INFLUENCE OF SEASONAL ENVIRONMENT, TOP AND BOTTOM DECK TRANSPORT, TRANSPORT DURATION, AND TIME IN LAIRAGE ON OVERALL PORK QUALITY AND BLOOD SERUM CORTISOL CONCENTRATIONS OF MARKET HOGS

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

INFLUENCE OF SEASONAL ENVIRONMENT, TOP AND BOTTOM DECK TRANSPORT, TRANSPORT DURATION, AND TIME IN LAIRAGE ON OVERALL PORK QUALITY AND BLOOD SERUM CORTISOL CONCENTRATIONS OF MARKET HOGS

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INFLUENCE OF SEASONAL ENVIRONMENT, TOP AND BOTTOM DECK TRANSPORT, TRANSPORT DURATION, AND TIME IN LAIRAGE ON OVERALL PORK QUALITY AND BLOOD SERUM CORTISOL CONCENTRATIONS OF MARKET HOGS

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Objectives of this study were to determine effects of seasonal environment, top and bottom deck transport, transport duration, and time in lairage on overall pork quality and blood serum concentrations of the stress hormone cortisol of market hogs. Mixed commercial crossbred market hogs (PIC, Franklin, KY) were harvested at dates representing traditional seasonal environments in the Midwestern United States: February 14 and 16, 2006 (n = 599), May 16 and 18, 2006 (n = 660), August 1 and 3, 2006 (n = 649), and October 17 and 19, 2006 (n = 661). Within season, pigs were randomly assigned to one of 8 treatments in a 2 x 2 x 2 factorial arrangement, with two transport durations; short (3 hours) or long (6 hours), two trailer deck locations; top or bottom, and two lairage durations; short (3 hours) or long (6 hours). Environmental conditions (temperature and relative humidity) in the trailer were monitored at one minute intervals using portable data loggers located in the three compartments (front, middle, rear) of both decks. All pigs originated from the same commercial source and identical transport procedure, data collection, and harvest procedure was repeated on Tuesday and Thursdays within the same week within season. Blood was collected from each carcass at exsanguination on the bleed table for analysis of serum cortisol concentration. Fresh pork loin quality parameters were evaluated on boneless pork loins for color (L*, a*, and

b*), pH, and drip loss. Loins were classified as pale, soft, and exudative (PSE) if 24h drip loss exceeded 5% and L* was greater than 55. Least-squares means were generated and tested for least significant difference across all main effects and appropriate interactions for fresh pork quality parameters and serum cortisol concentration. Cortisol levels were the greatest during the summer and fall seasons and interacted significantly (P < 0.05) between lairage, deck, and haul. Pigs transported in the bottom deck (regardless of duration traveled or time spent in lairage) had a higher rate of PSE loins (6.94%) in the winter compared to loins from pigs transported in the winter on the top deck (3.58%). Furthermore, pigs removed from the bottom deck entering short lairage generated 5.28% PSE loins while the pigs that came off the bottom deck into a long lairage generated 2.86% PSE loins.

Chapter I

REVIEW OF LITERATURE

Introduction

The 1993 Pork Chain Quality Audit (Cannon et al., 1995) reported the average United States market hog live weight was 111 kg, had a last rib backfat thickness of 2.95 cm, and a predicted carcass lean of 49.5%. In comparison, the 2002 National Pork Benchmarking Study revealed the market hog average live weight had increased to 116 kg, last rib backfat had remarkably decreased to 1.78 cm, and predicted carcass lean had increased to 55.5% (Scanga et al., 2003). The industry strategy of carcass merit pricing had clearly worked as the price incentive for leaner, heavier muscled hogs resulted in carcasses possessing a higher cutablity. This improvement, however, did not come without a cost. The 2002 audit also revealed a 5.2% increase in pale, soft, and exudative (PSE) pork over the 10 year span between surveys (Scanga et al., 2003). The value of PSE pork is much lower than normal pork and therefore is harmful to the economic efficiency of a pork processor. In fact, the 2002 audit reported economic loss associated with PSE pork at \$0.90 per carcass (Scanga et al., 2003). According to the USDA, the daily market hog harvest is reported at an estimated 410,000 pigs per day (USDA, 2007). Therefore PSE pork has the potential to cost the US pork industry approximately \$369,000 a day.

Several factors have been identified as playing a role in the occurrence of PSE pork. Genetics (relative to the Porcine Stress Syndrome) and use of growth promotants (PayleanTM) in swine diets have been extensively studied and their effects are well

documented (Allison et al., 2005; Armstrong et al., 2004). Hambrecht et al. (2005) stated the greatest improvements in pork quality could be achieved by decreasing stress in the immediate preslaughter period. Several programs initiated by the National Pork Board enable U.S. pork producers to make informed production decisions relative to improving genetic selection, selection of feed components, and animal handling on-farm and during transport as a means to minimize the risk of inducing PSE pork. Despite the research and educational programs to better inform producers, many questions remain with regard to the influence of ante-mortem stress and its effect on lean quality.

Pork Lean Quality

The term "quality" can mean several things to a wide spectrum of people. Pork quality can include yields realized by the packer, nutritional value of the meat, appearance of retail product, eating satisfaction by the final consumer, and a perspective of the welfare of the animal and environment in which the animal was raised. Regardless of the definition, pork quality in the United States over the past 15 years has become an increasingly important parameter relative to carcass value. With regard to the economic functionality of pork for fresh sale or further processing, pork quality can be lean color, ultimate pH (24 hour pH or after the conversion of muscle to meat), water-holding capacity, lean firmness, percentage intramuscular fat, wholesomeness or safety of the final product, and the consistency to which the aforementioned components are met. From the above description, the two parameters which primarily determine retail market value and functional (processing) pork quality are lean color and water-holding capacity.

Brewer and McKeith (1999) found lean color was the primary decisive factor

relative to consumer purchase of fresh pork. Consumers tend to prefer more reddish-pink lean, which possesses a lower light reflectance (L * value), than pork that is pale gray to white and has a high light reflectance (Table 1; NPB, 1999; Norman et al., 2002). Cravens (2000) noted Japanese consumers, relative to US consumers, prefer darker colored meat and more marbling with little variation in lean color. Moreover, some U.S. pork processors include an assessment of lean color in their value-based pricing system. According to the Pig Improvement Company (PIC; 2003), pork loins must exhibit an L* value lower than 50 (suggesting a relatively dark lean color) to be eligible for the premiums associated with Japanese export.

Water-holding capacity is the most important concern of further processing because the ability of fresh pork to retain water has a direct impact on cooking and processing yield. The value of water-holding capacity is emphasized when recognizing that 75 % of domestically produced pork is further processed (Cannon, 1995). Furthermore, water-holding capacity is a concern for the appearance of retail product because the amount of purge (water escaping the meat) displayed within the package contributes to the consumer's negative perception of quality of that product. Table 1 illustrates ideal ranges for different pork loin quality parameters (NPB, 1999).

The metabolism of intramuscular glycogen plays the primary role in the conversion of muscle to meat and the expression of different quality attributes of fresh pork (Berg, 1999). Glycogen, the muscle sugar, is comprised of several glucose molecules linked together. Intramuscular enzymatic reactions degrade the highly branched glycogen molecule back to glucose monomers via the process of

glycogenolysis. The subsequent glucose "fragment" is metabolized via glycolysis to either pyruvate (for aerobic entry into the Krebs cycle) or to lactate (anaerobic metabolism) (Brooks et al., 1996). During postmortem anaerobic glycolysis, two lactate molecules are produced per glucose molecule. In living muscle, lactate is transported in the blood to the liver where through the Cori cycle, it ultimately returns to the muscle cell as glucose. In postmortem muscle metabolism, the animal is exsanguinated and therefore the capacity for transport of lactate to the liver is eliminated, resulting in a build-up of lactic acid within the muscle. It is the accumulation of lactic acid that results in the natural pH decline of muscle tissue and the postmortem conversion of muscle to meat (Brooks et al., 1996).

The initial rate of acidification is primarily determined by the rate of muscle metabolism immediately before, during, and immediately after humane harvest (Warriss, 1987). Activation of the glycolytic system immediately before exsanguination leads to heat production which will elevate the pig's body temperature (Berg, 1999). Any preharvest stress could instigate and accelerate ante-mortem muscle metabolism and intramuscular heat production, thereby prolonging postmortem muscle glycolysis and ultimately generating detrimental effects on the functional lean quality. Increasing muscle metabolism ante-mortem can be attributed to a number of factors within the pork production chain such as genetic predisposition, pig excitability, pre-slaughter stress on the farm, during transport, and at the plant, and (or) interactions of all of the aforementioned factors (Meisinger, 2002). Pearson (1987) wrote that the ultimate condition of pork muscle is influenced by skeletal muscle pH drop as a function of time,

in vivo temperature patterns, carcass chilling rate, and the conditions at the onset of rigor mortis (Figure 1).

If the pH declines rapidly while muscle temperature is high, the resulting pork can become PSE. The phenomenon known as PSE pork was first characterized by Briskey (1964) as a rapid acidification of the postmortem muscle (pH < 6.0 within 1 h). This rapid decline in pH, combined with elevated postmortem muscle temperatures, denatures the integrity of the muscle structure resulting in myofibrillar shrinkage which can lead to a dramatic loss of water-binding capacity (Figure 2) and excessive light reflection. Generally, acute, short-term stress immediately before stunning and exsanguination can lead to the production of PSE pork. Pork classified as prototype PSE has distinctly reduced processing and cooking yields (Boles et al., 1991; Person et al., 2005), as well as decreased tenderness and juiciness (Bennett et al., 1973).

