12-2018

Study of Three Potential Variables that May Impact the Maximum Shear Force and Shear Variation During the Growth and Transport of Mixed Sex Broilers Grown in Commercial Poultry Houses

Mark Christie
University of Arkansas, Fayetteville

Study of Three Potential Variables that May Impact the Maximum Shear Force and Shear Variation During the Growth and Transport of Mixed Sex Broilers Grown in Commercial Poultry Houses

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Poultry Science

by

Mark A Christie
University of Arkansas
Bachelor of Science in Agricultural Engineering, 1985
University of Arkansas
Bachelor of Science in Mechanical Engineering, 1988
University of Arkansas
Master of Science in Biological and Agricultural Engineering, 2000

December 2018
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council

_____
John Marcy, PhD
Dissertation Director

_____
Craig Bacon, PhD
Committee Member

_____
Casey M. Owens-Hanning, PhD
Committee Member

_____
Jason K. Apple, PhD
Committee Member

_____
Navam S. Hettiarachchy, PhD
Committee Member

ABSTRACT

Temperature conditions during the transportation to the processing facility of commercial broilers have a direct impact on the meat texture, specifically maximum shear force and shear variation of cooked breast meat. Analysis of a calendar year of flock data (N=346), reveals that the largest impact of transport conditions, specifically temperature, is on the standard deviation of the flocks range of cooked shear values. The optimal transport temperature that yields broiler breast meat with average flock minimum shear and standard deviation values is approximately 60°F. The shear values for flocks at or near 60°F are on average 1 kg less than during temperatures less than 40°F. The location of the test was not subject to an extreme number of high temperature days preventing reporting the impact of high transport temperatures on meat texture. Chemical analysis of the raw *Pectoralis Major* (PM) muscle revealed no significant correlation between key minerals and the maximum shear force of the cooked PM muscle. There was a weak correlation of 8% between calcium and maximum shear force. The analysis was intended to lead further tests around intentional water chemistry modifications that would have a positive impact on meat texture. Based on the lack of correlation between minerals found in the raw tissue and cooked meat texture no further actions were pursued.

Cortisone accumulations in the flight feathers are a proven indication of the stress history of the bird. Ultra high performance gas liquid chromatography (UHPGLC) detectible levels of cortisone in the flight feathers were found to range from 2 to 8 ppb in a limited number of samples. Norepinephrine levels ranged from 220 ppb to 1872 ppb. The highest levels of norepinephrine were found in the section of the broiler house at the service entry point. This section of the house also had the lowest sample shear and shear variation of the four sections samples. This may indicate a potential relationship between chronic stresses and improved cooked meat texture.

ACKNOWLEDGEMENTS

My sincere appreciation to the individuals at Tyson Foods, Inc. that assisted in data collection and sample processing leading to the information presented in this document.

DEDICATION

This dissertation is dedicated to:

Dr. Joel Walker whose guidance and direction during my Master's program led me to have the desire to pursue a Doctorate, Dr. Navam S. Hettiarachchy who taught me the desire to learn beyond a grade, and to Dr. John Marcy, whose guidance through this doctorial process taught me that even the most finite aspects of science are important, and always demonstrating that he had my best interest at heart.

TABLE OF CONTENTS

INTRODUCTION

This paper will cover three potentially controllable aspects of broiler grow-out practices that may have a quality impact on the breast fillet from the commercial broiler. These topics are temperature and weather conditions the birds are exposed to during the transport phase of the production cycle, the chemical composition of the raw breast fillet, and finally, the potential impact of life-cycle stress (chronic stress) that a flock of birds are exposed to in a modern, well managed house.

The quality impact discussed in this document is the maximum shear force of the cooked fillet as measured in kilograms. The broilers used for tests throughout this research were Cobb 500, a fast growing, high-yielding meat bird. In 2018, it is projected that 41.8 billion pounds of ready to cook poultry will be produced in the United States. Based on the National Chicken Council, this is almost a 2% increase from the previous year's projections [1]. Tenderness of the product is a key quality metric that could influence the consumer's choice of brand and cut of meat. There are many factors that can influence the quality of the meat in commercial broilers, stress, transport conditions are the primary items under investigation in this document, but it is generally accepted that sex and breed are not significant factors in tenderness of commercial product [2].

Toughness tends to increase with the birds age in birds over 60 days of age [3]. Shear values of the fillet have been reported to increase with the age of the bird. In controlled studies, five week old birds were reported to have a lower average shear force in relation to the same breed at seven weeks of age [2]. The shear values of the older birds are still within an acceptable level for most consumer's needs. Shear value range levels and classification of cooked breast meat and consumer acceptance vary based on the study. Poole et al. (1999) stated that poultry products with shear values of 8 kg is acceptable for consumer acceptance [2].

The specific diet for the birds tested was primarily corn and soy based, the exact diet is a proprietary blend and was unavailable for publication. It is not felt that the diet would significantly influence the outcome of the shear testing values since all of the birds under testing were from the same corporation and feed formulations are relatively consistent, however, variations in the primary components in broiler diets can have an impact on shear values [4]. There have been many singular tests conducted regarding the effect of transport temperatures on average shear values of the cooked fillet; there has not been documented tests that involved data from multiple flocks throughout a calendar year. Looking at the average shear values as well as the standard deviation of cooked fillets from these flocks provides an interesting picture of the impact of weather, temperature and shift on the measured tenderness of the modern broiler. Data used in this research on average shear corresponds to most published studies, however the impact on the standard deviation of the flocks measured throughout a year provides another view into how flocks respond to acute stress caused by changes in environment. During certain environmental conditions the shear standard deviation values of flocks consistently almost double in value. This increase in such a critical quality variable of the raw material entering a manufacturing facility is a significant challenge to manage. Management of tenderness and tenderness variability can only be accomplished through traditional aging of the meat, marinade enhancements or electrical stimulation of the pre-rigor carcass.

With similar feed formulations across the tested birds, investigating if there were a relationship between the mineral component of the raw muscle and the corresponding shear values of the cooked fillet would be informative. Considering the feed process as nearly constant, the only remaining input that would be a variable is the water supply for the growing birds. If any relationships were found between raw fillet mineral concentrations and associated cooked shear

values the following step would be water sampling and trials designed to safely manipulate water chemistry to the house so not to have a negative impact on the flock's welfare, but to increase consistency in cooked fillet shear values. Ultimately, we didn't find any significant relationships between fillet composition and the relative cooked fillet shear at the level of granularly the mineral analysis provided.

Initial findings with the impact of transport environmental conditions on flock shear consistency led to further investigation on life cycle stress and the respective impact on meat tenderness. The catch and transport process leads to acute stress in the flocks; we were interested in if there were any implications of chronic stress during the active growing cycle and if it was potentially location specific by house zone. The scope and cost of this test afforded us the opportunity to test a single house to determine if there was a potential relationship between chronic stress and cooked fillet shear values as caused by, but not limited to air quality, air temperature, and human contact. To determine this I extracted specific hormones from the flight feathers collected from the post-kill carcasses. Previous work from Bortolotti et al. (2008) [5] led me to a basic extraction process that I ultimately had to modify for successful hormone extraction with broiler feathers. Not knowing what hormones we would find in broiler feathers, I elected to test for a variety of hormones commonly found in the pathway towards cortisol, a recognized indication of stress [6]. I also looked for norepinephrine as an indication of the sympatric nervous system activating. There were few findings of the traditional stress hormone cortisol in the flight feathers, however, I was successful in capturing a significant number of samples with detectable levels of norepinephrine.

When taking into consideration all of the conditions that have a measurable impact on how meat from a flock will perform from a tenderness perspective, it is apparent that there are some

potential management practices that can reduce the average shear for a flock as well as the shear standard deviation.

The literature review will cover a variety aspects of live production that have been shown to either impact or not impact final meat quality as defined as cooked fillet tenderness.

LITERATURE REVIEW

Tenderness and Age

Broiler populations in modern poultry houses grow to different ages to accommodate specific market requirements and typically represent a normal distribution of sexes and in most production cases, a limited variety of breeds. The impact of age on shear values of spent hens is directly associated to collagen development and collagen cross-linking [7]. Collagen development on young broilers (age < 60 days) is not a factor in tenderness levels [3], because the collagen associated with age has not had enough time to develop to the extent resulting in a perceived negative impact on meat texture. Shear values tend to increase as the bird's age; shear values on birds 5 weeks of age were reported at an average shear force value of 2.73 kg compared to birds of similar genetics and grow-out conditions reported shear values of 4.63 kg at 7 weeks of age [2].

Tenderness and Sex

Small differences in fillet shear values between males and females in early postmortem fillet shear values were detected with the shear variation due to sex on the PM muscle aged from 0 to 2 h post chill showed male birds tested on a Warner-Bratzler Shear Force (WBSF) device was measured at 29.5 N maximum shear having a slightly lower shear value than the PM sample from a similarly aged female counterpart of 31.7 N. The shear variations between sexes at 4 to 24 h post chill aging were no longer significantly different [8]. Abdullah, et al. (2010) [8], reported that a slight difference in shear values between sexes existed in broiler Pectoralis Major muscles immediately following chilling the carcass and up to 2 hours.

This relationship between PM shear values and sex on 56 day old broilers was also found where male broilers had an average shear of 10.2 kg. /gm and females with an average shear of 10.7 kg. /gm demonstrating a interaction between broiler sex and tenderness ($P < 0.05$) [9]. Handling and

the cooking processes will have a significant impact on the shear force of the PM muscle. The female birds had higher shear values but only approached statistical significance; the sex of the broiler had no significant difference on shear [10]. Sex was shown not to play a role in meat color during tests associated with varying growing temperatures [11].

Tenderness and Breed

The breed of 12 strains of 56 day old broilers was reported to be insignificant with regards to shear values [9]. Shear values between two unnamed broiler strains slaughtered at 42 days were also found to have no significant difference [12]. From the literature, we conclude there is no significant relationship of shear values from young birds (age < 52 days) regardless of breed. Lipid content in the breast meat of broilers was found to be dependent on the genetic cross where the protein levels show little difference [13] , indicating fat levels in broiler fillets do not have an impact on tenderness. The broilers used in testing throughout the course of this research have been of similar stock therefore, typical bird variations in flocks should not be considered a significant influence on the results of the described experimental data as the birds were from similar stock.

Temperature Stress Conditioning

Broiler thermal tolerance to cold temperatures can be altered by an early exposure to cold temperatures which reduces the associated stress response, as measured by the difference in blood cortisone levels between an initial exposure to cold and a subsequent exposure and indicates the animal's ability to manage cold stress can increase [14]. Repeated broiler exposure to stress may condition the broiler so that subsequent exposures to human contact [15] and temperature extremes minimize the animal's internal chemical response to that specific stress. This may indicate that a broiler house that is intensely managed by the farmer may reduce the

natural stress response of the broiler when exposed to other humans. Desensitizing the broilers to human contact may lead to lower stress hormones released during normal animal husbandry practices. Broilers are sensitive to visual contact with humans; consistent slow and deliberate movements reduce the fear and subsequent stress that would arise from quick erratic human movement [15].

Broiler Migration

In a study to assess broiler migration in commercial houses, some broilers were seen to migrate throughout the house both singularly and in groups [16]. The migration was described as random movements in both a straight line and a zig-zag manner. Not all of the marked birds moved in the same patterns or the same distance or direction. It was described that the movement was either random or deliberate with intentional movements postulated to be around environmental comfort. Birds moving through dense accumulations of the flock showed disinterest in the surrounding birds; the stationary population also showed disinterest in the nomads [16]. Barriers placed in commercial poultry to assist in controlling zone densities will assist in maintaining uniform social groupings but will at times not hinder the animal with intentional travel plans.

Air Quality

Air quality at the bird level as defined as head height in modern poultry houses degrades with time. Concentrations of CO_2 and NH_3 and have been described mathematically. For Carbon Dioxide, $Y= 340 – 40.7x + 5.59x^2 – 0.0683x^3$ where Y is the CO_2 production levels in L/h. 1000 birds, and where x is bird age in days [17]. Increases of carbon dioxide levels are from the respiratory function of the birds; house heaters are typically not used on older broilers as the house is heated by the broilers. Only in extreme cold is supplemental heat used and is a minor

contributor to carbon dioxide levels in older broilers where respiration is the main contributor to CO_2 levels [18].

Dust particles in a broiler house vary in size and volume depending on the age of the bird, litter conditions and ventilation practices. In tunnel houses, dust emission levels increase linearly with the age of the bird [19]. Invariably, litter becomes a primary component in broiler bedding and provides the foundational materials for dust and ammonia production by bacterial decomposition [20]. Smaller dust particles (< 5 µM) are more prevalent than larger particles [17]. Dust in combination with fungi, pathogens, and high ammonia levels can be responsible for lesions in the respiratory tract and inflammation of the lungs [21].

Ammonia levels were defined as $Y = 0.81\ e^{0.78x}$ in µL/(h.m^2. bird). Y is Ammonia production and x is bird age in days [17]. Modern broiler houses are equipped with automatic ventilation and heating controls that when managed correctly prevent concentrations of carbon dioxide and ammonia levels from reaching harmful levels while maintain the appropriate comfort level for the broiler. It is possible that in order to save energy during cold periods, farmers will alter ventilation programs causing a decrease in air quality; [21] this is a self-limiting practice as the farmer is forced to breathe the air during daily house inspections. Routine farm visits from field production technicians can help insure against excessive overriding ventilation systems for the sake of excessive energy costs during winter months. Ammonia levels between 0 and 25 ppm are considered normal, levels between 25 and 50 ppm begin to have a negative impact feed efficiency, and birds exposed to levels at 100 ppm had lower weight gains compared to a control population [21].

Al Homidan et al. (2003) [21], described research that relative humidity in broiler houses are directly associated with increased ammonia production from litter beds having a pH > 8. Proper

ventilation programs that keep the litter dry was also described as critical in managing the relative humidity of the broilers environment. Normal broiler respiration is a primary contributor to introducing moisture into the air [22].

High ambient temperatures during grow out have been reported to lower feed digestibility of broilers. This lowered feed conversion efficiency may be the result of an increased consumption of water during higher grow out temperatures [23]. Lower feed intake at high ambient temperatures results in a significant increase in blood glucose levels; conversely lower ambient temperatures result in significantly lower blood glucose levels [24]. These variations of glucose levels may be one of the contributing factors in variation of tenderness seen in accordance with seasonal temperatures. Higher blood glucose levels and lower breast meat glycogen levels and heat stress had no impact on PM tenderness due to collagen levels on birds reared under heat stress compared to control birds was reported by Aksit et al. (2006) [11]. This could indicate that variation in tenderness as seen in modern processing facilities is due in part to reasons other than collagen levels. Lower feed intake during high ambient temperatures leading to a decrease in weight gain in multiple breeds has also been reported [25]. Shear values of the PM muscle were not found to have a significant difference on broilers reared in open sided or closed sided houses during hot or cooler months [26].

Diets Impact on Tenderness

Dry Distillers grains (DDGS) are more available due to increased production of ethanol. A diet of 8% DDGS with soy and corn compared to a soy corn control diet showed no impact on the tenderness of broiler breast meat [27]. Breast meat from wheat fed broilers was significantly tougher than broilers fed a corn diet. Similar birds fed milo were no different than either birds

fed wheat [4]. Even though the shear values of the fillets were staticically different it was reported that there would be little practical differnce between the diets.

Supplements of various stains of *Saccharomyces cerevisiae* added to a corn soy based diet has been shown to improve tenderness of raw poultry meat. This decrease in toughness was most likely attributed to the prevention of glycogen depletion [28]. Ascorbic acid levels administered at four levels (0, 100, 200, 300 ppm) in the drinking water of during hot and cold seasons had no significant impact on meat quality [28]. Modifications to broiler diets are known to have physiologic effects in the resulting meat leading to the conclusion that there may be a potential relationship between the composition of feed and water regarding tenderness. It is known that zinc, manganese, and copper have an impact on bird health and growth rates [29]. Diets low in available zinc are associated with low growth rates when compared to birds fed diets not deficient in this mineral. Broilers fed diets with excessive zinc were shown to store the mineral in the tibia, but not in the liver. Conversely birds grown with a diet deficient in zinc had higher levels of this mineral in the liver than birds grown with a diet high in zinc, suggesting that the animal will regulate the availability of zinc to address vital bodily functions ahead of growth rate [30]. Increased levels of organically complexed copper, iron, manganese in broiler tissue was also found in broilers with diets deficient in this mineral when compared to birds grown with an available abundance of these minerals in their diets [30].

Tested feed supplements not found in commercial feed stocks such as medicinal herbs such as St. John's wort, small-flowered willow herb, sage and other herbs were found to have varying effects on mineral accumulations in the liver and meat tissues of broilers demonstrating that supplemental broiler diet ingredients can serve as a means for bioaccumulation of minerals in the protein structure of broilers [31].

Dietary supplements of vitamin E (α-tocopheryl acetate) when fed to broilers at varying levels throughout the growth cycle was found to have a positive impact on reducing lipid oxidation as indicated by measurements of thiobarbituric acid values. (TBA) Feeding high levels of α-tocopheryl acetate were correlated with increased levels of α-tocopheryl acetate in tissue samples [32].

Arginine and methionine additions to broiler diets increased the soluble collagen content without increasing the total collagen content in test broilers. This change in collagen ratios resulted in lower shear values that were attributed to a reduction in cross bridge development in the collagen structure [33].

Water Supply

Water treatments can have an impact on meat quality; broilers treated with a 0.5% Sodium Bicarbonate during periods of cyclical heat stress produced breast fillets with lower shear values than water treated with a 0.5% Potassium Chloride, a combination of 0.5% KCl and 0.5% $NaHCO_3$ water treatment or water receiving no treatment [34].

Improved weight gains and lower rectal temperatures were reported in birds reared in a hot environment with drinking water supplemented at a 0.6% KCl level. It was assumed that weight gain was associated to reduce panting sending the energy that would be used for body temperature maintenance towards growth [35].

Broilers are approximately 70% water by composition; this water is found intracellular, extracellular and in the broilers plasma. Water is introduced to the bird through consumption, and moisture from the feed. Moisture retention occurs through the reabsorption in the kidneys and the rectum. Moisture loss is through respiration, feces and urine [36]. Three causes of water balance in the broiler during periods of high temperatures was hypothesized as: 1) dry

oropharyngeal receptors, 2) systemic dehydration, and 3) changes in the temperature of the hypothalamus [37].

Drinking water temperature impacts feed consumption; warm water in the cooler months and cool water in the warmer months help increase feed consumption. Drinking water temperatures assist the bird in maintaining uniform body temperatures during periods of temperature extremes [36].

Feed / Water Withdrawal

Feed withdrawal practices allow the feed in the crop to be digested and mostly passed from the bird to reduce fecal contamination during evisceration. Typically, birds are removed from feed for a total of 8 hours prior to processing; water is withdrawn just prior to the catching process. This is not to say that all of the birds in the flock have exactly been off feed for 8 hours. Broilers eat on 4 hour cycles allowing for fewer feeders [3]. Modern houses are designed so that a percentage of the birds can feed at a single time. Within the flock it is expected that a portion of the birds will be off feed for approximately 12 hours prior to catch, another set of birds will be off feed for 8 hours with the balance of the flock somewhere in between. This variability of available feed stores in the bird's gastrointestinal tract translates to varying levels of glycogen stores in the liver. Feed withdrawal for 8 hours has been shown to cause glycogen depletion in the liver and the breast muscle within three hours [38]. Glycogen is the base molecule that fuels Adenosine Triphosphate (ATP) synthesis during the broilers life; up to 36 units of ATP are generated by one molecule of glycogen through each pass of the Krebs or citric acid cycle [39]. The Krebs cycle is halted once the bird is exsanguinated and oxygen is no longer available at the cellular level. Post mortem synthesis of ATP is through the inefficient process of glycolysis that drives increasing levels of lactic acid in the muscle that eventually halts the process after

approximately 4 hours, shutting down the synthesis of ATP [38]. The available energy levels in ante mortem broilers have a direct impact on the time of onset of rigor mortis [40].

Time off water (0 – 18h) has been reported not to have a significance influence on meat quality [41]. Mielnik et al. (1991) on the other hand found that meat tenderness decreased on extended periods (12 to 18 h) of feed and water withdrawal [42]. This could be interpreted that time off feed has increased significance in meat tenderness when compared to time off water.

Capture & Crating Stress

Cortisone levels in birds crated longer than 4 hours were lower than their cortisone levels prior to crating; peak cortisone levels occurred at approximately 3 hours of confinement [43]. Studies have demonstrated that there was no correlation between crate time and PM shear values [43]. Cortisone levels in the blood are the chemical indication of acute stress and continue to increase during transport and slaughter while glucose levels remained constant and are probably due to feed withdrawal and the higher levels of cortisone stimulating glycogenolysis and glyconeogenesis [44].

Crating alone does not appear to cause significant stress in broilers; however transportation caused a lower pH in the thigh muscle indicating an increased rate of glycolysis compared to birds that were not transported [43]. The density of the crates also influences the stress levels which in turn will influence glucose concentrations in the muscles. Broilers experience higher levels of heat stress during the warmer months when crated at a high density [45]. Crating densities by season are programs that are managed to eliminate transport death and yield loss in the transported flock. Transport times between 30 minutes and 4 hours were shown to have no impact on meat quality [43].

Transport and Temperature

Transport air conditions of temperature and humidity values change by season and by geographical region. The heat stress on the transported flocks is well documented specifically by Kannan et al. (1997) [43] and Delezie et al. (2007) [45]. The pH of the PM muscle is lower as indicated by measurements and an associated reduction in water holding capacity (WHC). Pre-slaughter heat stress is also reported to increase the rate and effect of rigor mortis but can also increase the incidence of pale, soft and exudative (PSE) meat [46]. Transportation during cold conditions cause a decrease in glucose levels in the PM muscle that is attributed to the birds natural response of regulating and maintaining body temperature [46]. A decrease in tenderness on birds held at higher temperatures was reported by several researchers [47]. However, other research indicates that extremes in temperature, both cold stress and heat stress caused an increase in toughness [48]. In all of these cases, electrical stimulation was not used as a part of the experimentation. Thermally stressed birds, both heat and cold stressed, enter the manufacturing process with depleted energy reserves [49]; electrical stimulation (ES), when managed correctly, works to deplete or minimize the remaining ATP in the muscle and shorten the delay phase of rigor.

Seasonality also has an impact on the tenderness of the meat; overall autumn months were reported to have the most tender meat [49]. Kadim, (2009) et al. [26] also showed that hot-season birds were slightly more tender as compared to cold-season birds; attributing this difference to glycogen levels and minimal rigor shortening.

Electrical Stunning

Electrical stunning on broilers, if done properly, electrically anesthetizes the animal allowing for a humane slaughter process. The practice of electrical stunning prior to exsanguination promotes

a calm death experience, minimizing involuntary wing movement and reducing bruising and breakage [50]. Electrical stunning was reported to decrease tenderness on the PM muscle from broilers sampled immediately after the chiller [51]. Conversely, as the aging time increased (2 hr. post chill) broilers that were processed with electrical stunning demonstrated a significant increase the tenderness of the PM muscle [51]. The variation in tenderness decreased in aged birds that were electrically stunned as opposed to birds where electrical stunning was not a part of the process [51]. Reports that stunning had no impact on tenderness on the *Pectoralis* muscle of turkeys are in conflict with previously mentioned research [52].

Lee et al. (1979) [51], also reported that birds processed using an electrical stunner had higher levels of ATP as compared to birds processed without the use of a stunner. Birds slaughtered without anesthetization, exhibit excessive wing flap until death overtakes the animal; this wing flap most likely causes ATP depletion in the PM muscle during the bleeding period [53]. Process control throughout in the receiving, kill and pick area may have an impact on the final meat quality, in the literature reviewed, regarding the influence of stunning on tenderness, there was no mention of stunner water temperature, the presence of salt in the stunner, shackle construction and the associated grounding efficiencies or if the stunner was completely loaded with birds during the testing; all of which will have an impact on the interaction between the stunner and the broiler. The efficiency of the stunner with regards to recovery time for the bird to regain consciousness was also not discussed. Recovery times are an equalizing factor in setting up the kill process leading to consistency in stunning multiple flocks with varying bird weights. Birds killed in the stunner will add an inconsistency in the process that may influence final meat quality.

Severing the spinal cord by either decapitation or too deep a cut by a kill machine specifically designed to cut the carotid and jugular veins will increase the likelihood of significant post kill bird movement [50], specifically wing flap which has an impact on the level of ATP depletion and the subsequent rate of rigor [51].

Electrical Stimulation (ES)

The effect of a post mortem electrical stimulation system has the opposite impact on tenderness as an electrical stunning device. There are three phases in the process of rigor mortis, the delay phase, the rigor phase, and lastly the resolution phase [54]. ES systems compress the delay phase (4-6 hr.) by electrically stimulating the breast muscles depleting the ATP reserves within the muscle structure. Commercial ES systems excite muscle contraction with either a cyclical or continuous current, depending on manufactured and are located after the stun and kill process. The carcass is stimulated during the bleeding process or after the bleeding process is completed; location of the ES system is dependent on the manufacturer. Excessive voltages can mechanically tenderize the muscle by creating tears throughout the muscle [55], however, these tears can lead to excessive yield losses in a deboning operation by leaving meat on the skeleton that can no longer be sold with the fillet.

Depletion of glycogen reserves in the PM muscle can be caused by thermal stress, catching at the farm, crating, transportation and other normal handling processes generating excitement in the bird [56]. As glycogen reserves are depleted and pre-mortem glycolysis declines, there is a buildup of lactic acid causing an increase in pH that is ultimately associated with tougher meat [40]. Previous research also reported that the pH was not correlated to shear forces in the PM muscle but that there was a correlation between low glycogen levels and high shear forces [57]. Lactic acid injected into the *Pectoralis profundus* muscles from culled cows caused an increase

in tenderness by weakening the perimysial connective tissue and increasing the activities of proteases [58]. The beef samples tested were injected with a 10% w/w 0.5M solution of lactic acid at 3 depths and vacuum packed. The samples were stored for 28 and 48 hours allowing time for the lactic acid to influence the textural properties of the beef muscle. The final pH of the 24 hour test beef was 4.9 as compared to the control sample pH of 5.5 [58]. Lee et al. (1979) [51], reported a 24 hour pH of broiler PM to be between 5.58 and 5.51 leading to the conclusion that lactic concentrations in the broiler muscle may not be high enough to have a significant impact on the tenderness of broiler breast meat. Rate of glycogen depletion in post mortem animals has more influence on meat quality than the total amount of glycogen available [59].

Life Cycle Stress

Broilers may be stressed throughout their growth cycle by many paths. Social stressors that are created from within the flock; thermal stress generated from poor animal husbandry practices; dietary stress from feeder failures to the feed storage bin running out of feed; physiological stresses from rapid growth, reaching sexual maturity, catching and crating stress and thermal stress created during transport [60].

When stress reaches a relative extreme, it becomes distress and the sympatric nervous system reacts. The hypothalamus-pituitary-adrenal axis reacts to stress by ultimately working to stimulate additional glucocorticoid steroids into the blood stream [5]. This chemical release increases the blood glucose levels through protein and fat metabolism providing available energy for the reactive animal [60].

Glucocorticoid steroids in the blood are stored as corticosterone in the feathers during keratinization. The levels of corticosterone in the feathers are an indication of relative stress the bird has seen during the growth of the feathers [5]. The stress responses of birds can be

conditioned during the life of the animal in a manner that subsequent stress events may not generate the same level of hypothalamus pituitary adrenal axis intensity as the previous event generated [5].

The oldest feathers on a bird are located at the end of the wings and are reference to as the flight feathers; these feathers will hold a cumulative history of the birds live cycle stress. Feathers that have fault bars or visually deformed by varying shades of colors are found to have high values or corticosterone in the deformity [61].

Extraction of corticosterone is accomplished through bathing ground feathers, less the calamus, in methanol with the sample tube placed in a warm agitated bath. Consistency in feather grinding is critical in achieving valid results [5].

There are two concepts in corticosterone level reporting. The first concept is to measure the length of the feathers sampled and report the corticosterone level on a unit length basis. Reporting the corticosterone levels on a unit length basis is thought to be the preferred method of corticosterone level reporting due to potential heavy keratinization of the feather that could dilute the reported corticosterone level [5]. The second is to weigh the sample and report on a unit weight. The latter is based on the concept that as feathers grow at different rates, reporting on a unit weight basis will provide a comparable data set when looking for a relationship with another variable [61]. The method used for this discussion was weighing the samples; the author feels this method allows for increased precision and accuracy in reporting the extracted hormone levels.

Validation work that acute stress due to controlled handling of the birds and ACTH pumps forcing elevated cortisol levels in the blood plasma eventually metabolized in the feathers as corticosterone was conducted by plucking flight feathers and allowing regrowth during periods

of controlled stress and comparing the levels of corticosterone before and after the stimuli [5]. Corticosterone is a stable compound so that feathers kept in a cool dry and covered can be tested years after sampling [5].

Meat Sampling and Sample Processing

Broiler PM muscle samples are generally taken from the left or right hand side of the animal. Whiting et al. (1991) [34] referenced data that the shear values in roasted turkey between sides were not significantly different. When sampling the PM muscle from any location, the samples were either all left hand or right hand fillets. Sampling a single side eliminates the risk of same bird sampling. Fillet samples collected from birds that had a wing removed prior to the chilling process and prior to rigor reaching completion showed the fillet associated to the wingless side on average had maximum shear values approximately 0.75 kg greater than the fillet of the same bird with a wing attached (M.A. Christie Unpublished Data). Collection of samples should be done with great care in that the shear data is not skewed by "wing off" birds.

The method of thermally processing the samples should also be consistent, Whiting et al (1991) [34], showed that boiling PM muscles resulted in significantly higher shear values when compared to oven roasting covered PM muscles from the same birds. All samples should be thermally processed using the same procedure regardless if boiled or roasted. Commercial microwaves were not found to be a good substitute for replicating the oven process where the samples are covered and cooked at 350 ^{0}F until the samples reached a temperature between 170 and 175^{0} F (M.A. Christie Unpublished Data).

Cooling samples prior to shearing is done for safety reasons; handling meat that is hot exposes the individual performing the tests to unnecessary burn risks. Hot samples will generate lower shear values when compared to sections sheared at room temperature. (M.A. Christie

Unpublished Data). During the time of sample processing, the hot meat will begin cooling and provide additional variability within the data due to moisture in the sample from the inevitable evaporation when the hot surface is exposed to the cooler surroundings (M.A. Christie Unpublished Data). Sample cooling can either be from natural convection or through conduction in a chilled water bath. The chilled water bath will generate samples higher in moisture and lower in maximum shear force (M.A. Christie Unpublished Data). Consistency in sample collection and processing is vital in understanding the relative toughness and variation of the sample set. Samples that vary in thickness will not cook at the same rate leading to overcooking or the thinner portions. These overcooked portions will shear at higher values (M.A. Christie Unpublished Data).

BIBLIOGRAPHY

1. Council, N. C. 2018. U.S. Broiler Production. Accessed 2/12/18 2018.

2. Poole, G. H., C. E. Lyon, R. J. Buhr, and L. L. Young. 1999. Evaluation of age, gender, strain, and diet on the cooked yield and shear values of broiler breast fillets. J. Applied Poultry Research 8:170-176.

3. Fletcher, D. L. 2002. Poultry meat quality. World's Poultry Science Journal 58:131-145.

4. Lyon, B. G., D. P. Smith, C. E. Lyon, and E. M. Savage. 2004. Effects of diet and feed withdrawal on the sensory descriptive and instrumental profiles of broiler breast fillets. J. Poultry Science 83:275-281.

5. Bortolotti, G. R., T. A. Marchant, J. Blas, and T. German. 2008. Coritcosterone in feathers is a long-term, integrated measure of avian stress physiology. Functional Ecology 22:494-500.

6. Bortolotti, G. R., T. Marchant, J. Blas, and S. Cabezas. 2009. Tracking stress: localisation, deposition, and stability of corticosterone in feathers. The Journal of Experimental Biology 212:1477-1482.

7. Iqbal, M., P. B. Kenney, and H. Klandorf. 1999. Age-related changes in meat tenderness and tissue pentosidine: Effect of diet restriction and aminoguanidine in broiler breeder hens. J. Poultry Science 78:1328-1333.

8. Abdullah, A. Y., and S. K. Matarneh. 2010. Broiler performance and the effects of carcass weight, broiler sex and postchill carcass aging duration on breast fillet quality characteristics. J. Applied Poultry Research 19:46-58.

9. Goodwin, T. L., L. D. Andrews, and J. E. Webb. 1969. The influence of age, sex and energy level on the tenderness of broilers. J. Poultry Science 48:548-552.

10. Lyon, C. E., and R. L. Wilson. 1986. Effects of sex, rigor condition, and heating method on yield and objective texture of broiler breast meat. J. Poultry Science 65:907-914.

11. Aksit, M., S. Yalcin, S. Ozkan, K. Metin, and D. Ozdemir. 2006. Effects of temperature during rearing and crating on stress parameters and meat quality of broilers. J. Poultry Science 85:1867-1874.

12. Lopez, K. P., M. W. Schilling, and A. Corzo. 2011. Broiler genetic strain and sex effects on meat characteristics. J. Poultry Science 90:1105-1111.

13. Lonergan, S. M., N. Deeb, C. A. Fedler, and S. J. Lamont. 2003. Breast meat quality and composition in unique chicken populations. J. Poultry Science 82:1990-1994.

14. Shinder, D., D. Luger, M. Rusal, V. Rzepakovsky, V. Bresler, and S. Yahav. 2002. Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. J. Thermal Biology 27:517-523.

15. Moberg, G. P., and J. A. Mench. 2000. The biology of animal stress: Basic principals and implications for animal welfare. 1st ed. CABI Publishing, New York, NY.

16. Preston, A., and L. B. Murphy. 1989. Movement of broiler chickens reared in commercial conditions. J. British Poultry Science 30:519-532.

17. Leonard, J. J., J. J. R. Feddes, and J. B. McQuitty. 1983. Air quality in commercial broiler housing. Canadian Agricultural Engineering 26:65-71.

18. Pedersen, S., V. Blanes-Vidal, H. Jorgensen, A. Chwalobog, A. Haeussermann, M. J. W. Heetkamp, and A. J. A. Aaenik. 2008. Carbon dioxide production in animal houses: A literature review. Agricultural Engineering International 10.

19. Lacey, R. E., R. E. Redwine, and J. Parnell, C. B. 2003. Particulate Matter and ammonia emission factors for tunnel-ventilated broiler production houses in the southern U.S. American Society of Agricultural Engineers May 2003:1203-1214.

20. Caberra, M. L., T. R. Kelly, O. C. Pancorbo, W. C. Merka, and S. A. Thompson. 1994. Ammonia volatilization and carbon dioxide emission from poultry litter. J. Communications in Soil Science and Plant Analysis 25:2341-2353.

21. Al Homidan, A., J. F. Robertson, and A. M. Petchey. 2003. Review of the effect of ammonia and dust concentrations on broiler performance. World's Poultry Science Journal 59:340-349.

22. Deaton, J. W., and F. N. Reece. 1980. Respiration in relation to poultry house ventilation. J. Poultry Science 59:2680-2685.

23. Bonnet, S., P. A. Geraert, M. Lessire, B. Carre, and S. Guillaumin. 1997. Effect of high ambient temperature on feed digestibility in broilers. J. Poultry Science 76:857-863.

24. Donkoh, A. 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol 33:259-265.

25. Cooper, M. A., and K. W. Washburn. 1998. The relationship of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. J. Poultry Science 77:237-242.

26. Kadim, I. T., B. H. A. Ai-Qamshui, O. Mahgoub, W. Ai-Marzooqi, and E. H. Johnson. 2009. The effects of ascorbic acid and seasonal temperatures on meat quality characteristics of broiler chickens maintained in open-sided and closed houses. International Journal of Poultry Science 8:733-739.

27. Corzo, A., M. W. Schilling, R. E. Loar II, V. Jackson, S. Kin, and V. Radhakrishnan. 2009. The effects of feeding distillers dried grains with solubles on broiler meat quality. J. Poultry Science 8:432-439.

28. Zhang, A. W., B. D. Lee, K. W. Lee, K. B. Song, G. H. An, and C. H. Lee. 2005. Effects of graded levels of dietary *Saccharomyces cerevisiae* on growth performance and meat quality in broiler chickens. Asian-Aust. Journal Animal Science 18:699-703.

29. Richards, J. D., J. Zhao, R. J. Harrell, C. A. Atwell, and J. J. Dibner. 2010. Trace mineral nutrition in poultry and swine. Asian-Australasian Journal of Animal Sciences 23:1527-1534.

30. Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Applied Poultry research 16:448-455.

31. Stef, D. S., and I. Gergen. 2012. Effect of mineral-enriched diet and medicinal herbs on Fe, Mn, and Cu uptake in chicken. J. Chemistry Central 6:1-9.

32. Winne, A. D., and P. Dirinck. 1996. Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. J. Agricultural Food Chemistry 44:1691-1696.

33. Jiao, P., Y. Guo, X. Yang, and F. Long. 2010. Effects of dietary arginine and methionine on broiler carcass traits and meat quality. J. of Animal and Veterinary Advances 9:1546-1551.

34. Whiting, T. S., L. D. Andrews, M. H. Adams, and L. Stamps. 1991. Effects of sodium bicarbonate and potassium chloride drinking water supplementation. 2. Meat and carcass characteristics of broilers grown under thermoneutral and cyclic heat-stress conditions. J. Poultry Science 70:60-66.

35. Ahmad, T., T. Khalid, T. Mushtag, M. A. Mirza, A. Nadeem, M. E. Barbar, and G. Ahmad. 2008. Effect of potassium chloride supplementation in drinking water on broiler performance under heat stress conditions. J. Poultry Science 87:1276-1280.

36. Leeson, S., J. D. Summers, and E. T. Moran. 1976. Avian water metabolism-A review. World's Poultry Science Journal 32:185-195.

37. Budgell, P. 1970. The effect of changes in ambient temperature on water intake and evaporative water loss. Psychonomic Science 20:275-276.

38. Savenije, B., E. Lambooij, M. A. Gerritzen, K. Venema, and J. Korft. 2002. Effects of Feed Deprivation and Transport on Preslaughter Blood Metabolites, Early Postmortem Muscle Metabolites, and Meat Quality. Poultry Science 81:699-708.

39. Kim, J., and C. V. Dang. 2006. Cancer's molecular sweet tooth and the warburg effect. Cancer research 66:8927-8930.

40. Khan, A. W., and R. Nakamura. 1970. Effects of pre- and postmortem glycolysis on poultry tenderness. J. Food Science 35:233-267.

