Investigating the role of genetics in breast meat quality and white striping in Canadian turkeys

by

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ABSTRACT

INVESTIGATING THE ROLE OF GENETICS IN BREAST MEAT QUALITY AND WHITE STRIPING IN CANADIAN TURKEYS

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The prevalence of meat quality defects, such as pale, soft, exudative (**PSE**) meat, and muscle myopathies, such as white striping (**WS**), have been of increasing interest to the poultry industry over the past few decades. The rise in prevalence of quality defects and myopathies are thought to be closely associated with the drastic changes in growth rate due to improvements in management, nutrition, and genetic selection. Research surrounding these traits in turkeys is limited, therefore, the objectives of this thesis were to provide a better understanding of the role genetics plays in breast meat quality and WS in Canadian turkeys and outline the relationships among these traits and other economically important traits such as body weight and feed efficiency. We first tested the reliability of a four-category severity scoring system for WS adapted from broiler chickens. After this system was found to be reliable within and between observers, it was used to score a large population of turkey toms for WS. In study 2, genetic parameters of breast meat colour, pH, and WS were estimated. These traits were estimated to be moderately heritable and various unfavorable correlations between these traits and economically

important traits were reported. Finally, given the moderate heritability of WS estimated in the second study, the genomic architecture of WS was further investigated by means of a genome-wide association study (**GWAS**) followed by functional analysis. The findings of this study supported that of previously published work in broiler chickens which suggests that the biological limits of the circulatory system have been reached resulting in ischemic conditions in the muscles and development of WS. Although continued research is needed to further understand the various aspects that influence turkey meat quality and the presence of myopathies, this thesis adds to the body of knowledge surrounding the genetic factors affecting these traits in turkeys.

DEDICATION

I dedicate this work to my amazing wife Alycia. There are no words to express how thankful I am that God placed you in my life. You are my best friend and I look forward to our next chapter together.

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LIST OF ABBREVIATIONS

a*	Redness
AC_2	Gwet's weighted agreement coefficient
b*	Yellowness
BMY	Breast meat yield
BP	Biological processes
BrW	Breast weight (cumulative sum of all breast meat)
BW	Body weight
CC	Cellular components
CT	Computed tomography
FCG	Functional candidate gene
FCR	Feed conversion ratio
Fillets	*Pectoralis major* muscle
gEBV	Genomic estimated breeding value
GO	Gene ontology
GWAS	Genome-wide association study
KEGG	Kyoto Encyclopedia of Genes and Genomes
L*	Lightness
LS	Least square

MF	Molecular function
MRI	Magnetic resonance imaging
pHu	Ultimate pH (measured 24h post-mortem)
PSE	Pale, soft, exudative
Slaughter weight	Body weight measured two days prior to slaughter
SNP	Single nucleotide polymorphism
Tenders	*Pectoralis minor* muscle
WS	White striping

Chapter 1: General Introduction

1.1 Turkey Meat Industry

Increases in meat consumption and production are expected over the next decade (OECD-FAO, 2021). As the cheaper and perceived healthier choice compared to other meat options, it is no surprise that poultry meat is expected to represent upwards of 40% of all consumed meat protein by 2030 (OECD-FAO, 2021). Turkey meat is the second most-consumed poultry meat globally with the majority of production taking place in Europe and North and South America (FAOSTAT, 2021). In 2020, 158 million kg of turkey meat was produced in Canada resulting in an annual farmgate value of $367 million CAD (Turkey Farmers of Canada, 2020). The demand for turkey meat is met through two main methods: production of a larger number of birds and production of larger birds with greater efficiency. Great progress has been made in the latter of these two production methods as seen by the ability of modern turkey lines to grow approximately twice as large, consume less feed per kg of body weight gained, and showing no significant difference in the livability of the birds (Havenstein et al., 2007). A large proportion of this success can be contributed to the carefully constructed breeding programs implemented by two major breeding companies which take into account several important traits such as growth rate, meat yield, fertility, egg production, and hatchability (Wood, 2009). However, the years of intense selection for growth rate and meat yield in some genetic lines is beginning to give rise to poor meat quality and the development of several muscle myopathies.

1.2 Meat Quality

The overall quality of a final meat product can be characterized by two main categories: appearance and texture. The major traits associated with these categories are colour, pH, drip loss, cooking loss, and shear force. Several factors ranging from bird characteristics (i.e., age, breed, sex) to management and processing procedures can play a major role in meat quality.

Raw meat colour is a large factor in consumer acceptance at the point of sale as products are almost always packaged or on display behind the counter and the only sensory characteristic able to be judged is visual perception of the product. Variations in product colour can lead to consumer rejection of the product or group of products (Droval et al., 2012). This variation can often be emphasized when products are packaged together (i.e., pack of multiple breast fillets) (Fletcher, 1999; Droval et al., 2012). Colour can be measured not only subjectively, but also quantitatively using reflectance colourimetry. With enhancements in technology over the past few decades, portable devices such as the Konica Minolta CR-400 Chroma Meter or Nix Pro Colour Sensor, have been manufactured that allow users to quickly and effectively measure the colour of a product. The most commonly expressed values are the trichromatic coordinates lightness (**L***), redness (**a***), and yellowness (**b***) (CIE, 2018).

A relationship that has been well document over the last few decades is that between colour and pH of meat. The rate and magnitude of post-mortem decline in muscle pH greatly affects the overall quality of the final meat product (Briskey, 1964; Wynveen et al., 1999; Barbut et al., 2008). Differences in post-mortem muscle pH among individual birds arise due to changes in rates of glycolysis which can be affected by several factors including pre-slaughter handling (stress), stunning method, muscle size, carcass chilling, nutrition, and genetics (Ma et al., 1971; Rathgeber et al., 1999; Wynveen et al., 1999; Velarde et al., 2000). A rapid decline in pH or an exceptionally low pH in the final meat product, can lead to pale, soft, exudative (**PSE**) meat. Characteristics of PSE meat include increased functional protein degradation leading to lighter coloured meat with decreased water-holding capacity and increased shear force of the cooked product (Barbut, 1993; Owens et al., 2002). A previous study by Owens et al. (2000) showed approximately 40% of turkey breasts were considered to be pale (1.5h post-mortem L* > 53). Additionally, these pale breast samples were associated with a 6% decrease in pH measured 1.5h post-mortem and a 2.5 fold increase in drip loss percentage (Owens et al., 2000). Not only is this difference in quality visually noticeable, but it also affects

sensory acceptability with panelists preferring cooked broiler breast meat classified as normal over meat classified as PSE (Droval et al., 2012).

In turkeys, breast meat colour and pH (both initial, taken 15 to 45 min post-mortem, and ultimate, taken 24h post-mortem) have been shown to be moderately heritable with published heritabilities ranging from 0.12 to 0.30 for colour (Le Bihan-Duval et al., 2003; Aslam et al., 2011) and 0.09 to 0.21 for pH (Le Bihan-Duval et al., 2003; Aslam et al., 2011). Genetic correlations between these traits and body weight have been shown to be moderate to strong and unfavorable (Le Bihan-Duval et al., 2003; Aslam et al., 2011) suggesting the prevalence of PSE meat will continue to rise with continued selection for growth traits. Emphasis should be placed on building the body of knowledge surrounding these traits to allow for their inclusion in selection indexes to minimize these negative characteristics in future populations.

1.3 Muscle Myopathies

The intense selection placed on growth traits in poultry is thought to have resulted in the rise of several muscle myopathies (Velleman and Nestor, 2003; Hocking, 2014). One of the most common growth-related myopathies seen in modern turkeys is white striping (**WS**). The WS myopathy presents itself as varying degrees of white striations on the surface of the muscle running parallel to the muscle fibers (Barbut, 2019). The prevalence of this myopathy has recently been shown to be as high as 60% in turkeys (Mudalal, 2019) and between 56-87% in heavy strain broiler lines (Lorenzi et al., 2014; Russo et al., 2015; Cruz et al., 2017; Golzar Adabi and Demirok Soncu, 2019). The presence of WS not only affects the quality of further processed products but also negatively affects consumer acceptance of broiler chicken whole muscle products with consumers associating affected breasts with extra or too much fat, discolouration, and not looking fresh (Kuttappan et al., 2012b; de Carvalho et al., 2020). Therefore, research into the biological mechanisms behind the condition and potential methods of prevention are of great importance to the poultry industry.

Although the amount of research conducted on WS in turkeys is limited, there is more known about WS in broiler chickens. Several studies have been conducted in broiler chickens that investigated WS on a microscopic level showing that breasts affected by WS have an increased presence of fat, connective tissue, and inflammatory cells, as well as necrotic muscle tissue (Kuttappan et al., 2013b; Russo et al., 2015; Baldi et al., 2018). While the exact mechanism for development of WS is still unknown, one of the main mechanisms proposed is ischemia in the affected muscle (Boerboom et al., 2018). With the magnitude and speed of growth in modern birds, the limits of supporting systems (e.g., circulatory, cardiovascular) might have been reached. Increases in muscle fiber hypertrophy, the major consequence of selection for muscle growth (Aberle and Stewart, 1983; Remignon et al., 1994; MacRae et al., 2006), can lead to insufficient vascularization and reduced blood supply to the muscles (Sosnicki and Wilson, 1991; Velleman and Clark, 2015; Kindlein et al., 2017). A restriction in the circulatory system can lead to the accumulation of metabolic byproducts, inducing oxidative stress likely leading to necrosis, and increases in hypoxic conditions potentially impairing muscle cell regeneration, ultimately leading to the development of WS.

On a genetics level, there have been no published heritability estimates for WS in turkeys, however, published estimates in broiler chickens range from 0.19 to 0.65 (Bailey et al., 2015; Alnahhas et al., 2016; Lake et al., 2021). Further research using various -omics technologies to investigate the genes and proteins relating to WS in broiler chickens have also been published. These include studies at the level of the transcriptome (Zambonelli et al., 2016; Malila et al., 2019; Marchesi et al., 2019), proteome (Kuttappan et al., 2017), and metabolome (Boerboom et al., 2018). In general, these studies further support the aforementioned mechanism of development by reporting the presence of increased muscle development, hypoxia, inflammation, fibrosis, calcium signaling and outline the polygenic nature of WS.

1.4 Objectives

This thesis aimed to provide a better understanding of the role genetics plays in breast meat quality and WS in Canadian turkeys and outline the relationships between these traits and other economically important traits such as body weight and feed efficiency. The specific objectives were to:

1) Test the reliability of a scoring system for WS severity to prove consistency in observation among a single observer and between several observers [Chapter 2]
2) Score WS severity in a large population of birds using a reliable scoring system and provide insight into the prevalence of WS and associations between WS scores and production traits (i.e., breast weight, slaughter weight, breast meat yield) in this population [Chapter 2]
3) Estimate genetic parameters of WS and key meat quality traits indicative of PSE meat in a large turkey population and determine their phenotypic and genetic correlations with key economic traits such as growth and feed efficiency giving insight into the effects of current selection strategies [Chapter 3]
4) Investigate the genomic architecture of WS in turkeys through execution of a genome-wide association study and functional analysis for detection of pathways and gene ontologies associated with the myopathy thus adding to the body of knowledge surrounding the biological development of the trait [Chapter 4]

Chapter 2: Reliability of a white striping scoring system and description of white striping prevalence in purebred turkey lines

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2.1 Abstract

To efficiently meet consumer demands for high-quality lean meat, turkeys are selected for increased meat yield, mainly by increasing breast muscle size and growth efficiency. Over time, this has altered muscle morphology and development rates which are believed to contribute to the prevalence of myopathies. White striping is a myopathy of economic importance which presents as varying degrees of white striations on the surface of skinless breast muscle and can negatively affect consumer acceptance at the point of sale. Breeding for improved meat quality may be a novel strategy for mitigating the development of white striping in turkey meat; however, it is crucial to have a reliable assessment tool before it can be considered as a phenotype. Six observers used a four-category scoring system (0-3) to score severity in several controlled rounds and evaluate intra- and inter-observer reliability of the scoring system. After sufficient inter-observer reliability (Kendall's W > 0.6) was achieved, 12,321 turkey breasts, from four different purebred lines, were scored to assess prevalence of the condition and analyze its relationship with important growth traits. Overall, the prevalence of white striping (score > 0) was approximately 88% across all genetic lines studied with most scores being of moderate-severe severity (score 1 or 2). As was expected, increased white striping severity was associated with higher slaughter weight, breast weight, and breast meat yield (**BMY**) within each genetic line. This study highlights the importance of training to improve the reliability of a scoring system for white striping in turkeys and was required to provide an updated account on white striping prevalence in modern turkeys. Furthermore, we showed that white striping is an important breast muscle myopathy in turkeys linked to heavily selected traits such as body weight and BMY. White striping should be investigated further as a novel phenotype in future domestic turkey selection through use of a balanced selection index.

2.2 Introduction

The consumption of turkey meat is increasing worldwide. It is the second most-consumed poultry meat globally, particularly in Europe and North and South America (FAOSTAT, 2021). With consumer preference for smaller, deboned, or further processed products typically sold in trays covered with clear plastic film, the visual appearance of skinless portions is the most important determinant of acceptance at the point of sale (Min and Ahn, 2012; Font-i-Furnols and Guerrero, 2014; Barbut, 2020).

Genetic selection in poultry has been geared toward increasing body weight, average daily gain, and breast muscle yield to meet consumer meat demand more efficiently (Havenstein et al., 2007). This selection pressure has increased myopathies of the *Pectoralis major*, such as white striping (**WS**) (Velleman and Nestor, 2003; Hocking, 2014). From a microscopic point of view, breasts affected by WS show an increased sign of muscle cell injury thought to be related to rapid growth of the breast muscle tissue leading to ischemia and myofibril necrosis with minimal time from cell repair (Russo et al., 2015; Kuttappan et al., 2016; Baldi et al., 2018; Barbut, 2019; Livingston et al., 2019). The necrotic muscle tissue is then infiltrated by fat and connective tissue leading to the macroscopic presence of white striations on the surface of the fillet and resulting in fillets with a higher lipid and lower protein content (Kuttappan et al., 2016; Soglia et al., 2018; Barbut, 2019). WS has been shown to negatively affect consumer acceptance of fresh, skinless broiler chicken meat (Kuttappan et al., 2012b; de Carvalho et al., 2020), and one would assume the same finding is likely in turkeys. This is because consumers associate these white striations with meat from older poultry (e.g., older chickens tend to show more striations than young broiler chickens). However, today we see these white striations also in young birds (Griffin et al., 2018). WS has also been shown to affect the quality of the meat by decreasing marinade uptake, increasing cooking loss, and increasing hardness of the meat as a result of decreased functional proteins (Kuttappan et al., 2013b; Petracci et al., 2013, 2014; Mudalal et al., 2015; Sanchez Brambila et al., 2016; Baldi et al., 2018).

Consequently, larger breast fillets that are commonly used for further processing will also be negatively affected by WS.