If glycogen has been excessively depleted before harvest (due to prolonged stress), postmortem lactic acid production will be insufficient to result in a normal pH decline, thus resulting in dark, firm, and dry (DFD) pork (Tarrant, 1989). Pork classified as prototype DFD will have a high 24 h postmortem pH (> 6.0) and will appear very dark and sticky due to the high water-binding capacity. Generally, chronic, long-term stress can lead to the production of DFD pork (Figure 1). Some consumers discriminate against DFD pork due to its dark color; however, DFD pork is predominantly discriminated against by retailers because of its high water activity, which leads to a reduced retail shelf-life and rapid spoilage (Dobrenov, 1990). Although DFD pork is certainly an undesirable lean defect, the incidence of PSE pork is certainly a more prevalent non-

conformity with a much more extensive cost to the United States pork industry (Scanga, 2003).

Stress Response

Stress can be defined as a condition in an animal that results from the action of one or more stressors that may be either external or internal of origin. A harmful stress can further be defined as the organism's ability to cope with a perceived threat as it regains a state of homeostasis (von Borell, 2001). In order to fully understand the impact production practices and environmental effects have on animals, we need to understand the physiology of stress within the animal. The initial response to a stressor was first categorized by Cannon (1914) as the emergency function of the adrenal medulla during stress. This short-acting stress response pathway includes sympathetic neural pathways innervated directly to the adrenal medulla, which release the catecholamines epinephrine and norepinephrine (Moberg, 2000). These hormones cause an acute increase in body metabolism (by increasing heart rate and blood pressure) and stimulate an increase in plasma glucose concentration via glycogenolysis (Knowles and Warriss, 2000). This response later became known as the fight-or-flight response. These hormones are very important measurements of immediate stress, however, they are difficult to quantify due to their short half-life (norepinephrine < 2 min) (Knowles and Warriss, 2000). Stress hormones with longer half-lives, such as cortisol, are utilized more frequently in research as a determination of stress.

A longer-termed, sustained response system to stress was first conceptualized by Hans Selye (general adaptation syndrome; Selye, 1946) and is known today as the hypothalamic-pituitary-adrenocortical (HPA) stress response system. The brain circuits that initiate and maintain the stress response include the parvocellular corticoptropin-releasing hormone (CRH) and agrinine-vasopressin (AVP) neurons of the paraventricular nuclei (PVN) of the hypothalamus (Tsigos and Chrousos, 2002). Primarily CRH stimulates the release of adrenocorticotropic hormone (ACTH) from anterior pituitary corticotrophs, which in turn stimulates glucocorticord release from the adrenal cortex (Matteri et al., 2000).

Cortisol is the primary glucocorticord in mammalian farm species. Cortisol plays an important role in gluconeogenesis by stimulating the liver to convert fat and protein to intermediate metabolites that are ultimately converted to glucose for energy (Matteri et al., 2000). Glucocorticords function to free energy for immediate use, are the final effectors of the HPA axis, and participate in the control of whole body homeostasis and the organism's response to stress. Through negative feedback, glucocorticords also limit the potentially damaging effect of chronically elevated levels of glucocorticords and therefore complete the stress response (Tsigos and Chrousos, 2002; Matteri et al., 2000). The degree of secretory activity of the adrenal gland, usually the degree of increase in cortisol concentration, is regarded as indicative of stressful conditions (Minton, 1994).

Lairage Recovery

According to Warriss (2003), lairage provides two functions to a swine harvest facility. First, reservoirs to allow the plant to accommodate variations in animal deliveries, as well as a place to gather, weigh, and sort animals before harvest. The second function is to allow the pigs opportunity to rest and recover from the stress of

loading, transport, and unloading. Various studies have been performed to reveal the optimal time requirement in lairage to both accommodate animal welfare and improve ultimate meat quality.

Warris et al. (1998) performed a study on the influence of lairage times of up to one hour, three hours, or overnight on pork lean quality. The authors found 24 h pH in all muscles tested increased (P < 0.05) progressively with longer periods of lairage and fiber optic probe (FOP) values decreased (P < 0.05) indicating darker color. Nanni Costa et al. (2002) found pigs given an overnight lairage (22 h) compared to a 2 h lairage had a significantly higher (P < 0.01) 1.5 h pH of 6.25 compared to 6.06, respectively. The ultimate meat quality data agreed with the initial data, in that overnight lairage significantly (P < 0.05) increased ultimate pH in the *Longissimus thoracis* (LT; 5.48 to 5.5) and *Biceps Femoris* (BF; 5.49 to 5.54) muscles. Overnight lairage also decreased L* values (i.e. darker) in both muscles significantly (P < 0.05) from 50.5 to 49.4 in the LT and 51.4 to 48.9 in the BF. With respect to drip loss values in the LT, overnight lairage significantly (P < 0.05) lowered drip loss % from 7.6 to 7.1% (Nanni Costa et al., 2002). In both studies, the percentage of potentially PSE carcasses progressively decreased, with an increased lairage time.

Milligan et al. (1998) found pigs allowed a 2 or 3 h lairage prior to slaughter had greater (P < 0.05) ultimate loin pH values, lower (P < 0.05) loin L* values (indicating darker lean), and lower (P > 0.05) loin purge loss percentages than pigs allowed a 0 or 1 h lairage. Perez et al (2002) found pigs given a 9 h lairage had the least dramatic pH decline within the *Longissimus thoracis* compared to 0 and 3 h lairage groups, and ended

with the highest (P < 0.05) 24 h pH of 5.89.

There are some inconsistencies within the literature. Hambrecht et al. (2005) found lairage length (3 h vs <45 min) did not affect (P > 0.05) ultimate pH values or electrical conductivity (an indication of water-holding capacity). However, they did find pigs given a shorter lairage, <45 min compared to 3 h, had a darker (P < 0.01) lean.

Ambient temperature in combination with various lairage times seems to have an effect on ultimate pork quality. Aaslyng and Barton-Gade (2001), performed a study on the effect of lairage time on lean quality in a low stress pre-slaughter handling environment. In their spring harvest (3-11°C), they found no lean quality differences (P > 0.10) if the pigs were allowed lairage periods of <0.5 h, 1.5 h, or >2.5 h. These data provide evidence that meat quality is independent of lairage time when pigs are subjected to a low stress environment and a temperate climate. However, during their summer harvest (15-20°C), they found pigs given a lairage period of <0.5 h had greater (P < 0.001) loin internal reflectance values (indicated paler color), and greater (P < 0.05) drip loss percentage, than pigs given >2 h of lairage. These data provide evidence that when pigs are subjected to higher temperatures; a longer lairage period can improve lean quality within a low stress environment. Fraquenza et al. (1998) found conflicting results when studying the effects of lairage temperature and time on pork quality. They found that when pigs were held at 20°C, an increased lairage time of 3 h compared to 0.5 h reduced the incidence of PSE meat from 32.9% to 23.7%. However, when the lairage temperature was raised to 35°C, the longer lairage period increased the incidence of PSE to 35.5 % from 31.1%. The authors, therefore, recommended that at high ambient temperature, it was best to slaughter pigs after at most 30 minutes of lairage to avoid a high incidence of PSE meat.

The association of blood cortisol levels with lairage time is consistent within the literature. Perez et al. (2002) found the group slaughtered immediately upon arrival (0h) had significantly higher (P < 0.05) cortisol levels than the group given a 3 h lairage. Warriss et al. (1992) found in two experiments, pigs given <1h lairage had greater (P < 0.05) cortisol levels at harvest than pigs given >2 h lairage. Cortisol levels were reported to return to pre-stressed levels after a 2 to 3 h lairage. Warriss et al. (1998) tested blood for cortisol and found they were similar in pigs rested for up to three hours, but lower in those held overnight. Collectively, this research provides evidence that the well-being of pigs given shorter lairage periods was more likely to be compromised than pigs given a longer lairage.

Transport Stress

The National Pork Benchmarking Study reported 51.8% of market hogs were transported less than 161 km, 32.6% were transported from distances of 162 to 322 km, and 15.6% were transported more than 323 km (Scanga et al., 2003). Grandin (1994) supports these findings by stating most hogs slaughtered within the United States are transported less than 4 h.

Apple et al. (2005), conducted a study of the effect of short-duration transportation on the stress response in pigs and found a dramatic increase (P < 0.05) in cortisol concentration of pigs during the first 30 min of transportation, which remained elevated (P < 0.05) above that of non-transported pigs. Geverink et al. (1998) found

transport increased salivary cortisol levels across preslaughter handling treatments of environmental exposure other than the pigs home, regular human contact, and a control of no human contact and no outside home environmental exposure (P < 0.01). Collectively, these data provide evidence that a marked stress response occurs in response to the action of transporting pigs.

Hambrecht et al. (2005) studied of the effect of preslaughter stress, transport length, and lairage length on blood stress parameters and ultimate meat quality. They found short transport increased (P < 0.01) cortisol levels when followed by short lairage, illustrating a transport by lairage interaction. The study revealed that pre-slaughter stress had the largest contribution to reductions in water-holding properties of pork. The combination of long transport and short lairage aggravated the negative aspects of pre-slaughter stress and therefore was a major factor in predicting ultimate pork quality.