41. Rasmussen, A. L., and M. G. Mast. 1989. Effect of feed withdrawal on composition and quality of broiler meat. J. Poultry Science 68:1109-1113.

42. Mielnik, M., and N. Kolstad. 1991. The influence of feed and water withdrawal time on post mortem biochemical changes and meat tenderness in broilers. Norwegian Journal of Agricultural Sciences 1-8:1-8.

43. Kannan, G., J. L. Heath, C. J. Wabeck, M. C. P. Souza, J. C. Howe, and J. A. Mench. 1997. Effects of crating and transport on stress and meat quality characteristics in broilers. J. Poultry Science 76:523-529.

44. Nijdam, E., E. Delezie, E. Lambooij, M. J. A. Nabuurs, E. Decuypere, and J. A. Stegeman. 2005. Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. J. Poultry Science 84:467-474.

45. Delezie, E., Q. Swennen, J. Buyse, and E. Decuypere. 2007. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. J. Poultry Science 86:1414-1423.

46. Dadgar, S., E. S. Lee, T. L. V. Leer, H. L. Burlinguette, H. L. Classen, T. G. Crowe, and P. J. Shand. 2010. Effect of microclimate temperature during transportation of broiler chickens on quality of the pectoralis major muscle. Poultry Science 89:1033-1041.

47. Petracci, M., D. L. Fletcher, and J. K. Northcutt. 2001. The effect of holding temperature on live shrink, processing yield, and breast meat quality of broiler chickens. J. Poultry Science 80:670-675.

48. Lee, Y. B., G. L. Hargus, E. C. Hagberg, and R. H. Forsythe. 1976. Effect of antemortem environmental temperatures on postmortem glycolysis and tenderness in excised broiler breast muscle. J. Food Science 41:1461-1465.

49. Ali, A. S. A., A. P. Harrison, and J. F. Jensen. 1999. Effect of some ante-mortem stressors on peri-mortem and post-mortem biochemical changes and tenderness in broiler breast muscle: a review. World's Poultry Science Journal 55:403-414.

50. McNeal, W. D., D. L. Fletcher, and R. J. Buhr. 2003. Effects of stunning and decapitation on broiler activity during bleeding, blood loss, carcass, and breast meat quality. J. Poultry Science 82:163-168.

51. Lee, Y. B., G. L. Hargus, J. E. Webb, D. A. Rickansrud, and E. C. Hagberg. 1979. Effect of electrical stunning on postmortem biochemical changes and tenderness in broiler muscle. J. Food Science 44:1121-1122.

52. Ma, R., and P. B. Addis. 1973. The association of struggle during exanguination to glycolysis, protein solubility and shear in turkey pectoralis muscle. J. Food Science 38:995-997.

53. Young, L. L., and R. J. Buhr. 1997. Effects of stunning duration on quality characteristics of early deboned chicken fillets. J. Poultry Science 76:1052-1055.

54. Li, C., P. Shi, C. Xu, X. Xu, and G. Zhou. 2010. Tracing processes of rigor mortis and subsequent resolution of chicken breast muscle using a texture analyzer. J. Food Engineering 100:388-391.

55. Sams, A. R. 1999. Meat Quality During Processing. J. Poultry Science 78:798-803.

56. Ali, M. S., G. Kang, and S. T. Joo. 2008. A review: Influences of pre-slaughter stress on poultry meat quality. Asian-Aust. J. Animal Science 21:912-916.

57. Mellor, D. B., P. A. Stringer, and G. J. Mountney. 1958. The influence of glycogen on the tenderness of broiler meat. J. Poultry Science 37:1028-1034.

58. Berge, P., P. Ertbjerg, L. M. Larsen, T. Astruc, X. Vignon, and A. J. Moller. 2001. Tenderization of beef by lactic acid injected at different times post mortem. J. Meat Science 57:347-357.

59. Hedrick, H. B. 1965. Influence of ante-mortem stress on meat palatability. J. Animal Science 24:255-263.

60. Puvadolpirod, S., and J. P. Thaxton. 2000. Model of physiological stress in chickens 3. Temporal patterns of response. J. Poultry Science 79:377-382.

61. Lattin, C. R., J. M. Reed, D. W. DesRochers, and L. M. Romero. 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J. Avian Biology 42:247-252.

Chapter 1

A One Year Study of Transport Air Temperature and the Associated Weather Conditions Impact on Cooked Broiler Pectoralis Major Maximum Shear Force and Shear Variation

ABSTRACT

The purpose of this study was to determine the impact of weather conditions on the flock's average maximum shear and the flocks associated SD throughout a sequential calendar year. Weather conditions were not limited to temperature, but weather conditions classified as sunny and rainy and the energy of the air to take into consideration RH was also investigated. Time of transport, as defined by shift, was also blocked in the analysis. The cooked fillet average shear and shear standard deviation of 346 flocks of Cobb 700 birds with an average live weight of 6.8 pounds across two shifts were examined. There with 172 flocks analyzed during early morning transport and 174 flocks analyzed during afternoon transport. Relationships between shift weather, cooked breast shear values and flock shear SD were realized. It is well documented in literature that transport temperatures impact average shear, what was discovered through this analysis was the degree of impact transport temperatures had on the flocks shear SD. Weather conditions did not show an interaction with the flocks' average shear, it did have a significant impact on the flocks' SD. It was also discovered there was a lack of interaction between the energy of the transport air with either the average flock shear but had an interaction the flocks' SD.

Transport environmental conditions of transport temperature and the energy of the transport air was also placed into bins of 4 ^{0}F and 2.4 btu for analysis against average bin shear and average bin shear standard deviation. Similar relationships between the dependent and independent variables were found.

INTRODUCTION

Consistency of textural qualities of meat in a poultry production facility allows the producer to maximize profits while meeting quality expectations of the consumer. There are several controllable factors in the manufacturing of tender breast meat that are available to the producer, the first being electrical stimulation, a process passing electrical current through a post mortem bird intending to use up latent energy stores in the muscle speeding up the process of rigor mortis, [1], the second is carcass aging, being the most desirable for consistently tender product. Seasonal transport temperature levels are an uncontrollable factor that add to the inconsistency of meat tenderness and the range of tenderness found in products produced in commercial poultry grow-out facilities. It is well documented that moderate temperatures during catch and transport yield more tender broiler breast meat than birds that were caught and transported during cold conditions [2, 3] Examination on the average flock shear differences and flock variation between and within flocks and the relationship with transport temperature, transport air energy and weather is the focus of this paper. Documenting the maximum flock shear values and the variation of the maximum shear values of cooked breast meat throughout all seasons provide insight into optimal environmental temperatures as related to tenderness of the resulting breast meat products. Understanding causes for extreme variation in a flock's breast meat tenderness could potentially provide the producer with specific management practices minimizing the probability of consumer complaints due to tough breast meat. Lyon et.al (1997) found that consumers classify cooked fillets as slightly to moderately tender with WB shear ranges from 3.62 – 6.61 Kg and slightly tender to slightly tough when the fillet sheared between 6.62 and 9.60 Kg. [4]

Temperature by itself is only part of an environmental stressor faced by the birds during transport. The relative humidity as expressed as an energy component may add an additional

descriptive component to environmental conditions when examining the relationship between the transport climate and the climates' relationship to tenderness and variation in tenderness values.

The birds under consideration throughout the test period were of a single breed. Bird genetics has been shown in previous research not to make a significant difference in breast tenderness values [5-7].

Understanding the relationship between transport environmental conditions and meat quality could provide the producer with preemptive manufacturing management strategies that could lead to higher quality poultry products.

LITERATURE REVIEW

Broiler populations in modern poultry houses grow to different ages to accommodate specific market place requirements and typically represent a normal distribution of sexes and in most production cases, a limited variety of breeds. The impact of age on shear values on spent hens is directly associated to collagen development and collagen cross linking [8]. Collagen development on young broilers (age < 60 days) is not a factor in tenderness levels. [9] Shear values tend to increase as the birds age, shear values on birds 5 weeks of age were reported at an average of 2.73kg, where birds of similar genetics and grow out conditions reported shear values of 4.63 kg at 7 weeks of age [10].

Shear variation due to sex on the PM muscle aged from 0 to 2 h post chill showed that the Pectorals Major (PM) muscle from a male bird tested on a Warner-Bratzler Shear Force (WBSF) device was measured at 29.5 N maximum shear having a slightly lower shear value than the PM sample from the female counterpart similarly aged (WBSF = 31.7 N). The shear variations between sexes at 4 to 24 h post chill aging were no longer significantly different [11]. This relationship between PM shear values and sex on 56 day old broilers were also found to be relevant where males had an average shear of 10.2 kg. /gm and females with an average shear of 10.7 kg. /gm [5].

The breed on 12 strains of 56 day old broilers was reported to be insignificant with regards to shear values [5]. Shear values between two unnamed broiler stains slaughtered at 42 days were also found to have no significant difference [6].

Broiler thermal tolerance to cold temperatures can be altered by a specific exposure to cold temperatures during the early portion of their lives by reducing the associated stress response as measured by the difference in blood cortisone levels between an initial exposure to cold and a subsequent exposure thus indicating the animal's ability to manage cold stress can increase [12].

Repeated exposure to stress may condition the broiler so that subsequent exposures to temperature extremes or human contact can minimize the animal's internal chemical response to that specific stress. This may indicate that a broiler house that is intensely managed by the farmer may reduce the natural stress response of the broiler when exposed to other humans. Desensitizing the broilers to human contact may lead to lower stress hormones released during the capture process. Broilers are sensitive to visual contact with humans; consistent slow and deliberate movements reduce the fear and subsequent stress that would arise from quick erratic human movement [13].

Air quality at the bird level in modern poultry houses degrades with time. Concentrations of CO_2 and NH_3 and have been described mathematically. For Carbon Dioxide, $Y= 340 – 40.7x + 5.59x^2 – 0.0683x^3$ where Y is the CO_2 production levels in L/h. 1000 birds, and where x is bird age in days. Increases of carbon dioxide levels are from the respiratory function of the birds; House heaters are typically not used as the house is heated by the broilers. Only in extreme cold is supplemental heat used and is a minor contributor to carbon dioxide levels. Ammonia levels were defined as $Y = 0.81\ e^{0.78x}$ in $\mu L/(h.m^2.\ bird)$. Y is Ammonia production and x is bird age in days [14]. Modern broiler house are equipped with automatic ventilation and heating controls that when managed correctly prevent concentrations of carbon dioxide and ammonia levels from reaching harmful levels while maintain the appropriate comfort level for the broiler. Routine farm visits from field production technicians help insure against overriding ventilation systems for the sake of excessive energy costs during winter months.

Feed withdrawal practices allow the feed in the crop to be digested and mostly passed from the bird to reduce fecal contamination during evisceration. Typically birds are removed from feed for a total of 8 hours prior to processing, water is withdrawn just prior to the catching process.

This is not to say that all of the birds in the flock have exactly been off feed for 8 hours. Broilers eat on 4 hour cycles allowing for fewer feeders [9]. Modern houses are designed so that only a portion of the birds can feed at a single time. Within the flock, it is expected that many of the birds will be off feed for approximately 12 hours prior to catch, with the balance of the flock somewhere in between 12 and 16 hours. This variability of available feed stores in the bird's GI tract translates to varying levels of glycogen stores in the liver. Feed withdrawal for 8 hours has been shown to cause glycogen depletion in the liver and PM muscle within three hours [15]. Glycogen usage through the Krebs cycle is the base molecule that fuels ATP synthesis during the broilers life, up to 38 units of ATP are generated by one molecule of glycogen through each pass of the cycle. The Krebs cycle is halted once the bird is exsanguinated and oxygen is no longer available at the cellular level. Post mortem synthesis of ATP is through the inefficient process of anaerobic glycolysis producing increasing levels of lactic acid in the muscle that eventually where the process halts after 4 hours shutting down the synthesis of ATP [15]. The available energy levels in ante mortem broilers have a direct impact on the time of onset of rigor mortis. High ambient temperatures have been reported to lower feed digestibility of broilers. This lowered feed conversion efficiency may be the result of an increased consumption of water during higher grow out temperatures [16]. Lower feed intake should result in lower energy stores in liver and may translate to a faster onset of rigor after exsanguination. Lower feed intake during high ambient temperatures leading to a decrease in weight gain in multiple breeds has also been reported [17]. Faster onset of rigor should translate to lower average PM shear values during periods of high ambient temperatures.

Baseline cortisone levels in birds were lower than birds crated for 3 hours with cortisone levels falling below the initial values after 4 hours of confinement [18], supporting the idea that

maximum cortisone levels on a broiler does not fully manifest itself until up to 3 hours after the initial stressor has occurred. Studies have demonstrated that there was no correlation between crate time and PM shear values [18]. Cortisone levels are the chemical indication of acute stress and continue to climb during transport and slaughter. Glucose levels remained constant and are probably due to feed withdrawal and the higher levels of cortisone stimulating glycogenolysis and glyconeogenesis [19].

Time off water (0 – 18h) has been reported not to have a significance influence on meat quality [20]. Mielnik et al. (1991) [21], on the other hand found that meat tenderness decreased on extended periods (12 to 18 h) of feed and water withdrawal [21]. This could be interpreted that time off feed has increased significance in meat tenderness when compared to time off water. Crating alone doesn't appear to cause significant stress in broilers; however transportation caused a lower pH in the thigh muscle indicating an increased rate of glycolysis compared to birds that were not transported [18]. The density of the crates also influences the stress levels which in turn will influence glucose concentrations in the muscles. Broilers experience higher levels of heat stress during the warmer months when crated at a high density [22]. Crating densities by season are programs that are managed to eliminate transport death and yield loss in the transported flock. Transport times between 30 minutes and 4 hours were shown to have no impact on meat quality [18].

Transport air conditions of temperature and humidity values change by season and by geographical region. The heat stress on the transported flocks is well documented specifically by Kannan et al. (1997), [18] and Delezie et al. (2007) [22]. The pH of the PM muscle is lower as indicated by measurements and an associated reduction in water holding capacity (WHC). Pre-slaughter heat stress is also reported to increase the rate and effect of rigor mortis but can

also increase the incidence of pale, soft and exudative (PSE) meat [23]. Transportation during cold conditions cause a decrease in glucose levels in the PM muscle that is attributed to the birds natural response of regulating and maintaining body temperature [23]. A decrease in tenderness on birds held at higher temperatures was reported by several researchers [2]. However, other research indicates that extremes in temperature, both cold stress and heat stress caused an increase in toughness [3]. In all of these cases electrical stimulation was not used as a part of the experimentation. Thermally stressed birds, both heat and cold stressed, enter the manufacturing process with depleted energy reserves, electrical stimulation (ES) when managed correctly works to deplete or minimize the remaining ATP in the muscle and compress the delay phase of rigor. The elimination or sever reduction of the delay phase of rigor mortis allows naturally occurring enzymatic reductions of the myofibrillar proteins along the H band and Z-line during the chilling and further processing phases of production.

Seasonality also has an impact on the tenderness of the meat; overall autumn months were reported to have the tenderest meat [24]. Increased oxidation of myofibular protein has been linked to increased toughness in meat. This increase in oxidized muscle fibers is linked to oxidized myoglobin. [25]

Electrical stunning on broilers, if done properly electrically anesthetizes the animal allowing for a humane slaughter process. The practice of electrical stunning prior to exsanguination promotes a calm death experience minimizing involuntary wing movement reducing bruising or breakage [26]. Electrical stunning was reported to decrease tenderness on the PM muscle from broilers sampled immediately after the chiller. Conversely, as the aging time increased (2 hr. post chill) broilers that were processed with electrical stunning demonstrated a significant increase the tenderness of the PM muscle [27]. The variation in tenderness decreased in aged birds that were

electrically stunned as opposed to birds where electrical stunning was not a part of the process [27]. Reports that stunning had no impact on tenderness on the Pectoralis muscle of turkeys are in conflict with previously mentioned research [28].

Lee et al. (1979) [27] also reported that birds processed using an electrical stunner had higher levels of ATP as compared to birds processed without the use of a stunner. Birds slaughtered without anesthetization exhibit excessive wing flap until death overtakes the animal, this wing flap most likely causes ATP depletion in the PM muscle during the bleeding period [29].

In the literature reviewed, regarding the influence of stunning on tenderness, there was no mention of stunner water, temperature, the presence of salt in the stunner, shackle construction and the associated grounding efficiencies or if the stunner was completely loaded with birds during the testing; all of which will have an impact on the interaction between the stunner and the broiler. The efficiency of the stunner with regards to recovery time for the bird to regain consciousness was also not discussed. Recovery times are an equalizing factor in setting up the kill process leading to consistency in stunning multiple flocks with varying bird weights.

Severing the spinal cord by either decapitation or too deep a cut by a kill machine specifically designed to cut the carotid and jugular veins will increase the likelihood of significant post kill bird movement [26], specifically wing flap which as discussed previously, has an impact on the level of ATP depletion and the subsequent rate of rigor [27].

The effect of a post mortem electrical stimulation system has the opposite impact on tenderness as an electrical stunning device. There are three phases in the process of rigor mortis, the delay phase, the rigor phase, and lastly the resolution phase [1]. ES systems compress the delay phase (4-6 hr.) by electrically stimulating the breast muscles depleting the ATP reserves within the muscle structure.

Commercial ES systems use either a cyclical or continuous current and are located after the stun and kill process. The carcass is stimulated during the bleeding process or after the bleeding process is completed, location of the ES system is dependent on the manufacturer. ES systems stimulate the muscle with either direct current or alternating current, again, depending on the ES manufacturer. Excessive voltages can mechanically tenderize the muscle by creating tears throughout the muscle [30], but these tears can lead to excessive yield losses in a deboning operation by leaving meat on the skeleton that can no longer be sold with the fillet.

Depletion of glycogen reserves in the PM muscle can be caused by thermal stress, catching at the farm, crating, transportation and other normal handling processes generating excitement in the bird [31]. As the glycogen reserves are depleted and pre-mortem glycolysis declines, there is a buildup of lactic acid causing an increase in pH that is ultimately associated with tougher meat [32]. Previous research also reported that the pH was not correlated to shear forces in the PM muscle but that there was a correlation between low glycogen levels and high shear forces [33]. Lactic acid injected into the *pectoralis profundus* muscles from culled cows caused an increase in tenderness by weakening the perimysial connective tissue and increasing the activities of proteases [34]. The beef samples tested were injected with a 10% w/w 0.5M solution of lactic acid at 3 depths and vacuum packed. The samples were stored for 28 and 48 hours allowing time for the lactic acid to influence the textural properties of the beef muscle. The final pH of the 24 hour test beef was 4.9 as compared to the control sample pH of 5.5 [34]. Lee et al. (1979), [27] reported a 24 hour pH of broiler PM to be between 5.58 and 5.51 leading to the conclusion that lactic concentrations in the broiler muscle may not be high enough to have a significant impact on the tenderness of broiler breast meat.

MATERIALS and METHODS

Sample Collection

Throughout the annual cumulative sampling process, 4,116 fillets were collected from 346 flocks from two production shifts in a commercial broiler operation. The samples were processed to measure the cooked maximum shear values during a full calendar year. There were some sporadic gaps in data due to unexpected downtime and scheduled plant shutdowns. Cobb 700 birds finished to an average live weigh of 6.5 pounds were processed for this test.

The first of the sample sets were collected early in the morning during the coolest part of the day, with the second sample set providing samples from birds transported in early afternoon hours, which typically associated to warmer portions of the day. Afternoon transport temperatures were warmer than morning transport temperatures ($P < 0.0001$).

Test sample size was typically twelve fillets, collected at the start of each shift for each production day. Care was taken that the samples collected were from the same production time periods; this was critical to have samples that represented a single population of animals representing a sample set that was transported under identifiable weather conditions and of populations representing samples raised under the same animal husbandry practices. Sample randomness of the birds was achieved in two ways. The first method is live birds are alternatingly unloaded from multiple trailers that hold birds collected from different locations in the same house. The second method is from with the mechanical action of water recirculation and air agitation in the water chillers that are processing carcasses from two evisceration lines. Production gaps in the bird chillers were placed to provide lot to lot separation in the event of an upstream quality issue. This practice allowed for separation of flocks corresponding to the time

the birds were transported allowed matching the sample with accurate weather conditions at the time of transport.

Chilled carcasses are sized for weight and distributed to deboning lines for processing, the deboned fillets are split along the keel, trimmed and packaged in a typical fresh chicken foam tray. Sample sets were collected after final package chilling and placed in a commercial refrigerator maintaining a temperature below 40 $^{\circ}$F for a 24 hour period before sample processing. The 24 hour wait period is designed to simulate the minimum time the consumer could use the product.

By nature of the packaging process of fillets from sized birds, shoulder thickness were consistent in thickness reducing the variability in cook times. Reduction in cook time variability reduced the opportunity for excessive cooking of unusually thin portions that could be mixed with thick portions. This process served to improve the precision of measured variation and maximum shear values throughout the sample sets. All samples were trimmed, eliminating excess fat and free from visual defect.

Sample Process Determination

Two methods of cooking the samples were evaluated to determine if there would be a tenderness difference associated with cooking on a metal tray covered with foil or in a covered ceramic dish. Three sets of 12 butterflies from three different farms were split longitudinal through the keel with one side processed using a metal tray covered with foil and the twin portion cooked in a covered ceramic cooking dish. The foil cover was intentionally fixed so contact with the portion was ensuring increased consistency in sample processing. Samples were cooled to room temperature and analyzed for maximum shear values. There was no apparent difference in

maximum shear values between the two cooking methods. This test defined that all future samples would be processed on a foil covered metal tray.

An analysis was also conducted to determine if one side of the butterfly was inherently tougher than the twin portion. Three sets of 12 butterflies from three different farms were longitudinally split down the keel with the genetically identical samples individually labeled and cooked in the same oven together on a foil covered metal tray. Left and right samples from the same butterfly were sheared in a Warner-Bratzler fixture and there was no apparent difference in shear between the opposing sides.

Sample Processing

After the 24 hour hold period, all fillet samples collected for sampling were placed on a standard teflon® coated metal cooking sheet that was sprayed with a non-stick coating and lined with aluminum foil. The food contact surface of the foil was also coated with a non-stick coating. The fillet sections were placed so that the shoulder of the thickest part was oriented at a known corner of the tray. This orientation was done so the technician could pierce the thickest sample through the foil for temperature measurement. This process kept the cook environment uniform and prevented the technician processing the samples from exposure to potential vapor burns. The trays were completely covered with aluminum foil sized and the foil securely sealed to the lips and edged of the cooking sheet to form a tight seal.

A conventional, non-convection oven was preheated to 350 °F (Frigidaire freestanding electric range/oven) with the oven racks placed so sample trays were evenly spaced in the center of the oven. Samples were cooked to temperatures ranging between 170 °F to 175 °F as measured in the center of the shoulder of the largest sample.

After reaching the aforementioned temperature, the cooked trays of covered product were removed from the oven and allowed to naturally cool in an open air environment to room temperature prior to sample preparation and testing. Trays were kept covered throughout the cooling process to reduce drying of the sample surface. Cooling the samples prior to processing eliminated the likelihood of burning the technician responsible for conducting the shear tests and prevented uncontrollable moisture loss due to product evaporation. This method of cooling the samples for processing improves shear precision compared to processing hot samples. Hot samples when sectioned would rapidly change temperature and add unnecessary variability to the process. Previous testing demonstrated a 3% to 5% sample moisture loss during shear processing by weight during open air cooling of sample sections. Samples that were cooked and cooled but not processed immediately were wrapped in plastic wrap and stored in a commercial refrigerator at a temperature less than 40 ^{0}F. Tests were conducted to determine if samples wrapped in a typical plastic wrap and held overnight yielded different shear values as samples not held overnight. Three sets of 12 butterflies from three different farms were longitudinally split down the keel with the genetically identical samples individually labeled and cooked on a foil covered metal tray. One side of the butterfly was sheared after cooling, the twin was held overnight in a plastic wrap and sheared using a Warner-Bratzler fixture after warming to room temperature. There was no difference in shear between the same sides of the butterfly. This leads to the conclusion that cooked meat samples if properly packaged and held at temperatures less than 40 ^{0}F will provide meaningful data if shear testing is conducted after allowing the sample to warm to room temperature.

I observed on numerous occasions that the samples with dry areas on the surface of the meat as indicated by muscled fiber separation would pull apart during the shearing process and wedge in

the Warner-Bratzler knife fixture causing excessive high shear readings that were not indicative of the samples' actual shear value. These false high shear values lead us to shear each sample twice, with the two values averaged to provide a representative result. In the event there was a shear value three times greater than the second cut from the same sample, the high value was removed from the analysis. Culling of data was used, but was an infrequent event throughout the dataset used throughout this project. The general rule used to remove what is considered erroneous data is defined as an unusually high shear value and a low shear value on the same cooked sample.

The logic for the removal of data is as follows: If $S_i * 2 < S_{ii}$ with $S_{ii} \geq 10$ the data point S_{ii} is removed from the data steam and the single data identified as S_i is the recorded data.

Where:

S_i is a unique maximum shear value.

S_{ii} is the second maximum shear value from the same fillet sample.

Shear Sample Preparation

A 20 mm wide stainless steel fixture was used to precisely section parallel samples cut with the grain of the cooked muscle. A single 20mm wide sample was removed from the humeral insertion and terminating at the anterior end of the keel. Those samples that were thicker than the opening of the Warner-Bratzler mandrel were trimmed by cutting with the grain of the meat on the non-membrane side of the sample. Testing of identically processed and identical genetic pectoralis major muscles were performed to verify that trimming the non-membrane side of the muscle did not have an impact on the maximum shear value; testing verified it was acceptable to trim the sample to fit the fixture. All samples used in shear testing were cooked, figure 1.1 depicting the portion of the fillet were the sample was removed. The solid lines represent the

section of fillet removed for evaluation, the dashed lines are the relative shear lines that provided maximum shear values for the sample. The sectioning of the cooked fillet was based on a similar model presented by Lyon et al. (1996) [35].

Individual cooked samples were placed approximately a third of the way into a benchtop Warner-Bratzler shear press and the maximum shear value recorded. The same sample was again shear 1/3 of the way from the opposing end of the sample. Figure 1.1 shows relative cut positions on a raw fillet; the two values were averaged and the average value recorded. All sample data was collected from cooked samples; the raw image provides a clear location for sample collection and cut location.

Care was taken that the samples collected were consistently from the same production time periods; this was critical to have samples that represented a single population of animals representing a sample set that was transported under identified weather conditions. Random collection of samples was indicative of the production process due to coop mixing as the birds are entered into the production system, mixing in the carcass chilling system, and weigh sorting in the deboning process. Shear data was recorded in Microsoft Excel and all statistical analysis conducted with SAS Version 9.3.

Environmental Data

The test data was collected from a manufacturing site located in Accomack County VA, approximately seven miles from the Atlantic Ocean, providing variation in seasonal temperatures and weather conditions. Shift one transport temperatures ranged from a low of 11 ^{0}F to high of 83 ^{0}F. Shift two transport temperatures ranged from a low of 27 ^{0}F to high of 100 ^{0}F. Transport times to the processing facility were typically held below 2 hours.

Environmental data was extracted from a historical database managed by an internet based weather channel with accessible historical data. Values for temperature and humidity from a local weather station were assigned for each respective shift and day of production. Relationships between the average individual flock maximum shear values and average flock standard deviation for the flocks were examined for possible interaction with transport temperature, production shift, weather that is classified as "rainy" or "sunny" and air energy. Historical values for temperature, humidity and weather conditions were collected for each respective transport time and day of production. The energy of the air was calculated using air temperature and relative humidity data and is expressed in British Thermal Units (btu).
The energy of the air was calculated using air temperature and relative humidity data and is expressed as BTU per pound of dry air (btu/lbm_{da}). The equation derived for this calculation is noted below:

Air Energy (btu) = (0.24*AIR TEMPERATURE)+(0.62198*(RELATIVE HUMIDITY /100)*(EXP((-10440.4/(AIR TEMPERATURE +459.67)-11.29465-0.02702235*(AIR TEMPERATURE +459.67)+0.00001289036*(AIR TEMPERATURE +459.67)^2-0.000000002478068*(AIR TEMPERATURE +459.67)^3+6.545967*(LN(AIR TEMPERATURE +459.67)))))/(14.7-(RELATIVE HUMIDITY /100)*(EXP((-10440.4/(AIR TEMPERATURE +459.67)-11.29465-0.02702235*(AIR TEMPERATURE +459.67)+0.00001289036*(AIR TEMPERATURE +459.67)^2-0.000000002478068*(AIR TEMPERATURE +459.67)^3+6.545967*(LN(AIR TEMPERATURE +459.67))))))*(1061+0.444*AIR TEMPERATURE)) [36]

Where air temperature is the dry bulb temperature of the transport air and relative humidity is the water content of the air at the respective dry bulb temperature expressed in decimal format. The

energy results from the aforementioned equation were validated on a psychrometric chart suitable for measurements at sea level.

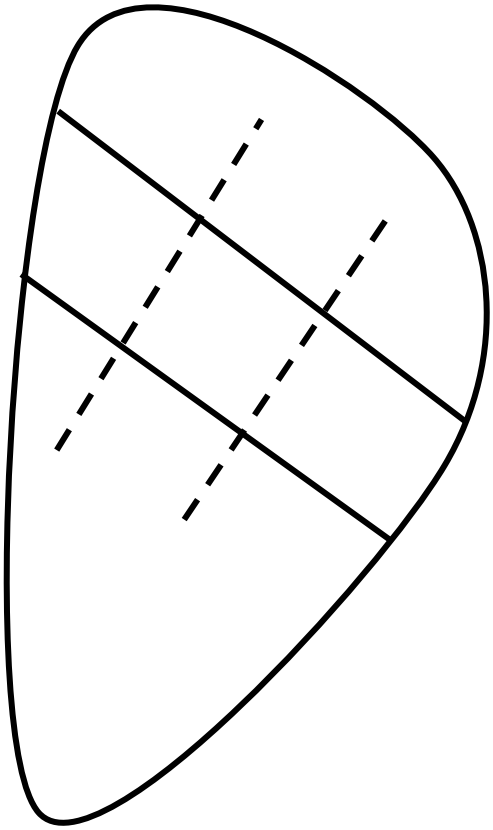

Figure 1.1 Typical Shear Sample Cut Detail of a Broiler Breast Fillet

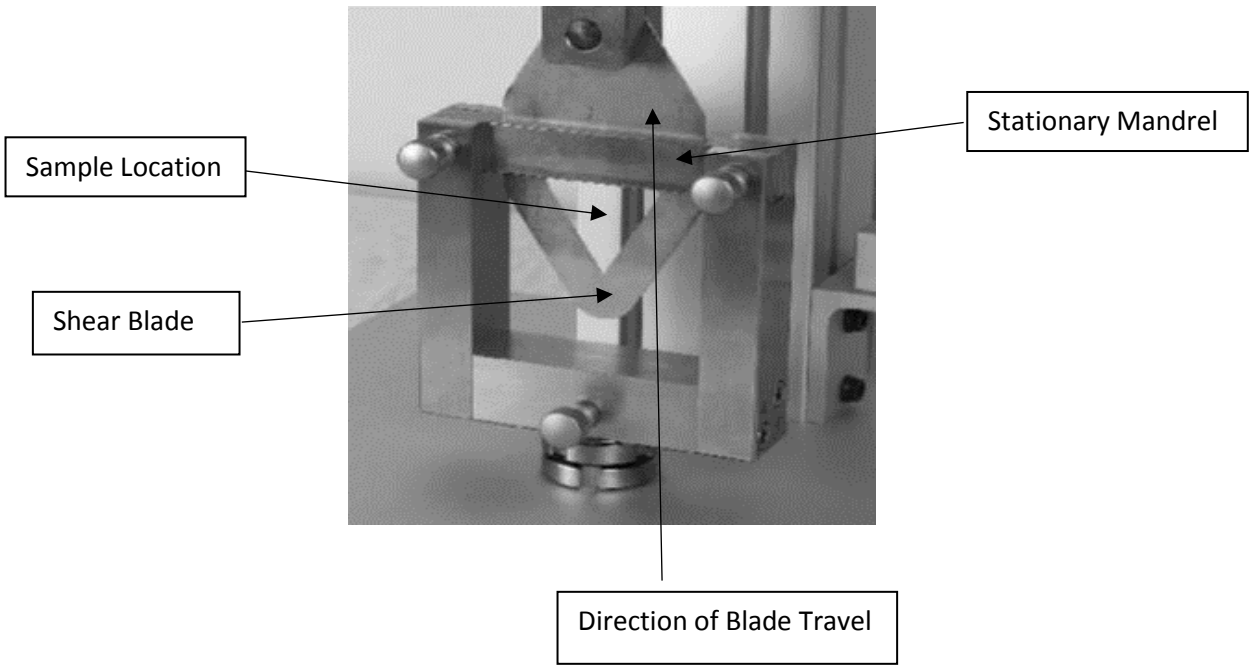

Figure 1.2 Example of a Warner-Bratzler Shear Fixture

RESULTS

The data sets analyzed in this discussion are from flock sample sets of approximately twelve fillet samples per flock. Samples from 346 unique flocks compose the data used in the analysis presented. There were 172 flocks sampled from the morning production shift and 174 flocks sampled from the afternoon production shift. There were a total of 4,116 individual samples sheared during a consecutive twelve month period. Individual sample shear data was investigated in looking for relationships between shear values and environmental conditions, it was seen that there was no significant correlation when this approach was used. Examining flock breast meat maximum shear averages and flock breast meat shear standard deviations it became apparent that there was a pattern between the transport environment and the tenderness quality attribute of toughness. This pattern directed analysis to be focused on flock average maximum shear force (kg) and the flocks' associated standard deviation (kg) data, focus was not placed on an individual bird data.

Initially flock data was examined without consideration to the time of transport or shift, it became apparent that with regards to maximum shear and maximum shear consistency of cooked breast meat sections, the flocks performed differently based on when they were transported and processed.

There is a statistically significant relationship between the time of transport (shift) and the maximum shear average for a flock ($P = 0.0092$). There is a statistically significant relationship between the standard deviation flocks' shear values and time of transport (shift) ($P < 0.0001$). The classification of weather as classified rainy and sunny and transport time was also considered as a factor on flock shear analysis. There is no interaction with weather and maximum flock shear within a shift ($P = 0.1630$). There is an interaction with weather and maximum flock shear between shifts ($P = 0.0074$). There is no interaction with weather and

maximum flock shear standard deviation within a shift (P = 0.2021). There is an interaction with weather and maximum flock standard deviation between shifts (P < 0.0001).

Table 1.0 provides a summary of the flock average shear annual values and flock annual standard deviation values. Table 2.0 provides a statistical summary of flock interaction between shear, shear standard deviation and transport air conditions.

Transport air temperature and air energy were binned into 4 0F and 2.5 btu increments respectively, for analysis with shear and shear variation under the aforementioned weather states combining data from both shifts. There was no interaction between transport air temperature during sunny conditions and shear values (P = 0.7775). There was an interaction between shear standard deviation and transport air temperature in sunny conditions (P < 0.001). There was interaction between transport air temperature during rainy conditions and shear values (P = 0.0371). There was an interaction between shear standard deviation and transport air temperature in sunny conditions (P < 0.0001). There was no interaction between transport air energy during sunny conditions and shear values (P = 0.6259). There was an interaction between shear standard deviation and transport air energy in sunny conditions (P < 0.0001). There was no interaction between transport air energy during rainy conditions and shear values (P = 0.9548). There was an interaction between shear standard deviation and transport air energy in sunny conditions (P = 0.0369).

Transport temperature

Interaction between maximum shear values and transport temperatures for the entire population of 346 flocks failed to reach significance at the 95% confidence level. (P = 0.2178).

Examining the flock shear by shifts indicated that there was an interaction between the transport environment and cooked breast meat shear values. The average annual maximum shear values

for shift 1 (N = 172) and shift 2 (N = 174) were 7.9 kg and 8.2 kg respectively. Examination of the data by shift indicates there is a significant relationship between flock average shear values and shift (P = 0.0092). Figures 1.3 and 1.4 are graphical representations by shift of transport temperatures and the flock maximum shear data respectively for shift one and shift two.

There is a strong indication that warmer transport temperatures appear to lower average flock shear standard deviation. Overall the annual afternoon temperatures were significantly warmer as compared to morning temperatures (P < 0.0001). The average first shift maximum shear standard deviation value for 12 months was calculated to be 3.45 kg (N= 172). Figure 1.5 provides a graphical representation of the interaction of transport temperature and flock standard deviation. Second shift maximum shear standard deviation value for the same 12 months was calculated to be 1.78 kg (N= 174). Figure 1.6 provides a graphical representation of the interaction of transport temperature and flock standard deviation for shift two. A Duncan Difference test indicated there was a significant difference between flock standard deviation values between the first and second shifts. A GLM test also indicated an interaction between maximum shear standard deviation and shift. (P < 0.0001) There was no significant interaction between transport air temperature and average flock shear when examining all data from shifts one and two (P = 0.2178). Significance was found within shift one flock shear data and transport air temperature (P = 0.0355). A significant relationship between flock shear standard deviation and transport air temperature was found when examining flock data from both shifts (P < 0.0001).

The response of shear and shear standard deviation with transport temperature was also analyzed by placing transport temperature into 4 ^{0}F bins. This 4 ^{0}F degree of separation provided 20 bins for evaluation. Average shear and average flock standard deviation response with their

respective bins are shown in figure 1.16. Regression analysis using binned data failed to reach a significant relationship between transport temperature and shear ($P = 0.3715$). There was a significant relationship found between the binned data of transport air temperature and shear standard deviation ($P < 0.0001$).

Air Energy

Transport air energy was significantly higher during shift two with respect to air energy during shift one ($P < 0.0001$). Figures 1.7 and 1.8 are graphical representations of the interaction of transport air energy and the standard deviation of flocks for shift one and shift two respectively. Even though there is a significant difference between shift and flock maximum shear, there is not an apparent interaction within either production shift between air energy levels and flock maximum shear values ($P = 0.8487$). Figures 1.9 and 1.10 are graphical representations of the interaction of transport air energy and the standard deviation of flocks for shift one and shift two respectively. There is not a significant relationship between air energy and flock shear variation of cooked breast meat. The statistical analysis using the GLM procedure indicates that an interaction between average flock tenderness standard deviation and the transport air energy for combined production shift exists ($P < 0.0001$).