The incidence of WS has been researched in broiler chickens, and flock prevalence between 56-87% has been reported in heavy strains (Lorenzi et al., 2014; Russo et al., 2015; Cruz et al., 2017; Golzar Adabi and Demirok Soncu, 2019). Research on WS in turkeys is limited compared to broiler chickens, with a recent study showing a flock-level prevalence of over 60% (Mudalal, 2019). Genetically, WS has been estimated to be moderate to highly heritable in broiler chickens (Bailey et al., 2015; Alnahhas et al., 2016; Pampouille et al., 2018; Lake et al., 2021), but to our knowledge, no estimates have been published for turkeys.

Although the development of WS is associated with microscopic changes to the muscle tissue (Kuttappan et al., 2013b), this myopathy is clearly observable at the macro level and can be scored without microscopes or other technologies. A four-category scoring scheme, primarily accounting for thickness of the striations, has been used to evaluate the severity of WS in broiler meat; this scheme classifies the appearance of breast meat samples as normal (no distinct white lines), moderate (small lines, <1mm), severe (large white lines, 1-2 mm), or extreme (thick bands, >2 mm) (Kuttappan et al., 2012b, 2016). This scheme has also been used in several turkey studies (Mudalal, 2019; Mudalal et al., 2020; Carvalho et al., 2021b). Zampiga et al. (2019) used a similar four-category scoring scheme based on the proportion of the breast fillets covered by stripes as opposed to the thickness of the bands.

As muscle myopathies are a rising problem in the North American meat industry (Bailey et al., 2015; Malchiodi et al., 2018; Santos et al., 2021) and consumer's awareness of these pathologies and their concerns about the animal's well-being arise (World Organization for Animal Health, 2008; Boerboom et al., 2018; Prisco et al., 2021), efforts are made by breeding companies to improve the health of the animal and the appearance and quality of meat products by reducing the occurrence of emerging muscle myopathies.

To do so, it is important to find a reliable method of identifying and classifying defects such as WS. There is a lack of evaluation of the reliability of visual scoring schemes for WS with multiple observers. Accurate recording of WS is important to ensure consistency over time and between observers, especially when considering the inclusion of a trait in breeding programs (Kapell et al., 2012). The objectives of this study were to: 1) evaluate the intra- and inter-observer reliability when using a WS scoring system adapted for turkeys, 2) evaluate the prevalence and severity of WS in turkey breast muscles, and 3) evaluate associations between WS scores and production traits (i.e., breast weight, slaughter weight, breast meat yield).

2.3 Materials and Methods

2.3.1 Ethics and Animal Care

All protocols complied with the guidelines of the Canadian Council on Animal Care and were approved by the University of Guelph Animal Care Committee (AUP 3782).

2.3.2 Animals

Adult male turkeys, 20-24 weeks old, from four purebred lines (A, B, C, and D) were processed over 44 weeks between 2018 and 2019 at a local commercial poultry processing plant as part of a larger project focusing on genomic selection in turkeys (Malchiodi et al., 2018). The number of birds included in this study was 12,321 with 2,839, 3,728, 2,034, and 3,720 from lines A, B, C, and D, respectively. Birds were reared under identical conditions according to Hybrid Turkeys (Hybrid Turkeys, 2020) and were weighed two days prior to processing (i.e., **slaughter weight**) (OHAUS scale, New Jersey, USA, accuracy to 0.01 kg). During processing at a commercial processing plant, the birds were electrically stunned and exsanguinated. Birds were scalded, defeathered, and eviscerated before moving to the chiller for 24 hours prior to deboning and collecting meat quality and breast muscle weights (OHAUS scale, New Jersey, USA, accuracy to

0.01 kg). Breast meat yield (**BMY**, %) was then calculated as a proportion of slaughter weight.

2.3.3 Scoring System

Both *Pectoralis major* muscles were photographed (Hero 6, GoPro, San Mateo, CA, USA) approximately 24 hours post-mortem. Photographs were taken approximately 40 cm from the surface of the fillets using the “normal” focal length setting of the camera to minimize distortion of the image. These photographs were used to evaluate a 0 – 3 scoring scale for WS which was adapted from a system originally used in broiler chickens (Kuttappan et al., 2016). As shown in Figure 1, a score of 0 represented no to minimal white striations, score 1 represented thin white striations visible on the breast, most of which tended to occur at the caudal end of the fillet, score 2 represented white striations visible on the breast spread between the caudal end and the main body of the fillet, and score 3 represented thick white striations visible on the breast covering majority of the outer surface. If the two breasts in each photograph differed in severity, observers were instructed to record the more severe score. All observers were blind to all additional recorded data of each image including genetic line, age at slaughter, slaughter weight, breast weight, and BMY.

2.3.4 Scoring Evaluation

The intra- and inter-observer reliability of the scoring system was assessed among six observers due to the high number of samples to be scored. One observer had previously tested the scoring system on a subset of photos to determine its feasibility, while others had no previous experience scoring WS severity. The scoring system was discussed among the observers before the first scoring session. A session consisted of all observers scoring 50 photographs in duplicate (two rounds per session). The initial subset of photographs was semi-randomly selected in that they had to give a clear visual of the breast muscle (e.g., without any damage due to trimming). Observers scored the same 50 photographs in an initial round and repeated the exercise several days later with

the same set of photographs in a different randomized order. Intra-observer reliability was determined based on the results of the two rounds within one session for each observer. In contrast, inter-observer reliability was determined based on the specific round across observers. Following the first session (session 1), all observers discussed the results to align their scoring before repeating another session with the same 50 photos (session 2). A final session (session 3) was conducted after further discussion with a new subset of 50 photos that were randomly selected to reflect photographs collected under more typical processing plant conditions (e.g., excessive fat/skin obscuring the muscle, damaged breast muscle).

2.3.5 Reliability Analysis

All agreement calculations were performed using SAS® statistical software, version 9.4 (SAS Institute Inc., Cary, NC, USA (SAS Institute Inc., 2016)).

Multiple measures of agreement were calculated for intra- and inter-observer reliability to comprehensively understand the reliability of the scoring system. Intra-observer reliability was assessed by calculating exact agreement between two rounds, Spearman's rank correlations, Fleiss-Cohen's kappa coefficient, linear weighted kappa, and quadratic weighted kappa. Kappa statistic estimates were calculated to determine the level of agreement corrected for chance. Weighted kappa coefficients (linear and quadratic) were calculated to attribute partial credit depending on the relative extent of the disagreement between scores using Fleiss-Cohen weights (Watson and Petrie, 2010). Kappa values were interpreted following the classifications suggested by Landis and Koch (1977) and presented in Petrie and Watson (2013) where $\kappa \leq 0.20$ is 'poor', $0.21 \leq \kappa \leq 0.40$ is 'fair', $0.41 \leq \kappa \leq 0.60$ is 'moderate', $0.61 \leq \kappa \leq 0.80$ is 'substantial', and $\kappa > 0.80$ is 'good'. It should be noted that these classifications are arbitrary (Landis and Koch, 1977), and the interpretation of kappa is dependent on the research field, however, a value closer to 1 indicates better agreement.

Inter-observer reliability was assessed by calculating estimates and tests of agreement among multiple observers using the %MAGREE macro (v3.8). The number of observers agreeing on each photograph was determined to calculate the percentage of cases in which 2, 3, 4, 5, or all 6 observers agreed on a score. Exact and weighted (linear and quadratic) kappa statistics were estimated (Watson and Petrie, 2010). Kendall's W coefficient of concordance was estimated to test agreement of the observers' ranking of the photographs. Finally, Gwet's weighted agreement coefficient (AC_2) for ordinal data was calculated to assess agreement on each score category while treating both observers and photographs as sampled rather than fixed to assess agreement of the entire populations of observers and photographs. For both Kendall's *W* and Gwet's agreement coefficient, the maximum value of 1 indicates perfect agreement.

2.3.6 White Striping Prevalence and Association with Production Traits

Upon completion of the three scoring sessions (six rounds), all 12,321 photos were divided among the six observers. Using R version 3.5.2 (R Core Team, 2020) and ggplot2 (Wickman, 2016), the score frequency within each line and within the entire population was plotted. To test the influence of genetic line on WS score, a Tukey's HSD test implemented in the stats package within R (R Core Team, 2020) was conducted on a linear model with genetic line as the only fixed effect. In addition to the WS scores, 12,282 birds had measurements for body weight two days before processing (**slaughter weight**), 12,190 had measures for breast weight (combined weight of both *Pectoralis major* and *minor* muscles), and 12,168 had measures for breast meat yield (**BMY**; breast weight as a percentage of slaughter weight). For each trait, outliers were excluded based on 3 standard deviations from the mean, and therefore, sample numbers differed slightly between the traits. Least square (**LS**) means of the three weight measures, for each score category, were calculated using a linear model with WS score and genetic line as fixed effects. LS means were then plotted for each genetic line. Statistical significance between the means was determined using Tukey's HSD test implemented in the stats package within R.

2.4 Results

2.4.1 Intra-observer Reliability

The results of the intra-observer reliability assessment in the three sessions for each observer are shown in Table 1. The percentage of exact agreement ranged between 62 – 78% in the first session for all observers, except observer 1, who acknowledged to have been influenced by discussion in between their rounds in the first session. As a result, kappa values ranged from poor to substantial in the first session, showing a substantial difference between observers in intra-observer reliability. These values improved in the second session, with all observers showing a moderate to good agreement. From sessions two to three, kappa values remained similar or showed a slight decrease which can likely be attributed to artifacts (e.g., excessive fat/skin obscuring the muscle, damaged breast muscle) in the new subset of photographs, which made scoring more difficult but represented more realistic examples. Considering the weighted kappa values, all observers had moderate to good agreement within themselves when considering the linear weighting. In contrast, all observers had good agreement when considering the quadratic weighting (session 2 and 3). In the second session, the increase in kappa when using the quadratic weighing was larger for observers 5 and 6 suggesting a larger difference in their scores (e.g., a score of 1 vs. 3 rather than 1 vs. 2). However, these were more similar across observers by the third session, suggesting only small differences in scores.

2.4.2 Inter-observer Reliability

The results of the inter-observer reliability assessment in the six rounds between all observers are shown in Table 2. In the initial round, there were no instances in which all observers agreed on the same score. Instead, in nearly half of the images, exactly three observers gave the same score. The agreement increased in subsequent rounds, with the best results observed in the third and fourth round where ≥ 4 observers agreed on most scores. In these rounds, we also observed all 6 observers agreeing; however,

this percentage was not higher than 32%, showing the difficulty in obtaining exact agreement on the scoring scale among 6 observers. This was also highlighted by the poor to fair kappa values for exact agreement and fair to moderate kappa values when using linear and quadratic weighing. In contrast, Kendall's *W* was relatively high (> 0.6) indicating good agreement for the ranking of the photographs. Similarly, Gwet's agreement coefficient for ordinal data showed substantial to good agreement overall, especially when considering the quadratic weighing.

2.4.3 Prevalence of White Striping

The WS prevalence in the population studied is shown in Figure 2. Descriptive statistics for the recorded weight measures for each genetic line are shown in Table 3. The frequency of affected breasts (i.e., breasts scored as 1, 2, or 3) within the entire population was 88.1%. The four genetic lines showed similar prevalence, with line A having the highest frequency at 90.5% and line D the lowest frequency at 84.1%. Line D was the only line to show a higher proportion of lower scores (0 and 1) than higher scores (2 and 3), with the lowest frequency of breasts scored as extreme (3.3% as score 3). In contrast, line B had 11.3% of breasts scored as extreme (score of 3). Consequently, genetic line was found to be a significant factor when analyzing WS score ($p < 0.05$). Although it has the largest average slaughter weight and breast meat weight of the four genetic lines, line D showed the lowest average WS score (2.30 ± 0.013, $p < 0.05$) compared to line A (2.57 ± 0.015), line B (2.61 ± 0.013), and line C (2.47 ± 0.017). Average WS score of all lines significantly differed ($p < 0.05$) with the exception of lines A and B, which tended to have a more similar average WS score ($p = 0.07$).

Figures 3, 4, and 5 show the average slaughter weight, breast weight, and BMY for each score within each line, respectively. Compared across lines, a similar trend was observed in that higher WS scores tended to be associated with higher average breast weight, slaughter weight, and BMY. For all lines, a WS score of 3 was associated with a significant larger average weight measurement than score 0 ($p > 0.05$). The only

exception was BMY of line A, which was numerically larger but not significantly different. The average percent increase between score 0 and score 3 was 4.6% for slaughter weight, 7.9% for breast weight, and 3.0% for BMY.

2.5 Discussion

The first objective of this study was to evaluate the reliability of a WS scoring scale for turkey breast muscles among multiple observers, followed by describing the prevalence and severity of WS in a turkey population consisting of birds from four genetic lines. Overall, we found that the scoring scale was reliable for multiple observers to score breasts for WS based on the cumulative information provided by the multiple coefficients estimated. Inter-observer reliability showed moderate to good agreement after completing three training sessions containing two rounds each. The inclusion of training is highly recommended for future studies attempting to score meat quality defects, such as WS, as the intra- and inter-observer reliability of the scoring system was improved after multiple sessions. Santos et al. (2021) reported a weighted kappa of 0.85 for intra-reliability of WS in broiler chickens, which is similar to the results of the observers with the highest intra-observer reliability in the current study. It was not reported how many training sessions were required to reach this value in their study. To the best of the authors' knowledge, no other studies have reported reliability measurements for WS in poultry. Reporting reliability measures is needed to evaluate and interpret data from such visual scoring systems. Observers in the current study appeared to stabilize their scoring and show the highest intra-observer reliability measures in session 3 (round 5 and 6), while the inter-observer reliability was strongest in session 2 (round 3 and 4). This is in line with research in other fields using discrete scoring systems to assess animal health. For example, D'Eath (D'Eath, 2012) reported that scoring reliability improved until the fifth scoring event. However, regular evaluation of reliability of scoring systems and retraining is typically recommended.

This study suggests that the most difficulty in reaching agreement between observers was found between score 1 and 2, which showed lower agreement coefficients. In contrast, the extremes of score 0 and 3 were more easily agreed upon among all observers. Intermediate scores in discrete scales have previously been reported to have poorer reliability (Garner et al., 2002; D'Eath, 2012; Schlageter-Tello et al., 2014). This difficulty in agreeing on scores 1 and 2 could show a need for clearer definitions and examples, a broader scoring scale (fewer categories), or a reduction in the overall number of observers. More detailed scoring systems with more categories have more room for disagreement and require more training (Garner et al., 2002; D'Eath, 2012; Schlageter-Tello et al., 2014; Decina et al., 2019). Depending on the purpose of the WS scoring, it could be recommended to reduce the scoring scale to combine scores 1 and 2. However, merging scores is also considered to lead to a loss of granularity and should be suitable for the intended purpose of the assessment (Garner et al., 2002; D'Eath, 2012; Schlageter-Tello et al., 2014; Decina et al., 2019). This seems relevant here, as scores 1 and 2 were associated with differences in breast muscle weight, slaughter weight, and breast muscle yield in some of the genetic lines in the current study (Figures 3-5). Ultimately, the development of a machine vision system capable of scoring breasts quantitatively, based on the number and thickness of the white striations would be optimal, however, the development and use of such a program was not considered here.

