Nutritional strategies for mitigating wooden breast and white striping myopathies of the *Pectoralis major* muscles in broiler chickens

by

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ABSTRACT

Throughout the last decade, broiler producers have increasingly noted the presence of wooden breast (WB) and white striping (WS) in the *Pectoralis majo*r muscles (breast fillet) of broilers. Non-genetic factors, such as environment and nutrition, may contribute a substantial portion of the observed variance in myopathy incidence, and could be suitable targets for practical interventions aimed at reducing the incidence of myopathy. Therefore, a series of 4 experiments was conducted to determine whether WB and WS may be ameliorated by modulating nutrient intake. The first experiment utilized a quantitatively controlled feeding program to obtain differences in growth trajectory aimed at reducing the incidence and severity of WB and WS. Linear decreases in feed intake resulted in linear decreases in WB and WS at 33, 43, and 50 d of age, indicating that controlled feeding programs have the potential to reduce myopathies. The second experiment attempted to replicate the nutrient intake of the quantitative feeding program in a qualitative manner by altering dietary AA and AME_n densities. However, broilers responded to the reduced density diets by over-consuming feed, eliminating the differences in dietary density. To mitigate compensatory intake, the third experiment investigated the use of diets that only reduced the concentrations of digestible Lys (dLys), but did not alter energy density or maintain AA ratios to dLys. Reducing dLys to 75% (18 to 26 d) and 85% (28 to 40 d) of primary breeder recommendations substantially decreased the incidence of severe WB and WS at 48 and 61 d of age, respectively. These

results indicated that the intended market weight of a flock must be taken into account when determining the timing and intensity of a reduction in dLys density. The fourth experiment utilized a diet formulated at 75% of primary breeder recommendations for dLys concentrations from 15 to 25 d of age in order to produce broilers within the same flock that were differentially affected by WB as a suitable model for an *in vivo* comparison of myofiber CSA and the relative populations of myogenic cells known to play critical roles in muscle growth and repair. The size and activity of Myf-5+ and Pax7+ myogenic cell populations were increased in the presence of severe WB. Additionally, broilers affected by severe WB had different distributions of myofiber CSA. Altogether, these results indicated that targeted modification of the growth trajectory using reduced dietary dLys is a viable strategy to reduce the severity of WB and WS. Future research should strive to establish a basic understanding of the etiology of WB and WS in order to develop practical nutrition and management interventions aimed at reducing the prevalence of these myopathies in the broiler industry.

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I. INTRODUCTION

Consumer demand for chicken has risen substantially, with per capita consumption more than doubling since 1975 (National Chicken Council, 2013). Much of this increase in consumption has been attributed to the low cost of chicken, as well as consumer perceptions regarding health benefits, versatility, and ease of preparation. The *Pectoralis major* **(PM)** muscles, or breast fillet, garner a premium in most markets as they are considered a desirable source of lean protein. Recently, the broiler industry has observed the concurrent appearance of 2 novel breast quality defects, wooden breast **(WB)** and white striping **(WS)**. Fillets affected by WB are typically pale in color and have a bulging appearance (Mudalal et al., 2014). The condition is characterized histologically by abnormal accumulation of connective tissue (Sihvo et al., 2017), which contributes to its tough texture. White striping is characterized by the macroscopically visible deposition of adipose tissue in parallel striations along the muscle fibers.

Retail consumers base their purchasing decisions primarily on the physical appearance of packaged raw poultry products. Discoloration and the presence of visible fat have been shown to negatively influence consumer perceptions of product quality (Kennedy et al., 2004; Kuttappan et al., 2012c).The presence of WS reduces consumer acceptability due to its effects on fillet color and the perception of health value associated with lean breast fillets (Kuttappan et al., 2012c). Fillets affected by WB have the potential to provoke consumer complaints due to noticeably altered textural properties.

These issues may also alter the functional qualities of affected fillets when incorporated into further processed products (Petracci et al., 2013). High volume fast-food establishments in particular must consider that isolated incidents of customer dissatisfaction may rapidly become amplified into public controversy in the age of social media. Indeed, these defects have recently become publicized as an animal welfare issue by action groups in the European Union. Depending on the market, affected fillets may be down-graded or even condemned, resulting in significant losses in yield and revenue.

The increased demand for white meat has motivated genetics companies to pursue the development of high-yielding strains of broilers. It has been reported that breast muscle myopathies are more common in these high-yielding broilers (Bauermeister et al., 2009; Kuttappan et al., 2009; Trocino et al., 2015). However, anecdotal evidence from producers of product mixes intended for fast-food and tray-pack markets indicate that WS, as well as WB, are not confined exclusively to heavier broilers. Analysis of the heritability and genetic correlation of these traits to growth performance indicated that the majority of the observed variation in WB and WS is attributable to non-genetic factors, such as environment, management, and nutrition (Bailey et al., 2015). Strategies that target these non-genetic factors may yield the most benefits in the short term.

Although effects of dietary amino acid **(AA)** density (Bilgili et al., 2014; Meloche et al., 2014) and antioxidant supplementation (Kuttappan et al., 2012b) on WB and WS have been individually investigated to some extent, currently there is little published data concerning the influence of nutrition on the development of these myopathies in broilers. Furthermore, existing studies have confined their assessment of WB and WS to

postmortem visual evaluation at the processing plant, and have not adequately addressed the lack of available *in vivo* methods to detect the presence of myopathies.

A few strategies to mitigate these conditions through nutrition and management have been investigated; however, the paucity of information regarding the mechanism of these myopathies has impeded the development of targeted practical interventions. In order to elucidate the mechanism by which these myopathies arise, it is first necessary to identify measures that accurately indicate the presence of myopathy. Increased breast yield correlates with greater cross-sectional areas **(CSA)** of individual muscle fibers (Hoving-Bolink, 2000; Joiner et al., 2014). It has been postulated that the increased muscle fiber diameter in high-yielding birds may result in decreased diffusion capacity for nutrient uptake and waste exchange (Velleman and Clark, 2015). The CSA distribution in PM muscle affected by WB and WS can be analyzed through histological examination. Researchers have also suggested that the increased rate of protein deposition in yield-selected broilers may surpass the intrinsic cellular maintenance systems of the muscle tissue, causing progressive degeneration of muscle fibers even during periods of rapid growth (Clark and Velleman, 2016). In addition to assessing the structural changes observed with these myopathies, it would be beneficial to determine if changes in activity among cell populations associated with myofiber growth and maintenance occur as well.

The growth trajectory of modern broilers can be greatly affected by nutritional status. It may be possible to alleviate the overload of cellular maintenance systems by strategically altering the growth curve during critical windows of muscle development through variations in dietary density. The effect of dietary density on growth is not only

dependent on the concentrations of nutrients themselves, but also on the timing of implementing a nutritional program during the growth period. A successful practical strategy for mitigating myopathies must also take into account any effects on the live performance and processing characteristics of the flock as well. However, few studies been conducted to assess the impact of specific nutrients on the development of WB and WS in a practical setting. Therefore, additional research is warranted to further characterize the effects of nutrition on the development of WB and WS in broilers.

The research reported herein focused on strategies to reduce the development of WB and WS by altering the growth curve through quantitative allocation of feed, qualitative reductions in AA and apparent metabolizable energy **(AME_n)** density, and timed reductions in digestible lysine **(dLys)**. The latter strategy was then implemented in a comparative study to assess differences in fiber CSA and myogenic cell activity among broilers affected by varying degrees of WB. Data generated by these experiments should provide a basis for practical nutritional recommendations to mitigate the presence of WB and WS, as well as aid in elucidating the cellular mechanisms involved in the development of myopathy.

II. LITERATURE REVIEW

SKELETAL MUSCLE DEVELOPMENT AND GROWTH

Development. In the embryo, striated skeletal muscle originates from uncommitted mesenchymal cells, which migrate from the paraxial somatic mesoderm to sites of muscle development (Muntz, 1990). At this point in myogenesis, mesenchymal cells may differentiate into precursor cells for muscle (myoblasts) or connective tissue (fibroblasts). Myogenic precursor cells are identified by their expression of Pax3 / Pax7 genes. Multiple types of myoblasts may be involved in the embryological development of avian skeletal muscle tissue (Stockdale and Miller, 1987). Fusion of many mononucleated myoblasts results in the formation of multinucleated myotubes (Boudjelida and Muntz, 1987). This committed step of myogenesis is characterized by the loss of mitotic capacity in the differentiated myoblasts participating in fusion (Stromer et al, 1974). Most myoblasts will fuse together to form multi-nucleated muscle fibers, but a small population will not fuse. These become satellite cells **(SC)**, which retain their ability to proliferate throughout life.

The processes of myofiber proliferation, differentiation, and fusion are governed by cascading waves of myogenic regulatory factor expression. The major myogenic regulatory factors are myogenic factor 5 (Myf-5), myogenic determination factor 1 (MyoD), and myogenin. In myogenic precursor cells, Pax3 and Pax7 expression stimulates the upregulation of MyoD, triggering the proliferation of the determined

myoblasts. Because its expression signals the commitment of myogenic precursor cells to a skeletal muscle fate, MyoD is considered to be the “master regulator” of myogenesis. In conjunction with MyoD, Myf-5 can have a co-regulatory effect on myoblast proliferation in some tissues. Myogenin is involved in myoblast differentiation (exiting the cell cycle) and subsequent fusion into primary myofibers. Proliferation and differentiation are inhibited by the expression of myostatin, an antagonist to MyoD and myogenin. Insulin-like growth factor-1 inhibits myostatin and promotes MyoD and myogenin activity.

During the fusion of myoblasts into primary myofibers, muscle-specific proteins are synthesized and organized into myofibrils. These proteins include the contractile elements myosin and actin as well as regulatory proteins such as tropomyosin and troponin (Devlin and Emerson, 1978). The production of cytosolic enzymes and neurotransmitter receptors also occurs. Innervation also occurs at the point of fusion, with each muscle fiber retaining its original innervations throughout life (Bechtel,1986). Fusing of myoblasts in the embryo initially produces primary myotubes, which provide the scaffolding for subsequent development of secondary myotubes in the embryo. The size and number of primary myotubes will limit the number of secondary fibers that will form before hatch. During early myogenesis, the nuclei of the fused myoblasts are located centrally, creating a tubular structure. As myofibrils accumulate, the nuclei are displaced to the surface of the myofibril bundle, resulting in a mature muscle fiber. In chickens, the initial formation of mature myofibers occurs at about 6 days of incubation (Stockdale, 1992; Stickland et al., 2004). Separate populations of myotubes will form different fiber types, beginning at 12 to 16 days of incubation in chickens (Stockdale, 1992; Stickland et al., 2004). Complete maturation and differentiation of all muscle fibers

occurs by day 16 of incubation (Stockdale and Miller, 1987). Each muscle fiber is surrounded by connective tissue, forming the endomysium. Bundles of myofibers form muscle fascicles, which are in turn surrounded by the perimysium. Lastly, the epimysium forms around the whole muscle (Bechtel, 1986).

Skeletal muscle fibers are classified into 3 major categories (Bechtel, 1986). Slow oxidative (Type I) fibers are predominantly located in postural muscles. These fibers utilize a myosin isoform that hydrolyzes ATP slowly, resulting in an efficient but less forceful contraction. Activities requiring higher contractile forces utilize fast-twitch Type II fibers, which are further divided by their preference for oxidative (Type IIa) or glycolytic (Type IIb) metabolism. Type IIb fibers are recruited for movements requiring a large force over a short period of time, whereas Type IIa fibers are better suited to endurance activities (Goldspink, 1996). The PM muscle in modern broilers is composed almost entirely of Type IIb glycolytic fibers, which give breast meat its characteristic white color (Remignon, 1996).

Growth. Skeletal muscle growth may be achieved through 2 processes, hyperplasia and hypertrophy. Hyperplasia of muscle progenitor cells only occurs during embryological development, with the total number of muscle cells fixed at hatch (Goldspink, 1972; Picard, 2002; Chen et al., 2013). Subsequent protein accretion and hypertrophy of existing myofibrils occurs after hatching (Mcfarland, 1999). Hypertrophy can be accomplished through the addition of sarcomeres along the myofibril or the synthesis of additional myofibrils for an increase in CSA of the myofiber (Williams and Goldspink, 1971). However, increased fiber size creates a challenge for myofibrils as the number of nuclei available for maintaining the cell decreases relative to CSA. Growth or

repair of the myofibers therefore requires the presence of additional nuclei. Indeed, over 90% of muscle DNA accumulation has been shown to occur after hatch, when muscle cell numbers are fixed (Allen et al., 1979). Because myofibers lack the ability to undergo mitosis, additional nuclei must be donated from SC, which are located on the periphery of the myofiber beneath the basal lamina (Campion, 1984).

Satellite cells are mononucleated myogenic stem cells characterized by their expression of Myf-5 and Pax7, as well as their location within the basal lamina (Zammit et al., 2006) Active SC have the capacity to proliferate mitotically and fuse with existing myofibers to donate additional DNA, increasing the protein accretion capability of that fiber. The potential for subsequent hypertrophy of the muscle fibers is directly proportional to the number of nuclei present on the myofibers after early SC proliferation ceases (Allen et al., 1979). The extent of postnatal SC proliferation is regulated by multiple growth factors, including fibroblast growth factors, insulin-like growth factors I and II, and hepatocyte growth factor (Mcfarland, 1999), which are secreted in response to positive energy balance or mechanical stimulation (exercise) promoting skeletal muscle growth (Hawke and Garry, 2001).

The effects of growth factors on SC proliferation and differentiation are coordinated through the Ras/Raf/MAP and phospoinositide-3-kinase pathways, respectively (Coolican et al., 1997). Proliferation increases the number of active SC available to donate nuclei to growing myofibrils. Some of these active SC will begin to express MyoD, signaling their differentiation into a committed myofiber. These committed cells can fuse with existing myofibers, increasing the number of nuclei

available to produce contractile proteins for additional sarcomeres, thus enabling an increase in myofiber size.

Some of the daughter cells resulting from proliferation will not differentiate, but will instead return to quiescence to maintain the SC pool (Yin et al., 2013). In rapidly growing young animals, SC are highly proliferative, but the majority of resultant cells undergo differentiation and fusion to support muscle accretion. As the early growth phase ends, SC numbers drop to a low but constant level and become quiescent (Hawke and Garry, 2001). Quiescent SC can become active and migrate in response to inflammatory cytokines and the presence of macrophages associated with muscle injury (Yin et al., 2013).

After activation by inflammatory cytokines, SC re-enter the cell cycle and proliferate, producing committed myogenic precursor cells. These precursors will exit the cell cycle and fuse together to form new myofibers or adhere to existing damaged myofibers (Wozniak et al., 2005). Increased expression of contractile proteins and creatine kinase **(CK)** activity coincide with the fusion of myoblasts. After regeneration has been carried out, remaining myogenic precursor cells may return to quiescence as SC. The efficacy of SC mediated regeneration may be dependent on the number of SC present, as well as the energy status of the muscle tissue.

Breast yield potential of modern broiler strains is largely dictated by the extent of SC activity in late incubation and the first week posthatch. During this time, maximization of SC activity requires adequate nutrition to sustain rapid muscle growth. Type II muscle fibers in the PM have been shown to undergo selective atrophy during late stages of incubation in poultry, in contrast to the continued hypertrophy observed in

late-term mammalian fetuses (Chen et al., 2013). This phenomenon is likely attributable to the reduced oxygen environment of late stage incubation, as well as the inability of the glycolytic fibers to adequately utilize lipids from the yolk (Moran, 2007). The process of hatching requires an extensive effort by the pipping muscles, and thus may reduce available energy for supporting hypertrophy in the PM. The diversion of energy to higher priority tissue may trigger the phosphorylation of AMP-activated kinase, which signals a cascade culminating in muscle degeneration (Chen et al., 2013). The mobilization of AA from protein degradation in the PM may also support the extensive development of the small intestine during late incubation (Uni et al., 2003). However, Uni et al. (2005) reported that atrophy of the PM during late incubation may be prevented by in-ovo injection of glucose, supporting the hypothesis that a deficiency of sufficient cellular energy is the primary cause for atrophy of the PM.

After hatching, atrophy of the PM may be reversed by immediate access to feed (Chen et al., 2013). However, chicks from commercial hatcheries may undergo a period as long as 48 h posthatch during which they are handled and transported without access to feed. Halevy et al. (2000) reported that a 48 h period of posthatch starvation resulted in permanent reductions in BW and breast muscle yield over a 41 day growth period. In contrast, chicks starved from day 4 to day 6 posthatch demonstrated pronounced compensatory growth. Both groups of chicks had similar numbers of active SC; however, muscle development in the early starvation group was permanently delayed (Halevy et al., 2000). This is likely due to the regulatory response of the transcription factors MyoD and myogenin (Velleman et al., 2010). In response to starvation, MyoD concentrations increase, stimulating the proliferation of SC. In contrast, expression of myogenin is

downregulated, preventing the efficient fusion of SC into the myofibers. Feeding reduced density diets in the first week of life has been shown to negatively impact PM morphology and the expression of myogenic regulatory factors (Velleman et al., 2014). However, these authors did not observe any negative effects of feeding a similarly reduced density diet after the first week posthatch. Adequate nutritional support for the proliferation of SC in this posthatch window is absolutely critical to develop a sufficient population of SC to sustain muscle growth later in life. For this reason, nutritional strategies that involve reduced dietary density should be postponed until after the first week posthatch.

NOVEL BREAST MEAT QUALITY DEFECTS

Both WB and WS have been observed in multiple commercially-available broiler genotypes reared under a variety of environmental conditions, management strategies, and nutritional programs all around the world. Neither of these quality defects is detrimental to the wholesomeness of affected meat, though their presence may reduce consumer acceptance of product on aesthetic grounds. The poultry industry is primarily interested in defining the meat quality properties of affected fillets and developing practical interventions for reducing incidence and severity in the field. However, it is important to consider the inherent cellular attributes of these myopathies in order to fully understand the consequences on the quality and functional characteristics of affected meat.

Wooden Breast. Fillets affected by WB are abnormally firm to the touch, tough in texture and have a pale, bulging appearance. In severe cases, a protruding ridge may be present along the caudal edge of the fillet (Sihvo et al., 2014). These macroscopic

characteristics are attributable to changes at a cellular level that are evident in the unique histological and gene expression profile of affected tissue.

Sihvo et al. (2014) first described the histologic features of WB. These authors defined the condition on the basis of increased variability in fiber diameter, rounded fibers, degenerating fibers, and the accumulation of loosely-organized connective tissue, as determined by hematoxylin and eosin staining as well as Masson's trichrome staining. Additionally, these authors observed substantial infiltration of the muscle with inflammatory cells, including hetrophils, macrophages, lymphocytes, and fibroblasts. Soglia et al. (2015) conducted a similar histological analysis of tissue from broilers affected with varying degrees of WB and also observed progressively increasing variability in fiber size, roundedness, and infiltration of connective tissue with increasingly severe WB.

Subsequent evaluations of cellular morphology in affected PM muscles have indicated that the primary location and structural organization of collagen deposition is dependent on genetic strain. These authors determined that in 2 strains of broilers, deposition of collagen may occur preferentially in the anterior or anteroventral regions of the fillet (Clark and Velleman, 2016). Additionally, the collagen appeared either tightly packed in a parallel formation or diffusely-distributed, depending on genetic strain (Velleman and Clark, 2015; Clark and Velleman, 2016). These results indicated that fillets with equally severe WB lesions from a histological perspective may vary substantially when palpated by hand due to strain-dependent differences in collagen deposition.

Because WB is a progressive degenerative myopathy, its severity will vary with bird age. Sihvo et al. (2017) assessed fillets by palpation and histological analysis at 10, 18, 24, 35, 38, and 42 d of age. By palpation and histology, these authors were unable to detect WB prior to 18 d of age. Approximately 30 and 50% of the birds were affected by WB, as detected by palpation, at 18 and 24 d of age, respectively. Histological evaluation of the affected birds revealed predominantly mild lesion scores. Incidence and severity increased with advancing bird age, with 86% of birds affected by WB at 42 d of age. Of these, approximately 70% had severe lesions as determined by histology.

Molecular techniques have provided supporting evidence for macroscopic and histological characterizations of WB. In conjunction with their use of histopathology to investigate the effects of genetic strain on the appearance of WB, Velleman and Clark (2015) also assessed the expression of regulatory factors for myogenesis and collagen cross-linking. Broilers affected by WB had increased expression of MyoD and myogenin, which supports the existing histological evidence of myofiber degeneration and regeneration. Additionally, presence of WB was associated with increased expression of decorin, a key regulator of collagen deposition. A subsequent study confirmed the increase of MyoD, myogenin, and decorin in broilers affected by WB; however, these results were dependent on genetic strain and sampling location (Clark and Velleman, 2016).

Molecular techniques such as gene microarrays and RNA-seq methodologies can provide broad pathway-level information regarding related genes that are differentially expressed in broilers affected by WB. Mutryn et al. (2015) utilized RNA-seq analysis to identify over 1,500 unique differentially expressed genes in PM tissue affected by WB.

From these data, the authors called attention to the differential expression of genes involved in the buildup of reactive oxygen species, reduced glycolytic metabolism, abnormal calcium homeostasis, and increased fibrosis. Zambonelli et al. (2016) used gene microarrays to categorize 204 differentially expressed genes in PM tissue affected by WB. These authors likewise identified pathway-level differences in calcium signaling, response to reactive oxygen species, and inflammatory responses. Collectively, these results support the prevailing consensus that WB is likely the result of a multifactorial mechanism rather than any single specific cause. However, it should be noted that these results do not necessarily distinguish whether the observed differences in expression contribute to the development of WB, or if they are the result of WB.

As a consequence of these microscopic differences, fillets with WB have altered nutritional composition as well as impaired functional characteristics. Mazzoni et al. (2015) correlated meat quality and functional characteristics with severity of myodegeneration as determined by histological evaluation. Fillets exhibited increased cook loss and decreased marinade uptake as the number of fibers classified as abnormal increased. Additionally, fillets with severe myodegeneration had increased moisture (75.4 vs. 74.4%), reduced crude protein (21.1 vs. 22.6%), and increased lipid content (2.94 vs. 2.36%) in comparison with the normal fillets. These authors did not observe any differences in color coordinates, drip loss, or Allo-Kramer shear force measurements.

Soglia et al. (2015) likewise observed increased lipid and moisture content, with reduced protein content. Additionally, these authors noted increased calcium (131 vs. 84 mg/kg) and sodium (741 vs. 393 mg/kg) in fillets affected by WB compared with those not affected. This result was attributed to increased expression of calcium-ATPase, which

facilitates transport of calcium into the myofiber, in severely affected fillets. Soglia et al. (2015) also reported increased final pH, reduced water-holding capacity, increased drip loss, and increased cooking losses with severely affected fillets. The concurrent observations of increased pH and reduced water-holding capacity are intriguing, as increased pH typically improves water-holding capacity. However, these authors attributed this unique meat quality property to the destabilization of the myofibrillar membranes due to degeneration and the overall reduction in number of fibers. Mudalal et al. (2014b) reported similarly high ultimate pH and reduced water-holding capacity in fillets affected by severe WB.

Although the ability to palpate WB by hand, as well as the histological confirmation of increased connective tissue content, should translate to increased toughness of raw and cooked product, researchers have encountered difficulties in quantifying these differences. Mazzoni et al. (2015) did not report any differences in Allo-Kramer shear force. Likewise, Mudalal et al (2014b) and Tasoniero et al. (2016) did not observe any differences in Warner-Bratzler shear force. However, studies that utilized texture profile analysis to gauge textural differences in fillets affected by WB were able to detect differences (Soglia et al., 2015; Cando, 2016). These results indicate that more sophisticated methods of texture analysis may be necessary to quantify the effects of WB on texture in raw and cooked fillets.

These differences in meat quality attributes not only affect the acceptability of whole fillets, but can also have negative consequences for further processed products. In order to avoid negative consumer reactions to WB, fillets are typically diverted in the processing product from raw product lines into further processed products, although this

practice requires time-consuming monitoring of fillets on the processing line. Little research exists regarding the effects of WB inclusion in processed products. Qin (2013) reported increased shear force, lower pH, and reduced a* and b* color coordinates with increasing inclusion of WB fillets. This author determined that fillets affected by WB may be incorporated into sausage or nugget products at 15 and 30% of lean meat content without detrimental effects (Qin, 2013). However, successful integration of affected meat may depend on specific product formulations and the severity to which the fillets are affected.

White Striping. White striping is characterized by the presence of white striations that occur between muscle fibers (Kuttappan et al., 2012a, 2012c, 2013a; Petracci et al., 2013). Histological evidence has confirmed that abnormal deposition of fat and connective tissue occurs as a response to muscle damage (Kuttappan et al., 2011, 2013b; Petracci and Cavani, 2011).

Fillets affected by WS have significantly increased fat content, lower protein content, and increased collagen content compared with normal fillets (Petracci et al., 2014). In breast fillets with severe WS, pH was significantly higher than normal breast fillets (Kuttapan et al., 2009; Petracci et al., 2013). No effect on L* color coordinates were detected for fillets with severe WS, although minor effects on b* (Kuttappan et al., 2009; Petracci et al., 2013) and a* (Petracci et al., 2013) have been reported. Severe WS was also associated with significantly higher purge losses, higher cook losses, lower Allo-Kramer shear force values, and reduced marinade uptake (Petracci et al., 2013). Changes in muscle composition associated with WS may render nutritional or percentage fat-free labels on affected products inaccurate, requiring product re-branding. The

alteration of meat quality attributes in breast fillets affected by WS may negatively impact the outcome of further processed products. Furthermore, WS has a negative effect on consumer acceptance of affected raw product (Kuttappan et al., 2012a). Incidence of WS in controlled experiments may exceed 50% (Kuttappan et al., 2012b, 2012c). Some evidence suggests that WS may be more prevalent in broiler strains selected for high breast yield (Petracci et al., 2013), large body weight (Bauermeister et al., 2009) or rapid growth (Kuttappan et al. 2012b). Although the incidence of WS and that of WB are frequently correlated (Kuttappan et al., 2016), they do not always occur concurrently.

PROPOSED MECHANISMS UNDERLYING THE DEVELOPMENT OF WOODEN BREAST AND WHITE STRIPING

Many potential mechanisms have been suggested as the underlying cause of WB and WS in modern broiler chickens. Each of these theories is predicated upon the assumption that genetic selection for a high-yielding, efficient, fast-growing chicken has resulted in muscle tissue that exists at the threshold of its capacity for maintaining homeostasis. Stressors to the tissue, of either internal or external origin, may overwhelm existing homeostatic systems, initiating a cascade in which myofibers are degenerated and gradually replaced with connective (WB) and adipose (WS) tissues.

Proposed stressors that are inherent to the bird include reduced vascular density, rapid increases in fiber diameter (Joiner et al., 2014), and saturation or deficiency of endogenous antioxidant systems (Mutryn et al., 2015; Sundekilde et al., 2017). The PM of modern broiler chickens is predominantly composed of fast-twitch Type IIb fibers. These fibers rely on glycolytic (anaerobic) energy metabolism for high-intensity short-duration activity, and therefore have low myoglobin content, resulting in their pale ‘white

meat' appearance. While the oxygen demand for muscle tissue of this type is lower than aerobic fiber types, a sufficient vascular supply is necessary to remove the waste products of anaerobic metabolism, specifically the buildup of lactate. Increased yield results from extensive hypertrophy of the myofibers. As myofiber diameter increases, the diffusion distance from the interior of the fiber to the nearest capillary also increases. If fewer capillaries per fiber are present and/or the fiber diameter has increased past a critical threshold, it is possible that waste buildup is creating microenvironments within the tissue in which cellular homeostasis (pH, ion exchange) has been compromised (Mutryn et al., 2015; Zambonelli et al., 2016). Additionally, the greater diffusion distance will inhibit the effective diffusion of oxygen, causing localized hypoxia (Velleman and Clark, 2015; Sihvo et al., 2017). Buildup of reactive oxygen species from metabolic processes (Mutryn et al., 2015), as well as hindered diffusion of nutrients, may result in insufficient antioxidant capacity and subsequent membrane destabilization.

External stressors in the rearing environment may also contribute to the development of myopathy. It has been proposed that high levels of ammonia or carbon dioxide due to poor ventilation practices may exacerbate any vascular insufficiencies, resulting in systemic hypoxia (Mutryn et al., 2015). Heat stress, whether from ambient temperature or from an interactive effect with coccidiostats such as nicarbazin that increase metabolic heat production, may also negatively impact the homeostasis of muscle tissue (Sandercock et al., 2006). The modern broiler is also prone to extended periods of sitting (Bokkers et al., 2003) as well as altered posture (Zuidhof et al., 2014) that places much of its weight on the PM when sitting. This behavior may cause the PM to succumb to the effects of heat stress more rapidly as heat becomes trapped between the

breast and the litter. Elevated body temperature will increase the rate of many oxidative reactions and will also depress feed intake, further restricting muscle access to nutrients. Oxidative stressors may enter the body through feed containing poor-quality fat sources, further taxing the antioxidant system.

The primary effects of these potential stressors are the induction of localized hypoxia in the muscle and destabilization of membranes. When membrane integrity is degraded, damaged muscle tissue will begin to leak enzymes, such as CK, into the systemic circulation (Mitchell, 1999; Sandercock and Mitchell, 2004; Sandercock et al., 2006). Additionally, membrane instability will impair the sequestering of calcium within the myofiber, negatively impacting cellular function. In response to local hypoxia and reduced capacity for normal function, inflammatory cytokines will be released. The inflammatory response activates transcription factors, such as MMPs, TIMPS, and TGFβ, that are known to upregulate degradation of myofibrillar proteins and promote the synthesis of collagen by fibroblasts (Mutryn et al., 2015).

Although none of these pathways has yet been conclusively proven as correct, each of the proposed pathways aligns well with the reported pathohistology, gene expression, and metabolic profile of affected tissue. It is likely that a combination of the proposed mechanisms, rather than any individual stressor, is responsible for the development of myopathy. Practical interventions should therefore target multiple internal and external stressors that may be predisposing factors for myodegeneration. Targets for nutritional strategies include the reduction of oxidative stressors in the feed, supplementation of antioxidants, and the investigation of anticoccidial pharmaceuticals that may contribute to metabolic heat production. Additionally, nutritional strategies may be applied to alter the

growth curve in a manner that balances the goal of increased muscle yield with the adequate development of cellular and vascular systems for maintaining accreted muscle tissue.

NUTRITIONAL INTERVENTIONS FOR WHITE STRIPING AND WOODEN BREAST

Nutritional strategies for reducing the prevalence of WB and WS have typically focused on either supplementing the diet with specific nutrients to combat external stressors or on the manipulation of overall dietary density with the goal of supporting homeostasis in the PM by reducing the metabolic load associated with accelerated growth.

Muscular dystrophy, presenting with white striated lesions of the PM, in broilers has been associated with deficiencies in vitamin E, selenium, or sulfur AA (Kuttappan et al., 2012b). Vitamin E functions as a powerful antioxidant, reducing damage to DNA and cellular membranes. The use of added dietary vitamin E has been investigated as an intervention for WS in broilers with no significant effect on the incidence or severity of WS at supplementation levels up to 400 IU/kg (Kuttappan et al., 2012b). Sihvo et al. (2017) compared the effects of diets containing inorganic and organic selenium sources at 0.12 and 0.31 mg/kg but did not observe any effects of selenium source or concentration on the incidence or severity of WB. Sirri et al. (2016) also investigated the supplementation of trace minerals on WS and WB. These authors supplemented 2 concentrations of organic and inorganic zinc, manganese, and copper from 1 to 51 d of age. However, no differences in the incidence or severity of WB and WS were observed.

The supply of sulfur AA has been shown to alter the *in vitro* proliferation, differentiation, and metaplasia of cultured SC (Powell et al., 2013; 2014). It is possible that a deficiency of sulfur AA may hinder proliferation and differentiation of SC, contributing to reduced muscle regeneration and the infiltration of connective tissue observed with WB. However, comparable *in vivo* studies of the effect of dietary sulfur AA on SC have not yet been reported.

Carcass yield and breast muscle accretion are dependent on AA and AME_n density (Kidd et al., 2005; Dozier et al., 2008, 2010; Powell et al., 2013). Strategies to reduce WB and WS by slowing muscle accretion during critical portions of the growth period have attempted to control nutrient intake through physical restriction of intake, as well as modulating nutrient density. Trocino et al. (2015) restricted broilers to 80% of *ad libitum* intake from 13 to 21 d of age. However, this program resulted in increased incidence of WS and no effects on the presence of WB at 46 d of age in comparison with an *ad libitum* control. These authors attribute their results to the short timing of the restricted feeding program, as well as the birds' ability to compensate with improved efficiency of nutrient utilization when returned to *ad libitum* feeding. Radaelli et al. (2017) repeated this feeding program but used histological analysis of degenerating fibers as a response, as opposed to the visual scoring of myopathies utilized by Trocino et al. (2015). In contrast to the results observed by the latter author, Radaelli et al. (2017) reported increased fiber degeneration in the *ad libitum* fed control group at 14 and 21 d of age, relative to the restricted group. However, these differences disappeared after the restricted group was returned to *ad libitum* feeding. From these studies, it appears that quantitative control of

feed intake may be a successful method of controlling myopathy. However, the beneficial effects are eliminated once birds return to an *ad libitum* program.

Other authors have investigated reductions in dietary density, rather than physically restricting access to feed. Kuttappan et al. (2012c) achieved a reduction in the incidence of severe WS (1.46% vs. 8.70%) by feeding broilers low energy (3,002; 3,025; and 3,063 kcal/kg for the starter, grower, and finisher, respectively) vs. high energy diets (3,206; 3,250; and 3,251 kcal/kg for the starter, grower, and finisher, respectively) from 1 to 54 d of age. These authors observed reduced BW gain and increased FCR for the broilers receiving low energy diets, as well as lower breast weights. Cruz et al. (2017) investigated the effects of reducing dLys from 12 to 28 and 28 to 40 d of age on the incidence of WB and WS at 35 and 42 d of age. These authors observed reductions in WB and WS when feeding diets formulated with dLys at approximately 70 and 63% of the recommended concentrations. Although this dietary strategy reduced the incidences of myopathy, the extreme reductions in dLys were deleterious to live performance and breast yield. Although reducing dietary AA and AME_n density may be effective in alleviating WS and WB, large deviations from the recommended nutrient concentrations can be detrimental to body weight gain and breast meat yield. Variations of these strategies may be successful if applied at a lower intensity or over a shorter duration of the growth period. However, the consequences of any such interventions on live performance and processing characteristics must be evaluated.