The response of shear and shear standard deviation with transport air energy was also analyzed by placing transport temperature into 2.5 Btu bins. This 2.5 Btu degree of separation provided 21 bins for evaluation. Average shear and average flock standard deviation response with their respective bins are shown in figure 1.17. Regression analysis using binned data failed to reach a significant relationship between transport temperature energy and shear ($P =0.5932$). There was a significant relationship found between the binned data of transport air energy and shear standard deviation ($P<0.0001$).

Table 1.0
Summary of shear, standard deviation, maximum, minimum and ranges with respect to environmental transport conditions by shift for a 12 month production cycle

Shift 1	*Cumulative Shear (kg)*	*Sunny Shear (kg)*	*Rainy Shear (kg)*	*Sunny St Dev (kg)*	*Rainy St Dev (kg)*	*Transport Air Energy (btu)*
N	172	123	49	123	49	172
Average	7.9	7.69	8.37	3.32	3.77	17.92
St Dev	3.5	1.33	1.54			7.72
Maximum	11.1	11.1	11.7	5.9	6.6	33.4
Minimum	1.2	3.2	5.6	1.2	1.2	5.17
Range	9.9	7.2	6.1	4.7	5.4	28.23

Shift 2	Cumulative Shear (kg)	Sunny Shear (kg)	Rainy Shear (kg)	Sunny St Dev (kg)	Rainy St Dev (kg)	Transport Air Energy (btu)
N	174	118	56	118	56	174
Average	8.2	8.19	8.20	1.71	1.8	34.08
St Dev	1.8	0.74	0.68			13.5
Maximum	10.8	10.8	9.9	3.5	3	60.16
Minimum	3.2	6.5	6.7	0.7	0.9	9.89
Range	7.6	4.3	3.2	2.8	2.1	50.27

Table 2.0

Overall statistical results of transport air temperature (^{0}F) and transport air energy (btu) by shift and transport weather condition and individual binned flock data segregated into 4 ^{0}F and 2.5 btu bins

Point to Point Analysis	*N (Flocks)*	*Air Temperature*	*Air Energy*	*Rain Air Energy*	*Sun Air Energy*	*Rain Air Temperature*	*Sun Air Temperature*
All Shifts Shear	346	P = 0.2178	P = 0.8487	P = 0.3279	P = 0.3083	P =0.0699	P = 0.8901
All Shifts SD	346	P < 0.0001	P < 0.0001	P = 0.0019	P < 0.0001	P =0.0005	P < 0.0001
Within Shift 1 Shear	172	P = 0.0355	P < 0.0997	P = 0.2278	P = 0.1561	P = 0.0602	P = 0.2992
Within Shift 2 Shear	174	P = 0.2481	P = 0.2102	P = 0.3033	P = 0.3632	P = 0.3752	P = 0.7962
Within Shift 1 Shear SD	172	P = 0.0996	P = 0.2484	P = 0.5029	P = 0.2956	P = 0.0853	P = 0.3033
Within Shift 2 Shear SD	174	P = 0.2104	P = 0.2517	P = 0.2351	P = 0.9604	P = 0.1467	P = 0.5822
Binned Analysis	N (Flocks)	Air Temperature	Air energy	Rain Air Energy	Sun Air Energy	Rain Air Temperature	Sun Air Temperature
All Shifts Shear	346	P = 0.3483	P = 0.5932	P = 0.9548	P = 0.6260	P = 0.0371	P = 0.7775
All Shifts SD	346	P < 0.0001	P < 0.0001	P = 0.0370	P < 0.0001	P = 0.0001	P = 0.001

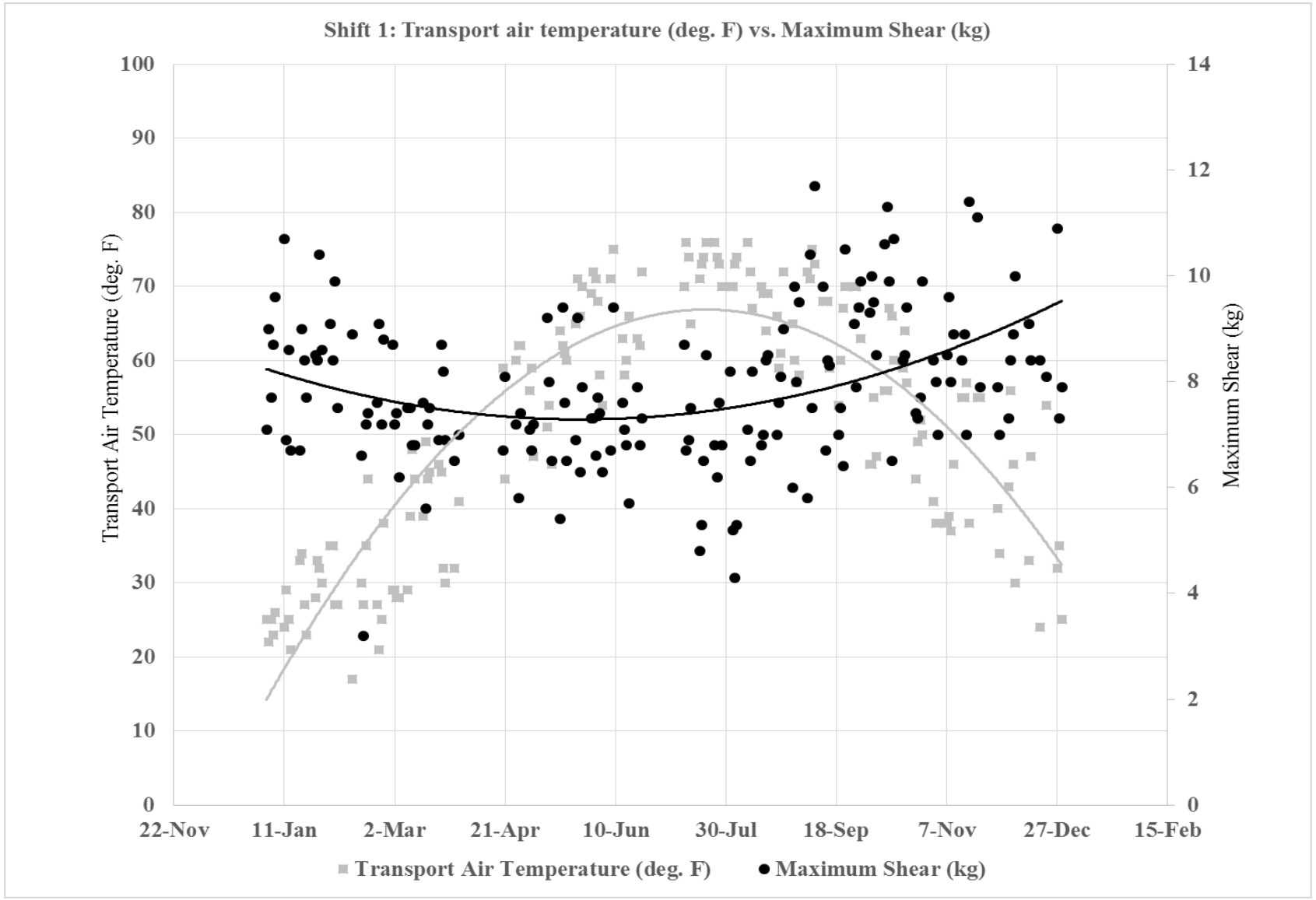

Figure 1.3 Shift 1 Transport air temperature relationship with maximum shear (kg) of cooked breast muscle

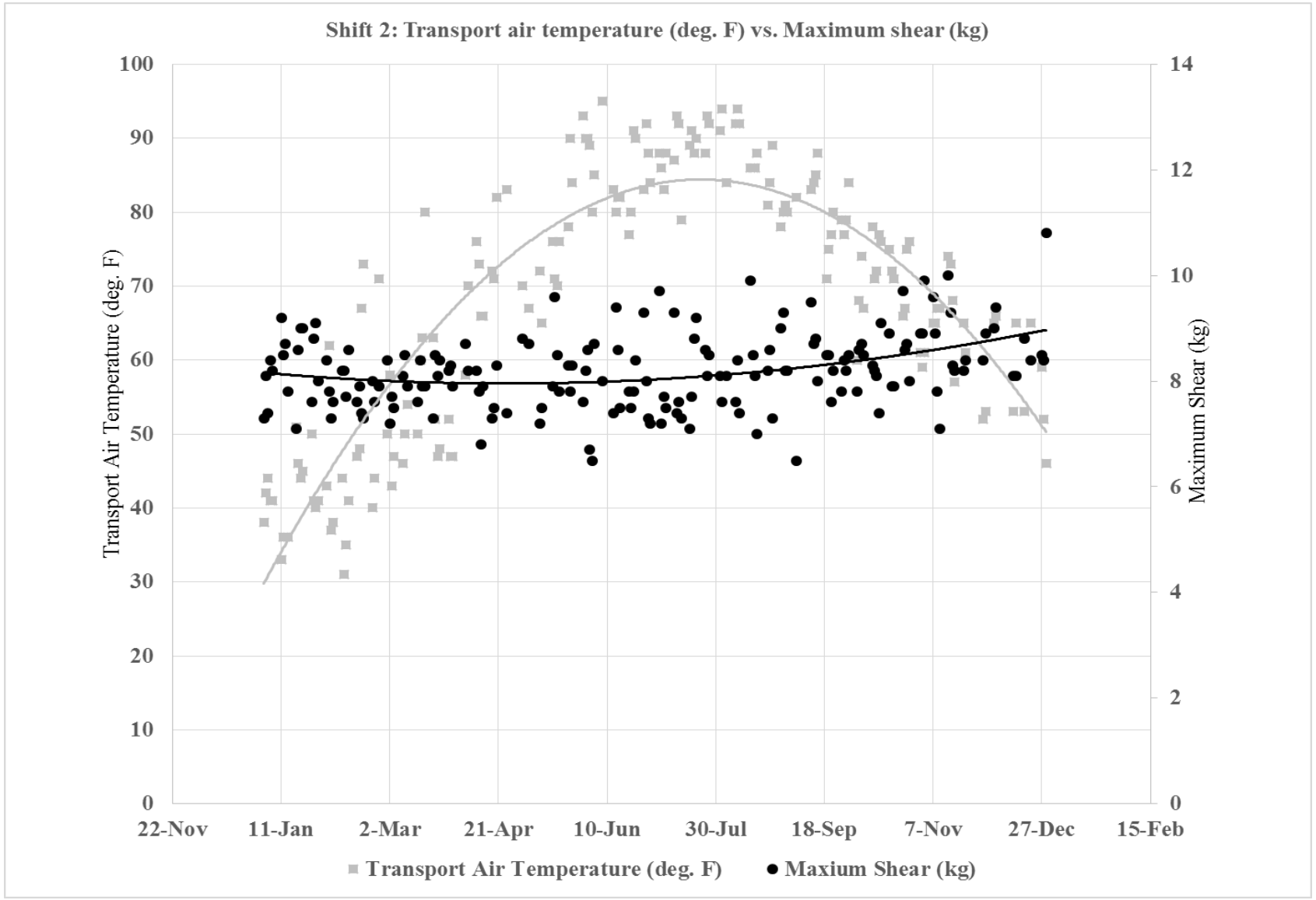

Figure 1.4 Shift 2 Transport air temperature relationship with maximum shear (kg) of cooked breast muscle

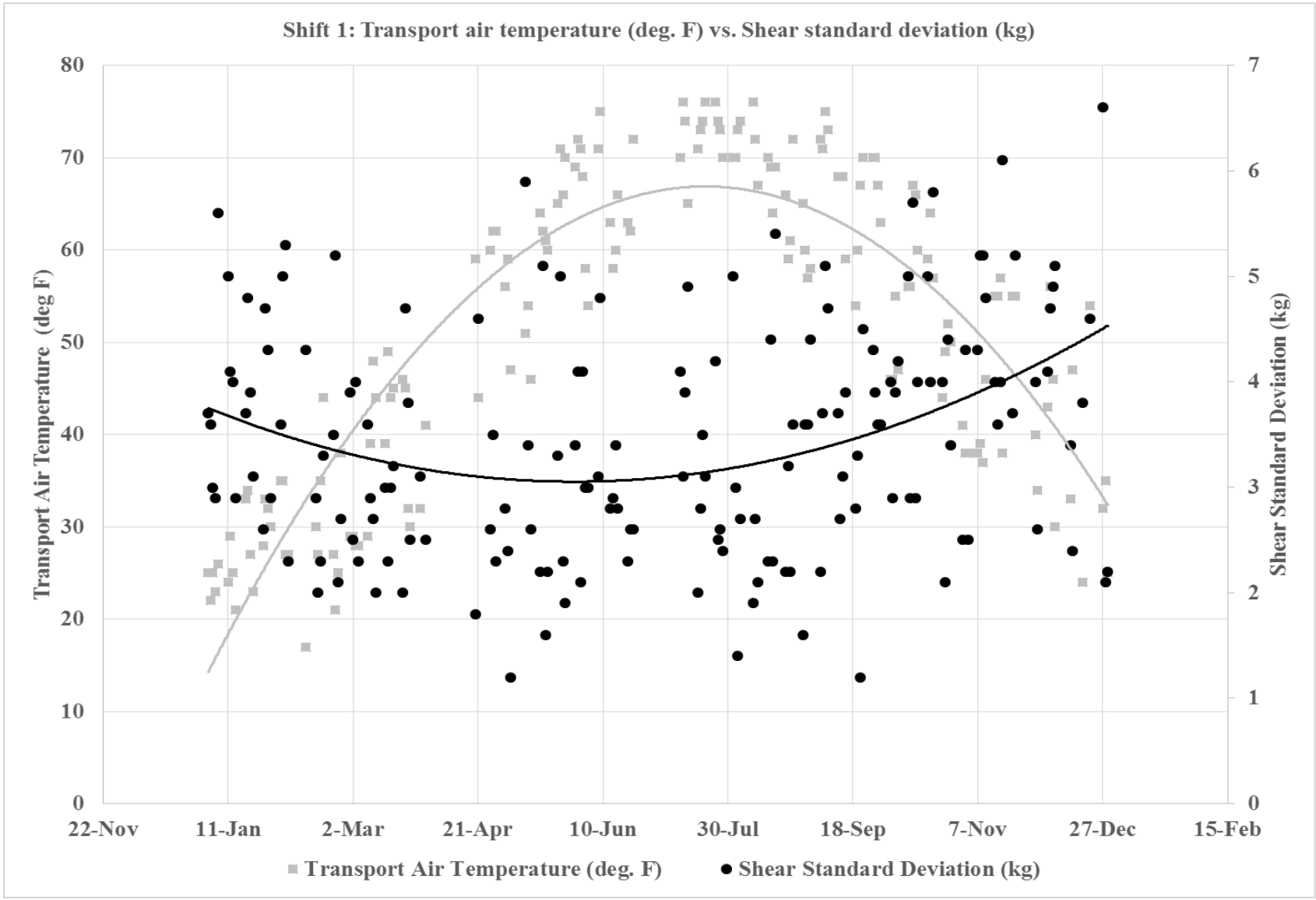

Figure 1.5 Shift 1 Transport air energy relationship with maximum shear (kg) of cooked breast muscle

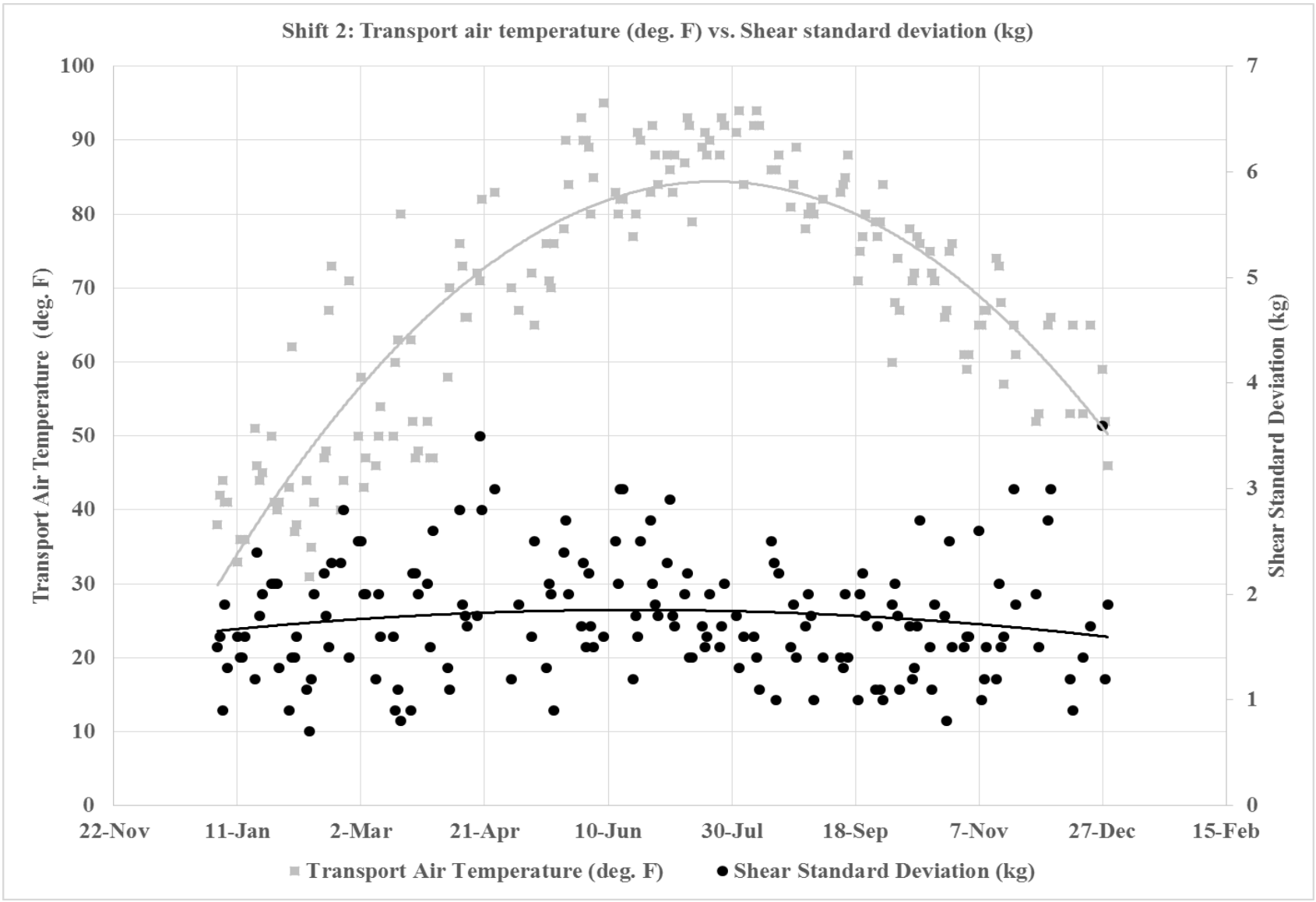

Figure 1.6 Shift 2 Transport air temperature vs. shear standard deviation

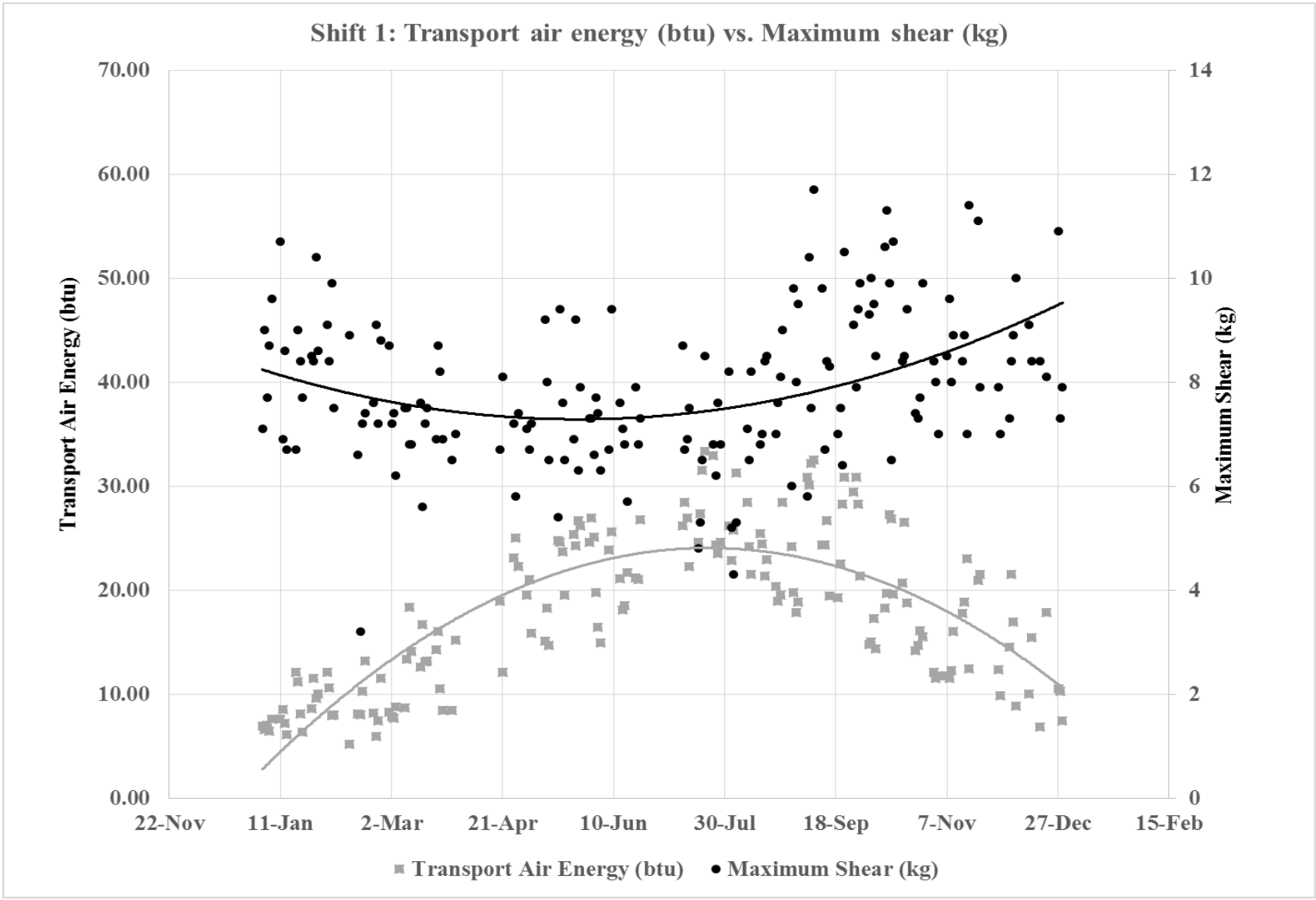

Figure 1.7 Shift 1 Transport air energy relationship with maximum shear standard deviation (kg) of cooked breast muscle

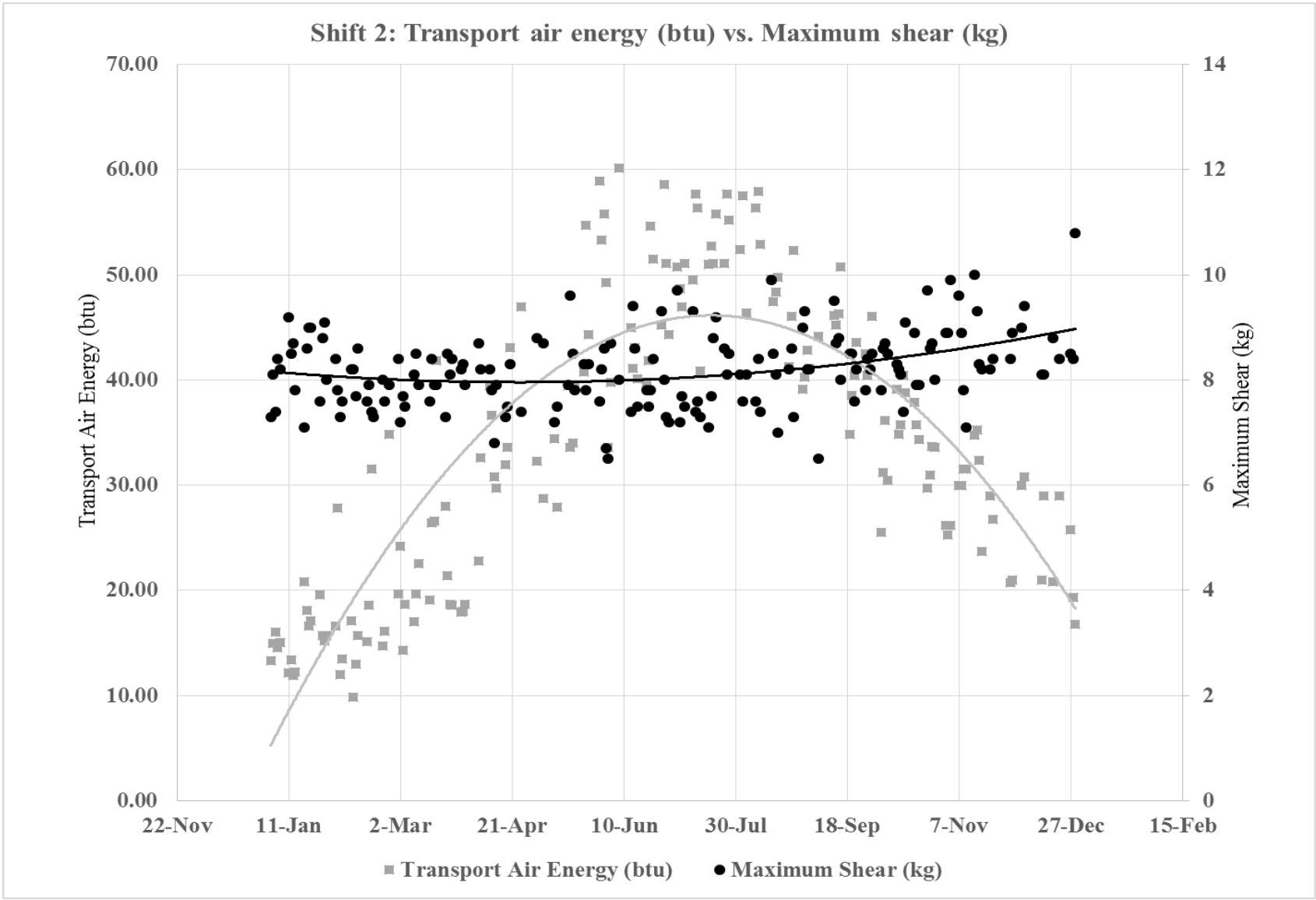

Figure 1.8 Shift 2 Transport air energy relationship with maximum shear (kg) of cooked breast muscle

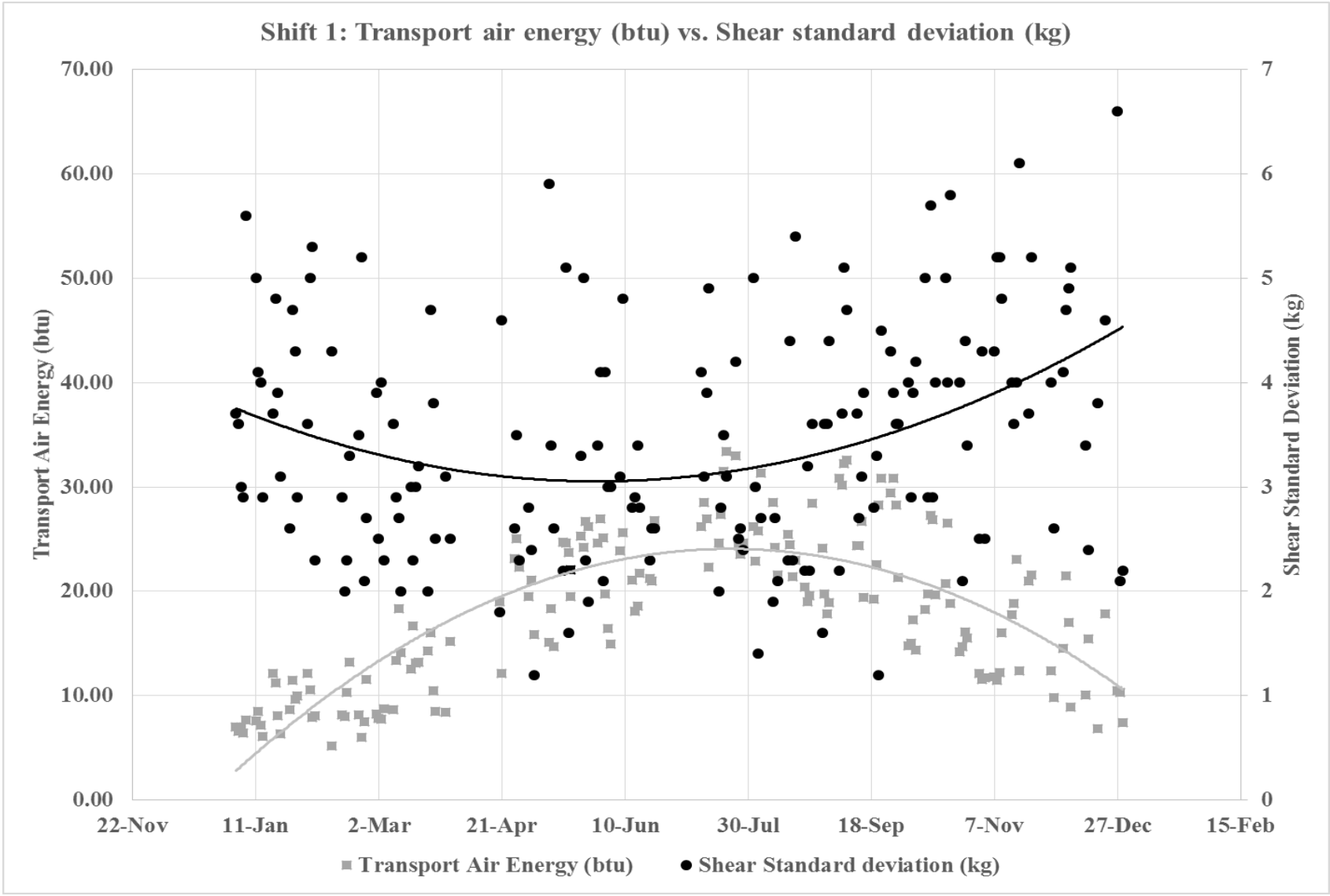

Figure 1.9 Shift 1 Transport air energy (btu) and maximum shear standard deviation (kg) of cooked breast meat

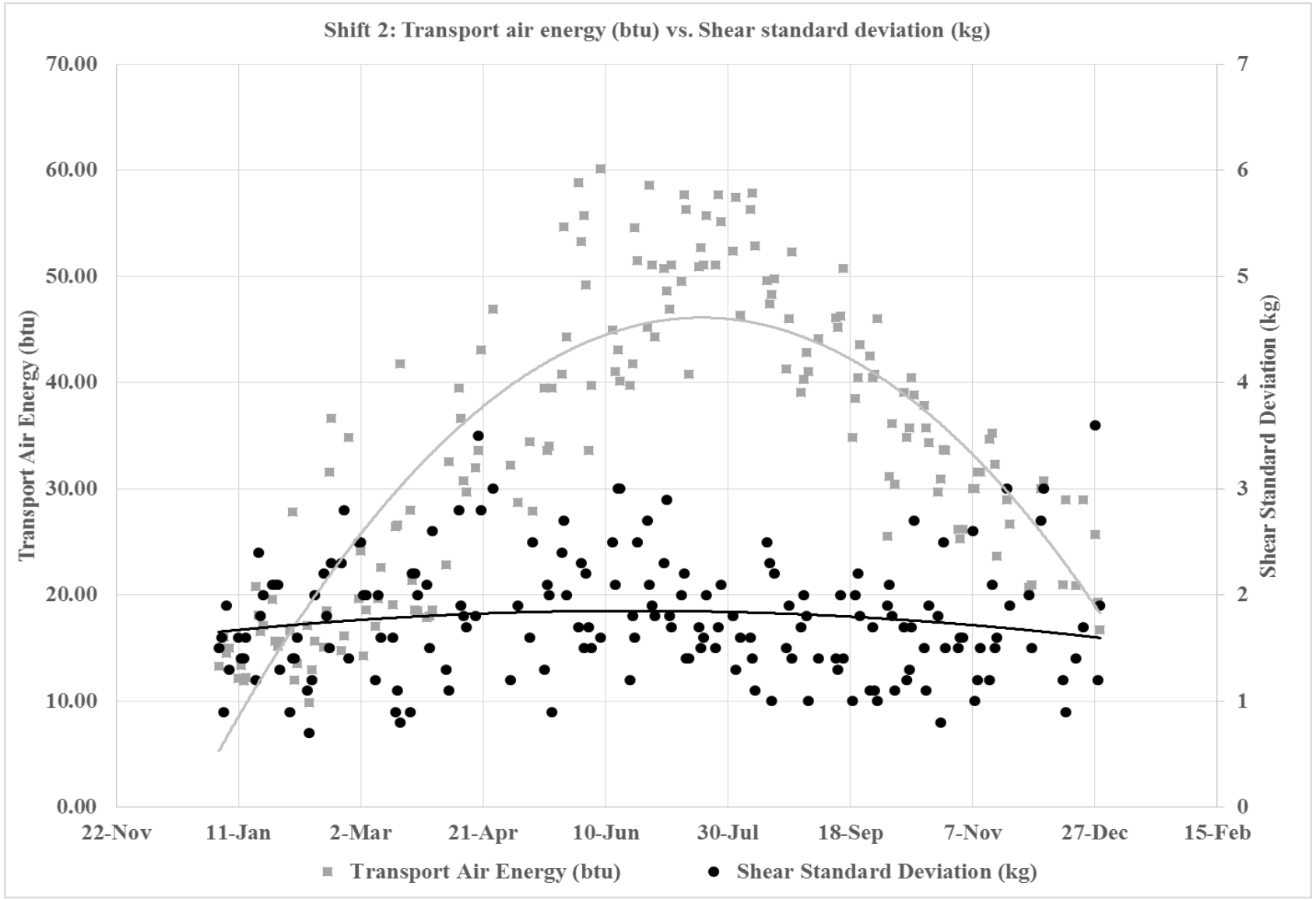

Figure 1.10 Shift 2 Transport air energy relationship with maximum shear standard deviation (kg) of cooked breast muscle

Weather as defined "Sunny" or "Rainy"

There was no interaction between weather and maximum flock shear within each shift (P = 0.1630). Figures 1.11 and 1.12 are graphical representations of the interaction of weather as classed as rainy and sunny with the average maximum shear of flocks for shift one and shift two respectively. There was an interaction between weather and maximum shear between shifts (P = 0.0074). Figures 1.13 and 1.14 are graphical representations of the interaction of weather as classed as rainy and sunny with the standard deviation of flocks for shift one and shift two respectively. There was no interaction between air temperature during rainy weather and maximum flock shear within shift one. (P = 0.0602). There was no interaction between air temperature during rainy weather and maximum flock shear within shift two (P = 0.3752). There was no interaction between air temperature during rainy weather and maximum flock shear standard deviation within shift one (P = 0.0602). There was no interaction between air temperature during rainy weather and maximum flock shear standard deviation within shift two (P = 0.0853). There was no interaction between air energy during rainy weather and maximum flock shear within shift one (P = 0.2278). There was no interaction between air energy during rainy weather and maximum flock shear within shift two (P = 0.3033). There was no interaction between air energy during rainy weather and maximum flock shear standard deviation within shift one (P = 0.5029). There was no interaction between air energy during rainy weather and maximum flock shear standard deviation within shift two (P = 0.2351). There was an interaction between standard deviation and weather between shifts (P < 0.0001).

Flocks transported during rainy weather had slightly higher shear values and slightly higher standard deviation values than flocks transported during no rain conditions. See table 1.0 for numeric details.

The response of shear and shear standard deviation with weather defined as sunny was also analyzed by placing transport temperatures into 4 ^{0}F bins. This 4 ^{0}F degree of separation provided 19 bins for evaluation of sunny conditions. Average shear and average flock standard deviation response with their respective bins are shown in figure 1.18. Regression analysis using binned data failed to reach a significant relationship between transport temperature during sunny conditions and shear ($P = 0.6260$). There was a significant relationship found between the binned sunny data of transport air temperature and shear standard deviation ($P < 0.001$). Regression analysis using binned data reached significance between transport temperature during rainy conditions and shear ($P = 0.0371$). There was a significant relationship found between the binned rainy data of transport air temperature and shear standard deviation ($P < 0.0001$). Shear and shear standard deviation with weather defined as rainy was also analyzed by placing transport air energy into bins of 2.5 Btu. This 2.5 Btu separation provided 12 bins for evaluation of rainy conditions. Average shear and average flock standard deviation response with their respective bins are shown in figure 1.19. Regression analysis using binned data failed to reach a significance between transport air energy during rainy conditions and shear ($P = 0.9548$). There was a significant relationship found between the binned data of rainy transport air energy and shear standard deviation ($P=0.0370$). There were 21 bins used in regression analysis for sunny weather between transport air energy, average bin shear and average bin standard deviation. The binned data failed to reach a significant relationship between transport temperature during sunny conditions and shear ($P = 0.6260$). There was a significant relationship found between the binned data of transport air energy and shear standard deviation ($P < 0.0001$).