It should be noted that this study used photographs to assess WS rather than live scoring due to practical constraints within the processing plant. Photographs or videos can be appropriate alternatives to live scoring (Schlageter-Tello et al., 2014; Palczynski et al., 2016). Technology is increasingly implemented in processing plants to take advantage of automated methods (e.g., scoring of footpad legions, grading broiler carcasses), which allow collection of larger datasets and benefits in terms of feasibility at high line-speeds (Barbut, 2020). However, disadvantages exist, and troubleshooting was required when initially setting up the camera system to ensure good quality photographs (e.g., adjustments for height, blur, and positioning). Feedback from observers showed

that glare, positioning of breast outside of the area of focus, surface colour (striations were less noticeable with lighter coloured breast) or damage of the breast muscle (e.g., by trimming) could influence the scoring. These scenarios were more present in session 3, which used less ideal photographs to mimic more realistic processing plant conditions. This could explain the slight dip in inter-observer reliability from session 2 to 3, as observers adjusted to these conditions. These issues may be less of a problem when assessing breast muscle in-person. In-person training might be useful on a small subset of breasts for aligning scores between observers and an initial discussion of these scenarios, while time consuming scoring of large samples sizes, may be more appropriate via photographs.

Compared to the results of this study, a lower prevalence of WS (61.3%) has previously been reported in turkeys using a 0-2 scoring system (Mudalal, 2019). A potential factor worth considering in future studies is the type of birds used since pedigree stock, used in the present study, tends to be larger and may show differing levels of WS compared to commercial birds that are typically a hybrid cross. The WS prevalence of the studied population was similar, if not slightly higher than that previously published in broiler chickens, ranging between 56-87% in heavy strains (Lorenzi et al., 2014; Russo et al., 2015; Cruz et al., 2017; Golzar Adabi and Demirok Soncu, 2019). A case has been made for WS being an emerging problem in broiler chickens over the past years and that this issue may also be present in turkeys.

Fast-growing broiler chickens and turkeys have been shown to have increased severity of WS (Russo et al., 2015; Baldi et al., 2018; Soglia et al., 2018; Carvalho et al., 2021b). The same finding was also observed within each line in the present study. However, this was not the case when comparing WS and average body weight between the four studied lines (i.e., the largest line, line D, showed the lowest WS severity). Increases in breast weight between normal breasts and breasts affected by WS in broilers range from 16% to 25% (Mudalal et al., 2015; Baldi et al., 2018), and an increase of 20%

was observed in turkeys (Soglia et al., 2018). These reported differences in breast muscle weight between normal and affected breasts were higher than those observed in the present study between normal and extreme breasts (average of 7.9% increase). However, it should be noted that the weight used in the present study was the total weight of both *Pectoralis major* and *minor* muscles, which may influence the correlation between breast weight and WS. Significant increases in both slaughter weight and BMY between normal and extreme breast fillets were also observed in this study (average of 4.6% and 3.0%, respectively). This negative association between larger birds (i.e., one of the major economic goals of poultry breeding), and increases in muscle myopathies, such as WS, may pose an issue for breeders that could be managed through a selection index with appropriate economic weights.

It is noteworthy that the studied four lines had differing average slaughter ages depending on the birds' size. The largest two lines, A and D, had an average slaughter age of 148.1 days and 143.8 days, respectively. The smaller two lines, B and C, had an average slaughter age of 153.7 days and 156.2 days. We do not believe this is a significant factor in the prevalence results as the range between the highest and lowest slaughter ages is minimal; however, it should be considered as age has been shown to affect the severity of WS (Radaelli et al., 2017).

2.6 Conclusions

The reliability of a WS scoring system for turkey breast muscles with multiple observers was developed and assessed. Later, the prevalence of WS in a large population of purebred turkey toms was determined. Overall, we report that the scoring system showed moderate to good reliability within and between the observers and that reliability improved after multiple training sessions. The prevalence of WS varied across the studied turkey lines and was 88.1% on average, with the majority of scores being moderate-severe (scores 1 and 2). This highlights that this breast muscle myopathy is quite prevalent in turkeys and should continue to be investigated due to its negative effect

on consumer preference and composition of the meat. In general, WS severity was found to be associated with higher slaughter weight, breast weight, and BMY, suggesting negative correlations that will have to be dealt with using a balanced selection index to reduce the presence of WS while continuing to maintain an increase in these economically significant traits.

2.7 Acknowledgements

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2.8 Figures

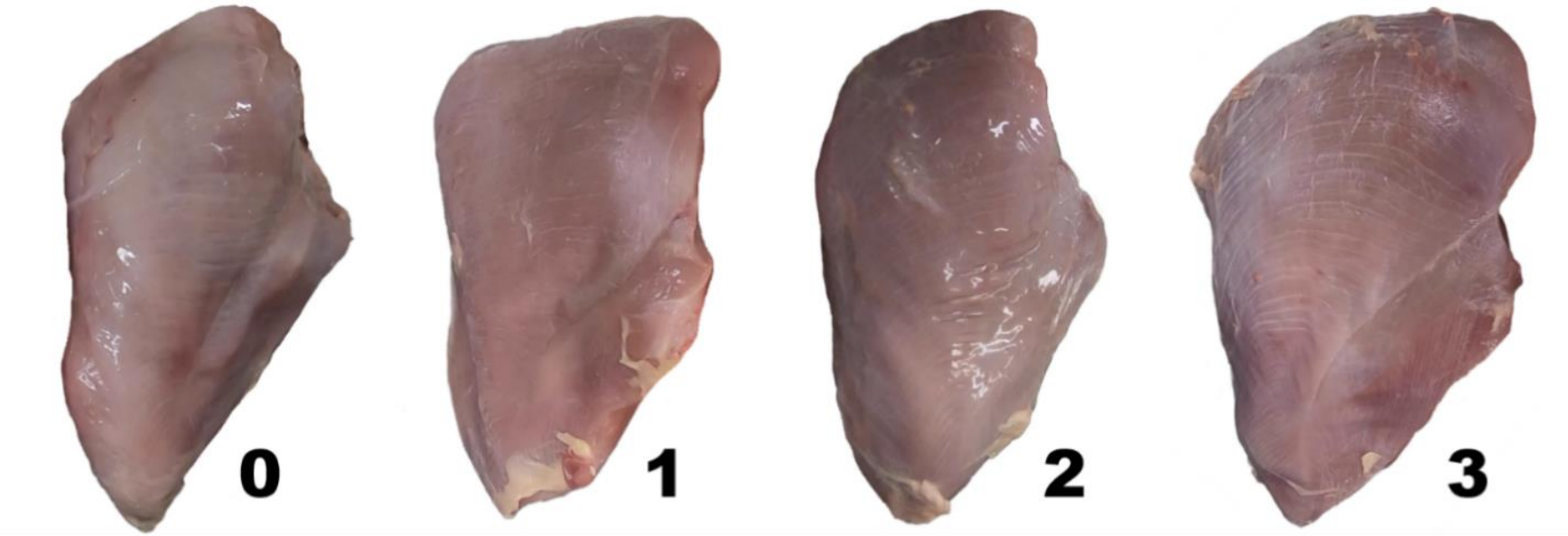

Figure 2.1: Visual scoring system used for scoring white striping severity of the Pectoralis major (fillet). Where 0 (normal) = no to minimal white striations; 1 (moderate) = thin white striations visible on the breast, most of which tended to occur at the caudal end of the fillet (bottom of pictured fillets); 2 (severe) = large white striations visible on the breast spread between the caudal end and the main body of the fillet (top of pictured fillets); 3 (extreme) = thick white striations visible on the breast covering majority of the outer surface. Pictures by Ryley Vanderhout.

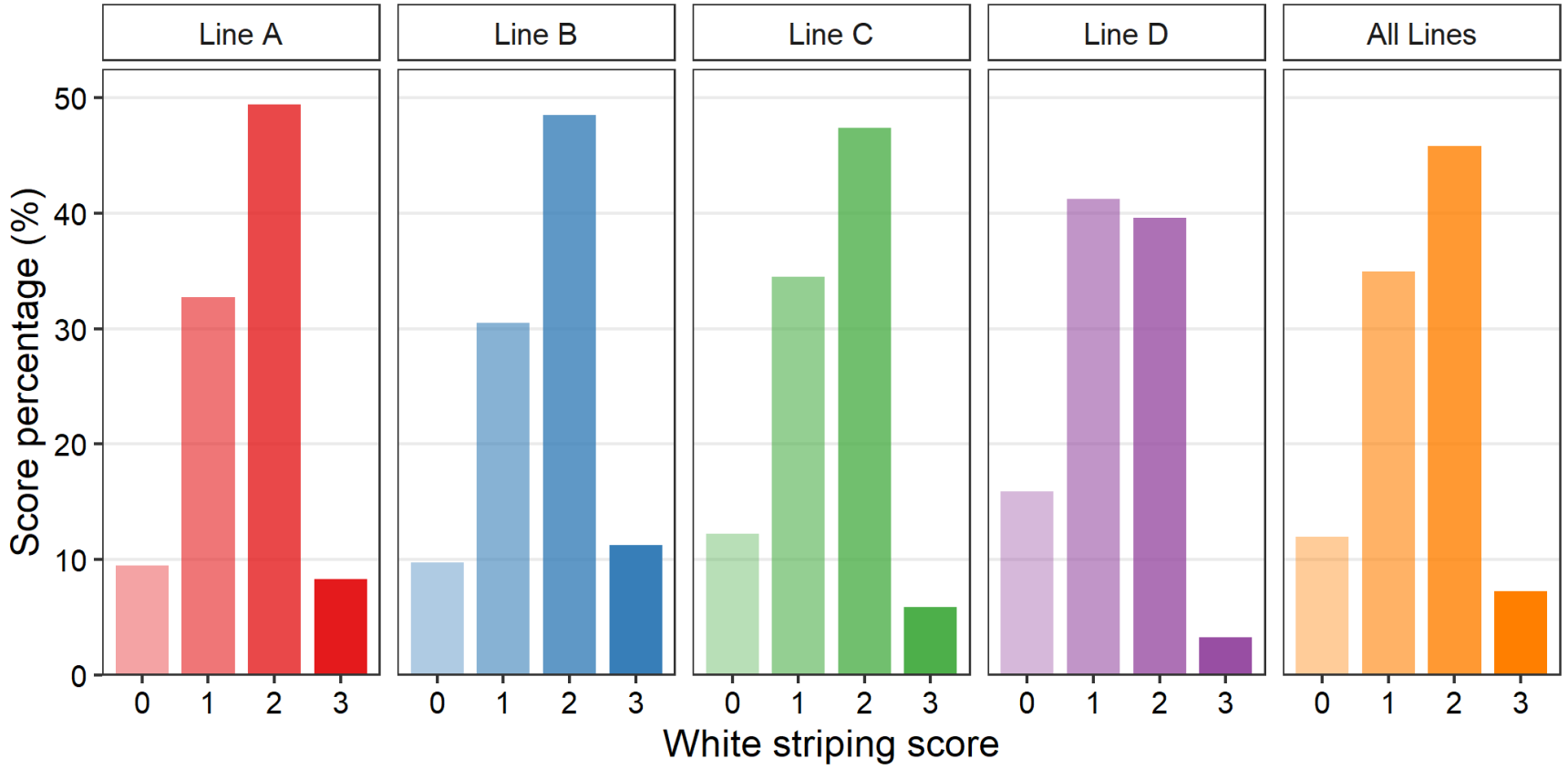

Figure 2.2: White striping severity score (score 0-3 with 0 representing normal, 1 moderate, 2 severe, and 3 extreme) percentage within purebred turkey toms of each genetic line, A (n = 2,839), B (n = 3,728), C (n = 2,034) or D (n = 3,720) and within the entire studied population (n = 12,321).

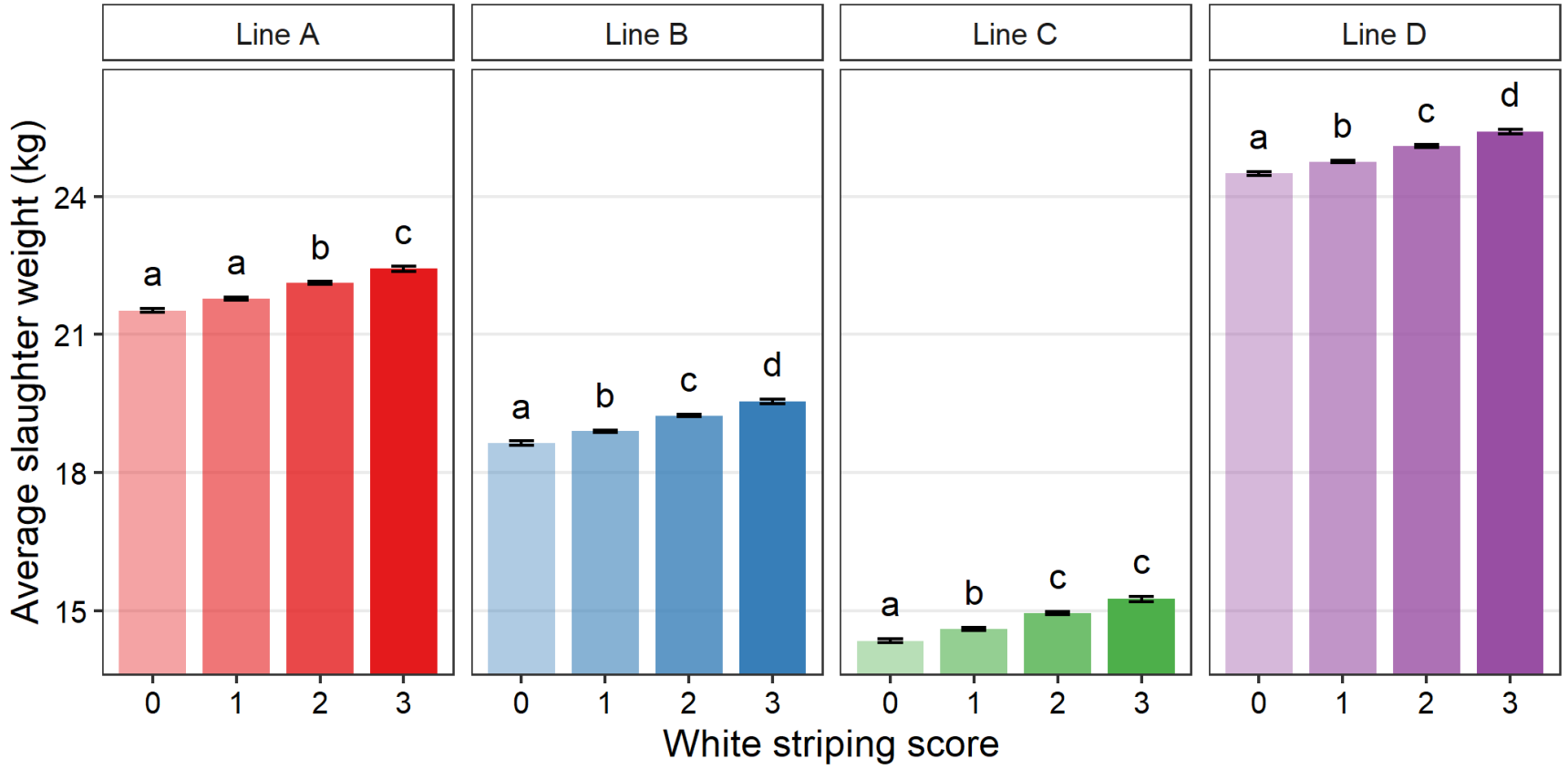

Figure 2.3: Least square means for slaughter weight (live weight of the bird 2 days prior to slaughter in kg) of purebred turkey toms for each white striping score (0-3) within each genetic line (A: n = 2,834, B: n = 3,715, C: n = 2,032, D: n = 3,701). Error bars show standard error. Means with different letters (a-d) within genetic line represent statistical significance ($p < 0.05$) as determined by Tukey's HSD test.

