees et al., 2002; Dilger et al., 2010). Other studies indicate that tenderization can extend to d 14, potentially up until d 21 (Clark et al., 2014). It was expected that these established differences in tenderness with postmortem aging would be detectable when chops were cooked using a sous-vide cooking method.

In 2011, the United States Department of Agriculture (USDA) lowered the recommended minimum cooking temperature for whole muscle pork cuts from 71°C (medium) to 63°C (medium-rare) (Pork Checkoff, 2011; USDA, 2013) because the safety of the product is maintained (Gamble et al., 2000) and eating quality might be improved (Moeller et al., 2010a; 2010b). Pork chops cooked to 63°C are more tender than those cooked to 71°C (Rincker et al., 2008; Klehm et al., 2018), though the majority of this work occurred using direct-heat cooking methods. Often, these differences in tenderness at varying degrees of doneness are attributed to the increased cook loss at higher temperatures (Klehm et al., 2018). It was expected that

differences in tenderness and cook loss between chops cooked to 63°C compared with 71°C would be detected in chops cooked using a sous-vide cooking method. Therefore, the objective was to determine cooking loss and WBSF of chops aged to 3, 7, and 14 d postmortem and cooked to 63°C or those aged 14d postmortem and cooked to 71°C. These values were compared to previous results to establish whether expected difference in tenderness are detectable using sous-vide cooking methods.

MATERIALS AND METHODS

Chop Selection

Loins (n=67) were sourced from pigs (HCW = 96 kg) slaughtered at the University of Illinois Meat Science Laboratory. Pigs were raised for a nutritional study with procedures reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois and fed a corn-soy diet. Left carcass sides were cut between the 10^{th} and 11^{th} rib, separating the loin into an anterior and posterior section. The posterior section was cut into a total of six 2.54 cm thick chops. Chops were standard in weight (CV = 5.42). The middle four chops were collected and randomly assigned to either 3 d aging and 63°C end-point cooking temperature, 7 d aging and 63°C end-point cooking temperature, 14 d aging and 63°C end-point cooking temperature, or 14 d aging and 71°C end-point cooking temperature. Samples were identified, individually vacuum packaged, and sorted into boxes by aging d. Once the designated aging period was achieved, chops were frozen at -20°C until further analysis. Once the 14 d aging was complete, chops were resorted into boxes by animal identification number such that all chops from the same loin were cooked and analyzed on the same d. Samples were stored frozen until further analysis.

Cook Loss

Chops were allowed to thaw at 4°C for at least 24 hours prior to analysis. Three 37 L plastic open containers were filled with approximately 25 L of hot water. An ANOVA precision immersion cooker (ANOVA Applied Electronics, Inc., San Francisco, CA, USA) was placed in the water and tightened to the rim of the tub. Two precision cookers were set to 63°C and the third was set to 71°C. Thawed chops were weighed in vacuum packaging for an initial weight. Once the water reached the set temperature, the chops were placed in the tub with the designated cooking temperature and allowed to cook for 90 min. Approximately 13 chops were cooked in each tub resulting in 6 groups of chops to complete cooking. A validation study was completed prior to analysis of experimental chops to ensure 90 min was sufficient to reach the desired internal temperature of the pork chops. After 90 min, chops were removed from their packaging and a temperature was verified using a digital thermometer to ensure complete cooking. If a chop was not at the appropriate temperature, it was re-packaged and placed back into the water bath. This was only required once. Chops were allowed to cool in ambient air (22°C) to approximately 22°C before the chops were weighed to determine the final weight. For every group of chops in a tub, the packaging was removed, dried, and weighed to determine the average packaging weight. This average was then subtracted from the initial weight of each chop within that tub to estimate initial chop weight. Cook loss was then determined using the following equation:

$$\textit{Cook loss (\%)} = \{[\textit{Initial wt (g)} - \textit{Cooked wt (g)}] / \textit{Initial wt (g)}\} \times 100$$

Warner-Bratzler Shear Force

After chops were cooled, 4 cores measuring 1.25 cm in diameter were removed. Cores were cut parallel to the orientation of the muscle fibers. Samples were sheared using a Texture

Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK) with a blade speed of 3.33 mm/s and a load cell capacity of 100 kg. The Warner-Bratzler shear force values for each core were averaged to yield a single shear force value for each chop.