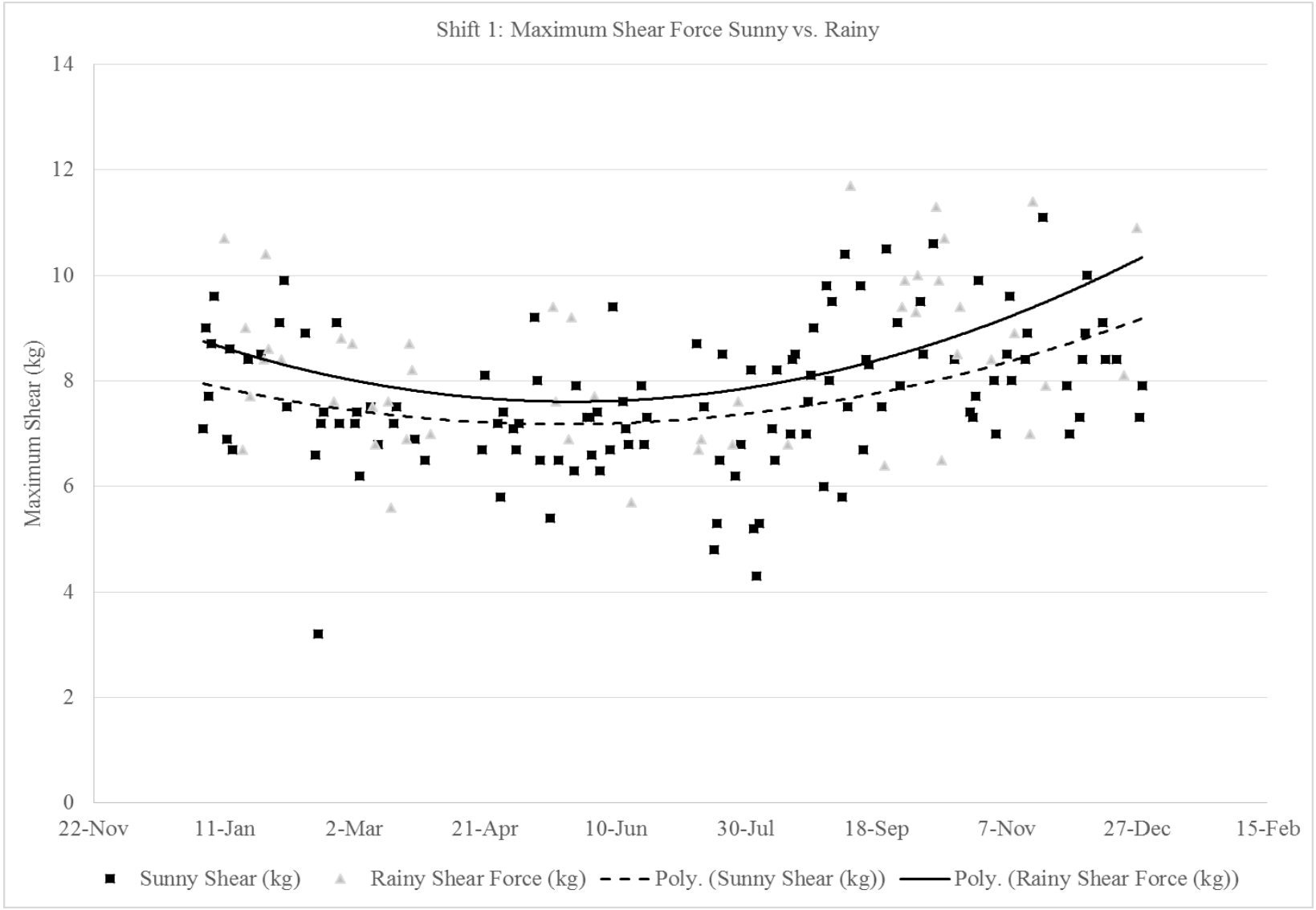

Figure 1.11 Shift 1 Maximum average shear force (kg) for cooked breast meat and weather

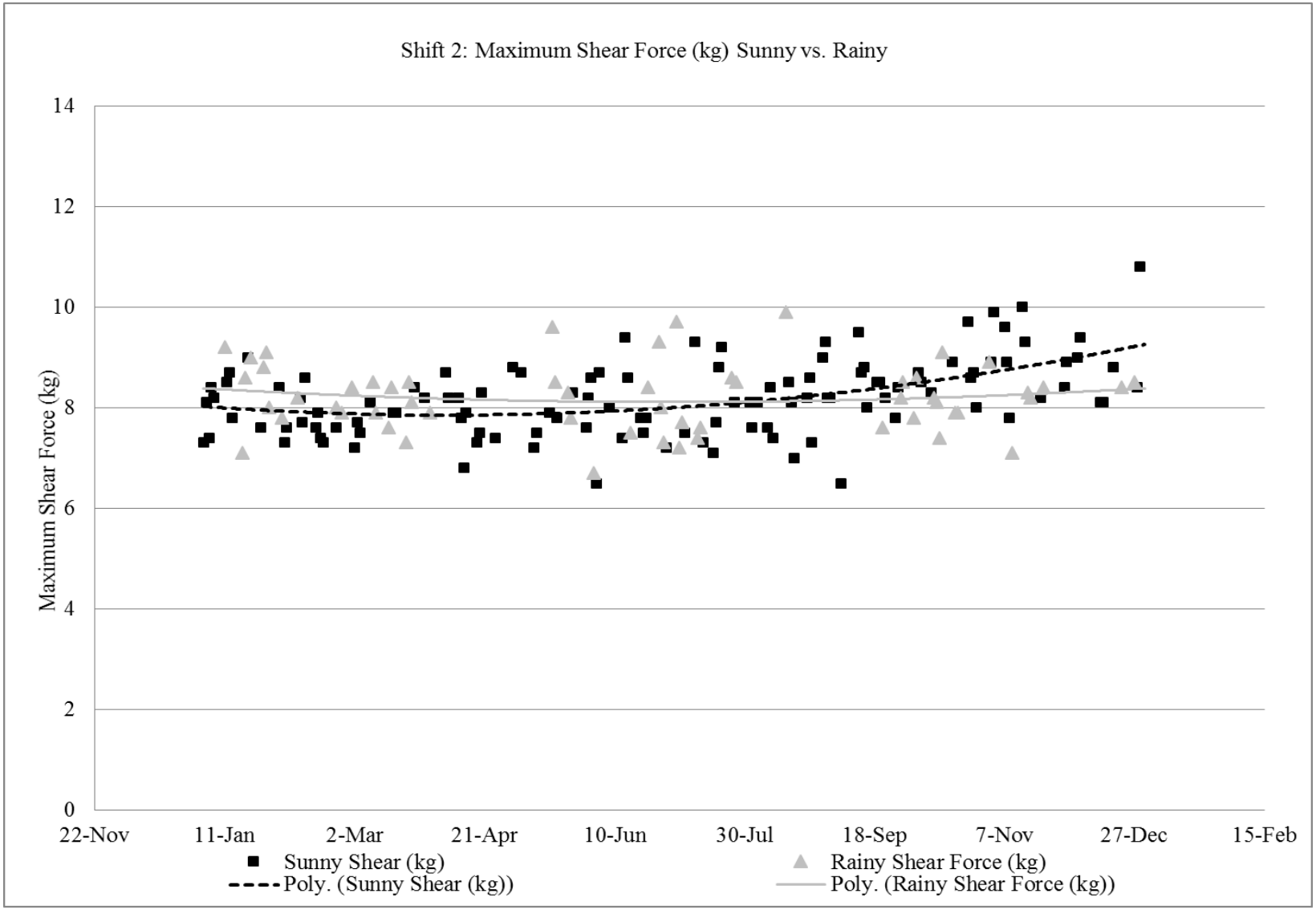

Figure 1.12 Shift 2 Maximum average shear force for cooked breast meat and weather

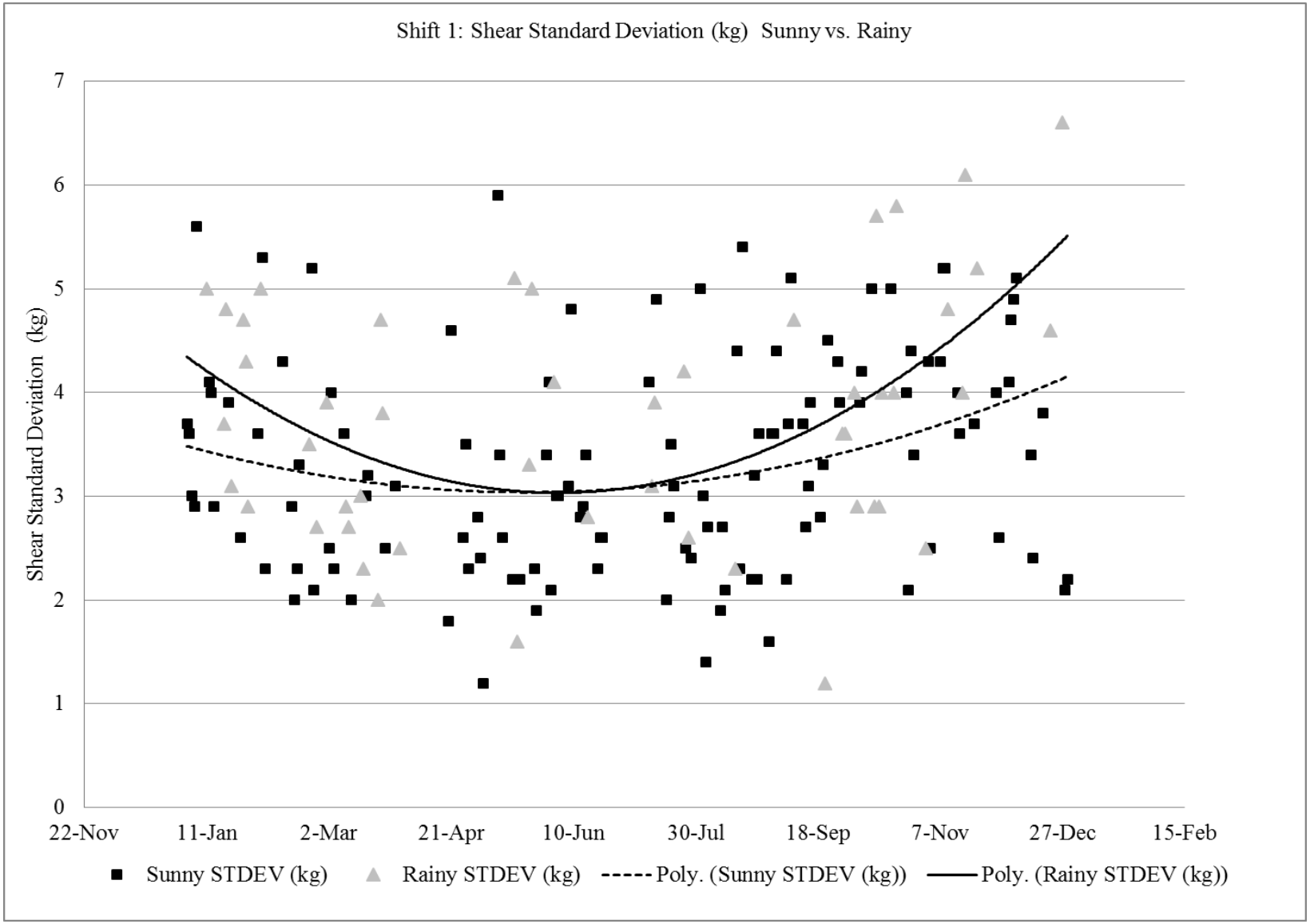

Figure 1.13 Shift 1 Maximum average flock standard deviation (kg) and weather

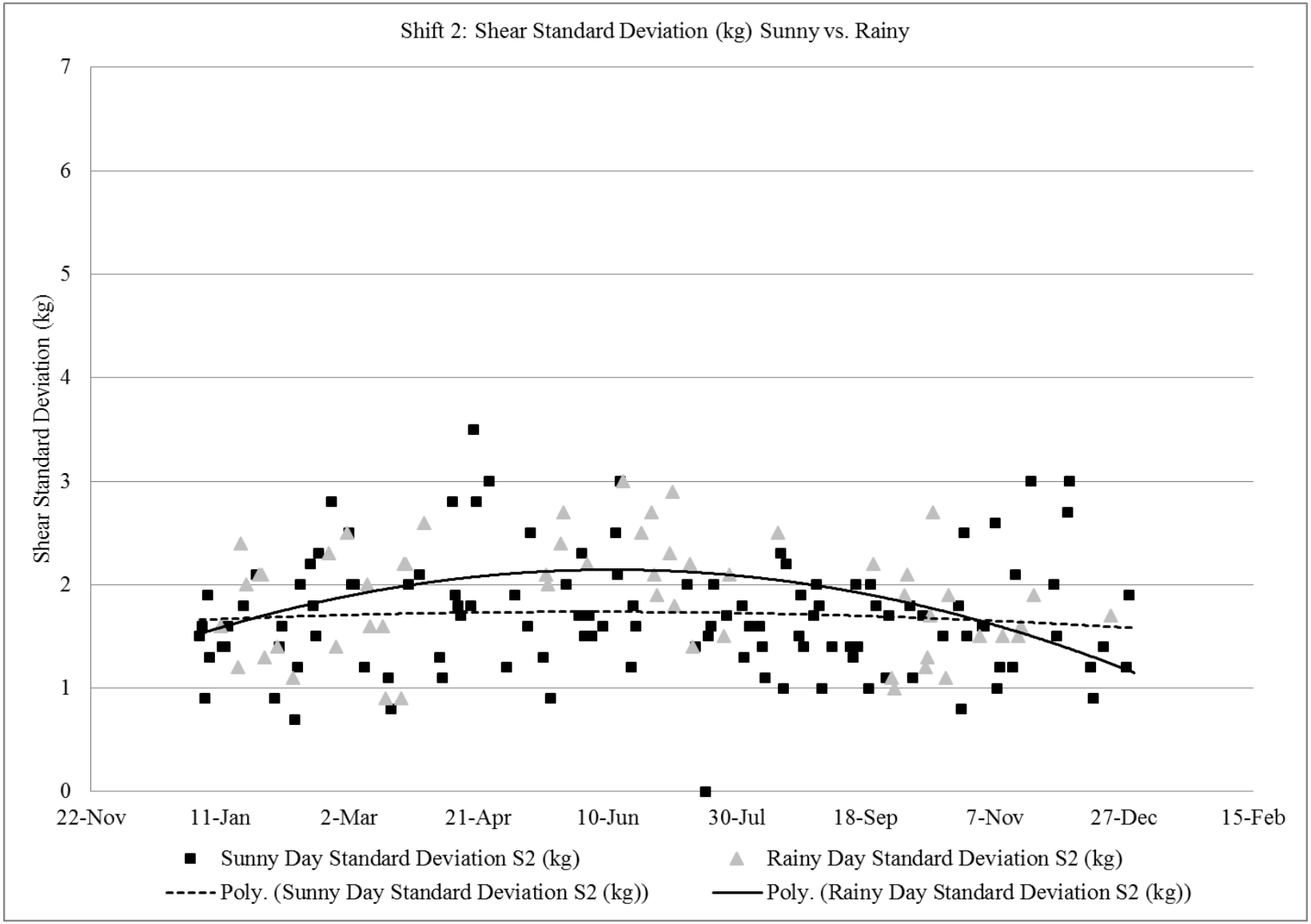

Figure 1.14 Shift 2 Maximum flock standard deviation (kg) and weather

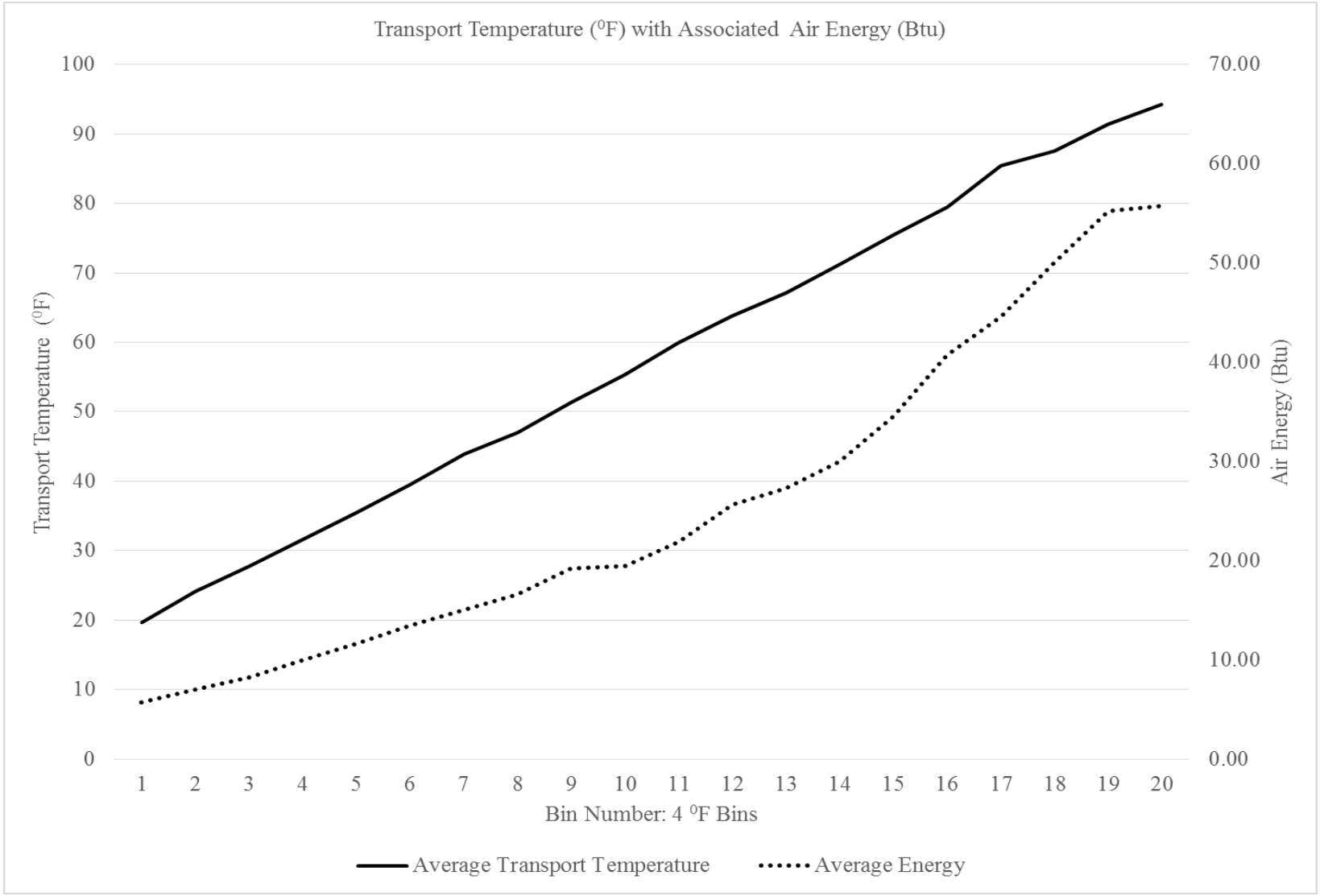

Figure 1.15 Relationship between Air Energy (Btu) and Air Temperature (4 ^{0}F Bins) and a Specific Set of Air Humidity Conditions

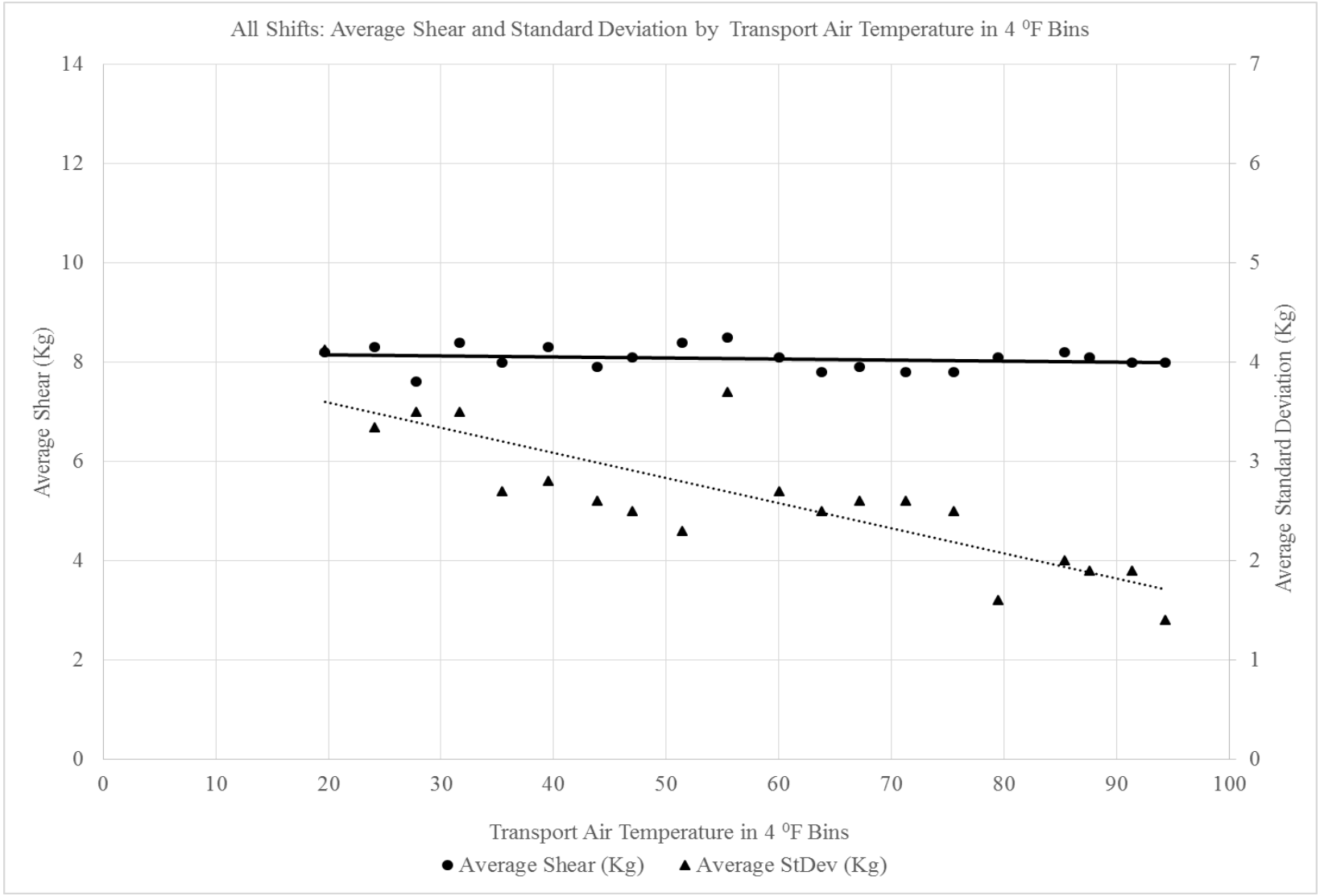

Figure 1.16 All Average Shift Shear (Kg) and Shear Standard Deviation (Kg) with Associated Transport Temperature Bins of 5 ^{0}F

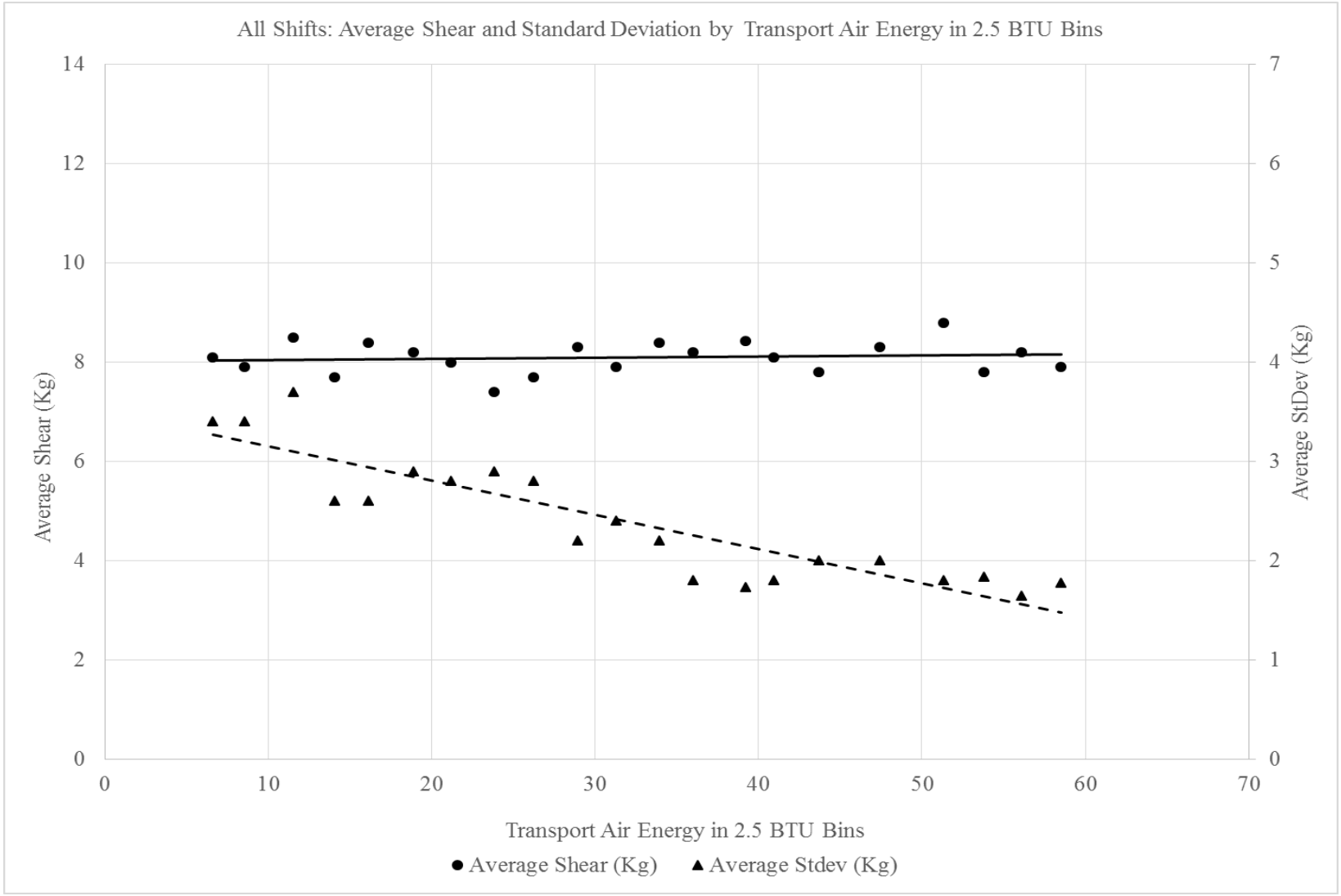

Figure 1.17 All Average Shift Shear (Kg) and Shear Standard Deviation (Kg) with Associated Transport Air Energy in 2.5 BTU Bins

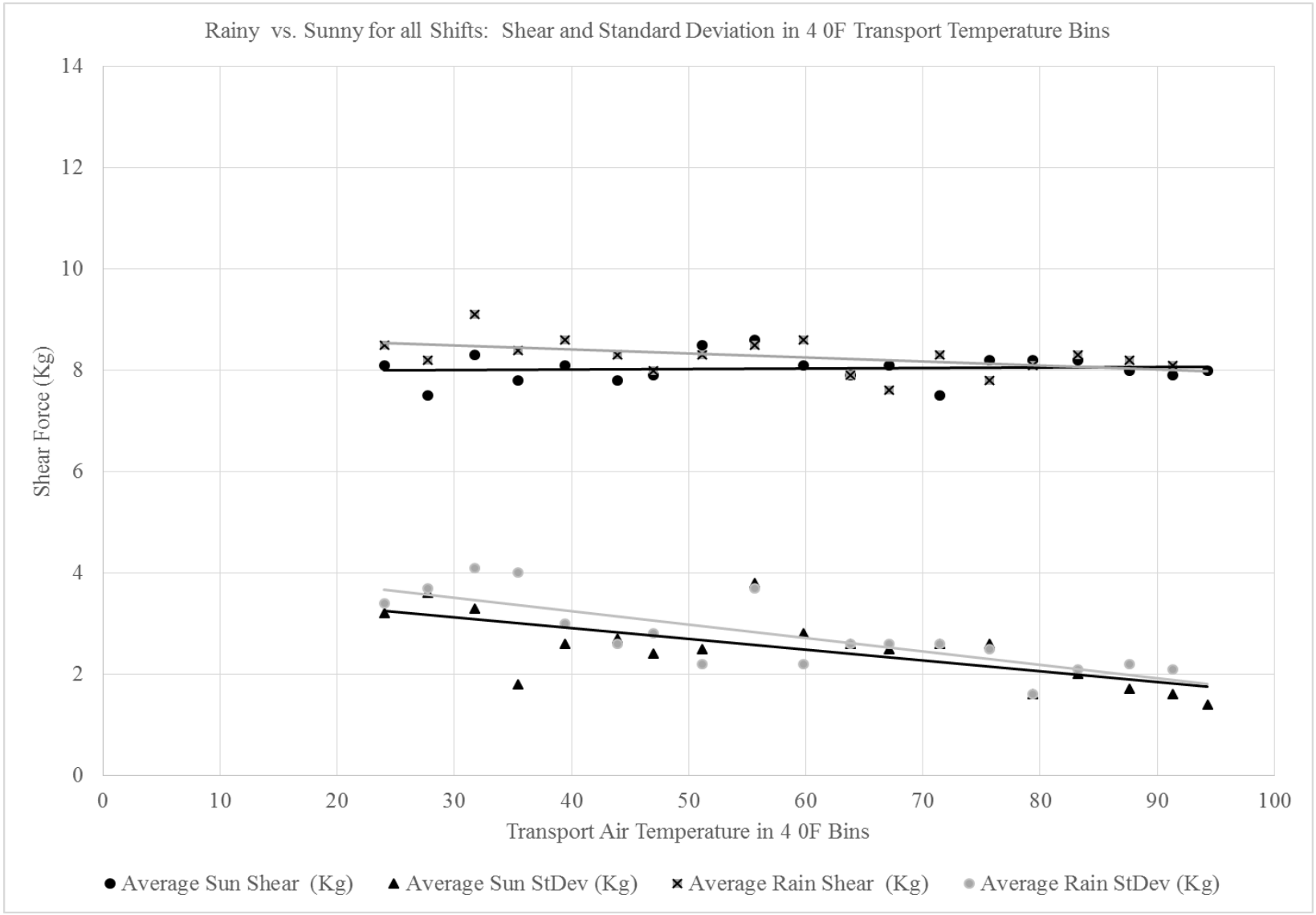

Figure 1.18 Weather classed in Rain vs. Sun for both Shifts Transport Temperature in 4^0F Bins impact on Shear (Kg) and Shear Standard Deviation (Kg)

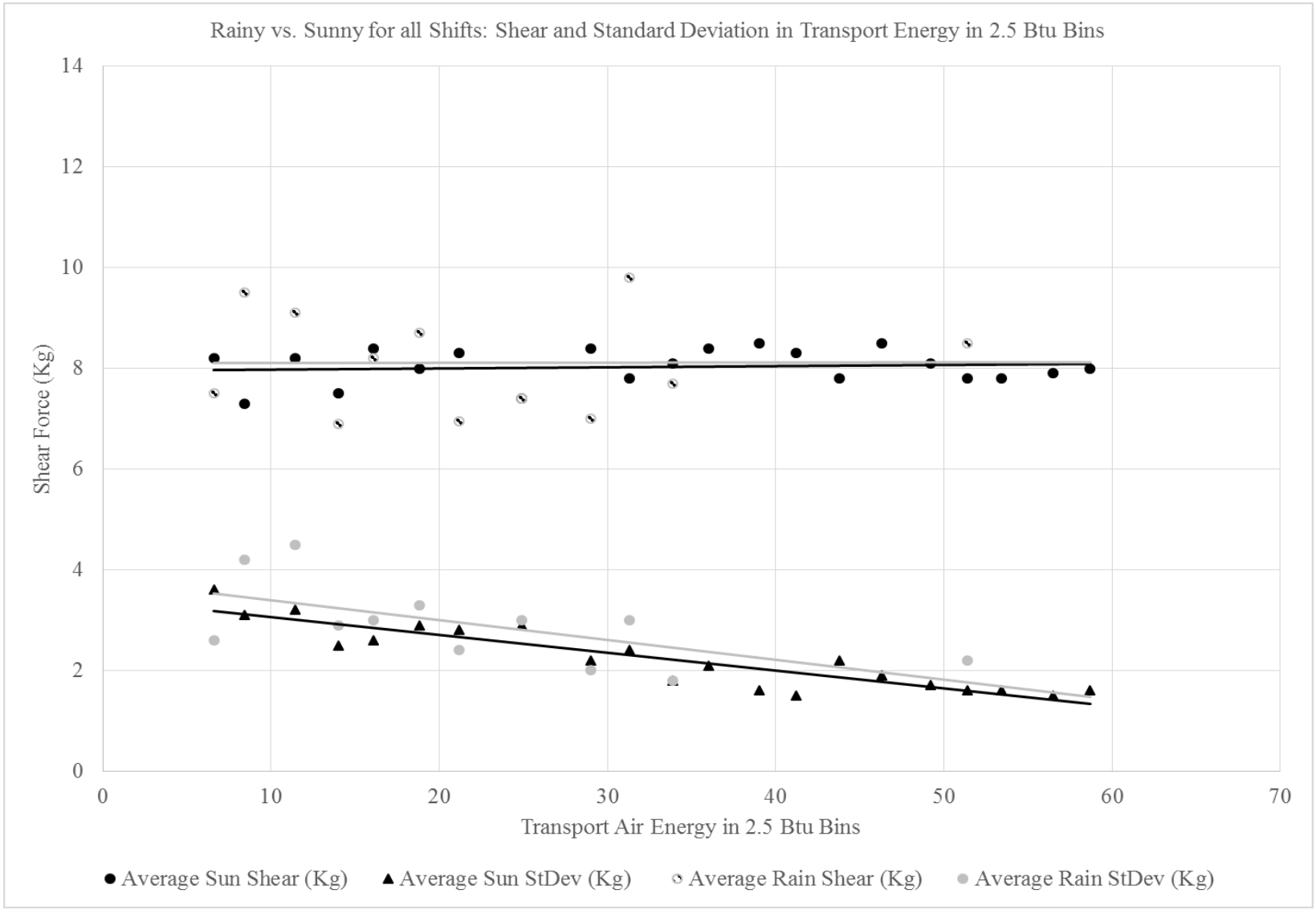

Figure 1.19 Weather classed in Rain vs. Sun for both Shifts Transport Air Energy in 2.5 Btu Bins impact on Shear (Kg) and Shear Standard Deviation (Kg)

DISCUSSION

The use of flock shear data across a consecutive calendar year was used as a means of determining if relationships exist between transport air temperatures, air energy, weather with the quality attribute of tenderness of cooked breast meat and tenderness variation between flocks. Flock shear data, not specific bird shear samples were used in all of the analysis expressed in this document to account for the impact of individual farm animal husbandry practices and housing conditions. The broiler houses used for growing birds that provided the shear data for this analysis were tunnel houses cycling flocks for production between four to five times per year. The frequency of turns indicate that flocks from available houses provided flocks through all seasons providing additional variation in the data. Production practices provided random samples from the flock populations by alternating cage loading to the bird dumper between trailers and through the mixing action of the bird spin chillers. Randomizing the population in production provides the best available representative sample of the population helping in filtering out data noise from bird location in the house during grow out, time on trailer after catch, time in house during catch. Twelve birds from each house is the best available representation of the population given the sample processing constraints. The overall number of samples throughout the testing combined with the random mixing in production aligned with consumer comments on meat that was perceived as tough. The alignment between qualitative and quantitative shear results indicate that the sampling process provides valuable information for analysis and management practices.

There were uncontrollable variables that occurred throughout the year such as transport time, transport noise, variations of inertia with the trailer during transport, relative position of the coop on the transport truck, and time the loaded trailer was required to wait in the production holding sheds prior to processing. The shear data collected was consistently processed in a controlled

environment by trained technicians; interactions between the dependent and independent variables should have a high degree of reliability that could potentially lead to solutions that will improve the quality of broiler meat.

Initial data analysis was done using the dependent variable of individual bird shear data with the specific independent variables of temperature, air energy, and weather. This strategy resulted in no meaningful data relationships leading to the conclusion that if relationships between shear and external stimuli existed, another method of examining the data would need to be used.

Analyzing the flock data indicated that there were significant levels of impact on flock shear performance indicated by maximum shear values and maximum shear standard deviation with specific transport condition stimuli. The impact of animal husbandry practices, transport times as well as other external conditions imposed on the flocks during catch and transport are not directly investigated in this research. Research results will be presented in a following chapter that show a potential interaction between the impact of the flocks' house micro environment and the flocks' cooked breast meat characteristics regarding tenderness.

Analysis of the data from 346 flocks across two production shifts clearly indicate that there is a relationship between transport temperature, weather and flock shear values. Exposure of birds to extreme temperatures have been found to cause high fillet shear values [23]. The results from the tests presented in this research indicate that birds exposed to colder transport temperatures are significantly tougher than birds handled at warmer temperatures. Petracci et al. (2001) [2], indicates that fillet shear values from two hour post mortem birds yield significantly more tender fillets than birds held at higher temperatures.

The test procedures from the data presented in this paper were held for 24 hours prior to processing; the increased hold time changed the characteristic of the meat with regards to

tenderness. The inverse relationship between transport air temperature and the standard deviation of the flocks is a key finding in this research. Decreased transport air temperatures are shown to cause a significant increase in the degree of variation of maximum shear values within a flock. The combined shift 1 and shift 2 flock data was further analyzed by creating flock data bins in increments of 4 ^{0}F for analysis. Analyzing the data with this method presented no significant difference between transport air temperature and shear ($P = 0.3483$) for 346 flocks. There was a significant relationship found between air temperatures and the average standard deviation for the binned flock data ($P < 0.0001$). Warmer air temperatures led to significantly lower flock shear standard deviations.

The energy of the transport air did not have a significant influence on the maximum flock average shear values regardless of shift. A Duncan Multiple Range Difference test was performed by grouping the calculated energy values of the transport air into increments of five, with air energy values ranging from 10 Btu's to 65 Btu's and comparing the maximum average flock shear and maximum flock shear standard deviation values indicated there was no interaction with the energy of the transport air with cooked breast meat tenderness for either shift one or shift two. Within both production shifts, the energy of the air as a function of both sensible and latent energy components had no significant impact on the flock's maximum shear values. The combined shift 1 and shift 2 flock data was further analyzed by creating flock data bins in increments of 2.5 Btu for analysis. Analyzing the data with this method presented no significant difference between transport air energy and shear ($P = 0.5932$). There was a significant relationship found between air energy levels and the average standard deviation for the binned flock data. Higher air energy levels led to significantly lower flock shear standard deviations.

This leads to the conclusion that warmer transport temperatures and associated higher energy air levels during transport is a primary environmental factor leading to an impact on the overall variation of cooked breast meat toughness. Lonergan et al. (2010), [25] proposed that oxidized myofibular protein potentially led to increased disulfide and di-tyrosine protein cross linking leading to increased toughness in meat. It is well known that cold air is denser and will contain an increased volume of oxygen. Increased levels of oxygen in the muscle [25] and reduced glucose levels synergistically may led to an increase in both average flock shear and flock shear SD values.

The impact of weather with regards to rain within a shift approached but failed to reach significance on shift one ($P = 0.0620$) on maximum average flock shear (kg) and failed to reach significance with average flock shear within shift two ($P = 0.3752$). The impact of weather regarding rain transport conditions within shift one on the flocks average shear standard deviation approached but failed to reach significance ($P = 0.0853$). Shear values for rainy conditions within shift two failed to indicate an interaction on flock shear standard deviation ($P = 0.1467$). This is an indication that the sensible temperature on the flock during transport may be a key component in the resulting flocks' tenderness performance after processing.