In respect to truck deck location (top or bottom), Nanni Costa et al. (2002) found that neither loading method nor truck deck location significantly influenced any carcass or meat quality parameter, nor did they interact with the other treatments of the study of low (0.4 m²) vs. high (0.6 m²) stocking density, long (22 h) vs. short (2 h) lairage, or with halothane genotype. Ritter et al. (2006) found trailer deck (top vs. bottom) onto which pigs were loaded and transported had no effect on transport losses; unfortunately they did not measure pork lean quality in their study.

Seasonal Stress

Carr (2006) found pigs from a seasonally hot environment (22 – 35°C) had higher (P < 0.02) cortisol levels than pigs from a temperate environment (6 – 13°C) or a cold

environment (-9 to 0° C) when given a tight stocking density. Heat stressed pigs given 3 h lairage had higher (P < 0.01) cortisol levels than temperate pigs given 3 h lairage. Hot and temperate environment pigs given 3 h of lairage had higher (P < 0.01) cortisol levels than those given 45 min of lairage. This suggests not only a marked heat stress response, but also that tight stocking density, and 3 h of lairage further exacerbated potential stressors. With regard to pork lean quality, pigs from the hot environment had paler loin muscle (P < 0.003) than cold and temperate pigs and greater (P < 0.003) loin purge loss percentages than cold stress pigs. Similarly Lefaucheur et al. (1991) found moisture loss percentage was lower (P < 0.05) in the Semispinalis muscle obtained from pigs raised in a temperate environment compared to pigs raised in a warm environment. This implies heat stress has a negative impact on pork lean quality.

Conversely, Witte (2000) conducted a study where pigs were finished in a thermoneutral (18°C) or hot (32°C) environment and found Hunter L* values were higher (P < 0.05) for pigs finished under thermoneutral conditions, indicating a paler color. However, there was no effect of environmental temperature on any of the other quality measurements of a*, b*, ultimate pH or drip loss (%). Lefaucheur et al. (1991) reared barrows in two different environmental temperatures, 12°C vs. 28°C, to determine their effect on meat quality. They found both 45 min pH and 24 h pH were significantly lower (P < 0.01) in the loin muscle of pigs reared in 12°C compared to loins obtained from pigs raised at 28°C. These data provide evidence that there is a negative effect of a temperate environment on meat quality (as measured by pH) compared to a hotter environment. Hicks et al. (1998) found heat stressed pigs (defined as stress resulting in an increased

respiration rate) had significantly lower (P < 0.05) cortisol levels than both cold stressed pigs and pigs experiencing the stress of a 4 h transport. Heat stressed pigs and the control group had statistically similar values for cortisol.

Justification for Research

The information provided within this literature review outlines the issues involved with the recent degradation of pork quality. While genetics, feeding regimes, and preslaughter stressors are recognized as being factors in producing high quality pork; preslaughter stress remains as the focus of this thesis and provides for the greatest variable for immediate improvement. Time of transport, time in lairage, ambient temperatures, and pre-slaughter handling of pigs, when held at ideal conditions, have all been associated with preserving ultimate pork quality.

Table 1. Targets for fresh pork loin quality, adapted from NPB (1999)

Variable	Target range
Subjective color score ^a	3.0 to 5.0
Lightness (L*) ^b	49 to 37
Subjective marbling score ^c	2.0 to 4.0
Ultimate pH ^d	5.6 to 5.9
Drip loss, %	≤ 2.5%

 $[^]a1$ = white to pale pinkish gray to 6 = dark purplish red (NPPC, 2000). $^bL^*$ = measure of darkness to lightness (larger value indicates a lighter color); a^* = a measure of redness (larger value indicates a redder color) c1 = 1% i.m. fat to 10 = 10% i.m. fat (NPPC, 2000). dM easured ≥ 24 h postmortem.

Figure 1. The relationship of postmortem pH decline with characteristics of pork muscle, adapted from Sellier and Monin (1994)

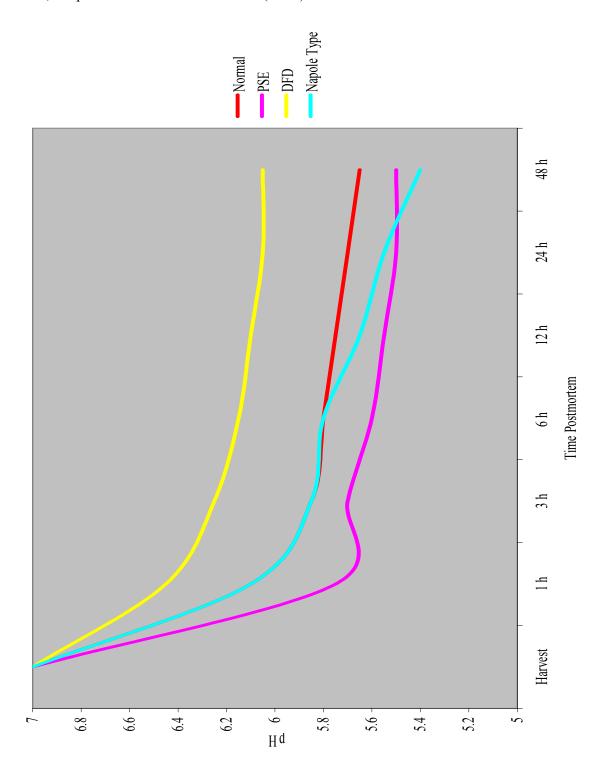
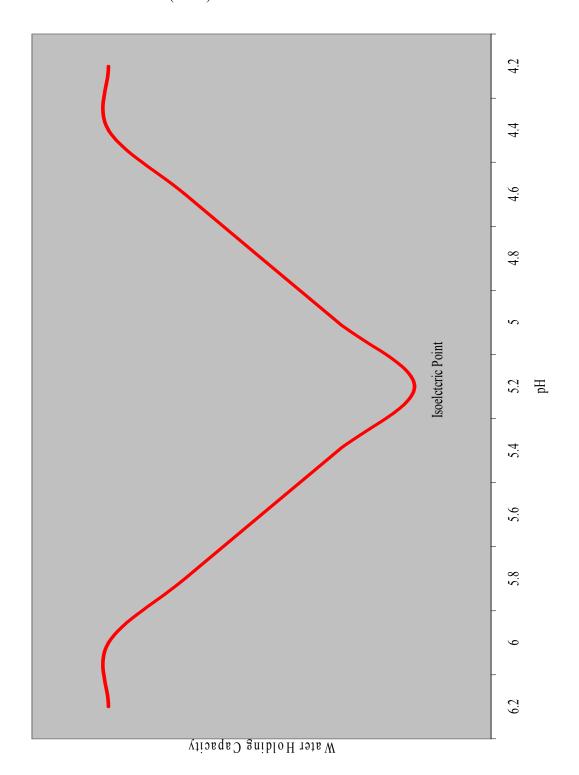


Figure 2. Relationship between pork muscle pH and water-holding capacity, adapted from Wismer-Pedersen (1987)



Chapter II

INFLUENCE OF SEASONAL ENVIRONMENT, TOP AND BOTTOM DECK TRANSPORT, TRANSPORT DURATION, AND TIME IN LAIRAGE ON OVERALL PORK QUALITY AND BLOOD SERUM CORTISOL CONCENTRATIONS OF MARKET HOGS

Abstract

Objectives of this study were to determine effects of seasonal environment, top and bottom deck transport, transport duration, and time in lairage on overall pork quality and blood serum concentrations of the stress hormone cortisol of market hogs. Mixed commercial crossbred market hogs (PIC, Franklin, KY) were harvested at dates representing traditional seasonal environments in the Midwestern United States: February 14 and 16, 2006 (n = 599), May 16 and 18, 2006 (n = 660), August 1 and 3, 2006 (n = 649), and October 17 and 19, 2006 (n = 661). Within season, pigs were randomly assigned to one of 8 treatments in a 2 x 2 x 2 factorial arrangement, with two transport durations; short (3 hours) or long (6 hours), two trailer deck locations; top or bottom, and two lairage durations; short (3 hours) or long (6 hours). Environmental conditions (temperature and relative humidity) in the trailer were monitored at one minute intervals using portable data loggers located in the three compartments (front, middle, rear) of both decks. All pigs originated from the same commercial source and identical transport procedure, data collection, and harvest procedure was repeated on Tuesday and Thursdays within the same week within season. Blood was collected from each carcass at exsanguination on the bleed table for analysis of serum cortisol concentration. Fresh pork loin quality parameters were evaluated on boneless pork loins for color (L*, a*, and b*), pH, and drip loss. Loins were classified as pale, soft, and exudative (PSE) if 24h

drip loss exceeded 5% and L* was greater than 55. Least-squares means were generated

and tested for least significant difference across all main effects and appropriate

interactions for fresh pork quality parameters and serum cortisol concentration. Cortisol

levels were the greatest during the summer and fall seasons and interacted significantly

(P < 0.05) with lairage, deck, and transport duration. Pigs transported in the bottom deck

(regardless of duration traveled or time spent in lairage) had a higher rate of PSE loins

(6.94%) in the winter compared to loins from pigs transported in the winter on the top

deck (3.58%). Furthermore, pigs removed from the bottom deck and entering short

lairage had 5.28% PSE loins while the pigs from the bottom deck entering into a long

lairage had 2.86% PSE loins.