Statistical Analysis

Chop served as the experimental unit, with loin serving as a block and cooking day as a random effect. The effects of aging period and degree of doneness on cook loss and Warner-Bratzler shear force value were analyzed as a 1-way ANOVA using the MIXED procedure in SAS. Least squares means were separated using a probability of difference (PDIFF) statement, and were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Cook loss was expected to increase with increased aging time (Dilger et al., 2010; Jones-Hamlow et al., 2015). Those expected differences were detected in the present study. Among chops cooked to 63°C, cook loss increased with increased postmortem aging (Figure 3.1a). Cook loss was increased by 1.13 units ($P < 0.01$) in chops aged 14 d compared with those aged 7 d. Cook loss was increased 1.14 units ($P < 0.01$) in chops aged 7 d compared with those aged 3 d. Dilger et al. (2010) reported a 0.55 unit decrease in cook loss in chops aged 2 d compared with those aged 7 d, but only a 0.06 unit decrease in cook loss in chops aged 7 d compared to those aged 14 d. Similarly, Ellis et al. (1998) reported a 0.7 unit decrease in cook loss in chops aged 2 d compared to chops aged 9 d, and a 0.5 unit decrease from chops aged 9 d compared to chops aged 16 d. The lower magnitude of difference reported by Dilger et al. (2010) and Ellis et al.

(1998) compared to the present study may be a result of increased degree of doneness (70°C) in those previous works.

Additionally, cook loss was expected to increase with increased cooking temperature. Among chops aged 14 d, cook loss was increased 10.04 units ($P < 0.001$; Fig. 3.1a) in chops cooked to 71°C compared with those cooked to 63°C. Protein denaturation decreases water holding capacity (Wagner, 2007), and cook loss increases as end-point temperature increases (Wood et al., 1995; Aaslyng et al., 2003; Klehm et al., 2018). Klehm et al. (2018) reported reduced cook loss for chops cooked to both 63°C (11.29%) and 71°C (12.93%) compared to the current study (15.35% and 25.39%, respectively). Because in the present study, chops were aged in the same packaging they were cooked in, cook loss represents both purge loss during aging and actual loss during cooking. In the previous study (Klehm et al., 2018), purge loss during aging was 4.81 and 4.76% for chops cooked to 63°C and 71°C, respectively. This makes total water loss 16.1 and 17.69% for each group, respectively. Thus, cook loss of 25.39% in the current study appears increased compared with previous work. However, others (Lonergan, et al. 2007; Arkfeld et al., 2015, Harsh et al., 2017) have reported cook loss of chops cooked to 71°C ranging from 20.73-23.96%, similar to the current study. Thus, meaningful differences can be detected in cook loss using sous-vide cooking but care needs to be taken when comparing between studies when meat is aged postmortem in the same packages as used for cooking.

Warner-Bratzler shear force was expected to decrease with increased aging time (van Laack et al., 2001; Channon et al., 2003; Dilger et al., 2010; Clark et al., 2014; Jones-Hamlow et al., 2015). This expected difference was detected early in the aging curve. In chops cooked to 63°C, shear force values decreased in early aging (Figure 3.1b). Shear force was decreased by 0.27 kg ($P = 0.02$) in chops aged 3 d compared to chops aged 7 d. Shear force decreased

numerically by 0.16 kg but was not different ($P = 0.15$) in chops aged 7 d compared to chops aged 14 d. Chops aged 14 d and cooked to 71°C had the greatest WBSF value, requiring 0.67 kg more force than chops aged 7 d and cooked to 63°C ($P < 0.001$) and 0.83 kg more force than chops aged 14 d and cooked to 63°C ($P < 0.001$; Figure 3.1b).

