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EFFECT OF LOWERING DIETARY LYSINE OR ENERGY AND AMINO ACID DENSITY DURING DIFFERENT GROWTH PHASES ON INCIDENCE AND SEVERITY OF WHITE STRIPING AND FOOTPAD DERMATITIS IN BROILERS

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APPROVAL

We, the jury members, certify the contents and form of the doctoral thesis titled "Effect of Lowering Dietary Lysine or Energy and Amino Acid Density During Different Growth Phases on Incidence and Severity of White Striping and Footpad Dermatitis in Broilers" prepared by Umair AHSAN, student at Department of Animal Nutrition and Nutritional Diseases, Institute of Health Sciences, Aydın Adnan Menderes University, Republic of Turkey.

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ABBREVIATIONS

AA	:	Amino acid
a*	:	Redness
AME_n	:	Apparent metabolizable energy corrected for nitrogen
ANOVA	:	Analysis of variance
ATP	:	Adenosine triphosphate
b*	:	Yellowness
BW	:	Body weight
CHOL	:	Total cholesterol
cm	:	Centimeter(s)
d	:	Day(s)
DNA	:	Deoxyribonucleic acid
dLys	:	Digestible lysine
FCR	:	Feed conversion ratio
FI	:	Feed intake
FPD	:	Footpad dermatitis
g	:	Gram
h	:	Hour(s)
kcal	:	Kilocalorie
kg	:	Kilogram
Lys	:	Lysine
L*	:	Lightness
ME	:	Metabolizable energy
min	:	Minute(s)
mm	:	Millimeter(s)
MyoD1	:	Myogenic differentiation factor 1
nm	:	Nanometer
rpm	:	Rotations per minute
SEM	:	Standard error of the mean
TCA	:	Tricarboxylic acid
TG	:	Triglycerides
WHC	:	Water holding capacity
wk(s)	:	Week(s)
WS	:	White striping
ºC	:	Degree celsius
>	:	Greater than
<	:	Less than
%	:	Percent

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ÖZET

ETLİK PİLİÇLERDE FARKLI BÜYÜME DÖNEMLERİNDE RASYONDA LİZİN DÜZEYİ VEYA ENERJİ İLE AMİNO ASİT YOĞUNLUĞUNUN DÜŞÜRÜLMESİNİN BEYAZ ÇİZGİ VE AYAK TABANI YANGISI OLUŞUM SIKLIĞI VE ŞİDDETİ ÜZERİNE ETKİSİ

Ahsan U. Aydın Adnan Menderes Üniversitesi Sağlık Bilimleri Enstitüsü Hayvan Besleme ve Beslenme Hastalıkları Programı, Doktora Tezi, Aydın, 2020.

Bu çalışmada etlik piliçlerde büyütme veya büyütme ve bitirme dönemlerinde rasyonda sindirilebilir lizin (dLys) veya metabolize edilebilir enerji (ME) ve amino asit (AA) yoğunluğunun azaltılmasının beyaz çizgi (WS) ile ayak tabanı yangısı (FPD) oluşum sıklığı ve şiddeti üzerine etkilerinin incelenmesi amaçlanmıştır. Toplam 390 Ross 308 erkek etlik civciv her birinde 13 hayvan bulunan 6 tekrar grubundan oluşan 5 deneme grubuna rastgele dağıtılmıştır. Gruplarından biri kontrol grubu olarak başlangıç, büyütme, bitirme 1 ve bitirme 2 dönemleri için Aviagen (2019)'da belirtilen besin madde gereksinimlerine göre beslenmiştir. Kalan gruplar ise %15 dLys veya %5 ME ve AA yoğunluğu içeren büyütme (GRO low Lys ve GRO low ME AA) vey büyütme ve bitirme 1 (GROFIN low Lys ve GROFIN low ME AA) rasyonlarla beslenmiştir. 0-49. günler arasında, GROFIN low Lys grubu GRO low Lys hariç diğer gruplara kıyasla daha düşük (P = 0,010) canlı ağırlık artışı göstermiştir. Göğüs eti randımanı GROFIN low Lys grubunda en düşük iken, GRO low Lys rasyonu ile beslenen etlik piliçlerde kontrol ve GROFIN low ME AA gruplarına göre daha düşük (P < 0,001) bulunmuştur. Göğüs eti ham protein düzeyi, GRO low ME AA ve GROFIN low ME AA rasyonları ile beslenen etlik piliçlerde GRO low Lys grubuna göre düşük (P = 0,045) bulunmuştur. GRO low Lys ve GROFIN low Lys rasyonlar ile beslenen etlik piliçlerde göğüs eti ham yağ düzeyi daha az (P = 0,006) bulunmuştur. GRO low Lys ve GROFIN low Lys gruplarındaki hayvanların diğer gruplara kıyasla daha düşük (P < 0,001) WS insidensi ve şiddetine sahip olduğu belirlenmiştir. Etlik piliçlerde büyütme döneminde rasyon dLys düzeyinin kısıtılanması canlı ağırlığı, yem tüketimi, göğüs eti ham yağ düzeyi ve yağ depozisyonu azaltarak WS oluşumunu azalttığı sonucuna varılmıştır.

Anahtar kelimeler: Amino asit, ayak tabanı yangısı, beyaz çizgi, lizin, metabolize edilebilir enerji

ABSTRACT

EFFECT OF LOWERING DIETARY LYSINE OR ENERGY AND AMINO ACID DENSITY DURING DIFFERENT GROWTH PHASES ON INCIDENCE AND SEVERITY OF WHITE STRIPING AND FOOTPAD DERMATITIS IN BROILERS

Ahsan U. Animal Nutrition and Nutritional Diseases Program, Institute of Health Sciences, Aydın Adnan Menderes University, Ph. D. Thesis, Aydın, 2020.

The objective of this study was to assess the effect of reducing dietary digestible lysine (dLys) or metabolizable energy (ME) and amino acid (AA) density during grower or grower and finisher growth phases on incidence and severity of white striping (WS) and footpad dermatitis (FPD) in broiler chickens. A total of 390 one-d-old Ross 308 male broilers were randomly distributed into 5 groups (6 replicate per group; 13 chicks per pen). Control group was fed diets according to the nutrient requirements for starter, grower, finisher, and withdrawal phases. Remaining groups received diets 15% low in dLys or 5% ME and AA density during grower (GRO low Lys and GRO low ME AA groups) or grower and finisher phases (GROFIN low Lys and GROFIN low ME AA groups). At 0-49 d, GROFIN low Lys group had lower body weight (BW) gain in comparison with other dietary treatments except for GRO low Lys (P = 0.010). Breast yield was lowest in GROFIN low Lys group, whereas broilers fed GRO low Lys diets had lower breast yield than those in control and GROFIN low ME AA groups ($P < 0.001$). Crude protein was lower in broiler chickens fed diets with low ME and AA density than those fed GRO low Lys diets (P = 0.045). Crude fat content of breast meat decreased in broiler chickens fed diets low in dLys content (P = 0.006). Birds in GRO low Lys and GROFIN low Lys groups had lower incidence and severity of WS in comparison with other dietary treatments ($P < 0.001$). It is concluded that reducing the dietary dLys levels during grower phase reduces the development of WS in broiler chickens by lowering the BW, FI, fat content of breast meat, and fat deposition.

Keywords: Amino acid, footpad dermatitis, lysine, metabolizable energy, white striping

1. INTRODUCTION

Advances in nutrition and genetic selection of chicken have improved the global food security. Simultaneously, these developments have increased the issues of animal welfare, meat quality, and economical losses due to the problems like breast myopathies and footpad dermatitis, the modern broiler industry is facing today. White striping (WS) is defined by the macroscopic appearance of fatty white striations between muscle fibers of breast muscle that traverse parallel to the course of muscle fibers (Kuttappan et al., 2016). The appearance of WS has adverse effects on the physical and sensory properties of meat (Petracci and Cavani, 2012), thereby reducing consumer acceptance (Kuttappan et al., 2012a), suitability for processing (Petracci et al., 2013a; Mudalal et al., 2015; Alnahhas et al.., 2016), and profitability (Kuttappan et al., 2016). The precise cause of WS was unclear until recently, although the existence of markers for dietary deficiencies, switching of fiber-type, hypoxia, and oxidative stress had been reported (Kuttappan et al., 2012b; 2013a; Russo et al., 2015). Metabolomics of WS confirmed that hypoxia is the main root cause of WS that initiates the development of white striations. Localized hypoxia disrupts the oxidation of fatty acids, arginine and taurine metabolism, osmolytes, and tricarboxylic acid (TCA) cycle that results in the accumulation of harmful fatty acids in the breast muscles of fast growing or heavy broiler chickens (Boerboom et al., 2018). Petracci et al. (2015) outlined the various predisposing factors, including genotype, sex, dietary energy levels, body weight (BW) at slaughter, and growth intensity.

Footpad dermatitis (FPD) is a condition of the skin in broilers and turkeys, primarily characterized by irritation and inflammation of footpads on the plantar surface of the feet. Several internal and external risk factors pose threat to the footpads of broilers and turkeys causing FPD lesions that are important not only from an economic perspective, these are significant as food safety, product quality, and animal welfare concerns (Shepherd and Fairchild, 2010). It has been reported that male broiler chickens are more vulnerable to develop FPD lesion due to their heavier body weight (Nagaraj et al., 2007). Similarly, diets with low protein and amino acids (AA) reduce the occurrence of FPD in broiler chickens by lowering the nitrogen content of feces and litter (Shao et al., 2018).

Several attempts have been made to prevent the appearance of WS and FPD that have been unable to fully prevent these conditions in the broiler chickens. Since the WS occurs because of fast growth of breast muscles, and FPD due to nutritional factors and heavier body

weight of broiler chickens, therefore, the manipulation of growth trajectory of broiler chickens is the suitable method to prevent the development of WS and FPD. The incidence of WS can be reduced through quantitative feed restriction (Meloche et al., 2015), however, it is an arduous task due to lack of facilities at farms. Consequently, qualitative nutrient allocation seems to be a practical solution to overcome this issue. Reduction in dietary energy has reduced the incidence of WS in breast meat of broiler chickens (Kuttappan et al., 2012b). Nevertheless, intermittent or continuous feeding of diets with lower apparent metabolizable energy corrected for nitrogen (AME_n) and AA density affects the growth performance of broiler chickens (Kuttappan et al., 2012b; Meloche et al., 2018a). In addition, intermittent feeding of low AME_n and AA density diets increased the incidence of WS (Meloche et al., 2018a). Similarly, continuous reduction of dietary allocation of digestible lysine (dLys) or AA density deteriorates the growth performance, carcass yield, and prevents the development of WS in broiler chickens (Cruz et al., 2017; Pekel et al., 2020). It suggests that intermittent decrease in dietary allocation of dLys or nutrient density might be helpful to prevent the development of WS and FPD without disturbing the growth performance.

We hypothesized that intermittent reduction in dietary dLys or ME and AA density might reduce the occurrence of WS and FPD by lowering the BW and litter deterioration. Therefore, the present study was conducted to prevent the incidence of WS and FPD in broiler chickens by decreasing the dietary dLys or metabolizable energy (ME) and AA density during different phases without affecting the growth performance through the manifestation of compensatory growth concept. The objective of this study was to slow down the growth trajectory of fast-growing broiler chickens at different phases followed by compensatory growth in later phases in order to reduce or prevent the development of WS and FPD in broiler chickens.