Key Words: Pork, Quality, Stress, Cortisol, Lairage, Seasonal

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Introduction

After the 1992 Checkoff-funded Pork Quality Audit was released, the National Pork Board (NPB) used the information to propose dramatic changes to the pork production chain. Positive changes were made in the 10 years following the 1992 Audit. Comparisons of the 1992 data with the more recent 2002 Pork Quality Audit revealed that pork carcass backfat was dramatically decreased from 1.07 (1992) to 0.69 inches (2002). An industry focus on reducing carcass fat and the subsequent implementation of carcass merit price incentives were the primary drivers of the reduction in backfat and consequently the industry saw an equally remarkable change in percent carcass lean; increasing from 49.5 to 55.5% carcass lean.

Not all changes were positive. In 1992 the estimated occurrence of PSE (pale, soft, and exudative) pork at the packing plant was 10.2% and in 2002 it was reported to have increased to 15.5%. This increase in PSE pork may be the unfortunate byproduct of the push for market hogs with less fat and more muscle. The relatively sudden change in phenotype may have led to physiological changes that influence muscle metabolism and muscle development.

Muscle growth is enhanced by a certain level of stress. This is well documented in training athletes, whereby training (an environmental stimulus) combined with genetics, generate maximum muscular size and (or) performance. Within the pork industry, genetic selection for muscular rate of gain and efficient use of dietary calories for lean gain (not fat gain), has resulted in 5.2% more muscle in the average market hog. Data collected by the USDA (presented by Ellis and McKeith at the 2004 Pork Quality

and Safety Summit) illustrated a steady increase in the number of dead on arrival (DOA) hogs from 1992 to 2002. This time period corresponds with the industry's emphasis on the use of lean swine genetics to enhance changes in swine body composition. An extrapolation could be made that muscular, metabolically efficient market hogs were more sensitive to the stress of loading and transport resulting in more DOA pigs at the packing plant.

Loading, transport, and unloading has been identified as a major stress for pigs (Meisinger, 2002). The worst time to stress pigs is in the antemortem period where physiological changes can result in PSE pork (Hambrecht, et al, 2005). There are two distinct trailer environments in the popular double-deck livestock trailers. Anecdotal observational evidence provides that pigs in certain locations within these trailers are more subject to certain transport stressors. Therefore, the objective of this study was to determine effects of seasonal environment, top and bottom deck transport, transport duration, and time in lairage on overall pork quality and blood serum concentrations of the stress hormone cortisol in market hogs.

Materials and Methods

Animals and Experimental Design

Mixed commercial crossbred market hogs (PIC, Franklin, KY) were harvested at dates representing traditional seasonal environments in the Midwestern United States: February 14 and 16, 2006 (winter; n = 599), May 16 and 18, 2006 (spring; n = 660), August 1 and 3, 2006 (summer; n = 649), and October 17 and 19, 2006 (fall; n = 661). Within season, pigs were randomly assigned to one of 6 treatments in a 2 x 2 x 2 factorial

arrangement, with two transport durations; short (3 hours) or long (6 hours), two trailer deck locations; top or bottom (Figure 2) and two lairage durations; short (3 hours) or long (6 hours). Environmental conditions in the trailer were monitored at one minute intervals using portable data loggers (HOBO Pro Series RH/Temp. Onset Computer Corp., Pocasset, MA, USA) located in the three compartments (front, middle, rear) of both the top and bottom decks. The two different lairage durations required slaughter at two different times (Table 2). All pigs originated from the same commercial source and identical transport procedure, data collection, and harvest procedure was repeated on Tuesday and Thursdays within the same week within each season.

Preslaughter and slaughter

All pigs were fed the same commercial diet and withheld from feed 12 h prior to slaughter. Upon arrival at the commercial slaughterhouse (QPP, Austin, MN), pigs from the long duration transport were unloaded first, beginning with the bottom deck. All pigs were unloaded in small groups of 20 and alternately assigned to long or short lairage pens. Each pig in the small subgroup was tattooed with a unique tattoo number to specify transport duration, top or bottom deck, and lairage length. Pigs were given free access to water during lairage. All pigs were subjected to humane head-to-heart electrical stunning procedures in compliance with the standard industry practices and the Humane Slaughter Act.

At exsanguination, blood was collected from each pig in pre-numbered disposable 15 ml tubes (Cat. No. 362695; Nalge NUNC International). Self-piercing "round post" metal eartags sequentially numbered to correspond with the blood collection tubes were

then tagged in the ear of each pig while still on the gambrel table. After all blood samples had been collected, blood tubes were allowed to clot under refrigeration in the laboratory. Centrifugation of blood samples began eight to 10 hours after collection. Samples were centrifuged for 10 minutes at 2,500 X g. After centrifugation, serum (5 ml) was transferred from tubes into 48 well plates and stored at –20°C until analysis for cortisol concentration. For the analysis of cortisol levels, 25µl of serum was assayed in duplicate using the procedures described by the technical manual from Diagnostic Systems Laboratories, Inc (Webster, TX). Values were reported in ng/ml.

Ear tag numbers were then transferred to the carcass by writing the number onto the left shoulder of each carcass using an edible blue carcass crayon. Carcass order (upon entry into the cooler), eartag number (blood tube number) and tattoo number were documented to match transport, deck, and lairage treatments with blood data. Once carcasses were sorted to a separate rail in the cooler, carcass eartag ID was transposed onto the left side dorsal (chine bone) thoracic vertebrae with an edible carcass crayon. After a 20 h chill (1°C), carcasses proceeded to the wholesale fabrication floor where loins were removed from the line for fresh pork quality analysis. Marking the chine allowed for identification and removal of the fresh loins from the processing line.

Pork quality measurements

Left-side loins were collected from the fabrication line and moved to the loin boning line. Boneless loins were cut at the 10th/11th rib interface, tagged with the original eartag ID, and moved to a remote table for ultimate pork quality assessment. Ultimate *longissimus* muscle (LM) pH (MPI pH-Meter, Meat Probes, Inc. Topeka, KS) and

objective lean color [L* (lightness), a*(redness), b*(yellowness)] was evaluated on the cut surface (10th rib surface) of the loin after allowing for a 10 minute blooming period (Brewer and McKeith, 1999). All objective color measurements were obtained using a Konica-Minolta portable Chroma Meter (Model CR 410, Minolta, Osaka, Japan) with an illuminant setting of D65/10 and calibrated to a white tile. Hue angle (arctangent ((b*/a*) * 360° / (2 * 3.14)) and chroma (square root (a*)²+(b*)²) were calculated. Hue angle is a measure of true red, where 0 is "true red" and chroma (color saturation) is a measurement of how vivid or concentrated a color appears, where by the higher the value the more vivid the color (Minolta, 1994).

A one-inch thick LM chop was fabricated from the 10th/11th rib interface, then a one-inch circumference core sample was removed from the center for determination of drip loss as described by Rasmussen and Stouffer (1996). Briefly, one-inch circumference core samples were removed from the chop, weighed to the nearest 0.01 g, and then placed into a specialized drip loss tube (meat juice containers; C. Christensen Laboratory, Denmark; Figure 4). The filled tubes were then placed in drip loss rack and the entire rack moved to a 39°F cooler for 24 h. After 24 h, samples were reweighed. The percentage of moisture loss (24 h drip loss) was calculated by dividing the difference between weights by the initial sample weight, multiplied by 100. Classification of PSE pork was determined through identification of those pork loins possessing a 24 hour drip loss > 5% and an L* value > 55.

Statistical Analysis

Data were analyzed as a general linear model (PROC GLM) of SAS (version

8.02; SAS Inst., Inc., Cary, NC). Least-squares means were generated by the LSMEANS statement and tested for least significant difference across all main effects and appropriate interactions for fresh pork quality parameters and serum cortisol concentration. Tests of multiple comparisons of LSMEANS were considered significant at a level of P < 0.05. The model applied included the fixed effects of season, transport length, transport deck location, lairage duration, as well as their interactions. The random effect was slaughter day nested within season.

Results and Discussion

Transport climatic conditions & pig welfare

According to Scanga et al. (2003) and the results of the 2002 NPB U.S. Pork Quality Audit, 51.8% of all pigs delivered to market traveled less than 100 miles to the point of destination, while 32.6% of pigs were transported 101 – 200 miles (approximately three hours duration) and 15.6% of pigs were transported over 200 miles. The transport duration treatments in the present study evaluated the median and longest range of transport duration as reported by Scanga and associates (2003).