KNOWLEDGE GAPS IN THE LITERATURE

The substantial negative impact of these myopathies on the acceptability of affected product is a major challenge facing the poultry industry worldwide. Considering a

hypothetical price of boneless-skinless breast fillets ($1.80/lb) and the amount of breast meat produced annually in the United States (8.75 billion broilers × 6 lbs average live weight × 25% breast yield = 13.1 billion lbs of breast meat), even a 1% loss of saleable product due to these quality defects amounts to an annual loss of approximately $236 million in revenue alone. Moreover, this value does not account for the additional costs of lost production inputs, quality assurance monitoring, production line inefficiency, and product diversion associated with birds affected by these quality defects. The reduction of myopathies to minimal levels through genetic selection, while possible, will likely require several years to accomplish, given the low heritability of these traits. Therefore, it will be necessary to develop short-term, practical strategies for reducing the incidence and severity of WB and WS.

Nutritional strategies for ameliorating WB and WS have thus far been unsuccessful in reducing the incidence and severity of these myopathies without detrimental effects on performance and processing characteristics. However, the current body of research represents a small portion of the possible combinations of dietary density and timing that could be investigated as potential interventions. It is possible that implementing similar quantitative or qualitative nutrient allocation strategies with more moderate reductions in density or over shorter periods of time may suffice to reduce myopathies without these negative effects.

Establishing a basic understanding of the etiology of these myopathies is critical to provide direction for future research aimed at developing practical nutrition and management interventions to reduce their prevalence in the broiler industry. However, the identification of affected birds in the literature has been largely dependent on postmortem visual or histological evaluation. Manual palpation is sufficient to identify severe WB in live birds,

but less severe manifestations of WB and the presence of WS cannot be distinguished without necropsy. Removal of birds for necropsy precludes the collection of data on the progression of myopathy in individuals over extended periods of time. For this reason, it may be advantageous to identify in vivo diagnostic markers for myopathy.

Additionally, it has been suggested that current rates of breast muscle accretion in modern broilers exceed the regenerative capacity of the SC population present at hatch, predisposing the muscle tissue to chronic inflammation and degradation. The upregulation of myogenic regulatory factors in PM tissue affected by WB has been supported by gene expression data. Yet, to our knowledge, data have not been reported concerning the actual populations of myogenic cells associated with these myodegenerative quality defects.

In order to address these knowledge gaps, a series of experiments was conducted to evaluate strategies to reduce the development of WB and WS by altering the growth curve through quantitative allocation of feed, qualitative reductions in AA and AME_n density, and reduced dLys concentrations in the grower phase. Lastly, the reduced dLys diets were used to produce broilers affected by varying degrees of WB as part of a comparative study to assess differences in fiber CSA and myogenic cell activity. The results of these experiments should provide a basis for practical nutritional recommendations to mitigate the presence of WB and WS in the short term, as well as guide future investigations into the underlying mechanisms of these myopathies.

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III. EFFECTS OF QUANTITATIVE NUTRIENT ALLOCATION ON MYOPATHIES OF THE PECTORALIS MAJOR MUSCLES IN BROILER CHICKENS AT 32, 43, AND 50 DAYS OF AGE

ABSTRACT

An experiment was conducted to determine if myopathies of the PM muscles are influenced by differences in growth trajectory achieved through a controlled feeding program. Male Yield Plus × Ross 708 broiler chicks were placed into 28 pens (25 birds/pen) equipped with plastic slats to prevent coprophagy. All birds received identical starter (1 to 10 d), grower (11 to 32 d), finisher (33 to 42 d), and withdrawal (43 to 50 d) diets that were formulated to meet or exceed nutrient recommendations of the primary breeder. Each pen of birds was randomly assigned to 1 of 4 pair-feeding programs (TRT 1: *ad libitum*; TRT 2: 95% of TRT 1 intake; TRT 3: 90% of TRT 1 intake; TRT 4: 85% of TRT 1 intake) with 7 replicate pens per treatment. Feed intake and mortality were recorded daily. Individual BW was recorded at 31, 42, and 49 d of age. Blood samples were collected from 4 birds per pen at 31, 41, and 48 d of age and subsequently analyzed for plasma CK and lactate dehydrogenase (LDH). At 32, 43, and 50 d of age, 4 birds per pen were euthanized for necropsy. The right breast fillet of each bird was visually scored for WS and WB. Linear decreases ($P \leq 0.01$) in feed intake, BW gain, feed conversion ratio and mortality were observed with decreasing feed allocation. Linear decreases ($P \leq 0.01$) in severity were observed for WS and WB at 33, 43, and 50 d with decreasing feed

allocation. Severity of WB at 33 and 43 d, as well as that of WS at 43 and 50 d, decreased ($P \leq 0.05$) quadratically with decreasing feed allocation. Reduced feed allocation produced quadratic decreases ($P \leq 0.05$) in CK and LDH concentrations at 31, 41, and 48 d. These results indicate that the incidence of breast fillet myopathies in broilers may be reduced through controlled feeding programs.

INTRODUCTION

Degenerative myopathies of the PM muscles manifest at processing as visible and palpable quality defects in the breast fillet. Recently, attention has focused on the increased occurrence of WS and WB in modern broiler strains (Kuttappan et al. 2012a; Sihvo et al., 2014). Whereas WS has been characterized as the abnormal deposition of intermuscular fat (Kuttappan et al., 2013b) resulting in parallel striations, WB is distinguished by excessive toughness due to the accumulation of connective tissue (Sihvo et al., 2014). Although these myopathies are predominantly aesthetic in nature and do not affect the wholesomeness of product, they may negatively impact consumer perception of affected meat (Kuttappan et al., 2012b). Furthermore, the presence of myopathy may alter nutritional and functional characteristics, causing issues for further processed products and labeling (Qin, 2013; Mudalal et al., 2014, 2015; Mazzoni et al., 2015). Each of these myopathies has been observed in multiple commercially available modern broiler strains (Meloche et al., 2014; Trocino et al., 2015) grown under a variety of conditions worldwide. Although numerous anecdotal reports indicate that these issues are associated with a sudden acceleration during critical windows of the growth trajectory, limited formal research has been conducted to investigate this relationship. Therefore, an experiment was conducted to determine if myopathies of the PM muscles are influenced by differences in growth trajectory obtained through quantitative feed restriction. In

conjunction with this paired-feeding trial, plasma CK and LDH were also investigated as potential *in vivo* markers for the presence of myopathy in broilers.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2014-2404).

Bird Husbandry

An experiment was conducted from 1 to 50 d of age. Seven hundred Yield Plus × Ross 708 (Aviagen Inc., Huntsville, AL) male broiler chicks were obtained from a commercial hatchery and placed into floor pens (0.11 m^2/bird; 25 birds per pen) equipped with plastic slats raised above the litter to prevent the recycling of nutrients through coprophagy in the pair-fed treatments. Chicks were vaccinated at the hatchery for Marek's disease, Newcastle disease, and infectious bronchitis. Each pen was equipped with a hanging feeder and a nipple drinker line (5 nipples per pen). Birds consumed water on an *ad libitum* basis. Experimental diets were weighed and allocated daily. Ambient temperature set points consisted of 33°C at placement until 4 d of age, 32°C from 5 to 9 d of age, 29°C from 10 to 14 d of age, 27°C from 15 to 23 d of age, 25°C from 24 to 28 d of age, 23°C from 29 to 33 d of age, 21°C from 34 to 38 d of age, and 19°C from 39 to 50 d of age. Birds were exposed to a 23L:1D photoperiod from placement to 7 d of age, followed by an 20L:4D photoperiod for the remainder of the experiment. Light intensity was set at 30 lux from 1 to 7 d of age, 10 lux from 8 to 13 d of age, 5 lux from 14 to 27 d of age, and 3 lux from 28 to 50 d of age. Light intensity settings were verified at bird level (30 cm) using a photometric sensor with NIST-traceable calibration (403125,

Extech Instruments, Waltham, Mass) for each intensity adjustment. Mortality was recorded daily.

Dietary Treatments

Each pen of birds received identical starter (1 to 10 d), grower (11 to 32 d), finisher (33 to 42 d), and withdrawal (43 to 50 d) diets that were formulated to meet or exceed nutrient recommendations from the primary breeder (Table 3.1). Diets were corn-soybean meal-based and contained distillers dried grains with solubles. No ingredients of animal origin were used in formulation of experimental diets. Feed was provided in crumble form during the starter phase, and were pelleted thereafter. Treatments consisted of an *ad libitum* control and 3 experimental feeding programs in which birds were pair-fed at 95%, 90%, and 85% of TRT 1 intake. All pens received feed *ad libitum* for the first 24h after placement. Beginning at 2 d of age, feed intake was recorded at the same time each morning and allocations were calculated as a percentage of the average TRT 1 intake for the previous day.

Measurements

Individual BW was recorded at 31, 42, and 49 d of age. At 31, 41, and 48 d of age, 4 mL of blood was collected from the ulnar vein of 4 birds per pen using 21 gauge 25.4 mm needles and heparinized (16 IU/mL) monovette syringes. Blood samples were retained on ice until sampling was completed, then centrifuged at 4,000 × *g* for 10 min at 4°C in order to obtain 1.5 mL of plasma which was stored at −20°C until subsequent analysis for plasma CK and LDH. Analyses for CK and LDH were conducted using a Roche/Hitachi Cobas c311 (Roche Diagnostics, Indianapolis, IN) automated blood analyzer at Auburn University College of Veterinary Medicine. At 32, 43, and 50 d of

age, the same 4 birds per pen which were utilized for blood sample collection were euthanized by carbon dioxide asphyxiation. The PM muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for WS and WB. All fillets were scored by the same evaluator. For WS, the defect was characterized as "mild" if visible striping comprised less than half of the total fillet surface area or was less than 2 mm wide on average, but was considered "severe" if the striping exceeded these limits. For WB, the defect was considered "mild" if palpable hardness was present in less than half the total fillet surface area, but was considered "severe" if it exceeded this limit.

Statistical Analyses

Each of the 4 dietary treatments was represented by 7 replicate pens arranged in a randomized complete block design with pen as the experimental unit and pen location as the blocking factor. Treatment effects on live performance were subject to analysis of variance using PROC MIXED of SAS 9.3 (SAS Institute, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu_{..} + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are the fixed factor level effects corresponding to the *j*th treatment such that $\Sigma\tau_j = 0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ. Additionally, linear and quadratic responses to dietary treatment were assessed using CONTRAST statements to evaluate the above model in PROC MIXED.

Relationships between myopathy scores and other variables were investigated using Spearman's rank correlation and logistic regression. Spearman's rank correlation (**ρ**) is more appropriate for ordinal categorical variables such as myopathy scores because it is a nonparametric measure of dependence between variables with a monotonic relationship and it does not assume a normal distribution as Pearson's correlation does for continuous variables. Likewise, logistic regression is more appropriate for categorical responses, as it utilizes maximum likelihood estimates rather than ordinary least squares. In order to accommodate a myopathy scoring system with more than 2 ordinal outcome levels, the following cumulative logit model was utilized:

$$\ln \frac{P(y_i \leq k)}{1 - P(y_i \leq k)} = \alpha_k + \boldsymbol{x_i'\beta},\ k = 0,1,\ldots,m$$

where y_i is the response variable (myopathy score), $\boldsymbol{x_i'}$ is the vector [1, x_{i1}, x_{i2}, …, x_{ij}] of j predictor variables, **β**' is the vector [β_0, β_1, β_2, …, β_l] of partial regression coefficients representing the expected change in response y per unit change in x_i when all the remaining regressor variables, x_i (i≠l) are held constant; and α_k is the unique intercept for outcome level k, where the outcomes are represented by 0,1, 2, …, m (Montgomery et al., 2012). For each fitted model, the assumptions of a binomially distributed response and proportional odds among outcome levels were met. Where applicable, residuals were visually assessed to ensure normality and nonnormal data were transformed prior to analysis. For all hypothesis tests, statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth Performance

Pair-feeding at specified percentages of *ad libitum* intake resulted in a linear decrease ($P < 0.0001$) in feed intake among the treatments as expected according to the experimental design (Table 3.2). Reducing feed intake through quantitative feed allocation resulted in a concomitant linear decrease in cumulative BW gain ($P < 0.0001$), with broilers pair-fed at 95, 90, and 85% of *ad libitum* intake respectively achieving 95, 93, and 89% of the BW gain observed for the *ad libitum* control. Reducing feed allocation resulted in a linear decrease ($P < 0.0001$) in feed conversion ratio **(FCR)**, with particularly notable improvement obtained at the 90% feed allocation program. However, this improved efficiency does not account for differences in BW among the reduced allocation treatments. A longer growth period would be required for these birds to obtain a similar final BW to the control, eliminating the benefits of reduced FCR due to the additional maintenance cost and increasing mortality over time. Previous research has demonstrated the capacity of broilers to exhibit reduced FCR under conditions of limited nutrient availability (Washburn, 1990; Ballay et al., 1992). Additionally, a linear reduction ($P < 0.0001$) in mortality was observed with decreasing feed allocation, an outcome which has been previously reported for broilers on reduced feed allocation programs (Ballay et al., 1992). These results indicate that the pair-feeding regimen utilized in this experiment was sufficient to alter the growth rate and efficiency of modern broilers.

Myopathy Scoring

Linear decreases ($P < 0.01$) in the average score for WB and WS were observed with decreasing feed intake at 33, 43, and 50 d of age (Table 3.3). Additionally, quadratic reductions in average score were observed for WB at 33 and 43 d of age and for WS at 50 d of age. These quadratic responses indicate a diminishing marginal return for reductions in feed intake, particularly below 95% of *ad libitum*. Figure 3.1 illustrates the quadratic effect of decreasing feed intake on the proportion of fillets scored as having normal, mild, or severe WB (Figure 3.1A) and WS (Figure 3.1B) at 43 d of age. In this case, the greatest benefit of reduced feed intake was observed between the *ad libitum* control and the 95% intake treatment, for which severe scores for each myopathy decreased substantially, with little additional reduction beyond the 95% feeding program. In contrast to these results, Trocino et al. (2015) observed increased incidence WS and no effects on the presence of WB at 46 d of age in broilers restricted to 80% of ad libitum intake from 13 to 21 d of age. These authors attribute their results to the short duration of the restricted feeding program, as well as the birds' ability to compensate with improved efficiency of nutrient utilization when returned to *ad libitum* feeding. Both the timing and intensity of a feed allocation program must be taken into account in order to manipulate the growth curve favorably for the reduction of myopathies.

In logistic regression, the overall χ^2 *P*-value corresponds to the hypothesis test for overall significance of the model. At each of the ages evaluated, logistic relationships between dietary treatment and myopathy severity were observed ($P < 0.05$) for both WB and WS. The strength of these relationships is reflected in the corresponding odds ratios for each logistic model (Table 3.3). Odds ratios represent the proportional increase in the

odds of obtaining a myopathy score of 0 vs. a score of 1 or 2 (or the odds of obtaining a score of 0 or 1 vs. a score of 2) for each change in feed allocation program. For example, at 33 d of age the odds of observing a WB score of 0 (normal) for broilers assigned to the 95% intake program were 11.7 times higher than the odds of observing a normal fillet for broilers fed *ad libitum*. Reducing feed allocation to 95% of *ad libitum* intake up to 43 d of age produced a more distinct effect on the odds of observing fillets unaffected by WB, as the odds ratios associated with WB are consistently higher than those for WS for all feeding programs relative to the *ad libitum* control. In contrast, higher odds ratios were observed for treatment effects on WS at 50 d of age.

Although Spearman's correlation indicated the presence of an association between feed intake and myopathy score ($P < 0.01$) at all ages evaluated, correlation coefficients ranging from 0.27 to 0.60 denote the modest strength of this relationship (Table 3.3). The highest correlation between feed intake and myopathy (0.60) was observed for WB at 43 d of age. Taken in conjunction with the higher odds ratios for dietary treatment effects on WB scores, these data indicate that feed allocation programs aimed at reducing myopathy scores may elicit the greatest effect at 43 d of age. Previous research has indicated that the severity of WB and WS increases with age at slaughter (Bauermeister et al., 2009; Petracci et al., 2013). However, in the current study, the severity of WB appeared to decrease with advancing bird age. This result may be a consequence of the sampling method, as the serial harvest of 4 birds per pen at each necropsy date inherently reduces the sampling pool. The impact of any feeding strategy may depend on both the timing of implementation and the age at harvest. Broilers processed at earlier ages may not benefit as substantially from a shorter duration feeding

program, whereas older birds may be affected by advanced myopathy that can no longer be ameliorated through nutritional means.

Blood Chemistry

Experiments aimed at investigating the development of myopathies over time are complicated by the necessity of scoring for the presence of the myopathy postmortem. Although manual palpation may suffice to identify severe WB in live birds, less severe manifestations of WB and the presence of WS cannot be distinguished without necropsy. Removal of birds for necropsy precludes the collection of data on the progression of myopathy in individuals over extended periods of time. For this reason, it may be advantageous to identify in vivo diagnostic markers for myopathy. Creatine kinase is an intracellular enzyme that catalyzes the transfer of high-energy phosphate groups. Although multiple isoforms of CK are expressed in various tissues, Mitchell and Sandercock (1995) determined that the major isoform present in chicken plasma originated from skeletal muscle. Plasma CK has been previously utilized as an indicator of compromised membrane integrity in the presence of myopathy (Mitchell, 1999; Sandercock and Mitchell, 2004; Sandercock et al., 2006). In the current study, plasma CK concentrations decreased ($P < 0.05$) quadratically with decreasing feed intake at 32 and 41 d of age. No differences were observed in plasma CK concentrations at 48 d of age (Table 3.4).

Similarly, plasma LDH concentrations decreased ($P < 0.01$) quadratically with decreasing feed intake at 32 and 41 d of age, but no differences among treatments were observed at 48 d (Table 3.4). Five isoenzymes of LDH are expressed ubiquitously, each catalyzing the conversion of pyruvate to lactate (Brancaccio et al., 2010). Plasma LDH is

commonly used as an indirect marker for rhabdomyolysis in humans (Sanchez-Navarro et al., 1998) and has been investigated as a potential indicator of heat stress in laying hens (Melesse et al., 2011) and broilers (Xing et al., 2015). In broilers, both CK and LDH have been shown to inherently increase with age (Mitchell and Sandercock, 1994; Macrae et al., 2006). This effect may not be truly dependent on absolute age, but rather due to the ability of modern broilers accrete more muscle mass at a given age than their predecessors.

Each of these enzymes has been associated with the presence of various myopathies (Mitchell, 1999). Kuttappan et al. (2013a) observed elevated serum CK and LDH in broilers with severe WS. Additionally, microarray gene expression profiling has demonstrated increased abundance of LDH in the PM tissue of broilers concurrently affected by WS and WB (Zambonelli et al., 2016). In the current study, Spearman's correlation indicated ($P < 0.001$) moderate relationships for both CK and LDH with each myopathy at 32 and 41 d of age (Table 3.5). Furthermore, there was a logistic relationship ($P < 0.05$) between these blood measures and the severity of each myopathy score at 32 and 41 d of age, with odds ratios indicating that, for example, each unit/mL increase in LDH at 32 d of age would correspond to a 64.5% decrease in the probability of obtaining a normal score for WB. The relationship between observed LDH concentrations and severity scores for WB in this experiment can be illustrated by plotting the change in probabilities for each individual score vs. LDH concentration (Figure 3.2). As plasma LDH concentration increases, particularly in excess of approximately 2 units/mL, the likelihood of obtaining a normal score for WB decreases substantially, with concomitant increases in the likelihood of abnormal scores. Overall, the lower odds ratios determined

for LDH signify that this enzyme may have greater value as a predictor of myopathy. This is likely due to the fact that CK concentrations in broilers inherently increase with growth, even in the absence of myopathy (Mitchell and Sandercock, 1994), confounding its utility as an indicator of muscle damage in a rapidly growing animal.

These results indicated that altering the growth trajectory through quantitative reductions in feed allocation to 95% of *ad libitu*m intake decreased average scores for WB at 33 and 43 d as well as decreased WS at 43 and 50 d at the cost of live performance. Further reductions in feed intake did not lead to substantial reductions in breast myopathy scores. It is possible that controlled feeding at an intermediate level, between 95 and 100% ad libitum, may reduce the incidence of myopathy without substantially impacting performance. However, such a feeding program would require further investigation. Elevated plasma CK and LDH concentrations are associated with the presence of myopathy and therefore may have utility for in vivo detection of myopathy. As quantitative allocation of feed is often not achievable in many areas of the due to logistical constraints, practical feeding programs which might reduce the incidence of breast myopathies with minimal impact on live performance require further evaluation. Additionally, research aimed at elucidating the underlying mechanism of these myopathies is warranted in order to support the development of practical interventions.

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Table 3.1 Ingredient and calculated nutrient composition of diets fed to male Yield Plus × Ross 708 broilers from 1 to 50 d of age

	Starter 1 to 10 d	Grower 11 to 32 d	Finisher 33 to 42 d	Withdrawal 43 to 50 d
Ingredient (%)				
Corn	48.16	54.07	60.85	62.83
Soybean Meal (48% CP)	38.50	31.23	23.81	20.98
Distillers Dried Grains with Solubles	6.00	7.00	8.00	9.00
Corn Oil	3.02	3.85	3.63	3.64
Dicalcium Phosphate	1.87	1.63	1.50	1.39
Calcium Carbonate	1.23	1.05	1.08	1.04
DL-Methionine	0.31	0.27	0.22	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.08	0.08	0.08
Sodium Chloride	0.43	0.42	0.42	0.41
L-Lys·HCl	0.16	0.16	0.18	0.19
L-Thr	0.08	0.07	0.07	0.07
Choline Chloride[3]	0.05	0.06	0.07	0.06
Calculated nutrient composition (%)[4]				
AME_n (kcal/kg)	3,025	3,150	3,200	3,225
CP	23.57	20.86	18.15	21.41
Digestible Lys	1.27	1.10	0.94	0.89
Digestible Met	0.62	0.56	0.48	0.45
Digestible TSAA	0.94	0.84	0.73	0.69
Digestible Thr	0.83	0.73	0.63	0.60
Digestible Val	0.95	0.84	0.73	0.69
Digestible Ile	0.88	0.76	0.65	0.61
Digestible Arg	1.42	1.23	1.03	0.96
Digestible Trp	0.25	0.21	0.18	0.16
Ca	1.05	0.90	0.85	0.80
Non-phytate P	0.50	0.45	0.42	0.40
Na	0.20	0.20	0.20	0.20

[1]Vitamin premix provided the following per kilogram of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopheryl acetate), 66 IU; Vitamin B12 (cyanocobalamin), 0.03 mg; D-biotin (biotin), 0.18 mg; menadione (menadione sodium bisulfate complex), 4 mg; thiamine (thiamine mononitrate), 5.5 mg; riboflavin (riboflavin), 22 mg; D-pantothenic acid (calcium pantothenate), 31 mg; pyridoxine (pyridoxine hydrochloride), 7.7 mg; niacin (niacinamide), 88 mg; folacin (folic acid), 2.6 mg.

[2]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite), 0.3 mg.

[3]Choline chloride-60 (Balchem Corporation, New Hampton, NY)

[4]Values reported as percentages unless noted otherwise. Digestible amino acid values were determined from digestible coefficients and calculated total amino acid content of the ingredients (Ajinomoto, 2004).

Table 3.2 Growth performance of Yield Plus × Ross 708 male broilers on a paired-feeding program from 1 to 50 d of age[1]

Treatment[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	FCR (kg:kg)	Mortality (%)
Control (ad libitum)	3.748	3.707	6.757	1.823	4.0
95%	3.579	3.538	6.312	1.785	3.4
90%	3.516	3.475	5.996	1.725	0.5
80%	3.347	3.306	5.665	1.714	0.0
SEM[3]	0.038	0.038	0.047	0.012	0.9
Source of Variation[4]	----------Probabilities----------				
Linear	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	0.99	0.99	0.24	0.28	0.66
Linear R^2_{Adj}	0.67	0.67	0.91	0.63	0.30
Quadratic R^2_{Adj}	0.66	0.66	0.92	0.64	0.27

[1]Broilers received diets that were identical in composition and nutrient content. Feed was provided in 4 phases: starter (1 to 10 d), grower (11 to 32 d), finisher (33 to 42 d), and withdrawal (43 to 50d). Values represent least-square means of 7 replicate pens per treatment (25 birds per pen)

[2]Treatments consisted of an *ad libitum* control and 3 controlled feeding programs that received the specified proportion of the control group intake on the previous day. Each of the 4 treatments was represented by 7 replicate pens.

[3]SEM = pooled standard error of the mean.

[4] R^2_{Adj} is the adjusted coefficient of determination.

Table 3.3 Average breast myopathy scores of male broilers on a paired-feeding program at 33, 43, and 50 d of age[1]

	33 days		43 days		50 days	
Treatment[2]	WB	WS	WB	WS	WB	WS
TRT 1: 100%	0.643	0.786	1.593	1.851	0.702	1.593
TRT 2: 95%	0.107	0.500	0.536	1.321	0.393	0.857
TRT 3: 90%	0.143	0.429	0.107	1.000	0.143	1.071
TRT 4: 80%	0.107	0.250	0.143	0.964	0.143	0.821
SEM[3]	0.096	0.109	0.106	0.112	0.109	0.119
Source of Variation[4]	----------Probabilities----------					
Linear	< 0.001	0.008	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic	0.010	0.62	< 0.001	0.027	0.14	0.011
Linear R^2_{Adj}	0.10	0.09	0.43	0.23	0.12	0.14
Quadratic R^2_{Adj}	0.14	0.08	0.55	0.26	0.13	0.17
Logistic χ^2 P[5]	0.001	0.011	< 0.001	< 0.001	0.001	< 0.001
Odds Ratios						
TRT 2 vs. 1	11.7	2.9	18.4	9.9	2.6	20.6
TRT 3 vs. 1	5.7	3.3	117.6	23.7	10.1	8.1
TRT 4 vs. 1	7.9	6.6	85.4	26.9	7.6	24.1
Spearman's P[6]	0.004	0.006	< 0.001	< 0.001	< 0.001	< 0.001
Spearman's ρ	0.27	0.32	0.60	0.44	0.35	0.39

[1]Broilers (25 birds per pen; 28 pens) received diets that were identical in composition and nutrient content. Feed was provided in 4 phases: starter (1 to 10 d), grower (11 to 32 d), finisher (33 to 42 d), and withdrawal (43 to 50d). At 33, 43, and 50 d of age, 4 birds per pen were euthanized for necropsy. The right breast fillet of each bird was assigned a subjective score (normal = 0; mild = 1; severe = 2) based on the incidence and severity of wooden breast (WB) and white striping (WS).

[2]Treatments (TRT) consisted of an *ad libitum* control and 3 controlled feeding programs that received the specified proportion of the average control group intake from the previous day. Each of the 4 treatments was represented by 7 replicate pens.

[3]SEM = pooled standard error of the mean.

[4] R^2_{Adj} is the adjusted coefficient of determination.

[5]The overall χ^2 *P*-value corresponds to the hypothesis test for overall significance of the logistic regression model. Odds ratios represent the proportional increase in the odds of obtaining a myopathy score of 0 vs. a score of 1 or 2 (or the odds of obtaining a score of 0 or 1 vs. a score of 2) for each change in feed allocation program.

[6]Spearman's rank correlation coefficient (ρ) and its associated *P*-value provide a measure of dependence between continuous and categorical variables, such as that between feed intake and myopathy scores.

Table 3.4 Effect of paired-feeding programs on plasma creatine kinase (CK) and lactate dehydrogenase (LDH) concentrations in male broilers at 32, 41, and 48 d of age[1]

	CK (U/mL)			LDH (U/mL)		
Treatment[2]	32 d	41 d	48 d	32 d	41 d	48 d
Control (100%)	26.549	54.863	35.509	2.100	2.815	1.917
95%	9.890	21.415	43.560	0.883	1.389	2.768
90%	5.934	11.454	42.295	0.656	0.805	2.006
80%	4.560	13.670	50.610	0.694	0.973	2.232
SEM[3]	2.847	7.862	6.128	0.207	0.264	0.384
Source of Variation[4]	----------Probabilities----------					
Linear	< 0.001	< 0.001	0.11	< 0.001	< 0.001	0.92
Quadratic	0.008	0.022	0.98	0.002	0.003	0.42
Linear R^2_{Adj}	0.21	0.12	0.01	0.17	0.18	0.01
Quadratic R^2_{Adj}	0.25	0.14	0.01	0.23	0.24	0.01

[1]Broilers (25 birds per pen; 28 pens) received diets that were identical in composition and nutrient content. Feed was provided in 4 phases: starter (1 to 10 d), grower (11 to 32 d), finisher (33 to 42 d), and withdrawal (43 to 50d). At 32, 41, and 48 d of age, blood was collected from 4 birds per pen for subsequent analysis of plasma CK and LDH.

[2]Treatments consisted of an *ad libitum* control and 3 controlled feeding programs that received the specified proportion of the control group intake on the previous day. Each of the 4 treatments was represented by 7 replicate pens.

[3]SEM = pooled standard error of the mean.

[4] R^2_{Adj} is the adjusted coefficient of determination.

Table 3.5 Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) concentrations in male broilers affected by breast fillet myopathies at 32, 41, and 48 d of age[1]

	CK (U/mL)			LDH (U/mL)		
Myopathy[2]	32 d	41 d	48 d	32 d	41 d	48 d
Wooden Breast (WB)						
0	7.457[c]	13.916[b]	43.444	0.803[c]	1.041[b]	2.272
1	16.239[b]	41.021[a]	39.420	1.414[b]	1.354[b]	2.197
2	64.334[a]	46.603[a]	48.023	4.404[a]	3.080[a]	1.824
SEM[3]	4.554	9.192	12.392	0.370	0.306	0.776
White Striping (WS)						
0	7.591[b]	7.834[b]	44.199	0.846[b]	0.645[b]	2.235
1	14.805[b]	17.255[b]	46.170	1.274[ab]	1.242[b]	2.317
2	33.722[a]	40.588[a]	32.749	2.220[a]	2.075[a]	1.972
SEM	6.529	11.366	8.095	0.479	0.406	0.513
Source of Variation	----------Probabilities----------					
WB	< 0.001	0.002	0.79	< 0.001	< 0.001	0.85
WS	< 0.001	0.008	0.21	0.011	0.003	0.77
Logistic χ^2 P[4]						
WB	< 0.001	0.009	0.83	< 0.001	< 0.001	0.67
WS	< 0.001	< 0.001	0.20	0.012	0.002	0.62
Odds Ratios						
WB	0.911	0.975	1.00	0.355	0.353	1.00
WS	0.960	0.945	1.00	0.668	0.769	1.00
Spearman's P[5]						
WB	< 0.001	< 0.001	0.70	< 0.001	< 0.001	0.86
WS	< 0.001	< 0.001	0.10	0.001	< 0.001	0.48
Spearman's ρ						
WB	0.45	0.52	---	0.40	0.48	---
WS	0.38	0.44	---	0.30	0.43	---

[1]Broilers (25 birds per pen; 28 pens) received diets that were identical in composition and nutrient content. Feed was provided in 4 phases: starter (1 to 10 d), grower (11 to 32 d) finisher (33 to 42 d), and withdrawal (43 to 50d). At 32, 41, and 48 d of age, blood was collected from 4 birds per pen for subsequent analysis of plasma CK and LDH.

[2]At 33, 43, and 50 d of age, 4 birds per pen were euthanized for necropsy. The right breast fillet of each bird was assigned a subjective score (normal = 0; mild = 1; severe = 2) based on the incidence and severity of WB and WS.

[3]SEM = pooled standard error of the mean;

[4]The overall χ^2 P-value corresponds to the hypothesis test for overall significance of the logistic regression model. Odds ratios (OR) represent the proportional increase (OR > 1) or decrease (OR < 1) in the odds of obtaining a myopathy score of 0 vs. a score of 1 or 2 (or the odds of obtaining a score of 0 or 1 vs. a score of 2) for each U/mL increase in CK or LDH.

[5]Spearman's rank correlation coefficient (ρ) and its associated P-value provide a measure of dependence between continuous and categorical variables, such as that between CK concentration and myopathy scores.

[a-c]Means within each myopathy not sharing a common superscript within a column differ significantly ($P < 0.05$).