2. REVIEW OF LITERATURE

The world population grew incessantly at a faster pace over the past decades and is expected to rise above 9 billion people by 2050. Parallel with the growth of the world population, the demand for broiler meat increased owing to its shorter production cycle and being a relatively cheaper source of animal protein in addition to the absence of religious and cultural considerations. Chicken industry, simultaneously, has witnessed swift changes, including nutrition and productivity per animal over the past few decades. The revolutionary changes in nutrition account for evolution in nutrient requirements of egg and meat-type chickens from feeding for prevention of chickens from any nutrient deficiency to nutrient intake for optimization of production responses (Applegate and Angel, 2014). Chicken nutrition is expected to advance further with optimal efficiency, quality products, profitability, and environmental safety coming into the picture as in precision animal nutrition. Carcass yield, especially the breast yield, has been desirable along with lowering the yield of less desirable parts and organs. Traditional quantitative genetic selection has significantly increased the productivity per broiler chicken over the past few decades. It is evident from the fact that body weight (BW) of commercial broiler strains of 1957 and 1978 represents only 20 and 40%, respectively, of BW of commercial broiler strain of 2005 (Zuidhof et al., 2014). In addition, *Pectoralis minor* yield of commercial broilers increased 30 to 37%, whereas that of *Pectoralis major* increased 79 to 85% over a period of five decades from 1957 to 2005 (Zuidhof et al., 2014). In short, genetic selection has resulted in fast growth of commercial broiler chickens under the influence of controlled environment and balanced nutrients for optimal growth and production. Although, these advancements enhanced the global food security, they have also increased issues of animal welfare, meat quality, and economic losses. The modern broiler industry is facing the issues like breast myopathies and leg health deterioration.

2.1. White Striping

WS is characterized by the appearance of white lines between muscle fibers of breast muscle that traverse parallel to the course of muscle fibers (Kuttappan et al., 2016). The appearance of WS has adverse effects on the physical and sensory properties of meat (Petracci and Cavani, 2012), thereby reducing consumer acceptance (Kuttappan et al., 2016), suitability

for processing (Petracci et al., 2013a; Mudalal et al., 2015; Alnahhas et al.., 2016), and profitability (Kuttappan et al., 2016). Therefore, it requires specific measures to be taken to contain this issue. In this context, it is necessary to understand this issue in its entirety to devise the strategies for preventing the development of WS in broiler chickens.

The development of WS is governed by several factors. These factors involve those associated with the chickens (or intrinsic factors in other words), and nutrition and environment (extrinsic factors).

2.1.1. Intrinsic Factors

Reports indicate that broiler chickens are more prone to the development of WS who have higher body weight (Kuttappan et al., 2013b; Bailey et al., 2015; Dalle Zotte et al., 2015; Russo et al., 2015), greater daily weight gain (Russo et al., 2015), heavier breast and greater breast yield (Kuttappan et al., 2013b; Petracci et al., 2013a; Mudalal et al., 2014; 2015; Dalle Zotte et al., 2015; Alnahhas et al., 2016; Sanchez Brambilla et al., 2016; Baldi et al., 2018), older age (Dalle Zotte et al., 2015; Kuttappan et al., 2017a), and male gender (Trocino et al., 2015; Alnahhas et al., 2016). The development of WS in breast muscles occurs in all the commercially used broiler hybrids. Therefore, it is not associated with any specific broiler hybrids.

2.1.2. Extrinsic Factors

Among the extrinsic factors, the environment does not affect the development of WS. A recent study reported that subjecting the broiler chickens to high stocking density as an environmental stress had no effect on the incidence and severity of WS in breast fillet (Pekel et al., 2020).

Studies investigating the effect of nutrition on the development of WS in broiler breast muscles are available. Dietary vitamin E levels has no effect on the incidence and severity of WS in broiler breast fillets (Kuttappan et al., 2012c). Similarly, occurrence of WS in breast fillets remained unaffected in response to phase feeding (Kuttappan et al., 2013b) and feed restriction (Trocino et al., 2015) whereas WS incidence and severity decreased due to feed restriction investigated by Meloche et al. (2018b). However, the development of WS in broiler

chickens responds to the qualitative feed restriction or dietary nutrient allocation such as energy (Kuttappan et al., 2012b; Kindlein et al., 2017), ME and AA density (Meloche et al., 2018a), dietary AA density (Pekel et al., 2020), and dietary dLys levels (Cruz et al., 2017; Meloche et al., 2018c). Nutrition related factors impact the development of WS by influencing the body weight, breast weight, and breast yield. Therefore, the effect of dietary dLys and ME and AA density on growth performance and breast yield will be discussed in detail in the upcoming sections.

2.2. Muscle Growth

Based on the functional and metabolic traits, there are 3 distinct types of skeletal muscle fibers in poultry i.e. 1) slow oxidative (Type I); 2) fast oxidative (Type IIa); and 3) fast glycolytic (Type IIb) (Bechtel, 1986; Velleman and McFarland, 2015). Type I muscle fibers accomplish effective and less robust contraction due to slower hydrolysis of adenosine triphosphate (ATP) using an isoform of myosin; therefore, these muscles are situated mostly in postural muscles. Type II muscle fibers are fast-twitching muscle fibers used in activities that require robust forces of contraction in shorter periods. Type II muscle fibers are further categorized into oxidative (Type IIa) and glycolytic (Type IIb) on the basis of metabolism. Activities involving endurance are carried out by Type IIa muscle fibers, whereas movements that require a stronger force in a shorter period recruit Type IIb fibers (Goldspink, 1996). Type IIb muscle fibers make the entire breast fillet (*Pectoralis major*), thus giving a characteristic white color to the breast fillet (Remignon, 1996; Velleman and McFarland, 2015). Type IIb muscle fibers meet their energy needs through glycogen and phosphocreatine (Westerblad et al., 2010) and are less vascularized compared to red muscle fibers (Clark and Harding, 2017).

Embryological development of progenitor cells of muscle occurs through hyperplasia; thus, the number of muscle fibers remains constant after hatch, whereas the growth of skeletal muscle fibers is attained as a result of hypertrophy (Goldspink, 1996; Picard et al., 2002). Hypertrophy or growth of muscles occurs after hatching in terms of protein accretion of myofibrils for accumulating new sarcomeres or through synthesizing supplementary sarcomeres to increase the cross-sectional area of muscle fiber (Williams and Goldspink, 1971). The greater size of muscle fibers requires more nuclei for the maintenance of cell (in terms of cell repair or growth) eventually posing a challenge for myofibrils as the number of muscle cells is fixed and most of DNA accrual (> 90%) is attained after hatch (Allen et al., 1979;

Velleman, 2007; Al-Musawi et al., 2011; Petracci et al., 2015). Satellite cells, mononucleated cells located in the basal lamina from the proximity of muscle fibers, contribute their deoxyribonucleic acid (DNA; nuclei) to the growing myofibers since muscle fibers are unable to undergo mitotic division (Campion, 1984). Satellite cells act as stem cells for myogenesis that are expressed by Pax7 and Myf-5 genes (Zammit et al., 2006). These cells undergo mitosis for proliferation and contribute their DNA to muscle fibers in order to maintain the protein accretion and cell repair abilities of myofibers. Once the earlier proliferation of satellite cells is accomplished, the number of nuclei (amount of DNA) per myofiber determine the potential for further growth of myofiber (Allen et al., 1979). The regulation of proliferation of satellite cells during the growth of birds occurs to the extent of expression of different growth factors like hepatocyte growth factor, fibroblast growth factor, insulin-like growth factor I and II, platelet-derived growth factor, epidermal growth factor, and transforming growth factor beta (McFarland, 1999; Velleman and McFarland, 2015). The expression of these growth factors is further influenced by mechanical stimuli or positive energy balance, thereby supporting the growth of muscles (Hawke and Garry, 2001). Growth factors activate two distinct pathways i.e. Ras/Raf/MAP and phosphoinositide-3-kinase pathways for proliferation and differentiation of satellite cells, respectively (Coolican et al., 1997). Satellite cells are activated via Ras/Raf/MAP pathways that start proliferation to generate a substantial number of nuclei to be donated to the myofibrils for muscular growth. Differentiation of satellite cells starts as soon as they express myogenic differentiation factor 1 (MyoD1), thus differentiating into a muscle fiber (Coolican et al., 1997) that fuses with already present myofibers. Consequently, the number of nuclei is increased that is important for protein accretion giving rise to more sarcomeres, thereby increasing the size of muscle fiber (Velleman, 2007; Picard et al., 2010). While the majority of satellite cells proliferate, differentiate, and fuse to support the protein accretion for muscle growth, some satellite daughter cells do not undergo differentiation after proliferation. These daughter cells become quiescent to maintain the reserves of satellite cells that can be activated for further muscle growth or migrate under the influence of inflammatory responses and muscle injury (Yin et al., 2013). The infiltration of inflammatory cytokines activates the proliferation of satellite cells yielding myogenic precursor cells that leave the cell cycle and form new muscle fibers by fusion or repair the damaged fibers by adherence (Wozniak et al., 2005). Consequently, the higher expression of the activity of creatine kinase and other contractile proteins occurs that is attributed to the formation of new muscle fibers through fusion.

2.3. Growth Trajectory and Development of White Striping

Cathepsins (lysosomal), calpains (calcium-dependent), and proteasome (ATP/ubiquitin-dependent) proteolytic systems are present in the muscles. While the cathepsins and calpains are consecutive (sequential) specific inhibitors, proteasome system governs the protein conversion rate largely (Dransfield and Sosnicki, 1999). In protein metabolism, the balance between protein synthesis (anabolism) and protein degradation (catabolism) decides the fate of muscle mass. A balance in the favor of anabolism directs the increase in protein accretion or muscle mass. In modern broiler lines, faster growth rate and muscle accrual is a result of declined protein catabolism (Dransfield and Sosnicki, 1999) that shifts the balance in the favor of anabolism compared with their slow growing counterpart.

Studies reported that musculoskeletal defects are attributed to genetic selection for faster growth, effective, and maximal yields (Sosnicki et al, 1991; Mahon, 1999). In view of these, it is considered that the muscles expend their full capacity in order to maintain homeostasis that can be exacerbated in response to any internal or external stress agents. This initiates a chain of muscle fiber degeneration in which fat (WS) and/or connective (wooden breast) tissues steadily replace the myofiber.

2.3.1. Internal Stress Factors

It is thought that the development of WS in broiler chickens increases under the influence of certain stress factors inherent to the birds. As broiler chickens grow, the diameter of muscle fibers and intercapillary space broadens quickly whereas the vascularization, capillary to fiber ratio, protein degradation rate declines, and overloading or insufficiency of endogenous antioxidant systems occurs (Joiner et al., 2014; Mutryn et al., 2015; Petracci et al., 2015; Velleman and Clark, 2015; Sundekilde et al., 2017).

As discussed earlier, Type IIb muscle fibers make the entire breast fillet (*Pectoralis major*) (Remignon, 1996; Velleman and McFarland, 2015). Type IIb muscle fibers meet their energy needs through anaerobic metabolism of glycogen and phosphocreatine (Westerblad et al., 2010) for forceful but short-lived activities. Due to the lower oxygen demand of Type IIb fibers, these fibers generate waste products (lactate) of intermediate metabolism. The lower protein degradation results in the growth (hypertrophy) of muscle fibers that increases the intercapillary space and lowers the capillary/fiber ratio. A decrease in the vascularization and

capillary/fiber ratio and increase in intercapillary space of muscle tissues disrupt the removal of products of intermediate metabolism and worsen the homeostatic balance such as pH and ion exchange (Mutryn et al., 2015; Petracci et al., 2015; Zambonelli et al., 2016). In addition, these factors interrupt the access of oxygen and nutrients to the muscle tissue creating localized hypoxic conditions, accumulation of reactive oxygen species, inadequacy of antioxidant system, and subversion of membrane stability (Mutryn et al., 2015; Petracci et al., 2015; Velleman and Clark, 2015; Sihvo et al., 2017).