The range of temperature and humidity recorded in the trailer throughout long transport duration (Table 3) was greatest during the winter season, with the bottom front compartment having the most volatility. Climatic conditions within the trailer during short transport duration (Table 4) had a wider range of temperature during the fall season transport. The Weather Safety Index is a tool that truckers can use to determine if temperature and humidity conditions reach a point during transport where animals could be in a "danger" or an "emergency" condition with regard to their welfare (if not

mortality). Figure 5 is a scatter plot of two compartments within the trailer during the summer, long haul transport. The red dots indicate the number of times the temperature and humidity reached a level of *Danger* or *Emergency* conditions. Climatic conditions in the bottom deck, front compartment were often the most climatically unfavorable while the top deck rear compartment was the most temperate during summer transport. According to the Scientific Committee on Animal Health and Animal Welfare (European Commission, 2002), market hog welfare during transport is highly dependent on vehicle design, driving method, and road quality. The European Commission also found that adequate ventilation of trailers was the most important criteria in maintaining proper market hog welfare during transport. Automated ventilation systems that opened or closed to maintain appropriate climatic conditions to maximize pig welfare reduced transport mortality by nearly 50% (Nielsen, 1981). Research by Christensen and Barton-Gade (1999) found that all DOA pigs in their experiment were removed from the front compartment on the lower deck where the temperature and humidity were highest and ventilation was the poorest. The number of market pigs classified as "slows" during unloading in the present study was highest in the winter and summer seasons (n = 7 each season; Table 5), yet the fall season had the most DOA pigs (n = 3). It is important to note that all three DOA pigs were removed from the bottom deck. Long hauls resulted in a greater number of DOA hogs (n = 6 versus 2 for short haul) while five deads were removed from the bottom deck versus 3 from the top deck over the course of the trial (Table 5). The number of slows reported in this study are well below the United States average of approximately 1% (Ellis et al., 2004). This could be attributed to the fact that all truckers were certified under the NPB Trucker Quality Assurance Program (NPB, 2004) or the fact that all pigs used in the study were of similar genetics and from the same production site. The observations of Christensen and Barton-Gade (1999) are consistent with the findings in our study relative to trailer climatic conditions; however, tracking of pigs from each trailer compartment was beyond the capabilities of this project. More research is necessary to quantify the influence of location within trailer, pig welfare, and pork quality.

Serum cortisol concentration

The stress hormone cortisol plays a major role in regulating energy (glucose) metabolism in livestock species (Knowles and Warriss, 2000). Cortisol remains active in the body longer than the more potent "fight or flight" hormones epinephrine and norepinephrine (Gregory, 1998) and is generally regarded as an indication of an animal's psychological state, as well as an index of its physiological reaction to environmental conditions and (or) welfare situation (Knowles and Warriss, 2000). Therefore, assessment of circulating cortisol is common in research which evaluates preharvest stress. If the precept is true that higher concentrations of cortisol in the blood are an indication of elevated stress, we observed that pigs transported in the summer and fall seasons were experiencing the most stress as indicated by higher serum cortisol concentration collected at slaughter (Table 6). Furthermore, pigs that experienced long transport duration had higher cortisol concentrations than those pigs transported for a shorter duration (103.01 vs. 95.16 ng/ml, respectively; Table 6). While the main effect of lairage duration was not significant as a main effect, differences were observed for

lairage time within season of the year (Figure 6). Short or long lairage was inconsequential during the spring, yet in the winter and fall seasons, short lairage (3) hours) appeared to be more stressful (i.e. higher cortisol levels were observed). In contrast, a longer lairage in the summer season resulted in higher cortisol levels. Martoccia et al. (1995) concluded that transport distance alone did not determine levels of severe stress in pigs and found that transport stress may be offset by the amount of time pigs spent in lairage before stunning. It is generally accepted that time spent in lairage allows a stressed animal to recover from loading, transport, and unloading stress. Temple Grandin, animal welfare specialist (Grandin, 1994), recommended that pigs be rested 2 to 4 hours before entering the stunning chute. The findings of the present study are consistent with Carr et al. (2006) who found that during the heat of the summer months, pigs given a shorter lairage (45 minutes) had lower circulating cortisol concentrations than pigs held in longer lairage (three hours). It is also interesting to note that short lairage after a long haul in our study appeared to induce a much greater stress response in pigs (cortisol concentration = 105.42 ng/ml) than those held in short lairage after a short haul (cortisol concentration = 92.3 ng/ml) as shown in Figure 7, providing evidence that market hogs require a longer duration lairage after a longer transport duration.

Transport duration and season of the year had the most significant influence on serum cortisol levels (Table 6). Statistical comparison of top or bottom trailer deck revealed no difference as a main affect (comparison of all pigs transported on the top or bottom of the trailer throughout the entire year did not differ), yet deck location within season and length of transport did differ, revealing a significant three-way interaction;

deck × haul × season (Figure 8). Long hauls within season were numerically higher moving through the calendar year from winter to fall. Pigs transported the long distance on the top deck in the fall season had the highest cortisol levels of any group of pigs (Figure 8). During the summer month, pigs on the bottom deck during the long haul had a significantly higher cortisol concentration than those on the top. In winter, pigs on top had higher cortisol levels.

It is interesting and perhaps surprising to note that historical (unpublished) data provided by industry contacts reveals that the highest incidence of slows and DOA pigs are removed from trailers during the fall of the year. The cortisol data from the present study provides evidence that the pigs are indeed experiencing a higher physiological stress response in the fall season that is exacerbated by transport duration and deck location. Traditionally, heat and humidity are thought of as the primary drivers of stress during transport, yet findings in this study reveal only moderate climatic conditions during the fall of the year. More research is necessary to determine the factors that deteriorate market hog well-being during the fall season.

Longissimus muscle pH

Traditional meat science studies have long attributed intramuscular pH as the principal, quantitative measurement which drives fresh and further processing pork quality conditions. It is generally known that pork with a low pH has less water-holding capacity and a paler color. In the present study, season of the year and time in lairage were the two main effects (Table 6) significantly influencing intramuscular pH. Consistent with the cortisol data, pigs transported to harvest in the summer had higher pH

after a long lairage and those transported the short duration in the fall had a higher pH (Figure 9). This suggests that the higher cortisol was influencing muscle glycogen stores. As previously stated, cortisol plays a major role in regulating energy (glucose) metabolism. Therefore, intramuscular concentrations of glycogen (glucose) may be metabolized in response to the elevated cortisol concentrations. We can presume this would then result in less intramuscular glucose being available for conversion to lactate at time of harvest and therefore hinder the normal drop in pH considered for traditional conversion of muscle to meat. A significant deck × haul interaction was also noted (Figure 10) with pigs transported for six hours (long haul) on the bottom deck having a higher loin pH than those on the top deck.

Longissimus muscle percentage drip loss

Season of the year (summer) had the most significant influence on loin muscle water-holding capacity. Drip losses collected in the summer were much lower than any other season of the year (Table 6). Furthermore, deck location played a role in influencing drip loss during season of the year with higher drip losses observed in loins from pigs transported on the bottom deck in the winter and the top deck in the fall (Figure 11). No differences were observed between long or short hauls within the winter or summer season (Figure 12). Loins from pigs transported a shorter duration in the spring had significantly lower drip loss than the long transport, while the opposite affect was observed in the fall (Figure 12).

The season effect on pH is perplexing at best. Traditional meat science research would indicate that pH measurements are higher and drip losses lower in the winter

months as pigs utilize intramuscular glycogen to maintain body temperature through shivering. Therefore, muscle glucose is "burned up" prior to harvest leaving less substrate to convert to lactic acid during postmortem metabolism. The opposite was observed in the present study with winter and spring seasons possessing the highest moisture losses (Table 6). Cortisol levels were the lowest in the winter and spring season (Table 6) which would provide evidence that the physiological status of the pigs was such that the endocrine system was not calling for a greater need for glucose. In contrast, cortisol concentrations were the second highest in the summer season when drip loss percentages were the lowest. Furthermore, cortisol concentrations were not significantly correlated to drip loss (Table 7) so it would appear that in this present study, stress was not related to drip loss. In fact, there was no significant correlation between cortisol concentration and percentage drip loss when data were analyzed separately for each season (data not presented). We must therefore conclude that the data collected in the present study cannot explain the seasonal variation in loin muscle drip loss and must be attributed to some undocumented preharvest or packing plant variable.

Longissimus muscle color; L^* , saturation, and hue angle

Objective measurement of fresh pork color variability has been limited to observations recorded by colorimeter or spectrophotometer equipment that generates L* (lightness), a* (redness), b* (yellowness) data points. Measurement of paleness (L*) and (or) redness (a*) of a pork chop are relatively easily understood, however, many meat science research papers find differences in b* values (yellowness) are not easily explained. The naked eye cannot readily discern the level of "yellow" in a fresh pork

sample, so when treatment influences this color observation, it cannot be readily explained. In the present study, we opted to report two lesser used color variables; color saturation and hue angle. These two color measurements are calculated using the a* and b* color variables as described in the Materials and Methods section of this paper. Hue angle is a measure of true red, where 0 is "true red" and chroma (color saturation) is a measurement of how vivid or concentrated a color appears, whereby the higher the calculation the more vivid the color (Minolta, 1994).

The main effects of season and lairage had a significant influence on L* value (Table 6) as was the interaction term of haul within season. Though significant differences were noted between treatments, the true physical differences capable of being discerned by the naked eye (as in the case of a consumer rejecting a pork chop based on pale color) are minimal. Brewer et al. (2001) reported that consumers evaluating the lightness of fresh pork chops were able to discern differences if the L* value difference was greater than three L* units. The greatest comparable statistical difference was observed between winter and spring season L* values (Table 6) which was under three L* units difference. Therefore, care must be taken in interpretation of the data with regard to the practical industry value of L* observations reported in Table 6 and Figure 13.