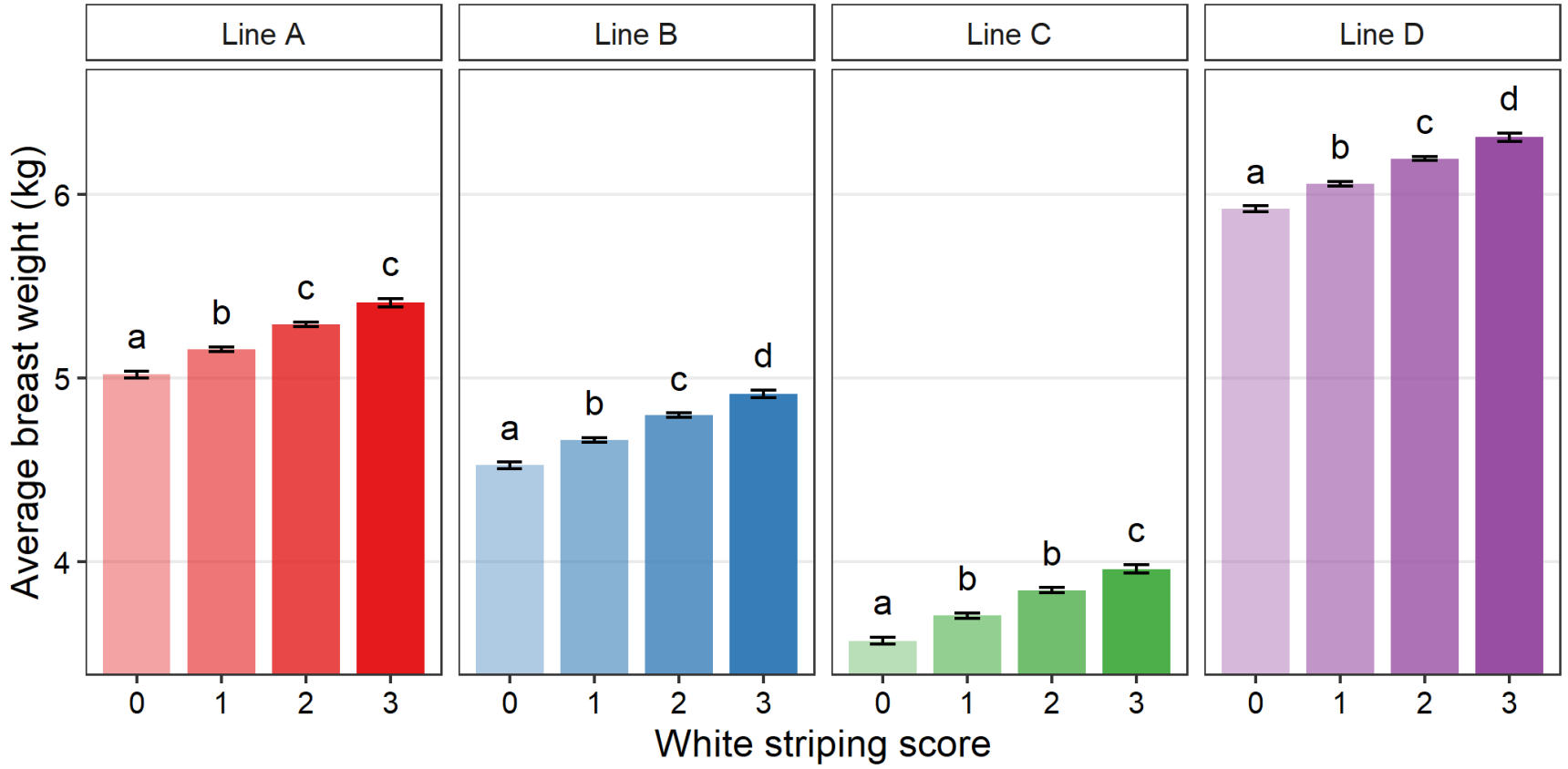

Figure 2.4: Least square means for breast weight (combined weight of Pectoralis major and minor muscles in kg) of purebred turkey toms for each white striping score (0-3) within each genetic line (A: n = 2,830, B: n = 3,677, C: n = 2,031, D: n = 3,652). Error bars show standard error. Means with different letters (a-d) within genetic line represent statistical significance ($p < 0.05$) as determined by Tukey's HSD test.

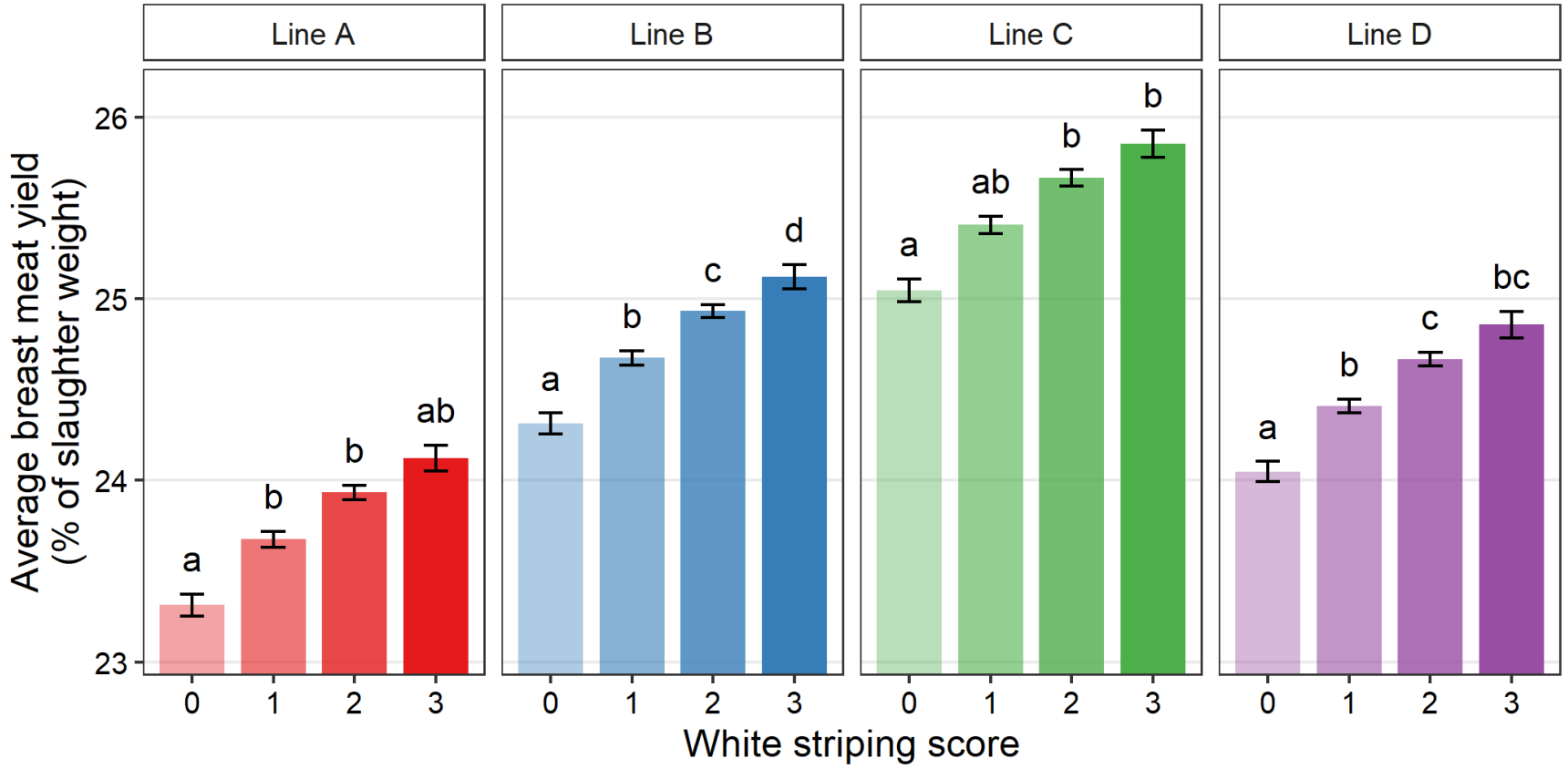

Figure 2.5: Least square means for breast meat yield (breast meat weight as a percentage of slaughter weight) of purebred turkey toms for each white striping score (0-3) within each genetic line (A: n = 2,827, B: n = 3,666, C: n = 2,028, D: n = 3,647). Error bars show standard error. Means with different letters (a-d) within genetic line represent statistical significance ($p < 0.05$) as determined by Tukey's HSD test.

2.9 Tables

Table 2.1: Intra-observer reliability coefficients of white striping scoring of turkey breast muscle (0-3 scale) to assess agreement within 6 observers over multiple training sessions. 95% confidence interval for kappa, linear weighted kappa, and quadratic weighted kappa are given below the coefficients in brackets.

	Observer 1	**Observer 2**	**Observer 3**	**Observer 4**	**Observer 5**	**Observer 6**	*Average*
			Session 1 (round 1 and 2)[1]				
Exact agreement (%)	38	74	68	78	62	74	*66*
Spearman correlation	0.48**	0.68***	0.69***	0.79***	0.63***	0.81***	*0.68*
Kappa	0.12 (-0.05-0.29)	0.51 (0.28-0.73)	0.43 (0.21-0.65)	0.64 (0.45-0.82)	0.41 (0.21-0.61)	0.62 (0.44-0.80)	*0.45*
Linear weighted kappa	0.20 (0.06-0.34)	0.58 (0.37-0.78)	0.54 (0.35-0.73)	0.70 (0.54-0.86)	0.51 (0.33-0.69)	0.72 (0.58-0.86)	*0.54*
Quadratic weighted kappa	0.31 (0.17-0.46)	0.68 (0.50-0.86)	0.68 (0.53-0.83)	0.78 (0.66-0.91)	0.62 (0.45-0.80)	0.82 (0.72-0.92)	*0.65*
			Session 2 (round 3 and 4)[1]				
Exact agreement (%)	80	86	88	74	68	72	*78*
Spearman correlation	0.73***	0.87***	0.86***	0.67***	0.70***	0.74***	*0.76*
Kappa	0.62 (0.41-0.83)	0.77 (0.61-0.93)	0.80 (0.64-0.95)	0.53 (0.31-0.75)	0.47 (0.27-0.68)	0.56 (0.36-0.76)	*0.63*
Linear weighted kappa	0.66 (0.46-0.85)	0.82 (0.68-0.95)	0.83 (0.70-0.96)	0.59 (0.39-0.79)	0.57 (0.40-0.75)	0.65 (0.49-0.82)	*0.69*
Quadratic weighted kappa	0.71 (0.50-0.91)	0.87 (0.77-0.97)	0.88 (0.78-0.98)	0.67 (0.49-0.84)	0.70 (0.56-0.83)	0.76 (0.63-0.89)	*0.76*
			Session 3 (round 5 and 6)[2]				
Exact agreement (%)	80	86	88	76	80	76	*81*
Spearman correlation	0.65***	0.88***	0.86***	0.65***	0.81***	0.71***	*0.76*
Kappa	0.59 (0.37-0.81)	0.80 (0.66-0.94)	0.80 (0.65-0.95)	0.52 (0.29-0.76)	0.69 (0.51-0.86)	0.58 (0.38-0.78)	*0.66*
Linear weighted kappa	0.62 (0.41-0.83)	0.84 (0.72-0.96)	0.83 (0.69-0.96)	0.57 (0.36-0.79)	0.75 (0.61-0.90)	0.63 (0.46-0.81)	*0.71*
Quadratic weighted kappa	0.67 (0.47-0.88)	0.88 (0.77-0.99)	0.87 (0.76-0.97)	0.64 (0.45-0.83)	0.83 (0.72-0.94)	0.71 (0.55-0.86)	*0.77*

[1] Sessions 1 and 2 were conducted with the same set of 50 photographs

[2] Session 3 was conducted with a new set of 50 photographs

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2.2: Inter-observer reliability coefficients of white striping scoring of turkey breast muscle (0-3 scale) to assess agreement between 6 observers over multiple training sessions. 95% confidence interval for kappa, linear weighted kappa, quadratic weighted kappa, and Gwet's AC_2 are given below the coefficients in brackets.

	Session 1[1]		Session 2[1]		Session 3[2]	
	Round 1	**Round 2**	**Round 3**	**Round 4**	**Round 5**	**Round 6**
			Percentage agreement (%)			
All 6 observers agree	0	6	32	22	12	10
5 observers agree	24	24	28	36	32	28
4 observers agree	24	46	36	26	26	40
3 observers agree	48	24	4	16	26	22
2 observers agree	4	0	0	0	4	0
			Exact agreement			
Kappa	0.17 (0.10-0.25)	0.27 (0.20-0.34)	0.33 (0.26-0.39)	0.29 (0.22-0.36)	0.21 (0.15-0.28)	0.24 (0.16-0.31)
Kendall's *W*	0.61***	0.68***	0.74***	0.70***	0.61***	0.66***
			Linear weighting			
Kappa	0.32 (0.20-0.44)	0.45 (0.36-0.54)	0.52 (0.45-0.59)	0.48 (0.39-0.56)	0.38 (0.28-0.47)	0.41 (0.31-0.51)
Gwet's AC_2						
Overall	0.54 (0.28-0.81)	0.65 (0.56-0.74)	0.79 (0.69-0.89)	0.76 (0.66-0.87)	0.67 (0.58-0.77)	0.68 (0.58-0.79)
Score 0	0.88 (0.80-0.96)	0.86 (0.77-0.94)	0.93 (0.88-0.99)	0.96 (0.91-1.00)	0.88 (0.78-0.97)	0.89 (0.80-0.97)
Score 1	0.47 (0.28-0.66)	0.51 (0.32-0.70)	0.72 (0.59-0.84)	0.70 (0.51-0.89)	0.57 (0.41-0.72)	0.60 (0.43-0.78)
Score 2	0.49 (0.26-0.72)	0.58 (0.45-0.72)	0.68 (0.53-0.84)	0.61 (0.46-0.77)	0.58 (0.44-0.73)	0.56 (0.38-0.73)
Score 3	0.81 (0.58-1.00)	0.92 (0.86-0.98)	0.93 (0.87-0.99)	0.89 (0.81-0.96)	0.91 (0.84-0.98)	0.89 (0.81-0.97)
			Quadratic weighting			
Kappa	0.32 (0.20-0.44)	0.45 (0.36-0.54)	0.52 (0.45-0.59)	0.48 (0.39-0.56)	0.38 (0.28-0.47)	0.41 (0.31-0.51)
Gwet's AC_2						
Overall	0.74 (0.53-0.95)	0.83 (0.77-0.88)	0.91 (0.85-0.96)	0.89 (0.83-0.95)	0.83 (0.76-0.90)	0.85 (0.78-0.92)
Score 0	0.88 (0.80-0.96)	0.86 (0.77-0.95)	0.93 (0.88-0.99)	0.96 (0.91-1.00)	0.88 (0.78-0.97)	0.89 (0.80-0.97)
Score 1	0.47 (0.28-0.66)	0.51 (0.32-0.70)	0.72 (0.59-0.84)	0.70 (0.51-0.89)	0.57 (0.42-0.72)	0.60 (0.43-0.78)
Score 2	0.49 (0.26-0.72)	0.58 (0.45-0.72)	0.68 (0.53-0.84)	0.61 (0.46-0.77)	0.58 (0.44-0.73)	0.56 (0.38-0.73)

Score 3	0.81 (0.58-1.00)	0.92 (0.86-0.98)	0.93 (0.87-0.99)	0.89 (0.81-0.96)	0.91 (0.84-0.98)	0.89 (0.81-0.97)

[1] Sessions 1 and 2 were conducted with the same set of 50 photographs

[2] Session 3 was conducted with a new set of 50 photographs

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2.3: Descriptive statistics (count, mean, and standard deviation) for white striping score, slaughter weight, breast weight, and breast meat yield (BMY) for the four genetic lines of turkeys studied.