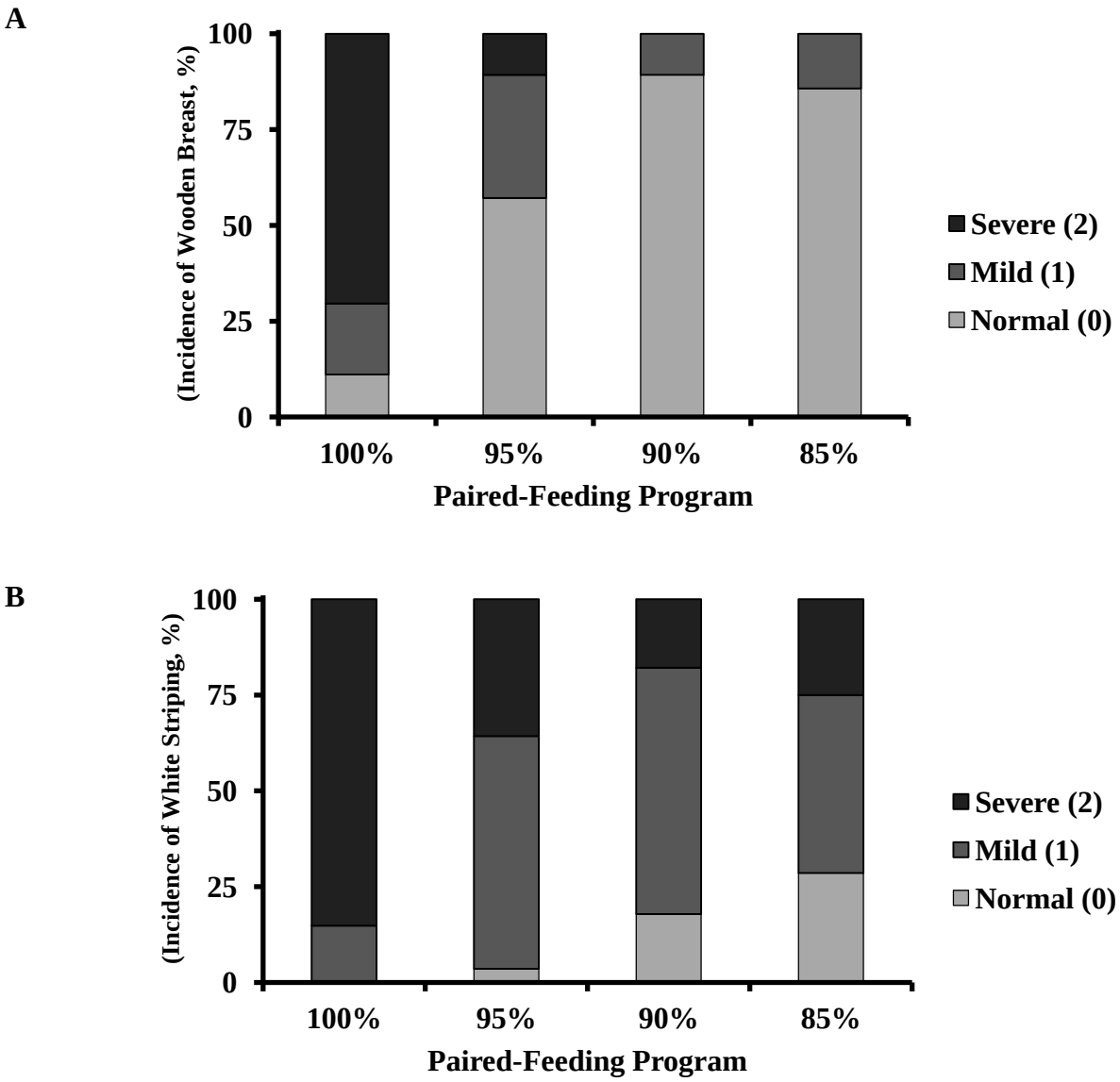

Figure 3.1 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast (A) and white striping (B) among male Yield Plus × Ross 708 broiler chickens receiving identical diets at specified percentages (100, 95, 90, and 85%) of ad libitum intake. Four birds per pen (7 pens per treatment) were euthanized for necropsy at 43 d of age and subsequently scored for the presence of wooden breast and white striping (0 = Normal; 1 = Mild, 2 = Severe).

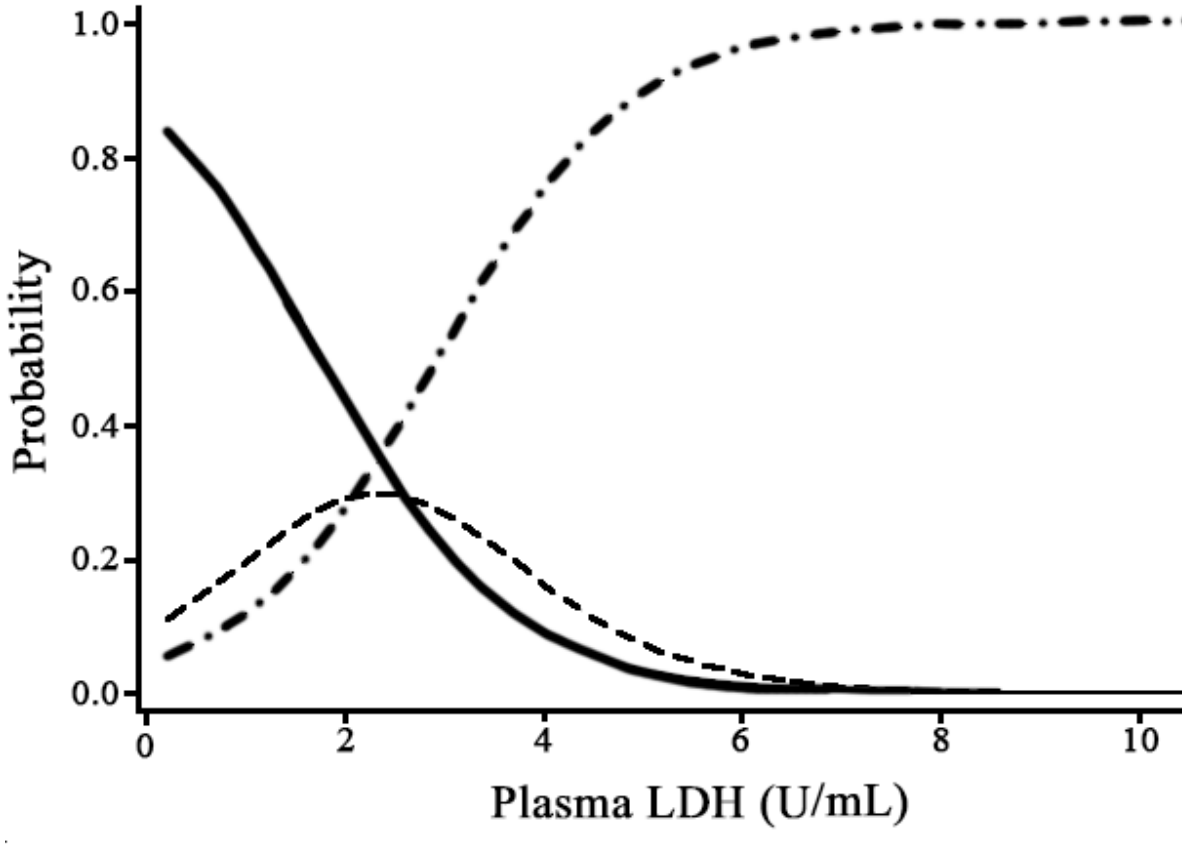

Figure 3.2 Probability of obtaining a score of 0 (Normal; solid line), 1 (Mild; dashed line), or 2 (Severe; dotted and dashed line) for wooden breast with increasing concentrations of plasma lactate dehydrogenase (LDH). Probability plot was generated using PROC LOGISTIC in SAS 9.3 to evaluate wooden breast scores and plasma LDH concentrations for a total of 112 male Yield Plus × Ross 708 broiler chickens at 32 d of age.

IV. EFFECTS OF REDUCED DIETARY ENERGY AND AMINO ACID DENSITY ON *PECTORALIS MAJOR* MYOPATHIES IN BROILER CHICKENS AT 36 AND 49 DAYS OF AGE

ABSTRACT

Two experiments were conducted to determine if reductions in the incidence and severity of WB and WS may be obtained by modulating dietary nutrient density. In each Exp, Yield Plus × Ross 708 male broiler chicks were placed into 63 pens (22 birds/pen). All birds received an identical prestarter diet until 7 d of age, after which time each pen was randomly assigned to 1 of the following 7 dietary treatments (TRT) for the starter (8 to 14 d), grower (15 to 25 d), finisher 1 (experiment 1: 26 to 35 d; Exp 2: 26 to 42 d), and withdrawal (experiment 2: 43 to 48 d) phases: 1) 100% of primary breeder recommendations for digestible AA and AME_n density throughout the experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout the experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout the experiment. At 36 d (experiment 1) and 49 d (experiment 2), 18 birds per pen were processed and evaluated for WS and WB. In Exp 1, reduced dietary density in the starter phase (TRT 2 and TRT 5) resulted in increased ($P \leq 0.05$) incidences of severe WB (32.9% and 34.7%) relative to TRT 1 (18.2%). In Exp 2, broilers assigned to TRT 7 had reduced ($P < 0.01$) incidences of severe WB (20.8%) and WS (42.3%) relative to the

control (WB: 36.5%; WS: 64.5%). In both Exp, plasma CK and LDH increased ($P \leq 0.05$) with increasing scores for WB and WS. Reducing dietary nutrient density from 8 to 14 d may exacerbate fillet myopathies in broilers reared to 35 d of age. Although reducing dietary AME_n and AA density to 90% of recommendations from 1 to 48 d reduced the severity of myopathies, these reductions occurred with substantial compromises in live performance. Altogether, these results indicated that concurrent manipulation of dietary AA and AME_n density is not a viable practical solution for reducing the incidence and severity of breast myopathies.

INTRODUCTION

Throughout the last decade, broiler producers have increasingly noted the presence of WB and WS in the PM muscles (breast fillet) of broilers. Although the macroscopic, microscopic, and meat quality characteristics of these myopathies have been well established, their underlying causes remain unknown. On the microscopic level, abnormal accumulation of connective tissue and fluid gives WB its eponymous hardness, which is physically palpable in live birds (Sihvo et al., 2014, 2017). White striping is characterized by the deposition of intramuscular fat parallel to the muscle fibers in a visible striated pattern (Kuttappan et al., 2013). These myopathies often appear concurrently in the same muscle, but can also occur independently (Kuttappan et al., 2016).

Each of these myopathies has been observed in both sexes of multiple commercially available modern broiler strains worldwide (Meloche et al., 2014; Trocino et al., 2015; Bailey, et al., 2015). Although WB and WS have been anecdotally associated with higher breast meat yield, strain comparisons have indicated relatively poor heritability and genetic correlations between these myopathies and performance characteristics (Bailey et al., 2015). Indeed, these authors determined that non-genetic factors contribute to more than 90 and 65% of the observed variance in WB and WS, respectively. Non-genetic factors, such as environment, nutrition, and management, likely

affect the development of myopathies by modulating the growth curve (Trocino et al., 2015).

Quantitative control of nutrient intake may decrease the incidence of common breast muscle myopathies with potential impairment in live performance at a given age. The paired-feeding program utilized by Meloche et al. (2015) to influence growth trajectory, and subsequently reduce the incidence of myopathies, resulted in broilers consuming approximately 93, 88, and 83% of the cumulative AME_n and AA intake of the *ad libitum* control. However, quantitative allocation strategies would have limited practical applicability in many areas of the world. For example, most broiler growers in the U.S. are not equipped to allocate feed by weight on a daily basis. Therefore, it may be beneficial to identify a means of altering the growth trajectory through reductions in nutrient allocation obtained qualitatively through the manipulation of dietary AME_n and AA density. Previous research has demonstrated reductions in the incidence of severe WS when feeding low energy diets from 1 to 54 d of age (Kuttappan et al., 2012). However, the magnitude of the reduction in dietary energy utilized by these authors also negatively impacted live performance and processing characteristics.

It is possible that these detrimental effects on performance may be mitigated by making concomitant small-magnitude changes in the densities of both AME_n and AA. Furthermore, the impact of reduced dietary density on the incidence and severity of WB has not yet been investigated. Therefore, 2 Exp were conducted to determine if similar decreases in WB and WS may be obtained by modulating dietary AA and AME_n concentrations to mimic the reductions in nutrient intake previously obtained through paired-feeding by Meloche et al. (2015).

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2015-2629).

Bird Husbandry

Two similarly designed Exp were conducted from 1 to 35 (Exp 1) and 1 to 49 d of age (Exp 2). In each Exp, one thousand three hundred eighty-six Yield Plus × Ross 708 (Aviagen Inc., Huntsville, AL) male broiler chicks were obtained from a commercial hatchery and placed into 63 floor pens (0.10 m^2/bird; 22 birds per pen) in a solid-sided house equipped with cross-ventilation system. This house was equipped with vent boards, exhaust fans, cooling pads, and an electronic controller to adjust temperature. Each pen contained a hanging feeder, a nipple drinker line, and used litter. Chicks were vaccinated at the hatchery for Marek's disease, Newcastle disease, and infectious bronchitis. Birds consumed feed and water on an *ad libitum* basis. Ambient temperature was set at 33°C at placement and was reduced to maintain comfort with advancing bird age to a final setpoint of 20°C. Birds were exposed to a 23L:1D photoperiod from placement to 7 d of age, followed by an 18L:6D photoperiod for the remainder of the Exp. Light intensity was set at 30 lux from 1 to 7 d of age, 10 lux from 8 to 14 d of age, 5 lux from 15 to 24 d of age, and 3 lux from 25 to 48 d of age. Light intensity settings were verified at bird level (30 cm) using a photometric sensor with National Institute of Standards and Technology-traceable calibration (403125, Extech Instruments, Waltham, Mass) for each intensity adjustment.

Dietary Treatments

All birds received an identical prestarter diet until 7 d of age, after which time each pen was randomly assigned to 1 of the following 7 dietary treatments (TRT; Table 4.1) for the starter (8 to 14 d), grower (15 to 25 d), finisher 1 (Exp 1: 26 to 35 d; Exp 2: 26 to 42 d), and withdrawal (Exp 2: 43 to 48 d) phases: 1) 100% of primary breeder recommendations for AA and AME_n density throughout the Exp; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout the Exp; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout the Exp. All AA concentrations were modulated concurrently, maintaining digestible AA to dLys ratios of 51, 77, 67, 78, and 71 for dMet, dTSAA, dThr, dVal, and dIle, respectively. Digestible AA values were calculated by multiplying digestibility coefficients (Ajinomoto, 2009) with the calculated total AA content of each AA-contributing ingredient. Diets were corn-soybean meal-based and contained distillers dried grains with solubles and wheat middlings as needed to obtain the desired nutrient dilutions. No ingredients of animal origin were used in formulation of experimental diets. Feed was provided in crumble form during the starter phase, and pelleted thereafter. Crude protein content of the TRT diets was calculated by multiplying percentage N by a correction factor (6.25). Nitrogen content was determined by the Dumas method (method 990.03; Association of Official Analytical Chemists **(AOAC)** International, 2006) using an N analyzer (Rapid N Cube, Elementar Analysensyteme GmbH, Hanau, Germany). Ether extract **(EE)** concentrations were estimated by boiling diet samples in hexane (method 2003.06; AOAC International,

2006) in a fat extractor (Soxtec model number 2043, Foss North America Inc., Eden Prairie, MN).

Measurements

Body weight and feed intake for each pen was determined at 7, 14, 25, and 35 days of age in Exp 1, as well as at 42 and 48 days of age in Exp 2. Mortality was recorded daily and FCR were corrected for mortality and excluded placement weights. At 33 (Exp 1) and 43 (Exp 2) d of age, 4 mL of blood was collected from the ulnar vein of 4 birds per pen using 21 gauge 25.4 mm needles and heparinized (16 IU/mL) monovette syringes. Blood samples were retained on ice until sampling was completed, then centrifuged at 4,000 × *g* for 10 min at 4°C in order to obtain 1.5 mL of plasma which was stored at −20°C until subsequent analysis for plasma CK and LDH. Analyses for CK and LDH were conducted using a Roche/Hitachi Cobas c311 (Roche Diagnostics, Indianapolis, IN) automated blood analyzer at Auburn University College of Veterinary Medicine.

At 36 (Exp 1) and 49 (Exp 2) d of age, after a feed withdrawal period of 12 h, 18 birds per pen were randomly selected for processing at the Auburn University Pilot Processing Plant. Selected birds were placed in coops, transported to the processing facility, electrically stunned, exsanguinated, scalded, picked, and manually eviscerated. Carcasses were chilled on ice for 3 hours prior to measuring carcass and abdominal fat pad weights. The front-halves of the carcasses were then packed in ice for 18 hours. The weights of the PM (boneless breast) and *Pectoralis minor* (tender) muscles were recorded after manual excision by experienced personnel from a commercial processing plant. Carcass and breast yields, as well as abdominal fat percentage, were calculated relative to BW at 35 (Exp 1) and 48 (Exp 2) d of age. The PM muscles of each bird were visually

assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for WS and WB. All fillets were scored by the same evaluator. For WS, the defect was characterized as "mild" if visible striping comprised less than half of the total fillet surface area or was less than 2 mm wide on average, but was considered "severe" if the striping exceeded these limits. For WB, the defect was considered "mild" if palpable hardness was present in less than half the total fillet surface area, but was considered "severe" if it exceeded this limit.

Statistical Analyses

Each of the 7 feeding programs was represented by 9 replicate pens arranged in a randomized complete block design with pen as the experimental unit and pen location as the blocking factor. Feeding programs with identical nutrient densities during the starter and grower phase were pooled for analysis. Treatment effects on live performance and processing characteristics were subject to analysis of variance using PROC MIXED of SAS 9.3 (SAS Institute, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu_{..} + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are the fixed factor level effects corresponding to the *j*th treatment such that $\Sigma\tau_j = 0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ.

Relationships between myopathy scores and blood chemistry measures were investigated using Spearman's rank correlation (PROC CORR; SAS Institute, 2009) and logistic regression (PROC LOGISTIC; SAS Institute, 2009). Spearman's rank correlation (**ρ**) is more appropriate for ordinal categorical variables such as myopathy scores because

it is a nonparametric measure of dependence between variables with a monotonic relationship and it does not assume a normal distribution as Pearson's correlation does for continuous variables. Likewise, logistic regression is more appropriate for categorical responses, as it utilizes maximum likelihood estimates rather than ordinary least squares. In order to accommodate a myopathy scoring system with more than 2 ordinal outcome levels, the following cumulative logit model was utilized:

$$\ln \frac{P(y_i \leq k)}{1 - P(y_i \leq k)} = \alpha_k + \boldsymbol{x_i'\beta},\ k = 0,1, \ldots, m$$

where y_i is the response variable (myopathy score), $\boldsymbol{x_i'}$ is the vector [1, x_{i1}, x_{i2}, …, x_{ij}] of j predictor variables, **β**' is the vector [β_0, β_1, β_2, …, β_l] of partial regression coefficients representing the expected change in response y per unit change in x_i when all the remaining regressor variables, x_i (i≠l) are held constant; and α_k is the unique intercept for outcome level k, where the outcomes are represented by 0,1, 2, …, m (Montgomery et al., 2012). For each fitted model, the assumptions of a binomially distributed response and proportional odds among outcome levels were met.

Proportions of affected fillets in each scoring category were analyzed by PROC GLIMMIX (SAS Institute, 2009) using the events/experiments syntax with a binomial distribution and R-side covariance structure. Where applicable, residuals were visually assessed to ensure normality and nonnormal data were transformed prior to analysis. For all hypothesis tests, statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

In each Exp, there were no differences ($P > 0.05$) among treatment groups prior to the experimental period (1 to 7 d).

Diet Analysis

Crude protein analysis can provide an approximation of overall variation in dietary AA density. Dietary CP content decreased with decreasing calculated AA density for all diets in all phases (Table 4.2). On average, analyzed CP contents of Diets 2 and 3 were 93% and 88% of the CP content of control Diet 1 in each TRT phase. Although these results are slightly lower than the designed TRT differences of 95% and 90% AA density, they nevertheless reflect an acceptable decrease in density given typical analytical variation. The lower CP values were likely due to ingredient composition, rather than formulation or manufacturing issues, as both diets differed from the planned reduction in density by 2%. Likewise, the determination of dietary AME_n content requires conducting an in vivo assay. The diets utilized in these Exp varied substantially in added fat as a result of the designed differences in calculated AME_n. Therefore, ether extract content was used as a proxy to confirm differences in overall energy density. Dietary EE content decreased with decreasing calculated AA density for all diets in all phases (Table 4.2). The reductions in dietary EE corresponded well with the decreases in added fat for each diet (Table 4.1).

Growth Performance

From 8 to 14 d of age, the experimental design resulted in 3 groups of broilers that each received an identical prestarter followed by starter diets formulated at 100% (TRT 1), 95% (TRT 2, 3, and 4), and 90% (TRT 5, 6, and 7) of primary breeder recommendations for AA and AME_n density with a total of 9, 27, and 27 replicate pens for each respective density (Table 4.3). During this time, no differences ($P > 0.05$) were observed among treatments in either Exp for BW gain. In Exp 1, broilers receiving

reduced density diets consumed more ($P < 0.001$) feed than the 100% density control. However, this compensatory intake was not sufficient to offset the formulated differences in dietary density beyond 95% of recommendations. When broilers were fed at the 90% density level, they had reduced dLys ($P < 0.001$) and AME_n ($P < 0.001$) intakes from 8 to 14 d of age. In Exp 2, broilers receiving diets formulated to 90% of recommendations likewise had reduced dLys ($P < 0.001$) and AME_n intakes ($P < 0.001$), with no differences ($P = 0.19$) in feed intake due to compensatory feeding. Although broilers are known to increase feed intake as a compensatory response to reduced dietary AME_n (Leeson et al., 1996), young broilers may have a reduced physiological capacity for increased intake (Dozier et al., 2008).

At 14 d of age, TRT 2 and 5 returned to 100% of recommended AA and AME_n concentrations for the duration of each Exp, resulting in 5 different grower feeding programs (Table 4.4). In Exp 1, broilers that returned from a reduced density diet to the 100% density diet (TRT 2 and 5) from 8 to 25 d had similar ($P > 0.05$) BW gain to the control group, whereas broilers continuing on the 95% (TRT 3 and 4) and 90% (6 and 7) diets had reduced ($P = 0.002$) BW gain in comparison to TRT 2 and 5. Feed intake increased ($P < 0.001$) proportionally as dietary density decreased, with broilers receiving the 100% control consuming the least (1.596 kg/bird) feed and the broilers receiving the 90% density diets from 8 to 25 days of age consuming the most (1.706 kg/bird) feed. Increased feed intake among the intermediate density treatments was sufficient to overcome the differences in dietary density, resulting in similar ($P > 0.05$) dLys and AME_n intakes relative to the control. Broilers receiving the 90% density diets from 8 to 25 days of age (TRT 6 and 7) consumed less ($P < 0.001$) dLys (16.8 vs. 17.6 g/bird) and

AME_n (4,779 vs. 4,974 kcal/bird) than the control, despite their increased intake. Additionally, broilers on reduced density diets throughout the starter and grower (TRT 3, 4, 6, and 7) had increased ($P < 0.001$) FCR relative to the control. In contrast, reduced dietary density in the starter alone (TRT 2 and 5) resulted in similar ($P > 0.05$) FCR to that of the control.

In Exp 2, no BWG differences ($P = 0.06$) were observed from 8 to 25 d of age. Feed consumption of broilers receiving reduced density diets was similar to that of the control. Because their intake was similar to that of the 100% control, broilers consuming the 90% density diets from 8 to 25 days of age (TRT 6 and 7) consumed less ($P < 0.001$) dLys (16.8 vs. 18.0 g/bird) and AME_n (4,822 vs. 5,150 kcal/bird) than the control. Reduced dietary density throughout the starter and grower resulted in increased ($P < 0.001$) FCR, similar to the results observed in Exp 1. In Exp 2, broilers receiving reduced density diets in the starter only (TRT 2 and 5) likewise had similar ($P > 0.05$) FCR to that of the control, as observed in Exp 1.

At 26 d of age, TRT 3 and 6 returned to 100% of recommended AA and AME_n density for the duration of each Exp, resulting in 7 different finisher feeding programs represented by 9 replicate pens per program (Table 4.5). No differences ($P > 0.05$) in BW gain were observed in either Exp from 1 to 35 d of age. In Exp 1, broilers assigned to TRT 2, 3, 4, and 5 did not ($P > 0.05$) consume significantly more feed than the control. However, the mild reduction in dietary density for these programs, as well as numerical increases in intake, nevertheless allowed these birds to achieve similar ($P > 0.05$) dLys and AME_n intakes relative to the control. Broilers receiving diets formulated at 90% of recommended density through the grower or finisher phases (TRT 6 and 7) consumed

more (P < 0.001) feed from 1 to 35 days of age than those receiving the 100% control. The return of TRT 6 to 100% of recommended density from 26 to 35 d of age enabled these birds to consume a similar ($P > 0.05$) quantity of dLys and AME_n relative to the control. In contrast, broilers assigned to TRT 7 consumed less ($P < 0.001$) dLys (33.7 vs. 34.9 g/bird) and AME_n (10,167 vs. 10,546 kcal/bird) than the control, despite their increased intake.

These differences in nutrient allocation for TRT 7 correspond to an approximately 3.5% decrease in actual nutrient intake, as opposed to the 10% decrease in formulated dietary density compared to the control diet. Broilers assigned to TRT 7 had increased ($P < 0.001$) FCR relative to the control. All other programs resulted in similar FCR relative to the control, with the lowest FCR observed for those broilers receiving reduced density diets in the starter phase only (TRT 2 and 5). This result may indicate that feeding reduced density diets for short periods of time may stimulate subsequent compensatory intake and improved nutrient utilization.

In Exp 2, only TRT 6 consumed increased ($P = 0.008$) feed relative to the control from 1 to 35 d of age. Because broilers receiving diets formulated at 95% (TRT 4) and 90% (TRT 7) of the control from 1 to 35 d of age did not ($P > 0.05$) have increased feed consumption, their intakes of dLys (34.8 and 33.7 g/bird) and AME_n (10,572 and 10,247 kcal/bird) were lower ($P < 0.001$) than that of the control (35.8 g dLys/bird and 10,880 kcal AME_n/ bird). These differences represent 2.8% and 5.9% reductions in dietary density for TRT 4 and 7, respectively. Although the difference in feed intake between these treatments was not significantly different, it was nevertheless sufficient to partially compensate for the designed 5% and 10% reductions in nutrient density at 35 d of age.

Reduced nutrient density at 95% (TRT 4) and 90% (TRT 7) of the control from 1 to 35 d of age or at 90% of the control from 1 to 26 d and 100% of the control thereafter (TRT 6) resulted in increased ($P < 0.001$) FCR relative to the control.

Broilers from Exp 2 continued to receive diets according to the 7 feeding programs until 48 d of age. At 42 d of age, no differences ($P > 0.05$) in BW gain existed among the 7 feeding programs (Table 4.6). Broilers assigned to TRT 7 had increased ($P < 0.001$) feed intake relative to the control. This compensatory intake was sufficient to obviate the designed differences in dLys intake or AME_n intake between the reduced density treatments and the control. However, those birds which engaged in compensatory feeding did have higher ($P < 0.001$) FCR compared with those receiving the recommended dietary density. Similar results occurred at 48 d, with broilers assigned to TRT 7 consuming more ($P = 0.008$) feed relative to the control, eliminating differences in dLys and AME_n intake at the cost of increased ($P < 0.001$) FCR (Table 4.7).

It is well established that broilers may adapt to diets of varying density by increasing feed intake to meet their AME_n or AA requirements, with older broilers responding markedly to dietary AA density (Dozier et al., 2007). Compensatory gain achieved through increased intake of low energy diets occurs less efficiently (Leeson et al., 1996; Dozier et al., 2007; Butzen et al., 2013), as demonstrated by the increased FCR of broilers receiving reduced density feed in the current study. Although it was expected that a 10% reduction in dietary density would be sufficient to offset any compensatory intake, these data suggest that a selection which includes a variety of challenging growing conditions has substantially enhanced the modern broiler's ability to modulate its voluntary feed intake in response to diets with reduced nutrient density.

Carcass Characteristics

No treatment differences ($P > 0.05$) in carcass weights or yields were observed relative to the control, although broilers receiving diets formulated to 95% of the recommended density for the entirety of the experimental feeding period (TRT 4) had reduced ($P = 0.001$) carcass weights in comparison with those receiving diets formulated at 95% or 90% of the recommended density for the starter period only (TRT 2 and 5; Table 4.8). Abdominal fat weights (18.8 g) and percentages (0.81%) were reduced ($P < 0.001$) relative to the control (22.8 g, 0.98%) when feeding diets formulated at 90% of the recommended density throughout the Exp period (TRT 7). Reduced deposition of abdominal fat in response to reduced dietary energy intake, as observed for broilers assigned to TRT 7, has been well established (Leeson et al., 1996; Dozier et al., 2007) .There were no treatment differences ($P > 0.05$) in breast weights relative to the control, however, broilers receiving the lowest density diets (TRT 7) had increased ($P = 0.030$) breast yield relative to the control. Insufficient dietary Lys will limit breast muscle protein accretion (Tesseraud et al., 1996; 2001). However, the lack of differences in breast weight between broilers assigned to TRT 7 and the 100% density control indicates that these birds were not deficient in their overall intake of dLys. In Exp 2, there were no observed differences ($P > 0.05$) in carcass weights or yields. However, broilers receiving diets formulated at 90% in the starter (TRT 5) had reduced ($P<0.001$) abdominal fat weights and percentages relative to the other 6 treatments. This group of birds also achieved similar breast weights as those receiving the 100% diet, but had increased ($P = 0.019$) breast yields relative to the control.

Myopathy Scoring

In Exp 1, compensatory feeding in Exp 1 largely eliminated the desired treatment differences in AA and AME_n intake, with the exception of TRT 7. As may be expected, there was no reduction in the incidence of any given scoring category for WB (Figure 4.1A) or WS (Figure 4.2A) relative to the control. Interestingly, broilers receiving reduced density diets in the starter alone (TRT 2 and 5) had increased ($P = 0.05$) incidences of severe WB relative to the control. Broilers assigned to these treatments had numerically greater dLys and AME_n intake, as well as numerically lower FCR relative to the control. Trocino et al. (2015) observed an increase in the occurrence of WS and no differences in WB among broilers pair-fed at 80% of *ad libitum* intake from 13 to 21 d of age. These authors suggested that compensatory growth during the post-restriction refeeding period may have resulted in an acceleration of breast muscle accretion, exacerbating the severity of myopathy. Reduced nutrient intake early in life has also been demonstrated to impact the expression of adipogenic genes, resulting in increased deposition of fat within the breast muscle (Velleman et al., 2014). Similar patterns in performance and myopathy incidence in the current study further support the existence of a causal link between compensatory growth acceleration and the presence of myopathies.

In Exp 2, broilers fed at 90% of the recommended dietary density (TRT 7) from 1 to 48 d had reduced incidence of severe WB ($P = 0.002$; Figure 4.1B) and WS ($P = 0.007$; Figure 4.2B) relative to the control. Although broilers assigned to TRT 7 engaged in compensatory feed intake sufficient to overcome the 10% reduction in dietary dLys and AME_n density and obtained a final weight similar to the control, they did so at the cost of increased FCR. It is likely that the reduction in growth efficiency for broilers on

TRT 7 limited the rate of breast muscle accretion during portions of the growth trajectory critical to the impediment of myopathies.

Kuttappan et al. (2012) achieved a reduction in the incidence of severe WS (1.46% vs. 8.70%) by feeding broilers a low energy vs. a high energy diet from 1 to 54 d of age. Although feed intake was not reported, these authors nevertheless observed reduced BW gain and increased FCR for the broilers receiving low energy diets, indicating insufficient compensation for reduced energy density. The low energy diets utilized by Kuttappan et al. (2012) were higher in AME_n (3,002; 3,025; and 3,063 kcal/kg) than the 90% density diets (2,723; 2,835; 2,880; and 2,903 kcal/kg) used in the current study. Furthermore, the low energy diet fed in the starter (0 to 18 d) phase by Kuttappan et al. (2012) was similar in energy content to the highest density starter (8 to 14 d) diet in the current study (3,003 vs. 3,025 kcal/kg). These differences in formulation may account for the more pronounced effects of qualitative nutrient allocation in the current study, which reduced the incidence of severe WS by 22.2 percentage points.

Blood Chemistry

Feeding program did not affect concentrations of CK or LDH in either Exp (Table 4.9). Spearman's correlation indicated a highly significant ($P < 0.001$), although moderately strong relationship between both CK and LDH with each myopathy in both Exp (Table 4.10). Furthermore, in each Exp there was also a logistic relationship ($P < 0.001$) between these blood measures and the severity of scores for WB and WS, with the 95% confidence intervals for the odds ratios indicating that, for example, each 1 unit/mL increase in LDH corresponds to a 40 to 60% decrease in the probability of obtaining a normal score for WB at 43 d of age. Similar to results from previous Exp, the odds ratios

indicate that LDH may have greater value as a predictor of myopathy (Meloche et al., 2015). This may be attributable to the inherent increase in CK concentrations observed even with normal muscle growth (Mitchell and Sandercock, 1994), which may obscure the impact of muscle damage in a rapidly growing broiler.

In conclusion, these results illustrate the concept that the development of myopathies is not necessarily dependent on final BW or even the absolute size of the breast muscles. Rather, myopathies appear to be more directly related to the slope of the growth trajectory for each individual bird during critical windows of its development to a given final BW. In each of the current Exp, no differences in final BW were observed among treatments. And yet, feeding program nevertheless resulted in a differential impact on the incidence of severe WB at 36 and 49 d of age. In Exp 1, TRT 2 and 5 triggered an acceleration of the growth curve due to compensatory intake in the refeeding period after reduced nutrient allocation in the starter, exacerbating the severity of WB at 35 d. In contrast, reduced nutrient allocation at 90% density throughout the rearing period resulted in less efficient growth and consequently reduced the severity of WB at 49 d. Therefore, qualitative nutrient allocation programs require further evaluation to identify the appropriate intensity and timing to reduce the incidence of breast myopathies while maintaining optimal performance. In order to develop practical interventions, continued research to determine the underlying mechanisms of these myopathies is warranted.

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Table 4.1 Ingredient composition of diets fed to male Yield Plus × Ross 708 broilers from 1 to 35 and 1 to 48 d of age

	Pre-Starter	Starter		Grower			Finisher 1			Finisher 2		
Experiment 1 (d)	1 to 7	8 to 14		15 to 25			26 to 35			---		
Experiment 2 (d)							26 to 42			43 to 48		
		Dietary Density (%)[1]										
Ingredient (%)		100	95	90	100	95	90	100	95	90	100	95	90
Corn	46.49	51.12	56.99	46.07	55.59	61.56	55.10	60.71	66.07	59.24	61.89	67.24	59.59
Soybean Meal	39.86	33.98	31.01	34.63	28.15	25.17	24.84	23.26	20.95	20.88	19.43	17.05	17.75
Distillers Grains	5.00	7.00	7.00	7.00	8.00	8.00	8.00	8.00	8.00	8.00	11.00	11.00	11.00
Wheat Middlings	---	---	---	8.00	---	---	8.00	---	---	8.00	---	---	8.00
Corn Oil	4.00	3.22	0.30	---	4.11	1.78	---	4.05	1.06	---	3.94	0.95	---
Dicalcium Phosphate	2.12	2.13	2.15	2.03	1.76	1.12	1.70	1.67	1.68	1.60	1.49	1.51	1.42
Calcium Carbonate	1.20	1.25	1.26	1.27	1.11	1.09	1.15	1.07	1.08	1.10	1.07	1.08	1.10
Sodium Chloride	0.42	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.42	0.40	0.40	0.40
DL-Methionine	0.35	0.31	0.29	0.23	0.26	0.24	0.22	0.24	0.21	0.20	0.20	0.18	0.15
L-Lys·HCl	0.17	0.17	0.18	0.01	0.21	0.23	0.18	0.21	0.21	0.17	0.23	0.24	0.18
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Thr	0.07	0.09	0.06	---	0.07	0.07	0.06	0.07	0.06	0.06	0.06	0.06	0.06
Choline Chloride (60%)	0.06	0.06	0.10	0.09	0.07	0.08	0.09	0.07	0.08	0.08	0.09	0.10	0.10
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	---	---	---	---	---	---

[1] Diets were formulated to the specified percentage of primary breeder recommendations for amino acids and apparent metabolizable energy.
[2]Vitamin premix provided the following per kilogram of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopheryl acetate), 66 IU; Vitamin B12 (cyanocobalamin), 0.03 mg; D-biotin (biotin), 0.18 mg; menadione (menadione sodium bisulfate complex), 4 mg; thiamine (thiamine mononitrate), 5.5 mg; riboflavin (riboflavin), 22 mg; D-pantothenic acid (calcium pantothenate), 31 mg; pyridoxine (pyridoxine hydrochloride), 7.7 mg; niacin (niacinamide), 88 mg; folacin (folic acid), 2.6 mg.
[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite), 0.3 mg.