2.3.2. External Stress Factors

Although there is no report describing that the external stress factors account for the development of WS in broiler chickens, it has been proposed that certain factors might increase or initiate the development of WS.

The use of rancid or low-quality fat sources in broiler diets may exacerbate the inherent antioxidant system, perhaps initiating the accumulation of adipose tissue (WS) in breast muscles. Similarly, heat stress either from elevated environmental temperature or heat increment due to diet or drug interaction may destabilize the homeostatic balance in the breast muscles (Sandercock et al., 2006). In addition, poor or defective ventilation results in the failure to remove ammonia, carbon dioxide, and heat from the poultry house that may aggravate the systemic hypoxic conditions due to reduced vascularization (Mutryn et al., 2015). Fast growing modern broiler chickens may become more vulnerable to the development of WS owing to sitting (Bokkers and Koene, 2003) and transformed recumbent posture (Zuidhof et al., 2014) comprising longer durations that exerts pressure on the *pectoralis major* due to its weight in this sitting position. It confines the heat within the microclimate between the pectoral muscles and the litter, thereby creating more pronounced localized heat stress. Heat stress may accelerate the redox reactions, consequently lowering the feed consumption and limiting the access of muscles to nutrients.

2.3.3. Underlying Mechanisms in the Development of White Striping

The precise cause of WS was inconclusive until recently, despite the existence of markers for dietary deficiencies, switching of fiber-type, hypoxia, and oxidative stress had been reported (Kuttappan et al., 2012b; 2013a; Russo et al., 2015). It was thought that the onset of

development of WS in broiler chickens occurs due to internal and external stress factors induced localized hypoxic conditions and insults of membrane integrity. Consequently, muscle cell injury starts the outflow of enzymes towards systemic circulation, for instance creatine kinase (Mitchell, 1999; Sandercock and Mitchell, 2004; Sandercock et al., 2006). In addition, destabilization of membrane integrity impedes the calcium sequestration in the muscle fibers that is harmful for normal cell function. Subsequently, this causes the release of inflammatory cytokines thereby eliciting the inflammatory response by activating the transcription factors responsible for the upregulation of myofibrillar protein degradation (Mutryn et al., 2015). The degraded fibers are gradually replaced by the fat (adipose) tissue appearing as WS on the breast muscles running parallel to the muscle fibers.

The mystery was solved using metabolomics. Metabolomics of WS confirmed that hypoxia is the main root cause of WS that initiates the development of white striations. Localized hypoxia disrupts the oxidation of fatty acids, arginine and taurine metabolism, osmolytes, and TCA cycle that results in the accumulation of harmful fatty acids in the breast muscles of fast growing or heavy broiler chickens (Boerboom et al., 2018).

2.4. White Striping and Meat Quality

Sensory and physical quality of chicken and chicken products are associated with body composition and growth rate (Duclos et al., 2007). Genetic studies to meet the heightened demand for broiler meat increased for maximal growth and maximal efficiency that have amplified the metabolic stress (Kindlein et al., 2017). This stress in animals exacerbates histomorphological and biochemical changes in the muscles (Petracci and Cavani, 2012). Therefore, meat quality has deteriorated, and quality issues have increased in response to genetic selection for these criteria (Dransfield and Sosnicki, 1999). Excessive hypertrophy of muscle fibers, and development of giant muscle fiber and frequent increase in number of abnormal muscle fibers are suggestive of increased meat quality defects (Petracci et al., 2013a).

Nutritional and physiological status of muscle at slaughter determine the meat quality as they play a role in the development of rigor mortis (Zhao et al., 2012). Muscles utilize the ATP produced by anaerobic glycolysis of intramuscular glycogen reserves. This anaerobic glycolytic pathway is prompted by hypoxia post-slaughter or post-mortem. Consequently, it accumulates lactic acid in the muscle tissue causing acidification and decline in pH that is dependent on the glycogen storage levels in the muscle (Duclos et al, 2007). Meat pH is the

most important quality trait of meat that determines the other meat quality traits including color, water holding capacity (WHC), drip loss, cooking loss, tenderness, juiciness, and shelf life in addition to the taste. Fall in pH of meat denatures the proteins, declines the solubility of proteins, and lowers the positive and negatively charged water binding reactive groups on the muscle proteins that reach an isoelectric point. At this point, the opposite charges only attract each other, thus the water binding capacity of muscle proteins is diminished. Loss of water retention occurs as the space between proteins shrinks due to attraction between opposite charges. Water retention is further decreased as the divalent Ca^{2+} and Mg^{2+} sarcoplasmic cations start neutralization of anions on adjoining protein chains causing diminution of electrostatic repulsion among the protein chains (Wismer-Perdersen, 1986; Mir et al., 2017). In short, higher pH increases the WHC, lowers the cooking loss, and supports microbial proliferation or vice versa.

Reduced capacity for carbohydrate storage and utilization in breast fillets is linked with the breast myopathies in broiler chickens. This notion is supported by greater pH of WS breast fillets 6 to 8-h (Bowker and Zhuang, 2016) and 24-h (Petracci et al, 2013a; Mudalal et al, 2015; Baldi et al, 2018) post-slaughter in comparison with those without WS. Lipidosis and fibrosis in WS breast meat also affect the quality traits of breast fillets in addition to glycogen level and pH (Kuttappan et al., 2013c).

Color is a quality trait of meat considered primarily by the consumers in their preference (Fanatico et al. 2005). Meat color varies in conjunction with the presence of heme pigments (hemoglobin, myoglobin, and ligands forming compounds with heme pigments), pre-slaughter conditions (climate, diet, genetics, and catching and transport conditions), slaughter, post-slaughter holding duration, and processing conditions (pH, water temperature during scalding, and presence of nitrates and additives). Broiler meat possesses a higher degree of variation in lightness (L*) and is negatively correlated with pH (Fletcher, 2002). Degree of yellowness (b*) is associated with fat level in chicken meat (Fanatico et al, 2005). The presence of WS in chicken breast meat affects the degree of L* (Alnahhas et al. 2016), redness (a*) (Petracci et al., 2013a), and b* (Petracci et al., 2013a; Kuttappan et al., 2009; 2017b; Baldi et al., 2018). Other studies reported that L* remained unaffected, protein percentage decreased whereas pH, drip loss, cooking loss, and fat percentage were greater in breast fillets with WS compared with those without WS (Kuttappan et al., 2009; Petracci et al., 2013a).

2.5. Lysine, Growth Performance, and Breast Yield

Lys is known to be an essential AA. It is the second limiting AA following methionine in corn-soybean meal-based broiler diets. The analysis of Lys in feedstuffs is easy and straightforward, a handsome body of data is available for dLys requirements of poultry, and absorbed Lys is directly associated with protein accretion in broilers that establish the Lys as a reference AA (Baker and Han, 1994). Therefore, ideal AA requirements as a percentage (ratio) of Lys was coined as ideal protein concept. The idea behind this concept is attributed to the fact that the ratio of essential amino acids to Lys is not affected due to the dietary, environmental, and genetic factors. It implies that changing Lys requirement also dictate the requirements of other AAs while the ratios of AAs to that of Lys remain unaffected. Different independent researchers, bodies, and government organizations have recommended the ideal AA profiles as a percentage of Lys for poultry. Dietary Lys requirements of poultry have been traditionally determined using dose-response method and diet-dilution method each carrying its own merits and demerits. Dietary Lys requirements vary according to the criteria of response (maximal weight gain, maximal feed efficiency), sex, and growth period (age). For instance, many researchers reported that lysine requirements are higher for male birds than that of females, and maximal feed efficiency have higher dietary requirement of lysine than maximal body weight gain does (Dozier et al., 2008; Garcia et al., 2006; Han and Baker, 1993; 1994; Mack et al., 1999).

Several studies were conducted to establish the dietary dLys requirement of broiler chickens. Han and Baker (1991) reported that gradual lowering of dietary dLys levels (1.41, 1.31, 1.21, 1.11, 1.01, 0.91, 0.81, 0.71, 0.61, and 0.51%) linearly decreased the BW gain and feed efficiency. Tesseraud et al. (1992) also noted that broiler fed diets deficient in Lys than the requirement had lower BW, FI, and poor FCR. Similarly, graded reduction in dietary dLys levels from 1.11 to 0.51% (1.11, 1.01, 0.91, 0.81, 0.71, 0.61, and 0.51%) fed during 3 to 6 wks of post-hatch quadratically reduced the live weight, BW gain, FI, feed efficiency, and breast yield in broiler chickens (Han and Baker, 1994). Likewise, broilers fed lowering dietary true dLys levels from 11.78 to 7.28 g/kg (11.78, 10.88, 9.98, 9.08, 8.18, and 7.28 g/kg) exhibited lowered daily gain and poor FCR (Leclercq, 1998). Feeding low levels of Lys during starter (95% of NRC 1994 requirement) and grower-finisher (85% of NRC 1994 requirement) resulted in poor growth performance, and carcass and breast meat yield in broiler chickens (Kidd et al., 1998). Similar results were reported by Kerr et al. (1999) who noticed lowered BW gain, poor FCR, and lowered yield in broiler chickens fed gradually decreasing dietary Lys levels from

1.33 to 0.93% (1.33, 1.23, 1.13, 1.03, and 0.93%). Similarly, lowered BW and breast yield were reported in broiler in response to low dietary dLys levels (Cruz et al., 2017).

Everson et al. (1989) confirmed that lowering or restriction of any essential AA impairs the initiation stage in the translation process thereby inhibiting the protein synthesis in the hepatocytes. The relationship between dietary Lys levels and growth performance of broilers was delineated by Tesseraud et al. (1992). They described that low Lys content in diets than the bird's requirement increases fractional proteolysis rate and fractional protein synthesis rate in *Pectoralis major* muscle (Tesseraud et al., 1992; 1996a; 1996b; 2001). In contrast, fractional rate of proteolysis in *Pectoralis major* in broiler chickens declines with increasing dietary Lys content shifting the equilibrium in favor of protein synthesis thus increasing protein accretion that triggers the growth. In addition, efficiency of protein deposition is lowered in broiler chickens fed diets with low Lys content. Moreover, *Pectoralis major* muscles are more sensitive to low Lys induced fractional rate of proteolysis than liver (Tesseraud et al., 1996a) and other skeletal muscles like *Anterior latissimus dorsi* and *Sartorius* (Tesseraud et al., 1996b). Also, broiler chickens selected for faster growth and breast muscle development exhibit increased responsiveness of *Pectoralis major* protein turnover to low Lys levels in diet (Tesseraud et al., 2001). In nutshell, protein turnover does not help the muscle accretion at low dietary Lys levels. Consequently, carcass and breast meat yields in broiler chickens are low or minimum at lower Lys levels than the requirement.

