Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels

R. F. A. Cruz,* S. L. Vieira,*[,1] L. Kindlein,† M. Kipper,* H. S. Cemin,* and S. M. Rauber*

*Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 7712, Porto Alegre, RS, 91540-000, Brazil; and †Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 8834, Porto Alegre, RS, 91540-000, Brazil

ABSTRACT Two experiments were conducted to evaluate the prevalence and severity of white striping (WS) and wooden breast (WB) in breast fillets from broilers fed diets with increasing digestible Lysine (dLys) from 12 to 28 d (Exp. 1) and from 28 to 42 d (Exp. 2). Trials were sequentially conducted using one-d-old male, slow-feathering Cobb 500 × Cobb broilers, both with 6 treatments and 8 replicates. Increasing dLys levels were equally spaced from 0.77 to 1.17% in Exp. 1 and from 0.68 to 1.07% in Exp. 2. The lowest dLys diet was not supplemented with L-Lysine (L-Lys) in either one of the studies and all other essential amino acid (AA) met or exceeded current commercial recommendations such that their dietary concentrations did not limit broiler growth. Four birds per pen were randomly selected from each replication and processed at 35 and 42 d in Exp. 1 and Exp. 2, respectively. Deboned breast fillets (*Pectoralis major*) were submitted to a 3 subject panel evaluation to detect the presence of WS and WB, as well as to provide scores of WS (0-normal, 1-moderate, 2-severe) and WB (0-normal, 1-moderate light, 2-moderate, 3-severe). Increasing the level of dLys had a positive effect on BW, carcass, and breast weight, as well as breast yield. White striping and WB prevalences were 32.3 and 85.9% in Exp. 1 and 87.1 and 89.2% in Exp. 2. Birds fed diets not supplemented with L-Lys had the lowest average WS and WB scores (0.22 and 0.78 in Exp. 1 and 0.61 and 0.68 in Exp. 2). White striping and WB presented linear responses to performance variables in Exp. 1, whereas quadratic responses were observed for all variables in Exp. 2. In conclusion, increasing the level of dLys improved growth performance and carcass traits as well as induced the occurrence and severity of WS and WB lesions.

Key words: broiler, breast myopathy, digestible lysine, white striping, wooden breast

2017 Poultry Science 96:501–510
http://dx.doi.org/10.3382/ps/pew310

INTRODUCTION

Constant increases in the world demand for white meat have been spurring the broiler industry towards practices that increase its production. Breast meat as a proportion of total chicken meat has been significantly increasing mainly due to improvements in genetic selection, but also due to advances in health, farm management practices, and nutrition (Havenstein et al., 2003a,b; Zuidhof et al., 2014).

As the most valuable cut from broiler chickens, breast meat must meet high quality market presentation standards. Therefore, it is of great importance to breeding companies, as well as to broiler producers, that breast muscle growth is such that the white meat quality delivered from it is not compromised. Recent reports of increased cases of breast muscle myopathies have brought concerns to the broiler meat industry because affected carcasses can be downgraded or less frequently condemned, leading to economic losses (Bailey et al., 2015). This is the case with white striping (**WS**) and wooden breast (**WB**) conditions, which appear to affect only the *Pectoralis major* as opposed to the deep breast myopathy (also known as green muscle disease) (Bailey et al., 2015) which only affects the *Pectoralis minor*.

White striping is characterized by white striations appearing in parallel to the direction of muscle fibers in broiler breast fillets (Kuttappan et al., 2013b). While breast meat that is visibly affected by WS contains slightly higher levels of fat and may be visually unappealing to the consumer, there are no known health or safety concerns associated with consuming breast meat with WS. Furthermore, recent research shows that WS is not related to any specific commercial broiler strains (Kuttappan et al., 2012a,b; Kuttappan et al., 2013a,b; Petracci et al., 2013; Ferreira et al., 2014; Sihvo et al., 2014; Mudalal et al., 2015). Histologic reports of WS demonstrated alterations with loss of cross striations between muscle fibers, variability in muscle fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, mononuclear cell infiltration, lipidosis,

Received February 18, 2016.
Accepted July 28, 2016.
[1]Corresponding author: slvieira@ufrgs.br

interstitial inflammation, and fibrosis (Kuttappan et al., 2013b; Ferreira et al., 2014). The etiology of WS is unknown, however broilers with higher growth rate and heavier breast weight have greater incidence of WS (Kuttappan et al., 2012a; Ferreira et al., 2014).