Genetic Line	White striping score (0-3)			Slaughter weight (kg)			Breast weight (kg)			BMY (% BW)		
	N	***Mean***	***SD***	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***
A	2,839	2.57	0.776	2,834	21.98	1.548	2,830	5.23	0.583	2,827	23.81	1.966
B	3,728	2.61	0.810	3,715	19.11	1.286	3,677	4.74	0.499	3,666	24.81	1.789
C	2,034	2.47	0.782	2,032	14.77	1.005	2,031	3.77	0.394	2,028	25.51	1.704
D	3,720	2.30	0.771	3,701	24.87	1.801	3,652	6.10	0.757	3,647	24.47	2.108

Chapter 3: Genetic parameters of white striping and meat quality traits indicative of pale, soft, exudative meat in turkeys (Meleagris gallopavo)

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Key words: breast meat, correlation, heritability, meat quality, poultry

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3.1 Abstract

Due to the increasing prevalence of growth-related myopathies and abnormalities in turkey meat, the ability to include meat quality traits in poultry breeding strategies is an issue of key importance. In the present study, genetic parameters for meat quality traits and their correlations with body weight and meat yield were estimated using a population of purebred male turkeys. Information on live body, breast, thigh, and drum weights, breast meat yield, feed conversion ratio, breast lightness (**L***), redness (**a***), and yellowness (**b***), ultimate pH, and white striping (**WS**) severity score were collected on 11,986 toms from three purebred genetic lines. Heritability and genetic and partial phenotypic correlations were estimated for each trait using an animal model with genetic line, hatch week-year, and age at slaughter included as fixed effects. Heritability of ultimate pH was estimated to be 0.34 ± 0.046 and a range of 0.20 ± 0.02 to 0.23 ± 0.02 for breast meat colour (L*, a*, and b*). White striping was also estimated to be moderately heritable at 0.15 ± 0.02. Unfavorable genetic correlations were observed between body weight and meat quality traits as well as white striping, indicating that selection for increased body weight and yield may decrease pH and increase the incidence of pale meat with more severe white striping. The results of this analysis provide insight into the effect of current selection strategies on meat quality and emphasizes the need to include meat quality traits into future selection indexes for turkeys.

3.2 Introduction

With an increasing desire for lean, quality poultry meat products emphasis needs to be placed on improving technological characteristics and functional properties of the meat (i.e., pH, colour, water-holding capacity) (Barbut, 2015; Petracci et al., 2015). However, it is suggested that intense selection for growth and yield in poultry could be associated with a greater occurrence of growth-related myopathies and abnormalities, consequently increasing the number of downgraded carcasses and leading to an overall reduction of meat quality (Sosnicki and Wilson, 1991; Updike et al., 2005; Owens et al.,

2009; Zampiga et al., 2020). The rate and magnitude of post-mortem decline in muscle pH greatly affects the overall quality of the final meat product (Briskey, 1964; Wynveen et al., 1999; Barbut et al., 2008). Differences in post-mortem muscle pH among individual birds arise due to changes in rates of glycolysis which can be affected by several factors including pre-slaughter handling (stress), stunning method, muscle size, carcass chilling, nutrition, and genetics (Ma et al., 1971; Rathgeber et al., 1999; Wynveen et al., 1999; Velarde et al., 2000). When there is a rapid decline in pH or an exceptionally low pH in the final meat product, the result is pale, soft, exudative (**PSE**) meat. Characteristics of PSE meat include increased functional protein degradation leading to lighter coloured meat with decreased water-holding capacity and sometimes an increased shear force of the cooked product (Barbut, 1993, 1997; Owens et al., 2002). Not only is this difference in quality visually noticeable in broiler chicken meat, but it also affects sensory acceptability, with panelists preferring cooked meat classified as normal over the PSE meat (Droval et al., 2012).

In addition to the rise in PSE meat observed in the poultry industry over the past decades, an increase in the incidence of the growth-related myopathy white striping (**WS**) has been observed in the turkey industry (Mudalal, 2019; Vanderhout et al. 2021, *In submission*). This myopathy presents itself as thin white striations on the surface of the muscle running parallel to the muscle fibers. These white striations are a result of muscle tissue necrosis and subsequent infiltration of fat and connective tissue into the muscle (Kuttappan et al., 2013b; Baldi et al., 2018; Barbut, 2019). The resulting product exhibits an increase in lipid content while decreasing myofibrillar protein content (Kuttappan et al., 2016; Soglia et al., 2018; Barbut, 2019). This not only affects the quality of further processed products but also negatively affects consumer acceptance of broiler chicken whole muscle products (Kuttappan et al., 2012b; de Carvalho et al., 2020). Estimates for heritability of WS in broiler chickens ranges from 0.19 to 0.65 (Bailey et al., 2015; Alnahhas et al., 2016; Lake et al., 2021), however, there are no published estimates for turkeys.

Estimation of genetic parameters of meat quality traits is crucial to evaluate the possibility of genetic selection and more importantly, the magnitude of indirect selection on these traits. Due to the complexity of measuring these traits, research on their genetic parameters in turkeys is limited in comparison to other species and when conducted, generally have small sample sizes (Le Bihan-Duval et al., 2003; Aslam et al., 2011). However, the initial published estimates do show low to moderate heritabilities for breast pH and colour along with moderate to strong unfavorable genetic correlations among these traits and body weight (Le Bihan-Duval et al., 2003; Aslam et al., 2010). Therefore, the objectives of this study were to measure WS and key meat quality traits indicative of PSE meat in a large turkey population and to estimate genetic parameters for these traits. Additionally, we determined their phenotypic and genetic correlations with key economic traits such as growth and feed efficiency.

3.3 Materials and Methods

3.3.1 Ethics and Animal Care

All protocols complied with the guidelines of the Canadian Council on Animal Care and were approved by the University of Guelph Animal Care Committee (AUP 3782).

3.3.2 Animals

Data were collected on male turkeys from three purebred genetic lines (A, B, and C) over 44 weeks between July 2018 and November 2019. There were 11,986 birds included in this study; 2,569 were from line A, 5,299 from line B, and 4,118 from line C. The genetic lines included a dam-line that was selected primarily for body weight and reproductive traits (line A), a dam-line selected mainly for reproductive traits (line B), and a sire-line with selection focused on body weight, meat yield, and feed efficiency (line C). All genetic lines were raised under the same husbandry conditions (Hybrid Turkeys, 2020). Processing occurred between 20-24 weeks of age (average body weight of 21.5 kg) at a commercial poultry processing facility in Ontario, Canada. During processing,

birds were electrically stunned and exsanguinated. Birds were scalded, defeathered, and eviscerated before moving to the chiller for 24h prior to deboning and collecting meat quality measurements and carcass component weights.

3.3.3 Production Traits

Body weight (**BW**) was collected two days prior to slaughter (20-24 weeks of age). A real-time automated system was used to record individual feed intake (Tu et al., 2011) and feed conversion ratio (**FCR**) was calculated as total feed intake divided by weight gain (Abdalla et al., 2019). Carcass component weights were collected approximately 24h post-mortem. Randomly selected carcasses were broken down into the major components, and *Pectoralis major* (**fillets**), *Pectoralis minor* (**tenders**), thighs (bone-in, skin on), and drums (bone-in, skin on) were individually weighed. All remaining carcasses were processed separately, and the weight of total breast meat (fillets and tenders; **BrW**) were measured. All weights were measured in kg. Breast meat yield (**BMY**) was calculated as a percentage of BW.

3.3.4 Meat Quality Measurements

Meat quality measurements included ultimate pH and color. A pH measurement was taken from the dorsal side of an intact, deboned fillet from the randomly selected, broken down carcasses at 24 hours post-mortem (**pHu**; Portable pH meter HI98163, Hanna Instruments, Woonsocket, RI, USA). Lightness, redness, and yellowness (**L***, **a***, and **b***; CIE, 2018) were measured on the skinless dorsal side of the fillet of all birds using a colorimeter with D50 illumination (Nix Pro Colour Sensor, Hamilton, ON, CA). All fillets were also photographed (Hero 6, GoPro, San Mateo, CA, USA) approximately 24h post-mortem. Photographs were used to evaluate white striping (**WS**) on a 0-3 scoring scale (Kuttappan et al., 2016; Vanderhout et al., *In submission*).

3.3.5 Statistical Models

Univariate and bivariate linear animal models were used to estimate (co)variance components through restricted maximum likelihood carried out using the BLUPf90 family of programs (Misztal et al., 2018). The linear animal models used can be described as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

where **y** is the vector of observations sorted within animals; **b** is a vector of fixed effects including genetic line (3 levels: A, B, and C), hatch week-year (58 levels), and age at slaughter (7 levels; 141 to 163 days) for all models and the addition of score observer (6 levels) for WS; **a** is a vector of additive genetic effects distributed as $\mathbf{a} \sim N(0, \mathbf{A} \otimes \mathbf{G})$, where **A** is the numerator relationship matrix including the inbreeding coefficients and **G** is the additive genetic variance-covariance matrix between traits; **e** is the vector of residual effects which has a distribution of $\mathbf{e} \sim N(0, \sum_{\mathrm{i}}^{+} \mathbf{E}_{iy})$ where $\mathbf{E}_{iy}$ is an m_i x m_i matrix corresponding to the traits that were present for animal *i* and m_i is the number of traits present for animal *i*; and **X** and **Z** are design matrices relating the observations to the fixed and random effects, respectively. Heritability (h^2) was estimated as the proportion of phenotypic variance (sum of the additive genetic variance and residual variance) explained by additive genetic variance estimated from the univariate models. Genetic correlation coefficients ($\mathbf{r_g}$) were calculated using the following equation:

$$\mathrm{r}_g = \sigma_{xy} / \sqrt{\sigma_x^2 \sigma_y^2},$$

where σ_{xy} is the additive genetic covariance of traits x and y, and σ_x^2 and σ_y^2 are the additive genetic variances for traits x and y, respectively. Pearson partial phenotypic correlation coefficients ($\mathbf{r_p}$) were calculated using PROC GLM in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) to account for the fixed effects included in the genetic (co)variance estimates.

3.4 Results and Discussion

3.4.1 Heritability Estimates

Analysis of the 13 traits (Table 1) resulted in 78 bivariate combinations. The heritability estimates (Table 2) for all traits were moderate to high (0.15 to 0.63). Estimates for BW (h^2 = 0.44 ± 0.03) and BMY (h^2 = 0.41 ± 0.02) were high and within the range of previously published estimates in turkeys, which ranged from 0.23 to 0.45 for BW (Le Bihan-Duval et al., 2003; Case et al., 2012a, 2012b; Willems et al., 2013; Abdalla et al., 2019) and 0.27 to 0.43 for BMY (Le Bihan-Duval et al., 2003; Aslam et al., 2011; Case et al., 2012b; Abdalla et al., 2019). Carcass component weights (i.e., fillets, tenders, thighs, and drums) all showed high heritabilities similar to those previously published in both turkeys ranging from 0.45 to 0.49 (Case et al., 2012b) and broiler chickens ranging from 0.38 to 0.61 (Felício et al., 2013; Alnahhas et al., 2016). Breast weight (BrW), fillets, and tenders had similar (co)variance estimates and therefore, the remainder of the discussion will focus on BrW. A moderate heritability estimate was observed for FCR (h^2 = 0.18 ± 0.03) which was slightly higher than previously published estimates in turkeys which ranged from 0.05 to 0.16 (Case et al., 2012a; Willems et al., 2013; Abdalla et al., 2019). The heritability estimates presented in the present study for body and carcass component weights and FCR, along with previously published estimates, emphasize the strong genetic aspect of weight and efficiency in turkeys. It is to no surprise that considerable gains have been made in these areas over time (Havenstein, 2006).

Heritability estimates for the trichromatic coordinates (L*, a*, and b*) were similar in magnitude, showing all three traits to be moderately heritable. The heritability estimates for L* (h^2 = 0.20 ± 0.02) and a* (h^2 = 0.22 ± 0.02) were similar to the previous estimates reported in turkeys. Estimates for L* and a* have been reported to range between 0.12 to 0.27 and 0.21 to 0.30, respectively (Le Bihan-Duval et al., 2003; Aslam et al., 2011). However, the estimate for b* (h^2 = 0.23 ± 0.02) was higher than previous estimates of 0.15 and 0.14 in turkeys (Le Bihan-Duval et al., 2003; Aslam et al., 2011). In comparison

to estimates for breast meat colour in broilers, the heritability of L* from the present study was lower than the published range of 0.29 to 0.59 (Le Bihan-Duval et al., 2001, 2008; Gaya et al., 2006, 2011; Felício et al., 2013; Alnahhas et al., 2016). However, h^2 estimates for a* and b* were within the published ranges of 0.21 to 0.57 and 0.12 to 0.55, respectively (Le Bihan-Duval et al., 2001, 2008; Gaya et al., 2006, 2011; Felício et al., 2013; Alnahhas et al., 2016). Similar to breast meat colour, only two estimates for pHu have been reported for turkeys. The heritability estimate for pHu ($h^2 = 0.34 \pm 0.05$) in the present study was much greater than the previous estimates (h2 = 0.09, Le Bihan-Duval et al., 2003; h2 = 0.16, Aslam et al., 2011). Since h2 is a population specific parameter, there are several factors that can lead to the observed difference in h2 estimates between these studies including the genetic line used, use of purebred or commercial birds, or the sex of the birds. However, this estimate was similar to the majority of previously published estimates in broiler chickens reporting an average heritability of 0.34 (Le Bihan-Duval et al., 2001, 2008; Gaya et al., 2006; Gaya, et al., 2011; Felício et al., 2013; Alnahhas et al., 2016). Overall, these estimates suggest that there is a moderate genetic component to breast colour and pHu. The development of highly automated measurement techniques may allow for accurate measurements of these traits without sacrificing processing line speed. This would allow for the collection of many phenotypes necessary for genetic selection for improved meat quality.