2.6. Footpad Dermatitis

Footpad dermatitis (FPD) is a condition of the skin in broilers and turkeys, primarily characterized by irritation and inflammation of footpads on the plantar surface of the feet. Several internal and external risk factors pose a threat to the footpads of broilers and turkeys, causing FPD that is important not only from an economic perspective, and these are significant as food safety, product quality, and animal welfare concerns (Shepherd and Fairchild, 2010). Among the internal factors, sex (Bilgili et al., 2006), breed or strain-cross (Bilgili et al., 2006), nutrition (Mayne, 2005), and body weight and size (Nagaraj et al., 2007) are associated with FPD. Wet litter (Mayne et al., 2007), drinker design (Ekstrand and Algers, 1997), litter material (Hester et al., 1997), stocking density (McIlroy et al., 1987), and season (Haslam et al., 2007) are the known external factors. Paw quality of broilers and turkeys can be judged at farm using

different scoring methods or at the processing line using different grading methods (Martland 1984; 1985; Ekstrand et al., 1997; 1998; Bilgili et al., 2006; Nagaraj et al., 2007).

Dietary protein fulfils the amino acid requirements of broilers and it can affect the footpad health in different ways. Dietary protein can increase the litter moisture by increasing the heat increment and poor energy utilization due to protein metabolism that increase the water intake of broiler chickens (Emmans, 1994; Musharaf and Latshaw, 1999). Similarly, all vegetable protein sources cause increased occurrence of FPD in broiler chickens by increasing the water intake (Cengiz et al., 2013). Moreover, the protein sources also possess certain antinutritional factors that initiate the development of FPD by enhancing the litter moisture (Leeson and Summers, 2005). Reducing the dietary protein has shown to reduce the incidence of FPD by lowering the nitrogen excretion through feces into the litter thus lowering the ammonia volatilization from litter (Shao et al., 2018).

2.7. Hypothesis

In view of the literature above, this study was aimed to depress the growth of fast-growing broiler chickens during grower or grower and finisher phases by lowering the dietary dLys or ME and AA density. In addition, it was expected that the broiler chickens may compensate the depression in growth in the next phase following the phase of growth depression. Also, we assumed that the reduction in nitrogen excretion and litter moisture due to low dietary dLys or ME and AA density may result in better litter quality. Therefore, it was hypothesized that the slowdown in growth trajectory of broiler chickens in response to low dietary dLys or ME and AA density in grower or grower and finisher phases followed by compensatory growth may reduce the occurrence of WS and FPD in broiler chickens.

3. MATERIALS AND METHODS

3.1. Ethical Statement

This study was conducted at Poultry Research Unit of Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Turkey. All the methods, procedures, and practices involved were in line with the guidelines of local animal care and use committee of the university and approved prior to the commencement of the study vide letter no. 64583101/2018/138 dated December 25, 2018.

3.2. Study Design and Experimental Groups

The study consisted of a completely randomized design with five different experimental groups, each group comprising of 6 replicates. One experimental group was designated as control group fed diets according to their nutrient requirements throughout the experiment. Second group was fed 15% low Lys diet in grower phase only denoted as GRO low Lys group. Third group received 5% low ME and AA diet in grower phase only abbreviated as GRO low ME AA group. Fourth group was fed 15% low Lys diets in grower and finisher phases (GROFIN low Lys group) whereas the fifth group received 5% low ME and AA diets in grower and finisher phases (GROFIN low ME AA group). GRO low Lys and GROFIN low Lys diets were same in the grower phase. Similarly, GRO low ME AA and GROFIN low ME AA diets were same in the grower phase. The reduction in dietary dLys and ME and AA density was based on careful observations from available literature. Previous studies have reported that continuous feeding of low ME diets severely affect the growth performance of broiler chickens. Therefore, a reduction of 5% ME along with AA density was selected so that the compensatory growth may take place. The experiment consisted of 49 days. The experimental design has been explained in the table below (Table 1).

Table 1. Details of experimental design and groups

Groups	Diets			
	Starter	**Grower**	**Finisher**	**Withdrawal**
Control	Common Starter Diet*	Ross 308 Specifications*	Common Finisher Diet*	Common Withdrawal Diet*
GRO Low Lys		15% Low dLys**, §		
GRO Low ME AA		5% Low ME & AA***, †		
GROFIN Low Lys		15% Low dLys**, §	15% Low dLys**	
GROFIN Low ME AA		5% Low ME & AA***, †	5% Low ME & AA***	

*According to the nutrient requirements recommended by Aviagen (2019)
**Diets with 15% lowered dLys than recommended by Aviagen (2019)
***Diets with 5% lowered ME and AA than recommended by Aviagen (2019)
§Same diets in the grower phase
†Same diets in the grower phase

3.3. General Management of Birds

A total of 30 floor pens were setup following thoroughly cleaning and disinfection. Each pen served as a replicate. Each pen provided a floor space of 1 m^2 exclusive of the space occupied feeder and drinkers. Approximately 5 to 8 cm layer of pine-wood shavings was laid as litter material. Each pen was equipped with one feeder and three nipple drinkers. A 23L:1D lighting program was implemented. Experimental station was heated to maintain the temperature of 32 ºC during the first week. The temperature was reduced afterwards 3ºC per week (0.5 ºC per day) until a constant temperature of 24 ºC was attained that was maintained throughout the remaining experiment. The experiment lasted for 49 days in four phases such as starter, grower, finisher, and withdrawal. The chickens had *ad libitum* access to feed and water.

3.4. Experimental Diets

The ingredients were analyzed to estimate the individual total AA content in each feed ingredient using NIR analyzer (Perten DA 7200, Perkin Elmer Inc., MA, US). Digestibility coefficients of each AA for each feed ingredient were ascertained from AMINODat® (Evonik Industries, Essen, Germany). A representative diet was formulated for control group in accordance with the recommendations of Aviagen (2019) to meet or exceed the nutrient requirements of Ross 308 broilers in starter (d 0-10), grower (d 11-24), finisher (d 25-39), and withdrawal (d 40-49) phases (Table 2). The inclusion levels of ingredients were adjusted in order to lower the 15% dLys levels in the diets. The ME and AA levels were lowered in GRO low ME AA and GROFIN low ME AA groups such that the dLys to ME and AA to dLys ratios remained constant. Wheat bran was included in 5% low ME and AA diets to allow lowering of ME in the diets. Diets were prepared in mash form by mixing the feed ingredients with a small portion of ingredients with the mineral and vitamin supplements. Diets were chemically analyzed on as fed basis according to AOAC (2000) for dry matter (method 934.01), crude protein (method 954.01), ether extract (method 920.39), crude fiber (method 978.10), crude ash (method 942.05). Calcium content of diets were measured with direct flame photometric method (Heckman, 1960) using a flame photometer (Jenway PFP7, Cole-Parmer Ltd., Staffordshire, UK). Vanadomolybdophosphate colorimetric method was used to measure the phosphorus content of diets (APHA, 2005). Colors were developed by adding 5% ammonium heptamolybdate tetrahydrate and 0.25% ammonium monovanadate. Phosphorus content was measured at 430 nm wavelength in a spectrophotometer (Shimadzu UV-160A, Kyoto, Japan).

Table 2. Composition (g/kg) of starter (d 0-10), grower (d 11-24), finisher (d 25-39), and withdrawal (d 40-49) diets (as fed basis)

Item	Starter	Grower			Finisher			Withdrawal
		Control	15% low dLys	5% low ME AA	Control	15% low dLys	5% low ME AA	
Corn	530.94	557.12	569.31	600.00	611.52	615.06	624.10	600.00
Soybean meal	366.89	360.10	351.80	321.78	302.70	302.06	274.11	260.00
Corn gluten meal	11.26	-	-	-	-	-	-	35.00
Fish meal (salmon)	21.68	-	-	-	-	-	-	6.00
Wheat bran	-	-	-	15.94	-	-	40.00	21.47
Sunflower oil	31.35	45.71	43.36	20.18	51.62	50.48	27.75	46.45
Salt	3.23	3.22	3.21	02.02	3.20	3.20	3.20	3.23
Sodium bicarbonate	0.18	1.08	1.09	5.43	1.12	1.13	1.11	0.81
Dicalcium phosphate	17.36	17.54	17.61	18.57	15.67	15.67	15.65	14.36
Limestone	10.00	8.54	8.55	8.89	7.82	7.82	7.95	7.65
DL-Methionine	1.84	1.91	1.94	1.93	1.74	1.74	1.57	1.10
L-Lysine HCl	2.02	1.76	-	2.12	1.77	-	1.77	1.60
L-Threonine	1.31	1.09	1.18	1.19	0.90	0.90	0.83	0.38
Vitamin premix*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

* Each kg contained: Vitamin A (as acetate) 12,000,000 IU; vitamin D_3 (as cholecalciferol) 5,000,000 IU; vitamin E (as α-tocoferol acetate 50%) 100,000 mg, vitamin K_3 (as menadione sodium bisulphate 51%) 4,000 mg; vitamin B_1 (as thiamine mononitrate 98%) 3,000 mg; vitamin B_2 (as riboflavin 80%) 8,000 mg, niacin (as nicotinic amide 99%) 70,000 mg; vitamin B_5 (as calcium D-pentothenate 98%) 20,000 mg; vitamin B_6 (as proxidine hydrochloride 99%) 5,000 mg; vitamin B_{12} (as cobalamin 1%) 30 mg; folic acid (91%) 2,000 mg; vitamin H_2 (as D-(+)-biotin 2%) 200 mg; calcium carbonate (as carrier) 63%

** Each kg contained: Manganese (as manganese oxide 62%) 150,000 mg; iron (as iron sulphate monohydrate 31%) 120,000 mg; zinc (as zinc oxide 72%) 120,000 mg; copper (as copper sulphate pentahydrate 25%) 12,000 mg; iodine (as calcium iodide 62%) 3,000 mg; selenium (as sodium selenite 4.5%) 225 mg; molybdenum (as sodium molybdate 39%) 750 mg; calcium carbonate as carrier 13%

Table 3. Chemical composition (g/kg) of starter (d 0-10), grower (d 11-24), finisher (d 25-39), and withdrawal (d 40-49) diets (as fed basis)

Item	Starter	Grower			Finisher			Withdrawal
		Control	15% low dLys	5% low ME AA	Control	15% low dLys	5% low ME AA	
Chemical composition (calculated)								
Crude protein	230.00	208.20	205.30	196.40	186.00	185.90	179.90	193.30
ME (kcal/kg)	12.56	12.98	12.98	12.37	13.39	13.39	12.72	13.39
Ca	9.90	8.70	8.70	9.00	7.80	7.80	7.80	7.50
Available P	4.80	4.40	4.40	4.50	3.90	3.90	3.90	3.80
Na	1.60	1.60	1.60	2.30	1.60	1.60	1.60	1.60
K	9.20	8.90	8.80	8.40	7.90	7.90	7.80	7.50
Cl	2.30	2.30	2.30	1.60	2.30	2.30	2.30	2.30
dLys	12.80	11.50	9.93	11.00	10.20	87.90	9.70	9.60
dMet	5.10	4.70	4.70	4.60	4.30	4.30	4.07	4.00
dTrp	2.40	2.30	2.20	2.10	2.00	2.00	1.90	1.90
dThr	8.60	7.70	7.70	7.40	6.80	6.80	6.50	6.40
dVal	9.60	8.70	8.60	8.20	7.80	7.80	7.50	8.00
dArg	13.90	12.90	12.70	12.00	11.30	11.30	10.80	10.90
dIle	8.80	8.00	7.90	7.50	7.10	7.10	6.80	7.20
Chemical composition (analyzed)								
Dry matter	890.60	881.90	883.90	880.30	881.10	880.70	878.20	881.90
Crude protein	231.10	208.40	205.80	197.70	183.80	181.10	178.50	192.30
Ether extract	51.20	68.90	69.60	42.70	74.60	77.40	52.50	74.70
Crude fiber	38.00	34.70	33.10	31.90	30.20	28.80	35.30	32.20
Crude ash	64.10	58.60	57.90	60.30	55.60	53.10	52.20	53.60
Nitrogen-free extract (calculated)	505.80	511.30	517.50	547.70	536.90	540.30	559.70	529.10
Total carbohydrates (calculated)	543.80	546.00	550.60	579.60	567.10	569.10	595.00	561.30
Ca	10.70	8.90	9.10	8.70	8.40	8.40	8.50	8.00
Total P	7.60	6.90	7.80	7.60	6.00	6.50	6.00	5.80

3.5. Growth Performance

Growth performance of broiler chickens was assessed in terms of BW gain, feed intake (FI), and feed conversion ratio (FCR). The birds were weighed on days 0, 10, 24, 39, and 49 of experiment and BW gain was calculated by difference method. Similarly, the difference between the amounts of feed offered and leftover was computed to determine the FI for starter, grower, finisher, and withdrawal phases. The ratio of amount of feed consumed to BW gain was calculated as FCR for all the phases. No adjustment was made in growth performance traits due to the absence of mortality.