Wooden breast is characterized by variable degrees of hardness in the *Pectoralis major* showing bulging and pale expansive areas (Sihvo et al., 2014). Wooden breast is characterized by concurrent polyphasic myodegeneration and regeneration, which leads to a variable amount of interstitial connective tissue accumulation and fibrosis (Sihvo et al., 2014). Lesions are seen as early as at 3 wk-of-age and can affect a high proportion of birds in a flock (Mutryn et al., 2015). Depending on the severity of the condition, WB may have surface hemorrhaging and produce a sterile, gelatinous exudate between the breast surface and the skin. So far, WS and WB are thought to be distinct myopathies since they are found independently of each other; however, they can be observed together (Bailey et al., 2015).

Increased growth rate as well as breast meat yields resulting from genetic selection have been suggested as leading causes for the increased presence of WS and WB in broiler chickens (Petracci and Cavani, 2012; Kuttappan et al., 2012a; Sihvo et al., 2014); however, the analysis of data from two broiler lines that differed in terms of selection for breast yield showed that there is also a strong non-genetic component for all the breast muscle myopathy traits (Bailey et al., 2015).

Post hatching muscle growth is mostly related to muscle cell hypertrophy instead of muscle hyperplasia (Sklan and Noy, 2003). Hypertrophy is attained by increasing cell diameter instead of length. Therefore, broilers with greater breast proportions have increased muscle cell diameters (Zheng et al., 2009). However, the full expression of the genetic potential for growth and meat yields of the modern broiler can only be fulfilled by adequate nutrition. The implication of dietary Lys on broiler muscle cell hypertrophy has been well established (Tesseraud et al., 1996; Eits et al., 2003; Sklan and Noy, 2003). Breast muscles are particularly sensitive to dietary concentration of Lys since it is its main essential amino acid (**AA**) representing approximately 7% of the total protein content (Munks et al., 1945). Concentration of Lys in feed affects growth, but also carcass yield. Therefore, broilers fed diets with increased Lys have thicker myofibers regardless of genetics (Holsheimer and Veerkamp, 1992; Roy et al., 2006; Sakomura et al., 2015). By itself, Lys can modulate breast growth due to a higher synthesis to degradation ratio (Urdaneta-Rincon and Leeson, 2004; Mehri et al., 2012).

Usual determination of AA requirements target the optimization of growth rate, FCR, and breast meat yields; however, AA concentrations that optimize breast meat yields have shown to be higher than for the other responses (Moran and Bilgili, 1990; Holsheimer and Veerkamp, 1992; Huyghebaert et al., 1994; Schutte and Pack, 1995). Because dietary Lys is such an important factor for breast muscle growth, it is possible that its concentration in feed is capable of triggering, or at least modulating, the appearance of breast muscle myopathies. The objective of this study was to evaluate the prevalence of WB and WS in broilers fed grower or finisher diets with increasing digestible Lysine (**dLys**) levels.

MATERIALS AND METHODS

Bird Husbandry

All procedures throughout the current study were approved by the Ethics and Research Committee of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Two experiments (**Exp.**) were conducted using 1,200 one-d-old male, slow-feathering Cobb 500 x Cobb broilers. Chicks were vaccinated for Marek's and infectious bursal diseases at the hatchery and then randomly distributed into 48 pens of 1.65 × 1.65 m (9.2 birds/m^2, 25 birds per pen). The pens were bedded with rice hulls, one 15 kg capacity tube feeder, and 3 nipple drinkers. Mash feeds and water were available for ad libitum consumption. Mortality was recorded daily. Initial temperature was set to 32°C being reduced by 1°C every two days until 22°C. A continuous lighting schedule was used until 7 d-of-age, whereas a 20L:4D cycle with constant intensity was used thereafter.