Table 4.2 Nutrient content of diets fed to male Yield Plus × Ross 708 broilers from 1 to 35 (experiment 1) and 1 to 48 (experiment 2) d of age

	Pre-Starter	Starter		Grower			Finisher 1			Finisher 2		
Experiment 1	1 to 7 d	8 to 14 d		15 to 25 d			26 to 35 d			---		
Experiment 2							26 to 42 d			43 to 48 d		
							Dietary Density (%)[1]					
Calculated[2]	100	95	90	100	95	90	100	95	90	100	95	90
AME_n (kcal/kg)	3,025	3,025	2,874	2,723	3,150	2,993	2,835	3,200	3,040	2,880	3,225	3,064	2,903
CP	24.7	22.9	22.0	21.5	20.8	19.9	20.2	18.9	18.3	18.6	18.02	17.4	18.0
dLys	1.31	1.18	1.12	1.06	1.07	1.02	0.96	0.95	0.90	0.86	0.89	0.85	0.80
dMet	0.67	0.61	0.58	0.53	0.54	0.51	0.48	0.49	0.46	0.44	0.45	0.42	0.39
dTSAA	0.97	0.90	0.86	0.81	0.81	0.77	0.73	0.74	0.70	0.67	0.69	0.66	0.62
dThr	0.85	0.78	0.74	0.72	0.71	0.67	0.64	0.64	0.61	0.59	0.60	0.57	0.56
dVal	0.98	0.90	0.86	0.90	0.81	0.77	0.74	0.73	0.70	0.68	0.69	0.66	0.65
dIle	0.93	0.84	0.80	0.84	0.75	0.70	0.68	0.66	0.63	0.61	0.62	0.58	0.58
dArg	1.47	1.32	1.24	1.32	1.16	1.08	1.05	1.02	0.96	0.94	0.92	0.87	0.86
dTrp	0.25	0.21	0.23	0.23	0.20	0.19	0.18	0.17	0.16	0.16	0.16	0.15	0.15
Ca	1.05	1.05	1.05	1.05	0.90	0.90	0.90	0.85	0.85	0.85	0.80	0.80	0.80
Available P	0.53	0.53	0.53	0.53	0.45	0.45	0.45	0.43	0.43	0.43	0.40	0.40	0.40
Na	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline (ppm)	1,700	1,700	1,700	1,700	1,500	1,500	1,500	1,400	1,400	1,400	1,400	1,400	1,400
Analyzed[2]													
	---	23.8	21.8	21.2	23.1	21.3	20.4	20.7	19.9	18.2	19.1	17.7	17.1
	---	5.1	3.1	2.6	4.4	4.0	2.8	6.0	4.1	2.9	6.6	4.0	3.3

[1] Diets were formulated to the specified percentage of primary breeder recommendations for amino acids and apparent metabolizable energy.

[2]Analyzed composition Crude protein content of the treatment diets was calculated by multiplying percentage N [Dumas method (method 990.03; Association of Official Analytical Chemists (AOAC) International, 2006) using an N analyzer (Rapid N Cube, Elementar Analysensyteme GmbH, Hanau, Germany)] by a correction factor (6.25). Ether extract concentrations were estimated by boiling diet samples in hexane (method 2003.06; AOAC International, 2006) in a fat extractor (Soxtec model number 2043, Foss North America Inc., Eden Prairie, MN).

Table 4.3 Effect of reduced dietary energy and amino acid density on the growth performance of male Yield Plus × Ross 708 broilers from 8 to 14 d of age[1]

Feeding Program[2]		BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg/kg)	Mortality (%)
Density (%)[3]					Exp 1[4]			
1)	100	0.539	0.332	0.437[b]	5.2[a]	1,322[a]	1.321[b]	0.0
2)								
3)	95	0.553	0.340	0.451[a]	5.1[a]	1,297[a]	1.326[ab]	0.2
4)								
5)								
6)	90	0.549	0.337	0.454[a]	4.8[b]	1,237[b]	1.352[a]	0.0
7)								
	SEM[5]	0.553	0.340	0.451[a]	5.1[a]	1,297[a]	1.326[ab]	0.2
					Exp 2[4]			
1)	100	0.477	0.282	0.361	4.3[a]	1,091[a]	1.281	0.0
2)								
3)	95	0.483	0.284	0.371	4.2[a]	1,065[a]	1.307	0.2
4)								
5)								
6)	90	0.477	0.280	0.369	3.9[b]	1,005[b]	1.316	0.3
7)								
	SEM[5]	0.0066	0.0053	0.0082	0.06	16.6	0.0243	0.33
		---------- Probabilities ----------						
Exp. 1		0.06	0.10	< 0.001	< 0.001	< 0.001	0.026	0.52
Exp. 2		0.42	0.43	0.37	< 0.001	< 0.001	0.33	0.63

[1]Values are least-square means separated using Tukey's Honestly Significant Difference test. Feeding programs with identical densities from 1 to 14 d were pooled for analysis, resulting in 9, 27, and 27 replicate pens (22 birds/pen) for the 100, 95, and 90% density levels, respectively.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT) :1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Starter diets were formulated to the specified density relative to primary breeder recommendations for AA and AME_n density.

[4]Exp = Experiment; Experiment 1 = 1 to 35 d of age; Experiment 2 = 1 to 49 d of age.

[5]SEM = pooled standard error of the mean.

[a-b]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.4 Effect of reduced dietary energy and amino acid density on the growth performance of male Yield Plus × Ross 708 broilers from 8 to 25 d of age[1]

Feeding Program[2]	Density (%)[3]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg/kg)	Mortality (%)
		Exp 1[4]						
1)	100, 100	1.369^{abc}	1.161^{ab}	1.596^{c}	17.6^{a}	4,974^{a}	1.377^{c}	0.0
2)	95, 100	1.407^{ab}	1.192^{a}	1.633^{bc}	17.7^{a}	5,020^{a}	1.370^{c}	0.0
3) 4)	95, 95	1.365^{bc}	1.151^{b}	1.659^{b}	17.3^{a}	4,895^{ab}	1.442^{b}	1.3
5)	90, 100	1.415^{a}	1.199^{a}	1.663^{ab}	17.7^{a}	5,034^{a}	1.388^{c}	0.5
6) 7)	90, 90	1.359^{c}	1.147^{b}	1.706^{a}	16.8^{b}	4,779^{b}	1.489^{a}	0.5
	SEM[5]	0.0136	0.0126	0.0141	0.14	41.4	0.014	1.0
		Experiment 2[4]						
1)	100, 100	1.378	1.222	1.649^{ab}	18.0^{a}	5,150^{a}	1.390^{c}	1.0
2)	95, 100	1.363	1.208	1.650^{ab}	17.8^{a}	5,097^{a}	1.414^{c}	0.5
3) 4)	95, 95	1.336	1.176	1.690^{a}	17.6^{a}	5,014^{a}	1.484^{b}	1.8
5)	90, 100	1.333	1.177	1.614^{b}	17.2^{ab}	4,930^{ab}	1.438^{c}	1.5
6) 7)	90, 90	1.333	1.175	1.716^{a}	16.8^{b}	4,822^{b}	1.528^{a}	1.3
	SEM	0.0161	0.0150	0.0210	0.22	62.6	0.0134	0.9
		Probabilities						
Exp 1		0.003	0.002	< 0.001	< 0.001	< 0.001	< 0.001	0.88
Exp 2		0.09	0.06	0.002	< 0.001	< 0.001	< 0.001	0.83

[1]Values are least-square means separated using Tukey's Honestly Significant Difference test. Feeding programs with identical densities from 1 to 24 d were pooled for analysis, resulting in 9, 9, 18, 9, and 18 replicate pens (22 birds/pen) for TRT 1, 2, 3 and 4, 5, or 6 and 7, respectively. SEM = pooled standard error of the mean.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT) 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower) relative to primary breeder recommendations for AA and AME_n density. Ideal amino acid ratios to digestible Lys (dLys) were maintained.

[4] Exp = Experiment; Experiment 1 = 1 to 35 d of age; Experiment 2 = 1 to 49 d of age.

[a-c]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.5 Effect of reduced dietary energy and amino acid density on the growth performance of male Yield Plus × Ross 708 broilers from 1 to 35 d of age[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg/kg)	Mortality (%)
Density (%)[3]				Experiment 1[4]			
1) 100, 100, 100	2.270	2.229	3.367^{c}	34.9^{ab}	10,546^{ab}	1.514^{bcd}	0.0
2) 95, 100, 100	2.316	2.275	3.401^{bc}	35.2^{a}	10,643^{a}	1.495^{d}	0.5
3) 95, 95, 100	2.308	2.266	3.443^{bc}	35.0^{ab}	10,554^{ab}	1.513^{bcd}	2.0
4) 95, 95, 95	2.234	2.193	3.417^{bc}	33.9^{bc}	10,199^{bc}	1.555^{abc}	1.0
5) 90, 100, 100	2.339	2.297	3.460^{abc}	35.3^{a}	10,679^{a}	1.505^{cd}	1.5
6) 90, 90, 100	2.286	2.243	3.525^{ab}	34.7^{abc}	10,507^{abc}	1.565^{ab}	1.5
7) 90, 90, 90	2.279	2.237	3.584^{a}	33.7^{c}	10,167^{c}	1.602^{a}	0.0
SEM[5]	0.0247	0.0338	0.0457	0.39	121.2	0.0190	1.02
				Experiment 2[4]			
1) 100, 100, 100	2.352	2.313	3.450^{b}	35.8^{a}	10,880^{a}	1.492^{c}	3.3
2) 95, 100, 100	2.359	2.319	3.472^{ab}	35.8^{a}	10,895^{a}	1.497^{c}	2.3
3) 95, 95, 100	2.346	2.306	3.530^{ab}	35.8^{a}	10,864^{a}	1.533^{abc}	4.3
4) 95, 95, 95	2.294	2.253	3.519^{ab}	34.8^{b}	10,572^{b}	1.562^{ab}	3.3
5) 90, 100, 100	2.331	2.291	3.449^{b}	35.3^{a}	10,769^{a}	1.505^{bc}	3.3
6) 90, 90, 100	2.344	2.304	3.597^{a}	35.4^{a}	10,803^{a}	1.561^{ab}	3.3
7) 90, 90, 90	2.284	2.244	3.590^{ab}	33.7^{b}	10,247^{b}	1.600^{a}	2.8
SEM	0.0330	0.0330	0.0479	0.48	146.5	0.0170	1.73
	---------- Probabilities ----------						
Exp 1	0.07	0.08	< 0.001	< 0.001	< 0.001	< 0.001	0.31
Exp 2	0.17	0.16	0.008	< 0.001	< 0.001	< 0.001	0.96

[1]Values are least-square means of 9 replicate pens (22 birds/pen) separated using Tukey's Honestly Significant Difference test. SEM = pooled standard error of the mean.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower, finisher) relative to primary breeder recommendations for AA and AME_n density. Ideal amino acid ratios to digestible Lys (dLys) were maintained.

[4]Exp = Experiment; Experiment 1 = 1 to 35 d of age; Experiment 2 = 1 to 49 d of age.

[a-d]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.6 Effect of reduced dietary energy and amino acid density on the growth performance of male Yield Plus × Ross 708 broilers from 1 to 42 d of age (Exp 2)[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg/kg)	Mortality (%)
Density (%)[3]							
1) 100, 100, 100	3.162	3.123	4.938^{b}	49.9^{ab}	15,679^{ab}	1.582^{b}	3.2
2) 95, 100, 100	3.183	3.143	4.983^{b}	50.1^{a}	15,769^{a}	1.586^{b}	2.8
3) 95, 95, 100	3.171	3.130	5.018^{b}	49.9^{ab}	15,662^{ab}	1.605^{b}	6.3
4) 95, 95, 95	3.124	3.084	5.076^{ab}	48.8^{ab}	15,342^{ab}	1.646^{ab}	4.3
5) 90, 100, 100	3.140	3.101	4.904^{b}	49.1^{ab}	15,462^{ab}	1.582^{b}	4.8
6) 90, 90, 100	3.176	3.136	5.085^{ab}	49.5^{ab}	15,599^{ab}	1.622^{b}	4.3
7) 90, 90, 90	3.132	3.092	5.248^{a}	48.0^{b}	15,062^{b}	1.698^{a}	2.8
SEM	0.0424	0.0424	0.0669	0.65	206.1	0.0215	2.61
	---------- Probabilities ----------						
Feeding Program	0.73	0.73	< 0.001	0.024	0.020	< 0.001	0.83

[1]Exp = Experiment. Values are least-square means of 9 replicate pens (22 birds/pen) separated using Tukey's Honestly Significant Difference test. SEM = pooled standard error of the mean.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower, finisher, withdrawal) relative to primary breeder recommendations for AA and AME_n density. Ideal amino acid ratios to digestible Lys (dLys) were maintained.

[a-b]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.7 Effect of reduced dietary energy and amino acid density on the growth performance of male Yield Plus × Ross 708 broilers from 1 to 48 d of age (Exp 2)[1]

	Feeding Program[2] Density (%)[3]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg/kg)	Mortality (%)
1)	100, 100, 100, 100	3.792	3.752	6.382[b]	62.1	20,047	1.675[c]	4.3
2)	95, 100, 100, 100	3.827	3.787	6.432[b]	62.5	20,231	1.684[c]	3.3
3)	95, 95, 100, 100	3.766	3.726	6.558[ab]	61.8	20,054	1.715[bc]	6.8
4)	95, 95, 95, 95	3.777	3.736	6.676[ab]	61.1	19,828	1.749[ab]	5.8
5)	90, 100, 100, 100	3.772	3.732	6.435[b]	61.6	19,987	1.701[bc]	5.3
6)	90, 90, 100, 100	3.798	3.578	6.607[ab]	62.0	20,144	1.730[bc]	4.8
7)	90, 90, 90, 90	3.789	3.749	6.832[a]	60.1	19,432	1.806[a]	3.3
	SEM	0.048	0.0484	0.1247	0.83	273.5	0.0204	2.67
		---------- Probabilities ----------						
	Feeding Program	0.91	0.90	0.008	0.11	0.10	< 0.001	0.95

[1]Exp = Experiment. Values are least-square means of 9 replicate pens (22 birds/pen) separated using Tukey's Honestly Significant Difference test. SEM = pooled standard error of the mean.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower, finisher, withdrawal) relative to primary breeder recommendations for AA and AME_n density. Ideal amino acid ratios to digestible Lys (dLys) were maintained.

[a-b]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4 8. Effect of reduced dietary energy and amino acid density on abdominal fat percentage and carcass characteristics of male Yield Plus × Ross 708 broilers at 36 (Exp 1) and 49 (Exp 2) d of age[1]

Feeding Program[2]	Abdominal Fat (g)	Abdominal Fat (%)	Carcass (kg)	Carcass (%)	Breast (kg)	Breast (%)
Density (%)[3]			Exp 1 – 35 d			
1) 100, 100, 100	22.8^{ab}	0.98^{ab}	1.645^{ab}	70.8	0.551^{ab}	23.72^{b}
2) 95, 100, 100	22.2^{ab}	0.94^{ab}	1.673^{a}	71.0	0.565^{ab}	23.91^{ab}
3) 95, 95, 100	22.6^{ab}	0.97^{ab}	1.646^{ab}	70.5	0.560^{ab}	23.96^{ab}
4) 95, 95, 95	21.5^{ab}	0.95^{ab}	1.609^{b}	71.0	0.544^{b}	23.99^{ab}
5) 90, 100, 100	23.4^{a}	0.98^{a}	1.678^{a}	70.7	0.567^{a}	23.89^{ab}
6) 90, 90, 100	20.6^{bc}	0.89^{bc}	1.649^{ab}	71.0	0.561^{ab}	24.14^{ab}
7) 90, 90, 90	18.8^{c}	0.81^{c}	1.644^{ab}	70.7	0.567^{a}	24.34^{a}
SEM	0.08	0.032	0.0163	0.19	0.0074	0.019
			Exp 2 – 49 d			
1) 100, 100, 100, 100	49.0^{a}	1.26^{a}	2.913	75.1	1.025^{ab}	26.43^{b}
2) 95, 100, 100, 100	48.9^{a}	1.27^{a}	2.891	75.1	1.026^{ab}	26.65^{b}
3) 95, 95, 100, 100	51.5^{a}	1.33^{a}	2.918	75.0	1.027^{ab}	26.39^{b}
4) 95, 95, 95, 95	51.8^{a}	1.35^{a}	2.871	74.9	1.009^{b}	26.35^{b}
5) 90, 100, 100, 100	39.1^{b}	1.01^{b}	2.913	75.4	1.054^{a}	27.36^{a}
6) 90, 90, 100, 100	52.2^{a}	1.36^{a}	2.887	75.1	1.019^{ab}	26.46^{b}
7) 90, 90, 90, 90	49.3^{a}	1.27^{a}	2.912	75.0	1.030^{ab}	26.58^{b}
SEM	1.62	0.041	0.0272	0.23	0.0123	0.218
			Probabilities			
Exp 1	< 0.001	< 0.001	0.001	0.06	0.007	0.030
Exp 2	< 0.001	< 0.001	0.52	0.38	0.019	< 0.001

[1]Exp = Experiment. Values are least-square means of 9 replicate pens with 18 birds/pen randomly selected at 35 (Exp1) and 49 (Exp 2) d of age. Means were separated using Tukey's Honestly Significant Difference test. SEM = pooled standard error of the mean

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower, finisher, withdrawal) relative to primary breeder recommendations for AA and AME_n density.

[a-b]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.9 Effect of reduced dietary energy and amino acid density on plasma creatine kinase (CK) and lactate dehydrogenase (LDH) concentrations in male Yield Plus × Ross 708 broilers at 33 (Exp 1) and 43 (Exp 2) d of age[1]

Feeding Program[2]	Density (%)[3]	CK (U/mL)	LDH (U/mL)
		Exp1 – 35 d	
1)	100, 100, 100	16.68	1.160
2)	95, 100, 100	19.02	1.661
3)	95, 95, 100	25.89	1.921
4)	95, 95, 95	18.22	1.486
5)	90, 100, 100	21.63	1.486
6)	90, 90, 100	15.99	1.133
7)	90, 90, 90	20.35	1.651
	SEM[4]	2.946	0.2083
		Exp 2 – 43 d	
1)	100, 100, 100, 100	43.49	2.332
2)	95, 100, 100, 100	38.98	2.396
3)	95, 95, 100, 100	44.03	2.629
4)	95, 95, 95, 95	47.89	2.700
5)	90, 100, 100, 100	53.24	2.916
6)	90, 90, 100, 100	47.29	2.403
7)	90, 90, 90, 90	45.25	2.430
	SEM[4]	5.891	0.3538
		---------- Probabilities ----------	
Exp 1		0.22	0.08
Exp 2		0.73	0.89

[1]Exp = Experiment. Values are least-square means of 9 replicate pens with 4 birds/pen randomly selected for blood collection at 33 (Experiment 1) and 43 (Experiment 2) d of age. Means were separated using Tukey's Honestly Significant Difference test. SEM = pooled standard error of the mean.

[2]Birds were fed an identical prestarter diet until 7 d of age. At 8 d, each pen (22 birds /pen) was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid (AA) density and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

[3]Diets were formulated to the specified density (starter, grower, finisher, withdrawal) relative to primary breeder recommendations for AA and AME_n density. Ideal amino acid ratios to digestible Lys (dLys) were maintained.

[a-b]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.10 Plasma creatine kinase (CK) and lactate dehydrogenase (LDH) concentrations in male Yield Plus × Ross 708 broilers affected by wooden breast (WB) and white striping (WS) at 33 (Exp1) and 43 (Exp 2) d of age[1]

	33 d			43 d	
Myopathy[2]		CK (U/mL)	LDH (U/mL)		CK (U/mL)	LDH (U/mL)
WB	n			n		
0	115	15.25[b]	1.218[b]	66	28.29[b]	1.582[b]
1	71	19.46[b]	1.538[b]	61	41.18[b]	2.434[a]
2	47	32.79[a]	2.363[a]	109	55.47[a]	3.104[a]
SEM		2.484	0.1786		4.109	0.2577
WS	n			n		
0	37	21.47	1.658[ab]	21	36.54[b]	2.229[ab]
1	126	20.48	1.507[b]	115	36.31[b]	2.058[b]
2	70	25.56	1.956[a]	100	52.10[a]	2.832[a]
SEM		2.819	0.1748		6.599	0.2714
Source of Variation		----------Probabilities----------				
WB		< 0.001	<0.001		<0.001	<0.001
WS		0.12	0.038		0.001	0.018
Logistic χ^2 P[4]						
WB		< 0.001	< 0.001		< 0.001	< 0.001
WS		< 0.001	< 0.001		< 0.001	< 0.001
Odds Ratios						
WB		(0.929, 0.963)	(0.364, 0.602)		(0.946, 0.969)	(0.402, 0.618)
WS		(0.944, 0.977)	(0.416, 0.706)		(0.952, 0.974)	(0.465, 0.692)
Spearman's P[5]						
WB		< 0.001	< 0.001		< 0.001	< 0.001
WS		< 0.001	< 0.001		< 0.001	< 0.001
Spearman's ρ						
WB		0.43	0.39		0.56	0.55
WS		0.30	0.30		0.49	0.44

[1]Exp = Experiment.Values are least-square means of blood was collected from 4 randomly selected birds per pen (63 pens) for subsequent analysis of plasma CK and LDH at 35 (Exp 1) and 49 (Exp 2) d of age. SEM = pooled standard error of the mean;

[2]The right breast fillet of each bird was assigned a subjective score (normal = 0; mild = 1; severe = 2) for wooden breast (WB) and white striping (WS). Means were separated using Tukey's Honestly Significant Difference test. Missing values for any measure resulted in exclusion of 19 and 16 birds from the analysis of Exp 1 and Exp 2, respectively.

[4]The overall χ^2 P-value corresponds to the hypothesis test for overall significance of the logistic regression model. Odds ratios (OR) represent the proportional increase (OR > 1) or decrease (OR < 1) in the odds of obtaining a myopathy score of 0 vs. a score of 1 or 2 (or the odds of obtaining a score of 0 or 1 vs. a score of 2) for each U/mL increase in CK or LDH. Displayed as 95% confidence interval.

[5]Spearman's rank correlation coefficient (ρ) and its associated P-value provide a measure of dependence between continuous and categorical variables

[a-c]Means within each myopathy not sharing a common superscript within a column differ significantly ($P < 0.05$).

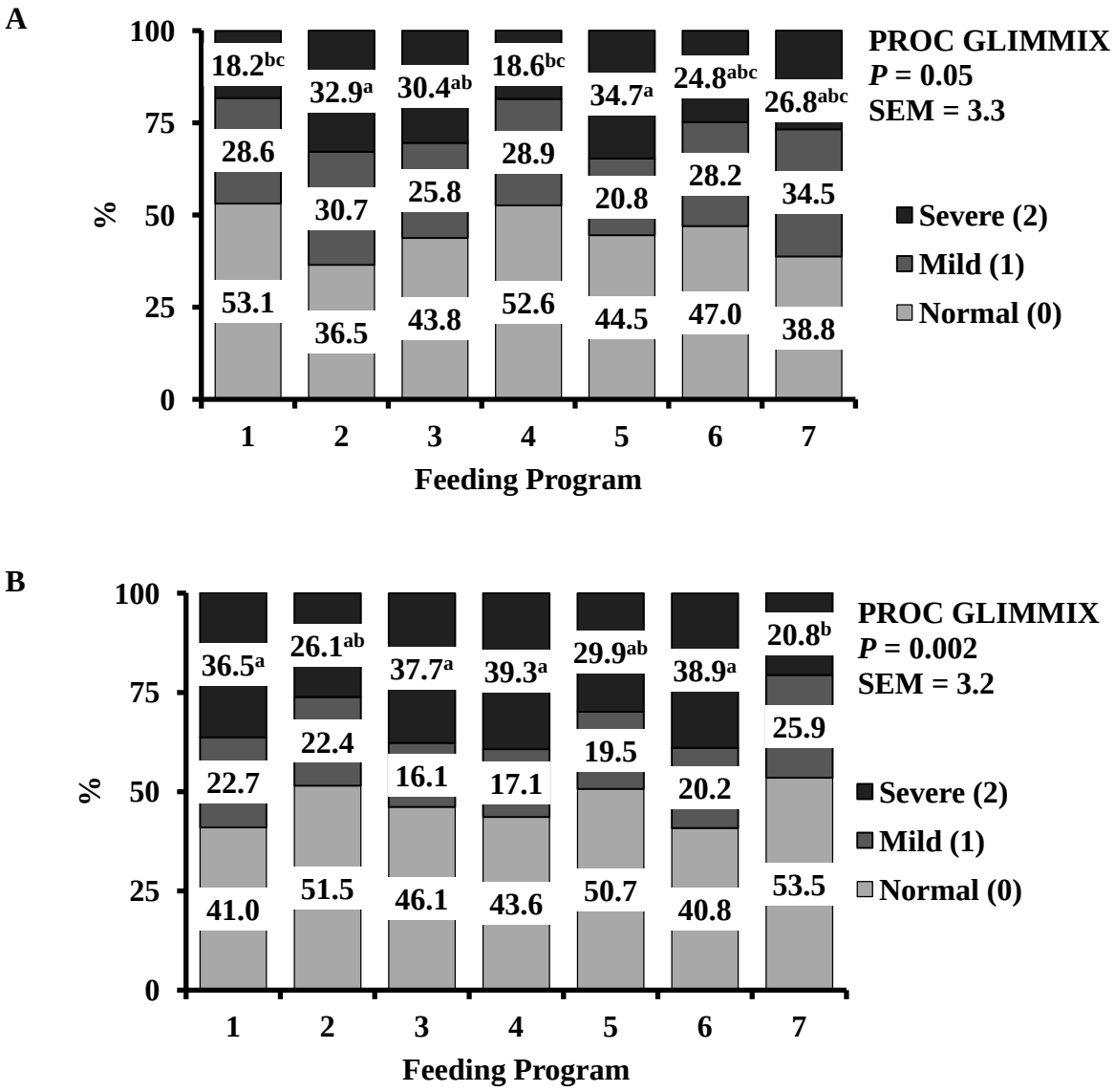

Figure 4.1 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast among male Yield Plus × Ross 708 broiler chickens**.** Values represent 9 replicate pens with 18 birds per pen randomly selected for processing and myopathy scoring at 35 (Experiment 1; A) and 49 (Experiment 2; B) d of age. Means were separated using Tukey's Honestly Significant Difference test. Birds received identical prestarter diet until 7 d of age. At 8 d, each pen was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

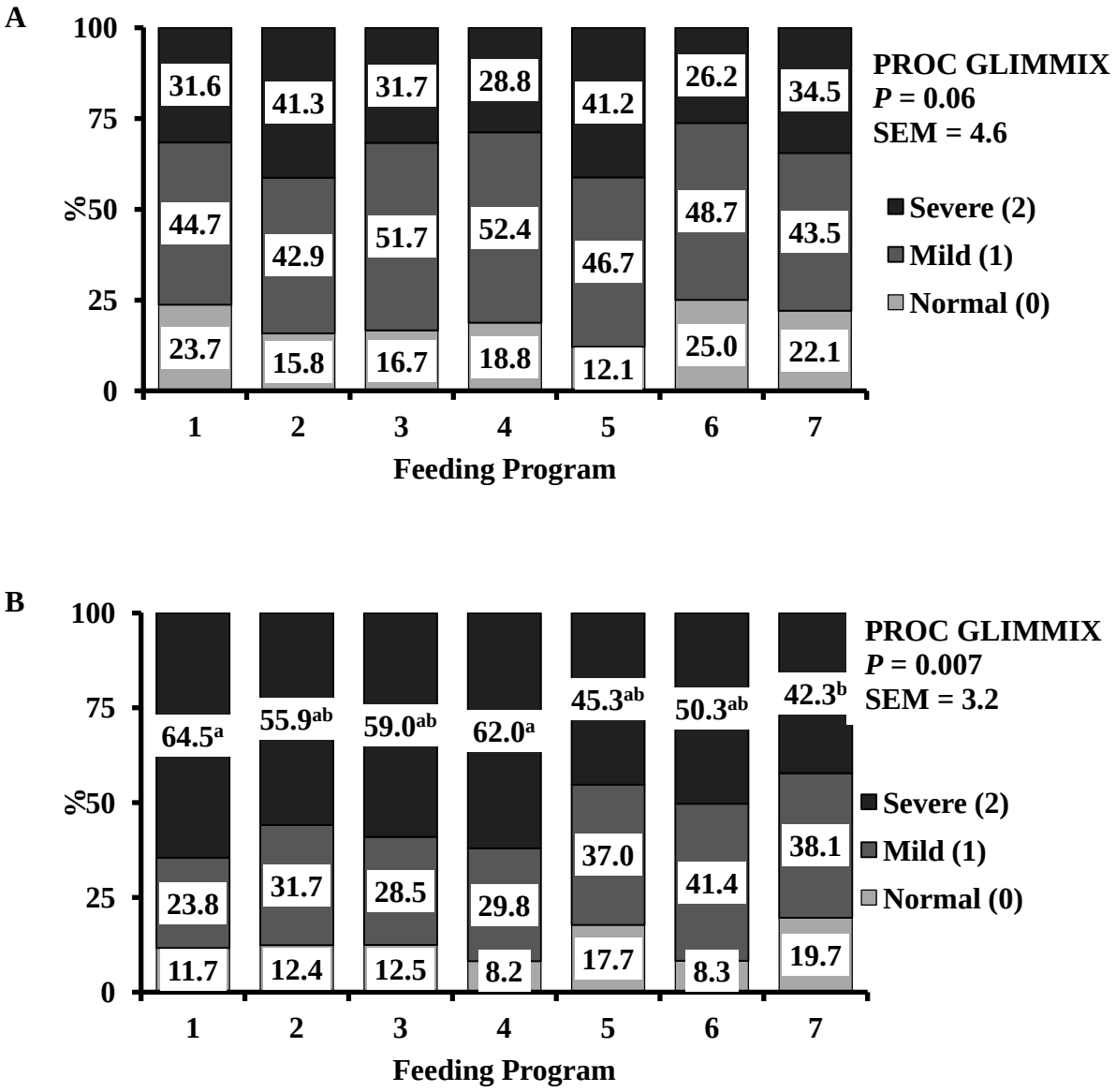

Figure 4.2 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for white striping among male Yield Plus × Ross 708 broiler chickens. Values represent 9 replicate pens with 18 birds per pen randomly selected for processing and myopathy scoring at 35 (Experiment 1; A) and 49 (Experiment 2; B) d of age. Means were separated using Tukey's Honestly Significant Difference test. Birds received identical prestarter diet until 7 d of age. At 8 d, each pen was randomly assigned to 1 of the following 7 feeding programs (TRT): 1) 100% of primary breeder recommendations for amino acid and apparent metabolizable energy (AME_n) density throughout experiment; 2) 95% of TRT 1 until 14 d of age, then as TRT 1; 3) 95% of TRT 1 until 24 d of age, then as TRT 1; 4) 95% of TRT 1 throughout experiment; 5) 90% of TRT 1 until 14 d of age, then as TRT 1; 6) 90% of TRT 1 until 24 d of age, then as TRT 1; 7) 90% of TRT 1 throughout experiment.

V. EFFECTS OF REDUCED DIGESTIBLE LYSINE DENSITY ON MYOPATHIES OF THE *PECTORALIS MAJOR* MUSCLES IN BROILER CHICKENS AT 48 AND 62 DAYS OF AGE

ABSTRACT

Quantitative control of nutrient intake may decrease the incidence of WB and WS myopathies with some impairment of live performance. Two Exp utilizing Yield Plus × Ross 708 male broilers were conducted to determine if a similar reduction in myopathies may be obtained using a qualitative approach by reducing dLys density. All birds received an identical starter diet until 11 d. In Exp 1 (63 pens; 22 birds/pen), each pen was then randomly assigned to 1 of the following 7 dietary treatments (TRT) for the grower 1 (G1; 12 to 18 d) and grower 2 (G2; 19 to 26 d) phases: 1) 100% of primary breeder recommendations for dLys throughout Exp; 2) 85% of TRT 1 dLys for G1; 3) 85% of TRT 1 dLys for G2; 4) 85% of TRT 1 dLys for G1 and G2; 5) 75% of TRT 1 dLys for G1; 6) 75% of TRT 1 dLys for G2; 7) 75% of TRT 1 for G1 and G2. In Exp 2 (24 pens; 30 birds/pen) birds were randomly assigned to 1 of the following 4 dietary TRT (Table 2) for the grower (G; 12 to 28 d) and finisher 1 (F1; 29 to 40 d) phases: 1) 100% of primary breeder recommendations for dLys 2) 85% of TRT 1 dLys for G; 3) 85% of TRT 1 dLys for F1; 4) 85% of TRT 1 dLys for G and F1; thereafter, birds received common finisher 1 (Exp 1: 27 to 42 d), finisher 2 (Exp 2: 41 to 48 d) and withdrawal (Exp 1: 43 to 47 d; Exp 2: 49 to 61 d) diets. Ideal AA ratios were not maintained in

reduced dLys diets in either Exp. At 48 (Exp 1; 18 birds/pen) and 62 (Exp 2; 30 birds/pen) d of age, selected birds were processed and fillets were visually scored for WB and WS. No differences ($P > 0.05$) in cumulative live performance responses between TRT 1 and the remaining TRT were observed in either Exp. In Exp 1, the incidence of severe WB (20.8%) and WS (42.3%) at 48 d of age among birds receiving TRT 7 was reduced ($P < 0.01$) compared with TRT 1 (WB: 36.6%; WS: 64.3%), at the expense of reduced ($P = 0.003$) breast weights and yield. In Exp 2, the incidence of severe WB (18.8%) and WS (17.8%) at 62 d of age for birds receiving TRT 4 was reduced ($P < 0.05$) compared with TRT 1 (WB: 39.3%; WS: 38.3%), without any detrimental effects on processing characteristics. These results indicate that altering dietary dLys during critical periods of the growth trajectory may be a viable strategy for reducing the incidence and severity of WB and WS.