Pigs held in long lairage in the summer produced loins that were more vivid (higher color saturation) than loins from pigs held in short lairage (Figure 14). The higher saturation value of loins from long lairage pigs complies with the higher cortisol concentration (Figure 6) and intramuscular pH (Figure 9) of the same pigs. Higher

cortisol concentrations during long lairage may have led to intramuscular glycogen reduction, the lower pH, and the subsequent improvement in the saturation of the muscle pigment.

As was observed with the L* values, the practical applicability of hue angle observations (Table 6, Figures 15 and 16) must also be evaluated with care as the significant differences may not discriminate a practical difference.

Longissimus muscle PSE characterization

Scanga et al. (2003) reported that the yearly industry average for the occurrence of pale, soft, and exudative (PSE) pork was 15.5%. Berg (2006) conducted a follow-up survey to further clarify the occurrence of PSE pork and found that 3.34% of the loins fabricated in U.S. packing plants exhibited all three conditions of classic PSE pork (they were pale and soft and exudative). The range in occurrence of PSE reported by the respondents to the Berg (2006) survey was from 0.1 to 10% occurrence. In the present study, PSE classification was objectively determined by using the percentage drip loss and L* thresholds as classification criteria whereby PSE= 24h drip loss > 5% and L* value > 55. These values were chosen for PSE classification based on their relationship with visible characteristics of fresh pork. The amount of moisture loss and the paleness (L*) of a fresh pork chop are characteristics that a consumer can observe when choosing pork at the retail case. The levels were selected based on data accumulated by the NPB in various pork quality studies. The threshold for drip loss set in the Pork Quality Targets is "not to exceed 2.5%" (NPB, 1998); therefore this value was doubled as an indication of extreme purge or drip loss. The L* level was chose as it corresponds to the NPB color standard of 2 (NPB, 1999). Acceptable pork quality targets for color are a color score of 3.0 to 5.0 utilizing a 6-point scale (NPB, 1998).

No statistical differences (P > 0.05) were observed for the main effects of season, deck location, transport duration, or lairage duration on occurrence of the PSE condition. Winter conditions were associated with the highest numeric occurrence of PSE (5.26%) of any season, followed by spring (4.26%), fall (3.78%), and summer (3.21%, Table 6). Trailer deck within season (Figure 17) had a significant influence on percentage occurrence of PSE (P = 0.05). In the winter, there was a significantly greater occurrence of PSE loins originating from pigs transported on the bottom trailer deck (6.94%) versus the top deck (3.58%). The classification of PSE in this case may be driven by similar differences observed for percentage drip loss reported for bottom vs. top deck in the winter season (Figure 11). Stress condition for winter transport of pigs (comparing the top and bottom compartments of the trailer) is inconclusive as cortisol concentrations did not differ between top or bottom transported pigs in the winter season. evaluation of the influence of deck location illustrates a higher percentage of PSE loins manifest from pigs transported on the bottom deck are then slaughtered after a short lairage (Figure 18). Of the loins from bottom deck pigs provided a short lairage, 5.28% were PSE loins compared to 2.86% of loins from bottom deck/ long lairage. This provides evidence that at least part of the potential for developing PSE loins may be alleviated by providing pigs with a longer lairage.

Implications

Pork producers have often been told that stressing pigs can not only have negative

consequences on the pig's welfare but also on the quality of pork that they generate. Removing pigs from their familiar environment, loading them on a trailer, trucking them to a packing plant, unloading them, and leaving them in an unfamiliar pen most definitely introduces stress on the animal. Cortisol levels were the greatest during the summer and fall seasons and interacted significantly (P < 0.05) between lairage, deck, and haul. Pigs transported in the bottom deck (regardless of duration traveled or time spent in lairage) had higher rates of PSE loins (6.94%) in the winter compared to loins from pigs transported in the winter on the top deck (3.58%). Furthermore, pigs removed from the bottom deck entering short lairage had 5.28% PSE loins, while the pigs that moved from the bottom deck into a long lairage had 2.86% PSE loins. Unpublished industry data provides evidence that the highest incidence of DOA pigs and pigs slow to exit the trailer are removed from trailers during the fall of the year. The cortisol data from the present study indicates that the pigs are indeed experiencing a higher physiological stress response in the fall season that is exacerbated by transport duration and deck location. Traditionally, heat and humidity are thought of as the primary drivers of stress during transport, yet findings in this study reveal that climatic conditions during the fall of the year were moderate. More research is necessary to determine the factors that deteriorate market hog welfare during the fall season.

Figure 3. Diagram of experimental trailer design and compartment location within trailer.

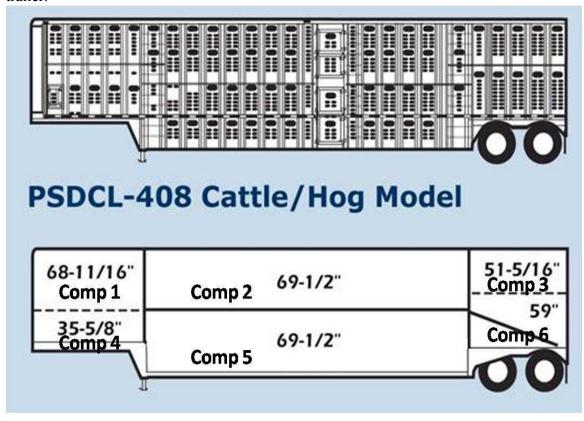


Figure 4. Example of specialized drip loss tube (manufactured and distributed by C. Christensen Laboratory, Denmark) and storage rack.



Figure 5. Scatter plot of the occurrence of temperature and relative humidity reaching the "danger" and "emergency" zones in the bottom front (warmest) and top rear (coolest) compartments within the trailer during the long haul (6 hours) summer transport.

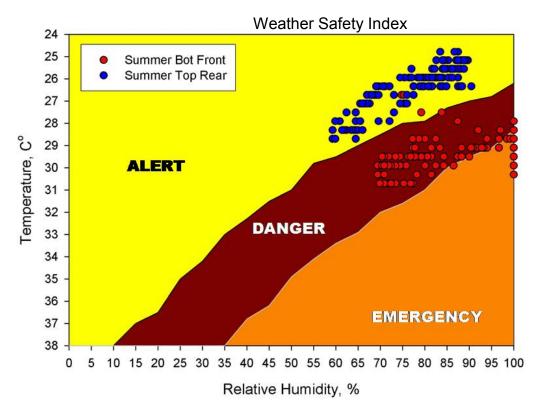


Figure 6. Least squares means and standard error bars for cortisol concentrations for short (3 hours) or long (6 hours) time in lairage within season; lairage \times season.

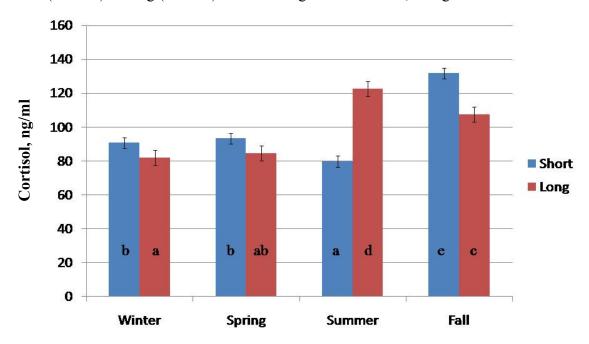


Figure 7. Least squares means and standard error bars for cortisol concentrations for long (6 hours) or short (3 hours) lairage within long (6 hours) or short (3 hours) haul; lairage × haul.

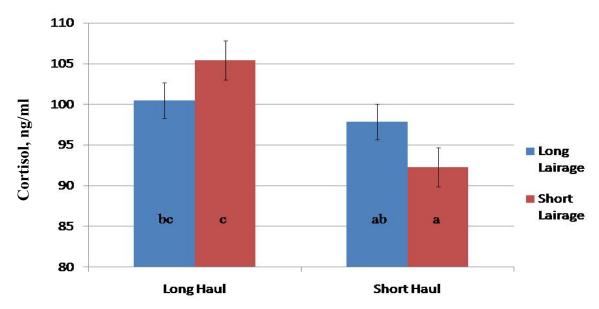


Figure 8. Least squares means and standard error bars for cortisol concentrations for top or bottom (**Bot**) trailer deck and short (**SH**; 3 hours) or long (**LH**; 6 hours) haul within season; haul × deck × season.

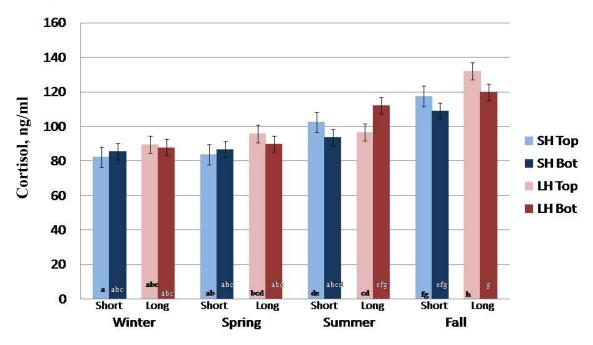


Figure 9. Least squares means and standard error bars for loin muscle ultimate pH for long (6 hours) or short (3 hours) lairage within season; lairage × season.