The objective of the present experiment was to demonstrate that sous-vide cooking is an acceptable method for meat science experiments given that expected differences in tenderness with aging or endpoint cooking temperature differences could be detected. Therefore, results from the present study are displayed on Table 3.1 along with those from previous studies. Among these previous studies, Warner-Bratzler shear force decreased between 7.10-21.29% when comparing early (1-3 d) and mid (7 or 9 d) aging and decreased between 3.53-15.38% when comparing mid and late (14-21 d) aging. In the present study, Warner-Bratzler shear force decreased 9.12% from early to mid aging and 5.95% from mid to late aging. This is consistent with the finding of van Laack et al. (2001), who reported a decrease in shear force value when chops were aged 7 d compared to 2 d and cooked to 70°C in an oven. Channon et al. (2003) reported chops aged 2 d were less tender than those aged 7 d when cooked to 80°C in a water bath. The agreement between these studies and the results from the current study indicate that differences in tenderness due to early aging are detectable in chops both cooked in a water bath and to a lower degree of doneness. The importance of this is not so much the differences reported between chops, but rather that those differences can be detected using the method and cooking temperature described here (Table 3.1).

However, the results from the current study regarding later aging is inconsistent with other studies (van Laack et al., 2001; Dilger et al., 2010) that reported increased tenderness in chops cooked to the same degree of doneness, but aged 14 d compared to 7 d. The discrepancy

between previous studies and the results presented here may be due to the difference in end-point cooking temperature. These previous studies cooked chops to 70-71°C, which may accentuate small changes in tenderization between aging periods. A lower internal end-point temperature allows the chop to retain more moisture resulting in an increase in juiciness and tenderness (Klehm et al., 2018).

Based on the results of this study, sous-vide style cooking of meat for experiments is a viable alternative to conventional direct-heat methods historically used in meat science. Given sufficient time in the water bath, all samples reach the desired end-point temperature and, based on the method, it is not possible for samples to exceed this temperature. Additionally, constant monitoring of temperature during cooking is not needed. Based on the results of the present study, sous-vide can be used as an acceptable method to cook pork chops in meat science experiments as expected differences in tenderness and cook loss when chops were aged differently or cooked to different degrees of doneness were detected and were similar to those of previous works. Therefore, sous-vide cooking is a method that can be used in meat science experiments when tenderness measurement is an objective of the research.

TABLES AND FIGURES

Figure 3.1a: Effect of aging period and degree of doneness on cook loss of boneless pork chops. Data are depicted as least squared means (reported) ± the standard error of the mean. [a-c]Values depicted with differing superscripts are considered significantly different ($P < 0.05$).

Figure 3.1a.