INTRODUCTION

Recently, broiler producers worldwide have reported increasing occurrence of the breast quality defects known as WB and WS. Wooden breast is easily recognized by its abnormal rigidity, which not only can be palpated by hand on live birds, but also persists through processing as an undesirable texture in raw and further processed products. Histological evidence suggests that the characteristic toughness of WB is caused by abnormal accumulation of connective tissue and fluid (Sihvo et al., 2014, 2017). Fillets affected by WS have visible deposits of intermuscular fat in a pattern of striations that appear parallel to the muscle fibers (Kuttappan et al., 2013). Although it has been proposed that these myopathies are related to genetic selection for increased breast meat yield (Kuttappan et al., 2016), non-genetic factors, such as environment and nutrition, contribute the majority of the observed variance in myopathy incidence (Bailey et al., 2015), and therefore may be more suitable targets for practical interventions aimed at reducing the incidence and severity of these myopathies.

Prior research investigating the effects of quantitative nutrient allocation has indicated that the incidence of these quality defects may be reduced through quantitative feed control (Meloche et al., 2015). Quantitative allocation strategies may have limited practical applicability in certain areas of the world, as most broiler growers in the U.S., for example, are not typically equipped to allocate feed by weight on a daily basis. For

this reason, studies have also been conducted with the aim of altering growth trajectories through reductions in nutrient allocation obtained qualitatively through the simultaneous manipulation of dietary AME_n and AA density (Meloche et al., 2016a,b). However, in these Exp, broilers receiving the lower density diets displayed a notable capacity for compensatory feed intake, eliminating the designed reduction in nutrient intake. Indeed, the compensatory response to the reduced density diets actually resulted in these broilers consuming more dLys than their unrestricted counterparts.

However, it may be possible to alter the growth trajectory without stimulating compensatory intake by altering the concentration of a single nutrient, rather than dietary density as a whole. Digestible Lys is an obvious target for single nutrient deletion because Lys comprises the largest proportion of any essential AA in breast muscle as a percentage of CP (Munks et al., 1945). Breast muscle exhibits pronounced responses to differences in dietary dLys, from regulation of protein turnover at the cellular level (Tesseraud, et al., 1996; 2001) to yield responses at the processing plant (Dozier et al., 2010). Furthermore, feed-grade Lys is frequently supplemented in poultry diets and therefore dietary concentrations may be easily modified.

Cruz et al. (2017) investigated the effects of single feeding-phase reductions (12 to 28 and 28 to 40 d of age) in dietary dLys on the incidence of WB and WS in Cobb × Cobb 500 male broilers at 35 and 42 d of age. These authors observed reductions in WB and WS when feeding diets formulated with dLys at approximately 70 and 63% of the recommended concentrations. Although this dietary strategy reduced the incidences of myopathy, the extreme reductions in dLys were deleterious to live performance and breast yield. However, it is possible that implementing a similar strategy with more

moderate reductions in dLys over shorter periods of time may suffice to reduce myopathies without these negative effects. Therefore, 2 Exp were conducted to evaluate the effects of short-term reductions in dLys concentrations on performance, processing characteristics, and the incidence of WB and WS in broilers processed at 48 and 62 d of age.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in Exp 1 (PRN 2015-2629). Experiment 2 was conducted at the Aviagen Inc. (Huntsville, AL) research facility in Albertville, AL in accordance with National Chicken Council welfare guidelines (National Chicken Council, 2014). Two similarly designed Exp were conducted from 1 to 48 (Exp 1) and 1 to 62 d of age (Exp 2).

Experiment 1

Bird Husbandry. One-thousand three hundred eighty-six Yield Plus × Ross 708 (Aviagen Inc., Huntsville, AL) male broiler chicks were obtained from a commercial hatchery and placed into 63 floor pens (0.10 m^2/bird; 22 birds per pen) in a solid-sided house equipped with a cross-ventilation system. Experimental facility was equipped with air inlets, exhaust fans, evaporative cooling pads, forced-air heaters, and an electronic controller to maintain optimum ventilation and temperature. Each pen contained a hanging feeder, a nipple drinker line, and used litter. Chicks were vaccinated at the hatchery for Marek's disease, Newcastle disease, and infectious bronchitis. Birds were provided feed and water on an *ad libitum* basis. Ambient temperature was set at 33°C at placement and reduced to maintain comfort with advancing bird age with a final set point of 20°C at 28 d of age. Birds were exposed to a 23L:1D photoperiod from placement to 7

d of age, followed by an 18L:6D photoperiod for the remainder of the Exp. Light intensity was set at 30 lux from 1 to 7 d of age, 10 lux from 8 to 14 d of age, 5 lux from 15 to 24 d of age, and 3 lux from 25 to 48 d of age. Light intensity settings were verified at bird level (30 cm) using a photometric sensor with National Institute of Standards and Technology-traceable calibration (403125, Extech Instruments, Waltham, Mass) for each intensity adjustment.

Dietary Treatments. All birds received an identical starter diet from placement until 11 d of age, after which time each pen was randomly assigned to 1 of the following 7 dietary treatments (TRT; Table 5.1) for the grower 1 (G1; 12 to 18 d) and grower 2 (G2; 19 to 26 d) phases: 1) 100% of primary breeder recommendations for dLys 2) 85% of TRT 1 dLys for G1; 3) 85% of TRT 1 dLys for G2; 4) 85% of TRT 1 dLys for G1 and G2; 5) 75% of TRT 1 dLys for G1; 6) 75% of TRT 1 dLys for G2; 7) 75% of TRT 1 for G1 and G2. Thereafter, all birds received common finisher (27 to 42 d) and withdrawal (43 to 47 d) diets which met or exceeded primary breeder recommendations for all nutrients. Digestible AA values were calculated by multiplying digestibility coefficients (Ajinomoto, 2009) to the calculated total AA content of each ingredient. Control diets for G1 and G2 were formulated to maintain digestible AA to dLys ratios of 51, 76, 67, 76, and 68 for dMet, dTSAA, dThr, dVal, and dIle, respectively (Table 5.2). Ideal AA ratios to Lys were not maintained in the reduced dLys diets. Differences in dLys among the TRT diets were created by replacing L-Lys·HCl with builder's sand. This formulation strategy allowed for the deletion of Lys from the diets without altering the concentrations of any other nutrients. Diets were corn-soybean meal-based and contained corn gluten meal and peanut meal as needed to force adequate concentrations of L-Lys·HCl into the

diet such that its replacement with sand would produce the desired concentrations of dLys in the TRT diets. Corn gluten meal and peanut meal were also utilized in the common starter, finisher, and withdrawal diets to minimize the effects of changing ingredients between phases. Feed was provided in crumble form during the starter phase, and pelleted thereafter. Grower 1 and G2 treatment diets were analyzed for concentrations of total Lys (AOAC, 2006; 982.30 E(a)) and free Lys concentrations (AOAC, 2000; method 999.13) at University of Missouri–Columbia Experiment Station Chemical Laboratory.

Measurements. Body weight and feed intake for each pen was determined at 11, 18, 26, 42, and 47 d of age. Mortality was recorded daily and FCR were corrected for mortality and excluded placement weights. At 48 d of age, after a feed withdrawal period of 12 h, 18 birds per pen were randomly selected for processing at the Auburn University Pilot Processing Plant. Selected birds were placed in coops, transported to the processing facility, electrically stunned, exsanguinated, scalded, picked, and manually eviscerated. Carcasses were chilled on ice for 3 h prior to measuring carcass and abdominal fat pad weights. The front-halves of the carcasses were then packed in ice for 18 h. The weights of the PM (boneless breast) and *Pectoralis minor* (tender) muscles were recorded after deboning by trained personnel from a commercial processing plant. Carcass and breast yields, as well as abdominal fat percentage, were calculated relative to live BW at 47 d of age. The PM muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for WS and WB. All fillets were scored by the same evaluator. For WS, the defect was characterized as “mild” if visible striping comprised less than half of the total fillet surface area or was less than 2 mm wide on average, but

was considered “severe” if the striping exceeded these limits. For WB, the defect was considered “mild” if palpable hardness was present in less than half the total fillet surface area, but was considered “severe” if it exceeded this limit.

Experiment 2

Bird Husbandry. Seven hundred twenty Yield Plus × Ross 708 (Aviagen Inc., Huntsville, AL) male broiler chicks were obtained from a commercial hatchery and placed into 24 floor pens (0.11 m^2/bird; 30 birds per pen) in concrete floor pens in an environmentally controlled research facility. Each pen contained a hanging feeder, a nipple drinker line, and used litter that was top-dressed with fresh shavings. Chicks were vaccinated at the hatchery for Marek’s disease, Newcastle disease, and infectious bronchitis. Birds were provided feed and water on an *ad libitum* basis. Ambient temperature was set at 33°C at placement and was reduced to maintain comfort with advancing bird age with a final set point of 20°C at 28 d of age. Birds were exposed to a 23L:1D photoperiod from placement to 3 d of age, followed by an 18L:6D photoperiod for the remainder of the Exp. Light intensity was set at 20 lux from 1 to 3 d of age and 3 lux thereafter.

Dietary Treatments. All birds received an identical starter diet from placement until 11 d of age, after which time each pen was randomly assigned to 1 of the following 4 dietary TRT (Table 5.3) for the Grower (G; 12 to 28 d) and Finisher 1 (F1; 29 to 40 d) phases: 1) 100% of primary breeder recommendations for dLys 2) 85% of TRT 1 dLys for G; 3) 85% of TRT 1 dLys for F1; 4) 85% of TRT 1 dLys for G and F1; Thereafter, all birds received common finisher 2 (41 to 48 d) and withdrawal (49 to 61 d) diets which met or exceeded primary breeder recommendations for all nutrients. Digestible AA

values were calculated by multiplying digestibility coefficients (Ajinomoto, 2009) to the calculated total AA content of each ingredient. Control diets for G1 and G2 were formulated to maintain digestible AA to dLys ratios of 51, 77, 67, 77, and 68 for dMet, dTSAA, dThr, dVal, and dIle, respectively (Table 5.4). Ideal AA ratios to Lys were not maintained in the reduced dLys diets. Differences in dLys among the TRT diets were created by replacing L-Lys·HCl with builder's sand. This formulation strategy allowed for the deletion of Lys from the diets without altering the concentrations of any other nutrients. Diets were corn-soybean meal-based. Feed was provided in crumble form during the starter phase, and pelleted thereafter. Treatment diets (G and F1) were analyzed for concentrations of total Lys by AMINOLab® (Evonik Industries, Kennesaw, GA).

Measurements. Body weight and feed intake for each pen was determined at 11, 28, 40, 48, and 61 d of age. Mortality was recorded daily and FCR were corrected for mortality and excluded placement weights. At 62 d of age, 4 randomly selected replicate pens (30 birds/pen) per TRT were processed at the Aviagen Albertville Research Processing Plant. Selected birds were individually identified with wingbands and placed in a holding pen for a feed withdrawal period of 12 h. The following morning, birds were placed in coops, transported to the processing facility, electrically stunned, exsanguinated, scalded, picked, and eviscerated. Abdominal fat pads were returned to the body cavity. Carcasses were air-chilled for 3 h prior to measuring carcass and abdominal fat pad weights. The weights of the PM (boneless breast) and *Pectoralis minor* (tender) muscles were recorded after deboning by trained personnel. Carcass and breast yields, as well as abdominal fat percentage, were calculated relative to live BW at 61 d of age. The

PM muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for WS and WB by a group of trained evaluators. Defects were assigned scores as described above for Exp 1.

Statistical Analyses

In each Exp, TRT were arranged in a randomized complete block design with pen as the experimental unit and pen location as the blocking factor. Treatment effects on live performance and processing characteristics were subject to analysis of variance using PROC MIXED of SAS 9.3 (SAS Institute, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu_{..} + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are the fixed factor level effects corresponding to the *j*th treatment such that $\Sigma\tau_j = 0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ. For live performance, TRT were represented by 9 replicate pens (22 birds/pen) in Exp 1 and 6 replicate pens (30 birds/pen) in Exp 2. Treatment effects on processing characteristic were evaluated using 9 replicate pens per TRT (18 birds/pen) in Exp 1 and 4 replicate pens per TRT (30 birds/pen) in Exp 2.

Proportions of affected fillets in each scoring category were analyzed by PROC GLIMMIX (SAS Institute, 2009) using the events/experiments syntax with a binomial distribution and R-side covariance structure. Where applicable, residuals were visually assessed to ensure normality and nonnormal data were transformed prior to analysis. For all hypothesis tests, statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Diet Analysis

Experiment 1. Analysis of total Lys content of the G1 diets indicated that a reduction in Lys occurred, with total Lys contents of 1.45, 1.28, and 0.99% for TRT 1, 2, and 3, respectively (Table 5.2). For the G2 diets, TRT 1, 2, and 3 contained 1.29, 1.14, and 1.15% total Lys, respectively. The reduction in Lys between TRT 2 and 3 was not as evident in the total Lys analysis for the G2 diets. Because the diets were formulated to omit added L-Lys·HCl, analysis of free Lys is more informative. For the G1 diets, TRT 1, 2, and 3 contained\ 0.27, 0.13, and 0.02% free Lys, respectively. Analysis of the G2 diets indicated that they contained 0.25, 0.12, and 0.03% free Lys for TRT 1, 2, and 3, respectively. These analyses correspond well with the values utilized in formulation.

Experiment 2. Grower diets contained 1.37 and 1.25% analyzed total Lys Table 5.4). These values were higher than the target values for formulation, but nevertheless represent a substantial reduction in Lys content between treatment diets. Likewise, the F1 diets contained 1.18 and 1.01% total Lys, which were higher values than the target for these diets but still indicate a reduction in Lys content.

Growth Performance

In each Exp, there were no differences ($P > 0.05$) among treatment groups prior to the experimental period (1 to 11 d).

Experiment 1. During the G1 phase (12 to 18 d of age), TRT 2, 4, 5 and 7 received diets with reduced dLys density, while the other TRT remained on the 100% dLys control diet (Table 5.5). Broilers receiving TRT 5 and 7, which were formulated to 75% of recommended dLys concentrations, had reduced ($P < 0.001$) BW gain relative to

all other TRT. In contrast, broilers receiving diets formulated to 85% of dLys recommendations (TRT 2 and 4) had similar ($P > 0.05$) BW gain to that of the control. These results indicate that reduction of dLys to 75% of the recommended density from 12 to 18 is sufficient to alter the growth trajectory, whereas a reduction to 85% did not lower BW gain. Perryman et al. (2013) observed no differences in BW gain between Ross × Ross 708 male broilers fed diets formulated to weighted dLys concentrations of 1.23 and 1.17% from 1 to 28 d of age, which corresponds well to similar BW gain observed among broilers receiving TRT 1 vs. TRT 2 and 4, with 1 to 15 d weighted dLys concentrations of 1.24 and 1.17% respectively, in the current study.

Previous attempts to prevent the development of myopathies through feeding reduced density diets have diluted AME_n concurrently with all AA (Meloche et al., 2016a,b). These authors observed increased feed intake sufficient to compensate for the designed differences in nutrient density, due to the innate propensity of broilers to consume feed until they meet their AA or AME_n needs, depending on their age. In the current study, no differences ($P > 0.05$) in feed intake from 12 to 18 d of age were observed relative to the control, although TRT 2 and 4 had increased ($P < 0.001$) feed intake relative to TRT 5 and 7. This observation may be attributable to the reported appetite depressant effect of reduced dLys diets in young broilers (Picard et al., 1993; Noble et al., 1993), which is mediated by altered plasma Lys concentrations (Alam et al., 2014). The lack of feed intake differences between the reduced dLys diets (TRT 2, 4, 5, and 7) and those receiving the 100% dLys control (TRT 1, 3, and 6) from 12 to 18 d of age resulted in reduced ($P < 0.001$) dLys intakes. Broilers receiving diets formulated at 85% of the control (TRT 2 and 4) consumed 6.7 g dLys/bird vs. 7.7 g/bird consumed by

those receiving the control (TRT 1, 3, and 6). In contrast to the current study, Meloche et al. (2016a,b) observed that broilers responded to diets varying in both AME_n and AA density by increasing feed intake. The results of the current study indicate that broilers may be less sensitive to differences in dietary dLys content only, allowing for more effective control of the growth curve.

Additionally, broilers receiving diets formulated at 75% of the control (TRT 5 and 7) consumed less dLys (averaging 9.35 g dLys/ bird) than those receiving either the control or the diets formulated to 85% of the control. The single-nutrient deletion strategy utilized in this study circumvents the issue of compensatory feeding by maintaining concentrations of other dietary AA, as well as AME_n in the reduced dLys diets, resulting in no differences ($P > 0.05$) in AME_n intake relative to the control. Broilers assigned to the 75% dLys diets (TRT 5 and 7) had increased ($P < 0.001$) FCR in comparison to the control. These treatments were formulated to a dLys concentration of 0.88%, which is substantially lower than the reported optimum of 1.10% dLys for performance responses in Ross × Ross 708 male broilers (Dozier et al., 2009). Growth performance results from 12 to 18 d of age indicated that reducing dLys to 75% of recommendations during G1 altered the growth curve of birds assigned to TRT 5 and 7.

At 26 d of age, all broilers receiving diets formulated at 85% of the dLys content of the control had similar ($P > 0.05$) BW gain, feed intake, AME_n intake, and FCR compared with the control (5.6). However, broilers receiving these diets for any duration had reduced ($P < 0.001$) dLys intake (17.3, 16.6, and 16.0 g/bird for TRT 2, 3, and 4, respectively) compared with the control (18.7 g/bird). Feeding diets formulated at 75% of recommended dLys concentrations from 12 to 18 (TRT 5) resulted in decreased ($P <$

0.001) BW gain and dLys intake, but similar ($P > 0.05$) feed intake, AME_n intake, and FCR relative to the 100% control diet. However, broilers consuming diets formulated at 75% of recommended dLys concentrations from 12 to 26 d of age had reduced ($P < 0.001$) feed intake and AME_n intake, as well as increased ($P < 0.001$) FCR relative to the control. These results indicated that a single-nutrient deletion strategy targeting dLys, even at reductions as low as 75%, bypasses the compensatory intake issues previously observed by Meloche et al. (2016a,b) when implementing a reduction in overall AME_n and AA density.

Cruz et al. (2017) fed 6 diets varying solely in dLys (0.77 to 1.17% in increments of 0.08%) to male Cobb × Cobb 500 broilers from 12 to 28 d of age. A weighted dLys concentration across phases can be calculated by multiplying the dLys concentration fed in each phase by the number of days each diet was fed as a proportion of the period of interest. For example, these authors fed a common starter diet formulated at 1.34% dLys prior to applying the treatment diets from 12 to 28 d of age, resulting in weighted dLys concentrations of 0.99 to 1.24% in increments of 0.05% for the entire 1 to 28 d period. Three of the intermediate dLys density diets utilized by Cruz et al. (1.04, 1.09, and 1.19% weighted dLys for diets 2, 3 and 5, respectively) are comparable to the 1 to 26 d weighted dLys concentrations for the reduced dLys diets fed throughout G1 and G2 in the current study (1.19, 1.09, and 1.03 % for TRT 1, 4, and 7, respectively). These authors only discussed growth performance in terms of BW at 35 d of age, with reduced ($P < 0.001$) BW in broilers receiving diets with a weighted dLys of 1.04 vs. 1.19% from 1 to 28 d of age, with the 1.09% weighted dLys diet intermediate between them. This same pattern in BW was observed with the comparable treatments in the current Exp at 26 d of age,

indicating the effectiveness of short term modifications in dLys concentration aimed at altering the growth trajectory.

There were no differences ($P > 0.05$) in 1 to 47 d BW gain, feed intake, AME_n intake, or FCR between any of the reduced dLys treatments relative to the 100% control (Table 5.7). At 29 d of age, all birds were returned to the 100% control diet for the remainder of Exp 1, allowing some recovery from the reduced density diets. Although broilers assigned to reduced dLys density diets for were able to recover similar cumulative growth performance to that of the control, those assigned to TRT 3 (57.4 g dLys/bird), TRT 5 (57.1 g dLys/bird), and TRT 7 (54.4 g dLys/bird) consumed less ($P < 0.001$) dLys than those assigned to the 100% control (59.7 g dLys/bird). Feeding common finisher (28 to 41 d of age) and withdrawal (42 to 47 d of age) diets that were adequate in dLys following the reduced dLys treatment period allowed for a modification of the growth curve during early development without negatively impacting cumulative growth performance at 47 d of age. Additionally, the single-nutrient deletion strategy was successful in reducing the dLys intake of certain treatments, which may have further consequences on the development of myopathy due to the critical role of dLys in breast muscle accretion (Tesseraud et al.,2001). A similar feeding strategy of reducing dLys exclusively from 12 to 28 d followed by a nutritionally adequate common finisher was employed by Cruz et al. (2017). Yet, in contrast to the current study, these authors observed significant differences in BW at the end of the Exp (35 d of age). These results are likely attributable to Cruz et al. (2017) feeding reduced dLys diets throughout the grower for all treatments, as well as the limited duration of the finisher phase (28 to 35 d) vs. the split grower phase and later processing date utilized in the current study (28 to 47

d). Clearly, the timing of a short-term reduced dLys treatment, as well as the remaining days to market weight thereafter, will affect the ability of the birds to recover performance.

Experiment 2. During the G phase (12 to 28 d of age), TRT 2 and 4 received diets formulated at 85% of recommended dLys concentrations, while TRT 1 and 3 remained on the 100% control diet (Table 5.8). As a result, similar ($P > 0.05$) outcomes were observed within the pairs of duplicated treatments for all measured performance responses. There were no differences ($P > 0.05$) in BW gain, feed intake, or AME_n intake due to diet relative to the 100% control. Broilers receiving the 100% diet in the G phase as part of TRT 3 exhibited increased ($P = 0.024$) BWG relative to the birds assigned to TRT 2. Broilers receiving TRT 2 and 4 had reduced ($P < 0.001$) dLys intake (16.0 and 16.3g dLys/bird, respectively) compared with the 100% control (18.9 g dLys/bird). Additionally, these birds had increased ($P < 0.001$) FCR (1.527 and 1.512 for TRT 2 and 4, respectively) relative to TRT 1 (1.471), indicating that the reduction to 85% of recommended dLys from 12 to 28 d did not optimize FCR.

From 12 to 40 d of age, there were no differences ($P > 0.05$) in feed intake or AME_n intake due to diet relative to the 100% control (Table 7). Broilers receiving diets formulated to 85% of the recommended dLys concentrations from 12 to 28 d of age had reduced BW gain from 12 to 40 d of age, relative to the control. Intake of dLys decreased ($P < 0.001$) relative to the control (41.8 g dLys/bird) as the duration of reduced dLys feeding increased, with the lowest intake (36.0 g dLys/bird) among broilers receiving reduced dLys diets throughout the G and F1 phases (TRT 4) and intermediate intakes (38.0 and 38.5 g dLys/bird) for broilers receiving reduced dLys diets for a single phase

(TRT 2 and 3). Similarly, FCR was higher (P = 0.005) relative to the control, among broilers receiving reduced dLys diets during both phases (TRT 4), with the single-phase reduced dLys diets having intermediate FCR.

In addition to their Exp from 1 to 35 d of age, Cruz et al. (2017) also fed 6 diets varying solely in dLys (0.77 to 1.17% in increments of 0.08%) to male Cobb × Cobb 500 broilers from 28 to 42 d of age. These birds received common starter and grower diets formulated at 1.34 and 1.18% dLys, resulting in weighted dLys concentrations of 1.06 to 1.19% in increments of 0.027% from 1 to 42 d. Two of the intermediate dLys density diets utilized by Cruz et al. (1.11 and 1.16% weighted dLys for diets 3 and 5, respectively) were comparable to the 1 to 40 d weighted dLys concentrations for the control (TRT 1; 1.15%) and the reduced dLys diet fed throughout G and F1 (TRT 4; 1.10%) in the current study. Although 42 d BW was the only growth performance response reported, these authors observed no differences BW in broilers receiving reduced dLys diets from 28 to 42 d of age, as likewise observed in the current study.

At 61 d of age, there were no differences ($P > 0.05$) in BW gain, feed intake, AME_n intake, or FCR due to diet (Table 5.10). However, all broilers assigned to a reduced dLys feeding programs had lower ($P < 0.001$) dLys intakes relative to the control. These results illustrate that short-term reductions of dLys will result in a persistent deficit in cumulative dLys intake, despite prolonged access to adequate concentrations of dLys in the common diets from 40 to 61 d of age. In contrast, Meloche et al. (2016b) reported that reducing overall AME_n and AA density from 8 to 24 d of age as a strategy to manipulate the growth trajectory resulted in broilers engaging in

compensatory feeding sufficient to overcome the differences in dietary density when returned to 100% density diets.

Carcass Characteristics

Experiment 1. No treatment differences ($P > 0.05$) in abdominal fat pad weights and percentages or carcass weights and yields were observed (Table 5.11). Broilers receiving diets formulated at 75% of recommended dLys concentrations from 12 to 26 d of age (TRT 7) had reduced ($P < 0.01$) breast weights (0.944 kg) and yields (26.3%) relative to the 100% control (0.995 kg and 27.3%). Insufficient dietary Lys has been repeatedly shown to increase protein turnover, and thereby reduce muscle tissue accretion, in broilers (Tesseraud et al., 2001; 2009). Furthermore, selection of broilers for breast yield has resulted in the PM muscles becoming particularly sensitive to dietary dLys concentrations in comparison to other muscles (Tesseraud et al., 2001). Broilers assigned to TRT 7 had reduced dLys intake relative to all other TRT, which specifically limited their accretion of breast muscle, but did not prevent them from achieving a similar BW to broilers assigned to other TRT. Other portions of the carcass that were not measured in this study, such as the thighs and drumsticks, may have increased in weight as these muscles are not impacted by reduced dLys to the same extent as breast muscle (Tesseraud et al., 1996). Interestingly, broilers receiving the 75% dLys dietary program from 12 to 18 d of age (TRT 5) had reduced ($P = 0.001$) breast weights (0.973 kg) in comparison with broilers receiving the 85% dLys diet from 12 to 26 d of age (TRT 4). Not only did these TRT consume similar amounts of dLys, but the longer duration of reduced dLys feeding in TRT 4 actually created a lower weighted 1 to 47 d average dLys concentration (1.05%) than that of TRT 5 (1.07%). This result may indicate that the

timing of a reduced dLys diet program impacts breast weight more so than the duration or intensity of the reduction in dLys averaged over the whole lifespan. Implementing a reduced dLys diet earlier in the growth period may limit the birds' potential for breast muscle accretion, even when allowed a substantial recovery period on adequate diets.

In contrast to the current study, Cruz et al. (2017) observed reduced carcass weights and yields as well as reduced breast weights and yields at 35 d of age for broilers fed a diet with comparable weighted dLys content to TRT 7 in the current study (1.04 %) during the experimental period. As with the live performance results, the differences in processing characteristics between these 2 studies are likely attributable to the longer interval on adequate dietary dLys between the end of the experimental period and processing in the current study.

Experiment 2. No TRT differences ($P < 0.05$) were observed for abdominal fat weights, carcass weights, or carcass yields (Table 5.11). However, abdominal fat percentages were higher ($P = 0.022$) in broilers receiving reduced dLys diets in G alone (TRT 2) or throughout G and F1 (TRT 7). Although dLys effects on lipid deposition have been demonstrated in younger broilers (Mack et al. 1999; Dozier et al. 2010), abdominal fat percentages are typically not affected by reduced dLys in broilers older than 50 d of age (Corzo et al., 2006; Dozier et al., 2008). In contrast to Exp 1, no differences ($P > 0.05$) in breast weights or yields occurred in Exp 2. However, in Exp 1 these differences were observed for broilers receiving diets formulated at 75% of the recommended dLys concentration for both G1 and G2, a more extreme reduction in Lys, which was not utilized in Exp 2.

Myopathy Scoring.

Experiment 1. At 48 d of age, the overall incidences of normal, mild, and severe scores for WB were 46.8, 20.4, and 32.7% respectively (Figures 5.1A and 5.1B). Though incidence of WB varies substantially in the existing literature, flock incidences greater than 50% have been reported (Mutryn et al., 2015; Cruz et al., 2017). For WS, the overall incidences of normal, mild, and severe scores at 48 d of age were 12.8, 32.9, and 54.2%, respectively. Broilers receiving diets formulated at 75% of recommended dLys concentrations from 12 to 26 d of age (TRT 7) had reduced ($P < 0.05$) incidence of severe scores for WB (Figure 5.1A) and WS (Figure 5.1B) relative to the control. Reduced cumulative dLys intake observed among broilers assigned to TRT 7 resulted in reduced breast weights and yields, which likely resulted in the observed decrease in WB and WS. Cruz et al. (2017) fed reduced dLys diets from 12 to 28 d of age with comparable weighted dLys content to TRT 1 and TRT 7, but did not report any differences in WB or WS incidences or scores among broilers receiving the reduced dLys diet. These authors reported that the incidences of WB and WS increased with increasing dietary dLys at 35 d of age. In the current study, no such relationship was evident at 48 d of age.

Experiment 2. Similar to Exp 1, the incidences of normal, mild, and severe scores for WB across all treatments at 62 d of age were 46.4, 22.5, and 31.0%, respectively (Figure 5.2A). Feeding 85% of the recommended dLys concentrations from 12 to 40 d (TRT 4) decreased ($P = 0.005$) the incidence of severe WB relative to the control (Figure 5.2A). This reduction (18.8% vs. 39.3%) was obtained without observing any detrimental effects on live performance or processing characteristics. The overall incidences of normal, mild, and severe scores for WS were 34.8, 37.0, and 27.8%, respectively. The incidence of severe scores for WS was reduced in broilers receiving diets formulated at

85% of recommended dLys from 12 to 28 d (TRT 2; 22.3%) or from 12 to 40 d (TRT 4; 17.8%) in comparison with the control (38.3%; Figure 5.2B). Cruz et al. (2017) fed reduced dLys diets from 28 to 40 d of age with comparable weighted dLys content to TRT 1 and TRT 4, but did not report any differences in WB or WS incidences or scores at 42 d among broilers receiving these diets.

From these studies, it can be concluded that short-term reductions in dLys density are effective at reducing the severity of WB and WS. However, the magnitude of the reduction in dLys, as well as its timing relative to the total length of the growth period, may impact live performance and processing characteristics. Furthermore, it is worth noting that the dLys concentrations utilized in these studies were based upon primary breeder recommendations. Similar percentage reductions applied to dLys concentrations representative of those used in the U.S. broiler industry may not provide comparable results. The mechanism by which reducing dietary dLys may alleviate the development of myopathy without detrimentally impacting breast yield is not yet known. Identifying the critical periods during which manipulation of the growth trajectory effectively reduces myopathy with the least compromise on performance warrants additional research.

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Table 5.1. Ingredient composition of diets fed to male Yield Plus × Ross 708 broilers from 1 to 47 d of age, experiment 1[1]

Days of age	Starter 1 to 11	Grower 1 12 to 18			Grower 2 19 to 26			F 27 to 42	W 43 to 47
Ingredient (%)		Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3		
Corn	52.07	58.86	58.86	58.86	62.33	62.33	62.33	62.07	64.03
Soybean Meal	36.27	25.64	25.64	25.64	23.54	23.54	23.54	25.51	23.67
Corn Gluten Meal	2.00	5.00	5.00	5.00	4.00	4.00	4.00	2.00	2.00
Peanut Meal	2.00	3.00	3.00	3.00	3.00	3.00	3.00	2.00	2.00
Poultry Fat	3.15	2.98	2.98	2.98	2.63	2.63	2.63	4.53	4.58
Dicalcium Phosphate	2.02	1.86	1.86	1.86	1.88	1.88	1.88	1.64	1.55
Calcium Carbonate	1.05	0.98	0.98	0.98	0.99	0.99	0.99	0.88	0.84
Sodium Chloride	0.44	0.29	0.29	0.29	0.24	0.24	0.24	0.32	0.28
DL-Methionine	0.33	0.30	0.30	0.30	0.26	0.26	0.26	0.28	0.24
L-Lys·HCl	0.23	0.38	0.15	---	0.35	0.14	---	0.23	0.21
L-Thr	0.10	0.11	0.11	0.11	0.10	0.10	0.10	0.07	0.05
L-Val	0.00	0.02	0.02	0.02	0.01	0.01	0.01	---	---
Sodium Bicarbonate	0.00	0.23	0.23	0.23	0.29	0.29	0.29	0.19	0.25
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Chloride	0.07	0.10	0.10	0.10	0.11	0.11	0.11	0.08	0.08
TBCC	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	---	---
Sand	---	---	0.23	0.37	---	0.21	0.35	---	---

[1]F = Finisher; W = Withdrawal; TBCC = Tribasic copper chloride

[2]Vitamin premix provided the following per kilogram of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopheryl acetate), 66 IU; Vitamin B12 (cyanocobalamin), 0.03 mg; D-biotin (biotin), 0.18 mg; menadione (menadione sodium bisulfate complex), 4 mg; thiamine (thiamine mononitrate), 5.5 mg; riboflavin (riboflavin), 22 mg; D-pantothenic acid (calcium pantothenate), 31 mg; pyridoxine (pyridoxine hydrochloride), 7.7 mg; niacin (niacinamide), 88 mg; folacin (folic acid), 2.6 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite), 0.3 mg.