To the best of our knowledge, this is the first published heritability estimate for WS in turkeys. In the population studied, WS was found to be moderately heritable ($h^2 = 0.15 \pm 0.02$) suggesting the potential for genetic selection against WS in turkeys. This estimate was low compared to reported estimates for broiler chickens which ranged from 0.19 to 0.50 when using a similar 0-3 scoring system (Bailey et al., 2015; Lake et al., 2021) and 0.65 when using a 0-2 scoring system (Alnahhas et al., 2016). With this being the first estimate of heritability in turkeys, the only comparable estimates are those of broiler chickens. The goal of comparison is therefore not to arrive at the same estimate but express the potential difference between the species. Due to longer intense directional

selection for growth and meat yield in broiler chickens and the well documented relationship between WS and growth, a larger genetic variation in chickens would not be surprising. The difference in heritability observed between these species could also suggest that WS is still in its infancy in turkeys resulting in the reduced genetic variance observed in the present study. If this is the case, that only emphasizes the importance of introducing genetic selection against WS in hopes to prevent further development of the myopathy.

3.4.2 Genetic and Partial Phenotypic Correlations

Additive genetic and partial phenotypic correlation coefficients between the traits are presented in Table 2. As expected, the genetic and partial phenotypic correlations between the body and carcass component weights were favorable. The strongest correlations were observed between BW and BrW ($r_g = 0.74 \pm 0.02$, $r_p = 0.78$), BW and thighs ($r_g = 0.73 \pm 0.04$, $r_p = 0.62$), and BW and drums ($r_g = 0.66 \pm 0.04$, $r_p = 0.58$). These are the three largest muscle groups which make up the largest portion of overall body weight. Moderate, favorable genetic and partial phenotypic correlations were observed between BrW and thighs ($r_g = 0.33 \pm 0.06$, $r_p = 0.38$) and BrW and drums ($r_g = 0.24 \pm 0.06$, $r_p = 0.29$), which is logical as birds with larger and heavier breast muscles require stronger leg muscles to support walking ability. However, since the genetic correlation estimates are not perfect, stronger emphasis placed on selection for breast traits may still lead to an unbalanced development of the leg muscles. Correlations between body and carcass component weights and FCR were all weak ($-0.04 < r_g < 0.04$), except for fillets ($r_g = 0.16 \pm 0.10$, $r_p = -0.04$), suggesting a moderate, unfavorable relationship between fillet weight and FCR in the studied population. This was not the case in previously published studies in turkeys which showed a genetic correlation of BW and FCR ranging from 0.10 to 0.19 (Case et al., 2012a; Abdalla et al., 2019). All correlations between the studied traits and FCR were also weak ($-0.04 < r_g < 0.04$) with the exception of FCR and L* ($r_g = 0.14 \pm 0.10$, $r_p = 0.05$) which showed a moderate, favorable genetic correlation and FCR and a* ($r_g = -0.26 \pm 0.09$, $r_p = -0.15$) which showed moderate, favorable genetic

and phenotypic correlations. This suggests that selection for FCR may affect meat quality, however, this may be due to the connection between FCR and fillet weight and should be investigated further.

Several correlations were observed in the present study that support the mechanism for the development of PSE meat. Moderate, unfavorable correlations were observed between BW and pHu (r_g = -0.18 ± 0.08, r_p = -0.17) as well as BrW and pHu (r_g = -0.19 ± 0.08, r_p = -0.18). The correlation between size of the bird and pHu of the breast muscle has been documented with larger, faster-growing lines showing a tendency to have a faster pH decline and lower pHu (Wang et al., 1999; Owens et al., 2002; Updike et al., 2005; Aslam et al., 2011). Another important relationship is between muscle temperature during the chilling process and colour of the final meat product. Rathgeber et al. (1999) showed that delayed chilling of turkey breasts led to an increase in L*, a*, and b*. Similarly, Mckee and Sams (1998) reported increases in L* when turkey breasts were held at 40°C for 2h compared to breasts held at lower temperatures. A similar relationship was found in the current study with heavier BrW measurements showing an unfavorable genetic correlation with L* and b* (r_g = 0.43 ± 0.06 and r_g = 0.19 ± 0.06, respectively) potentially due to the increased chilling time required for larger carcasses resulting in higher carcass temperatures and functional protein degradation. Finally, the relationship between pHu and L* was strong and negative in the present study (r_g = -0.47 ± 0.09, r_p = -0.19) similar to previously published genetic correlation estimates in turkeys which ranged from -0.42 to -0.53 (Le Bihan-Duval et al., 2003; Aslam et al., 2011). Decreases in pH of the breast muscle lead to degradation of the functional proteins causing increased light refraction (increased L*) in the final meat product (Warriss and Brown, 1987; Barbut, 1993) as supported by our results. The strong genetic correlations between pHu and colour support the suggestions of Barbut (1997) for future use of non-destructive, easier to automate colour measurements over more invasive pH measurements in selection against PSE meat.

Several studies have reported the relationship between growth and muscle myopathies, including WS, in turkeys and broiler chickens (Mudalal et al., 2015; Russo et al., 2015; Baldi et al., 2018; Soglia et al., 2018; Carvalho et al., 2021b). In the present study, a moderate, unfavorable genetic correlation was observed between WS and BW ($r_g = 0.26 \pm 0.07$, $r_p = 0.12$) and WS and BrW ($r_g = 0.25 \pm 0.07$, $r_p = 0.05$), suggesting that selection for growth will also lead to an increase in WS prevalence if additional traits are not used to balance correlated effects. WS also showed weak to moderate, unfavorable correlations with the studied meat quality traits, the strongest of which was with b* ($r_g = 0.24 \pm 0.08$, $r_p = 0.00$). Moderate genetic correlations were also estimated with pHu ($r_g = 0.16 \pm 0.10$, $r_p = -0.14$) and a* ($r_g = 0.17 \pm 0.09$, $r_p = 0.19$), while a weak genetic correlation was estimated between WS and L* ($r_g = 0.10 \pm 0.089$, $r_p = -0.05$). Varying results have been previously published regarding the relationship between WS and meat quality in poultry. Carvalho et al. (2021) reported that turkey breasts affected by WS showed increased L* and b* but no difference in pHu. In contrast, Soglia et al. (2018) reported no significant difference between WS severity and pH or breast meat colour in turkeys, while Mudalal (2019) reported a significant increase in b* and pHu in raw turkey breasts affected by WS. In broiler chickens, significant differences have been observed in various meat quality traits, such as pHu, L*, a*, and b*, between normal breast and breast affected by WS, however, with varying degrees of consistency (Kuttappan et al., 2013a; Petracci et al., 2013; Trocino et al., 2015; Baldi et al., 2018). Future research should focus on understanding the biological mechanism and relationship between WS and meat quality to better understand these inconsistent results. Development of machine vision algorithms to quantitatively score white striping would assist in increasing the amount and accuracy of data collection to support this research and provide a more robust trait for future selection programs.

3.5 Conclusions

In the present study, we estimated the genetic parameters for white striping severity, breast meat colour, and pHu alongside several body and carcass component weights and FCR in turkeys. We have reported the first estimate of heritability for white striping in turkeys and have added to the body of knowledge surrounding heritability of meat quality. The estimates show potential for future genetic selection for improved meat quality and reduced white striping severity. The estimates also provide insight into the unfavorable effects selection for growth, meat yield, and efficiency have on meat quality.

3.6 Acknowledgements

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3.7 Tables

Table 3.1: Descriptive statistics (count, mean, and standard deviation) for the 13 recorded traits. Traits were recorded on three purebred genetic lines (A, B, and C) and statistics are presented for each genetic line and all genetic lines combined.

Trait	Abbreviation	Trait Unit	Line A		Line B		Line C		All Lines
			N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	Mean (SD)
Body weight[1]	BW	kg	2,564	21.77 (1.61)	5,285	19.12 (1.35)	4,102	24.57 (1.73)	21.56 (2.85)
Breast weight	BrW	kg	2,553	5.14 (0.60)	5,215	4.74 (0.50)	4,033	6.04 (0.75)	5.27 (0.84)
Fillets	-	kg	659	4 .47 (0.49)	1,431	4.06 (0.42)	1,011	5.13 (0.63)	4.50 (0.69)
Tenders	-	kg	660	0.83 (0.09)	1,426	0.77 (0.07)	1,009	1.01 (0.11)	0.86 (0.14)
Breast meat yield	BMY	% BW	2,550	23.61 (1.94)	5,203	24.77 (1.71)	4,024	24.52 (2.05)	24.43 (1.93)
Thighs	-	kg	667	3.00 (0.26)	1,478	2.51 (0.21)	1,072	3.44 (0.29)	2.92 (0.48)
Drums	-	kg	646	2.34 (0.20)	1,460	1.94 (0.15)	1,050	2.59 (0.21)	2.24 (0.34)
Feed conversion ratio	FCR	kg/kg	827	2.37 (0.36)	1,604	2.44 (0.33)	3,318	2.51 (0.39)	2.47 (0.37)
Lightness	L*	-	1,921	37.40 (2.54)	3,671	38.05 (2.58)	2,957	37.48 (2.68)	37.71 (2.62)
Redness	a*	-	1,921	3.23 (0.66)	3,671	3.21 (0.70)	2,957	2.94 (0.65)	1.12 (0.69)
Yellowness	b*	-	1,921	5.09 (0.91)	3,671	4.90 (0.94)	2,957	4.92 (0.93)	1.95 (0.93)
Ultimate pH	pHu	-	643	5.75 (0.12)	1,340	5.77 (0.11)	1,041	5.79 (0.11)	5.77 (0.11)

White Striping (0-3)	WS	-	1,838	2.62 (0.77)	3,728	2.61 (0.81)	2,856	2.31 (0.77)	2.51 (0.80)

[1]Measured two days prior to slaughter (20-24 weeks)

Table 3.2: Heritability[1] (diagonal; mean of all bivariate estimates for the given trait), additive genetic correlation coefficients[2] (above diagonal), and partial phenotypic correlation coefficients (below diagonal).

	BW	BrW	Fillets	Tenders	BMY	Thighs	Drums	FCR	L*	a*	b*	pHu	WS
BW	**0.44**	0.74	0.73	0.49	0.18	0.73	0.66	-0.09	0.31	0.10	0.16	-0.18	0.26
BrW	0.78[b]	**0.46**	0.99	0.53	0.79	0.33	0.24	-0.03[a]	0.43	0.06[a]	0.19	-0.19	0.25
Fillets	0.76[b]	0.99[b]	**0.47**	0.45	0.78	0.37	0.27	0.16	0.38	0.08[a]	0.16	-0.22	0.42
Tenders	0.52[b]	0.61[b]	0.48[b]	**0.50**	0.32	0.48	0.41	0.03[a]	0.13	-0.09	0.17	-0.01[a]	-0.08[a]
BMY	0.29[b]	0.83[b]	0.82[b]	0.47[b]	**0.41**	-0.23	-0.27	0.04[a]	0.37	0.00[a]	0.14	-0.09	0.13
Thighs	0.62[b]	0.38[b]	0.35[b]	0.40[b]	0.03	**0.46**	0.85	0.00[a]	0.03[a]	-0.06[a]	0.12	-0.21	0.10
Drums	0.58[b]	0.29[b]	0.26[b]	0.29[b]	-0.08	0.55[b]	**0.64**	-0.03[a]	-0.06[a]	-0.04[a]	0.02[a]	0.01[a]	0.01[a]
FCR	-0.05	-0.04	-0.04	-0.03	-0.01	-0.04	-0.04	**0.18**	0.14	-0.26	-0.04[a]	-0.01[a]	-0.03[a]
L*	0.02	0.15	0.13	0.16[b]	0.21[b]	-0.03	0.06	0.05	**0.20**	-0.10	0.31	-0.47	0.10
a*	0.12	0.08	0.12	-0.16[b]	0.01	0.06	-0.11	-0.15[b]	-0.23[b]	**0.22**	0.17	-0.23	0.17
b*	0.09	0.11	0.10	0.13	0.09	0.06	0.01	-0.09	0.31[b]	0.27[b]	**0.23**	-0.24	0.24
pHu	-0.17[b]	-0.18[b]	-0.17[b]	-0.18[b]	-0.12	-0.11	-0.04	0.01	-0.19[b]	-0.32[b]	-0.19[b]	**0.34**	0.16
WS	0.12	0.05	0.06	-0.02	-0.02	0.04	-0.03	0.00	-0.05	0.19[b]	0.00	-0.14	**0.15**

1 Standard error for heritability estimates ranged from 0.02-0.05

[2] Standard error for the additive genetic correlation coefficients ranged from 0.00-0.12

[a] Additive genetic correlation coefficients with a superscript represent cases where the associated SE is larger than the coefficient

[b] Partial phenotypic correlation coefficients with a superscript represent coefficients that are significantly different from 0 ($p < 0.05$)

BW = body weight two days before slaughter (20-24w; kg), FCR = feed conversion ratio (kg/kg), BrW = breast weight (kg), BMY = breast meat yield (% BW), pHu = ultimate pH, WS = white striping (0-3), L* = fillet lightness, a* = fillet redness, b* = fillet yellowness

Chapter 4: Genomic architecture of white striping in turkeys (*Meleagris gallopavo*)

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Key words: functional analysis, GWAS, myopathy, pectoralis major, white striping

4.1 Abstract

White striping (**WS**) is a growth-related myopathy of increasing concern to the turkey industry due to its increasing prevalence and negative consequences for consumer acceptance and functional properties of the meat. However, limited research has been conducted on the genomic architecture of WS in turkeys. The objective of the present study was to conduct a genome-wide association study (**GWAS**) and functional analysis on white striping severity. White striping was scored on turkey breast fillets (N=8,422) by trained observers on a 0-3 scale based on severity (none to severe). Of the phenotyped birds, 4,667 genotypic records were available from a proprietary 65K single nucleotide polymorphism (**SNP**) chip. The SNP effects were estimated using a linear mixed model and a 30-SNP sliding window approach was used to express percent genetic variance explained. Positional candidate genes within 50kb of the top 1% of SNP windows explaining the most genetic variance were recorded. Of the 95 positional candidate genes, seven were further classified as functional candidate genes because of their association with both a significant gene ontology and molecular function term. The results of the GWAS emphasize the polygenic nature of the trait with no specific genomic region contributing a large portion to the overall genetic variance (maximum 1% variance explained by any given SNP window). Significant pathways relating to growth, muscle development, collagen formation, circulatory system development, cell response to stimulus, and cytokine production were highlighted. These results support published biological associations between WS and hypoxia and oxidative stress and provide information that may be useful for future -omics studies in understanding the biological associations with WS development in turkeys.