Experimental Diets

Dietary treatments in Exp. 1 were provided from 12 to 28 d-of-age and in Exp. 2 from 28 to 42 d-of-age (Tables 1 and 2). Diets in both experiments were based on corn, soybean meal, and corn gluten meal (Rostagno et al., 2011). Basal diets were formulated without supplemental L-Lysine (L-Lys) (0.77% dLys in Exp. 1 and 0.68% of dLys in Exp. 2, respectively), but contained all other essential AA that meet or exceed commercial recommendations to ensure dietary adequacy such that responses were only limited by dLys. Treatments were structured with the addition of increasing levels of dLys in 0.08% increments from 0.77 to 1.17% in Exp. 1 and from 0.68 to 1.07% in Exp. 2 by adding L-Lysine HCl at the expense of an inert filler (kaolin). Standard corn-SBM-based broiler diets were provided to all treatments in the periods before (starter diet from 1 to 12 d-of-age) and after (finisher diet from 28 to 35 d-of-age) experimental phases of Exp. 1 and before (starter diet from 1 to 12 d-of-age; grower from 12 to 28 d-of-age) the experimental phase of Exp. 2 (Table 3).

Broiler Performance Measurements

Birds and feeds were weighed 1, 12, 28, and 35 days in Exp. 1, and 1, 12, 28, and 42 days in Exp 2. Four birds per pen were randomly selected from each pen

Table 1. Ingredient and nutrient composition of experimental diets in the Experiment 1.

Item	Diets in the Experiment 1 (12 to 28 d)					
	0.77% dLys[1]	0.85% dLys	0.93% dLys	1.01% dLys	1.09% dLys	1.17% dLys
Ingredients, %						
Corn	67.97					
Soybean meal	21.54					
Soybean oil	0.94					
Corn gluten meal	5.50					
Sodium bicarbonate	0.39					
Dicalcium phosphate	0.94					
Limestone	1.06					
Salt	0.14					
Vitamin and mineral mix[2]	0.15					
Inert filler (kaolin)	0.50	0.40	0.30	0.20	0.10	–
DL-Methionine, 99%	0.26					
L-Lysine HCl, 78%	–	0.10	0.20	0.30	0.40	0.50
L-Leucine, 98.5%	0.03					
L-Threonine, 98.5%	0.14					
L-Arginine, 98%	0.14					
L-Isoleucine, 98.5%	0.08					
L-Valine, 96.5%	0.11					
L-Tryptophan, 98%	0.01					
Choline chloride, 60%	0.10					
Energy and nutrients, % or unless noted[3]						
AME_n, kcal/kg	3,108					
CP	19.50 (19.90)					
Ca	0.84 (0.84)					
Av. P	0.42					
Choline, mg/kg	1,550					
dLys	0.77	0.85	0.93	1.01	1.09	1.17
dMet + dCys	0.83					
dThr	0.73					
dVal	0.89					
Total AA						
Lys	0.86 (0.85)	0.94 (0.95)	1.02 (1.00)	1.10 (1.15)	1.16 (1.18)	1.26 (1.26)
Met + Cys	0.92 (0.89)					
Thr	0.84 (0.83)					
Val	0.99 (1.00)					

[1]Ingredients, energy and nutrients were the same for all diets, except for the inert and L-Lys HCl.
[2]Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamine, 0.012 mg, panthothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; phytase, 100 mg, monensin sodium, 100 mg.
[3]Values between parenthesis are analyzed; d = digestible.

at 35 and 42 d-of-age, respectively in Exp. 1 and 2. Birds were fasted for 6 h, individually weighed before electrical stunning (45 V for 3 s), bled for 3 min after carotid and jugular veins cut, scalded at 60°C for 45 s, and mechanically defeathered. Carcasses were manually eviscerated and then statically chilled in slush ice for 3 h before processing. Breast fillets were manually deboned from the carcasses. White striping and WB evaluations were immediately performed on boneless skinless breasts. Carcass yield was expressed as a percentage of live weight and breast yield was expressed as a percentage of the eviscerated carcass weight.