3.6. Slaughtering

At the end of experiment (d 49), all the broiler chickens were slaughtered by decapitation. The feathers were softened by scalding at 55 ºC for 2 min in an electric scalder (Cimuka Kuluçka Makinaları, Ankara, Turkey), feathers were removed using a feather removing machine (Cimuka Kuluçka Makinaları, Ankara, Turkey), and carcasses were opened by dissection.

Blood samples were collected in empty vacutainers from jugular vein at the time of slaughter from 5 birds per replicate (30 birds per group, 150 birds in total) at the time of slaughtering. The blood samples were allowed to clot, centrifuged at 4500 rpm for 15 minutes. The sera were separated into Eppendorf tubes and stored at -18 ºC until further analysis.

3.7. Carcass Yield and Characteristics

Carcasses (whole carcass, wings, breast, and thigh) and organs (heart, liver, and spleen) of 5 birds from each replicate (30 birds from each group) were separated and weighed. Parts and organ yields were calculated as percent of whole carcass whereas carcass yield was calculated as percent of live weight.

3.8. Breast Meat Quality

Breast meat samples were collected for the assessment of meat quality and composition. Breast meat samples were stored at +4 ºC for the meat quality 24-h post-slaughter. pH and color

of breast meat were measured 15 minutes after slaughter and 24-h post-slaughter. The left breast fillets were used for the measurements related to breast meat quality.

3.8.1. pH

The probe of a waterproof pH meter (Testo 205; Testo Inc., Lenzkirch, Germany) was inserted 2.5 cm deep into the pectoralis major muscle. Three readings were taken from different sites (cranial, cranio-axial, and cranio-abaxial) of the cranial 1/3rd of breast fillet.

3.8.2. Color

Meat color was recorded using a chromameter (Minolta CR400; Konica Minolta Sensing Inc., Osaka, Japan) from the cranial site of *Pectoralis major* muscle. Meat color was expressed as L*, a*, and b* in accordance with the Commission Internationale de l'Eclairage (CIE; International Commission on Illumination).

3.8.3. Drip Loss

Drip loss of breast meat of broiler chickens was measured 24-h post-slaughter using compression method, a procedure previously described by Barton-Gade et al. (1993). For this purpose, a known quantity of finely cut *Pectoralis major* muscle from the cranial portion was compressed under 2250 g weight for a duration of 5 min between two layers of Whatman filter paper no.1. The difference between the initial and final weights of sample was calculated and expressed as percent drip loss.

3.8.4. Cooking Loss

Cooking loss of breast meat was measured 24-h post-slaughter according to the method described by Honikel (1998). A known quantity of breast meat sample from the cranial portion of breast fillet wrapped in the plastic bag tied to the numbered tags was allowed to cook at 80 ^{o}C in a water bath for an hour. Later, the sample was dried using paper towel, and weighed. The difference between the initial and final weights of sample was calculated and expressed as percent cooking loss.

3.9. Composition of Breast Meat

Stored breast meat samples (5 samples from each replicate, 30 samples from each group, 150 samples in total) were analyzed for nutrient composition (moisture, crude protein, ether extract, and crude ash). Cranial 1/3rd portions of right breast fillets were used for the analyses related to breast meat composition. The samples were taken from the proximal 1/3 portion of the breast meat. Breast meat samples were dried at 65 ºC in a hot air oven until the persistence of dried weight and dry matter was calculated (method 934.01). Dried breast meat samples were ground for further analysis of crude protein using Kjeldahl method (method 954.01), ether extract using Soxhlet apparatus (method 920.39), and crude ash using muffle furnace (method 942.05) on dry matter basis. All the procedures were adopted from Association of Official Analytical Chemists (AOAC, 2000).

3.10. Serum Metabolites

Serum triglycerides (TG) and total cholesterol (CHOL) levels were analyzed biochemically using colorimetric and kinetic spectrophotometric method. Commercial reagent kits (Roche Diagnostics International Ltd., Risch-Rotkreuz, Switzerland) were used for this purpose according to manufacturer's recommendations followed by measurement of TG and CHOL levels in an automatic photometric plate reader (Roche cobas c501, Roche Diagnostics International Ltd., Risch-Rotkreuz, Switzerland).

3.11. Scoring of Paws for Footpad Dermatitis

The paws of broiler chickens were scored for the incidence and severity of FPD on days 24, 39, and 49 using a three-scale visual scoring method described by Bilgili et al. (2006). Briefly, the paws having no signs of lesion were scored as 0, whereas those having lesion size not exceeding 7.5 mm were scored as 1 (mild). Footpads exhibiting a lesion size exceeding 7.5 mm were assigned a score of 2 (severe).

3.12. Scoring of White Striping on Breast Meat

The breast of all the chickens (78 birds from each group, 390 birds in total) were scored for WS immediately after the slaughtering process. For this purpose, the skin from the breast

area of chickens was removed following decapitation and removal of feathers in order to expose the breast meat of broiler chickens. A three-scale visual scoring method described by Kuttappan et al. (2013c) was followed to score the incidence and severity of WS on the breast meat of broiler chickens. The absence of WS on the breast meat was scored as 0 whereas those having WS thickness less than or more than 1 mm were scored as 1 or 2, respectively.

3.13. Statistical Analysis

One-way analysis of variance (ANOVA) was applied to assess the effect of treatments during different growth phases on growth performance, carcass yield and characteristics, breast meat quality, and breast meat composition of broiler chickens in a completely randomized design. The data related to FPD were excluded due to the absence of FPD lesions. The data obtained by scoring (incidence and severity of WS) were analyzed using Kruskal-Wallis non-parametric analytical test and pairwise comparisons were conducted using Mann-Whitney U test with Bonferroni adjustment as post-hoc test. A computer-based statistical software package SPSS (version 22.0, Armonk, NY, US) was used for statistical analysis of the data. Normality of data was tested using Shapiro-Wilk's test followed by logarithmic or square root transformation in case of non-normalized traits. All the tests and analyses were conducted in SPSS assuming *P*-value lower than 0.05 (95% confidence interval) as significant using Duncan's multiple range test as post-hoc test to separate the significantly different means. The results were presented as mean ± SEM (pooled).

4. RESULTS

4.1. Growth Performance

4.1.1. Live Weight

Live weights of broiler chickens have been presented in Table 4. Live weights of broiler chickens were not different among the groups at days 0 and 10 of experiment.

At d 24, broiler chickens in control group had greater live weight compared to those in other groups ($P < 0.001$). Live weight of broilers in GROFIN low ME AA group was greater in comparison with those in GRO low Lys and GROFIN low Lys groups ($P < 0.001$) whereas broilers in GRO low ME AA group were heavier than GRO low Lys ($P < 0.001$).

At d 39, live weights of broiler chickens in control group were greater than GRO low Lys and GROFIN low ME AA groups ($P < 0.001$), which in turn, were greater compared to GROFIN low Lys group ($P < 0.001$). Broilers fed GRO low ME AA diets expressed higher live weight in comparison with those in GROFIN low Lys group ($P < 0.001$).

At d 49, birds in control group were heavier than those in GRO low Lys and GROFIN low Lys groups ($P < 0.001$) whereas broilers in GRO low ME AA and GROFIN low ME AA had greater live weight compared to those in GROFIN low Lys group ($P < 0.001$).

Table 4. Live weight (g) of broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases (n = 6)

Days	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
d 0	47.27	47.28	47.28	47.26	47.26	0.02	0.973
d 10	281.86	257.00	267.79	261.10	272.32	19.20	0.184
d 24	1190^{a}	995^{d}	1083^{bc}	1025^{cd}	1105^{b}	15.94	<0.001
d 39	2662^{a}	2487^{b}	2578^{ab}	2340^{c}	2493^{b}	26.52	<0.001
d 49	3562^{a}	3351^{bc}	3463^{ab}	3250^{c}	3457^{ab}	26.41	<0.001

$^{a, b, c, d}$ Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.1.2. Body Weight Gain

The BW gain of broiler chickens are shown in Table 5. There was no difference in BW gain of broiler chickens among the groups at d 0-10 and 40-49.

However, at d 11-24, control group had greater BW gain compared to GRO low ME AA and GROFIN low ME AA groups ($P < 0.001$), which in turn, had greater BW gain in comparison with GRO low Lys and GROFIN low Lys ($P < 0.001$).

At d 25-39, BW gain of birds in GRO low Lys and GRO low ME AA groups was greater than those in GROFIN low Lys and GROFIN low ME AA ($P < 0.001$). Moreover, birds fed GROFIN low Lys diets had lower BW gain in comparison with the control group ($P < 0.001$).

At d 0-24, all the groups had lower BW gain than control group ($P < 0.001$). The BW gain of broilers in GRO low Lys group suppressed in comparison with those in control, GRO low ME AA, and GROFIN low ME AA groups ($P < 0.001$). The BW gain decreased in GROFIN low Lys group compared to control and GROFIN low ME AA groups ($P < 0.001$). Similarly, GRO low ME AA and GROFIN low ME AA groups had lower BW gain than control group ($P < 0.001$).

At d 0-39, birds in GRO low Lys and GROM low ME AA groups had lower and greater BW gain compared to control and GROFIN low Lys groups ($P < 0.001$), respectively. In addition, BW of broiler chickens fed GROFIN low Lys diets was lower than those in GRO low ME AA group ($P < 0.001$).

Overall, broilers in GROFIN low Lys group had lower BW gain than those in control, GRO low ME AA, and GROFIN low ME AA groups ($P = 0.010$). The BW gain of GRO low Lys group was numerically greater than GROFIN low Lys group (3327 g vs 3193 g; $P > 0.05$).