4.2 Introduction

Great improvements have been made in meat yield and efficiency of turkeys as evident in Havenstein et al. (2007) where genetic lines from 1966 were compared to lines from 2003. Strategic improvements in management, nutrition, and genetic selection, has

led to turkey toms that can weigh upwards of 25 kg at 20 weeks of age. However, some negative consequences of this improvement in meat production are becoming apparent. White striping (**WS**) is a growth-related myopathy that is of increasing interest to the poultry industry. This myopathy presents itself as varying degrees of white striations on the surface of the muscle running parallel to the muscle fibers (Barbut, 2019). The prevalence of this myopathy has recently been shown to be as high as 88% in a population of Canadian purebred turkeys (Vanderhout et al., 2022) and as much as 60% in other turkey populations (Mudalal, 2019). Therefore, WS is highly prevalent, and it has known negative consequences for consumer acceptance (Kuttappan et al., 2012b; de Carvalho et al., 2020), nutritional and functional quality of the product (Kuttappan et al., 2016; Soglia et al., 2018; Barbut, 2019). These aspects make research into the biological mechanisms behind the condition and potential methods of prevention of great importance to the turkey industry.

Although the amount of research conducted on WS in turkeys is limited, there is more known about WS in broiler chickens. Several studies have been conducted in broiler chickens that investigated WS on a microscopic level showing that breasts affected by WS have necrotic muscle tissue and increased presence of fat, connective tissue, and inflammatory cells (Kuttappan et al., 2013b; Russo et al., 2015; Baldi et al., 2018). While the exact mechanism for development of WS is still unknown, one of the main mechanisms proposed is ischemia in the affected muscle (Boerboom et al., 2018). With the magnitude and speed of growth in modern birds, the limits of supporting systems (e.g., circulatory, cardiovascular) might have been reached. Increases in muscle fiber hypertrophy, the major consequence of selection for muscle growth (Aberle and Stewart, 1983; Remignon et al., 1994; MacRae et al., 2006), can lead to insufficient vascularization and reduced blood supply to the muscles (Sosnicki and Wilson, 1991; Velleman, 2015; Kindlein et al., 2017). A restriction in the circulatory system can lead to the accumulation of metabolic byproducts, inducing oxidative stress likely leading to necrosis, and

increases in hypoxic conditions potentially impairing muscle cell regeneration, ultimately leading to the development of WS.

The aforementioned mechanism of WS development has been supported through various -omics studies in broiler chickens on the level of the transcriptome (Zambonelli et al., 2016; Malila et al., 2019; Marchesi et al., 2019), proteome (Kuttappan et al., 2017), and metabolome (Boerboom et al., 2018). However, there is a lack of research in these areas for turkeys. Therefore, the objective of this study was to investigate the genomic architecture of WS in turkeys through the estimation of genomic heritability and execution of a genome-wide association study (**GWAS**) followed by functional analysis for detection of metabolic pathways and gene ontologies associated with the myopathy.

4.3 Materials and Methods

4.3.1 Ethics and Animal Care

All protocols complied with the guidelines of the Canadian Council on Animal Care and were approved by the University of Guelph Animal Care Committee (AUP 3782).

4.3.2 Animals

Adult male turkeys (20-24 weeks old) from three purebred genetic lines (A, B, and C) were processed over 44 weeks between July 2018 and November 2019. The genetic lines included a sire-line with selection focused on body weight, meat yield, and feed efficiency (line A), a dam-line that was selected primarily for body weight and reproductive traits (line B), and a dam-line selected mainly for reproductive traits (line C). Birds were reared under identical housing and management conditions (Hybrid Turkeys, 2020). During processing at a commercial poultry processing plant, birds were electrically stunned, exsanguinated, scalded, defeathered, and eviscerated before moving to the chiller. Upon completion of the 24 h chilling period (40 min in 5˚C water, 1.5-2 h in 1-2˚C water, and remainder of time layered in ice), birds were deboned, and meat quality and breast muscle weights were measured.

4.3.3 Phenotype and Genotype Data

Summary statistics of the data are presented in Table 1. Deboned *Pectoralis major* muscles (N = 8,422) were photographed (Hero 6, GoPro, San Mateo, CA, USA) approximately 24 hours post-mortem. Photographs were taken using the “normal” focal length setting from approximately 40 cm above the surface of the breast. The photographs were randomly assigned to six observers who scored the breasts for WS using a 0-3 scoring scale adapted from a system developed in broiler chickens after testing the reliability of the system (Kuttappan et al., 2016; Vanderhout et al., 2022). In brief, a score of 0 indicated no or minimal white striations whereas a score of 3 indicated the presence of thick white striations covering the breast. Genotypes were collected on 4,667 birds using a proprietary 65K single nucleotide polymorphism (**SNP**) array (65,000 SNP; Illumina, Inc.). PLINK software (Purcell et al., 2007) was used for quality control and SNP markers located on non-autosomal regions with minor allele frequency lower than 0.05, call rate lower than 90%, or significantly deviating from Hardy Weinberg proportions ($p < 1\times10^{-8}$) were removed. The quality control resulted in 54,407 markers retained for analysis.

4.3.4 Statistical Analysis

A linear mixed model was used to estimate variance components through restricted maximum likelihood carried out using the BLUPf90 family of programs (Misztal et al., 2018). The linear mixed model used can be described as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

where **y** is the vector of WS scores; **b** is a vector of fixed effects including genetic line (3 levels: A, B, and C), hatch week-year (58 levels), age at slaughter (7 levels; 141 to 163 days), and score observer (6 levels); **a** is a vector of additive genetic effects distributed as $\mathbf{a} \sim N(0, \mathbf{H}\sigma_a^2)$, where **H** is the combined pedigree-genomic relationship matrix as in Aguilar et al. (2010) constructed using the PREGSf90 program (Misztal et al., 2018) and

σ_a^2 is the additive genetic variance; **e** is the vector of residual effects which has a distribution of $\mathbf{e} \sim N(0, \sigma_e^2)$ where σ_e^2 is the residual variance; and **X** and **Z** are design matrices relating the observations to the fixed and random effects, respectively.

Estimates of SNP effects were derived from the estimated genomic breeding values (**gEBV**) following Wang et al. (2012), using a weighted genomic relationship matrix:

$$\hat{\mathbf{g}} = \mathbf{DZ}'[\mathbf{ZDZ}']^{-1}\hat{\mathbf{u}}_{\mathrm{g}},$$

where $\hat{\mathbf{g}}$ is a vector of SNP marker effects; **D** is a diagonal matrix of weights for variances of SNPs; **Z** is a matrix relating genotype of each locus; and $\hat{\mathbf{u}}_{\mathrm{g}}$ is the vector of gEBV. Due to the proposed polygenic nature of WS and the relatively poor annotation of the turkey genome, a 30-SNP sliding window approach was utilized. This approach allows for the information of nearby genes with smaller effects to combine thus revealing genes that ultimately would not be found significant in a single SNP analysis. These analyses were carried out using the BLUPf90 family of programs (Misztal et al., 2018).

4.3.5 Functional Analysis

Markers in the 99th percentile of variance explained, an arbitrary threshold, were considered significant. Using the Turkey 5.1 assembly (Dalloul et al., 2014), positional candidate genes within ±50kb of the significant SNP were retrieved using the Ensembl Genes database version 104 (https://useast.ensembl.org/Meleagris_gallopavo/Info/Index) implemented through the GALLO R package (Fonseca et al., 2020). Gene ontology (**GO**) enrichment analysis including biological processes (**BP**), cellular components (**CC**), and molecular functions (**MF**) as well as metabolic pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (**KEGG**) database were performed on the positional candidate genes using the WebGestaltR R package (Wang and Liao, 2020) and the *Gallus gallus* database.

4.4 Results and Discussion

4.4.1 Estimation of Genetic Parameters

Heritability of WS was estimated to be 0.20 ± 0.022. To the best of our knowledge, this is the first published genomic heritability estimate for WS in turkeys. The addition of genomic data resulted in a 33% increase in estimated heritability compared to pedigree information alone (Vanderhout et al., 2022). The present estimate was found to be within the range of previously published estimates of heritability in broiler chickens of 0.18 to 0.65 (Bailey et al., 2015; Alnahhas et al., 2016; Lake et al., 2021). The moderate heritability estimated in the present study suggests that there is a presence of genetic factors influencing WS that could potentially be exploited in selecting birds for reduced WS severity, however, environmental factors influence majority of the phenotypic variance observed in the population.

4.4.2 Significant SNP and Positional Candidate Genes

The percentage of genetic variance explained by each 30-SNP sliding window is presented in Figure 1. Each window explained 0.05% of the genetic variance on average with no more than 1.00% of the variance being explained by any given window. This suggests that the inheritance of the trait is largely polygenic in nature. A total of 544 SNP windows were classified as significant (top 1% of variance explained) resulting in 95 positional candidate genes found within 50 kb up or down stream of these SNP. This distance has been suggested by Do et al. (2014) to be used when dealing with lower quality assemblies like that of the turkey. The positional candidate genes were located on *Meleagris gallopavo* autosomal chromosomes (MGA) 2 to 9, 11, 14, 19, 20, and 24. The 95 positional candidate genes were significantly associated ($p < 0.05$) with four KEGG metabolic pathways (Table 1) and 31 GO terms (21 BP, 3 CC, and 7 MF; Table 2). Positional candidate genes were further considered functional candidate genes (**FCG**) if they were associated with both a significant metabolic pathway and a significant GO term. Seven FCG were found and were involved mainly in the Wnt signaling pathway (*NFATc1*),

RNA degradation (*LSM6* and *DHX36*), and focal adhesion (*COL6A3*, *FN1*, *VCL*, and *GRB2*).

Due to WS being a growth-related myopathy, it is not surprising that several growth and muscle development related BP terms (GO:0003012 muscle system process, GO:0040007 growth, and GO:0061061 muscle structure development) were found to be significantly overrepresented ($p < 0.05$) by the 95 positional candidate genes. The large selection pressure placed on growth for economically significant muscle groups (ex. breast) has resulted in meat producing birds that are likely reaching the limit of supporting systems, such as the circulatory system. Thus, the proposed mechanism of WS development is primarily thought to be related to poor blood flow in the breast muscle leading to hypoxia and oxidative stress (Boerboom et al., 2018; Malila et al., 2019). The highly conserved Wnt signalling pathway, one of the four significant metabolic pathways, plays an important role in both embryonic development, where it regulates processes such as differentiation and cell proliferation, polarity, and migration, as well as post-natal, where it regulates tissue homeostasis and biological processes involved in many disorders and cancers (Clevers, 2006; Ackers and Malgor, 2018; Noguchi et al., 2018). The FCG associated with the Wnt signalling pathway, nuclear factor of activated T cells 1 (*NFATc1*), is found on MGA 3 associated with the largest peak in variance explained. This gene has been shown to play a large role in cell cycle progression of human aortic smooth muscle cells (Karpurapu et al., 2010) and promotion of response to injury in arterial smooth muscle cells (Chow et al., 2008). The effect of *NFATc1* and the Wnt signalling pathway in the development and repair of the vascular system may be what leads to its significant relationship to WS. Some significant BP terms found in the present study were associated with FCG (including *NFATc1*, *DHX36*, and *FN1*), specifically GO:0009628 response to abiotic stimulus ($p = 0.01$) and GO:0072359 circulatory system development ($p = 0.04$), further supporting the relationship between hypoxia, oxidative stress, and WS.

Functional candidate genes collagen type VI alpha 3-chain (*COL6A3*) and fibronectin 1 (*FN1*) were also previously found to be significantly associated with WS in broiler chickens (Pampouille et al., 2018) and differentially expressed between broiler chicken breasts affected versus not affected by WS (Pampouille et al., 2019; Praud et al., 2020). The *COL6A3* gene produces collagen found in the extracellular matrix of cells that make up skeletal muscles, and mutations in the gene are associated with muscle weakness, atrophy, and necrosis in humans (Bertini and Pepe, 2002). The *FN1* gene encodes a glycoprotein which plays a role in the creation of extracellular matrix structures during tissue repair and increases in the expression of this gene have been linked with Duchenne muscular dystrophy in humans (Cynthia Martin et al., 2014). Given the increase in fat and connective tissue that replaces damaged muscle tissue in affected breast muscles, the link between these two genes and WS is understandable. Another gene of interest is cytosine and glycine rich protein 3 (*CSRP3*), a positional candidate gene found to be associated with several significant GO terms including 10 BP, one CC, and 1 MF. The *CSRP3* gene has been previously shown to be upregulated in broiler chicken breasts affected with WS (Marchesi et al., 2019; Marciano et al., 2021). This gene encodes a muscle LIM protein and overexpression of such protein can promote muscle differentiation, regeneration, and structural repair of skeletal muscle (Arber et al., 1994; Barash et al., 2005) further emphasizing the link between WS and muscle tissue damage.

The BP term, GO: 0001816 cytokine production, was found to be significantly overrepresented (p = 0.02) by the positional candidate genes in the present study, including three of the seven FCG (*NFATc1*, *FN1*, and *DXH36*). A microscopic characteristic consistently found in poultry breast tissue affected by WS is an elevated presence of inflammatory cells and cytokines (Kuttappan et al., 2013b; Carvalho et al., 2021b; Prisco et al., 2021). Cytokines are small proteins that play a large role in immune response and inflammation and the elevated presence of these molecules in the muscle of affected breasts is symbolic of muscle cell injury (Prisk and Huard, 2003; Smith et al., 2008). Whether these genes, and subsequent production of cytokines, was upregulated

in the affected breasts of the current study is unknown, however, the expression of inflammatory cytokine genes has been shown to increase with increasing severity of WS in broiler chickens (Prisco et al., 2021).

Overall, the results of the present study provide support for the oxidative stress and hypoxia theory in relation to WS development. Continued -omics research on the topic of WS in turkeys is recommended to further identify relationships between the myopathy and biological processes allowing for improved prevention methods. Future research should also focus on developing methods of quantitatively scoring WS using technologies such as machine vision algorithms. Such a measure would allow for much more power in future analyses.

4.5 Conclusions

This study provides the first published estimate of genomic heritability of WS in turkeys and provides the first look into the genomic architecture of WS in turkeys by means of a GWAS and functional analysis. The heritability estimate of WS was found to be 0.20 ± 0.022, and results of the GWAS emphasize the polygenic nature of the trait with no specific genomic region contributing a large portion to the overall genetic variance. Results of the functional analysis identified four significant KEGG metabolic pathways, 31 significant GO terms (21 BP, 3 CC, and 7 MF) and seven functional candidate genes associated with WS. Overall, pathways relating to growth, muscle development, collagen formation, circulatory system development, cell response to stimulus, and cytokine production were highlighted. These results support the primary proposed mechanism for WS development being hypoxia and oxidative stress leading to muscle tissue damage and provide information that may be useful in future studies in developing novel breeding strategies for turkeys.