The difference in shear standard deviations between shifts appear to be largely affected by the transport temperature and the energy of the transport air. There was not a strong relationship between flock standard deviation and weather as classified rainy or sunny. It appears that when a flocks' transport environment is significantly changed from the grow-out environment where temperature and humidity are controlled, there is a significant measurable impact on the level of tenderness variation as measured by the standard deviation of the samples.

Binned flock data was also analyzed combing data from both shifts; significance between flock shear and rainy weather and air temperature approached significance (P = 0.0699). A significant relationship between rainy weather, temperature and flock standard deviation (P = 0.0005). In both cases warmer air temperatures led to cooked fillets that were less tough with improved shear consistency. Binned flock data was also analyzed combing data from both shifts; no significance between flock shear and sunny weather and air temperature was found (P = 0.7775). A significant relationship between sunny weather, temperature and flock standard deviation (P = 0.001). Warmer air temperatures during sunny transport conditions led to cooked fillets with improved shear consistency.

It is reasonable to believe that other conditions such as transport time, impulsive loads to the transport trailer during live haul, road noise or others that deviate from the flock's growth environment will serve to stress a portion of the population to a degree where meat texture is negatively impacted as defined by high shear values and excessive shear variation in the packaged meat. It was reported that one possible explanation of increased stress is the impulsive movements of the birds in the cages during transport [37]. Interpretation that significance in maximum shear values was reached between the two production shifts leads to a potential relationship with other external stimuli on the flock's performance. External factors such as a different set of sounds that the bird is accustomed to or a difference in visual stimulation may contribute to observed differences in shear values.

As birds are handled, caged and transported a portion of the population move from being stressed to becoming distressed and the sympatric nervous becomes active, triggering a fight or flight reaction [18]. The birds location on the truck, specifically animals located on the outside of the trailer are exposed to higher wind speeds, moisture from fog or rain and traffic noise, all of

which are significantly different than the environment how the bird has experienced life. The results presented regarding relationships between transport temperatures and maximum shear values does not conflict with Dadgar et al. (2010) [23] who found that transport conditions of birds located in various location on the trailer and transported under temperature's ranging from < 0 C to 30 C had no significant impact on average maximum shear values of the pectoralis major muscle; there was no mention in these results of the impact on shear variation due to cage location on the trailer and transport temperature.

The normal industry process of catch and haul are stressful on a bird that has not experienced any significant level of stress during its life cycle. Once distress is reached, epinephrine as well as norepinephrine both hormones and neurotransmitters belonging to the chemical class of catecholamine will be released and are well known to break down glycogen to glucose at the tissue level and in the liver making immediate energy available to the muscle [13]. The increased available energy made available from the activation of a fight or fight response combined with the birds in a crate that is providing limited movement [18], will lead to the energy stores remaining available to the muscles during the transport phase of poultry production. The excess available energy in a post mortem bird may act to lengthen or delay completion of rigor mortis leading to breast meat with higher maximum shear values creating increased shear variation within a flock. The increased delay phase of rigor mortis and that some of the fillets are removed from the carcass without completing the conversion of muscle to meat will inherently have a higher probability of meat being perceived as tough.

Literature references that colder temperatures lead to the birds decrease of their energy stores in maintaining body temperature [23] leading to a possible conclusion that the flocks may be capable of completing rigor at a higher rate during processing thus lower maximum shear values.

This is contrary to the findings of this research; large sample size of the data presented inherently indicates increased accuracy of the findings presented in this document.

BIBLIOGRAPHY

1. Li, C., P. Shi, C. Xu, X. Xu, and G. Zhou. 2010. Tracing processes of rigor mortis and subsequent resolution of chicken breast muscle using a texture analyzer. J. Food Engineering 100:388-391.

2. Petracci, M., D. L. Fletcher, and J. K. Northcutt. 2001. The effect of holding temperature on live shrink, processing yield, and breast meat quality of broiler chickens. J. Poultry Science 80:670-675.

3. Lee, Y. B., G. L. Hargus, E. C. Hagberg, and R. H. Forsythe. 1976. Effect of antemortem environmental temperatures on postmortem glycolysis and tenderness in excised broiler breast muscle. J. Food Science 41:1461-1465.

4. Lyon, B. G., and C. E. Lyon. 1997. Sensory Descriptive Profile Relationships to Shear Values of Deboned Poultry. J. Poultry Science 62:885-887.

5. Goodwin, T. L., L. D. Andrews, and J. E. Webb. 1969. The influence of age, sex and energy level on the tenderness of broilers. J. Poultry Science 48:548-552.

6. Lopez, K. P., M. W. Schilling, and A. Corzo. 2011. Broiler genetic strain and sex effects on meat characteristics. J. Poultry Science 90:1105-1111.

7. Lonergan, S. M., N. Deeb, C. A. Fedler, and S. J. Lamont. 2003. Breast meat quality and composition in unique chicken populations. J. Poultry Science 82:1990-1994.

8. Iqbal, M., P. B. Kenney, and H. Klandorf. 1999. Age-related changes in meat tenderness and tissue pentosidine: Effect of diet restriction and aminoguanidine in broiler breeder hens. J. Poultry Science 78:1328-1333.

9. Fletcher, D. L. 2002. Poultry meat quality. World's Poultry Science Journal 58:131-145.

10. Poole, G. H., C. E. Lyon, R. J. Buhr, and L. L. Young. 1999. Evaluation of age, gender, strain, and diet on the cooked yield and shear values of broiler breast fillets. J. Applied Poultry Research 8:170-176.

11. Abdullah, A. Y., and S. K. Matarneh. 2010. Broiler performance and the effects of carcass weight, broiler sex and postchill carcass aging duration on breast fillet quality characteristics. J. Applied Poultry Research 19:46-58.

12. Shinder, D., L. Dror, M. Rusal, V. Rzepakovsky, V. Bresler, and S. Yahav. 2002. Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. Journal of Thermal Biology 27:517-523.

13. Moberg, G. P., and J. A. Mench. 2000. The biology of animal stress: Basic principals and implications for animal welfare. 1st ed. CABI Publishing, New York, NY.

14. Leonard, J. J., J. J. R. Feddes, and J. B. McQuitty. 1983. Air quality in commercial broiler housing. Canadian Agricultural Engineering 26:65-71.

15. Savenije, B., E. Lambooij, M. A. Gerritzen, K. Venema, and J. Korft. 2002. Effects of Feed Deprivation and Transport on Preslaughter Blood Metabolites, Early Postmortem Muscle Metabolites, and Meat Quality. Poultry Science 81:699-708.

16. Bonnet, S., P. A. Geraert, M. Lessire, B. Carre, and S. Guillaumin. 1997. Effect of high ambient temperature on feed digestibility in broilers. J. Poultry Science 76:857-863.

17. Cooper, M. A., and K. W. Washburn. 1998. The relationship of body temperature to weight gain, feed consumption, and feed utilization in broilers under heat stress. J. Poultry Science 77:237-242.

18. Kannan, G., J. L. Heath, C. J. Wabeck, M. C. P. Souza, J. C. Howe, and J. A. Mench. 1997. Effects of crating and transport on stress and meat quality characteristics in broilers. J. Poultry Science 76:523-529.

19. Nijdam, E., E. Delezie, E. Lambooij, M. J. A. Nabuurs, E. Decuypere, and J. A. Stegeman. 2005. Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. J. Poultry Science 84:467-474.

20. Rasmussen, A. L., and M. G. Mast. 1989. Effect of feed withdrawal on composition and quality of broiler meat. J. Poultry Science 68:1109-1113.

21. Mielnik, M., and N. Kolstad. 1991. The influence of feed and water withdrawal time on post mortem biochemical changes and meat tenderness in broilers. Norwegian Journal of Agricultural Sciences 1-8:1-8.

22. Delezie, E., Q. Swennen, J. Buyse, and E. Decuypere. 2007. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. J. Poultry Science 86:1414-1423.

23. Dadgar, S., E. S. Lee, T. L. V. Leer, H. L. Burlinguette, H. L. Classen, T. G. Crowe, and P. J. Shand. 2010. Effect of microclimate temperature during transportation of broiler chickens on quality of the pectoralis major muscle. Poultry Science 89:1033-1041.

24. Ali, A. S. A., A. P. Harrison, and J. F. Jensen. 1999. Effect of some ante-mortem stressors on peri-mortem and post-mortem biochemical changes and tenderness in broiler breast muscle: a review. World's Poultry Science Journal 55:403-414.

25. Lonergan, E. H., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle - Lessons on Mechanisms of meat tenderization. Meat Science 86:184-195.

26. McNeal, W. D., D. L. Fletcher, and R. J. Buhr. 2003. Effects of stunning and decapitation on broiler activity during bleeding, blood loss, carcass, and breast meat quality. J. Poultry Science 82:163-168.

27. Lee, Y. B., G. L. Hargus, J. E. Webb, D. A. Rickansrud, and E. C. Hagberg. 1979. Effect of electrical stunning on postmortem biochemical changes and tenderness in broiler muscle. J. Food Science 44:1121-1122.

28. Ma, R., and P. B. Addis. 1973. The association of struggle during exanguination to glycolysis, protein solubility and shear in turkey pectoralis muscle. J. Food Science 38:995-997.

29. Young, L. L., and R. J. Buhr. 1997. Effects of stunning duration on quality characteristics of early deboned chicken fillets. J. Poultry Science 76:1052-1055.

30. Sams, A. R. 1999. Meat Quality During Processing. J. Poultry Science 78:798-803.

31. Ali, M. S., G. Kang, and S. T. Joo. 2008. A review: Influences of pre-slaughter stress on poultry meat quality. Asian-Aust. J. Animal Science 21:912-916.

32. Khan, A. W., and R. Nakamura. 1970. Effects of pre- and postmortem glycolysis on poultry tenderness. J. Food Science 35:233-267.

33. Mellor, D. B., P. A. Stringer, and G. J. Mountney. 1958. The influence of glycogen on the tenderness of broiler meat. J. Poultry Science 37:1028-1034.

34. Berge, P., P. Ertbjerg, L. M. Larsen, T. Astruc, X. Vignon, and A. J. Moller. 2001. Tenderization of beef by lactic acid injected at different times post mortem. J. Meat Science 57:347-357.

35. Lyon, B. G., and C. E. Lyon. 1996. Texture evaluations of cooked, diced broiler breast samples by sensory and mechanical methods. J. Poultry Science 75:812-819.

36. Sawyer, J. T. 2010. Calculating the Enthalpy of Air. Pages Calculating Enthalpy of Air in Book Calculating the Enthalpy of Air. Editor ed.^eds. IEEE Global Spec, City.

37. Cashman, P. J., C. J. Nicole, and R. B. Jones. 1989. Effects of transportation on the tonic immobility fear reactions of broilers. British Poultry Science 30:211-221.

Chapter 2 Mineral analysis in broiler pectoralis major muscle and the relationship to maximum shear force and shear variation

ABSTRACT

This work investigated if there was any relationship between the mineral content of raw broiler breast meat and the resulting cooked shear levels of the same meat sample. Samples from three geographic locations including eastern Kentucky, south central Missouri and central Arkansas. Samples were collected in the early morning hours for consistency. Early morning sampling was done due to previous findings of higher flock SD shear values occurring during morning bird transport conditions. The minerals investigated (Ca, Mg, P, K, Cu, Fe, Mn, Zn) are those found in varying amounts of most water supply systems providing drinking water to broiler flocks. Broiler breast meat may contain up to 75% water; investigating if a surplus or deficit of a specific mineral impacts cooked breast meat shear values would be valuable to the producer. If a relationship were found, the drinking water could potentially be modified so the target mineral concentration in muscle could be controlled resulting in lower cooked breast meat shear values. From an individual analysis, the data from the three locations indicated weak positive relationships between Manganese, Potassium and Phosphorus with the cooked shear values of broiler breast meat.

Examining average values between minerals and shear and shear SD there was some indication that there may be a relationship between the level of calcium in the drinking water and the flocks average shear and shear SD results.

INTRODUCTION

Food and water intakes of the broiler have been identified as having an impact on the textural characteristics on the resulting meat. Lyon et al. (2004) [1] reported broilers diets consisting of corn were significantly tenderer when compared to broilers with a wheat diet. Minerals that are directly involved in muscle contraction are calcium, sodium, and magnesium. [2] Deficiencies or excesses in calcium, sodium and magnesium along with other minerals found in the pectoral major muscle were analyzed and their respective maximum shear values and examined to determine if there was a relationship with the textural properties of the meat, specifically maximum shear force and shear variation. Finding a relationship between a specific concentration of a mineral or groups of minerals in the raw portion and the cooked maximum shear values could provide an opportunity for the producer to treat the broilers drinking water with mineral supplements improving the quality of the final product through lower average breast meat maximum shear values, decreased shear variation within a flock delivering improved texture consistency. Conversely, if there were a negative relationship between a mineral or group of minerals, the producer could take action to reduce the identified mineral or mineral combination. Changes in water chemistry would be made only if there was no negative impact to the flocks' wellbeing with the result of potentially reducing high maximum shear values and excessive variation in populations maximum shear values.

LITERATURE REVIEW

Diets Impact on Tenderness

Dry Distillers grains (DDGS) are more available due to increased production of ethanol. A diet of 8% DDGS with soy and corn compared to a soy corn control diet showed no impact on the tenderness of broiler breast meat [3]. Breast meat from wheat fed broilers was significantly tougher than broilers fed a corn diet. Similar birds fed milo were no different than either birds fed wheat [1]. Even though the shear values of the fillets were staticically different it was reported that there would be little practical differnce between the diets.

Supplements of various stains of *Saccharomyces cerevisiae* added to a corn soy based diet has been shown to improve tenderness of raw poultry meat. This decrease in toughness was most likely attributed to the prevention of glycogen depletion [4]. Ascorbic acid levels administered at four levels (0, 100, 200, 300 ppm) in the drinking water of during hot and cold seasons had no significant impact on meat quality [4]. Modifications to broiler diets are known to have physiologic effects in the resulting meat leading to the conclusion that there may be a potential relationship to relationship between the composition of feed and water compositions regarding tenderness. It is known that zinc, manganese, and copper have an impact on bird health and growth rates [5]. Diets low in available zinc is associated with low growth rates when compared to birds fed diets not deficient in this mineral. Broilers fed diets with excessive zinc were showed to store the mineral in the tibia, but not in the liver. Conversely birds grown with a diet deficient in zinc had higher levels of this mineral in the liver than birds grown with a diet high in zinc leading that the animal will regulate the availability of zinc to address vital bodily functions ahead of growth rate [6]. Increased levels of organically complexed copper, iron, manganese in

broiler tissue was also found in broilers with diets deficient in this mineral when compared to birds grown with an available abundance of these minerals in their diets [6].

Medicinal herbs such as St. John's wort, small-flowered willow herb, sage and other herbs were found to have varying effects on mineral accumulations in the liver and meat tissues of broilers demonstrating that supplemental broiler diet ingredients can serve as a means for bioaccumulation of minerals in the protein structure of broilers [7]. To further the impact of diet additions, ginseng foliage added to a vegetable diet were also shown to have a positive impact on cook yields ranging from ½ to 1% on product cooked in a continuous steam oven compared to a dissimilar breed control. (M.A. Christie Unpublished Data)

Dietary supplements of vitamin E (α-tocopheryl acetate) when fed to broilers at varying levels throughout the growth cycle was found to have a positive impact on reducing lipid oxidation as indicated by measurements of thiobarbituric acid values. (TBA) Feeding high levels of α-tocopheryl acetate were responsible for increased levels of α-tocopheryl acetate in tissue samples [8].

Arginine and methionine additions to broiler diets increased the soluble collagen content without increasing the total collagen content in test broilers. This change in collagen ratios resulted in lower shear values that were attributed to a reduction in cross bridge development in the collagen structure [9].

Water Supply

Water treatments can have an impact on meat quality; Broilers treated with a 0.5% Sodium Bicarbonate during periods of cyclical heat stress produced breast meat meat with lower shear values than water treated with a 0.5% Potassium Chloride, a combination of 0.5% KCL and 0.5% $NaHCO_3$ water treatment or water receiving no treatment [10].

Improved weight gains and lower rectal temperatures were reported in birds reared in a hot environment with drinking water supplemented at a 0.6% KCL level. It was assumed that weight gain was associated to reduce panting sending the energy that would be used for body temperature maintenance towards growth [11].

Broilers are approximately 70% water by composition; this water is found intracellular, extracellular and in the broilers plasma. Water is introduced to the bird through consumption, and moisture from the feed. Moisture retention occurs through the reabsorption in the kidneys and the rectum. Moisture loss is through respiration, feces and urine [12]. Three causes of water balance in the broiler during periods of high temperatures was hypothesized as: 1) dry oropharyngeal receptors, 2) systemic dehydration, and 3) changes in the temperature of the hypothalamus [13].

Drinking water temperature impacts feed consumption; warm water in the cooler months and cool water in the warmer months help increase feed consumption. Drinking water temperatures assist the bird in maintaining uniform body temperatures during periods of temperature extremes [12].

Feed / Water Withdrawal

Feed withdrawal practices allow the feed in the crop to be digested and mostly passed from the bird to reduce fecal contamination during evisceration. Typically birds are removed from feed for a total of 8 hours prior to processing; water is withdrawn just prior to the catching process. This is not to say that all of the birds in the flock have exactly been off feed for 8 hours. Broilers eat on 4 hour cycles allowing for fewer feeders [14]. Modern houses are designed so that a percentage of the birds can feed at a single time. Within the flock it is expected that a portion of the birds will be off feed for approximately 12 hours prior to catch, another set of birds will be

off feed for 8 hours with the balance of the flock somewhere in between. This variability of available feed stores in the bird's GI tract translates to varying levels of glycogen stores in the liver. Feed withdrawal for 8 hours has been shown to cause glycogen depletion in the liver and breast muscle within three hours [15]. Glycogen is the base molecule that fuels ATP synthesis during the broilers life; up to 36 units of ATP are generated by one molecule of glycogen through each pass of the Krebs or citric acid cycle [16]. The Krebs cycle is halted once the bird is exsanguinated and oxygen is no longer available at the cellular level. Post mortem synthesis of ATP is through the inefficient process of glycolysis that drives increasing levels of lactic acid in the muscle that eventually halts the process after 4 hours, shutting down the synthesis of ATP [15]. The available energy levels in ante mortem broilers have a direct impact on the time of onset of rigor mortis [17].

Time off water (0 – 18h) has been reported not to have a significance influence on meat quality [18]. Mielnik et al. (1991), on the other hand found that meat tenderness decreased on extended periods (12 to 18 h) of feed and water withdrawal [19]. This could be interpreted that time off feed has increased significance in meat tenderness when compared to time off water.

MATERIALS and METHODS

The birds were grown in commercial tunnel-ventilated houses from 3 different geographic regions; western Kentucky, eastern Missouri, and central Arkansas. The target live weight for data set 1 and data set 2 was 6.8 pounds where the target live weigh for data set 3 was 5.4 pounds. Cobb 700 broilers were used in all tests. Sample size was consistent for each of the three sites, (N=106) with a collective sample size of 318.

Evaluation of a breast shear values of carcasses with and without the humerus attached was conducted at the eastern Kentucky facility. Thirty birds from 3 separate flocks that had a single wing removed in evisceration were collected and deboned off line. The breast lobes from the same carcass were identified as pairs and labeled with and without wing. These like pairs were cooked and shear tested in a Warner-Bratzler shear fixture and the results compared. On average, the side of the bird without a wing had higher cooked breast meat maximum shear values. Carcasses without the humerus attached were shown to correlate with cooked breast shear values 0.75 kg higher than a carcass with the humerus attached during the production process.

In all locations, typical poultry production procedures of catch, transport and yard management were utilized. The animals were hand captured and transported to the processing facility in cages on open air trailers. Samples from the respective flocks were naturally randomized at live receiving by typical production procedures. Birds were unloaded from alternating trailers providing a mix of birds captured from various locations at the farm. The birds were processed meaning live hang, stun, kill, bleed, pick, eviscerated and chilled using standard industry methods. Once the birds completed the chilling process with internal meat temperatures less

than 40 °F, they were immediately deboned. Samples were collected throughout the bird lot, with samples collected only from the right side of the bird from birds with the humerus intact. Breast meat from thirty carcass with both wings attached were collected from the production line exiting the spin chiller in three 15 minute intervals from each of the three plants, the nature of comingled birds in a water bath spin chiller transferred to a common hanging conveyor provided a high degree of random samples that were collected from at the start of a production period to ensure samples were collected from the same farm. Samples were deboned from carcasses within 15 minutes of exiting the water bath chiller. The samples were isolated in numbered sterile plastic bags and were layer packed in an ice chest in dry ice and transported to a laboratory for processing. Sample processing was accomplished within two weeks of slaughter. The numbered samples were allowed to thaw to 28 °F so that the origin section from the terminal portion of the keel bone of the PM muscle could be easily sectioned. This raw sample from each respective fillet was placed in a whirl pack bag and numbered to the corresponding fillet sample and immediately refrozen on a tray placed in a -10 °F commercial freezer. The raw samples were analyzed for mineral and metal content using the protocol described by **AOAC 968.08** and **AOAC 990.08**. The hardware used to conduct the analysis was a *Spectro* (Ametek) inductively coupled optical emission spectrometer (ICP-OES).

The tempered fillet samples were sized, with similar sized fillets were placed on a gridded metal rack preventing the meat from contacting the cooking tray. The fillets were completely covered and sealed to the cooking tray with aluminum foil to prevent moisture loss during processing. A freestanding commercial convection oven (*Rational* model SCC 62 G) preheated to 350 °F dry bulb and 200 °F wet bulb with the oven racks placed so sample trays were evenly spaced in the oven. Two trays were thermally processed at the same time; the trays were switched, top to

bottom and bottom to top as well as rotated approximately half way through the cooking process to provide even cooking. Samples were cooked to a minimum of 170 °F but no hotter than 175 °F. Cook temperatures were measured in the center of the shoulder of the largest sample through and integrated temperature connected to the ovens control system. The temperature probe was penetrating through the foil into the shoulder of the thickest fillet immediately after placing the racks in the oven providing for a consistent cooking environment.

Cooked trays of covered product were removed from the oven and allowed to naturally cool to room temperature prior to sample preparation and testing. Trays were kept covered throughout the cooling process to reduce drying of the sample surface. The cooling process typically takes approximately 2 hours for the samples to reach room temperatures. Due to the volume of samples the cooked fillets were cooked and cooled covered in a typical food grade plastic wrap and stored in a commercial refrigerator at a temperature less than 40^0 F for shear processing the following day. The chilled samples were removed from the cooled for approximately 4 hours allowing the samples to reach room temperature prior to cover removal and shear testing.

A 20 mm width sample was removed from the fillet using a rectangular stainless steel fixture. The sample cut was made starting at the insertion and ending at the origin portion of the fillet ensuring the cut was parallel with the directions of the muscle fibers. The individual tempered samples maximum shear force was measured twice on a *Stable Micro Systems* model *TA HD Plus Texture An*alyzer fitted with a Warner-Bratzler fixture and the resulting measurements averaged and the result recorded. The samples were cut at approximately one third down the length of the strip. The two sample shears were averaged to express a representative shear force. (See figure 2.0 in chapter 1 for the cut diagram of a cooked fillet used for shear analysis)

Corresponding raw tissue samples from the processed samples were measured for the following eight elements:

1) Calcium
2) Magnesium
3) Phosphorus
4) Potassium
5) Copper
6) Iron
7) Manganese
8) Zinc

These elements were selected specifically due to the ability of these minerals to vary with the water supplies of the farms. Table 2.0 is the data representation of the analysis of the broiler water supplies from the three farms where the samples were grown. Data was examined as a point to point comparison looking at specific cooked fillet shear values and the respective mineral concentration of a raw portion of the same fillet.

RESULTS

There was no significant relationship between any element specifically or combinations of elements. The strongest relationship was between calcium and shear; the R^2 value was calculated to be less than 10% leaving in excess of 90 % of any variation unexplainable. Shear data was recorded in Microsoft Excel and with statistical analysis conducted with SAS Version 9.3. Table 2.1 is a data summary expressed in averages, standard deviations, maximum and minimum values of the resulting chemical analysis for each of the raw samples (N=106 per farm) as well as the corresponding cooked fillet shear results for three plant locations. Jeon et al. (2010) [20], reported breast meat in native breeds analyzed (N=10) from North Korea and South Korea had higher levels of Ca, Fe, and Mg than that of a commercial broiler breed; the specific commercial breed was not identified. Zn levels in the commercial breed was nearly twice that of the native species analyzed. It is difficult to make comparisons between Jeons' mineral data and texture and the results with the data presented in this paper as Jeons' results were defined in terms of hardness, springiness, cohesiveness, gumminess, and chewiness and not from a comparative method used to measure texture in the data presented in this paper. This is mentioned as it was the single reference found between cooked meat texture and mineral content. Suchy et al. (2002) [21], tested for levels of Ca, P, and Mg in hybrid combinations (Ross 308, Cobb and Hybro). The levels if the aforementioned elements were similar in concentrations between the breeds. In the literature reviewed, there were no reported results between mineral data and textural properties of the samples.

Data was analyzed using the Pearson's correlation to determine if there was a relationship between the specific mineral content of the raw meat samples and the respective cooked shear values. The Pearson correlation coefficients (r) for shear vs. mineral content is reported in Table

2.2. Using the simple linear regression procedure (SAS v 9.3) with the shear held as the dependent variable no significant relationships were found for any mineral level and cooked shear values. There was no magnesium variation found in all raw samples tested. Figures 2.0 through 2.17 are graphical representations of the cooked shear values and mineral content. The data was sorted based on the shear values from highest to lowest with the respective mineral content for each sample assigned accordingly. This method graphically demonstrates the lack of relationship between the individual mineral content concentrations of the raw sample with the corresponding cooked shear value of like samples.

Table 2.0
Average mineral values of livestock drinking water by farm where the poultry samples were collected

	Plant 1	**Plant 2**	**Plant 3**
Mineral	ppm	ppm	ppm
Iron	0.06	<0.05	<0.05
Zinc	0.12	0.06	0.33
Calcium	5.26	2.09	22.84
Copper	<0.25	<0.25	<0.25
Magnesium	1.41	1.55	2.4
Manganese	<0.25	<0.25	<0.25
Phosphorus	5	5	5
Potassium	2.29	2.72	3.53
Sodium	5	7.42	5

Table 2.1
Data Summary of flock cooked breast meat shear (kg) and corresponding mineral content of the respective raw muscle

Plant 1	Shear (kg)	Calcium (%)	Magnesium (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)
Average	4.05	0.01	0.02	0.18	0.27	0.04	1.64	4.61	1.00	5.41
St-Dev	1.26	0.00	0.00	0.02	0.37	0.01	1.05	2.32	0.00	1.84
Max	8.64	0.01	0.03	0.22	0.32	0.08	6.00	16.00	1.00	11.00
Min	1.82	0.01	0.02	0.14	0.21	0.02	1.00	2.00	1.00	3.00
Plant 2										
Average	4.44	0.01	0.03	0.20	0.30	0.04	1.68	4.73	1.00	6.08
St-Dev	1.62	0.00	0.00	0.02	0.02	0.01	0.87	1.72	0.00	1.97
Max	9.94	0.01	0.03	0.23	0.35	0.08	4.00	13.00	1.00	15.00
Min	2.25	0.01	0.02	0.15	0.24	0.03	1.00	2.00	1.00	3.00
Plant 3										
Average	**6.91**	0.02	0.02	0.19	0.28	0.05	1.15	5.24	1.00	5.94
St-Dev	**3.01**	0.01	0.00	0.02	0.03	0.01	0.41	1.56	0.00	1.15
Max	**21.37**	0.07	0.03	0.22	0.33	0.06	3.00	15.00	1.00	11.00
Min	**2.44**	0.01	0.01	0.01	0.10	0.03	0.10	3.00	1.00	4.00

Table 2.2
Pearson's correlation values for tested minerals in raw samples compared with respective cooked fillet shear values

Independent Variable	Pearson's coefficient	Correlation
Calcium	-0.11	Very Weak Negative
Manganese	0.25	Weak Positive
Phosphorus	0.25	Weak Positive
Potassium	0.20	Weak Positive
Sodium	-0.005	Very Weak Negative
Copper	-0.12	Very Weak Negative
Iron	-0.15	Very Weak Negative
Magnesium	0	No significance in detectable levels
Zinc	0.06	Very Weak Positive

Table 2.3
Probability of Significance of Minerals on cooked shear values using a simple regression analysis

Independent Variable	Probability of a type 1 error (false positive)
Calcium	P = 0.4566
Manganese	P = 0.0803
Phosphorus	P = 0.0824
Potassium	P = 0.1685
Sodium	P = 0.9681
Copper	P = 0.4006
Iron	P = 0.9197
Magnesium	No significance in detectable levels
Zinc	P = 0.6752

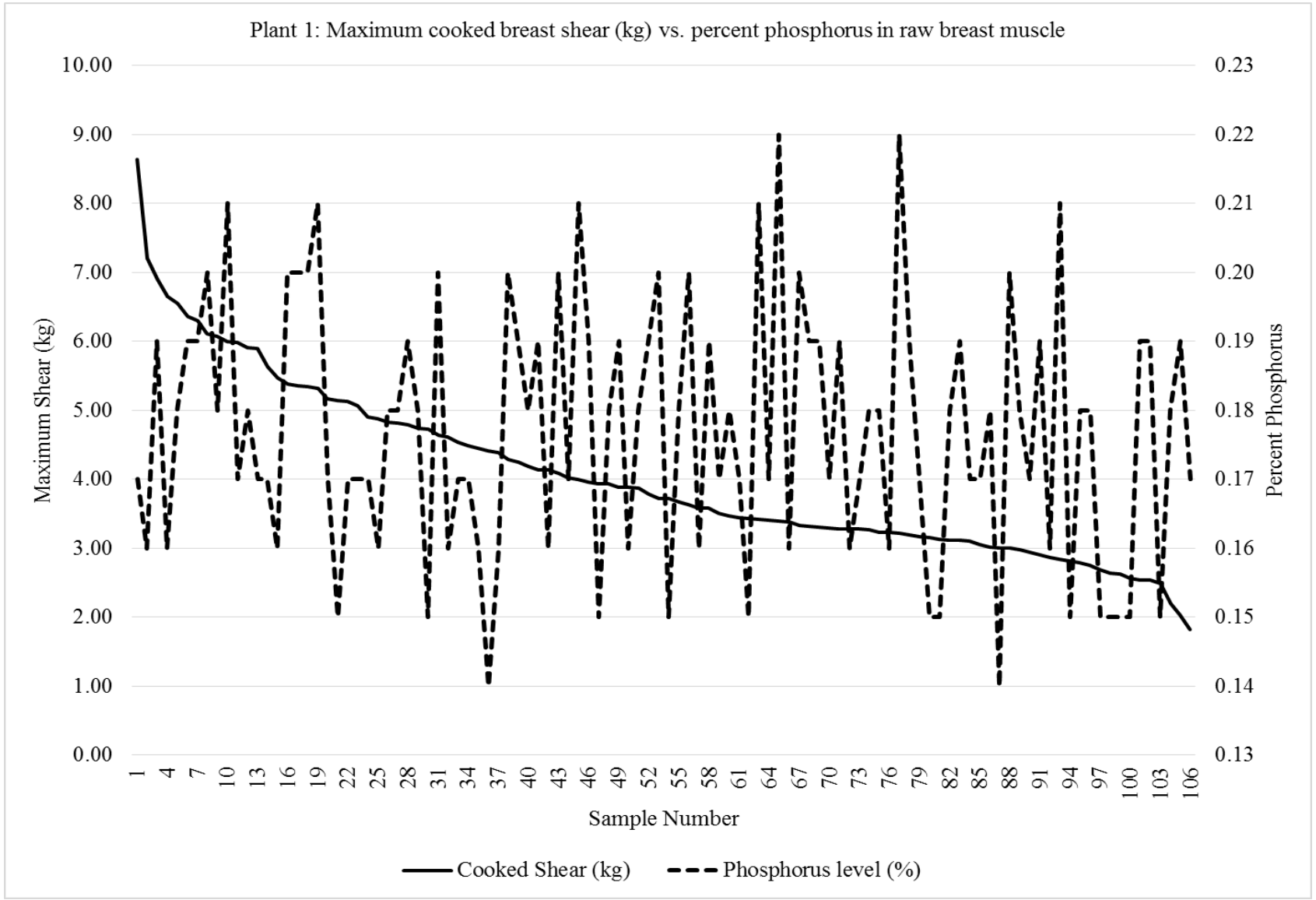

Figure 2.0 Plant 1: Maximum cooked breast shear (kg) vs. percent phosphorous in raw breast muscle

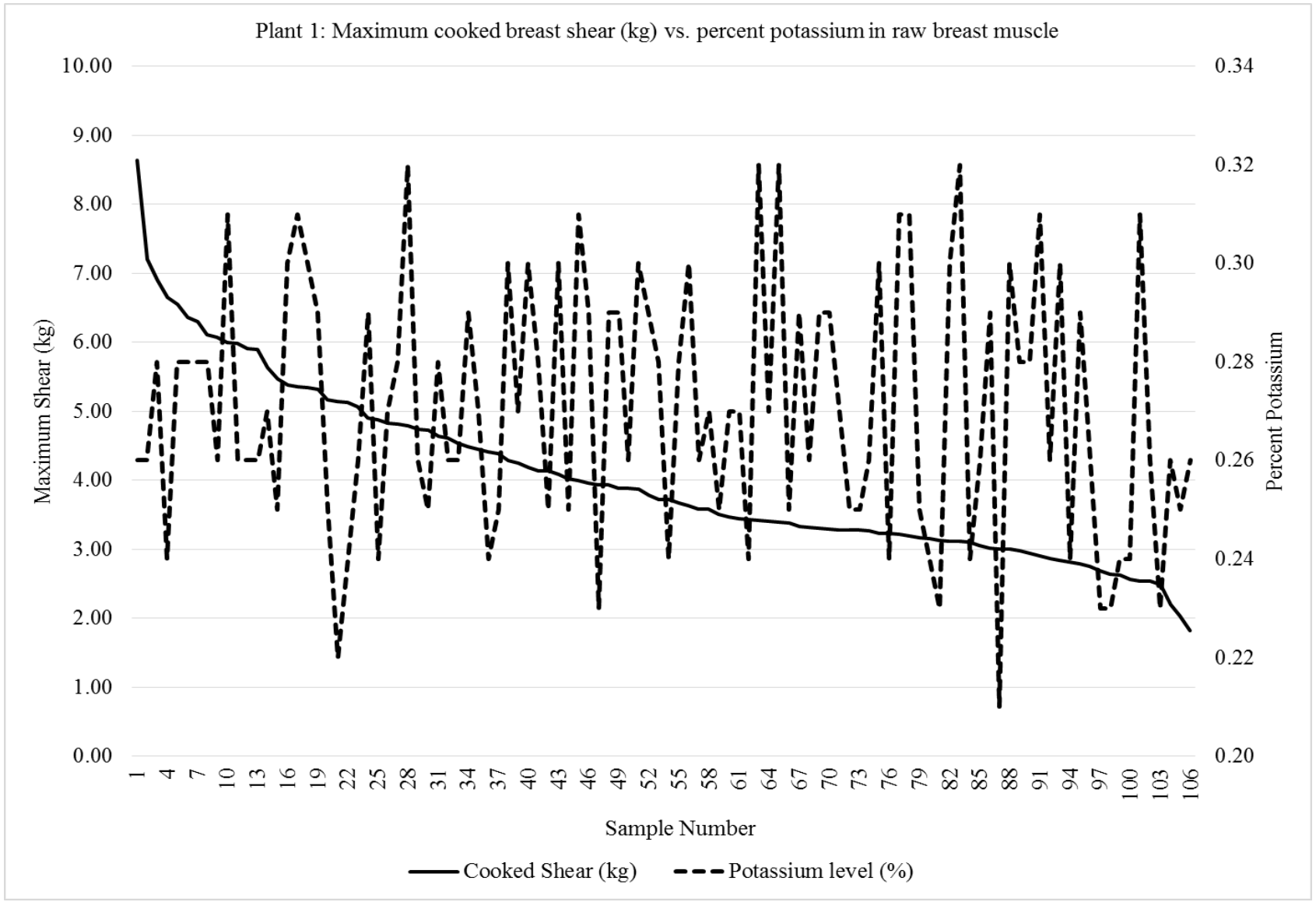

Figure 2.1 Plant 1: Maximum cooked breast shear (kg) vs. percent potassium in raw breast muscle