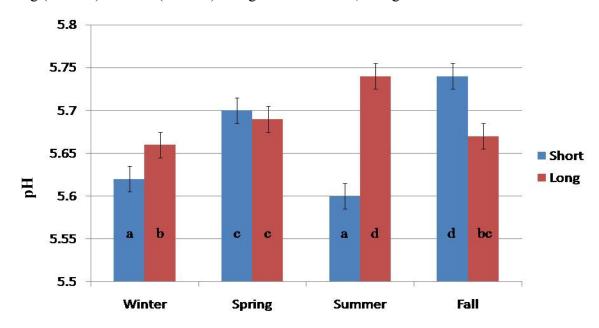


Figure 10. Least squares means and standard error bars for loin muscle ultimate pH for top or bottom trailer deck within short (3 hours) or long (6 hours) haul; deck × haul.

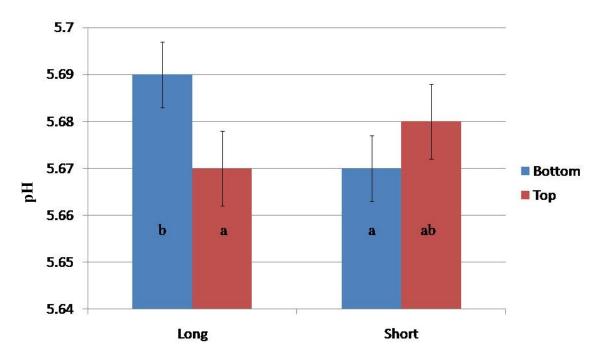


Figure 11. Least squares means and standard error bars for loin muscle percentage drip loss for top or bottom trailer deck within season; deck × season.

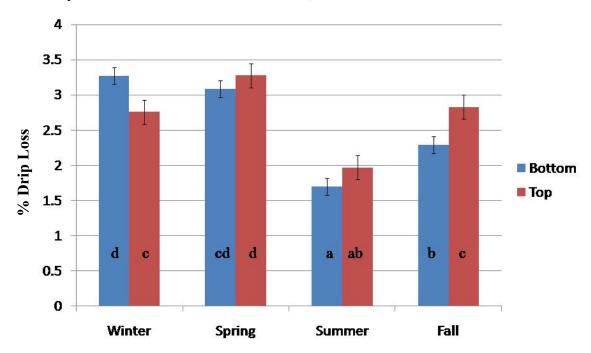


Figure 12. Least squares means and standard error bars for loin muscle percentage drip loss for short (3 hours) or long (6 hours) haul within season; haul × season.

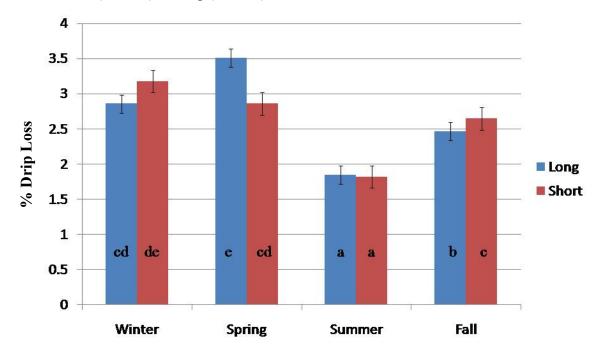


Figure 13. Least squares means and standard error bars for loin muscle L^* value (lightness) for short (3 hours) or long (6 hours) haul within season; haul \times season.

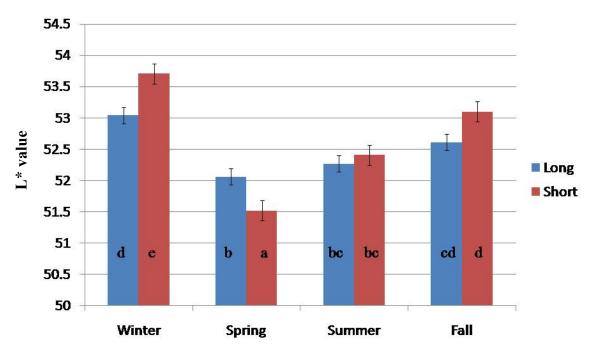


Figure 14. Least squares means and standard error bars for loin muscle color saturation (total pigment; higher is a more intense/ vivid color) for short (3 hours) or long (6 hours) lairage within season; lairage × season.

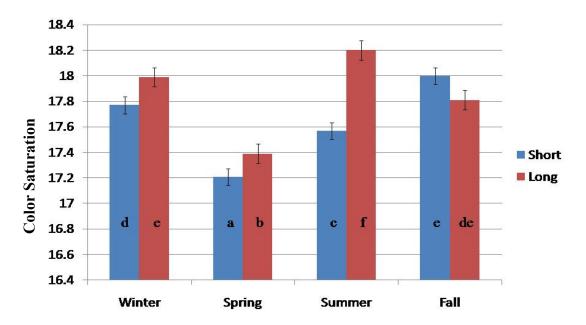


Figure 15. Least squares means and standard error bars for loin muscle hue angle (a value of zero equals "true" red) for short (3 hours) or long (6 hours) haul within season; haul × season.

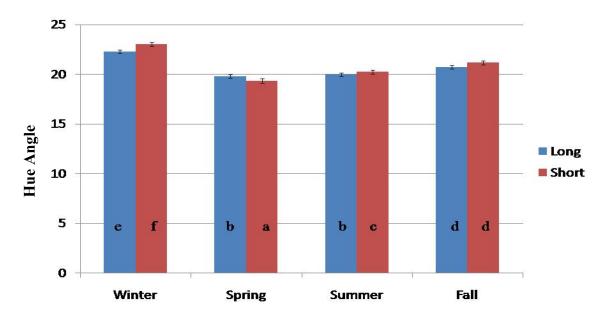


Figure 16. Least squares means and standard error bars for loin muscle hue angle (a value of zero equals "true" red) for short (3 hours) or long (6 hours) lairage within season; lairage × season.

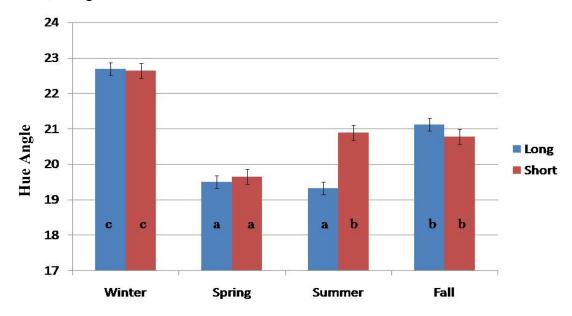
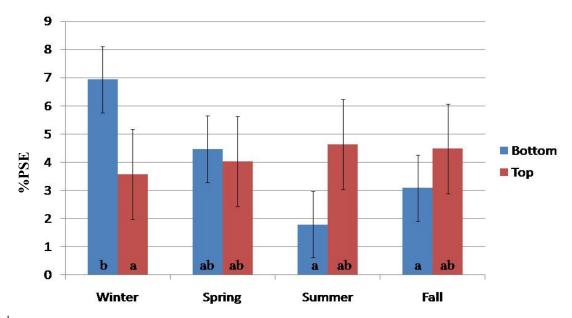
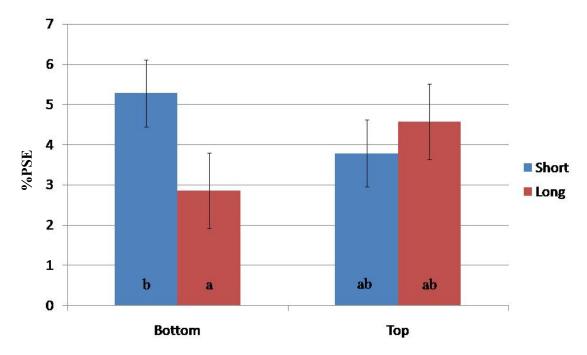


Figure 17. Least squares means and standard error bars for the frequency of occurrence of pale, soft, and exudative (PSE) classified loin muscles for top or bottom trailer deck within season; deck × season.



 1 PSE = 24h drip loss > 5% and L* value > 55

Figure 18. Least squares means and standard error bars for the frequency of occurrence of pale, soft, and exudative (PSE^1) classified loin muscles for short (3 hours) or long (6 hours) lairage associated with trailer deck location; deck \times lairage.



 1 PSE = 24h drip loss > 5% and L* value > 5

Table 2. Time schedule for pigs going to slaughterhouse.

Action	Time schedule			
	Short lairage Long lairag			
Loading pigs for long transport:	2300 - 2330h	2300 – 2330h		
Loading pigs for short transport:	0130 - 0200h	0130 - 0200h		
Arrival at slaughterhouse:	0500h	0500h		
Begin driving to stunner:	0755h	1055h		
Slaughter:	0800h	1100h		

Table 3. Average and ranges of temperatures (C°) (a) and relative humidity (b) for each trailer compartment of the top and bottom trailer deck for each season of the long (6 hour) transport duration.