4.6 Acknowledgements

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4.7 Figures

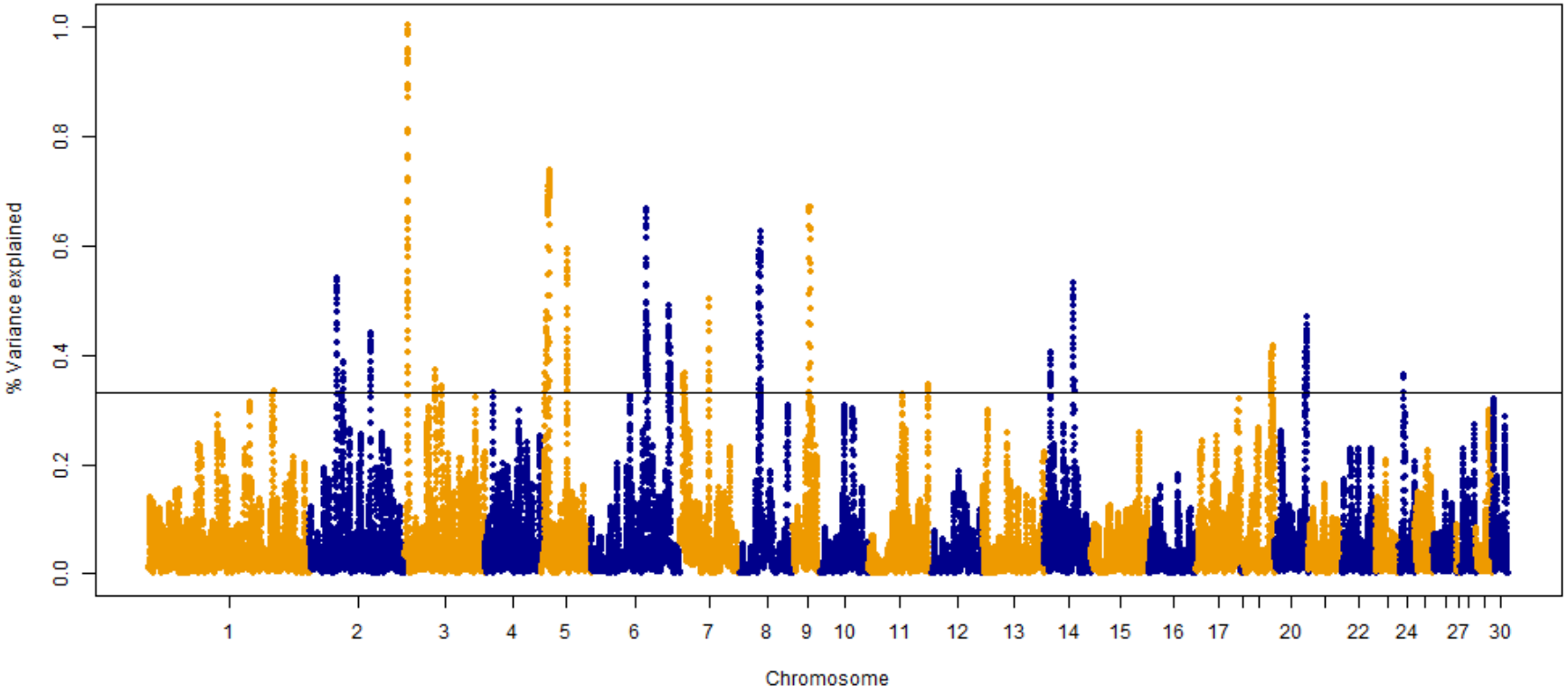

Figure 4.1: Manhattan plot for percentage of genetic variance explained by a 30-SNP sliding window across the genome for white striping severity score (0-3). The top 1% of SNP windows that explain the most genetic variance are located above the horizontal line (% of variance explained > 0.330%).

4.8 Tables

Table 4.1: Summary statistics of each genetic line (A, B, and C) of turkeys.

	Line A	**Line B**	**Line C**
Number of phenotypic records	2,856	1,838	3,728
White striping score 0 (frequency)	450 (0.16)	155 (0.08)	362 (0.10)
White striping score 1 (frequency)	1,181 (0.41)	565 (0.31)	1,137 (0.30)
White striping score 2 (frequency)	1,128 (0.40)	950 (0.52)	1,809 (0.49)
White striping score 3 (frequency)	97 (0.03)	168 (0.09)	420 (0.11)
Mean slaughter age in days (SD)	144.13 (3.95)	149.68 (2.10)	153.65 (3.63)
Number of animals in the pedigree	6,530	4,146	6,063
Number of genotyped birds (with phenotypic data)	963 (766)	477 (431)	1,185 (1,059)
Number of genotyped sires	126	153	183
Number of genotyped dams	623	354	583
Number of SNP markers		54,407	

Table 4.2: List of the KEGG metabolic pathways ($p < 0.05$) associated with the 95 positional candidate genes for white striping severity score (0-3) in turkeys.

Molecular pathway description	***p*-value**	**Gene names**
One carbon pool by folate	<0.01	*MTR*; *ATIC*
Wnt signaling pathway	0.01	*NFATC1**; *SFRP4*; *CAMK2G*; *PPP3CB*
RNA degradation	0.01	*LSM6**; *DHX36**; *DCP1A*
Focal adhesion	0.05	*COL6A3**; *FN1**; *VCL**; *GRB2**

* Denotes functional candidate genes (genes associated with both a significant KEGG metabolic pathway and significant GO term)

Table 4.3: List of gene ontology terms including biological processes, cellular components, and molecular functions ($p < 0.05$) associated with the 95 positional candidate genes for white striping severity score (0-3) in turkeys.

GO ID	GO term	*p*-value	Gene names
Biological processes			
GO:0030031	cell projection assembly	<0.01	*ACTN2*; *TMEM216*; *TMEM138*; *NME8*; *RAB17*; *VCL**; *ARHGEF26*
GO:0070925	organelle assembly	<0.01	*ACTN2*; *CSRP3*; *TMEM216*; *TMEM138*; *MLH1*; *NME8*; *RAB17*; *MYOZ1*; *BMP10*
GO:0003012	muscle system process	<0.01	*ACTN2*; *CSRP3*; *STAC*; *MYOZ1*; *BMP10*
GO:0060537	muscle tissue development	<0.01	*ACTN2*; *SMAD1*; *CSRP3*; *MYOZ1*; *BMP10*
GO:0030029	actin filament-based process	<0.01	*ACTN2*; *CSRP3*; *ELMO1*; *MYOZ1*; *MAPKAP1*; *GRB2**; *BMP10*
GO:0043900	regulation of multi-organism process	0.01	*CTDP1*; *TKFC*; *DDB1*; *GPR149*
GO:0009628	response to abiotic stimulus	0.01	*CSRP3*; *DDB1*; *ARPP21*; *STAC*; *DHX36**; *HSPA5*; *GRB2**
GO:0034394	protein localization to cell surface	0.02	*ACTN2*; *VCL**
GO:0120036	plasma membrane bounded cell projection organization	0.02	*ACTN2*; *TMEM216*; *TMEM138*; *NME8*; *RAB17*; *FN1**; *VCL**; *ZSWIM8*; *ARHGEF26*
GO:0032989	cellular component morphogenesis	0.02	*ACTN2*; *CSRP3*; *FN1**; *VCL**; *ZSWIM8*; *MYOZ1*; *ARHGEF26*; *BMP10*
GO:0001816	cytokine production	0.02	*NFATC1**; *CD6*; *TKFC*; *FN1**; *DHX36**
GO:0040007	growth	0.02	*ADNP2*; *SMAD1*; *FN1**; *VCL**; *MYOZ1*; *GPR149*; *BMP10*
GO:0097435	supramolecular fiber organization	0.02	*ACTN2*; *TPPP*; *CSRP3*; *MYOZ1*; *GRB2**; *BMP10*
GO:0007264	small GTPase mediated signal transduction	0.03	*ELMO1*; *RAB17*; *ARHGEF26*; *MAPKAP1*; *GRB2**
GO:0061061	muscle structure development	0.03	*ACTN2*; *NFATC1**; *CSRP3*; *MYOZ1*; *BMP10*
GO:0051640	organelle localization	0.04	*ABCE1*; *MLH1*; *STARD3NL*; *RAB17*; *AP3M1*
GO:0072359	circulatory system development	0.04	*ACTN2*; *NFATC1**; *SMAD1*; *CSRP3*; *FN1**; *DHX36**; *BMP10*
GO:0048646	anatomical structure formation involved in morphogenesis	0.04	*ACTN2*; *SMAD1*; *CSRP3*; *FN1**; *MYOZ1*; *GRB2**; *BMP10*
GO:0034330	cell junction organization	0.04	*ACTN2*; *FN1**; *VCL**

GO:0000003	reproduction	0.05	*ARID4B*; *TRIP13*; *SMAD1*; *MLH1*; *GPR149*; *DHX36**
GO:0104004	cellular response to environmental stimulus	0.05	*DDB1*; *DHX36**; *GRB2**
Cellular Components			
GO:0042383	sarcolemma	<0.01	*STAC*; *COL6A3**; *VCL**
GO:0099080	supramolecular complex	<0.01	*ACTN2*; *TPPP*; *CSRP3*; *FN1**; *VCL**; *MYOZ1*; *BMP10*
GO:0120114	Sm-like protein family complex	0.03	*TXNL4A*; *LSM6**
Molecular Functions			
GO:0051020	GTPase binding	0.02	*ELMO1*; *AP3M1*; *ARHGEF26*; *GAPVD1*; *MAPKAP1*
GO:0008092	cytoskeletal protein binding	0.02	*ACTN2*; *TPPP*; *CSRP3*; *NME8*; *VCL**; *MYOZ1*; *BMP10*
GO:0044877	protein-containing complex binding	0.02	*ACTN2*; *CTDP1*; *ABCE1*; *DDB1*; *MLH1*; *FN1**; *VCL**
GO:0019904	protein domain specific binding	0.03	*ACTN2*; *DDB1*; *ELMO1*; *FN1**; *GRB2**
GO:0044325	ion channel binding	0.04	*ACTN2*; *STAC*
GO:0003697	single-stranded DNA binding	0.05	*MLH1*; *DHX36**
GO:0060090	molecular adaptor activity	0.05	*DDB1*; *GRB2**

* Denotes functional candidate genes (genes associated with both a significant KEGG metabolic pathway and significant GO term)

Chapter 5: General Discussion and Conclusions

The main objectives of this thesis were to provide a better understanding of the genetics of breast meat quality and white striping (**WS**) in Canadian turkeys and outline the relationships between these traits and other economically important traits such as body weight and feed efficiency. To accomplish these objectives, three studies were conducted (Chapters 2-4).

Chapter 2 used images of turkey *Pectoralis major* muscles (fillets) to test the reliability of a WS scoring system. White striping severity scores recorded by six observers showed moderate to good reliability within and between observers. In the population studied, 88% of the scored breasts were found to be affected by WS to some degree, a prevalence higher than what has been previously published in turkeys (Mudalal, 2019). Key relationships between the severity of WS and economically important traits such as body weight and breast meat yield (BMY) were outlined suggesting unfavorable correlations that will have to be accounted for using a balanced selection index.

In Chapter 3, genetic parameters for breast meat colour, pH, and WS severity were estimated alongside several economically important traits. Moderate heritabilities were estimated for the meat quality traits and the first published heritability estimate for WS in turkeys ($h^2 = 0.15 \pm 0.019$) was reported. Continuing with the findings of the first study (Chapter 2), in Chapter 3 we estimated genetic and partial phenotypic correlations which quantified the unfavorable effects of selection for growth, meat yield, and efficiency on meat quality and WS.

Given the moderate heritability estimated for WS in the chapter 3, the final study (Chapter 4) further investigated the biological processes associated with the development of WS by performing a genome-wide association study (**GWAS**) and functional analysis. Pathways relating to growth, muscle development, collagen formation, circulatory system development, cell response to stimulus, and cytokine production were highlighted

supporting hypoxia and oxidative stress resulting from increased muscle growth as a primary mechanism for WS development (Boerboom et al., 2018; Malila et al., 2019).

5.1 Implications

The results of this thesis have implications in both the scientific community and turkey industry. Findings of chapter 2 provide confirmation of the reliability of scoring WS severity by multiple observers but also the highest reported prevalence of WS in turkeys. Confirming the reliability of WS severity scoring and providing techniques which could improve the reliability of scoring among several observers, such as the use of training sessions, will allow for improved confidence in future data collection. Furthermore, providing an up-to-date record of prevalence in turkeys allows the industry to better asses the present issue and begin to implement changes (i.e., adapting breeding schemes, adapting management techniques, implementing quality scoring at the processing plant level, etc.) that will hopefully better the life of the animal and meat quality. The findings of studies two and three further add to the body of knowledge required to implement these changes. Estimates of heritability for these traits provide scientists and industry members with a better understanding of the role genetics plays in these traits. Estimates of genetic correlations for these quality traits and other traits at the forefront of current breeding programs, such as body weight, meat yield, and feed efficiency, showcase the potential benefits and consequences of current selection strategies. In addition, understanding the biological processes taking place in relation to conditions like WS allow for the development of prevention methods beyond genetic selection such as nutrition advancements or alterations to management practices.

5.2 Future directions

A limitation of including meat quality traits in breeding schemes is the difficulty of measurement. Measuring these traits generally requires the slaughter of the animal which makes it impossible for this animal to be selected as a breeding candidate. Development

of a method to accurately measure meat quality traits and myopathies or other closely related indicator traits in live animals would be beneficial. Methods such as breast palpation in live birds, which is a current method used to classify severity of wooden breast in broiler chickens (Clark and Velleman, 2017), or the use of technologies such as ultrasound, computed tomography (**CT**) scanners or magnetic resonance imaging (**MRI**) could allow for a more depth view at the internal structure of live birds. In relation to the findings of study three, research into indicators of circulatory system structure and quantifying blood flow to large muscle groups using MRI (Lalande et al., 2008) or near-infrared spectroscopy (Hachiya et al., 2008) could provide insight into the future presence of WS in live birds.

When considering a trait for use in a selection index, methods are available to implement categorical traits (Dempster and Lerner, 1950; Finney, 1952; Aguilar et al., 2018). However, compared to quantitative measures, a much lower genetic gain can be expected (Meuwissen et al., 1995). Even with a reliable scoring system, it is a subjective measure with limited categories and therefore the information obtained from such a measure is not maximized. Development of an objective, or better yet, quantitative scoring system for WS using technologies such as machine vision would allow for the collection of large amounts of data that could be utilized in future selection indexes or in future studies surrounding WS in turkeys. Preliminary results of such methods in broiler chickens have been released (Kato et al., 2019; Carvalho et al., 2021a).

In addition to continued research into the genetic aspects of meat quality and WS, research into the environmental factors playing a role in these traits is important. Various environmental contributors have been investigated in previous studies including temperature stress (Leishman et al., 2021), stocking density (Pekel et al., 2020), nutrition (Kuttappan et al., 2012a; Bodle et al., 2018; Surai, 2020), and egg incubation (Livingston et al., 2019; Tejeda et al., 2021). The combination with carefully designed selection

programs and improved management and nutrition strategies should help with reducing myopathies and PSE meat in turkeys.

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