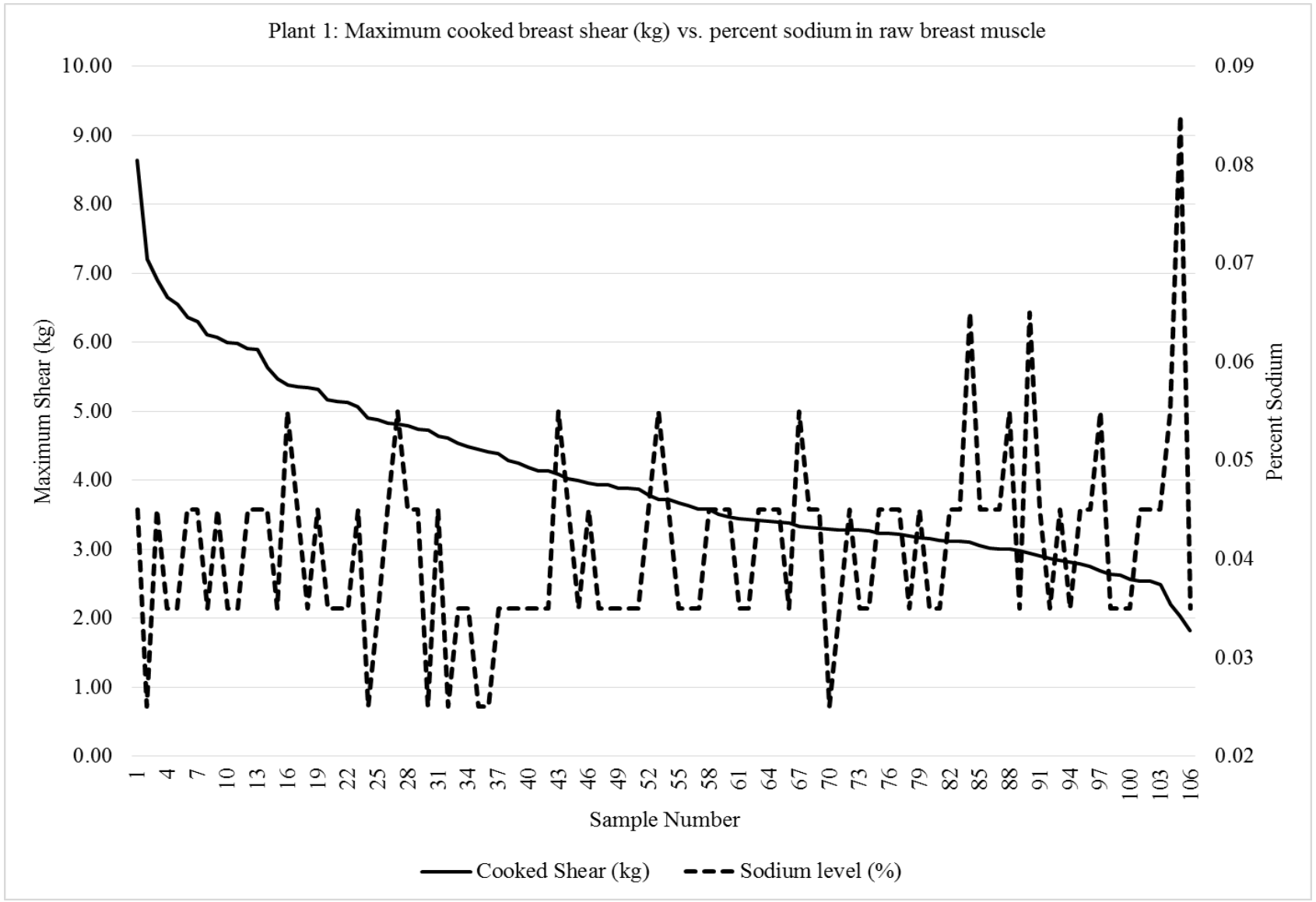

Figure 2.2 Plant 1: Maximum cooked breast shear (kg) vs. percent sodium in raw breast muscle

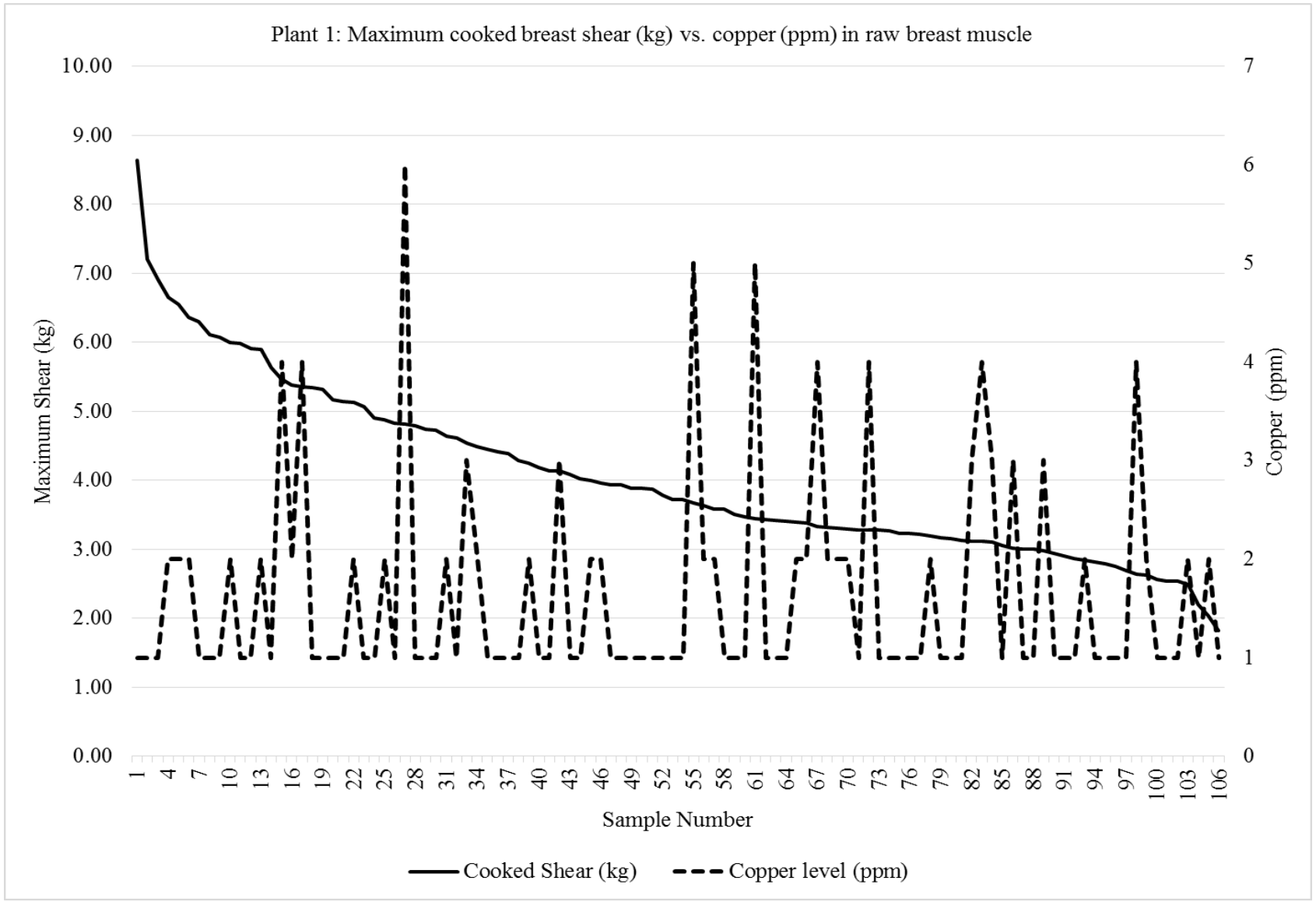

Figure 2.3 Plant 1: Maximum cooked breast shear (kg) vs. copper (ppm) in raw breast muscle

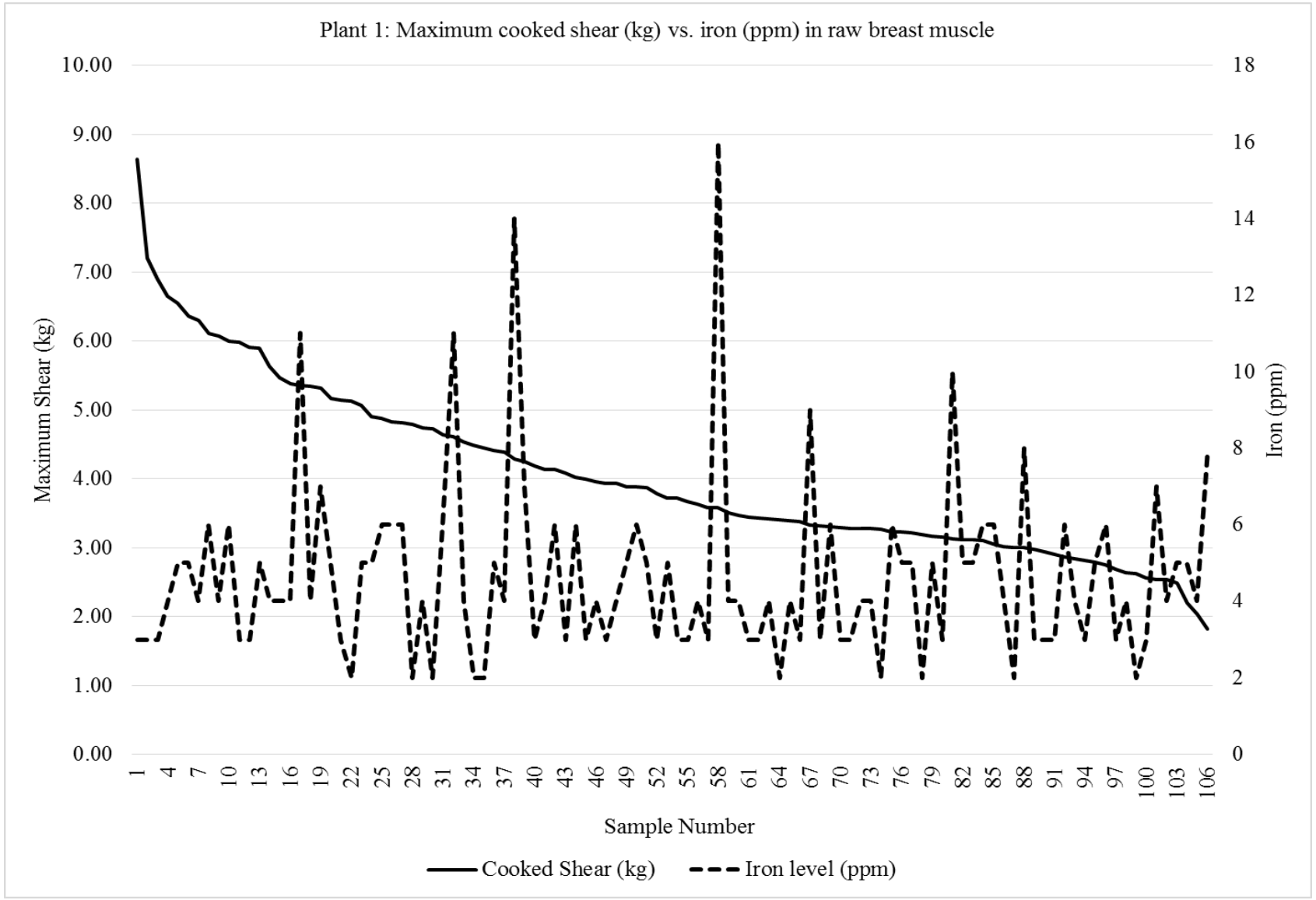

Figure 2.4 Plant 1: Maximum cooked breast shear (kg) vs. iron (ppm) in raw breast muscle

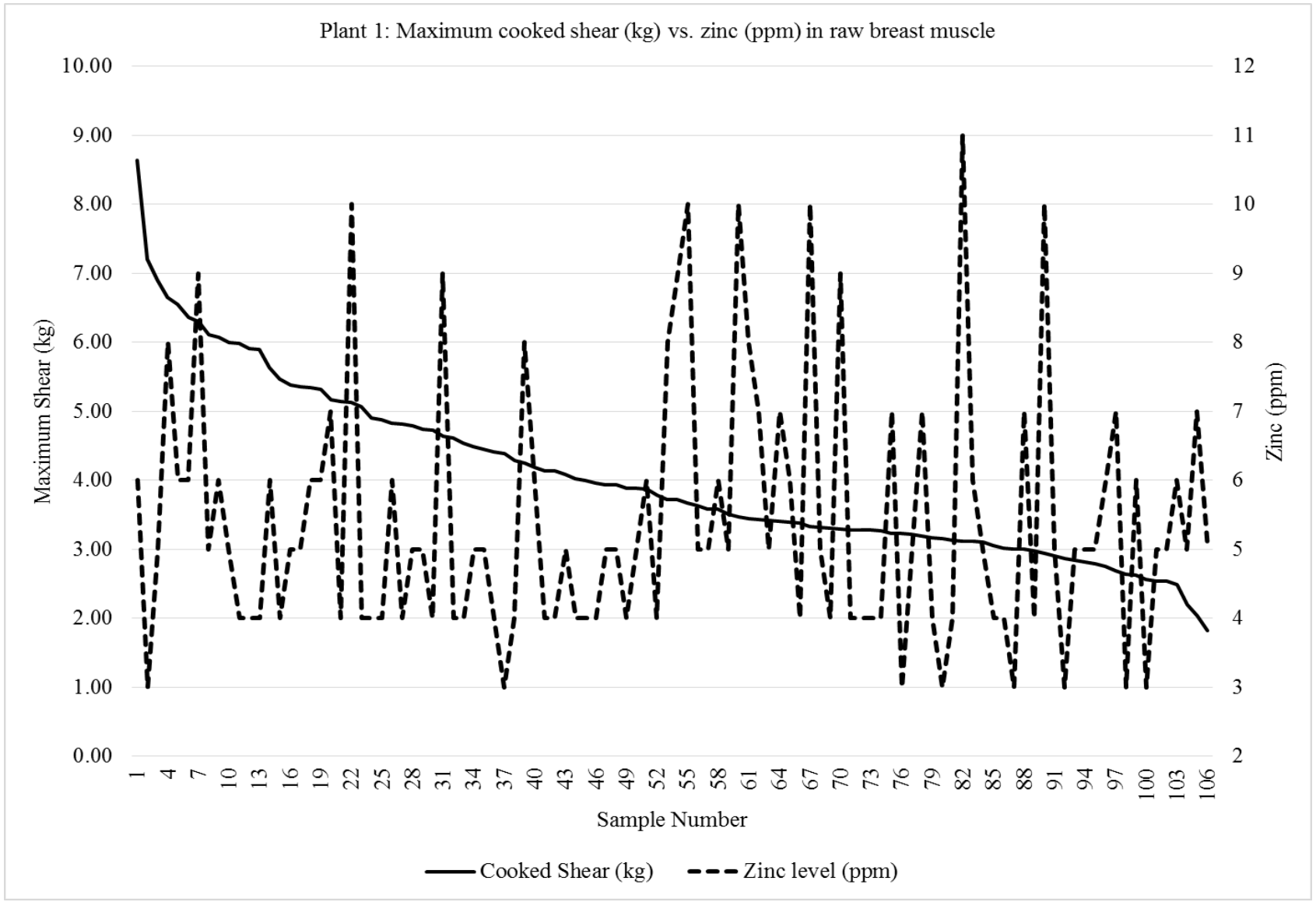

Figure 2.5 Plant 1: Maximum cooked breast shear (kg) vs. zinc (ppm) in raw breast muscle

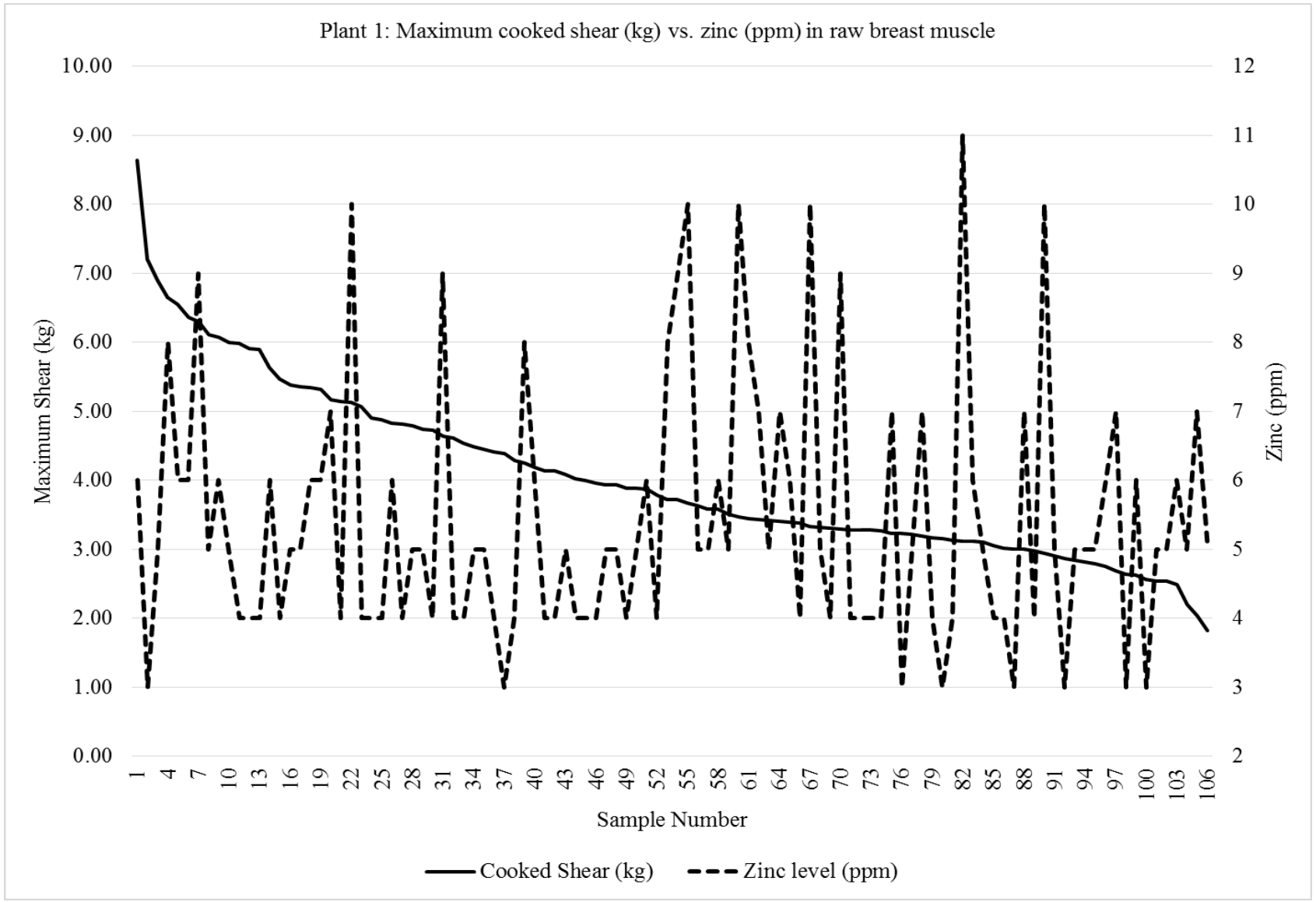

Figure 2.6 Plant 2: Maximum cooked breast shear (kg) vs. zinc (ppm) in raw breast muscle

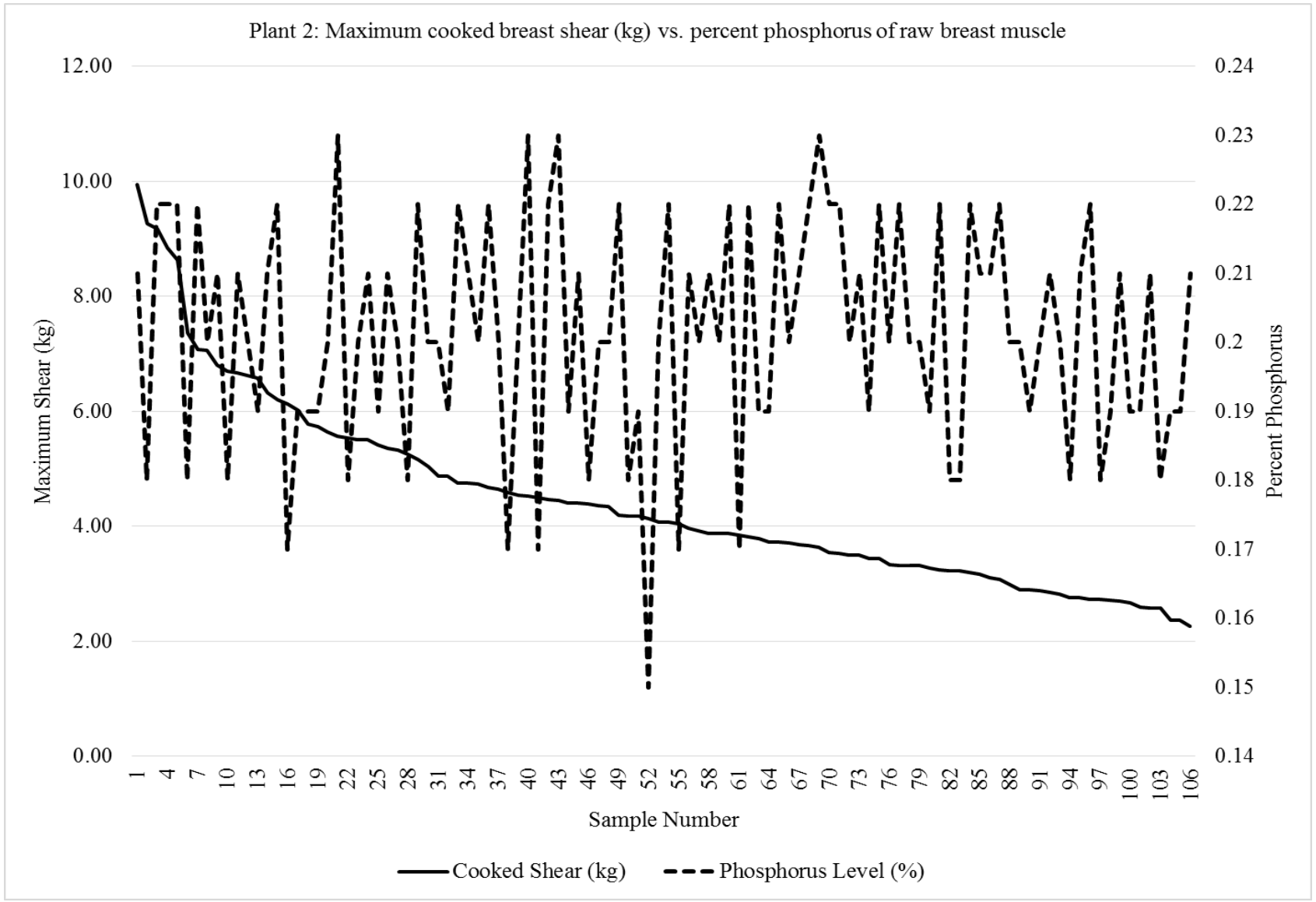

Figure 2.7 Plant 2: Maximum cooked breast shear (kg) vs. percent phosphorus in raw breast muscle

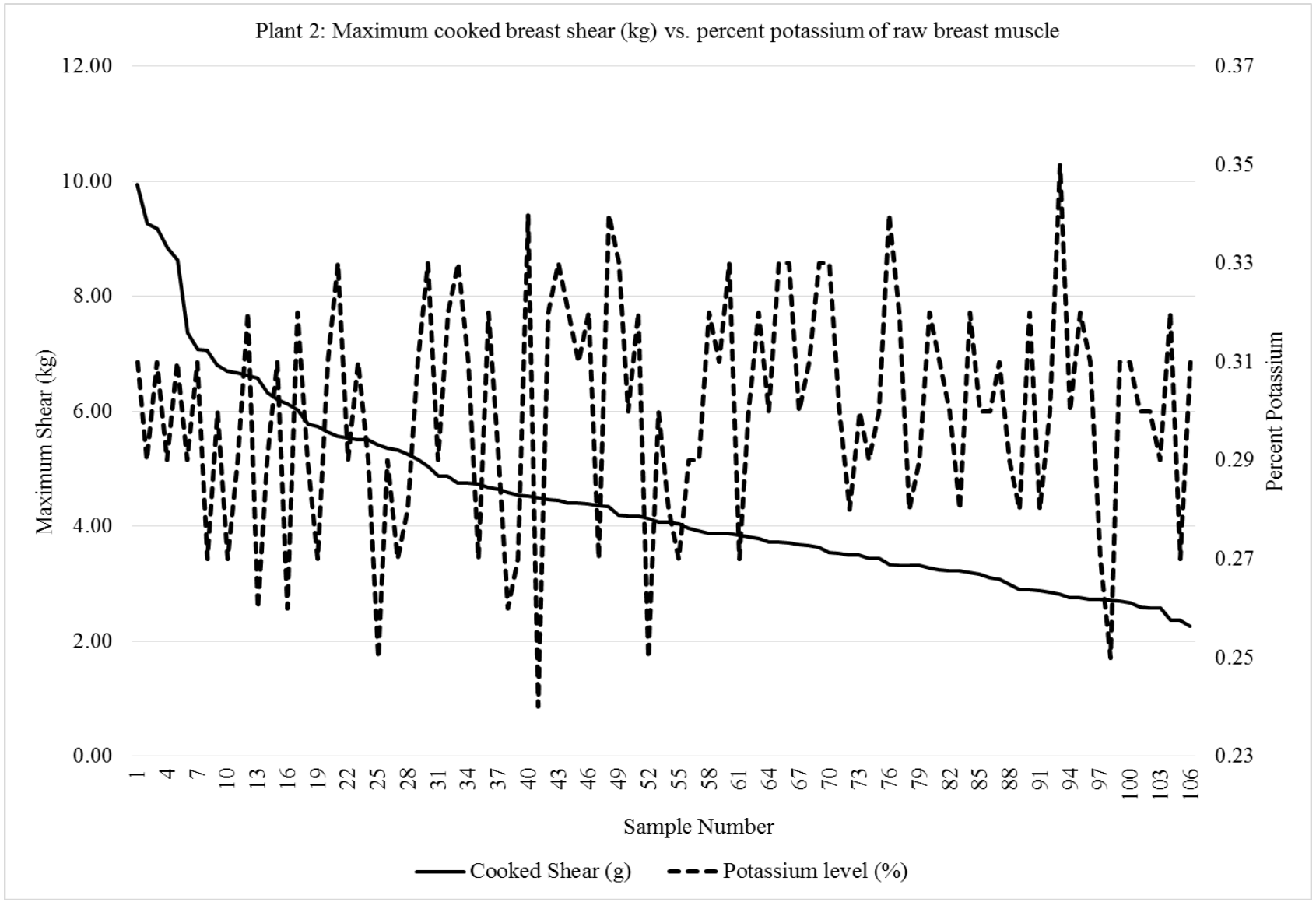

Figure 2.8 Plant 2: Maximum cooked breast shear (kg) vs. percent potassium in raw breast muscle

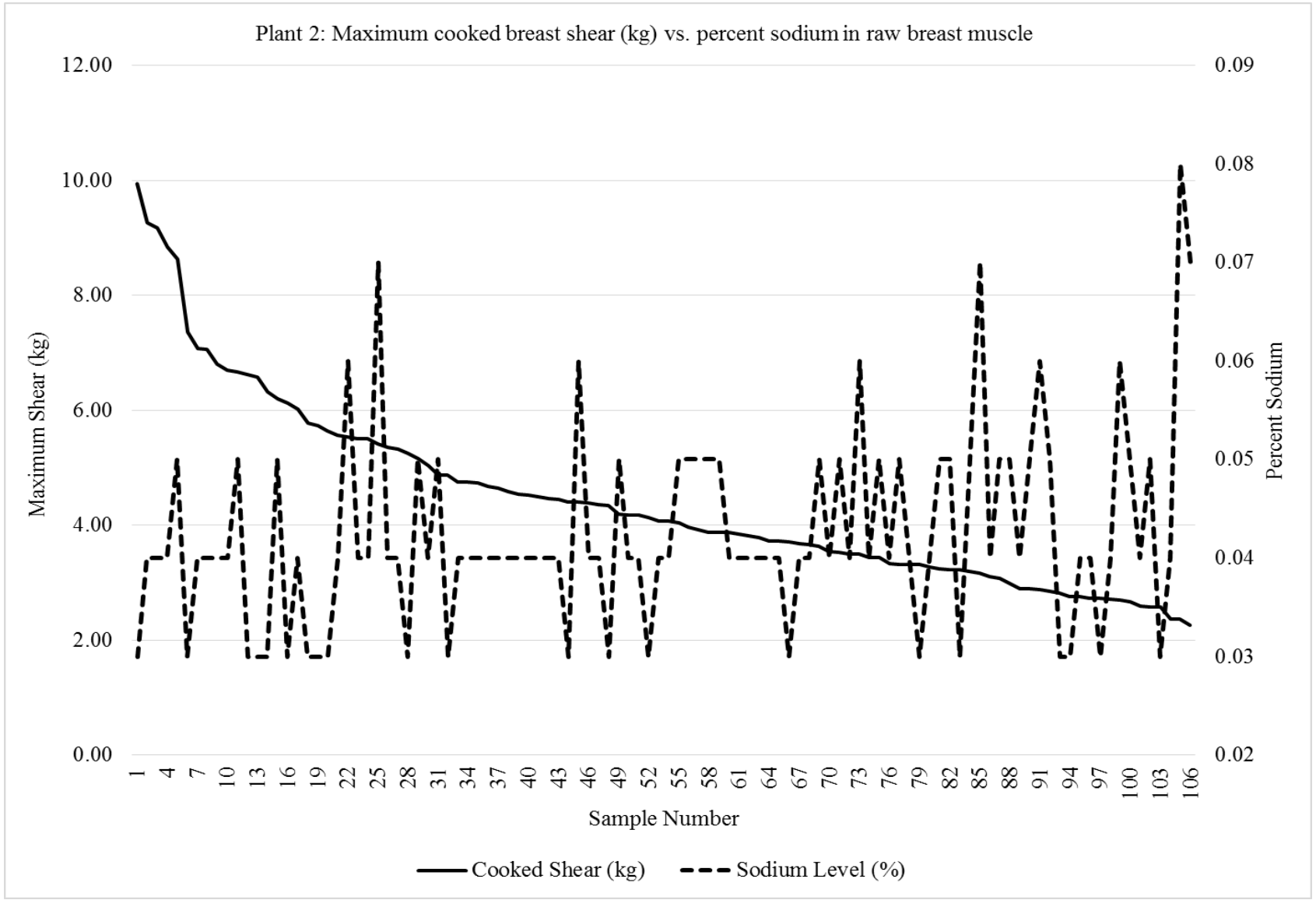

Figure 2.9 Plant 2: Maximum cooked breast shear (kg) vs. percent sodium in raw breast muscle

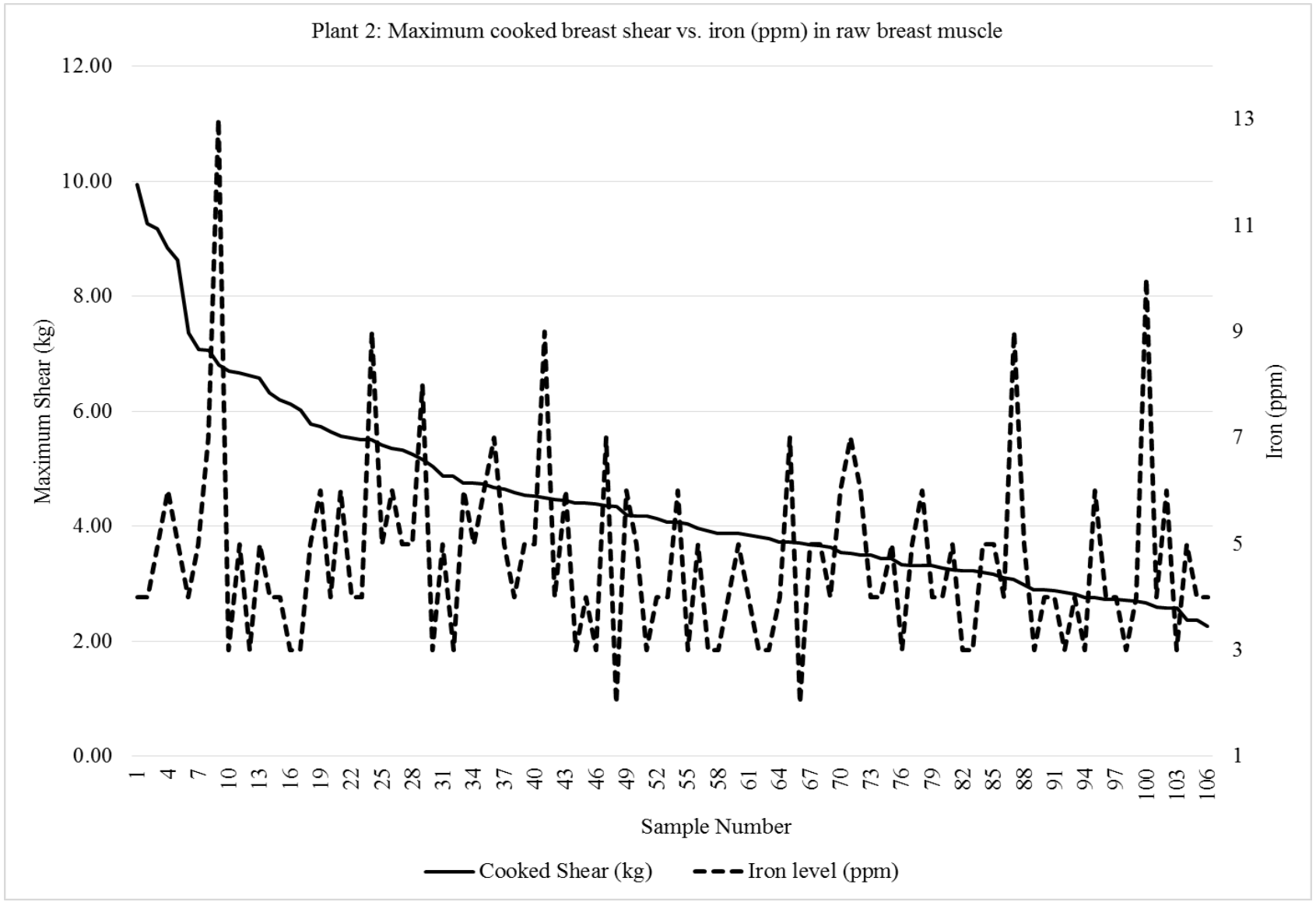

Figure 2.10 Plant 2: Maximum cooked breast shear (kg) vs. iron (ppm) in raw breast muscle

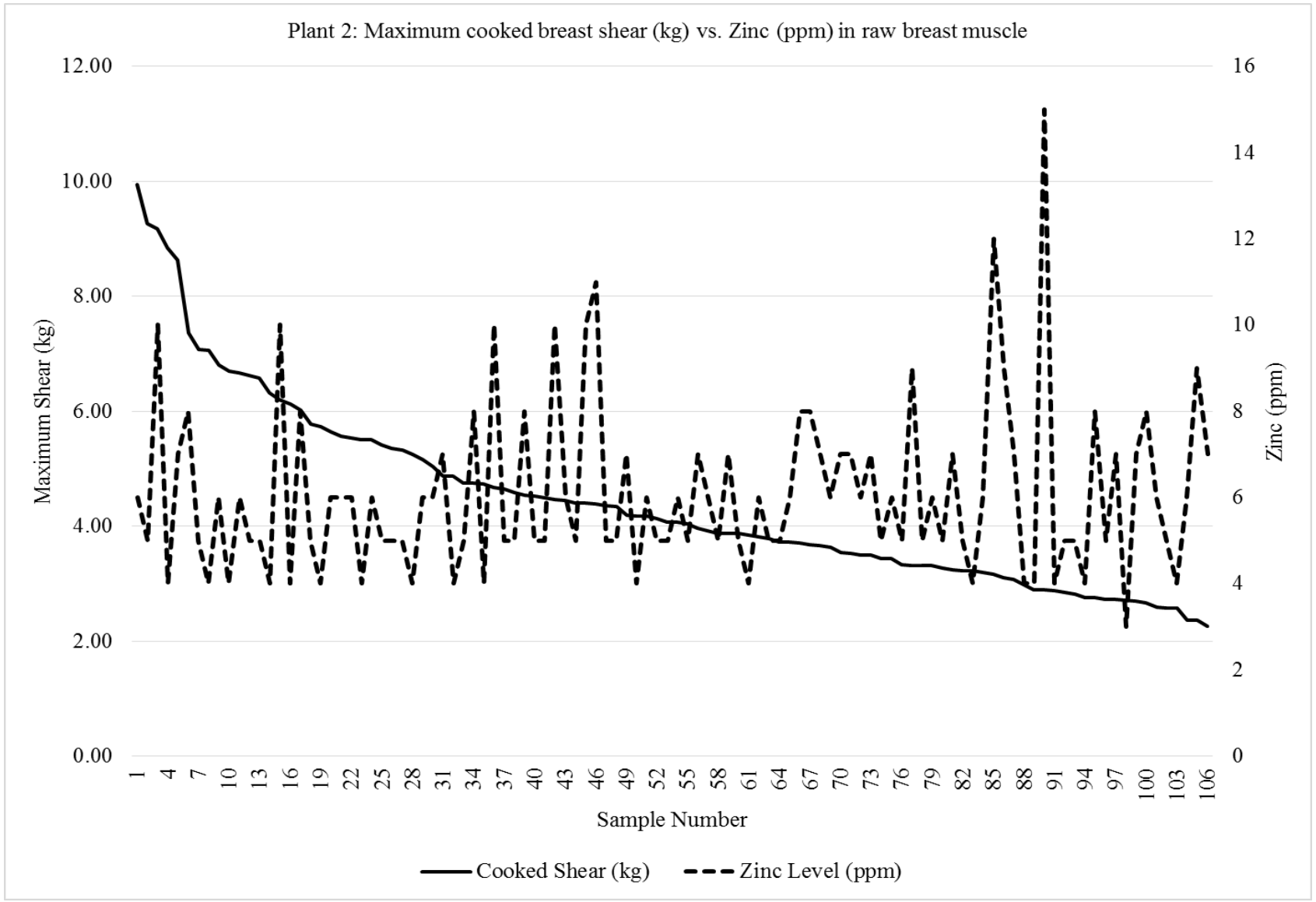

Figure 2.11 Plant 2: Maximum cooked breast shear (kg) vs. zinc (ppm) in raw breast muscle