<u>a)</u>

	Front	Middle	Back
Top	Winter: 2.28 (3.89 –10.6)	Winter: 1.56 (-5.6 – 11.1)	Winter: 1.5 (-4.4 – 11.1)
	Spring: 13.56 (11.1 – 16.1)	Spring: 13.11 (10.6 – 15.6)	Spring: 13.06 (10.0 – 16.7)
	Summer: 24.56 (18.3 – 29.4)	Summer: 24.0 (17.8 – 29.4)	Summer: 24.5 (18.9 – 29.4)
	Fall: 8.78 (2.2 – 15.6)	Fall: 13.11 (10.0 – 16.1)	Fall: 9.39 (2.2 – 20.6)
Bottom	Winter: 9.56 (-3.3 – 18.3)	Winter: 1.39 (-3.3 – 12.2)	Winter: 1.28 (-6.1 – 8.3)
	Spring: 18.06 (15.0 – 28.3)	Spring: 14.17 (11.1 – 17.2)	Spring: 14.5 (12.2 – 18.3)
	Summer: 25.89 (20.6 – 29.4)	Summer: 25.06 (20.0 – 28.9)	Summer: 25.33 (20.0 – 29.4)
	Fall: 6.33 (4.4 – 11.1)	Fall: 10.11 (3.9 – 19.4)	Fall: 9.89 (1.7 – 20.6)

<u>b)</u>

	Front	Middle	Back
Тор	Winter: 78.5 (63 – 95)	Winter: 80.1 (63 – 98)	Winter: 76.1 (56 – 93)
	Spring: 71.2 (48 – 90)	Spring: 72.4 (47 – 93)	Spring: 71.0 (49 – 91)
	Summer: 84.7 (59 – 104)	Summer: 83.8 (57 – 104)	Summer: 79.0 (56 – 97)
	Fall: 82.2 (67 – 104)	Fall: -	Fall: 73.8 (53 – 97)
Bottom	Winter: 72.0 (41 – 104)	Winter: 84.1 (73 – 95)	Winter: 76.4 (63 – 95)
	Spring: 68.6 (56 – 79)	Spring: -	Spring: 64.8 (48 – 84)
	Summer: -	Summer: -	Summer: 76.7 (59 – 96)
	Fall: -	Fall: 69.8 (61 – 78)	Fall: 79.7 (63 – 100)

Table 4. Average and ranges of temperatures (C°) (a) and relative humidity (b) for each trailer compartment of the top and bottom trailer deck for each season of the short (3 hour) transport duration.

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a	. 1
а	L.

	Front	Middle	Back
Top	Winter: 1.0 (6.7 – 11.1)	Winter:11 (6.7 – 8.3)	Winter: -1.08 (6.67 – 8.9)
	Spring: 12.78 (10.0 – 16.47)	Spring: 12.56 (9.4 – 15.0)	Spring: 12.39 (9.4 – 15.0)
	Summer: 24.28 (19.4 – 28.9)	Summer: 23.39 (18.9 – 28.9)	Summer: 24.94 (18.9 – 28.9)
	Fall: 12.67 (11.1 – 13.9)	Fall: 6.11 (2.2 – 18.9)	Fall: 6.67 (2.2 – 20.6)
Bottom	Winter: 2.28 (6.7 – 13.3)	Winter: 6.39 (-4.4 – 12.2)	Winter: -1.78 (6.7 – 7.2)
	Spring: 16.83 (13.9 – 26.1)	Spring: 12.94 (10.0 – 15.6)	Spring: 13.72 (10.0 – 18.9)
	Summer: 26.72 (22.2 – 30.6)	Summer: 23.83 (20.0 – 28.9)	Summer: 24.83 (19.4 – 28.9)
	Fall: 12.94 (2.2 – 23.3)	Fall: 11.33 (3.3 – 20.0)	Fall: 11.89 (2.2 – 20.0)

<u>b)</u>

	Front	Middle	Back
Top	Winter: 80.4 (66 – 95)	Winter: 79.3 (71 – 93)	Winter: 77.4 (59 – 93)
	Spring: 76.1 (59 – 92)	Spring: 73.6 (57 – 88)	Spring: 75.4 (57 – 92)
	Summer: 80.7 (57 – 97)	Summer: 82.3 (59 – 96)	Summer: 75.6 (59 – 90)
	Fall: 93.4 (87 – 102)	Fall: 65.2 (41 – 80)	Fall: 62.3 (38 – 77)
Bottom	Winter: 80.03 (60 – 98)	Winter: 73.9 (60 – 93)	Winter: 79.9 (58 – 92)
	Spring: 83.5 (74 – 88)	Spring: 73.5 (57 – 87)	Spring: 70.0 (53 – 89)
	Summer: 96.5 (69 – 104)	Summer: 81.3 (60 – 96)	Summer: 80.2 (61 – 98)
	Fall: 66.9 (38 – 91)	Fall: 61.3 (34 – 73)	Fall: 99.6 (46 – 83)

Table 5. Account of pig well-being during trailer unloading for the main effects of season, transport duration, trailer deck, and deck \times season.

Main effect	Slow to Exit	Dead on Arrival
Season		
Winter	7	2
Spring	3	2
Summer	7	1
Fall	3	3
Transport Duration		
Long haul (6 hours)	9	6
Short haul (3 hours)	11	2
Trailer deck		
Bottom	12	5
Тор	8	3
Deck × Season		
Winter – Bottom deck	4	1
Winter – Top deck	3	1
Spring – Bottom deck	1	1
Spring – Top deck	2	1
Summer – Bottom deck	6	0
Summer – Top deck	1	1
Fall – Bottom deck	1	3
Fall – Top deck	2	0

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Table 6. Least squares means (standard error) for main effects of season, top or bottom deck, long (6h) or short (3h) haul duration and short (3h) or long (6h) lairage.

		Seaso	on (S)		Deck		Hau	l (H)	Lairage (L)			
	Winter	Spring	Summer	Fall	Bottom	Top	Long	Short	Long	Short	Signif	Intrxn ¹
Cortisol, ng/ml	86.35 ^a (2.24)	89.05 ^a (2.62)	101.24 ^b (1.84)	119.69° (2.59)	98.13 (1.48)	100.03 (1.70)	103.01 (1.54)	95.16 (1.65)	98.92 (1.45)	99.24 (1.78)	<0.001 0.380 <0.001 0.888	S×L H×L S×D×H
pН	5.64 ^a (0.007)	5.69 ^{bc} (0.009)	5.67 ^b (0.009)	5.71° (0.009)	5.68 (0.005)	5.67 (0.006)	5.68 (0.005)	5.67 (0.006)	5.66 (0.006)	5.69 (0.006)	<0.001 0.491 0.315 0.002	D×H S×L
Drip, %	3.02° (0.09)	3.18° (0.08)	1.84 ^a (0.08)	2.56 ^b (0.11)	2.59 (0.06)	2.71 (0.07)	2.67 (0.06)	2.63 (0.07)	2.66 (0.06)	2.64 (0.06)	<0.001 0.163 0.596 0.859	S×D S×H
L*	53.38 ^d (0.12)	51.79 ^a (0.11)	52.34 ^b (0.11)	52.85° (0.15)	52.56 (0.08)	52.63 (0.09)	52.50 (0.08)	52.68 (0.09)	52.71 (0.08)	52.46 (0.09)	<0.001 0.579 0.104 0.037	S×H
Saturation	17.88 ^b (0.04)	17.30 ^a (0.04)	17.88 ^b (0.04)	17.91 ^b (0.05)	17.76 (0.03)	17.73 (0.03)	17.74 (0.03)	17.75 (0.03)	17.64 (0.03)	17.85 (0.03)	<0.001 0.511 0.781 <0.001	S×L
Hue Angle	22.66 ^a (0.12)	19.57 ^b (0.11)	20.12° (0.11)	20.95 ^d (0.15)	20.81 (0.08)	20.84 (0.09)	20.70 (0.08)	20.95 (0.09)	20.66 (0.08)	20.99 (0.09)	<0.001 0.796 0.036 0.004	S×H S×L
PSE, %	5.26 (0.87)	4.26 (0.78)	3.21 (0.78)	3.78 (1.04)	4.07 (0.57)	4.18 (0.65)	3.81 (0.58)	4.45 (0.64)	4.53 (0.89)	3.72 (0.62)	0.364 0.896 0.456 0.3288	S×D D×L

Significant (P < 0.05) interaction terms

Table 7. Simple correlation coefficients, level of significance, and number of observations (n) for serum concentration of cortisol and all fresh pork quality variables.

	pН	%Drip	L*	a*	b*	Saturation	Hue angle
Cortisol	0.1641	-0.0293	-0.0310	0.1294	0.0684	0.1306	0.0197
	< 0.001	0.186	0.159	< 0.001	0.002	< 0.001	0.370
	1994	2031	2063	2065	2067	2064	2064
pН		-0.2867	-0.4872	-0.0389	-0.3365	-0.1417	-0.3497
		< 0.001	< 0.001	0.073	< 0.001	< 0.001	< 0.001
		2080	2115	2117	2119	2116	2116
%Drip			0.3982	0.0713	0.2833	0.1532	0.2838
			< 0.001	0.001	< 0.001	< 0.001	< 0.001
			2333	2335	2337	2334	2334
L*				-0.1484	0.6167	0.0822	0.7213
				< 0.001	< 0.001	< 0.001	< 0.001
				2429	2430	2428	2428
a*					0.3560	0.9482	0.0021
					< 0.001	< 0.001	0.917
					2432	2432	2432
b*						0.6329	0.9328
						< 0.001	< 0.001
						2432	2432
Saturation							0.3163
							< 0.001
							2432

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