Table 5. Body weight gain (g) of broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases (n = 6)

Days	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
d 0-10	235	210	221	214	225	3.51	0.185
d 11-24	908[a]	738[c]	815[b]	764[c]	832[b]	23.29	<0.001
d 25-39	1471[ab]	1492[a]	1495[a]	1315[c]	1388[bc]	17.68	<0.001
d 40-49	876	897	915	907	935	14.18	0.781
d 0-24	1143[a]	947[d]	1036[bc]	978[cd]	1057[b]	15.94	<0.001
d 0-39	2614[a]	2439[b]	2531[ab]	2292[c]	2446[b]	26.52	<0.001
d 0-49	3490[a]	3327[ab]	3375[a]	3193[b]	3381[a]	28.24	0.010

[a, b, c, d] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.1.3. Feed Intake

Table 6 shows the FI of broiler chickens. The FI remained unaffected at 0-10 and 40-49 days of experiment.

The FI suppressed in birds fed GRO low Lys and GROFIN low Lys diets compared to other groups during the grower phase ($P = 0.001$) and at 0-39 days ($P = 0.003$).

At d 25-39, broilers fed GROFIN low Lys diets had lower FI in comparison with those in control, GRO low ME AA, and GROFIN low ME AA groups ($P = 0.042$).

At 0-24 days, FI decreased in broilers in GRO low Lys group compared with those in control, GRO low ME AA, and GROFIN low ME AA groups ($P < 0.001$).

Birds in GROFIN low Lys groups had lower FI at 0-49 days than those in control, GRO low ME AA, and GROFIN low ME AA groups ($P = 0.006$). Similarly, GRO low Lys group had lower FI than control and GROFIN low ME AA groups ($P = 0.006$).

Table 6. Feed intake (g) of broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases (n = 6)

Days	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
d 0-10	212.32	187.15	201.12	198.51	214.38	3.78	0.134
d 11-24	1243[a]	1084[b]	1202[a]	1106[b]	1222[a]	16.95	0.001
d 25-39	2355[a]	2275[ab]	2345[a]	2202[b]	2382[a]	21.52	0.042
d 40-49	1687	1705	1682	1678	1778	19.45	0.475
d 0-24	1456[a]	1271[b]	1403[a]	1304[ab]	1436[a]	17.89	<0.001
d 0-39	3810[a]	3546[b]	3748[a]	3506[b]	3818[a]	36.51	0.003
d 0-49	5497[a]	5251[bc]	5430[ab]	5184[c]	5596[a]	43.74	0.006

[a, b, c] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.1.4. Feed Conversion Ratio

The FCR of broiler chickens during different phases have been presented in Table 7. The FCR was not different among the groups in starter and withdrawal phases.

Control group had lower FCR compared with other groups during the grower phase ($P < 0.001$) and at 0-24 days ($P < 0.001$).

At d 25-39, broilers in GRO low Lys group had lower FCR in comparison with those in control, GROFIN low Lys, and GROFIN low ME AA groups ($P < 0.001$). GROFIN low Lys and GROFIN low ME AA groups had greater FCR compared to control and GRO low ME AA groups ($P < 0.001$).

Control and GRO low Lys groups had better FCR compared to other groups at 0-39 days ($P < 0.001$). Birds in GRO low ME AA group had lower FCR in comparison with those in GROFIN low Lys and GROFIN low ME AA groups ($P < 0.001$). The FCR of broiler chickens in GROFIN low Lys group was lower than those of GROFIN low ME AA group ($P < 0.001$).

At 0-49 days, birds in control, GRO low Lys, GRO low ME AA groups showed better FCR than other groups ($P < 0.001$).

Table 7. Feed conversion ratio (g:g) of broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases (n = 6)

Days	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
d 0-10	0.91	0.89	0.92	0.94	0.97	0.02	0.898
d 11-24	1.37^{b}	1.47^{a}	1.47^{a}	1.45^{a}	1.47^{a}	0.01	<0.001
d 25-39	1.60^{b}	1.53^{c}	1.57^{bc}	1.67^{a}	1.72^{a}	0.01	<0.001
d 40-49	1.95	1.91	1.89	1.88	1.92	0.02	0.797
d 0-24	1.27^{b}	1.34^{a}	1.36^{a}	1.33^{a}	1.36^{a}	0.01	<0.001
d 0-39	1.46^{d}	1.45^{d}	1.48^{c}	1.53^{b}	1.56^{a}	0.01	<0.001
d 0-49	1.58^{b}	1.57^{b}	1.59^{b}	1.64^{a}	1.66^{a}	0.01	<0.001

$^{a, b, c, d}$ Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.2. Carcass Yield and Characteristics

Carcass yield and characteristics of broiler chickens are shown in Table 8. Relative liver, heart, and spleen yields were not different among the treatments.

Slaughter weight was lower in GROFIN low Lys group in comparison with other groups ($P = 0.003$).

Carcass yield was lower in GRO low Lys and GROFIN low Lys groups than control and GROFIN low ME AA groups ($P = 0.004$).

Breast yield was lower in GROFIN low Lys group compared with other groups ($P < 0.001$). Broilers fed GRO low Lys diets had lower breast yield than those in control and GROFIN low ME AA groups ($P < 0.001$).

Thigh yield was lower in GROFIN low ME AA group than GROFIN low Lys groups ($P = 0.041$).

Wing yield tended to increase in GRO low Lys and GROFIN low Lys groups in comparison with other groups ($P = 0.057$).

Table 8. Carcass yield (%) and characteristics of broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases (n = 30)

Item	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
Slaughter weight (g)	3566[a]	3439[a]	3485[a]	3299[b]	3505[a]	22.71	0.003
Carcass	76.38[a]	75.66[b]	76.08[ab]	75.50[b]	76.44[a]	0.10	0.004
Breast	28.50[a]	27.30[b]	28.58[a]	25.88[c]	29.12[a]	0.15	<0.001
Thigh	21.04[ab]	21.32[ab]	21.18[ab]	21.62[a]	20.66[b]	0.05	0.041
Wing	6.78	6.87	6.80	7.02	6.71	0.04	0.057
Liver	1.96	2.02	1.96	2.00	1.97	0.02	0.732
Heart	0.58	0.60	0.57	0.62	0.59	0.08	0.068
Spleen	0.12	0.12	0.12	0.12	0.12	0.02	0.981

[a, b, c] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.3. Breast Meat Quality

4.3.1. Breast Meat Quality at Slaughter

Breast meat quality of broiler chickens at slaughter has been presented in Table 9. Lightness (L*), redness (a*), yellowness (b*), and pH of breast meat of broiler chickens at slaughter were similar regardless of the groups.

4.3.2. Breast Meat Quality 24-h Post-Slaughter

Color (L*, a*, b*), pH, and cooking loss of breast meat of broiler chickens remained unaffected 24-h post-slaughter among the groups. However, drip loss of breast meat was lower in broilers in control group in comparison with other groups (P = 0.001) whereas GROFIN low Lys group had lower drip loss of breast meat compared with GROFIN low ME AA group (P = 0.001).

Table 9. Meat quality of broiler chickens at slaughter fed diets low in lysine or metabolizable and amino acid density at different phases (n = 30)

Item	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
L*	50.92	50.61	50.90	51.71	51.15	0.19	0.278
a*	2.84	2.92	2.99	2.40	2.68	0.09	0.205
b*	9.22	9.01	9.04	8.72	9.78	0.13	0.139
pH	6.68	6.69	6.68	6.66	6.70	0.01	0.892

[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

Table 10. Meat quality of broiler chickens 24-h post-slaughter fed diets low in lysine or metabolizable and amino acid density at different phases (n = 30)

Item	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
L*	53.67	54.38	54.84	54.39	53.59	0.27	0.550
a*	3.75	3.56	3.43	2.92	2.90	0.13	0.147
b*	10.96	11.13	10.72	9.83	10.49	0.22	0.365
pH	5.78	5.64	5.67	5.72	5.63	0.02	0.053
Drip loss (%)	3.62[c]	5.04[ab]	5.06[ab]	4.61[b]	5.72[a]	0.14	0.001
Cooking loss (%)	29.44	31.10	29.76	30.37	29.87	0.35	0.610

[a, b, c] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.4. Breast Meat Composition

Dry matter and crude ash of breast meat were not different among the groups (Table 11). Crude protein was lower in broiler chickens fed GRO low ME AA and GROFIN low ME AA diets than those fed GRO low Lys diets (P = 0.045). Ether extract content of breast meat decreased in broiler chickens fed diets low in dLys (GRO low Lys and GROFIN low Lys) compared to other groups (P = 0.006).

Table 11. Breast meat composition (%, on dry matter basis) of broiler chickens fed diets low in digestible lysine or metabolizable and amino acid density at different growth phases (n = 30)

Item	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
Dry matter	25.85	25.58	25.63	25.58	25.50	0.08	0.672
Crude protein	87.22[ab]	87.85[a]	86.63[b]	87.50[ab]	86.67[b]	0.15	0.045
Ether extract	5.82[a]	4.66[b]	5.86[a]	4.84[b]	5.81[a]	0.14	0.006
Crude ash	4.50	4.50	4.48	4.44	4.46	0.01	0.457

[a, b] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.5. Serum Metabolites

Serum TG and CHOL in broiler chickens were different among the groups (Table 12). Serum TG decreased in broilers fed GRO low Lys diets compared to control, GROFIN low Lys and GROFIN low ME AA groups ($P < 0.001$). Broilers in GRO low ME AA group had lower serum TG compared to those in GROFIN low Lys and GROFIN low ME AA groups ($P < 0.001$). Feeding GROFIN low Lys diets decreased the serum TG levels in broiler chickens compared to those fed GROFIN low ME AA diets ($P < 0.001$).

Serum CHOL levels were lower in broiler chickens in GRO low Lys and GRO low ME AA groups in comparison with other dietary treatments ($P < 0.001$). Birds fed control diets had higher CHOL levels than those fed GROFIN low ME AA diets ($P < 0.001$).

Table 12. Serum triglycerides (mmol/L) and cholesterol (mmol/L) of broiler chickens fed diets low in digestible lysine or metabolizable and amino acid density at different growth phases (n = 30)

Item	Control	GRO Low Lys[1]	GRO Low ME AA[2]	GROFIN Low Lys[3]	GROFIN Low ME AA[4]	SEM	*P*-value
Triglycerides	0.71^{bc}	0.46^{d}	0.51^{cd}	0.79^{b}	1.06^{a}	0.04	<0.001
Total Cholesterol	2.33^{b}	1.49^{c}	1.64^{c}	2.84^{ab}	2.97^{a}	0.10	<0.001

[a, b, c, d] Means with different superscripts within the same row are significantly different
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the finisher phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases

4.6. Incidence and Severity of White Striping and Footpad Dermatitis

Table 13 shows the incidence and severity of WS in broiler chickens fed low dLys or low ME AA diets during grower and finisher phases. Birds in GRO low Lys and GROFIN low Lys groups had lower incidence and severity of WS in comparison with other dietary treatments ($P < 0.001$).

No lesions of FPD were observed in broiler chickens in any group on days 39 and 49 of the experiment.