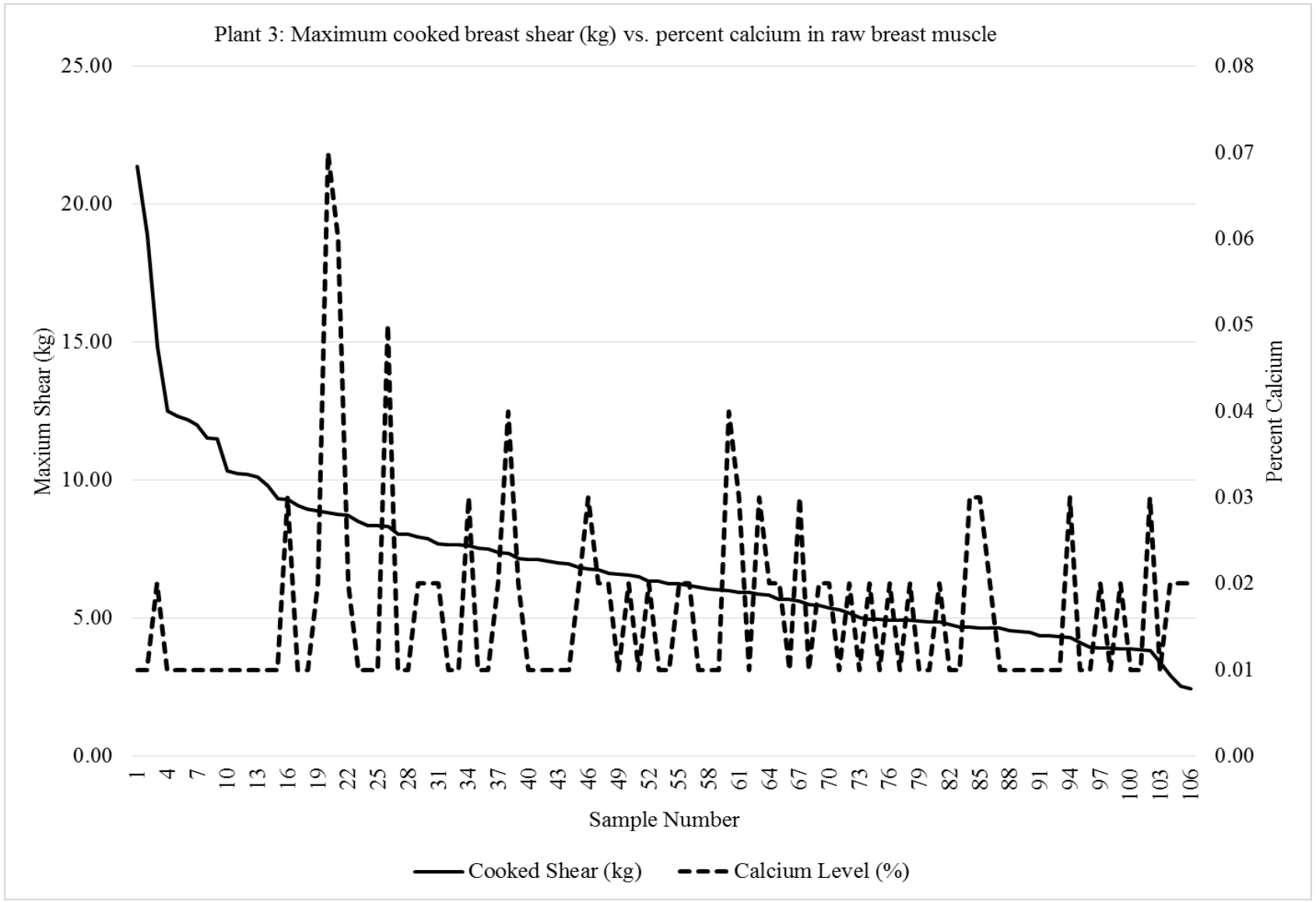

Figure 2.12 Plant 2: Maximum cooked breast shear (kg) vs. percent calcium in raw breast muscle

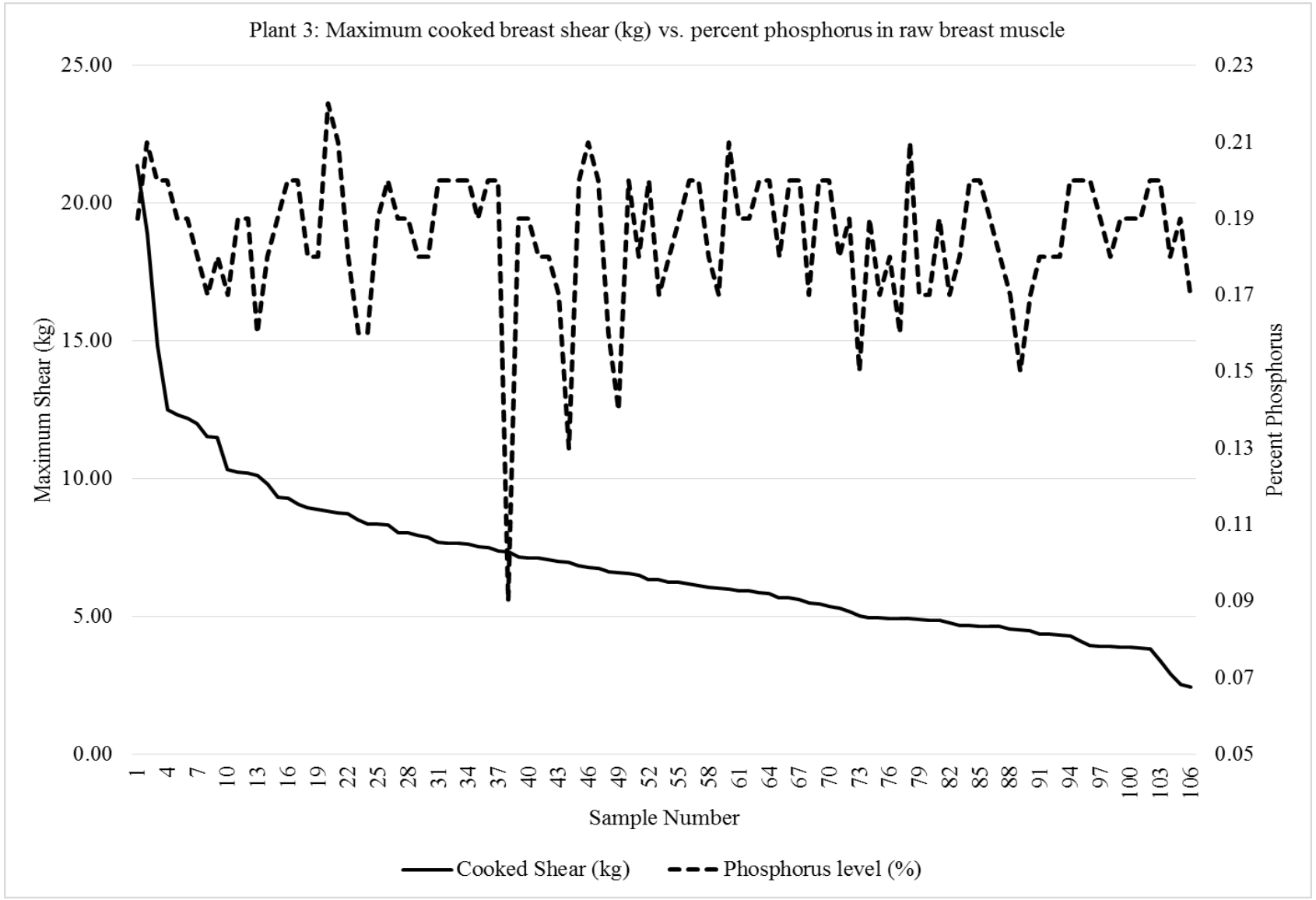

Figure 2.13 Plant 3: Maximum cooked breast shear (kg) vs. percent phosphorus in raw breast muscle

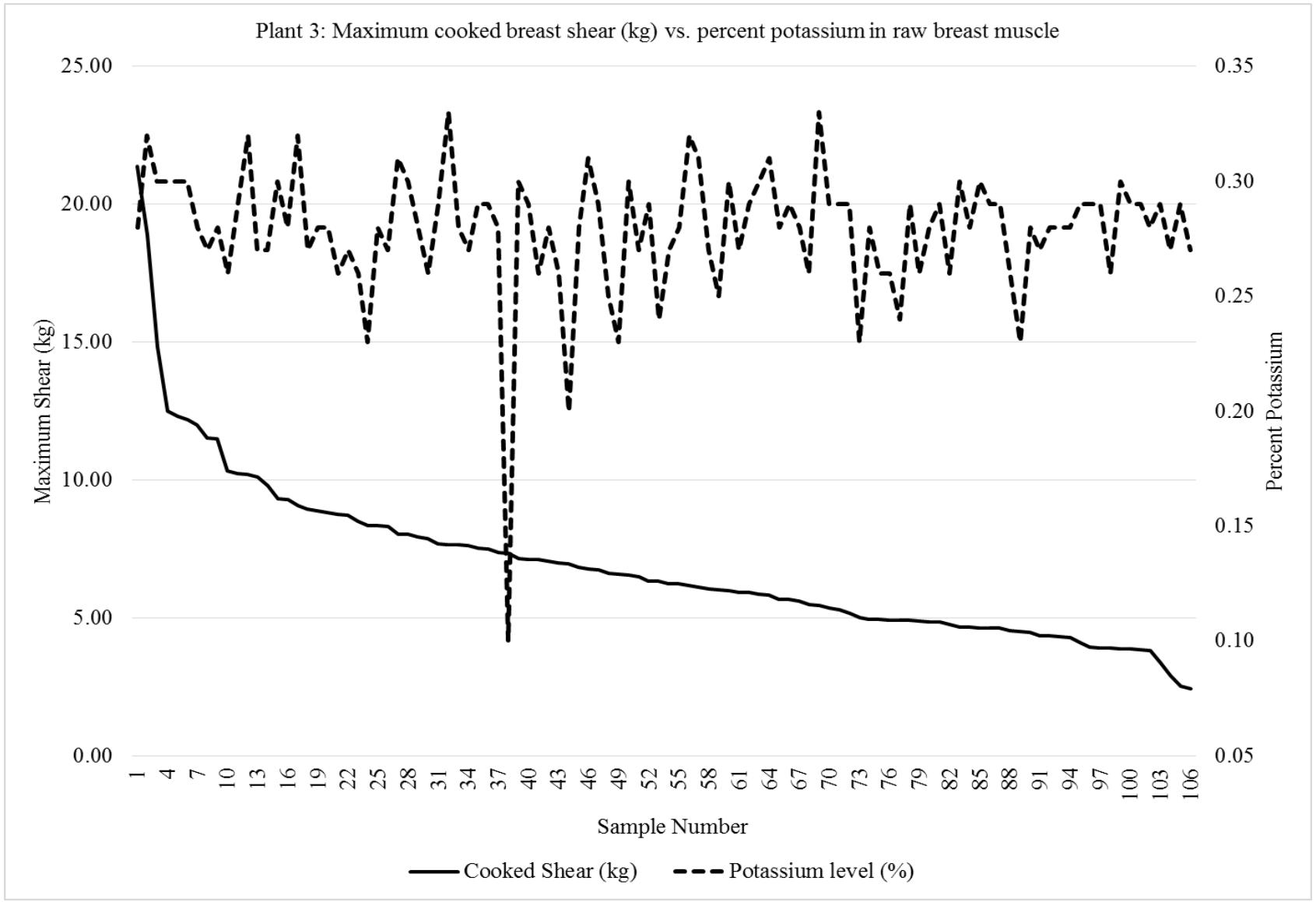

Figure 2.14 Plant 3: Maximum cooked breast shear (kg) vs. percent potassium in raw breast muscle

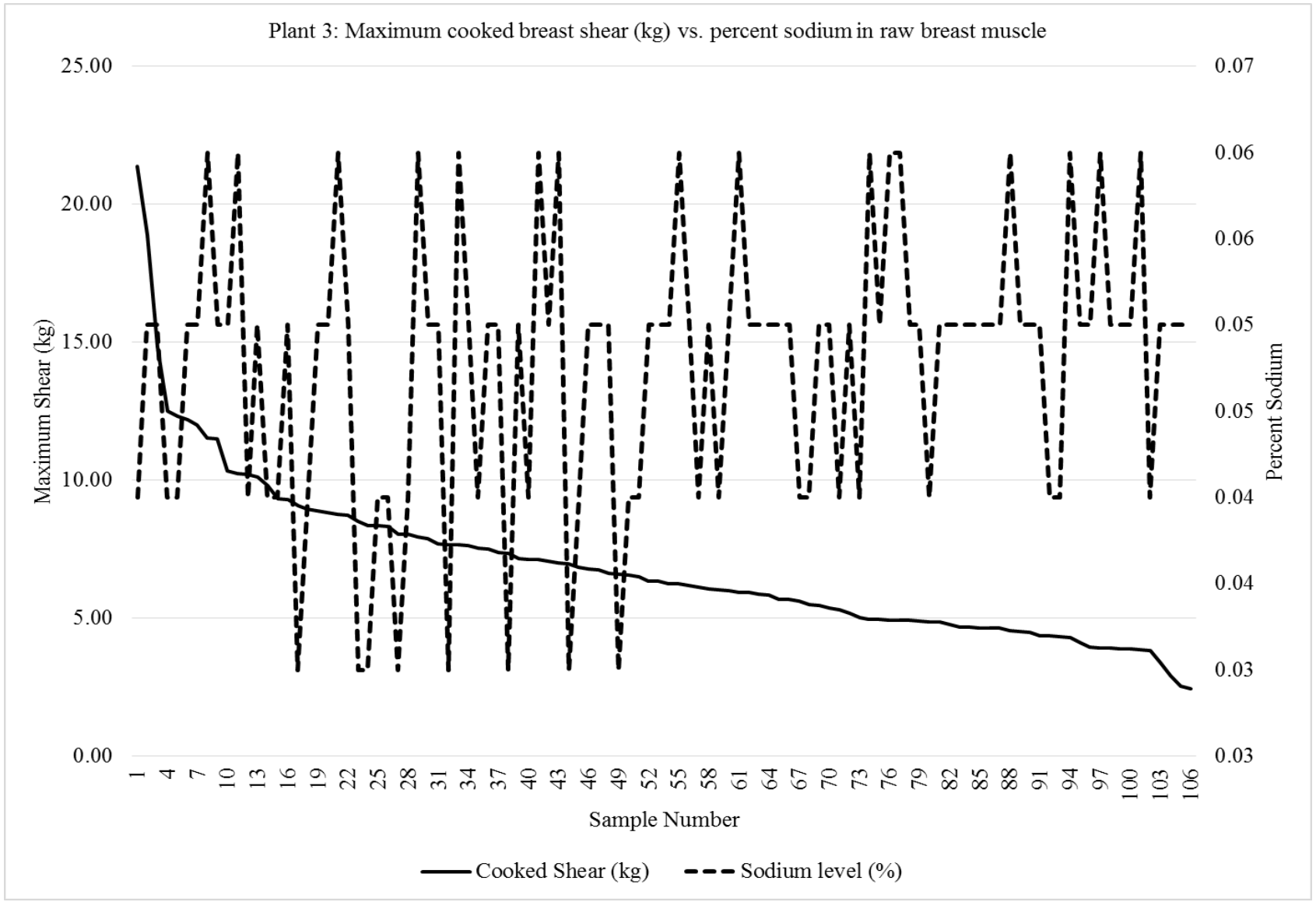

Figure 2.15 Plant 3: Maximum cooked breast shear (kg) vs. percent sodium in raw breast muscle

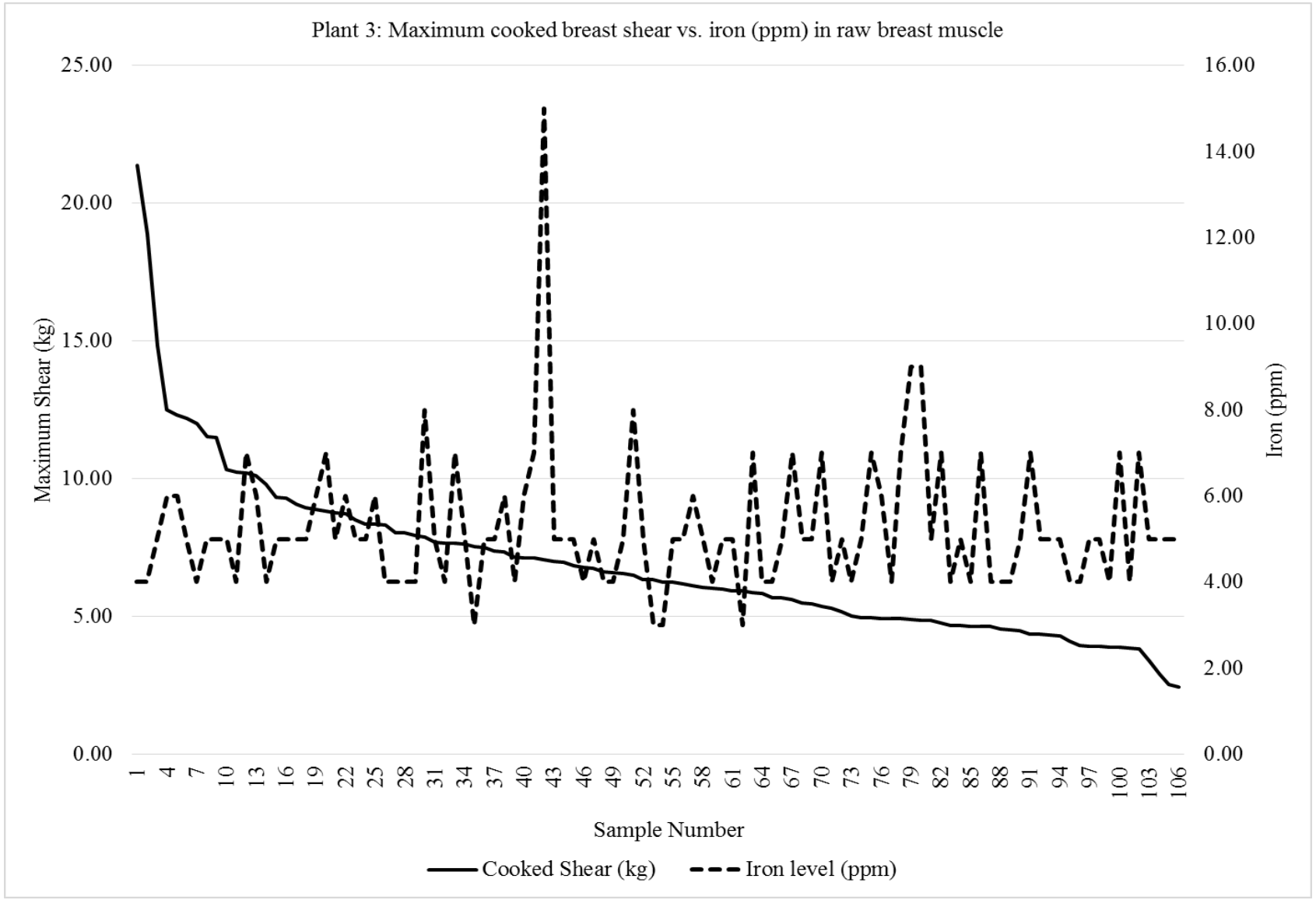

Figure 2.16 Plant 3: Maximum cooked breast shear (kg) vs. iron (ppm) in raw breast muscle

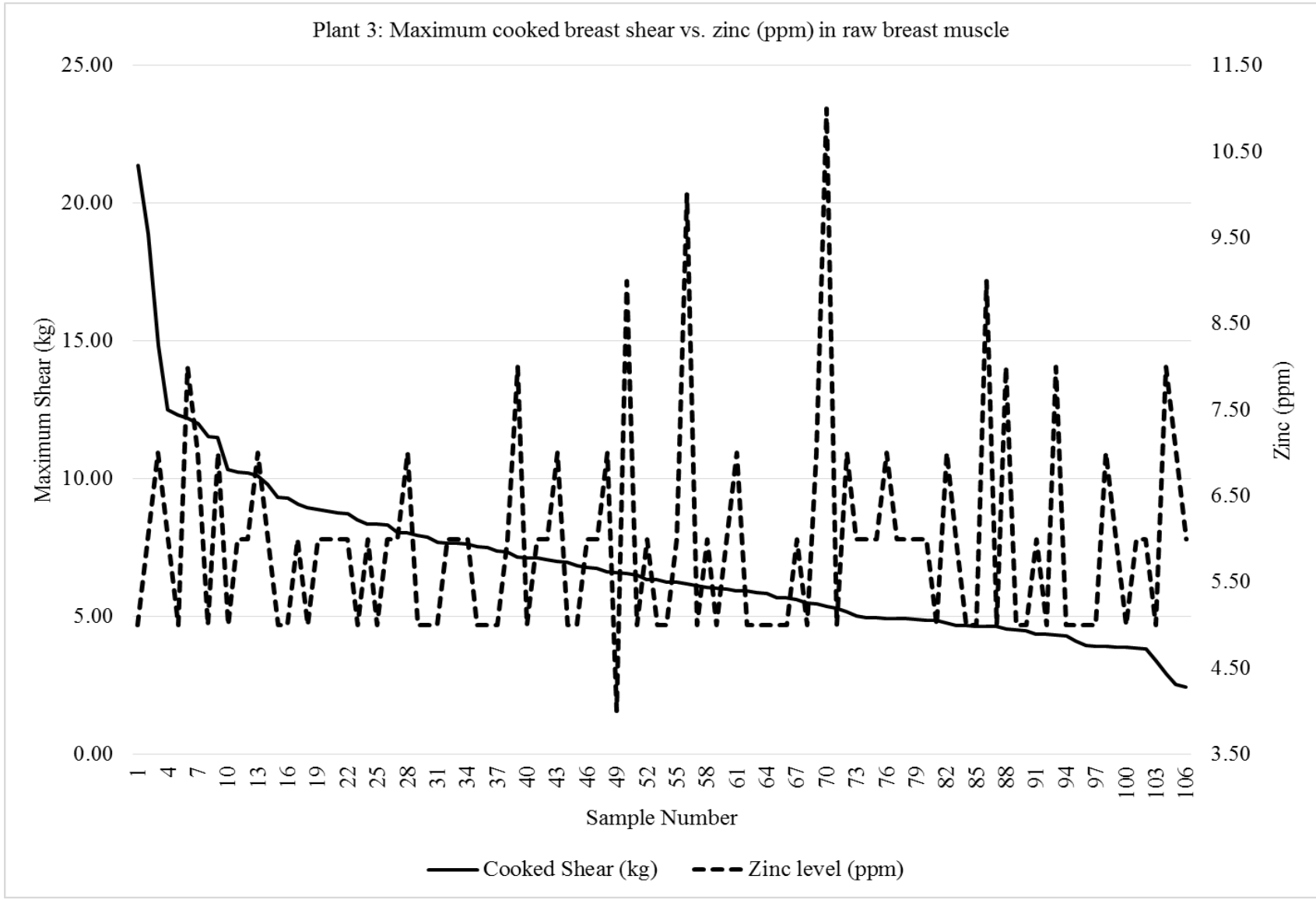

Figure 2.17 Plant 3: Maximum cooked breast shear (kg) vs. zinc (ppm) in raw breast muscle

DISCUSSION

There was no apparent significant relationship between the specific measured elements of the breast meat samples and the respective maximum shear values. Calkins et al. (1988) [22] discussed the importance of calcium dependent proteases on the aging process of muscle during the conversion to meat. This is not to say that there could be no specifically induced or reduced minerals in the broilers drinking water or the diet at times during grow out at levels that would not be harmful to the broiler that would lead to improved texture.

Drinking water from all three farms examined contained similar levels of the minerals under consideration with the exception of the birds processed in plant three. The drinking water from the farm supplying live birds to plant three had the highest measured level of calcium in the drinking water at 22.84 ppm compared to 5.26 ppm and 2.09 ppm for plants one and two respectively. Feed data was not available for this research; the proprietary nature of feed formulations are made specifically levels of micro-ingredients and supplement content and level. The feed may have played a role in the mineral concentrations of the raw tissue samples and may have been different from plant to plant precluding meaningful conclusions.

The associated average shear for plant 1 was 6.91 kg and an average SD of 3.01 kg. The average raw muscle calcium level for plant 3 was 0.2%, or double that of the calcium levels found in raw meat samples from plants one and two. Shear values for plants one and two were 4.05 kg and 4.44 kg respectively. The SD for plants one and two were 1.26 kg and 1.62 kg respectively. Through graphic and statistical analysis there is no clear relationship between individual cooked shear values and the associated raw muscle calcium levels, examining the results on average, would lead to a possible relationship between calcium levels in the drinking water and flock shear values. This potential relationship does not account for transport temperatures, location of the bird in the house or any other variable that could shift the shear values higher than the other

plants examined in this test. Table 2.3 states that the probability for a type I error for any relationship between calcium levels and cooked fillet shear values is P = 0.4566. This high chance for no interaction between the levels of calcium in the raw muscle and cooked fillet shear values does not take into consideration other calcium dependent biochemical needs of maintaining homeostasis in the broilers that may impact final meat quality. Further examination with a highly controlled study of the impact on calcium levels in drinking water and a potential relationship to fillet shear may be warranted.

Calcium ions are the initiator for muscle contraction [23] during rigor mortis there may be a link to an increased availability of calcium ions and increased muscle shortening during rigor mortis. A weak relationship was independently displayed between manganese and phosphorus and maximum shear. Data analysis represented in table 2.3 indicated the relationship of manganese to maximum shear (P = 0.0803) and the relationship of phosphorus to maximum shear (P = 0.0824). The Pearson correlation represented in table 2.2 indicated a weak positive relationship (r = 0.25) between magnesium levels and maximum shear values. The Pearson correlation in table 2.2 indicated a weak positive relationship (r = 0.25) between phosphorous levels and maximum shear values. The remaining minerals interaction with cooked fillet shear values were very weak or non-existent, indicating that there may be an issue with the resolution of the data around mineral concentration or these minerals have no impact on meat quality. The weak positive relationship of phosphorus levels in the raw samples may be associated with the level of phosphocreatine in the sample. It is well known that phosphocreatine is synthesized in mitochondria where it donates a high energy phosphate to form ATP from ADP during rigorous muscle activity [24].

Acute stress on animals are associated with the immediate release of energy stores from the liver is linked to the activation of the sympatric nervous system [25]. Further testing of water chemistry or diet manipulation focusing on adjusting levels of these elements may produce conclusive relationships one way or another.

It appears there may be a relationship between high levels of sodium in drinking water leading to a measurable impact on meat quality, further testing would have been conducted in a controlled environment to augment the farms water supply with varying levels of sodium to assess the impact on cooked maximum shear values.

BIBLIOGRAPHY

1. Lyon, B. G., D. P. Smith, C. E. Lyon, and E. M. Savage. 2004. Effects of diet and feed withdrawal on the sensory descriptive and instrumental profiles of broiler breast fillets. J. Poultry Science 83:275-281.

2. Lonergan, E. H., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle - Lessons on Mechanisms of meat tenderization. Meat Science 86:184-195.

3. Corzo, A., M. W. Schilling, R. E. Loar II, V. Jackson, S. Kin, and V. Radhakrishnan. 2009. The effects of feeding distillers dried grains with solubles on broiler meat quality. J. Poultry Science 8:432-439.

4. Zhang, A. W., B. D. Lee, K. W. Lee, K. B. Song, G. H. An, and C. H. Lee. 2005. Effects of graded levels of dietary *Saccharomyces cerevisiae* on growth performance and meat quality in broiler chickens. Asian-Aust. Journal Animal Science 18:699-703.

5. Richards, J. D., J. Zhao, R. J. Harrell, C. A. Atwell, and J. J. Dibner. 2010. Trace mineral nutrition in poultry and swine. Asian-Australasian Journal of Animal Sciences 23:1527-1534.

6. Bao, Y. M., M. Choct, P. A. Iji, and K. Bruerton. 2007. Effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and accumulation in tissues. J. Applied Poultry research 16:448-455.

7. Stef, D. S., and I. Gergen. 2012. Effect of mineral-enriched diet and medicinal herbs on Fe, Mn, and Cu uptake in chicken. J. Chemistry Central 6:1-9.

8. Winne, A. D., and P. Dirinck. 1996. Studies on vitamin E and meat quality. 2. Effect of feeding high vitamin E levels on chicken meat quality. J. Agricultural Food Chemistry 44:1691-1696.

9. Jiao, P., Y. Guo, X. Yang, and F. Long. 2010. Effects of dietary arginine and methionine on broiler carcass traits and meat quality. J. of Animal and Veterinary Advances 9:1546-1551.

10. Whiting, T. S., L. D. Andrews, M. H. Adams, and L. Stamps. 1991. Effects of sodium bicarbonate and potassium chloride drinking water supplementation. 2. Meat and carcass characteristics of broilers grown under thermoneutral and cyclic heat-stress conditions. J. Poultry Science 70:60-66.

11. Ahmad, T., T. Khalid, T. Mushtag, M. A. Mirza, A. Nadeem, M. E. Barbar, and G. Ahmad. 2008. Effect of potassium chloride supplementation in drinking water on broiler performance under heat stress conditions. J. Poultry Science 87:1276-1280.

12. Leeson, S., J. D. Summers, and E. T. Moran. 1976. Avian water metabolism-A review. World's Poultry Science Journal 32:185-195.

13. Budgell, P. 1970. The effect of changes in ambient temperature on water intake and evaporative water loss. Psychonomic Science 20:275-276.

14. Fletcher, D. L. 2002. Poultry meat quality. World's Poultry Science Journal 58:131-145.

15. Savenije, B., E. Lambooij, M. A. Gerritzen, K. Venema, and J. Korft. 2002. Effects of Feed Deprivation and Transport on Preslaughter Blood Metabolites, Early Postmortem Muscle Metabolites, and Meat Quality. Poultry Science 81:699-708.

16. Kim, J., and C. V. Dang. 2006. Cancer's molecular sweet tooth and the warburg effect. Cancer research 66:8927-8930.

17. Khan, A. W., and R. Nakamura. 1970. Effects of pre- and postmortem glycolysis on poultry tenderness. J. Food Science 35:233-267.

18. Rasmussen, A. L., and M. G. Mast. 1989. Effect of feed withdrawal on composition and quality of broiler meat. J. Poultry Science 68:1109-1113.

19. Mielnik, M., and N. Kolstad. 1991. The influence of feed and water withdrawal time on post mortem biochemical changes and meat tenderness in broilers. Norwegian Journal of Agricultural Sciences 1-8:1-8.

20. Jeon, H., J. Choe, Y. Jung, Z. A. Kruk, D. Lim, and C. Jo. 2010. Comparison of the chemical composition, textural characteristics, and sensory properties of North and South Korean chickens and commercial broilers. Korean Journal Food Science Animal Resources 30:171-178.

21. Suchy, P., P. Jelinek, E. Strakova, and J. Hucl. 2002. Chemical composition of muscles of hybrid broiler chickens during prolonged feeding. Chech Journal Animal Science 47:511-518.

22. Calkins, C. R., and S. C. Seideman. 1988. Relationships among calcium-dependent protease, cathepsins B and H, meat tenderness and the response of muscle to aging 1, 2. J. Animal Science 66:1186-1193.

23. Szent-Gyorgyi, A. G. 1975. Calcium regulation of muscle contraction. Biophysical Journal 15:707-723.

24. Meyer, R. A., H. L. Sweeney, and M. J. Kushmerick. 1984. A simple analysis of the phosphocreatine shuttle. American Journal of Physiology-Cell Physiology 246:C365-C377.

25. Bloom, S. R., P. M. Daniel, D. I. Johnston, O. Ogawa, and O. E. Pratt. 1973. Release of glucagon, induced by stress. Experimental Physiology 58:99-108.

Chapter 3

Commercial broiler life cycle stress as measured through norepinephrine levels in primary flight feathers and the relationship to maximum shear strength of cooked breast meat

ABSTRACT

This research is an initial look at the relationship between chronic stress and cooked breast meat shear values in a commercial broiler operation utilizing a modern tunnel closed wall grow out operation. Cooked breast meat shear was also evaluated by what section of the house the bird reached maturity during growth. Broilers exposed to acute stress exhibit physiological decreases in blood glucose levels, increased cortisol levels and higher H:L levels through activation of the HPA axis. Historical activation of the HPA axis or chronic stress are recorded in the growing feathers through the vascular system. Through extraction and detection of specific stress hormones is possible providing a historical image what level of stress each animal has experienced in its life. The extraction process used in this research extracted norepinephrine at a relatively high efficiency compared to other known stress hormones, more work needs to be done to determine if further modification to hormone extraction in flight feathers can lead to increased sensitivity in hormone detection. Installing fences after brooding in a modern grow out house isolates sub populations of the whole population in to groupings where the impact varying environmental conditions can be evaluated against chronic stress and cooked breast shear values. It was found that there is a significant difference in cooked shear values of the flocks in one of the four sections as well as significantly higher levels of norepinephrine levels in the primary flight feathers from the same section of the house.

INTRODUCTION

Broilers face lifecycle stress factors during the hatch, grow out, catch and transport, the cumulative stress history is captured in the feathers as cortisone as generated by the HPA axis during stressful events. The relationship to lifecycle stress and any relationship to meat quality is unknown. Stress events can range from thermal, either too cold or too hot, hunger, thirst, social, physiological, or poor animal husbandry practices. Early cold stress conditions have been shown to allow higher cold tolerance in adult birds demonstrating that some stressors have physiological effects on the broilers 21 days old [1]. It is reasonable to consider the possibility that chronic or acute stress may have an impact on the characteristic of the resulting meat potentially leading to increased meat toughness or increased tenderness.

Growing feathers are living structures supported by the birds' vascular system [2]. Feathers can record the stress history of the bird during growth by gradually accumulating hormones with hormone levels unaffected by the momentary stress associated with capture [3].

LITERATURE REVIEW

Acute Stress

Broilers grown in controlled environments face few stressors that would perceived as a threat to their life. Heat stress is a common stressor that is known to increase corticosterone levels and cause oxidative stress in five week old broilers [4]. Heat stress on broilers during transportation can cause higher mortality rates, mortality rates due to heat stress can be reduced by heat challenging five day old chicks. This stress conditioning is thought to be caused from a reduction of plasma triiodothyronine as well as hemodynamic changes in the chick [5]. Early age cold conditioning of chicks has also shown birds have a lower mortality rate ducting cold season transport. [1] These results of early age conditioning for both heat and cold conditions may indicate that birds can be conditioned against the stress generated due to the auditable influences of transportation. Currently there is no recorded research in sound conditioning the birds to elicit a change in how the bird responds to specific stimuli during catch and transport and any impact that stress reduction may have on meat quality.

Life Cycle Stress

Broilers may be stressed throughout their growth cycle by many paths. Social stressors that are created from within the flock, thermal stress generated from poor animal husbandry practices, dietary stress from feeder failures to the feed storage bin running out of feed, physiological stresses from rapid growth, reaching sexual maturity, catching and crating stress to thermal stress created during transport [6].

When stress reaches to a relative extreme it becomes distress and the sympatric nervous system reacts. The hypothalamus pituitary adrenal axis (HPA) reacts to stress by ultimately working to stimulate additional glucocorticoid steroids into the blood stream [7]. This chemical release

increases the blood glucose levels through protein and fat metabolism providing available energy for the reactive animal [6].

Glucocorticoid steroids in the blood are stored as corticosterone in the feathers during keratinization. The levels of corticosterone in the feathers are an indication of relative stress the bird has seen during the growth of the feathers [7]. The stress responses of birds can be conditioned during the life of the animal in a manner that subsequent stress events may not generate the same level of the HPA axis intensity as the previous event generated [7].

The oldest feathers on a bird are located at the end of the wings and are reference to as the flight feathers; these feathers will hold a cumulative history of the birds live cycle stress [8]. Feathers that have fault bars or visually deformed by varying shades of colors are found to have high values or corticosterone in the deformity [8], the broilers used in these tests are virtually white feathered.

Extraction of corticosterone is accomplished through bathing ground feathers, less the calamus, in a lower alcohol such as methanol in a warm agitated bath. Consistency in feather grinding is critical in achieving valid results [7].

There are two concepts in corticosterone level reporting. The first concept is to measure the length of the feathers sampled and report the corticosterone level on a unit length basis. Reporting the corticosterone levels on a unit length basis is thought to be the preferred method of corticosterone level reporting due to potential heavy keratinization of the feather that could dilute the reported corticosterone level [7]. The second is to weigh the sample and report on a unit weight. The latter is based on the concept that as feathers grow at different rates, reporting on a unit weight basis will provide a comparable data set when looking for a relationship with another variable [8].

Validation work that acute stress due to controlled handling of the birds and ACTH pumps forcing elevated cortisol levels in the blood plasma eventually metabolized in the feathers as corticosterone was conducted by plucking flight feathers and allowing regrowth during periods of controlled stress and comparing the levels of corticosterone before and after the stimuli [7]. Corticosterone is a stable compound so that feathers kept in a cool dry and covered can be tested years after sampling [7].

Feed / Water Withdrawal

Feed withdrawal practices allow the feed in the crop to be digested and mostly passed from the bird to reduce fecal contamination during evisceration. Typically birds are removed from feed for a total of 8 hours prior to processing; water is withdrawn just prior to the catching process. This is not to say that all of the birds in the flock have exactly been off feed for 8 hours. Broilers eat on 4 hour cycles allowing for fewer feeders [9]. Modern houses are designed so that a percentage of the birds can feed at a single time. Within the flock it is expected that a portion of the birds will be off feed for approximately 12 hours prior to catch, another set of birds will be off feed for 8 hours with the balance of the flock somewhere in between. This variability of available feed stores in the bird's GI tract translates to varying levels of glycogen stores in the liver. Feed withdrawal for 8 hours has been shown to cause glycogen depletion in the liver and also the breast muscle within three hours [10]. Glycogen is the base molecule that fuels ATP synthesis during the broilers life; up to 36 units of ATP are generated by one molecule of glycogen through each pass of the Krebs or citric acid cycle [11]. The Krebs cycle is halted once the bird is exsanguinated and oxygen is no longer available at the cellular level. Post mortem synthesis of ATP is through the inefficient process of glycolysis that drives increasing levels of lactic acid in the muscle that eventually halts the process after 4 hours, shutting down the

synthesis of ATP [10]. The available energy levels in ante mortem broilers have a direct impact on the time of onset of rigor mortis [12].

Time off water (0 – 18h) has been reported not to have a significance influence on meat quality [13]. Mielnik et al. (1991), on the other hand found that meat tenderness decreased on extended periods (12 to 18 h) of feed and water withdrawal [14]. This could be interpreted that time off feed has increased significance in meat tenderness when compared to time off water.

Capture & Crating Stress

Baseline cortisone levels in birds were lower than birds crated for 3 hours with cortisone levels falling below the initial values after 4 hours of confinement [15] supporting the idea that maximum cortisone levels on a broiler does not fully manifest itself until up to 3 hours after the initial stressor has occurred. Studies have demonstrated that there was no correlation between crate time and PM shear values [15]. Cortisone levels in the blood are the chemical indication of acute stress and continue to climb during transport and slaughter. Glucose levels remained constant and are probably due to feed withdrawal and the higher levels of cortisone stimulating glycogenolysis and glyconeogenesis [16].

Crating alone doesn't appear to cause significant stress in broilers; however transportation caused a lower pH in the thigh muscle indicating an increased rate of glycolysis compared to birds that were not transported [15]. The density of the crates also influences the stress levels which in turn will influence glucose concentrations in the muscles. Broilers experience higher levels of heat stress during the warmer months when crated at a high density [17]. Crating densities by season are programs that are managed to eliminate transport death and yield loss in the transported flock. Transport times between 30 minutes and 4 hours were shown to have no impact on meat quality [15].