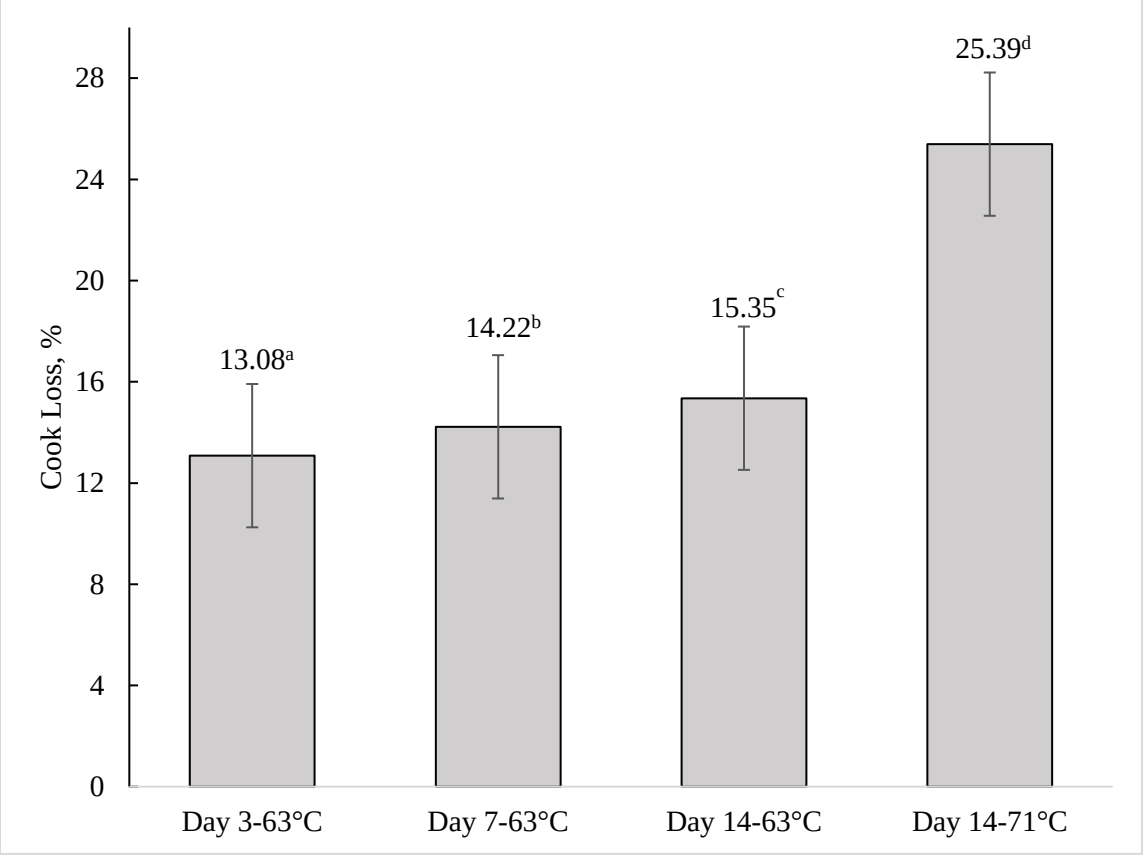

Figure 3.1b: Effect of aging period and degree of doneness on Warner-Bratzler shear force (WBSF) value of boneless pork chops. Data are depicted as least squared means (reported) ± the standard error of the mean. [a-c]Values depicted with differing superscripts are considered significantly different ($P < 0.05$).

Figure 3.1b.