Table 5.2. Calculated nutrient content of diets fed to male Yield Plus × Ross 708 broilers from 1 to 47 d of age, experiment 1[1]

Days of age	Starter 1 to 11	Grower 1 12 to 18			Grower 2 19 to 26			F 27 to 42	W 43 to 47
Calculated nutrient composition (%)[2]		Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3		
AME_n (kcal/kg)	3,000	3,100	3,100	3,100	3,100	3,100	3,100	3,200	3,225
CP	24.4	22.5	22.5	22.5	21.1	21.1	21.1	20.2	19.4
Digestible Lys	1.28	1.17	0.99	0.88	1.09	0.93	0.82	1.02	0.96
Digestible Met	0.65	0.61	0.61	0.61	0.55	0.55	0.55	0.54	0.50
Digestible TSAA	0.95	0.89	0.89	0.89	0.82	0.82	0.82	0.80	0.75
Digestible Thr	0.86	0.78	0.78	0.78	0.73	0.73	0.73	0.68	0.64
Digestible Val	0.96	0.89	0.89	0.89	0.82	0.82	0.82	0.78	0.75
Digestible Ile	0.91	0.79	0.79	0.79	0.74	0.74	0.74	0.72	0.69
Digestible Arg	1.46	1.25	1.25	1.25	1.18	1.18	1.18	1.15	1.10
Digestible Trp	0.24	0.20	0.20	0.20	0.18	0.18	0.18	0.18	0.18
Ca	0.96	0.87	0.87	0.87	0.87	0.87	0.87	0.78	0.74
Available P	0.48	0.44	0.44	0.44	0.43	0.43	0.43	0.39	0.37
Na	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed nutrient composition (%)[3]									
Total Lys	---	1.45	1.28	0.99	1.29	1.14	1.15	---	---
Free Lys	---	0.27	0.13	0.02	0.26	0.12	0.03	---	---

[1] F = Finisher; W = Withdrawal;

[2] Values reported as percentages unless noted otherwise. Digestible amino acid values were determined from digestibility coefficients and analyzed total amino acid content of the ingredients (Ajinomoto, 2009).

[3] Grower 1 and grower 2 treatment diets were analyzed for concentrations of total Lys (AOAC, 2006; 982.30 E(a)) and free Lys concentrations (AOAC, 2000; method 999.13) at University of Missouri–Columbia Experiment Station Chemical Laboratory.

Table 5.3 Ingredient composition of diets fed to male Yield Plus × Ross 708 broilers from 1 to 61 d of age, experiment 2[1]

Days of age	Starter 1 to 11	Grower 1 12 to 28		Grower 2 28 to 40		F 41 to 48	W 49 to 61
Ingredient (%)		Diet 1	Diet 2	Diet 1	Diet 2		
Corn	58.50	64.27	64.27	68.25	68.25	70.82	73.07
Soybean Meal (48% CP)	36.87	28.59	28.59	24.03	24.03	21.68	19.88
Poultry Meal	---	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium Phosphate	1.84	1.39	1.39	1.19	1.19	1.10	1.06
Calcium Carbonate	0.80	0.63	0.63	0.59	0.59	0.57	0.58
Poultry Fat	0.51	0.73	0.73	1.62	1.62	1.57	1.18
Sodium Chloride	0.31	0.25	0.25	0.26	0.26	0.26	0.20
DL-Methionine	0.26	0.27	0.27	0.24	0.24	0.20	0.19
L-Lys·HCl	0.25	0.24	0.02	0.21	0.02	0.19	0.20
L-Thr	0.10	0.08	0.08	0.06	0.06	0.05	0.04
Sodium Sesquicarbonate	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin Premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral Premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Chloride	0.11	0.10	0.10	0.10	0.10	0.10	0.05
Salinomycin	0.05	0.05	0.05	0.05	0.05	---	---
Sand	---	---	0.22	---	0.19	---	---

[1]F = Finisher; W = Withdrawal; TBCC = Tribasic copper chloride

[2]Vitamin premix provided the following per kilogram of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopheryl acetate), 66 IU; Vitamin B12 (cyanocobalamin), 0.03 mg; D-biotin (biotin), 0.18 mg; menadione (menadione sodium bisulfate complex), 4 mg; thiamine (thiamine mononitrate), 5.5 mg; riboflavin (riboflavin), 22 mg; D-pantothenic acid (calcium pantothenate), 31 mg; pyridoxine (pyridoxine hydrochloride), 7.7 mg; niacin (niacinamide), 88 mg; folacin (folic acid), 2.6 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite), 0.3 mg.

Table 5.4. Calculated nutrient content of diets fed to male Yield Plus × Ross 708 broilers from 1 to 61 d of age, experiment 2[1]

	Starter	Grower		Finisher 1		F2	W
Days of age	1 to 11	12 to 28		28 to 40		41 to 48	49 to 61
Calculated nutrient composition (%)[2]		Diet 1	Diet 2	Diet 1	Diet 2		
AME_n (kcal/kg)	3,000	3,100	3,100	3,200	3,200	3,225	3,225
CP	23.4	21.5	21.5	19.63	19.63	18.7	18.0
Total Lys	1.40	1.28	1.06	1.14	0.95	1.07	1.14
Digestible Lys	1.28	1.15	0.98	1.02	0.87	0.95	0.91
Digestible Met	0.63	0.58	0.58	0.53	0.53	0.48	0.46
Digestible TSAA	0.95	0.87	0.87	0.80	0.80	0.74	0.71
Digestible Thr	0.86	0.77	0.77	0.68	0.68	0.64	0.61
Digestible Val	0.96	0.87	0.87	0.79	0.79	0.75	0.72
Digestible Ile	0.88	0.78	0.78	0.70	0.70	0.66	0.63
Digestible Arg	1.40	1.25	1.25	1.12	1.12	1.06	1.01
Digestible Trp	0.24	0.21	0.21	0.18	0.18	0.17	0.16
Ca	0.96	0.87	0.87	0.78	0.78	0.74	0.72
Available P	0.48	0.44	0.44	0.39	0.39	0.37	0.36
Na	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed nutrient composition (%)[3]							
Total Lys	1.55	1.37	1.25	1.18	1.01	1.12	1.14

[1]F2 = Finisher 2; W = Withdrawal;

[2] Values reported as percentages unless noted otherwise. Digestible amino acid values were determined from digestibility coefficients and analyzed total amino acid content of the ingredients (Ajinomoto, 2009).

[3]Treatment diets (grower and finisher 1) were analyzed for concentrations of total Lys by AMINOLab® (Evonik Industries, Kennesaw, USA).

Table 5.5 Growth performance of male broilers from 12 to 18 d of age after receiving reduced digestible Lys diets during portions of the grower phase, experiment 1[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	0.791[a]	0.538[a]	0.657[ab]	7.7[a]	2,038[ab]	1.222[bc]	0.0
2 85% G1	0.786[a]	0.533[a]	0.669[a]	6.6[b]	2,072[a]	1.255[b]	0.0
3 85% G2	0.795[a]	0.543[a]	0.652[abc]	7.6[a]	2,023[ab]	1.202[c]	0.0
4 85% G1 + G2	0.781[a]	0.527[a]	0.674[a]	6.7[b]	2,090[a]	1.274[ab]	1.0
5 75% G1	0.731[b]	0.480[b]	0.627[c]	5.5[c]	1,943[b]	1.309[a]	0.0
6 75% G2	0.782[a]	0.532[a]	0.664[a]	7.8[a]	2,060[ab]	1.233[bc]	1.0
7 75% G1 + G2	0.727[b]	0.476[b]	0.630[bc]	5.5[c]	1,953[b]	1.323[a]	0.0
SEM[3]	0.0098	0.0078	0.0071	0.08	27.6	0.0125	0.39
	---------- Probabilities ----------						
Diet Effect	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.11

[1]Values are least-square means of 9 replicate pens with 22 birds per pen at placement.

[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower 1 (G1; 12 to 18 d), grower 2 (G2; 19 to 26 d), finisher (27 to 42 d), and withdrawal (43 to 46 d). Starter, finisher, and withdrawal diets were identical in composition and nutrient content for all birds. Grower 1 and G2 treatments consisted of a control formulated at 100% of the recommendation for dLys and 6 feeding programs that received the specified proportion of recommended dLys concentrations during either G1, G2, or the entirety of the grower phase (G1 + G2).

[3]SEM = pooled standard error of the mean.

[a-d]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.6 Growth performance of male broilers from 12 to 26 d of age after receiving reduced digestible Lys diets during portions of the grower phase, experiment 1[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	1.477[a]	1.181[a]	1.663[ab]	18.7[a]	5,156[ab]	1.416[b]	1.1
2 85% G1	1.456[ab]	1.160[ab]	1.645[ab]	17.3[b]	5,101[ab]	1.422[b]	0.5
3 85% G2	1.435[ab]	1.143[ab]	1.617[ab]	16.6[bc]	5,013[ab]	1.416[b]	0.0
4 85% G1 + G2	1.450[ab]	1.157[ab]	1.673[a]	16.0[cd]	5,186[a]	1.429[b]	1.5
5 75% G1	1.388[b]	1.094[b]	1.592[bc]	16.0[cd]	4,937[bc]	1.447[b]	1.0
6 75% G2	1.399[b]	1.111[b]	1.633[ab]	15.7[d]	5,062[ab]	1.459[b]	1.5
7 75% G1 + G2	1.280[c]	0.989[c]	1.523[c]	12.9[e]	4,722[c]	1.542[a]	0.0
SEM[3]	0.0182	0.0162	0.0175	0.19	57.7	0.0166	0.69
	---------- Probabilities ----------						
Diet Effect	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.45

[1]Values are least-square means of 9 replicate pens with 22 birds per pen at placement.
[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower 1 (G1; 12 to 18 d), grower 2 (G2; 19 to 26 d), finisher (27 to 42 d), and withdrawal (43 to 46 d). Starter, finisher, and withdrawal diets were identical in composition and nutrient content for all birds. Grower 1 and G2 treatments consisted of a control formulated at 100% of the recommendation for dLys and 6 feeding programs that received the specified proportion of recommended dLys concentrations during either G1, G2, or the entirety of the grower phase (G1 + G2).
[3]SEM = pooled standard error of the mean.
[a-e]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.7 Growth performance of male broilers from 1 to 47 d of age after receiving reduced digestible Lys diets during portions of the grower phase, experiment 1[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	3.594	3.552	5.742	59.7[a]	17,885[ab]	1.599	4.0
2 85% G1	3.566	3.524	5.728	58.4[ab]	17,841[ab]	1.600	3.5
3 85% G2	3.585	3.543	5.697	57.4[b]	17,669[b]	1.572	4.0
4 85% G1 + G2	3.692	3.649	6.004	58.2[ab]	18,319[ab]	1.589	8.1
5 75% G1	3.581	3.540	5.692	57.1[b]	17,671[b]	1.579	4.5
6 75% G2	3.606	3.564	5.840	57.8[ab]	18,126[ab]	1.610	5.1
7 75% G1 + G2	3.536	3.495	5.644	54.4[c]	17,597[b]	1.609	4.0
SEM[3]	0.0472	0.0471	0.0839	0.51	53.7	0.017	1.68
	---------- Probabilities ----------						
Diet Effect	0.10	0.11	0.040	< 0.001	0.010	0.44	0.46

[1]Values are least-square means of 9 replicate pens with 22 birds per pen at placement.
[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower 1 (G1; 12 to 18 d), grower 2 (G2; 19 to 26 d), finisher (27 to 42 d), and withdrawal (43 to 46 d). Starter, finisher, and withdrawal diets were identical in composition and nutrient content for all birds. Grower 1 and G2 treatments consisted of a control formulated at 100% of the recommendation for dLys and 6 feeding programs that received the specified proportion of recommended dLys concentrations during either G1, G2, or the entirety of the grower phase (G1 + G2).
[3]SEM = pooled standard error of the mean.
[a-c]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.8 Growth performance of male broilers from 12 to 28 d of age after receiving reduced digestible Lys diets during portions of the grower and finisher phases, experiment 2[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	1.444[ab]	1.163[ab]	1.646	18.9[a]	5,103	1.471[b]	0.5
2 85% G	1.395[b]	1.114[b]	1.637	16.0[b]	5,075	1.527a	0.0
3 85% F1	1.477[a]	1.197[a]	1.667	19.1[a]	5,167	1.445b	0.6
4 85% G + F1	1.422[ab]	1.138[b]	1.661	16.3[b]	5,150	1.152a	2.6
SEM[3]	0.0218	0.0195	0.0197	0.22	63.0	0.0112	0.84
	---------- Probabilities ----------						
Diet Effect	0.024	0.007	0.59	< 0.001	0.60	< 0.001	0.14

[1] Values are least-square means of 6 replicate pens with 30 birds per pen at placement.
[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower (G; 12 to 28d), finisher 1 (F1; 29 to 40 d), finisher 2 (40 to 48 d), and withdrawal (49 to 61 d). Starter, finisher 2, and withdrawal diets were identical in composition and nutrient content for all birds. Grower and finisher 1 treatments consisted of a control formulated at 100% of the recommendation for dLys and 3 feeding programs that received 85% of the recommended dLys concentrations during either G, F1, or the entirety of the both phases (G + F1).
[3]SEM = pooled standard error of the mean.
[a-b]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.9 Growth performance of male broilers from 12 to 40 d of age after receiving reduced digestible Lys diets during portions of the grower and finisher phases, experiment 2[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	2.744	2.421[a]	3.885	41.8[a]	12,268	1.608[b]	0.5
2 85% G	2.639	2.317[b]	3.786	38.0[b]	11,951	1.634[ab]	1.1
3 85% F1	2.719	2.396[ab]	3.893	38.5[b]	12,292	1.626[ab]	0.6
4 85% G + F1	2.697	2.370[ab]	3.926	36.0[c]	12,398	1.660[a]	3.8
SEM[3]	0.0290	0.0283	0.0389	0.38	122.5	0.0117	1.02
	---------- Probabilities ----------						
Diet Effect	0.06	0.042	0.06	< 0.001	0.06	0.010	0.09

[1] Values are least-square means of 6 replicate pens with 30 birds per pen at placement.
[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower (G; 12 to 28d), finisher 1 (F1; 29 to 40 d), finisher 2 (40 to 48 d), and withdrawal (49 to 61 d). Starter, finisher 2, and withdrawal diets were identical in composition and nutrient content for all birds. Grower and finisher 1 treatments consisted of a control formulated at 100% of the recommendation for dLys and 3 feeding programs that received 85% of the recommended dLys concentrations during either G, F1, or the entirety of the both phases (G + F1).
[3]SEM = pooled standard error of the mean.
[a-b]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.10 Growth performance of male broilers from 1 to 61 d of age after receiving reduced digestible Lys diets during portions of the grower and finisher phases, experiment 2[1]

Feeding Program[2]	BW (kg)	BW Gain (kg)	Feed Intake (kg)	dLys Intake (g)	AME_n Intake (kcal)	FCR (kg:kg)	Mortality (%)
1 Control (100%)	4.545	4.504	8.778	88.2[a]	27,970	1.954	4.4
2 85% G	4.483	4.442	8.609	83.7[b]	27,430	1.874	6.6
3 85% F1	4.474	4.432	8.632	83.5[b]	27,500	1.924	4.9
4 85% G + F1	4.457	4.415	8.752	81.8[b]	27,888	1.848	10.6
SEM[3]	0.0570	0.0571	0.0786	0.73	252.5	0.0415	1.89
	---------- Probabilities ----------						
Diet Effect	0.72	0.71	0.35	< 0.001	0.35	0.29	0.11

[1] Values are least-square means of 6 replicate pens with 30 birds per pen at placement.
[2]Broilers received diets provided in 5 phases: starter (1 to 11 d), grower (G; 12 to 28d), finisher 1 (F1; 29 to 40 d), finisher 2 (40 to 48 d), and withdrawal (49 to 61 d). Starter, finisher 2, and withdrawal diets were identical in composition and nutrient content for all birds. Grower and finisher 1 treatments consisted of a control formulated at 100% of the recommendation for dLys and 3 feeding programs that received 85% of the recommended dLys concentrations during either G, F1, or the entirety of the both phases (G + F1).
[3]SEM = pooled standard error of the mean.
[a-b]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 5.11 Processing characteristics of male broilers harvested at 48 (Exp 1) or 62 (Exp 2) d of age after receiving reduced digestible Lys diets during portions of the grower and finisher phases[1]

		Fat		Carcass		Breast	
Feeding Program		kg	%	kg	%	kg	%
				Experiment 1[2]			
1	Control (100%)	0.044	1.2	2.750	75.4	0.995^{ab}	27.3^{a}
2	85% G1	0.048	1.3	2.744	75.5	0.991^{ab}	27.3^{a}
3	85% G2	0.045	1.2	2.745	75.4	0.991^{ab}	27.2^{a}
4	85% G1 + G2	0.046	1.3	2.799	75.3	1.017^{a}	27.4^{a}
5	75% G1	0.045	1.2	2.722	75.0	0.973^{bc}	26.8^{ab}
6	75% G2	0.045	1.2	2.753	75.3	0.990^{ab}	27.1^{ab}
7	75% G1 + G2	0.048	1.3	2.709	75.6	0.944^{c}	26.3^{b}
	SEM[3]	0.0013	0.04	0.0303	0.26	0.0135	0.198
				Experiment 2[3]			
1	Control (100%)	0.072	1.6^{b}	3.593	80.4	1.277	28.6
2	85% G	0.074	1.7^{a}	3.546	80.4	1.280	28.9
3	85% F1	0.071	1.6^{b}	3.534	80.2	1.266	28.6
4	85% G + F1	0.078	1.8^{a}	3.519	80.5	1.227	28.0
	SEM[4]	0.0020	0.04	0.0416	0.21	0.0209	0.266
		---------- Probabilities ----------					
Exp 1		0.29	0.19	0.25	0.61	0.001	0.003
Exp 2		0.10	0.022	0.64	0.86	0.30	0.22

[1] Exp = Experiment. Values are least-square means of 9 replicate pens (18 birds per TRT) for experiment 1 and 4 replicate pens (30 birds per pen) for experiment 2.
[2]In experiment 1, broilers received diets provided in 4 phases: starter (1 to 11 d), grower 1 (G1; 12 to 18 d), grower 2 (G2; 19 to 26 d), finisher (27 to 42 d), and withdrawal (43 to 46 d). Starter, finisher, and withdrawal diets were identical in composition and nutrient content. Grower 1 and G2 treatments consisted of a control formulated at 100% of the recommendation for dLys and 6 feeding programs that received the specified proportion of recommended dLys concentrations during either G1, G2, or the entirety of the grower phase (G1 + G2).
[3]In experiment 2, broilers received diets provided in 5 phases: starter (1 to 11 d), grower (G; 12 to 28 d), finisher 1 (F1; 29 to 40 d), finisher 2 (40 to 48 d), and withdrawal (49 to 61 d). Starter, finisher 2, and withdrawal diets were identical in composition and nutrient content for all birds. In experiment 2, G and F1 treatments consisted of a control formulated at 100% of the recommendation for dLys and 3 feeding programs that received 85% of the recommended dLys concentrations during either G, F1, or the entirety of the both phases (G + F1).
[4]SEM = pooled standard error of the mean.
[a-c]Means within each experiment not sharing a common superscript within a column differ significantly ($P < 0.05$).

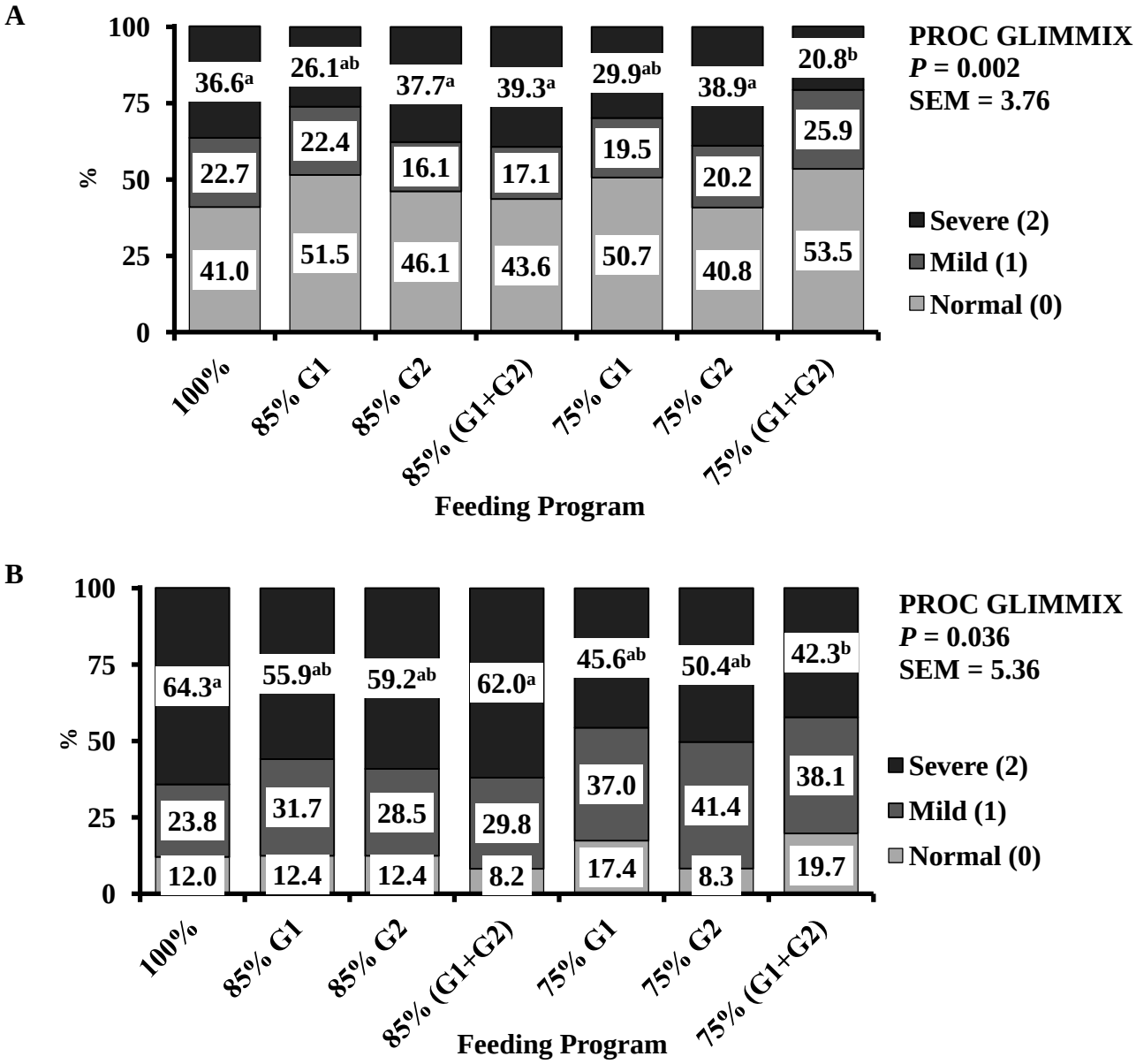

Figure 5.1 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast (A) and white striping (B) among male Yield Plus × Ross 708 broiler chickens at 48 d of age. Values are least square means of 9 replicate pens (18 birds per pen). Means were separated using Tukey's Honestly Significant Difference test. Broilers received diets provided in 4 phases: starter (1 to 11 d), grower 1 (G1; 12 to 18 d), grower 2 (G2;19 to 26 d), finisher (27 to 42 d), and withdrawal (43 to 46 d). Grower 1 and G2 treatments consisted of a Control formulated at 100% of the recommendation for dLys and 6 feeding programs that received the specified percentage (85% or 75%) of recommended dLys concentrations during either G1, G2, or the entirety of the grower phase (G1 + G2). Ideal amino acid ratios were not maintained in reduced dLys diets. Starter, finisher, and withdrawal diets were identical in composition and nutrient content for all treatments. SEM = pooled standard error of the mean. [a-b]Means within each panel not sharing a common superscript within across a scoring category differ significantly ($P < 0.05$).

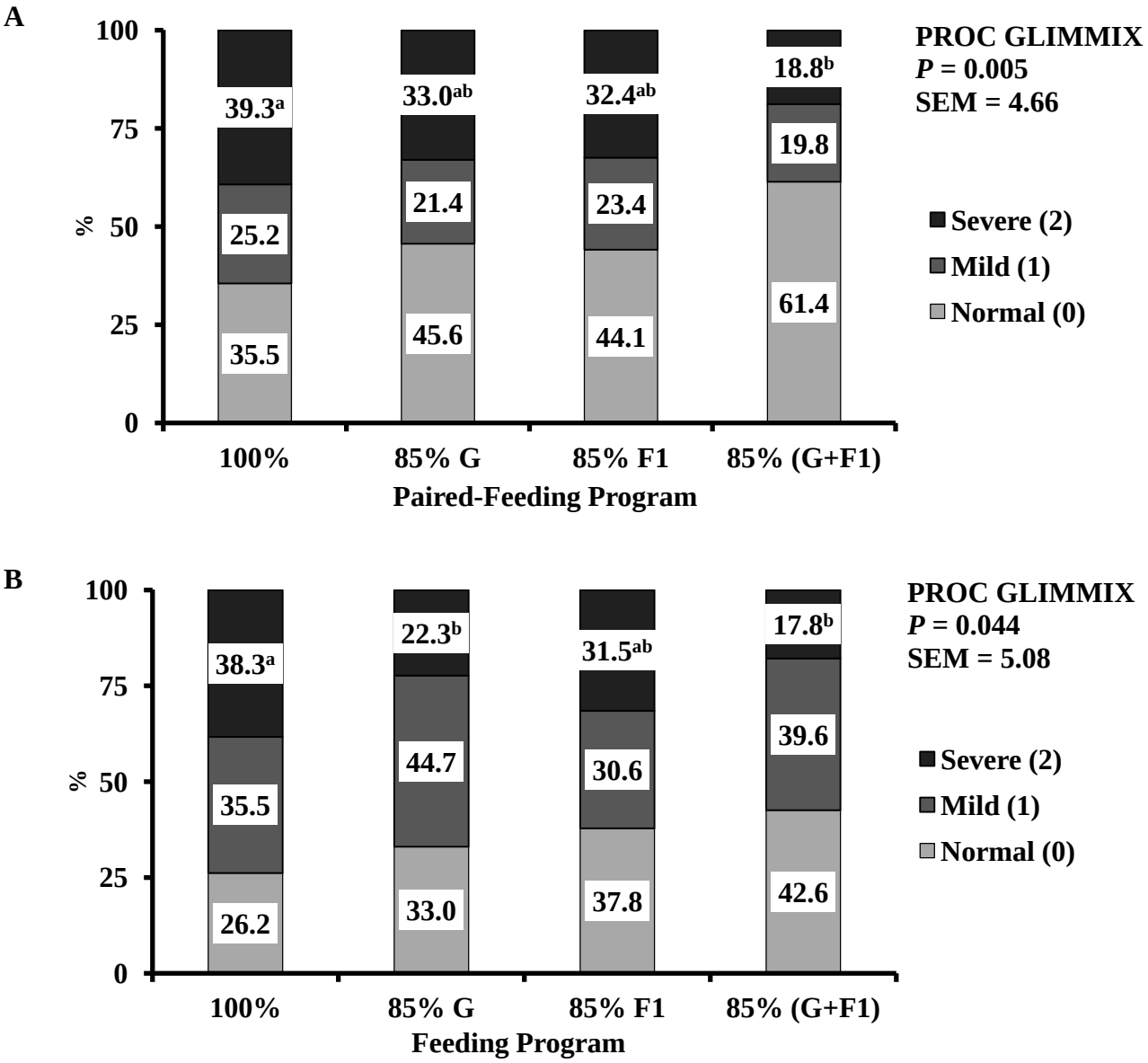

Figure 5.2 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast (A) and white striping (B) among male Yield Plus × Ross 708 broiler chickens at 61 d of age. Values are least square means of 4 replicate pens (30 birds per pen) at 62 d of age (Experiment 2; B). Means were separated using Tukey's Honestly Significant Difference test. Broilers received diets provided in 4 phases: starter (1 to 11 d), grower (G; 12 to 28 d), finisher 1 (F1; 29 to 40 d), finisher 2 (40 to 48 d), and withdrawal (49 to 61 d). Grower and F1 treatments consisted of a control formulated at 100% of the recommendation for dLys and 3 feeding programs that received 85% of the recommended dLys concentrations during either G, F1, or the entirety of the both phases (G + F1). Ideal amino acid ratios were not maintained in reduced dLys diets. Starter, finisher 2, and withdrawal diets were identical in composition and nutrient content for all treatments. SEM = pooled standard error of the mean. [a-b]Means within each panel not sharing a common superscript within across a scoring category differ significantly ($P < 0.05$).

VI. SKELETAL MUSCLE FIBER MORPHOMETRICS AND *IN VIVO* MYOGENIC STEM CELL MITOTIC ACTIVITY IN BROILER CHICKENS AFFECTED BY WOODEN BREAST

ABSTRACT

The degenerative myopathy known as WB has been increasingly observed in commercial broilers during the last decade. The presence of WB may be detrimental to raw product texture and further processing attributes, causing potential revenue losses to broiler integrators forced to downgrade affected breast fillets (PM). Although it has been suggested that WB may occur as a result of modern high-yielding broilers exceeding the capacity of breast muscle for hypertrophy, little information exists regarding heterogeneity and mitotic activity of myogenic stem cells known to play critical roles in muscle growth and repair. Previous research has demonstrated that WB may be induced or ameliorated by modulating dietary digestible Lys (dLys). Two concurrent experiments (Exp) were conducted to verify the effects of feeding 2 diets formulated to 75% and 100% of recommended dLys concentrations from 15 to 25 d of age on production responses and the incidence of WB (Exp 1), and to allow for characterization of stem cell populations and myofiber CSA on an individual basis in broilers affected by WB (Exp 2). At 25 and 43 d of age, 25 birds per treatment were injected intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU) 1 h prior to the collection of PM tissue to label mitotically active cells. The PM of each bird was weighed and processed for cryosectioning and immunofluorescence microscopy in order to determine myofiber

CSA, to enumerate Myf-5+ and Pax7+ myogenic stem cell populations, and to determine the mitotic activity (BrdU+) of these populations. The reduced dLys diet was successful in producing subpopulations of broilers with higher and lower incidences of WB within the same flock (Exp 1) with some detrimental effects on performance and processing characteristics. In Exp 2, broilers with severe WB had increased numbers ($P = 0.016$) and proportions ($P = 0.022$) of mitotically active myogenic stem cells, as well as increased proportions ($P < 0.05$) of large CSA myofibers relative to broilers unaffected by WB at 25 d of age. At 43 d of age, broilers affected by severe WB had a greater ($P = 0.011$) total population of myogenic stem cell types (Myf-5+, Pax7+, or Myf-5+:Pax7+) and a concurrent increase ($P = 0.007$) in the mitotic activity (Myf-5+:BrduU+, and Pax7+:BrduU+, and Myf-5+:Pax7+:BrduU+) of these cells. Additionally, a greater ($P < 0.05$) proportion of small CSA myofibers was observed in broilers with severe WB. These results provide evidence that myofiber CSA, as well as the heterogeneity and mitotic activity of myogenic stem cell populations were altered in the presence of WB. Results from this research may direct the focus of future investigations into the role of myogenic stem cells in the development of WB.

INTRODUCTION

Wooden breast is a novel myopathy of the PM (breast fillet) muscles in broiler chickens that is characterized by the replacement of functional skeletal muscle proteins with highly cross-linked collagen at a cellular level, leading to notable changes in the textural characteristics of the whole muscle (Sihvo et al., 2014). Breast meat not only accounts for as much as 31% of live bird weight in modern broilers, but is also a major source of revenue for broiler integrators. Although WB is a purely aesthetic quality defect, with no ramifications on the wholesomeness of breast fillets, it may negatively impact the sensory attributes of raw products, as well as cause issues in further processing (Kuttappan et al., 2016; Tijare et al., 2016).

Preliminary research concerning WB has focused predominantly on defining the meat quality (Mudal et al., 2014; Trocino et al., 2015; Tasoniero et al., 2016; Tijare et al, 2016) or histopathological (Sihvo et al., 2014; Soglia et al., 2015; Velleman and Clark, 2015; Clark and Velleman 2016) characteristics of affected tissue. Additionally, some researchers have begun to investigate the genetic basis of WB (Mutryn et al., 2015; Velleman and Clark, 2015; Bailey et al., 2015; Abasht et al., 2016, Clark and Velleman, 2016; Zambonelli et al.,2016). Velleman and Clark (2015) observed increased transcription of myogenic regulatory factors associated with the proliferation and differentiation of SC in breast muscle affected by WB. Satellite cells are myogenic stem

cells located on the periphery of myofibers, between the basement membrane and the sarcolemma (Mauro, 1961). Post-hatch hypertrophic growth of skeletal muscle requires the donation of nuclei from SC to existing myofibers. Additionally, SC mediate the regeneration of myofibers in response to muscle damage (Zammit et al., 2006). It has been suggested that the rate of breast muscle accretion in modern broilers may inhibit SC activation or exceed the regenerative capacity of the SC population present at hatch, predisposing the muscle tissue to chronic inflammation and degradation (Clark and Velleman, 2016). However, studies investigating this theory have focused exclusively on *in vitro* cell culture methodology. To our knowledge, no research has yet been conducted to assess the heterogeneity and mitotic activity of *in vivo* SC populations within the context of WB.

Although WB occurs spontaneously in multiple commercially available modern broiler stains under a variety of growing conditions worldwide, it can be reliably increased by feeding diets with high AA density (Meloche et al., 2014; Meloche et al., 2016a,b). Likewise, strategically reducing the concentration of AA critical to breast muscle accretion, such as dLys, during specific phases of growth can reduce the incidence of WB (Meloche et al., 2016c; Cruz et al., 2017). By modulating dietary density, it may be possible to produce broilers within the same flock that are differentially affected by WB as a suitable model for an *in vivo* comparison of SC populations in broilers reared under practical conditions. Therefore, 2 concurrent experiments **(Exp)** were conducted to verify the effects of the experimental diets on production responses and the incidence of WB (Exp 1) and to allow for characterization

of myogenic stem cell populations in the PM muscles of affected and unaffected birds on an individual basis (Exp 2).