Table 13. Incidence and severity of white striping in broiler chickens fed diets low in lysine or metabolizable and amino acid density at different phases

Groups	n	White Striping Score			
		0	1	2	Total
Control	78	27[b] (34.62%)	42[a] (53.85%)	9 (11.54%)	51[a] (65.38%)
GRO Low Lys[1]	78	48[a] (61.54%)	30[b] (38.46%)	0 (0.00%)	30[b] (38.46%)
GRO Low ME AA[2]	78	33[b] (42.31%)	38[ab] (48.72%)	7 (8.97%)	45[a] (57.69%)
GROFIN Low Lys[3]	78	47[a] (60.26%)	31[b] (39.74%)	0 (0.00%)	31[b] (39.74%)
GROFIN Low ME AA[4]	78	29[b] (37.18%)	43[a] (55.13%)	6 (7.69)	49[a] (62.82%)
P-value[5]		<0.001			

[a, b] WS scores with different superscripts within the same column are significantly different according to pairwise comparisons conducted using Mann-Whitney U test with Bonferroni adjustment
[1] GRO Low Lys = 15% low dLys than recommended in the grower phase
[2] GRO Low ME AA = 5% low ME and digestible AA than recommended in the grower phase
[3] GROFIN Low Lys = 15 low dLys than recommended in the grower and finisher phases
[4] GROFIN Low ME AA = 5% low ME and digestible AA than recommended in the grower and finisher phases
[5] Probability from non-parametric Kruskal-Wallis test

5. DISCUSSION

5.1. Growth Performance, Carcass Yield and Characteristics

In the present study, reduction in dietary dLys levels in different growth phases declined the growth performance of broiler chickens during the corresponding phases that reflected in the overall phases (days 0 to 24, 0 to 39, and 0 to 49) as well. On the other hand, although lowering dietary ME and AA density had no effect on live weight, BW gain, and FI comparable to that of control group, poor FCR was seen in these groups. In addition, carcass and breast meat yields decreased in response to lowering dietary dLys during grower phase (GRO low Lys) which further declined with decreasing dLys during grower and finisher phases (GROFIN low Lys). However, broiler chickens fed diets with low ME and AA density (GRO low ME AA and GROFIN low ME AA) had greater carcass and breast yield and lower thigh yield than those fed low Lys diets.

Previous studies have reported similar findings in response to low dietary Lys levels than the requirement. Han and Baker (1991) reported that gradual lowering of dietary dLys levels (1.41, 1.31, 1.21, 1.11, 1.01, 0.91, 0.81, 0.71, 0.61, and 0.51%) linearly decreased the BW gain and feed efficiency. Tesseraud et al. (1992) also noted that broiler fed diets deficient in Lys than the requirement had lower BW, FI, and poor FCR. Similarly, graded reduction in dietary dLys levels from 1.11 to 0.51% (1.11, 1.01, 0.91, 0.81, 0.71, 0.61, and 0.51%) fed during 3 to 6 wks of post-hatch quadratically reduced the live weight, BW gain, FI, feed efficiency, and breast yield in broiler chickens (Han and Baker, 1994). Likewise, broilers fed lowering dietary true dLys levels from 11.78 to 7.28 g/kg (11.78, 10.88, 9.98, 9.08, 8.18, and 7.28 g/kg) exhibited lowered daily gain and poor FCR (Leclercq, 1998). Feeding low levels of Lys during starter (95% of NRC 1994 requirement) and grower-finisher (85% of NRC 1994 requirement) resulted in poor growth performance, and carcass and breast meat yield in broiler chickens (Kidd et al., 1998). Similar results were reported by Kerr et al. (1999) who noticed lowered BW gain, poor FCR, and lowered yield in broiler chickens fed gradually decreasing dietary Lys levels from 1.33 to 0.93% (1.33, 1.23, 1.13, 1.03, and 0.93%). Similarly, lowered BW and breast yield were reported in broiler in response to low dietary dLys levels (Cruz et al., 2017). Lowering or restriction of any essential AA impairs the initiation stage in the translation process thereby inhibiting the protein synthesis in the hepatocytes (Everson et al., 1989). The relationship between dietary Lys levels and growth performance of broilers was further

delineated by Tesseraud et al. (1992). They described that low Lys content in diets than the bird's requirement increases fractional proteolysis rate and fractional protein synthesis rate in *Pectoralis major* muscle (Tesseraud et al., 1992; 1996a; 1996b; 2001). In addition, efficiency of protein deposition is lowered in broiler chickens fed diets with low Lys content. In contrast, fractional rate of proteolysis in *Pectoralis major* muscle in broiler chickens declines with increasing dietary Lys content shifting the equilibrium in favor of protein synthesis thus increasing protein accretion that triggers the growth. Therefore, the growth trajectory was slowed down during the phases when broiler chickens were fed diets low dLys content. The compensatory growth occurred in the next immediate phases although not to the extent comparable to control group. It is a well-known fact that FI in broiler chickens is driven by the ME content of the diet (Leeson et al., 1996a; 1996b). It seems that an increase in FI of broiler chickens fed GROFIN low ME AA diets occurred as a response in order to compensate for low ME content of the diets resulting in poor FCR. In addition, carcass and breast meat yields remained comparable to that of control group as a consequence of increased FI that augmented the dietary dLys intake in broilers fed low ME AA diets. This shows that low dLys caused a slowdown in the growth of broiler chickens that rebounded in the phases when dietary dLys content was restored according to the requirement. On the contrary, birds fed low ME AA diets compensated their FI in the same phases when low ME AA diets were fed.

In the present study, breast meat yield was lower whereas wing, thigh, and liver yields were higher in broiler chicken fed diets with low dLys content compared to other groups. Studies reported that *Pectoralis major* muscles are more sensitive to low Lys induced fractional rate of proteolysis than liver (Tesseraud et al., 1996a) and other skeletal muscles like *Anterior latissimus dorsi* (muscle in wings) and *Sartorius* (muscle in thighs) (Tesseraud et al., 1996b). Also, broiler chickens selected for faster growth and breast muscle development exhibit increased responsiveness of *Pectoralis major* protein turnover to low Lys levels in diet that their slow growing counterpart (Tesseraud et al., 2001). Therefore, it seems that breast yield decreased while liver, wing, and thigh yields increased in broiler chickens in GRO low Lys and GROFIN low Lys groups. Unlike these, increased dLys intake in control, GRO low ME AA, and GROFIN low ME AA groups increased the breast meat yields attributed to increased protein deposition in breast muscles whereas liver, wing, and thigh yields reduced due to low availability of dLys for protein accretion in these muscles. In nutshell, protein turnover did not help protein deposition in breast muscles at low dietary dLys levels whereas other muscle yields

were not affected. Consequently, carcass and breast meat yields in broiler chickens were low or minimum at lower dLys levels than the requirement.

5.2. Breast Meat Quality

Meat quality traits at slaughter (pH and color) and 24-h post-slaughter (color and cooking loss) remained unaffected among the groups. This is the first study reporting the breast quality of broiler chickens fed diets concurrently low in dietary dLys or ME AA density. In general, pre-slaughter conditions, and nutritional and physiological status of muscle at slaughter determines the meat quality as they play a role in the development of rigor mortis (Zhao et al., 2012). Since these conditions were same in all the groups at the time of slaughter, there was no difference in pH and color of breast meat of broiler chickens among the groups.

Muscles utilize the ATP produced by anaerobic glycolysis of intramuscular glycogen reserves. This anaerobic glycolytic pathway is prompted by hypoxia post-slaughter or post-mortem. Consequently, it accumulates lactic acid in the muscle tissue causing acidification and decline in pH that is dependent on the glycogen storage levels in the muscle (Duclos et al, 2007). Meat pH is the most important quality trait of meat that determines the other meat quality traits including color, WHC, drip loss, cooking loss, tenderness, juiciness, and shelf life in addition to the taste. Fall in pH of meat denatures the proteins, declines the solubility of proteins, and lowers the positive and negatively charged water binding reactive groups on the muscle proteins that reach an isoelectric point. At this point, the opposite charges only attract each other, thus the water binding capacity of muscle proteins is diminished. Loss of water retention occurs as the space between proteins shrinks due to attraction between opposite charges. Water retention is further decreased as the divalent Ca^{2+} and Mg^{2+} sarcoplasmic cations start neutralization of anions on adjoining protein chains causing diminution of electrostatic repulsion among the protein chains (Wismer-Perdersen, 1986; Mir et al., 2017). In short, higher pH increases the WHC, lowers the cooking loss, and supports microbial proliferation or vice versa. In our study, pH tended to be greater in control group followed by GROFIN low Lys group (P = 0.053). Therefore, drip loss was lower in control group followed by GROFIN low Lys group. In this study, the dietary amendments were made intermittently (i.e. during grower and finisher phases) followed by restoration to the same diets as in control group. Our findings are in line with those of Tang et al. (2007) who reported that broilers continuously fed dietary low Lys had lower drip loss whereas those fed diets low in ME had greater drip loss.

5.3. Breast Meat Composition

Birds fed low ME and AA diets (GRO low ME AA and GROFIN low ME AA) had no difference in ether extract crude protein contents of breast meat compared with control group. However, broiler chickens fed low dLys diets (GRO low Lys and GROFIN low Lys) had lower extract content in comparison with control group. However, dry matter and crude ash contents remined unaffected. Broiler chickens fed diets with low ME and crude protein had lower dry matter and crude protein, and higher fat content in breast meat in addition to no effect on crude ash content (Marcu et al., 2012). Similarly, low ME diets had no effect on dry matter, crude protein, and crude ash, however, these diets increased the fat content of breast meat in broiler chickens (Infante-Rodríguez et al., 2016). In addition, a study reported that Aseel chickens fed low Lys diets exhibited no effect on dry matter, crude protein, and crude fat contents although crude ash decreased with decreasing Lys in diets (Hussain et al., 2018).

These findings might be attributed to the pace of muscle growth, and incidence and severity of WS in broiler chickens. It was noted that breast meat showing moderate WS lesions have higher fat and lower protein percentage than those not having WS lesions and fat and protein content in case of severe WS lesions was even higher and lower than moderate WS breast meat (Mudalal et al., 2014; Petracci et al., 2014; Baldi et al., 2018; Kuter, 2018). Under normal circumstances, the muscle growth occurs at a faster pace in modern broiler chickens in response to diets formulated in accordance with their nutrient requirements. It causes the development of localized hypoxia that initiates inflammatory response resulting in muscle fiber degradation (Mutryn et al., 2015). The degraded muscle fibers (protein) are gradually replaced by fat/adipose tissue appearing as WS on the breast muscles running parallel to the muscle fibers. In the present study, the broiler chickens fed diets having low ME and AA density satiated their nutrient requirements through an elevated FI to support the faster growth of muscles. In contrast, birds fed low levels of dietary dLys exhibited lowered FI, slowdown in the growth of muscles, and a low incidence and severity of WS. Consequently, crude fat percentage was higher and crude protein content was lower in broilers fed control and low ME AA diets (GRO low ME AA and GROFIN low ME AA) than those fed low dLys diets.

5.4. Serum Metabolites

Serum TG and CHOL levels are indicative of the existing status of fat metabolism in poultry. This is the first study describing the effect of lowering dietary dLys or ME and AA

density during different growth phases on the blood metabolites of broiler chickens. CHOL is supplied through diets or synthesized in the body. Serum TG and CHOL levels in broiler chickens are governed by several factors like feeding, environmental conditions, genetic makeup, gender, and age (Meluzzi et al., 1992). Serum metabolites (TG and CHOL) were within the normal range reported by Meluzzi et al. (1992).