Transport

Transport air conditions of temperature and humidity values change by season and by geographical region. The heat stress on the transported flocks is well documented specifically by Kannan et al. (1997) [15] and Delezie et al. (2007) [17]. The pH of the PM muscle is lower as indicated by measurements and an associated reduction in water holding capacity (WHC). Pre-slaughter heat stress is also reported to increase the rate and effect of rigor mortis but can also increase the incidence of pale, soft and exudative (PSE) meat [18]. Transportation during cold conditions cause a decrease in glucose levels in the PM muscle that is attributed to the birds natural response of regulating and maintaining body temperature [18]. A decrease in tenderness on birds held at higher temperatures was reported by several researchers [19]. However, other research indicates that extremes in temperature, both cold stress and heat stress caused an increase in toughness [20]. In all of these cases electrical stimulation was not used as a part of the experimentation. Thermally stressed birds, both heat and cold stressed, enter the manufacturing process with depleted energy reserves [21], electrical stimulation (ES) when managed correctly works to deplete or minimize the remaining ATP in the muscle and compress the delay phase of rigor.

Seasonality also has an impact on the tenderness of the meat; overall autumn months were reported to have the tenderest meat [21]. Kadim, (2009) et al. [22] also showed that hot season birds were slightly tenderer as compared to cold season birds attributing this difference to glycogen levels and minimal rigor shortening.

Sampling and Sample Processing

Broiler PM Muscle samples are generally taken from the left or right hand side of the animal. There is no significant difference in shear values between the left and the right hand side of the bird (M.A. Christie Unpublished Data). Whiting et al. (1991) [23] referenced data that the shear values in roasted turkey between sides were not significantly different. When sampling the PM muscle from any location, the samples should be either all left hand or right hand fillets. Sampling a single side eliminates the risk of same bird sampling. Samples collected from birds that had a wing removed prior to the chilling process that is before rigor had reached completion showed the wingless side had maximum shear values measured on a Warner-Bratzler Shear device is approximately 0.75 kg greater than the side of the same bird with a wing attached (M.A. Christie Unpublished Data). Collection of samples should be done with great care in that the shear data is not skewed by "wing off" birds.

The method of thermally processing the samples should also be consistent, Whiting et al (1991) [23], also showed that boiling PM muscles resulted in significantly higher shear values when compared to oven roasting covered PM muscles from the same birds. All samples should be thermally processed using the same procedure regardless if boiled or roasted. Commercial microwaves were not found to be a good substitute for replicating the oven process where the samples are covered and cooked at 350 F until the samples reached a temperature between 170 and 175 F (M.A. Christie Unpublished Data). Cooling samples prior to shearing is done for safety reasons; handling meat that is hot exposes the individual performing the tests to unnecessary burn risks. Hot samples will generate lower shear values when compared to sections sheared at room temperature. (M.A. Christie Unpublished Data). During the time of sample processing the hot meat will begin cooling and provide additional variability within the

data is due to moisture in the sample from the inevitable evaporation when the hot surface is exposed to the cooler surroundings (M.A. Christie Unpublished Data). Sample cooling can either be from natural convection or through conduction in a chilled water bath. The chilled water bath will generate samples higher in moisture due to reduce evaporative loss from the samples rapid cooling resulting in lower maximum shear force values (M.A. Christie Unpublished Data). Consistency in sample collection and processing is vital in understanding the relative toughness and variation of the sample set. Samples that vary in thickness will not cook at the same rate leading to overcooking or the thinner portions. These overcooked portions will shear at higher values (M.A. Christie Unpublished Data).

MATERIALS and METHODS

Bird selection and identification was done in a 400’ long by 50’ wide modern tunnel-house that was fitted with migration prevention devices. Birds that were to be processed were tagged 24 hours before the catch process. The birds were randomly selected from five areas of four isolated sections for a total of 20 zones within the poultry house. The sampling pattern is shown in figure 3.0. Color coded tags were used to easily identify the specific zone of the house where the birds were sampled to minimize the effort in matching the feather samples to the bird number. A catch pen was used to isolate small clusters of birds for identification, the birds were surrounded by a collection fence to minimize handling. Birds were tagged on the floor of the house without lifting the animal in the air. During the tagging process, the birds displayed no visible or audible signs of distress.

The birds were tagged with unique identifying numbers around the leg in a manner that would not restrict circulation or would be lost during the manufacturing process. Birds were selected evenly from throughout the house. There were 100 birds sampled from each of the four sections of the house. The four sections were divided into 5 areas with 20 birds from each section tagged for random sampling and to determine if the relative position of the bird in the house made a difference in the meat texture as measured by maximum shear force from one of the breast muscles from each captured carcass. Figure 3.0 is a graphical layout of the sample locations. The pinned birds were all released back into the general population once the planned number of birds were tagged.

The farm was selected based on two criteria, the first requirement was a grower with a history of excellent performance based on feed conversion. The second criteria was proximity of the house to the production facility. The production facility was approximately 3 miles from the farm

where the birds were grown to minimize transport stress. Typical feed withdrawal practices were used with water withdrawal occurring just prior to the catching process. Transportation and yard hold times for the test flock were under 90 minutes prior to processing. The sampling and processing was accomplished during mid-September 2013. The birds were transported and processed during dark and low light conditions.

The electrically stunned and exsanguinated test birds were removed from the processing line prior to scalding. Primary flight feathers were collected from post mortem birds to give the longest history of lifecycle stress possible [8]. Feathers were manually removed from the tip of the postmortem bird and placed in a sterile single use plastic bags that had been marked with a corresponding tag number linking the flight feather sample to the carcass. The test carcasses were immediately returned to the production line following feather sample collection.

The birds were processed using standard industry methods; as the test birds completed the chilling process, they were immediately deboned and frozen with dry ice to halt potential enzymatic activity within 30 minutes of reaching a target temperature of 35 ^{0}F. The tags were removed from the bird and placed in the sample bag along with a single fillet. The fillet and tag were layer packed in dry ice to stop any further aging and any resulting tenderness changes. The identified samples were layer packed with dry ice for rapid sample freezing and placed in an ice chest for transportation to a laboratory for cooking and shearing, all samples were stored in a freezer and thawed prior cooked within 7 days of slaughter. The cooking The cooking process, shear test equipment and shear procedure were the same as described in Chapter 2 of this document.

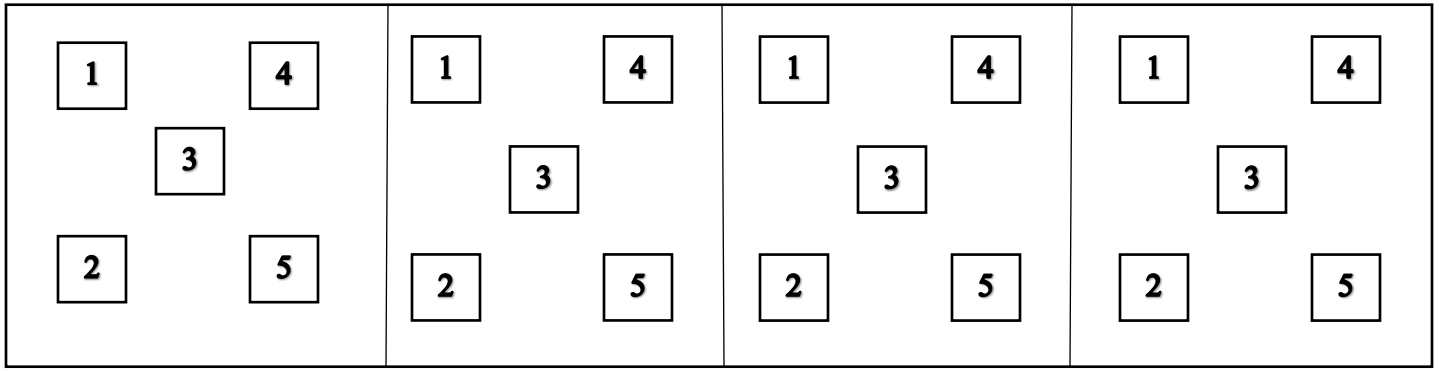

Figure 3.0 Live bird tag location by house zone

Feather Processing:

The surface used to process the feathers was a plastic cutting board that was wiped clean with paper towels and isopropyl alcohol between samples. A stainless steel surgical scalpel was used to process the samples. Between sample preparations, the blade of the scalpel was wiped clean with fresh paper towels saturated with isopropyl alcohol. The calamus of the feather was removed with a scalpel prior to obtaining the resulting feather sample. Feathers were processed by splitting the shaft of the feather to expose additional surface area and mincing the vanes and split shaft with a scalpel. The minced sample was placed in a weigh boat and weighed on a Mettler Toledo scale and transferred into a centrifuge tube for future hormone extraction. Feather weight was used to normalize the extracted hormone to weight for an absolute measurement when comparing hormone levels to maximum shear force of the cooked breast section.

A limited test was conducted to determine the efficiency of the methodology used by Davis; (2006). Hormone extraction methods were modified from previously mentioned literature to achieve measurable hormone extraction as the cited extraction process failed to provide any level of extraction of the selected hormones. There was no calibration issue with the equipment due to accurate identification of spiked reference samples. The methodology comparison between the Davis process and the procedure that was ultimately used for this experiment are graphically depicted in figure 3.1. A typical pathway for stress hormones was used as a basis for what we would be trying to identify in the flight feathers.

After washing and drying the samples, the method found to work was accomplished by immersion of the feather sample in 25 ml of methanol in a sealed 50 ml centrifuge tube that was placed in a 50 ^{0}C shaker bath for 24 hours. The feather-alcohol mix was centrifuged in a *Thermo*

Scientific Legend XTR centrifuge at 4200 RPM for 20 minutes. The sample tube was rinsed with 10 ml of methanol and the methanol poured over the feathers passing through the same # 4 filter into a fresh 50 ml centrifuge tube. The methanol solution was removed with clean pipettes and placed in a second set of tubes and the samples were dried under a 50 ^{0}C nitrogen cloud in a fume hood overnight. A 50% methanol and DI water was added back to the tubes and the solution was prepared for examination with a *Acquity* # 5 UHPLC feeding a *Acquity* UPLC liquid chromatography unit tuned to detect the following 6 hormones as indicated in Table 3.0. Three vials with spiked samples for each the reference hormones under consideration were introduced to the test to indicate that the HPGLC was correctly reading the correct level of magnitude of the test samples. Each spiked sample yielded the expected level of reference hormone. Two blank sample vials were also introduced to the test sequence to indicate false positive readings. All blanks did not produce any level of the respective hormone ensuring no false positives.

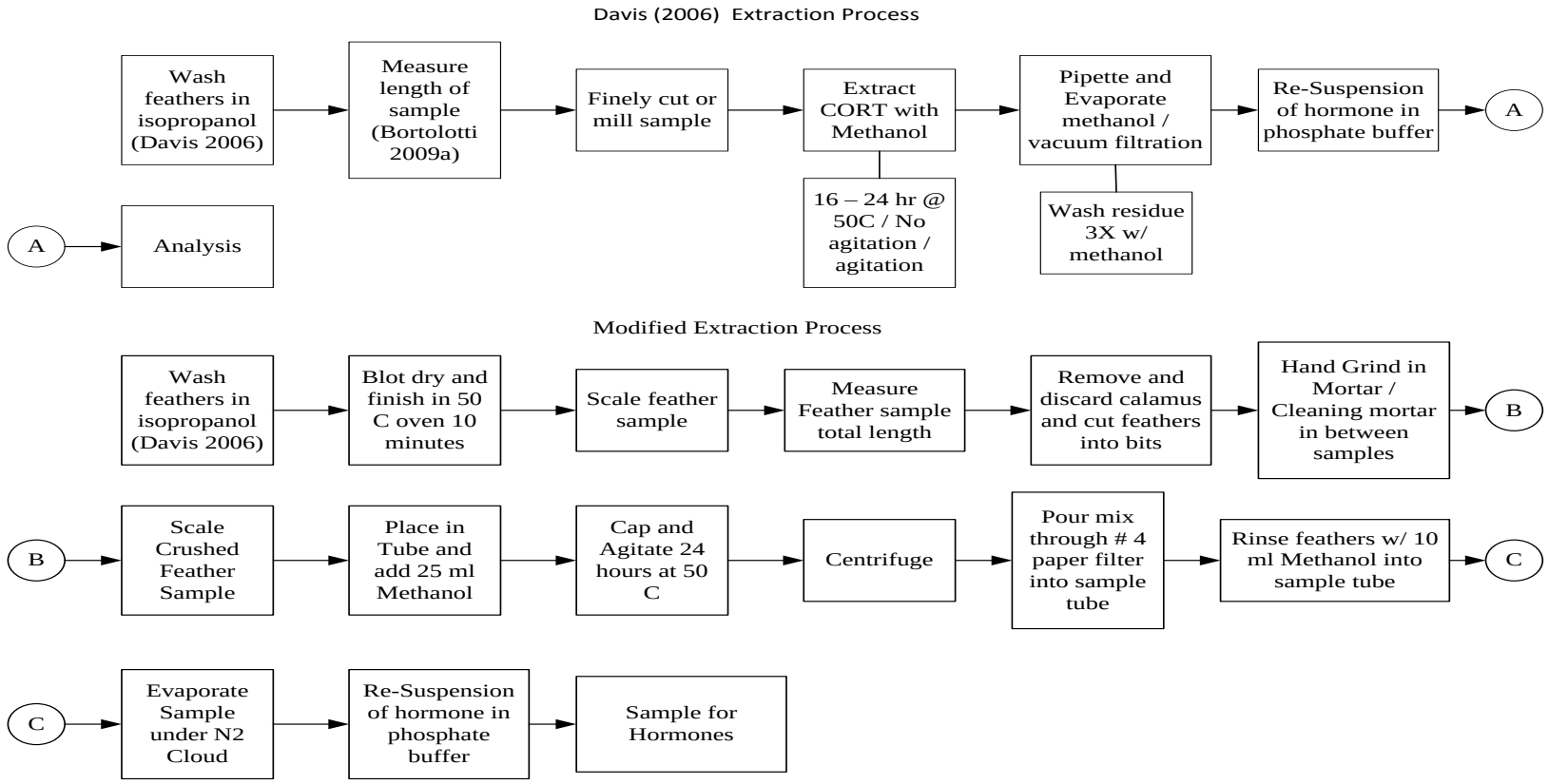

Figure 3.1 Davis (2006) and Modified Hormone Extraction Process Flow Diagram for broiler flight feathers

Table 3.0
Tested Hormones with Relative Information

Hormone	Detection level out of 65 samples	Classification	Relative Information
Norepinephrine	N = 65	Neurotransmitter and Hormone	Fight or Flight Hormone makes glucose available for metabolism (From Dopamine)
Deoxycorticosterone	N = 0	Mineralocorticoid	Precursor to aldosterone
Deoxycortisol	N = 0	Glucocorticoid	Oxidized to cortisone
Corticosterone	N = 1	Glucocorticoid	Precursor to aldosterone
Progesterone	N = 14	Mineralocorticoid	Precursor to corticosterone
Cortisone	N = 5	Glucocorticoid	Released as a reaction to stress

RESULTS

There was no significant difference in maximum shear value and standard deviation between the three of the four zones of the house indicating a uniform shear values of the sample population regardless of what area from each zone the birds were sampled. Out of the 400 birds that were tagged, 268 carcasses were recovered behind the chiller. Figure 3.3 describes the shear data and norepinephrine descriptions by house location of each sample sub population by location for the house that was sampled. Figure 3.4 is a graphical representation of the maximum fillet shear expressed in kilograms of force and norepinephrine levels expressed in ppb.

A Duncan multi range difference test indicated that populations from sections one, two and four demonstrated no difference between groups in texture as described by maximum shear values, but their shear values were significantly different from the shear values of birds from section three. The GLM procedure indicated a significant difference in shear results between sections one, two and four compared to bird shear results from section three at the 95% confidence level (P =.0191). Levels of norepinephrine at the entry point (section three) of the house were significantly higher than those levels in the other sections of the broiler house. Eight samples from section three of the house yielded measurable hormone levels visibly higher than levels found in the other sections of the house. Conclusions between the location of the sample and any relationship with texture is an indication there may be a relationship between bird location and hormone level would be speculation with the low positive sample count. Additional testing would be required to validate any claim that additional chronic stress is present at the entry point of a broiler house.

Detection levels for all of the hormones tested are found in table 3.0. The number of samples where hormone detection was found were too low to form any conclusions regarding relationships between hormonal levels and cooked breast meat shear. Norepinephrine was the only hormone consistently found throughout the test feathers.

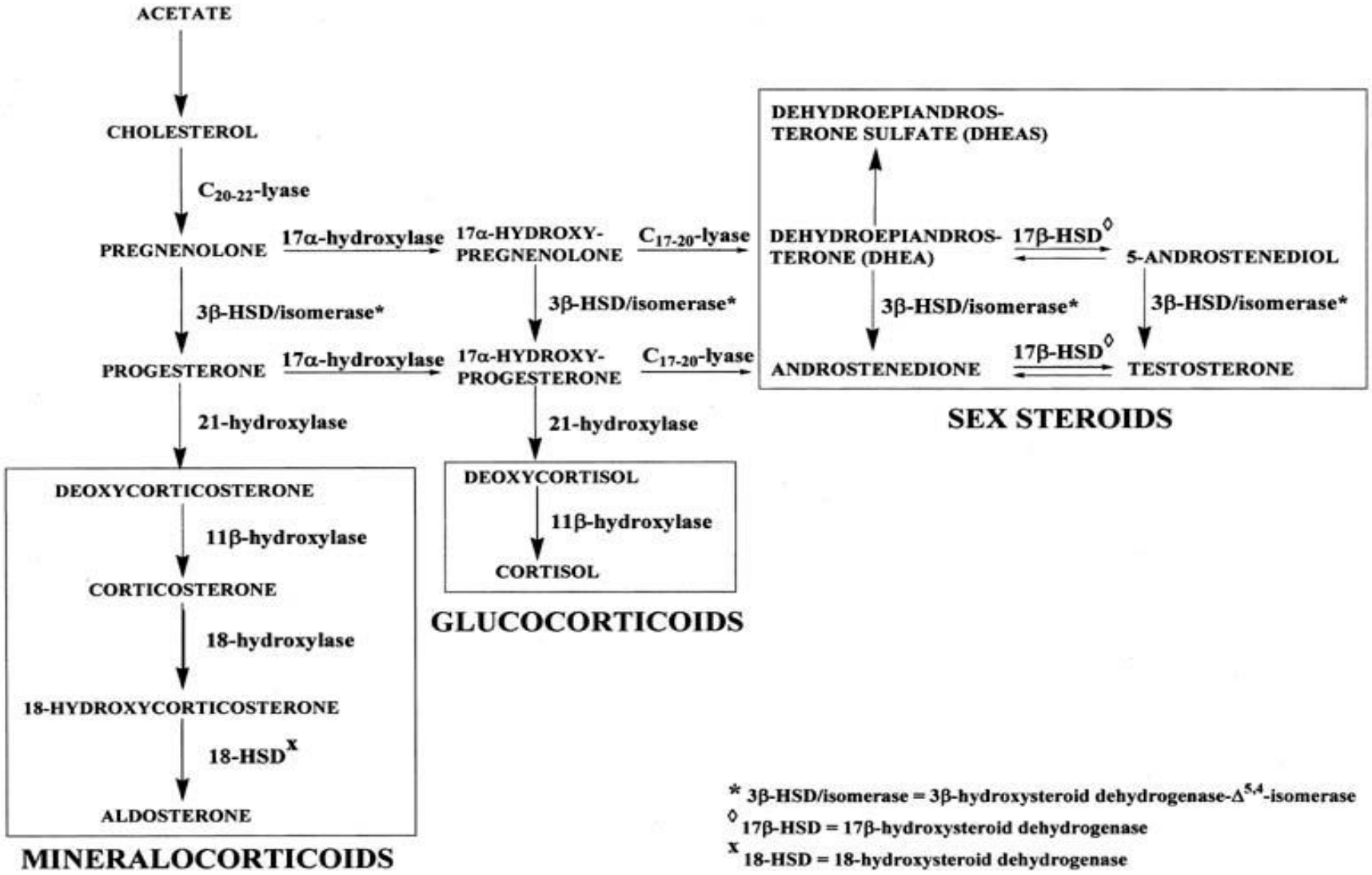

Figure 3.2 Steroid Hormone Pathway for mineralocorticoids, glucocorticoids and sex steroids Stanczyk (2009)

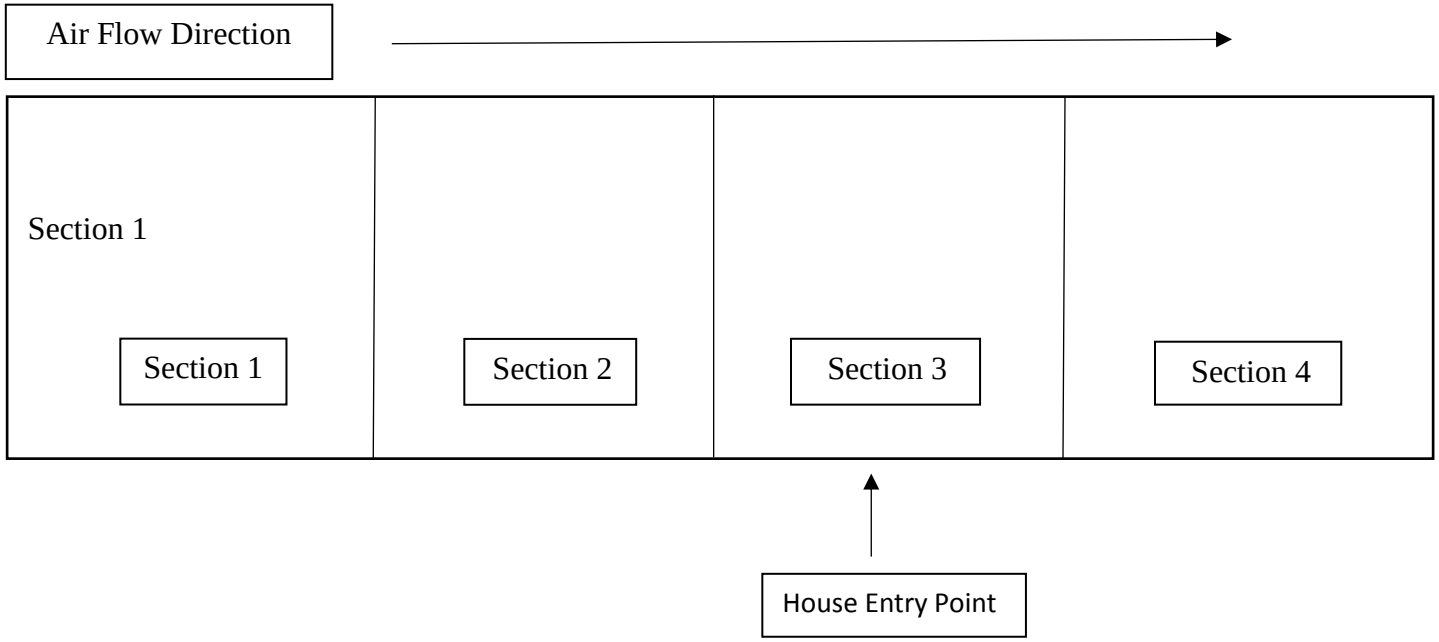

Figure 3.3 Sample section location with air direction and entry point

Table 3.1
Population Fillet Maximum Shear (kg) and Norepinephrine levels (ppb) by house location of each sample sub-population

	Section 1	Section 2	Section 3	Section 4
Shear (kg)	10.7	10.6	9.5	11.6
Shear Standard Deviation (kg)	4.2	3.8	3.4	3.6
Shear Sample Size	69	68	66	65
Average Norepinephrine Level (ppb)	870	548	1122	489
Hormone Sample Size	24	24	8	15

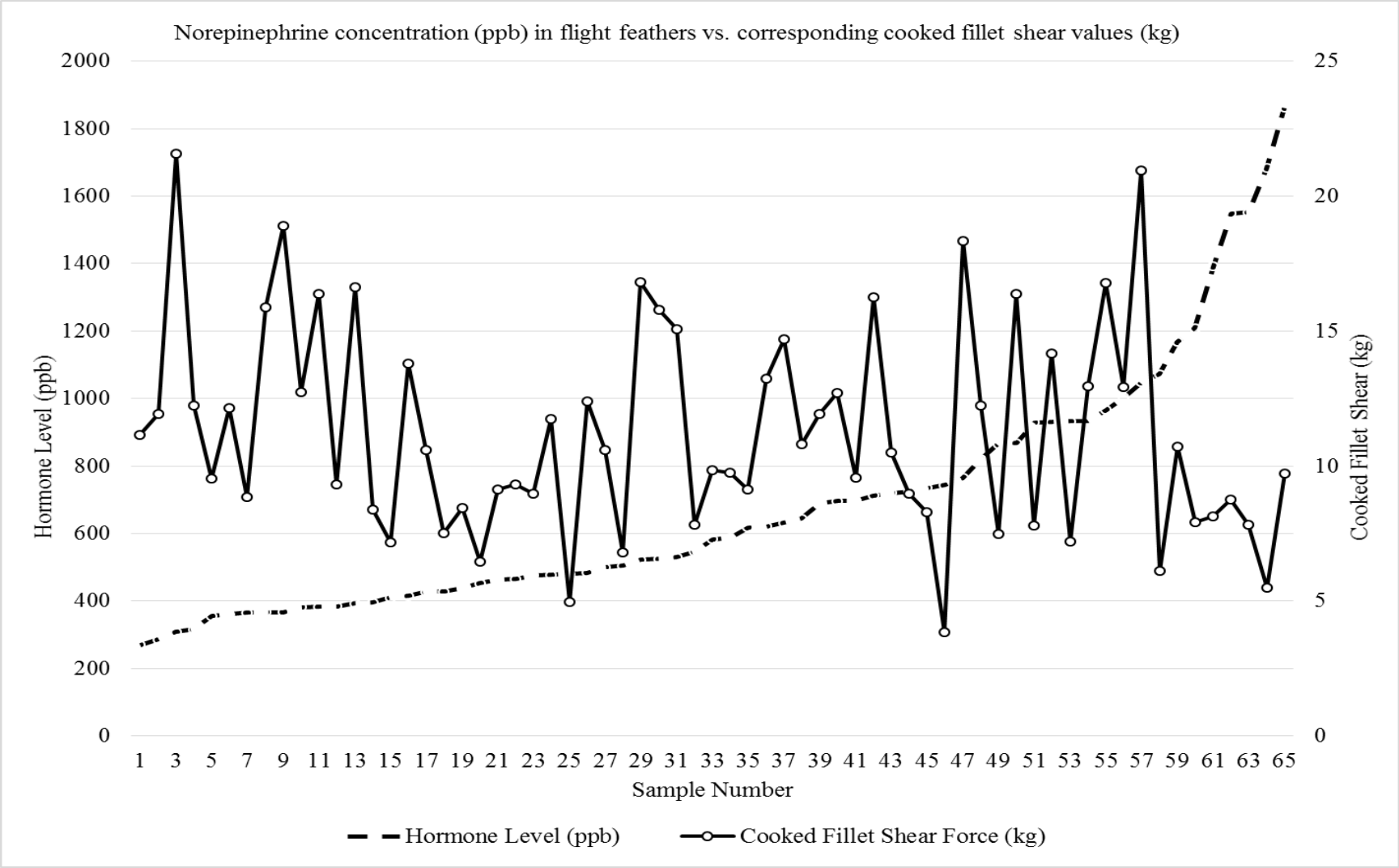

Figure 3.4 Norepinephrine Concentration in Flight Feathers and Associated Shear Values of Pectoralis Major Muscle

DISCUSSION

Shear values from section three of the broiler house tested were significantly different from the shear values for the remaining three sections. (P = 0.0191) Section three is the location of the daily entry point and the location of the house feed delivery system. Birds located in this section would be exposed to the random noise if the feed augers activating and the farmer entering and leaving during daily house check making passive contact with these birds twice as often as birds in the remainder of the flock. There was not enough extracted norepinephrine hormone data available (N = 8) to reliability determine if there was truly a significant relationship between corresponding norepinephrine levels in the flight feathers and the associated maximum shear values. Feather samples that do not yield measurable hormone data is not uncommon, Bortolotti et.al (2008) [7], also reported low measurable sample rates using similar recovery and sampling procedures for cortisol.

The results do indicate that there may be a relationship between house location and chronic stress and indicated by the bioaccumulation of norepinephrine in the flight feathers. Birds with higher accumulated levels of norepinephrine in the flight feathers were located at the entry to the house and are subject to twice the human interaction. This location is also near the entry point for feed entrance to the house and subject to unexpected auditory stimulation caused by activating the feeders. This noise could be associated with the pleasurable activity of eating leading the birds in this section of the house conditioned to be less stressed in random noise conditions. Noise and human contact conditioning of the birds may condition the bird and inhibit intense activation of the HPA axis during the catch and transport phase or modern poultry production operations. This potential mechanism could be in some way related to physiological changes in T3 levels improving thermos-tolerance at maturity due to the process of hot setting chicks lowering the mortality of birds during transport under hot weather conditions (Yahav 1996), [5]. Quinteiro-

Filho et.al. (2012), [24] reported that increasing bird environmental temperatures from 21 ^{0}C to 31 ^{0}C and 36 ^{0}C caused an increase in serum corticosterone levels decreasing feed intake and changing intestinal mucosa integrity. The increase in corticosterone levels indicate that the HPA-axis was activated which should also lead to elevated levels of norepinephrine in the blood, ultimately accumulating in the feathers. Scanes (2016), [25] reported that H:L ratios in commercial broilers indicate evidence that the birds are not stressed during the grow-out phase of broiler production. Based on the low number of samples testing positive for cortisol, our data agrees with this finding. With no preconditioning to stress, it would be easy to conclude that small changes in the birds' environment could cause varying levels of HPA activation during feed removal, catch and transport. HPA activation may lead to physiological changes in the broiler potentially having a negative impact on meat tenderness. Samanta et.al. (2008), [26] reported that increasing stress levels broilers led to increased blood glucose concentrations. Increased blood glucose levels were reported to influence the tenderness of broiler meat. Mellor et.al. (1958) [27]. Savenije et.al. (2002), [10] did not find an influence of glucose levels on breast meat shear values.

Fairhurst et al. (2011), [28] reported that using environmental enrichment techniques did manifest in changes in feather cortisol levels in feathers from wild caught Clark's nutcrackers (*Nucifraga columbiana*) indicating changing environmental conditions in captive animals can influence activation of increased hormonal activity.

The pattern of higher levels of norepinephrine in section three would indicate that additional testing focusing primality in this zone of the house would have merit and potentially lead to adjustments in animal husbandry practices that could potentially lead to improved bird performance with regards to meat tenderness. Additional work should be done to improve the

efficiency of hormone extraction from primary flight feathers as well as validating whether a relationship truly exists between chronic stress and the shear force of specific areas in a broiler house.

BIBLIOGRAPHY

1. Shinder, D., L. Dror, M. Rusal, V. Rzepakovsky, V. Bresler, and S. Yahav. 2002. Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. Journal of Thermal Biology 27:517-523.

2. Leeson, S., and T. Walsh. 2004. Feathering in commercial poultry I. Feather growth and composition. World's Poultry Science Association 60:42-51.

3. Koren, L., S. Nakagawa, T. Burke, K. K. Soma, K. E. Wynne-Edwards, and E. Geffen. 2012. Non-breeding feather concentrations of testosterone, corticosterone, and cortisol are associated with subsequent survival in wild house sparrows. Proceedings of the Royal Society of London B: Biological Sciences, rspb200112062 279:1560-1566.

4. Lin, H., E. Decuypere, and J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 144:11-17.

5. Yahav, S., and S. Hurwitz. 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. J. Poultry Science 75:402-406.

6. Puvadolpirod, S., and J. P. Thaxton. 2000. Model of physiological stress in chickens 3. Temporal patterns of response. J. Poultry Science 79:377-382.

7. Bortolotti, G. R., T. A. Marchant, J. Blas, and T. German. 2008. Coritcosterone in feathers is a long-term, integrated measure of avian stress physiology. Functional Ecology 22:494-500.

8. Lattin, C. R., J. M. Reed, D. W. DesRochers, and L. M. Romero. 2011. Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J. Avian Biology 42:247-252.

9. Fletcher, D. L. 2002. Poultry meat quality. World's Poultry Science Journal 58:131-145.

10. Savenije, B., E. Lambooij, M. A. Gerritzen, K. Venema, and J. Korft. 2002. Effects of Feed Deprivation and Transport on Preslaughter Blood Metabolites, Early Postmortem Muscle Metabolites, and Meat Quality. Poultry Science 81:699-708.

11. Kim, J., and C. V. Dang. 2006. Cancer's molecular sweet tooth and the warburg effect. Cancer research 66:8927-8930.

12. Khan, A. W., and R. Nakamura. 1970. Effects of pre- and postmortem glycolysis on poultry tenderness. J. Food Science 35:233-267.

13. Rasmussen, A. L., and M. G. Mast. 1989. Effect of feed withdrawal on composition and quality of broiler meat. J. Poultry Science 68:1109-1113.

14. Mielnik, M., and N. Kolstad. 1991. The influence of feed and water withdrawal time on post mortem biochemical changes and meat tenderness in broilers. Norwegian Journal of Agricultural Sciences 1-8:1-8.

15. Kannan, G., J. L. Heath, C. J. Wabeck, M. C. P. Souza, J. C. Howe, and J. A. Mench. 1997. Effects of crating and transport on stress and meat quality characteristics in broilers. J. Poultry Science 76:523-529.

16. Nijdam, E., E. Delezie, E. Lambooij, M. J. A. Nabuurs, E. Decuypere, and J. A. Stegeman. 2005. Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. J. Poultry Science 84:467-474.

17. Delezie, E., Q. Swennen, J. Buyse, and E. Decuypere. 2007. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. J. Poultry Science 86:1414-1423.

18. Dadgar, S., E. S. Lee, T. L. V. Leer, H. L. Burlinguette, H. L. Classen, T. G. Crowe, and P. J. Shand. 2010. Effect of microclimate temperature during transportation of broiler chickens on quality of the pectoralis major muscle. Poultry Science 89:1033-1041.

19. Petracci, M., D. L. Fletcher, and J. K. Northcutt. 2001. The effect of holding temperature on live shrink, processing yield, and breast meat quality of broiler chickens. J. Poultry Science 80:670-675.

20. Lee, Y. B., G. L. Hargus, E. C. Hagberg, and R. H. Forsythe. 1976. Effect of antemortem environmental temperatures on postmortem glycolysis and tenderness in excised broiler breast muscle. J. Food Science 41:1461-1465.

21. Ali, A. S. A., A. P. Harrison, and J. F. Jensen. 1999. Effect of some ante-mortem stressors on peri-mortem and post-mortem biochemical changes and tenderness in broiler breast muscle: a review. World's Poultry Science Journal 55:403-414.

22. Kadim, I. T., B. H. A. Ai-Qamshui, O. Mahgoub, W. Ai-Marzooqi, and E. H. Johnson. 2009. The effects of ascorbic acid and seasonal temperatures on meat quality characteristics of broiler chickens maintained in open-sided and closed houses. International Journal of Poultry Science 8:733-739.

23. Whiting, T. S., L. D. Andrews, M. H. Adams, and L. Stamps. 1991. Effects of sodium bicarbonate and potassium chloride drinking water supplementation. 2. Meat and carcass characteristics of broilers grown under thermoneutral and cyclic heat-stress conditions. J. Poultry Science 70:60-66.

24. Quinteiro-Filho, W. M., M. V. Rodrigues, A. Ribeiro, V. Ferraz-de-Paula, L. R. M. S. Pinheiro, A. J. P. Ferreira, and J. Palermo-Neto. 2012. Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hypothalamic-pituitary-adrenal axis activation. J. Animal Science 90:1986-1994.

25. Scanes, C. G. 2016. Biology of stress on poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. J. Poultry Science 95:2208-2215.

26. Samanta, S., S. Haldar, V. Bahadur, and T. Ghosh. 2008. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. J. of the Science of Food and Agriculture 88:787-796.

27. Mellor, D. B., P. A. Stringer, and G. J. Mountney. 1958. The influence of glycogen on the tenderness of broiler meat. J. Poultry Science 37:1028-1034.

28. Fairhurst, G. D., M. F. Frey, J. F. Reichert, I. Szelest, D. M. Kelly, and G. R. Bortolotti. 2011. Does environment enrichment reduce stress? An integrated measure of corticosterone from feathers provides a novel perspective. PLOS One 6:e17663.

SUMMARY

The production of poultry broiler breast meat that has acceptable tenderness attributes may be influenced by a variety of production practices, the most common is aging the carcass allowing the rigor mortis process to reach completion along with the enzymatic breakdown of the muscle fiber connections. Using electrical stimulation to artificially metabolize residual energy in the breast muscle is another method to speed up the natural process of aging but these systems effectiveness is limited to the design, length and management practices. Electrical stimulation systems also come with a potential yield loss during deboning. Over stimulating the carcass will cause breast meat to remain on the frame during a deboning operation that is only recoverable through the use of advanced meat separation systems producing a product that is significantly lower in value than a boneless fillet. A key finding in general was, looking at the response of an individual bird's relationship to shear and live side conditions produced little usable information. The potential relationships were manifested when looking at populations of birds and not the individual bird. Examining the flock's average fillet shear values and shear SD to the conditions discussed in this research did exhibit potential relationships between transport temperature, meat chemistry, drinking water chemistry, as well as the physical location in the house where the bird was grown with relationship to cooked shear values and more importantly, to the average shear standard deviation of the flock. There have been many studies that link transport conditions to average toughness, the results found in this research support these previous findings. One key finding in this research is the significance of weather, bird location during grow-out, and possibly the drinking water has on the shear SD of the flocks examined. There also needs to be additional research to determine if the relationship between tougher meat is related to lower transport air temperatures or air with higher oxygen concentrations due to air that is denser, containing more moles of O_2 per unit volume of each breath. The use of hormone extraction to

determine life cycle stress in broilers and a potential relationship to meat quality produced interesting results exposing a potential relationship between birds that have higher measureable levels of stress hormones in flight feathers produce meat with lower cooked fillet shear values along with lower shear SD values. This finding could lead to additional studies where the flock could be conditioned during the live side production phase that could lead to higher quality meat as related to tenderness attributes. This methodology could also be used as a method of proving commercially grown birds do not have a stressed life cycle during the majority of its life. Additional controlled studies regarding the level of calcium in the broilers drinking water to determine if there is a true relationship would need to be developed and thoroughly tested. If this proved to be a true relationship, intervention would be as simple as incorporating water softeners into the birds drinking water supply to those farms with high calcium levels in the drinking water and a history of producing broilers with consistently tough breast meat. It appears that many components in broiler production may impact production, more understanding on controllable management practices would benefit the broiler industry as a whole.