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2016-2829).

Dietary Treatments

In each Exp, a dietary treatment previously shown to reduce the incidence of WB (Meloche et al., 2016c) was applied from 15 to 25 d of age. Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). Starter, grower 1, and finisher diets were identical in composition and were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations (Table 6.1). The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Digestible AA values were calculated by multiplying digestibility coefficients (Ajinomoto, 2009) to the calculated total AA content of each ingredient. The control diet for grower 2 was formulated to maintain digestible AA to dLys ratios of 51, 76, 67, 76, and 68 for dMet, dTSAA, dThr, dVal, and dIle, respectively (Table 6.2). Ideal digestible AA ratios to Lys were not maintained in the reduced dLys diet. The reduced dLys diet was created by replacing L-Lys·HCl with builder's sand. This formulation strategy allowed for the deletion of Lys from the diets without altering the concentrations of any other nutrients. Diets were corn-soybean meal-based and contained corn gluten meal and peanut meal as needed to force

adequate concentrations of L-Lys·HCl into the diet such that its replacement with sand would produce a 25% reduction in dLys. Corn gluten meal and peanut meal were also utilized in the common starter, finisher, and withdrawal diets to minimize the effects of changing ingredients between phases. Feed was provided in crumble form during the starter phase, and pelleted thereafter.

Experiment 1

Broiler Husbandry. Six hundred newly-hatched Yield Plus × Ross 708 (Aviagen Inc., Huntsville) male broiler chicks were obtained from a commercial hatchery and vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis. Chicks were randomly distributed into 24 floor pens (25 birds per pen; 0.08m^2/bird) and reared in a solid-sidewall facility with thermostatically controlled ventilation. Each pen was equipped with a hanging feeder, a nipple drinker line, and used litter. Birds consumed feed and water on an *ad libitum* basis. Ambient temperature was set at 33°C at placement and reduced to maintain comfort with advancing bird age, with a final setpoint of 20°C. Birds were exposed to a 23L:1D photoperiod from placement to 7 d of age, followed by an 18L:6D photoperiod for the remainder of the experiment. Light intensity was set at 30 lux from 1 to 7 d of age, 10 lux from 8 to 14 d of age, 5 lux from 15 to 24 d of age, and 3 lux from 25 to 48 d of age. Light intensity settings were verified at bird level (30 cm) using a photometric sensor with National Institute of Standards and Technology-traceable calibration (403125, Extech Instruments, Waltham, Mass) for each intensity adjustment.

Measurements. Birds and feed were weighed by pen at 1, 15, 25, and 40 d of age for the determination of BW gain and feed intake. Mortality was recorded daily and FCR were corrected for mortality. Prior to the experimental period (15 d of age), broilers were

reallocated to achieve 24 birds per pen. At 40 d of age, broilers were wingbanded for individual identification and feed was removed for a 12-h withdrawal period prior to processing. The following day, all birds were placed in coops, transported to the Auburn University Pilot Processing Plant, electrically stunned, exsanguinated, scalded, picked, and manually eviscerated. Carcasses were chilled on ice for 3 hours prior to measuring carcass and abdominal fat pad weights. The front-halves of the carcasses were then packed in ice for 18 hours. The weights of the PM (boneless breast) and *Pectoralis minor* (tender) muscles were recorded after excision by professional commercial deboners. Carcass and breast yields, as well as abdominal fat percentage, were calculated relative to live BW at 40 d of age. The PM muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for WS and WB. All fillets were scored by the same evaluator. For WS, the defect was characterized as "mild" if visible striping comprised less than half of the total fillet surface area or was less than 2 mm wide on average, but was considered "severe" if the striping exceeded these limits. For WB, the defect was considered "mild" if palpable hardness was present in less than half the total fillet surface area, but was considered "severe" if it exceeded this limit.

***Statistical Analysis*.** Dietary treatments arranged in a randomized complete block design with pen as the experimental unit and pen location as the blocking factor. Treatment and WB effects on live performance and processing characteristics were subject to analysis of variance using PROC MIXED of SAS 9.3 (SAS Institute, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu_{..} + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are the fixed factor level effects corresponding to the *j*th treatment or WB severity categoty such that $\Sigma\tau_j = 0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ. For live performance and processing characteristics, each dietary treatment was represented by 12 replicate pens (24 birds/pen).

Proportions of affected fillets in each scoring category were analyzed by PROC GLIMMIX (SAS Institute, 2009) using the events/experiments syntax with a binomial distribution and R-side covariance structure. Where applicable, residuals were visually assessed to ensure normality and nonnormal data were transformed prior to analysis. For all hypothesis tests, statistical significance was considered at $P \leq 0.05$.

Experiment 2

Broiler Husbandry. Four hundred eighty Yield Plus × Ross 708 (Aviagen Inc., Huntsville) male broiler chicks were obtained from a commercial hatchery. These chicks were contemporaries of those utilized concurrently in Exp 1. Chicks were vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis at the hatchery. Until 6 d of age, chicks were housed in groups of 8 in raised pens (0.03 m^2/bird) located in a solid-sided research facility with thermostatically controlled ventilation. Each pen was equipped with individual feeders, 2 water nipples, and clean wood shavings. At 7 d of age, all chicks were weighed and the upper and lower 12% of the BW range were excluded from the Exp. The remaining 360 chicks were identified using wing bands and allocated by weight into the individual-housing pens (0.20 m^2/bird) previously described above. Birds consumed feed and water on an *ad libitum* basis. Ambient temperature was

set at 33°C at placement and reduced to maintain comfort with advancing bird age, with a final setpoint of 20°C. Birds were exposed to a 23L:1D photoperiod from placement to 7 d of age, followed by an 18L:6D photoperiod for the remainder of the experiment. Light intensity was set at 30 lux from 1 to 7 d of age, 10 lux from 8 to 14 d of age, 5 lux from 15 to 24 d of age, and 3 lux from 25 to 48 d of age. Light intensity settings were verified at bird level (30 cm) using a photometric sensor with National Institute of Standards and Technology-traceable calibration (403125, Extech Instruments, Waltham, Mass) for each intensity adjustment.

Growth Performance. Birds and feed were weighed on an individual basis at 1, 15, 25, and 40 days of age for the calculation of BW gain and feed intake. Mortality was recorded daily and FCR were corrected for mortality.

Bromodeoxyuridine Injection and Muscle Tissue Collection. At 25 and 43 d posthatch, 1 hour prior to harvest, 25 birds per treatment were weighed and injected intraperitoneally with an aqueous solution (25 mg/mL) of 5'-bromo-2'-deoxyuridine (**BrdU**; Sigma Aldrich, St. Louis, MO; pH of 8.0; 100 µg of BrdU per g of body weight) to label mitotically active cells as described by Hutton et al., 2014. After BrdU injection, birds were placed in disposable containers for no longer than 1 h to allow mitotically active cells to incorporate the BrdU and become labeled for subsequent detection by immunohistochemistry and fluorescence microscopy. Following the cell-labeling period, birds were euthanized by CO_2 asphyxiation followed immediately by cervical dislocation and the PM muscles were excised, weighed, and samples were taken from the anteroventral area and processed for the cryohistological analyses according to procedures adapted from Hutton et al. (2014).

Cryohistological Analysis. Samples collected from the left PM were coated in talc, snap frozen in liquid nitrogen, and subsequently stored at −80°C. After being held at −20°C for 24 hours, samples were embedded in frozen section compound (VWR International, Westchester, PA) and cryosectioned using a Leica CM 1950 cryomicrotome. Serial cross-sections (5-µm thickness) were cut from each PM sample, mounted on positively charged glass slides (VWR International), and stored at 4°C prior to immunofluorescence staining.

Immunofluorescence Staining. Cryosections were fixed and stained according to the methods of Day et al. (2009) with minor modifications as described below. All procedures were conducted at room temperature in unless otherwise noted. Slides were rehydrated in PBS (pH 7.4; Invitrogen, Carlsbad, CA) for 10 min, fixed in paraformaldehyde (4% in PBS; VWR International) for 10 min, and then rinsed twice in PBS. Cryosections were then exposed to 0.5% Triton X-100 (VWR International) to permeabilize the cell membranes. Tissue sections were incubated in a blocking solution of 10% horse serum (Sigma-Aldrich), 2% bovine serum albumin (VWR International), and 0.2% Triton X-100 in PBS for 30 minutes in order to block non-specific antibody binding. Antibody reactions were conducted in a dark, humidified box. Primary antibodies diluted with blocking solution were allowed to react with tissue samples for 1 hour, followed by rinsing 3 times in PBS, for 5 minutes each rinse. Likewise, secondary antibodies diluted with blocking solution were allowed to react with tissue for 30 min followed by rinsing 3 times in PBS, for 5 minutes each rinse. All slides were briefly exposed to 4’,6-diamidino-phenylindole (DAPI; 1 ug/mL; VWR International) and immediately rinsed twice in PBS. Slides were mounted using Fluoro-gel Mounting Media

(VWR International), covered with thin glass coverslips (VWR International), and allowed to dry at 4°C overnight. All cryosections were imaged within 72 h of immunofluorescence staining.

Primary and Secondary Antibodies. Control cryosections processed in the manner described above, but without adding either primary or secondary antibodies, were used to ensure that no fluorescence signal beyond natural autofluorescence was observed for the selected combination of antibodies described below.

Primary antibodies were as follows: rabbit polyclonal anti-C terminus human Myf-5 (1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA); mouse anti-chicken Pax7 hybridoma cell supernatant (1:10 dilution; Developmental Studies Hybridoma Bank, Iowa City, IA); rat monoclonal anti-BrdU (1:250 dilution, Cedarlane Laboratories, Burlington, Ontario, Canada). Secondary antibodies (Invitrogen) used at a 1:1000 dilution to detect the primary antibodies were as follows: AlexaFluor 488-conjugated Goat anti-rabbit IgG heavy and light chain, AlexaFluor 546-conjugated goat anti-mouse IgG1, and AlexaFluor 633-conjugated goat anti-rat heavy and light chain.

Image Analysis. Immunofluorescence-stained cryohistological slides were imaged at 200X magnification using an inverted fluorescence microscope (Nikon Eclipse, T*i*-U; Nikon Instruments, Inc. Mellville, NY) equipped with a UV light source (Nikon Intensilight). Images were captured and analyzed using an Evolve 512 EMCCF camera (Photometrics, Tuscon, AZ) and Elements Imaging software (Nikon Instruments, Inc.). A representative image was captured from each slide. Cross-sectional area was measured for every myofiber contained entirely within the image and expressed on a μm^2-basis. Myf-5+, Pax7+, (Myf-5+:Pax7+), (Myf-5+:BrdU+), (Pax7+:BrdU+), and

(Myf-5+:Pax7+:BrdU+) cells were enumerated for each image. The total number of DAPI-stained nuclei were counted in each image as a measure of nuclear density.

Statistical Analysis. Performance data were evaluated as a randomized complete block design with each pen (1 bird/pen) as the experimental unit and pen location as the blocking factor. Treatment and WB effects on live performance were subject to analysis of variance using PROC MIXED of SAS 9.3 (SAS Institute, 2009) using the mixed-effects model described above for Exp 1. When separating means using unbalanced data (i.e. WB scores), the highest standard error of the pairwise comparisons is given in lieu of the standard error of the mean. Dietary effects were represented by 25 replicate pens (birds) per treatment at each tissue collection date. Wooden breast scores of 0, 1, and 2 were replicated by 20, 23, and 6 birds, respectively at the 25 d of age and 12, 17, and 16 birds, respectively, at 43 of age. Analysis of treatment and WB effects on the absolute counts of specific cell types per mm^2 were conducted in PROC MIXED with BW as a covariate. Treatment and WB effects on the proportions of specific cell types were conducted in PROC GLIMMIX using the events/experiments syntax with a binomial distribution and BW as a covariate. Likewise, treatment and WB effects on the relative distribution of myofiber CSA were analyzed within each histogram bin in PROC GLIMMIX using the events/experiments syntax with a binomial distribution and BW as a covariate. Where applicable, residuals were visually assessed to ensure normality and nonnormal data were transformed prior to analysis. For all hypothesis tests, statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

In each experiment, there were no differences ($P > 0.05$) among treatment groups prior to the experimental period (1 to 11 d).

Experiment 1

Live Performance. At 40 d of age, birds achieved final BW of 3.03 and 2.85 kg for 100% control group and those consuming 75% reduced dLys diet, respectively (Table 6.3). Broilers receiving the reduced dLys diet from 15 to 25 d of age had reduced ($P = 0.008$) BW gain and feed intake relative to the control. No differences ($P > 0.05$) in FCR or mortality were observed between treatments. In contrast, Cruz et al. (2017) fed a common starter diet formulated at 1.34% dLys followed by 6 diets varying solely in dLys (0.77 to 1.17% in increments of 0.08%) to male Cobb × Cobb 500 broilers from 12 to 28 d of age. Multiplying the dLys concentration fed in each phase by the number of days each diet was fed as a proportion of the period of interest results in a weighted dLys concentration across phases. For example, the 6 diets utilized by Cruz et al. (2017) had dLys concentrations of 1.02 to 1.21% in increments of 0.04% for the entire 1 to 35 d period. These authors determined that feeding a weighted dLys concentration of 1.05 from 1 to 40 d, resulted in reduced BW at 35 d of age relative to broilers receiving a diets containing 1.13% weighted dLys. These diets are comparable to the weighted dLys content of the diets fed to the reduced density group (1.05%) and control group (1.12%) from 1 to 40 d in the current study. In contrast, Meloche et al. (2016c) fed identical diets (1.09 and 0.82% dLys) to broilers from 12 to 26 d of age, but did not observe any differences in BW gain or feed intake at 47 d of age. It is likely that the earlier processing (40 d of age) in the current study resulted in a greater impact of the reduced dLys diet on

live performance, as birds had a shorter interval to compensate during the common finisher phase.

Processing Characteristics. The reduced dLys group also had lower ($P < 0.01$) carcass weights and yields, as well as reduced ($P < 0.001$) breast weights and yields compared with the control (Table 6.3). However, broilers receiving diets formulated at 75% of recommended dLys concentrations had increased ($P < 0.05$) abdominal fat weights and percentages. The effects of reduced dietary dLys on abdominal fat deposition have been previously observed (Mack et al. 1999; Dozier et al. 2010). For processing characteristics, Cruz et al. (2017) likewise reported reduced carcass weights, breast fillet weights, and breast yields at 35 d of age for broilers receiving weighted dLys concentrations comparable to that of the reduced density diet fed from 1 to 41 d of age in the current study, relative to the processing characteristics of broilers receiving a diet similar in weighted dLys to that of the control group in the current study. As with live performance, Meloche et al. (2016c) did not observe any differences in carcass weights, carcass yields, or abdominal fat weights and percentages at 48 d of age between broilers fed diets identical (1.09 and 0.82% dLys) to those in the current study from 12 to 25 d of age. However, these authors did report reduced breast weights and yields when evaluating broilers fed the comparable diets. The disparity in the results of these 2 similar studies is likely attributable to the differences in phase length and target body weight at slaughter, as the dLys concentrations used in both studies were identical.

Myopathy Scoring. Figure 6.1 illustrates the incidence of each severity score for both treatments. The overall flock incidences of normal, mild, and severe scores for WB were 33.6, 27.6, and 38.8%, respectively. The application of the reduced dLys diet from

15 to 25 d of age was intended to alter the growth trajectory of the birds during that phase such that a reduction in WB incidence and severity would occur at 40 d of age. Broilers receiving diets formulated at 75% of recommended dLys concentrations from 15 to 25 d of age had reduced ($P < 0.001$) incidence of severe scores (58.6 vs. 19.7%) relative to the control. Meloche et al. (2016c) reported a more modest reduction in severe scores (36.6 vs. 20.8%) at 48 d of age in broilers receiving diets identical to those in the current study from 12 to 26 d of age. These authors attributed the reduction in severe WB to a loss of 1% breast yield as a result of reduced dLys intake. In the current study, reduced dLys diets resulted in a 1.5% loss of breast yield, perhaps contributing to the greater reduction in severe WB observed at 41 d of age. Interestingly, Cruz et al. (2017) did not observe any differences in WB score at 35 d of age among broilers receiving diets comparable in weighted dLys content to those of the current study. The lower overall incidence of WB observed by these authors, particularly severe scores, may have obscured the treatment effects at 35 d of age.

The results of Exp 1 indicate that the dietary treatment applied from 15 to 25 d of age was successful in producing subpopulations of broilers with higher and lower incidences of WB within the same flock. However, the diets did have a detrimental effect on performance and processing characteristics. Nevertheless, the ability to ensure the presence of a sufficient number of affected and unaffected birds within a given flock was necessary for tissue collections conducted to evaluate myogenic stem cell populations *in vivo* on an individual basis, as in Exp 2.

Experiment 2

Among the birds selected for tissue collection at 25 and 42 d of age, the diets utilized in Exp 1 were successful in altering the observed proportions of normal, mild, and severe WB (Figure 6.2). There were sufficient numbers of birds in each scoring category to allow for comparisons of myogenic stem cell heterogeneity, mitotic activity, and myofiber CSA on the basis of both dietary treatment and WB score.

Growth Performance. Broilers selected for tissue sampling at 25 d of age had reduced ($P < 0.001$) BW and average daily gain **(ADG)** and increased ($P < 0.001$) FCR from 15 to 25 d of age when they consumed diets formulated at 75% of recommended dLys concentrations (Table 6.4). Additionally, broilers assigned to the reduced dLys diet had decreased ($P < 0.001$) PM weights. Live performance also varied concurrently with WB score, with higher ($P < 0.05$) BW, ADG, and PM weights for birds affected by severe WB vs. those unaffected by WB. It should be noted that, due to the progressive degenerative nature of WB, at 25 d of age, broilers are less likely to exhibit symptoms of severe WB. In the current study, only 6 of the selected birds were assigned a severe score at 25 d of age. Sihvo et al. (2017) observed increasing macroscopic and histological severity of WB with increasing age from 10 to 42 d of age. These authors also noted that, similar to the current study, few of the affected fillets at 24 d of age or less were categorized as severe on the basis of histological findings.

Myogenic Stem Cell Mitotic Activity. At 25 d of age, PM samples collected from broilers receiving diets formulated at 75% of dLys recommendations had greater ($P = 0.013$) numbers of non-myogenic cells, which included those that were not positive for either Myf-5 or Pax7 (Table 6.5). In contrast, PM tissue from the control group had a

higher (P = 0.028) concentration of mitotically-active myogenic stem cells (Myf-5+:BrdU+, Pax7+:BrdU+, and Myf-5+:Pax7+:BrdU+) as a proportion to the total number of nuclei (DAPI+) (Table 6.6). Increased mitotic activity of myogenic stem cells in the control group may be due to the increased ADG in response to high dietary density.

Tissue from broilers with severe WB had increased (P < 0.05) numbers of all mitotically-active cells (BrdU+), mitotically active Myf-5+ cells, and total (Table 6.7) mitotically-active myogenic stem cells (Myf-5+:BrdU+, Pax7+:BrdU+, and Myf-5+:Pax7+:BrdU+) relative to PM samples from broilers without WB. Furthermore, mitotically-active myogenic stem cells were also increased as a proportion of total nuclei (DAPI+) and as a proportion of total myogenic (Myf-5+, Pax7+, or Myf-5+:Pax7+) cells in the PM of broilers with severe WB relative to those unaffected by WB (Table 6.8). These differences are predominantly attributable to the mitotic activity of Myf-5+ cells, as broilers affected by severe WB also had increased Myf-5+:BrdU+ cells as a proportion of all Myf-5+ cells. No such variations were observed in the number or activity of the Pax7+ cell population. These observations fit well with the proposed upregulation of myogenic cell activity in the regeneration of damaged myofibers in broilers affected by WB (Velleman and Clark, 2015).

At 43 d of age, broilers selected for tissue collection had similar (P > 0.05) BW, ADFI, and ADG, from 15 to 43 and 25 to 43 d of age, regardless of dietary treatment (Table 6.9). However, broilers consuming diets formulated to 75% of recommended dLys concentrations had increased (P = 0.029) FCR from15 to 43 d of age. This effect was not observed on FCR from 25 to 43 d of age, indicating that the detrimental effects of feeding reduced dLys concentrations was isolated to the 15 to 25 d experimental period, but

nevertheless negatively impacted overall FCR at 43 d of age. Although both treatment groups attained similar BW at 43 d of age, broilers consuming the reduced dLys diet had smaller (P = 0.041) PM muscles. Relative to other muscles, the PM in modern broilers is particularly sensitive to dietary dLys, with increased protein turnover occurring in response to reduced dLys concentrations (Tesseraud et al., 2001). Although other portions of the carcass were not assessed in the current study, it is likely that the broilers on the reduced dLys diets accreted greater proportions of other muscles, such as those in the thigh and leg, as these muscles are not as severely affected by dietary dLys concentrations.

No differences in live performance characteristics were observed among birds varying in WB score. Although WB is often associated with higher BW at slaughter (Kuttappan et al., 2016), it is possible that affected and unaffected broilers in the current study attained similar BW at 43 d of age, but broilers receiving reduced dLys diets from 15 to 25 d of age did so less efficiently. Temporarily altering the growth trajectory during this critical window may have slowed the progress of myopathy, resulting in broilers of a similar BW to the control without developing the severe WB observed in the control birds.

No differences in cell type counts or proportions at 43 d of age were observed as a result of dietary treatment (Table 6.10 and Table 6.11). Interestingly, despite no differences in live performance, there were substantial differences in the cell populations of interest among broilers affected by varying degrees of WB (Table 6.12). Broilers affected by severe WB had increased numbers of nuclei (DAPI+; P = 0.050). Additionally, PM muscles with severe WB had a greater number total number of cells

expressing Myf-5 (P = 0.034). However, these differences were due to increases in the number of double positive (Myf-5+:Pax7+) cells concurrently expressing Myf-5 and Pax7 (P = 0.017), as the number of non-proliferative myogenic cells expressing Myf-5 only was similar among all WB scores. Similarly, these PM samples also had a greater total number of cells expressing Pax7 (P = 0.006), with increases in non-proliferative myogenic cells expressing Pax7 only (P = 0.041) as well as the aforementioned (Myf-5+:Pax7+) cells.

Broilers affected by severe WB had increased total numbers of mitotically active cells (BrdU+, P = 0.010). The difference in mitotically active cells was attributable to active double positive (Myf-5+:Pax7+:BrdU+) cells. These cells not only increased (P = 0.008) in number, but also as a proportion (P = 0.049) of all double positive (Myf-5+:Pax7+) cells, indicating the activation of this population of myogenic cells in broilers affected by WB (Table 6.13). As a whole, these results indicate an increase (P = 0.011) in the total population of myogenic cell types (Myf-5+, Pax7+, or Myf-5+:Pax7+) and a concurrent increase (P = 0.007) in the mitotic activity of these cells (Myf-5+:BrdU+, Pax7+:BrdU+, and Myf-5+:Pax7+:BrdU+). Although there is relatively little information available about these populations of myogenic stem cells in broiler chickens, these results are in agreement with previously observed increases in the expression of the myogenic regulatory factors MyoD and myogenin in broilers affected with WB (Velleman and Clark, 2015). These regulatory factors are expressed by SC during proliferation and differentiation, respectively, and have been associated with regenerative responses to myofiber damage (Velleman and Clark, 2015). Increased activity of the myogenic stem

cell populations suggests that these cells are functioning in a reparative capacity in tissue affected by WB.

Myofiber Cross-Sectional Area. At 25 d of age, differences in the distribution of observed myofiber CSA in the broilers selected for tissue sampling occurred due to dietary treatment as well as the presence of WB (Figure 6.3A), even after including BW as a covariate to account for its significant influence. Broilers receiving the reduced dLys diet had a right-skewed CSA distribution with decreased spread relative to that of the control group. These results correspond with the live performance data, as broilers receiving the control diet were larger at the end of the 15 to 25 d experimental period and therefore had a greater ($P < 0.05$) proportion of myofibers in the higher CSA range. Broilers affected by severe WB had decreased ($P < 0.05$) proportions of smaller CSA myofibers, whereas broilers without WB had decreased ($P < 0.05$) proportions of larger CSA myofibers (Figure 6.3B). Muscle tissue affected by WB often has increased numbers of giant myofibers (Sihvo et al., 2017), although such myofibers may occur normally in modern broilers, as well. However, the broilers receiving reduced dLys diets in the current study had less accretion of breast muscle at slaughter in Exp 1 as well as at 25 d of age in Exp 2, reducing their likelihood of having giant myofibers and skewing the distribution due to a smaller average CSA.

The distribution of myofiber CSA in the PM of broilers selected for tissue collection at 43 d of age was not affected ($P > 0.05$) by dietary treatment (Figure 6.4A). At this point, the effects of the reduced dLys diets fed during the 15 to 25 d experimental period were no longer apparent, resulting in similar distributions of CSA. In contrast to the results at 25 d of age, broilers affected by severe WB had increased ($P < 0.05$)

proportions of smaller CSA myofibers relative to those unaffected by WB at 43 d of age (Figure 6.4B). These results are likely reflective of the degenerative progression of WB, which increases in severity over time. As myofibers degenerate, they are replaced by regenerating myofibers of smaller diameter, causing an increase in the proportion of small myofibers observed in broilers with severe WB. Increased regenerating fibers in severe WB, in combination with the aforementioned increases in myogenic stem cell activity, indicates that WB is likely not caused by a dysfunction of these cells.

In conclusion, manipulation of dietary density successfully produced broilers with varying degrees of WB as a model for investigating myogenic cell populations on an individual basis. The heterogeneity and mitotic activity of Myf-5+ and Pax7+ myogenic stem cell populations were altered in the presence of WB. Additionally, broilers affected by severe WB have different CSA distributions as a result of the widespread myofiber degeneration and regeneration associated with the progression of this myopathy. Although the mechanism by which these changes occur is not yet understood, these insights may direct the focus of future investigations into the role of other cell populations within the muscle during the development of WB. Establishing a basic understanding of the etiology of WB is critical to support future research aimed at developing practical nutrition and management interventions to reduce its prevalence in the broiler industry.

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Table 6.1 Ingredient composition of diets fed to male Yield Plus × Ross 708 broilers from 1 to 40 d of age, experiment 1 and experiment 2

	Starter	Grower 1	Grower 2	Finisher	
	1 to 10 d	11 to 14 d	15 to 25 d	26 to 40 d	
Ingredient (%)			Diet 1	Diet 2	
Corn	52.07	58.86	62.33	62.33	62.07
Soybean Meal	36.27	25.64	23.54	23.54	25.51
Corn Gluten Meal	2.00	5.00	4.00	4.00	2.00
Peanut Meal	2.00	3.00	3.00	3.00	2.00
Poultry Fat[1]	3.15	2.98	2.63	2.63	4.53
Dicalcium Phosphate	2.02	1.86	1.88	1.88	1.64
Calcium Carbonate	1.05	0.98	0.99	0.99	0.88
Sodium Chloride	0.44	0.29	0.24	0.24	0.32
DL-Methionine	0.33	0.30	0.26	0.26	0.28
L-Lys·HCl	0.23	0.38	0.35	---	0.23
L-Thr	0.10	0.11	0.10	0.10	0.07
L-Val	0.00	0.02	0.01	0.01	---
Sodium Bicarbonate	0.00	0.23	0.29	0.29	0.19
Vitamin premix[2]	0.10	0.10	0.10	0.10	0.10
Mineral premix[3]	0.10	0.10	0.10	0.10	0.10
Choline Chloride[4]	0.07	0.10	0.11	0.11	0.08
TBCC[5]	0.02	0.02	0.02	0.02	0.02
Salinomycin	0.05	0.05	0.05	0.05	---
Sand	---	---	---	0.35	---

[1]Poultry fat was added at 0.75% in the mixer, with the remainder spray-added post-pelleting.

[2]Vitamin premix provided the following per kilogram of diet: Vitamin A (Vitamin A acetate), 18,739 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopheryl acetate), 66 IU; Vitamin B12 (cyanocobalamin), 0.03 mg; D-biotin (biotin), 0.18 mg; menadione (menadione sodium bisulfate complex), 4 mg; thiamine (thiamine mononitrate), 5.5 mg; riboflavin (riboflavin), 22 mg; D-pantothenic acid (calcium pantothenate), 31 mg; pyridoxine (pyridoxine hydrochloride), 7.7 mg; niacin (niacinamide), 88 mg; folacin (folic acid), 2.6 mg.

[3]Mineral premix includes per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (stabilized ethylenediamine dihydriodide), 1.4 mg; Se (sodium selenite), 0.3 mg.

[4]Choline chloride-60 (Balchem Corporation, New Hampton, NY)

[5]Tribasic copper chloride (Micronutrients, Inc., Indianapolis, IN)

Table 6.2 Calculated nutrient content of diets fed to male Yield Plus × Ross 708 broilers from 1 to 40 d of age, experiment 1 and experiment 2

Calculated nutrient composition (%)[1]	Starter 1 to 10 d	Grower 1 11 to 14 d	Grower 2 15 to 25 d		Finisher 26 to 40 d
			Diet 1	Diet 2	
AME_n (kcal/kg)	3,000	3,100	3,100	3,100	3,200
CP	24.4	22.5	21.1	21.1	20.2
Digestible Lys	1.28	1.17	1.09	0.82	1.02
Digestible Met	0.65	0.61	0.55	0.55	0.54
Digestible TSAA	0.95	0.89	0.82	0.82	0.80
Digestible Thr	0.86	0.78	0.73	0.73	0.68
Digestible Val	0.96	0.89	0.82	0.82	0.78
Digestible Ile	0.91	0.79	0.74	0.74	0.72
Digestible Arg	1.46	1.25	1.18	1.18	1.15
Digestible Trp	0.24	0.20	0.18	0.18	0.18
Ca	0.96	0.87	0.87	0.87	0.78
Available P	0.48	0.44	0.43	0.43	0.39
Na	0.20	0.20	0.20	0.20	0.20

[1]Values reported as percentages unless noted otherwise. Digestible amino acid values were determined from digestible coefficients and calculated total amino acid content of the ingredients (Ajinomoto, 2004).

[2]AMEn = nitrogen-corrected apparent metabolizable energy

Table 6.3 Effects of reduced digestible Lys from 15 to 25 d of age on growth performance and processing characteristics of male broilers from 1 to 40 d of age, experiment 1[1]

Live Performance[2]	100% dLys	75% dLys	SE[3]	*P*-Value
BW (kg)	3.028	2.848	0.0536	0.008
BW Gain (kg)	2.984	2.807	0.0381	0.008
Feed Intake (kg)	4.574	4.402	0.0514	0.038
Feed Conversion Ratio (kg:kg)	1.494	1.532	0.0136	0.06
Mortality(%)	5.3	4.1	1.1	0.47
Carcass Characteristics[4]				
Carcass Weight (kg)	2.267	2.119	0.2319	< 0.001
Carcass Yield (%)	75.0	74.20	0.24	0.009
Breast Weight (kg)	0.764	0.679	0.0116	< 0.001
Breast Yield (%)	25.2	23.7	0.19	< 0.001
Abdominal Fat Weight (kg)	0.036	0.038	0.0009	0.013
Abdominal Fat Percentage (%)	1.2	1.3	0.03	< 0.001
Wooden Breast Score[5]	1.38	0.73	0.060	< 0.001

[1] Values are least-square means of 12 replicate pens per treatment with 24 birds per pen.

[2] Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). All birds received common starter, grower 1, and finisher diets that were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations. The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Ideal digestible amino acid ratios to Lys were not maintained in the reduced dLys diet.

[3]SE = standard error of the treatment difference.

[4]Carcass yield, breast yield, and abdominal fat percentage were calculated using 40d live weight.

[5]After deboning, the *Pectoralis major* muscles of each bird were palpated and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for severity of wooden breast.

Table 6.4. Effects of reduced digestible Lys and severity of wooden breast on growth performance of male broilers from 15 to 25 d of age, experiment 2[1]

	Dietary Treatment[2]				Wooden Breast[3]				
Growth Performance	100% dLys	75% dLys	SE[4]	*P*-Value	Normal (0)	Mild (1)	Severe (2)	SE[4]	*P*-Value
					n = 20	*n* = 23	*n* = 6		
BW (kg)	1.459	1.296	0.0241	< 0.001	1.308[b]	1.390[b]	1.555[a]	0.0488	< 0.001
BW Gain (kg)	0.845	0.693	0.0184	< 0.001	0.707[c]	0.783[b]	0.915[a]	0.0396	< 0.001
Average Daily Gain (kg)	0.085	0.069	0.0018	<0.001	0.071	0.078	0.092	0.0040	< 0.001
Feed Intake (kg)	1.270	1.231	0.0387	0.46	1.244	1.252	1.259	0.0790	0.98
Average Daily Feed Intake (kg)	0.127	0.123	0.0039	0.47	0.124	0.125	0.126	0.0079	0.98
Feed Conversion Ratio (kg:kg)	1.504	1.842	0.0956	0.015	1.830	1.613	1.369	0.1871	0.08
Pectoralis major Weight (kg)	0.209	0.181	0.0053	< 0.001	0.188[b]	0.196[ab]	0.214[a]	0.0097	0.040

[1]Values are least-square means of 23 individually housed broilers per dietary treatment and the specified *n* per wooden breast scoring category. Where applicable, means were separated using Tukey's Honestly Significant Difference Test.

[2]Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). All birds received common starter, grower 1, and finisher diets that were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations. The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Ideal digestible amino acid ratios to Lys were not maintained in the reduced dLys diet.

[3]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.

[4]SE = highest standard error of the pair-wise comparisons.

[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$).