Restriction or lowering the dietary dLys or ME and AA density during the grower phase reduced the TG and CHOL levels in comparison with their control or GROFIN counterparts. The exact reason of these findings is not known as the serum metabolites were analyzed only at the end of experiment and same diets were fed during the withdrawal phase. Serum metabolites are usually discussed along with growth rate and muscle yields (carcass). Broilers fed GRO or GROFIN low ME AA diets compensated their growth during the grower or grower and finisher phases, respectively. In contrast, GRO or GROFIN low Lys groups continuously had a declined growth during grower or grower and finisher phases, respectively, followed by compensatory growth in the next immediate phase after the restoration to diets as in control group. However, birds in GROFIN low Lys were unable to reach the extent of compensatory growth as exhibited by other groups. Consequently, they had the lowest breast yield among the groups. Zhai et al. (2016) reported a negative correlation between breast meat yield and serum CHOL levels. This might be one of the reasons in addition to increased fat metabolism to support increased fat deposition. This idea is further supported by the fact that low protein (AA) levels in diets tend to increase the fat deposition in poultry (Kassim and Suwanpradit, 1996a; Collin et al., 2003; Yalçin et al., 2010; Jlali et al., 2012).

Higher TG and CHOL levels in GROFIN low ME AA group might be due to continued low lipid metabolism for abdominal and muscle fat deposition during the grower and finisher phases supported by increased FI under the influence of low ME in diets to maintain the increased BW gain and breast yield. A surge in fat deposition in terms of increased serum TG and CHOL levels might have been due to a sudden transition in dietary ME and AA density from finisher phase to withdrawal phase. Studies have reported that low ME diets lead to lowered abdominal fat deposition in birds without any negative effect on growth performance and carcass characteristics (Kassim and Suwanpradit, 1996b; Rabie and Szilagyi, 1998; Fan et al., 2008).

Apparently, lowered serum TG and CHOL in GRO low Lys and GRO low ME AA groups might be attributed to sustained growth rate comprising of finisher and withdrawal

phases after the end of restriction of dLys or ME and AA density during the grower phase. A carryover effect might have kept the fat deposition at a lower level, thus lower serum TG and CHOL might have been observed.

5.5. Incidence and Severity of White Striping

Studies have reported that broiler chickens that have higher BW, greater BW gain, heavier breast and greater breast yield, faster growth rate, older age, and belonging to male gender are more prone to the development of WS (Russo et al., 2015; Dalle Zotte et al., 2015; Alnahhas et al., 2016; Baldi et al., 2018). In protein metabolism, the balance between protein synthesis (anabolism) and protein degradation (catabolism) decides the fate of muscle mass. A balance in the favor of anabolism directs the increase in protein accretion or muscle mass. In modern broiler lines, faster growth rate and muscle accrual is a result of declined protein catabolism (Dransfield and Sosnicki, 1999) that shifts the balance in the favor of anabolism compared with their slow growing counterpart. Studies reported that musculoskeletal defects are attributed to genetic selection for faster growth, effective, and maximal yields (Sosnicki et al, 1991; Mahon, 1999). In view of these, it is considered that the muscles expend their full capacity in order to maintain homeostasis that can be exacerbated in response to any internal or external stress agents. This initiates a chain of muscle fiber degeneration in which fat/adipose tissue steadily replaces the myofiber as in WS. In the present study, broiler chickens fed diets low in dietary dLys levels (GRO low Lys and GROFIN low Lys) slowed down the growth rate by lowering the BW, BW gain, and breast yields, unlike those fed control diets or diets with low ME and AA density (GRO low ME AA and GROFIN low ME AA). Consequently, incidence and severity of WS was lower in broiler chickens fed GRO low Lys and GROFIN low Lys diets in comparison with other groups. These findings are in conformity with those of Meloche et al. (2018c) who reported that reducing 25% or 15% dietary dLys during grower, or grower and finisher phases, respectively, reduced the incidence and severity of WS in Yield Plus × Ross 708 broiler breeders. Likewise, feeding low dLys diets continuously reduced the incidence and severity of WS in Cobb 500 × Cobb broilers, however, with severe reduction in BW and breast yield (Cruz et al., 2017). Moreover, concurrent reduction in dietary ME and AA density has no effect on the incidence and severity of WS in Yield Plus × Ross 708 broiler breeders (Meloche et al., 2018b).

5.6. Incidence and Severity of Footpad Dermatitis

It is known that dietary factors related to protein contribute to the development of FPD in poultry by giving rise to a condition known as wet litter (Furlan et al., 2004; Collett, 2006; Vieira and Lima, 2005; Cengiz et al., 2013; 2017). It has been reported that wet litter or high litter moisture alone is sufficient to initiate the development of FPD in poultry (Mayne et al., 2007).

In the present study, no FPD lesions were observed at 39 and 49 days of experiment. This might be due to better ventilation and temperature management that prevented the high litter moisture as the litter was dry in all the pens irrespective of the groups.

6. CONCLUSIONS AND SUGGESTIONS

Dietary restriction of 15% dLys in this study slowed down the growth trajectory of broiler chickens in the grower or grower and finisher phases of the respective groups. Feeding diets with low dLys or ME and AA density in grower and finisher phases (GROFIN low Lys and GROFIN low ME AA) showed poor growth performance in terms of FCR. Compensatory growth did not occur in any group. In addition, lowering ME and AA density in grower or grower and finisher phases did not slowdown the growth trajectory of broiler chickens as they compensated their ME and AA restriction by increased FI. Carcass and breast yield declined in broiler chickens fed low dLys diets whereas thigh yield increased in these groups. Decreasing ME and AA density in grower or grower and finisher phases had no effect on the carcass, parts, and organ yields compared to control groups. Breast meat quality remined unaffected except for drip loss that was lower in control group followed by GROFIN low Lys group. Lowering dietary dLys increased the crude protein whereas lowering dietary ME and AA density lowered the crude protein content of breast meat of broiler chickens. Dietary restriction of dLys or ME and AA density during grower phase may reduce the serum TG and CHOL levels. Reduction in dietary dLys levels also reduced the incidence and severity of WS in broiler chickens. Taken together, it is concluded that reducing the dietary dLys levels during grower phase reduces the development of WS in broiler chickens by lowering the BW, FI, fat content of breast meat, and lowered fat deposition.

This is the first study conducted in commercial broilers and is a torch bearer for further research in this domain. Under commercial settings in Turkey, rearing period is up to 42 days. Therefore, further experiments are recommended to chalk out the optimum dLys restriction levels during an appropriate growth phase to contain the issue of WS in broiler chickens without any adverse effect on growth performance.

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ANNEXURE

Annex. 1

T.C.
AYDIN ADNAN MENDERES ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
(AYDIN ADÜ-HADYEK)

Aydın, 25/12/2018

Oturum : Hayvan Deneyleri Yerel Etik Kurulu 2018 Yılı XII. Oturum

Sayı : 64583101/2018/138

Proje Başlığı :Etlik piliçlerde farklı büyüme dönemlerinde rasyonda lizin düzeyi veya enerji ile amino asit yoğunluğunun düşürülmesinin beyaz çizgi ve ayak tabanı yangısı oluşum sıklığı ve şiddeti üzerine etkisi.

Proje Yürütücüsü : Özcan CENGİZ

Proje Ekibi : Umair AHSAN, Ömer SEVİM

Bu çalışmanın hiçbir bölümünde:

İnsan embriyosu ve fötusu kullanılması

İnsan embriyosu ve fötusu dokularının kullanılması

Diğer insan doku ve hücrelerinin kullanılması

Hayvan Çalışması

İnsanlarda araştırma

İnsan olmayan primatların kullanılması

Transgenik hayvanların kullanılması

Hayvanlarda genetik modifikasyon öngörülmemiştir.

Bu çalışmanın yapılmasında etik açıdan bir sakınca bulunmamaktadır.

Prof. Dr. M. Dinçer BİLGİN
Başkan

(Yıllık İzinli)
Prof. Dr. Turhan DOST
Başkan Yardımcısı

Prof. Dr. Işıl SÖNMEZ
Üye

Prof. Dr. Deniz ÇOBAN
Üye

Prof. Dr. Yücel KOCA
Üye

Doç. Dr. Evrim DERELİ FİDAN
Üye

Vet. Hek. Dr. Serdar AKTAŞ
Üye

Vet. Hek. Dr. Birgül ÜNAL
Üye

(Toplantıya Katılmadı)
Yurdagül ALTINBAŞ
Üye

Bu rapor, sadece Adnan Menderes Üniversitesi'nde yapılacak çalışmalar için geçerlidir.

RESUMÉ

A. PERSONAL DETAILS

Family Name, First Name	**:**	AHSAN, Umair
Nationality	**:**	Pakistani
Birthplace & Date	**:**	Khanewal, Pakistan | 12-OCT-1990
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Foreign Languages	**:**	English, Turkish

B. EDUCATION

Degree	Institution	Completion
Bachelors Doctor of Veterinary Medicine	The Islamia University of Bahawalpur	2013
M.Sc Animal Nutrition and Nutritional Diseases	Aydın Adnan Menderes University	2016
Ph. D. Animal Nutrition and Nutritional Diseases	Aydın Adnan Menderes University	2020

C. SCHOLARSHIPS & AWARDS

1. Position holder scholarship from The Islamia University of Bahawalpur throughout the DVM tenure (2008-13)

2. Merit scholarship from Punjab Educational Endowment Fund (PEEF), Government of Punjab throughout the DVM tenure (2008-13)

3. Merit laptop award from the Government of Punjab (2012)

4. Gold medal from The Islamia University of Bahawalpur for securing the 1st position in DVM

5. Scholarship for pursuing master and Ph.D in Turkey from The Scientific and Technological Research Council of Turkey (**TÜBİTAK**) under the framework **BİDEB 2215** - Graduate Scholarship Programme for International Students vide letter No. 16698286-215.01-99618 during the term 2014-2016 (master) and 2016-2020 (Ph. D.)

D. ACADEMIC PUBLICATIONS

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10. Chacher MFA, Kamran Z, **Ahsan U,** Ahmad S, Qutab ud Din HG, Cengiz Ö. Use of MOS in broiler diets: an overview of underlying mechanisms. Worlds's Poultry Science Journal 2017, 73(4), 831-844.

11. Köksal BH, Cengiz Ö, **Ahsan U,** Sevim Ö, Tatlı O, Beyaz D, Büyükyörük S, Boyacıoğlu, Kuter E, Kızanlık PK, Kaya M, Önol AG. Effect of dietary prebiotics supplementation on growth performance, relative carcass and organ yields, gut microbiome, and blood malondialdehyde level of broilers subjected to post-hatch feed and water restriction. European Poultry Science 2018, 82. doi: 10.1399/eps.2018.234.

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Conference Proceedings

1. Ahsan U, Kuter E, Köksal BH, Güleş Ö, Raza I, Kızanlık PK, Beyaz D, Cengiz Ö. Effect of dietary supplementation of different levels of phytogenic feed additive on intestinal microbiota and gut morphology of broiler chickens. 4th International Poultry Meat Congress, Antalya, Turkey, 26/04/2017 to 30/04/2017.

2. Sevim Ö, Tatlı O, Kuter E, Karimiyan E, Kaya M, Karaaslan S, **Ahsan U,** Uçan U, İpek E, Köksal BH, Cengiz Ö, Önol AG. Effects of nano selenium on performance, egg quality, sperm quality and hatching parameters of breeding quails. 2nd International Animal Nutrition Congress, Antalya, turkey, 1/11/2018 to 4/11/2018.

3. Tatlı O, Sevim Ö, Karaaslan S, Kuter E, Kaya M, Karimiyan E, **Ahsan U,** Uçan U, İpek E, Köksal BH, Cengiz Ö, Önol AG. Effects of dietary supplementation of nano zinc on performance, egg characteristics, sperm quality and hatching parameters in breeding quails. 2nd International Animal Nutrition Congress, Antalya, Turkey, 1/11/2018 to 4/11/2018.

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