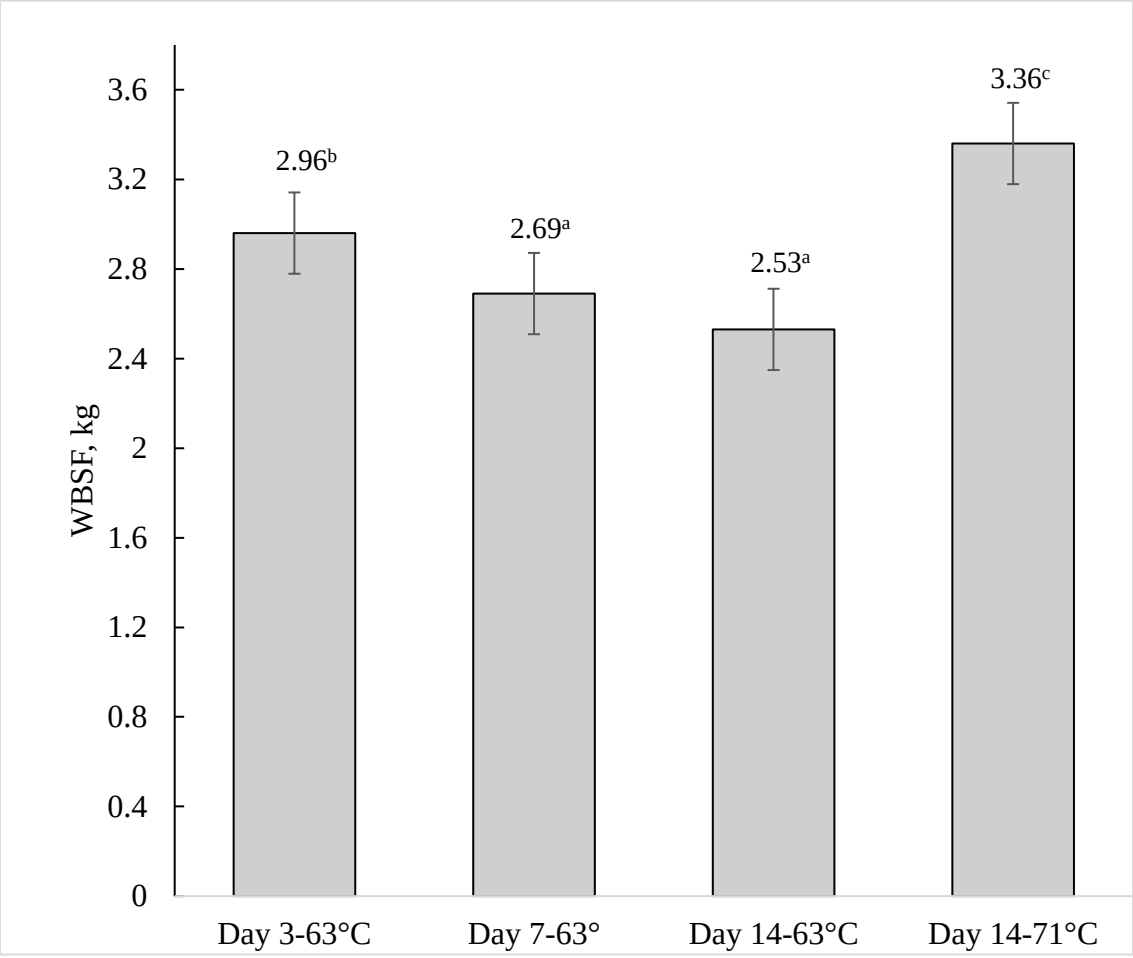

Table 3.1. Reported values for Warner-Bratzler shear force (WBSF) and cook loss in pork loin chops aged post-mortem and cooked to 63, 70, or 80 °C

Citation	Cooking Method	Degree of Doneness	Early Aged[1] Reported	Mid Aged Reported	Late Aged Reported	Early-Mid % Difference[2]	Mid-Late % Difference[3]	Early-Late % Difference[4]
WBSF, kg								
Ellis et al. (1998)	Open-hearth grill	70 °C	4.07	3.76	3.90	7.62	-3.72	4.18
van Laack, et al. (2001)	Oven	70 °C	4.89	4.16	3.52	14.93	15.38	28.02
Channon, et al. (2003)	Water bath	80 °C	4.65	3.66	.	21.29	.	.
Dilger, et al. (2010)	Open-hearth grill	70 °C	3.38	3.12	3.01	7.69	3.53	10.95
Clark, et al. (2014)	Open-hearth grill	70 °C	3.38	3.14	2.82	7.10	10.19	16.57
Jones-Hamlow, et al. (2015)	Open-hearth grill	70 °C	4.05	3.49	3.00	13.83	14.04	25.93
Present study	Water bath	70 °C	.	.	3.36	.	.	
Present study	Water bath	63 °C	2.96	2.69	2.53	9.12	5.95	14.53
Cook Loss, %								
Channon, et al. (2003)	Water Bath	80 °C	35.19	34.51	.	1.93	.	.
Dilger, et al. (2010)	Open-hearth grill	70 °C	21.52	20.97	21.65	2.56	-3.24	-0.60
Jones-Hamlow, et al. (2015)	Open-hearth grill	70 °C	24.29	23.62	23.70	2.76	-0.34	2.42
Present Study	Water Bath	70 °C	.	.	25.39	.	.	.
Present Study	Water Bath	63 °C	13.08	14.22	15.35	-8.72	-7.95	-17.35

[1]Early Aged chops aged 1 to 3 d, Mid Aged chops aged 7 or 9 d, Aged chops aged 14-21 d.

[2]Percent difference calculated between early- and mid-aged chops.

[3]Percent difference calculated between mid- and late-aged chops.

[4]Percent difference calculated between early- and late-aged chops.

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