Table 6.5 Effects of reduced digestible Lys from 15 to 25 d of age on density (per mm2) and mitotic activity of Myf-5+ and Pax7+ myogenic stem cells in the Pectoralis major at 25 d of age, experiment 2[1]

Population[1]	Immunofluorescence Profile[2]	100% dLys	75% dLys	SE[3]	*P*-Value
Total Nuclei	DAPI+	1,853	1,967	91.8	0.23
Total Active	BrdU+	733	650	65.6	0.21
Total Non-myogenic	DAPI+ only and DAPI:BrdU+ only	745	930	72.7	0.013
Non-myogenic, Inactive	DAPI+ only	590	775	62.0	0.005
Non-myogenic, Active	DAPI:BrdU+ only	155	155	24.3	0.88
Total Myogenic	Myf-5+, Pax7+, Myf-5:Pax7+, Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	1,108	1,031	74.5	0.32
Myogenic, Inactive	Myf-5+, Pax7+, and Myf-5:Pax7+	524	536	52.7	0.81
Myogenic, Active	Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	584	495	59.0	0.14
Total Myf-5+	Myf-5+	1,037	948	74.5	0.25
Myf-5+, Active	Myf-5:BrdU+ only	495	411	54.8	0.14
Myf-5+, Inactive	Myf-5+ only	441	447	50.1	0.92
Total Pax7+	Pax7+	137	143	11.3	0.46
Pax7+, Active	Pax7+:BrdU+ only	24	18	5.4	0.53
Pax7+, Inactive	Pax7+ only	48	66	8.4	0.10
Total Myf-5+:Pax7+	Myf-5+:Pax7+	89	101	10.7	0.36
Myf-5+:Pax7+, Active	Myf-5+:Pax7+:BrdU+ only	60	60	8.3	0.74
Myf-5+:Pax7+, Inactive	Myf-5+:Pax7+ only	36	30	4.8	0.18

[1]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof.

[2]DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.

[3]SE = highest standard error of the pair-wise comparisons.

Table 6.6 Effects of reduced digestible Lys from 15 to 25 d of age on relative density (%) and mitotic activity of Myf-5+ and Pax7+myogenic stem cells in the Pectoralis major at 25 d of age, experiment 2

Population[1]	100% dLys	75% dLys	SE[2]	*P*-Value
Myogenic, Active (%, Total DAPI)	31.5	25.0	1.91	0.028
Myogenic, Active (%, Total Myogenic)	52.6	47.7	2.4	0.21
Pax7+, Active (%, Total Pax7+)	16.5	13.3	2.4	0.35
Myf-5+, Active (%, Total Myf-5+)	48.0	43.6	2.5	0.26
Myf-5:Pax7+, Active (%, Total Myf-5:Pax7+)	63.4	67.0	2.8	0.48
Pax7+, Active (%, Total, Active)	3.1	2.9	0.5	0.89
Myf-5+, Active (%, Total Active)	67.7	63.7	2.3	0.25

[1]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof. DAPI = 4’6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5’-bromo-2’-deoxyuridine.

[2]SE = highest standard error of the pair-wise comparisons.

Table 6.7 Effects of wooden breast severity on density (per mm2) and mitotic activity of Myf-5+ and Pax7+ myogenic stem cells in the Pectoralis major at 25 d of age, experiment 2[1]

Population[2]	Immunofluorescence Profile[3]	Normal (0)	Mild (1)	Severe (2)	SE[4]	*P*-Value
		n = 20	*n* = 23	*n* = 6		
Total Nuclei	DAPI+	1,865	1,931	1,973	143	0.66
Total Active	BrdU+	608[b]	715[ab]	876[a]	94.8	0.018
Total Non-myogenic	DAPI+ only and DAPI:BrdU+ only	852	840	793	119.8	0.90
Non-myogenic, Inactive	DAPI+ only	709	679	632	103.7	0.76
Non-myogenic, Active	DAPI:BrdU+ only	143	161	161	37.5	0.68
Total Myogenic	Myf-5+, Pax7+, Myf-5:Pax7+, Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	1,013	1,091	1,180	113.2	0.31
Myogenic, Inactive	Myf-5+, Pax7+, and Myf-5:Pax7+	548	537	465	80.5	0.54
Myogenic, Active	Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	465[b]	554[ab]	715[a]	85.2	0.016
Total Myf-5+	Myf-5+	936	1,013	1,097	113.2	0.30
Myf-5+, Active	Myf-5:BrdU+ only	381[b]	471[ab]	638[a]	78.1	0.007
Myf-5+, Inactive	Myf-5+ only	453	453	375	69.1	0.53
Total Pax7+	Pax7+	143	137	143	17.3	0.74
Pax7+, Active	Pax7+:BrdU+ only	18	24	18	7.7	0.65
Pax7+, Inactive	Pax7+ only	60	54	66	13.1	0.32
Total Myf-5+:Pax7+	Myf-5+:Pax7+	101	95	83	16.7	0.64
Myf-5+:Pax7+, Active	Myf-5+:Pax7+:BrdU+ only	60	60	60	13.1	0.99
Myf-5+:Pax7+, Inactive	Myf-5+:Pax7+ only	36	36	24	7.7	0.18

[1]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.

[2]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof.

[3]DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.

[4]SE = highest standard error of the pair-wise comparisons.

[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$).

Table 6.8 Effects of wooden breast severity on relative density (%) and mitotic activity of Myf-5+ and Pax7+myogenic stem cells in the Pectoralis major at 25 d of age, experiment 2[1]

Population[2]	Normal (0)	Mild (1)	Severe (2)	SE[3]	*P*-Value
	n = 20	*n* = 23	*n* = 6		
Myogenic, Active (% of Total DAPI)	24.9[b]	28.5[b]	36.9[a]	3.9	0.03
Myogenic, Active (% of Total Myogenic)	45.8[b]	50.7[b]	61.2[a]	4.3	0.022
Pax7+, Active (% of Total Pax7+)	13.7	16.6	11.9	3.9	0.45
Myf-5+, Active (% of Total Myf-5+)	41.0[b]	46.2[b]	58.9[a]	4.3	0.007
Myf-5:Pax7+, Active (% of Total Myf-5:Pax7+)	63.4	65.7	72.4	4.9	0.36
Pax7+, Active (% of Total, Active)	3.2	3.2	1.9	0.7	0.39
Myf-5+, Active (% of Total Active)	62.9	65.6	73.2	3.5	0.11

[1]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.

[2]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof. DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.

[3]SE = highest standard error of the pair-wise comparisons.

[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$)..

Table 6.9 Effects of reduced digestible Lys from 15 to 25 d of age and severity of wooden breast on mitotic activity of Myf-5+ and Pax7+ cells in the Pectoralis major at 43 d of age, experiment 2[1]

		Dietary Treatment[2]				Wooden Breast[3]				
Growth Performance[4]		100% dLys	75% dLys	SE[5]	*P*-Value	Normal (0)	Mild (1)	Severe (2)	SE[5]	*P*-Value
						n = 12	*n* = 17	*n* = 16		
BW (kg)		3.543	3.481	0.0655	0.51	3.559	3.542	3.444	0.0910	0.56
	BW Gain (kg)	2.949	2.874	0.0625	0.40	2.962	2.932	2.852	0.0872	0.60
	ADG (kg)	0.105	0.103	0.0022	0.39	0.106	0.105	0.102	0.0031	0.60
15 to 43	Feed Intake (kg)	4.695	4.848	0.0962	0.27	4.784	4.821	4.704	0.1189	0.76
	ADFI (kg)	0.168	0.173	0.0034	0.26	0.171	0.172	0.168	0.0050	0.78
	FCR (kg:kg)	1.590	1.699	0.0340	0.029	1.632	1.653	1.643	0.0515	0.95
	BW Gain (kg)	2.074	2.170	0.0537	0.21	2.208	2.213	2.050	0.0743	0.28
	ADG (kg)	0.742	0.775	0.0019	0.23	0.079	0.076	0.073	0.0026	0.29
25 to 43	Feed Intake (kg)	1.270	1.231	0.0387	0.46	3.422	3.385	3.445	0.0876	0.85
	ADFI (kg)	0.118	0.124	0.0025	0.11	0.122	0.121	0.120	0.0037	0.84
	FCR (kg:kg)	1.594	1.603	0.0314	0.84	1.561	1.598	1.628	0.0442	0.52
Pectoralis major Weight (kg)		0.686	0.636	0.0161	0.004	0.625	0.672	0.675	0.0214	0.06

[1]Values are least-square means of 23 individually housed broilers per dietary treatment. Where applicable, means were separated using Tukey's Honestly Significant Difference Test.

[2]Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). All birds received common starter, grower 1, and finisher diets that were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations. The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Ideal digestible amino acid ratios to Lys were not maintained in the reduced dLys diet.

[3]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.

[4]ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio

[5]SE = highest standard error of the pair-wise comparisons.

[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$).

Table 6.10 Effects of reduced digestible Lys from 15 to 25 d of age on density (per mm2) and mitotic activity of Myf-5+ and Pax7+ myogenic stem cells in the Pectoralis major at 43 d of age, experiment 2[1]

Population[1]	Immunofluorescence Profile[2]	100% dLys	75% dLys	SE[3]	*P*-Value
Total Nuclei	DAPI+	1,913	1,830	131.7	0.53
Total Active	BrdU+	918	894	74.5	0.71
Total Non-myogenic	DAPI+ only and DAPI:BrdU+ only	739	703	78.1	0.63
Non-myogenic, Inactive	DAPI+ only	578	524	64.4	0.43
Non-myogenic, Active	DAPI:BrdU+ only	161	179	23.8	0.56
Total Myogenic	Myf-5+, Pax7+, Myf-5:Pax7+, Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	1,150	1,126	75.1	0.55
Myogenic, Inactive	Myf-5+, Pax7+, and Myf-5:Pax7+	393	411	34.3	0.90
Myogenic, Active	Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	757	715	59.6	0.49
Total Myf-5+	Myf-5+	1,085	1,049	71.5	0.53
Myf-5+, Active	Myf-5:BrdU+ only	620	584	50.7	0.42
Myf-5+, Inactive	Myf-5+ only	340	340	31.6	0.94
Total Pax7+	Pax7+	185	179	7.2	0.80
Pax7+, Active	Pax7+:BrdU+ only	30	36	7.7	0.46
Pax7+, Inactive	Pax7+ only	54	48	7.7	0.41
Total Myf-5+:Pax7+	Myf-5+:Pax7+	125	119	19.1	0.73
Myf-5+:Pax7+, Active	Myf-5+:Pax7+:BrdU+ only	101	95	17.3	0.70
Myf-5+:Pax7+, Inactive	Myf-5+:Pax7+ only	24	24	4.8	0.99

[1]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof.

[2]DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.

[3]SE = highest standard error of the pair-wise comparisons.

Table 6.11 Effects of reduced digestible Lys from 15 to 25 d of age on relative density (%) and mitotic activity of Myf-5+ and Pax7+myogenic stem cells in the Pectoralis major at 43 d of age, experiment 2

Population[1]	100% dLys	75% dLys	SE[2]	*P*-Value
Myogenic, Active (%, Total DAPI)	39.5	39.1	1.54	0.88
Myogenic, Active (%, Total Myogenic)	64.6	63.6	1.69	0.67
Pax7+, Active (%, Total Pax7+)	17.5	21.1	1.7	0.12
Myf-5+, Active (%, Total Myf-5+)	57.3	55.9	1.83	0.58
Myf-5:Pax7+, Active (%, Total Myf-5:Pax7+)	80.5	80	2.6	0.89
Pax7+, Active (%, Total, Active)	3.5	4.3	0.55	0.32
Myf-5+, Active (%, Total Active)	67.7	65.2	2.12	0.41

[1]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof. DAPI = 4’6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5’-bromo-2’-deoxyuridine.

[2]SE = highest standard error of the pair-wise comparisons.

Table 6.12 Effects of wooden breast severity on density (per mm2) and mitotic activity of Myf-5+ and Pax7+ myogenic stem cells in the Pectoralis major at 43 d of age, experiment 2[1]

Population[2]	Immunofluorescence Profile[3]	Normal (0)	Mild (1)	Severe (2)	SE[4]	*P*-Value
		n = 12	*n* = 17	*n* = 16		
Total Nuclei	DAPI+	1,651[b]	1,847[ab]	2,056[a]	159.7	0.05
Total Active	BrdU+	757[b]	882[ab]	1037[a]	86.4	0.01
Total Non-myogenic	DAPI+ only and DAPI:BrdU+ only	667	697	793	99.5	0.40
Non-myogenic, Inactive	DAPI+ only	513	548	590	83.4	0.65
Non-myogenic, Active	DAPI:BrdU+ only	155	149	203	29.2	0.09
Total Myogenic	Myf-5+, Pax7+, Myf-5:Pax7+, Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	983[b]	1,150[ab]	1,263[a]	87.6	0.011
Myogenic, Inactive	Myf-5+, Pax7+, and Myf-5:Pax7+	381	417	429	43.9	0.53
Myogenic, Active	Myf-5:BrdU+, Pax7:BrdU+, and Myf-5:Pax7:BrdU+	602[b]	733[ab]	834[a]	69.1	0.008
Total Myf-5+	Myf-5+	930[b]	1,067[ab]	1,162[a]	85.8	0.034
Myf-5+, Active	Myf-5:BrdU+ only	507	614	656	62.0	0.07
Myf-5+, Inactive	Myf-5+ only	328	340	352	40.5	0.87
Total Pax7+	Pax7+	125[b]	173[ab]	238[a]	32.2	0.006
Pax7+, Active	Pax7+:BrdU+ only	24	36	42	9.5	0.09
Pax7+, Inactive	Pax7+ only	36[b]	48[ab]	60[a]	9.5	0.041
Total Myf-5+:Pax7+	Myf-5+:Pax7+	89[b]	113[ab]	155[a]	22.1	0.017
Myf-5+:Pax7+, Active	Myf-5+:Pax7+:BrdU+ only	72[b]	83[b]	131[a]	19.7	0.008
Myf-5+:Pax7+, Inactive	Myf-5+:Pax7+ only	18	30	24	6.0	0.44

[1]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.
[2]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof.
[3]DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.
[4]SE = highest standard error of the pair-wise comparisons.
[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$).

Table 6.13 Effects of wooden breast severity on relative density (%) and mitotic activity of Myf-5+ and Pax7+myogenic stem cells in the Pectoralis major at 43 d of age, experiment 2[1]

Population[2]	Normal (0)	Mild (1)	Severe (2)	SE[3]	*P*-Value
	n = 12	*n* = 17	*n* = 16		
Myogenic, Active (% of Total DAPI)	36.5	39.8	40.4	2.2	0.37
Myogenic, Active (% of Total Myogenic)	61.3	64.0	65.9	2.5	0.34
Pax7+, Active (% of Total Pax7+)	17.5	21.1	18.8	2.7	0.53
Myf-5+, Active (% of Total Myf-5+)	55.0	57.6	56.6	2.7	0.75
Myf-5:Pax7+, Active (% of Total Myf-5:Pax7+)	77.6[ab]	75.5[b]	85.0[a]	3.9	0.049
Pax7+, Active (% of Total, Active)	2.9	4.1	4.3	0.7	0.38
Myf-5+, Active (% of Total Active)	67.1	69.6	63.3	3.1	0.18

[1]The *Pectoralis major* muscles of each bird were visually assessed and scored on a 3-point scale (0 = none; 1 = mild; 2 = severe) for wooden breast.

[2]Total = Every cell positive for the specified immunofluorescence target, regardless of the status of other targets; Non-myogenic = not positive for Myf-5, Pax7,or any combination thereof; Inactive = mitotically inactive as indicated by lack of BrdU; Active = mitotically active as indicated by BrdU+; Myogenic = positive for Myf-5, Pax7,or any combination thereof. DAPI = 4'6-diamindino-phenylindole. All subsequent populations may be assumed to be DAPI+; BrdU = 5'-bromo-2'-deoxyuridine.

[3]SE = highest standard error of the pair-wise comparisons.

[a-b]Means within the same row that do not share a common superscript are significantly different ($P < 0.05$)..

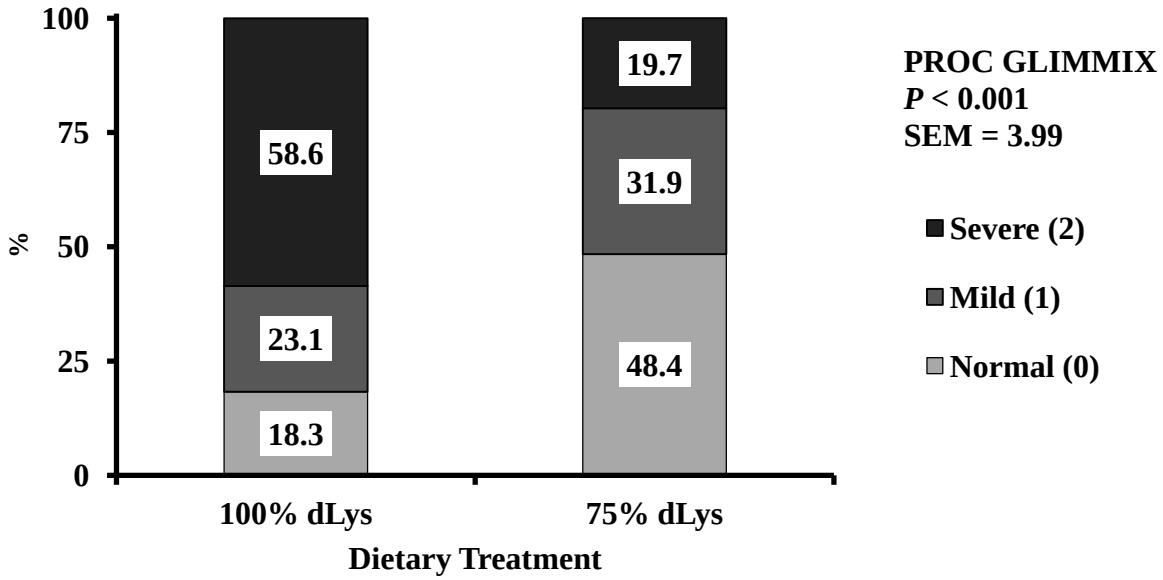

Figure 6.1 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast among male Yield Plus × Ross 708 broiler chickens at 41 d of age (experiment 1). Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). All birds received common starter, grower 1, and finisher diets that were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations. The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Ideal digestible amino acid ratios to Lys were not maintained in the reduced dLys diet. SEM = pooled standard error of the mean.

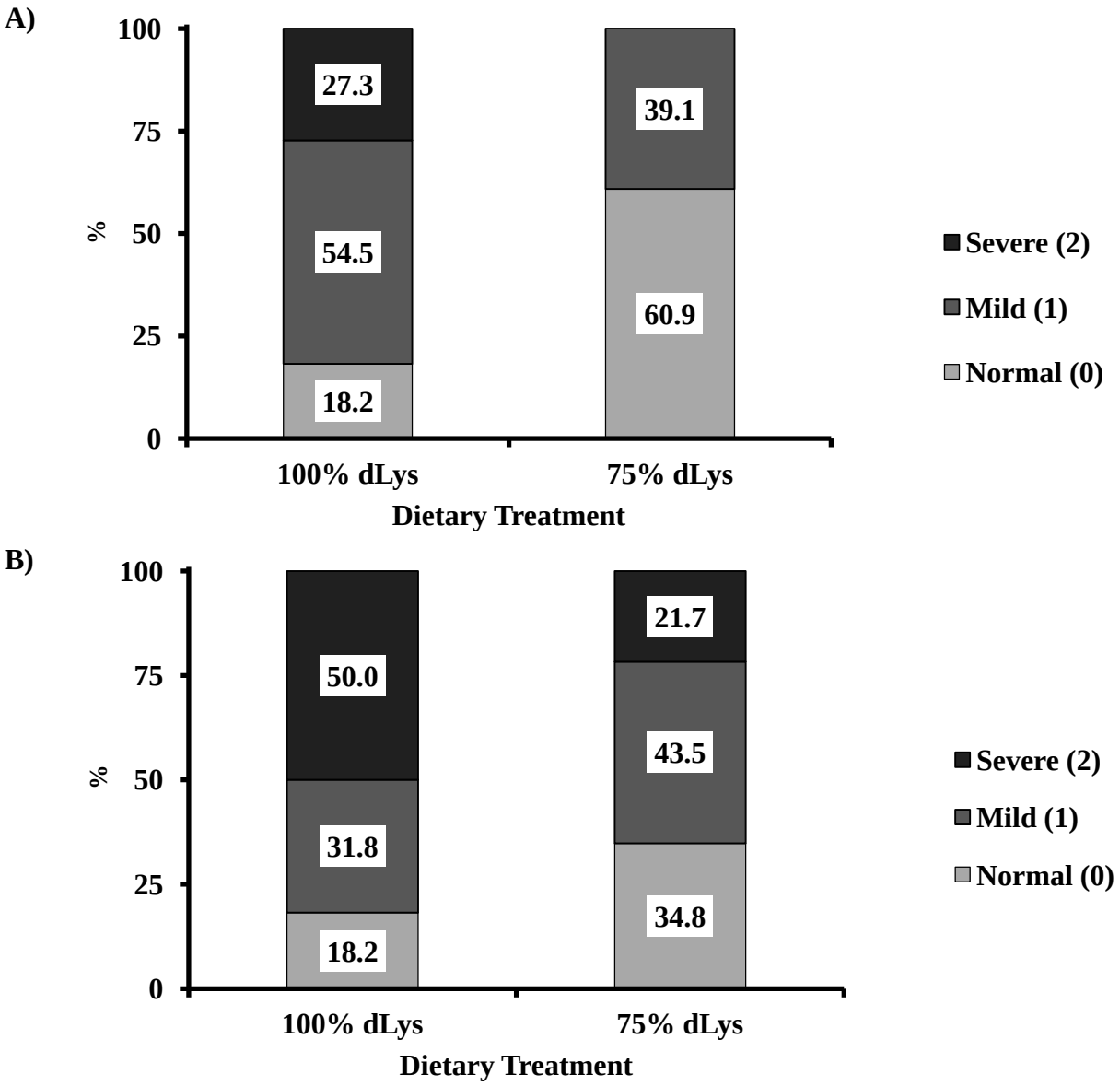

Figure 6.2 Proportions of observed Normal (0), Mild (1), and Severe (2) scores for wooden breast among male Yield Plus × Ross 708 broiler chickens at 25 (A) and 43 (B) d of age reared in individual raised pens for subsequent use in tissue sampling (experiment 2). Broilers received diets provided in 4 phases: starter (1 to 10 d), grower 1 (11 to 14 d), grower 2 (15 to 25 d), and finisher (26 to 40 d). All birds received common starter, grower 1, and finisher diets that were formulated to meet or exceed the primary breeder recommendations for all nutrients. Grower 2 treatments consisted of a control formulated at 100% of the primary breeder recommendation for dLys and a reduced density diet formulated at 75% of recommended dLys concentrations. The experimental grower 2 diets were identical in concentrations of all other nutrients except dLys. Ideal digestible amino acid ratios to Lys were not maintained in the reduced dLys diet.

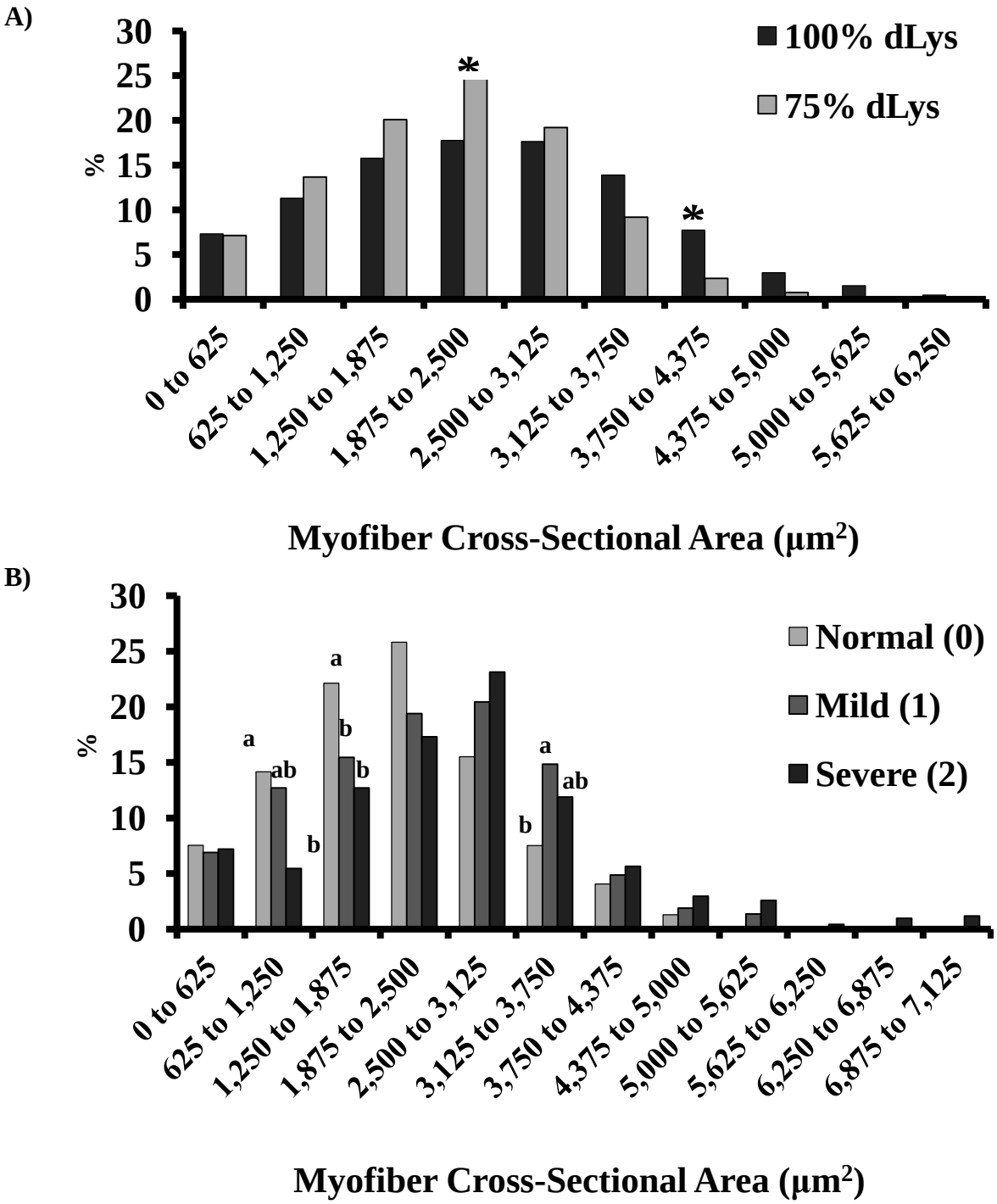

Figure 6.3 Distribution of myofiber cross-sectional areas in *Pectoralis major* muscles of male Yield Plus × Ross 708 broiler chickens selected for tissue sampling at 25 d of age after receiving diets formulated at 100% or 75% of recommended dLys concentrations from 15 to 25 d of age, experiment 2. Myofibers are clustered in bin intervals of 500 µm^2. Treatment effects (A) and effects of wooden breast (B) were evaluated using PROC GLIMMIX on the proportion of total myofibers in each bin with BW as a covariate (*P < 0.05). $^{a-b}$Means within the same bin that do not share a common superscript are significantly different (P < 0.05).

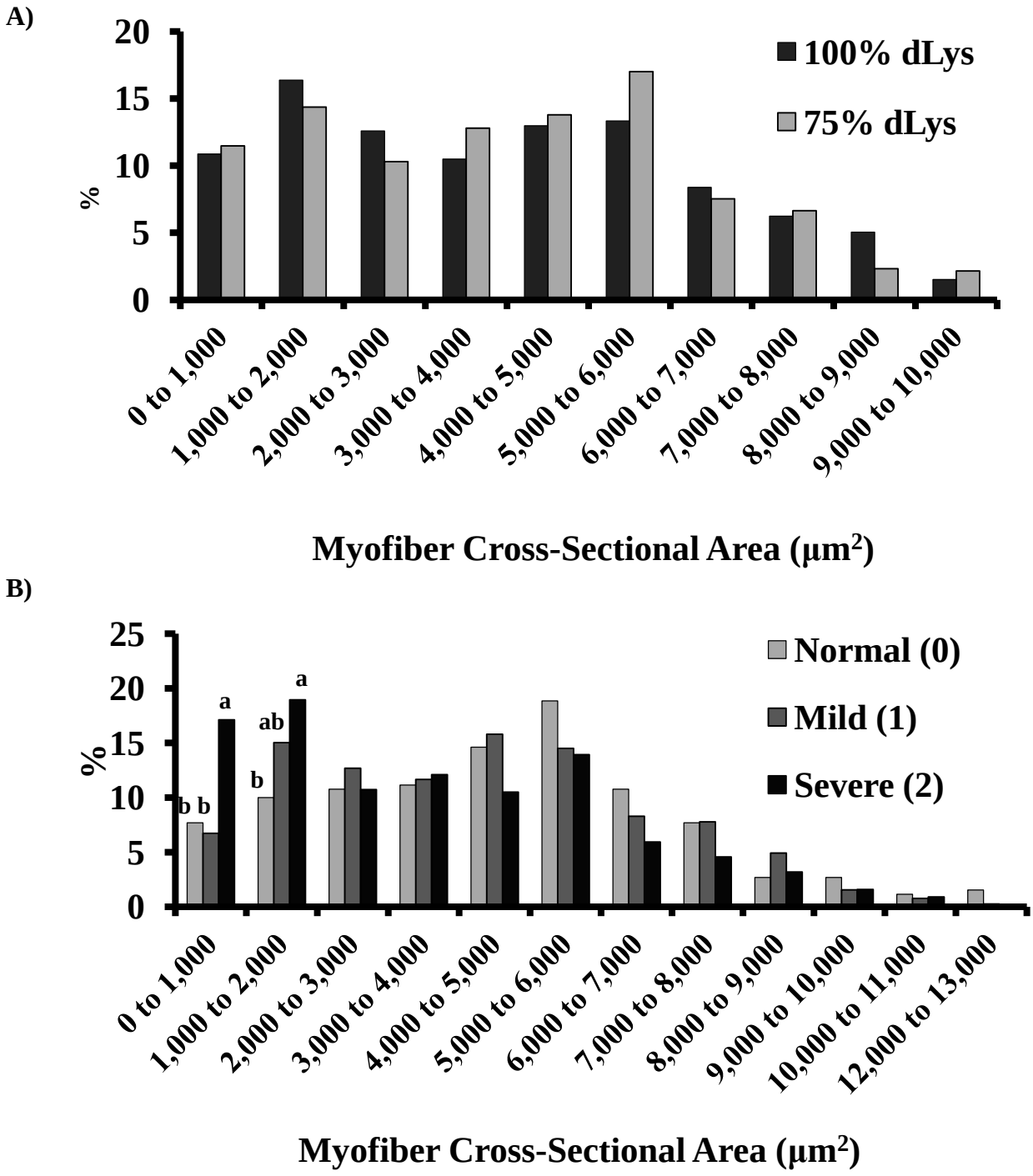

Figure 6.4 Distribution of myofiber cross-sectional areas in *Pectoralis major* muscles of male Yield Plus × Ross 708 broiler chickens selected for tissue sampling at 43 d of age after receiving diets formulated at 100% or 75% of recommended dLys concentrations from 15 to 25 d of age, experiment 2. Myofibers are clustered in bin intervals of 1,000 µm^2. Treatment effects (A) and effects of wooden breast (B) were evaluated using PROC GLIMMIX on the proportion of total myofibers in each bin with BW as a covariate (*P < 0.05). $^{a-b}$Means within the same bin that do not share a common superscript are significantly different ($P < 0.05$).

VII. CONCLUSIONS

Wooden breast and WS continue to challenge broiler integrators worldwide. Although it has been proposed that these myopathies are related to genetic selection for increased breast meat yield, the poor heritability and weak genetic correlations for these myopathies indicate that practical nutritional and management interventions may be more effective at reducing the incidence of myopathy in the short term.

Experiment 1 tested the hypothesis that differences in growth trajectory during critical windows of development may account for some of the observed variation in WB and WS. A paired-feeding program was used to create linear decreases in feed intake, which subsequently resulted in linear decreases BW and concomitant decreases in WB and WS at 33, 43, and 50 d of age. However, similar quantitative allocation strategies may have limited practical applicability in some locations, as most broiler growers in the U.S. are not equipped to allocate feed by weight on a daily basis. Therefore, experiment 2 attempted to alter the growth trajectory through reductions in nutrient allocation obtained qualitatively through the manipulation of dietary AME_n and AA density. However, in these experiments, broilers receiving the lower density diets displayed a notable capacity for compensatory feed intake, overcoming the formulated reductions in dietary density. Indeed, the compensatory response to the reduced density diets actually resulted in these broilers consuming more dLys than their unrestricted counterparts, exacerbating the incidence and severity of WB in some cases.

To circumvent the confounding compensatory intake, the third experiment investigated the use of diets that modulated the concentration of a single nutrient, rather than dietary density as a whole. In this experiment, dLys was reduced relative to the recommendations of the primary breeder, without altering the density of AME_n. Additionally, ratios of digestible AA to dLys were not maintained in the diets containing reduced dLys. Reducing dLys to 75% of primary breeder recommendations from 18 to 26 d or to 85% of primary breeder recommendations from 28 to 40 d substantially decreased the incidence of severe WB and WS at 48 or 61 d of age, respectively. These results demonstrate that the intended market weight of a flock must be considered a factor when applying a timed reduction in dLys density. It has been suggested that WB may occur as a result of modern high-yielding broilers exceeding the capacity of breast muscle for hypertrophy. However, little information exists in the literature regarding the activity of specific populations of myogenic cells important to muscle growth and repair. Within the same flock, broilers that were differentially affected by WB were produced using the reduced dLys diets identified in experiment 3. These birds provided a suitable model for comparing myofiber CSA and the relative populations of myogenic cells in tissue that was affected or not affected by WB. Increased populations Myf-5+ and Pax7+ myogenic cell populations, as well as increased mitotic activity, were observed in the presence of severe WB. Furthermore broilers affected by severe WB had increased proportions of large CSA myofibers at 25 d of age and increased proportions of abnormally small CSA myofibers at 43 d of age.

Collectively, these results indicated that the growth trajectory can be modulated by reducing dietary dLys as a strategy to reduce the severity of WB and WS. However, it

should be noted that the reduction in dietary dLys utilized in these Exp were based upon the recommendations of the primary breeder. Application of a similar strategy to diets used in the U.S. broiler industry may not result in comparable reductions in myopathies, depending on the dLys concentrations used. The results of these experiments also provide evidence that although myogenic stem cell populations do function differently in tissue affected by WB, their role is likely reparative rather than causative. Future research should focus on elucidating the underlying mechanism by which WB and WS develop in order to further investigate viable nutrition and management interventions to decrease the prevalence of these myopathies in the broiler industry.