White Striping and Wooden Breast Scores

Occurrence and severity of WS and WB were assessed by a 3 subject panel evaluation. First, deboned fillets were visually separated into groups by the presence or absence of WS and WB. Breast fillets presenting WS were classified and scored according to Kuttappan et al. (2013b) as: normal (score 0) without any distinct white lines; moderate (score 1) presenting white lines in parallel to muscle fibers and that were <1 mm thick; and severe (score 2) exhibiting white lines in parallel to muscle fibers and that were >1 mm thick. Breast fillets presenting WB were classified as: normal (score 0) without any hardness or paleness areas; moderate light (score 1) mildly affected in cranial and/or caudal areas; moderate severe (score 2) moderately affected throughout the fillets; and severe (score 3) with surface hemorrhaging and the presence of sterile exudate on the muscle surface.

Statistical Analysis

The study was conducted in a completely randomized design. Data were tested for normality prior to analysis and values that were not normal were square root transformed to maintain a normal distribution of residuals. Live performance data were submitted to ANOVA using GLM procedures of SAS (SAS Institute, 2009) and, when significant, means were compared by Tukey test at 5%. Scores of WS and WB were analyzed using the non-parametric Kruskal-Wallis test

Table 2. Ingredient and nutrient composition of experimental diets in the Experiment 2.

Item	Diets in the Experiment 2 (28 to 42 d)					
	0.68% dLys[1]	0.76% dLys	0.84% dLys	0.92% dLys	1.00% dLys	1.08% dLys
Ingredients, %						
Corn	75.54					
Soybean meal	14.32					
Soybean oil	0.80					
Corn gluten meal	5.80					
Sodium bicarbonate	0.57					
Dicalcium phosphate	0.52					
Limestone	0.91					
Salt	0.05					
Vitamin and mineral mix[2]	0.15					
Inert filler[2]	0.50	0.40	0.30	0.20	0.10	–
DL-Methionine, 99%	0.27					
L-Lysine HCl, 78%	–	0.10	0.20	0.30	0.40	0.50
L-Threonine, 98.5%	0.09					
L-Arginine, 98%	0.20					
L-Isoleucine, 98.5%	0.06					
L-Valine, 96.5%	0.07					
L-Tryptophan, 98%	0.01					
Choline chloride, 60%	0.14					
Energy and nutrients, % or unless noted[3]						
AME_n, kcal/kg	3,180					
CP	18.90 (19.00)					
Ca	0.68 (0.67)					
Av. P	0.33					
Choline, mg/kg	1,500					
dLys	0.68	0.76	0.84	0.92	1.00	1.08
dMet + dCys	0.80					
dThr	0.72					
dVal	0.87					
Total AA						
Lys	0.75 (0.77)	0.84 (0.84)	0.92 (0.95)	1.00 (1.03)	1.08 (1.11)	1.16 (1.19)
Met + Cys	0.87 (0.81)					
Thr	0.80 (0.81)					
Val	1.02 (1.08)					

[1]Ingredients, energy and nutrients were the same for all diets, except for the inert and L-Lys HCl.
[2]Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamine, 0.012 mg, panthothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; phytase, 100 mg, monensin sodium, 100 mg.
[3]Values between parenthesis are analyzed; d = digestible.

(PROC NPAR1WAY), and means were separated using the Bonferroni test ($P < 0.05$). Linear and quadratic polynomial regressions were estimated (PROC REG) for WS and WB using dLys, BW, carcass, and breast fillet weight as well as yield as independent variables.

RESULTS

Growth Performance and Processing Data

Growth performance and broiler processing data from Exp. 1 and 2 are presented in Table 4. Increasing dietary dLys levels positively affected ($P < 0.01$) BW gain and carcass weight in Exp. 1 and 2. Birds fed diets without Lys supplementation had the lowest BW and carcass weight. Body weight, carcass weight, and breast yield increased quadratically ($P < 0.01$) when broilers were fed diets with increasing levels of dLys. In Exp. 1 maximum responses at 35 d for BW, carcass weight, and breast weight were obtained using 1.08%, 1.07%, and 1.07% of dLys, respectively. In Exp. 2, maximum responses at 42 d for BW gain, carcass weight, and breast weight of broilers were obtained using 0.99%, 0.98%, and 0.98% of dLys, respectively. Quadratic increases ($P < 0.01$) were observed for breast meat yields at 35 and 42 d, with maximum quadratic responses obtained with 1.08% and 1.01% dLys, respectively.

White Striping and Wooden Breast Occurrence and Severity

Occurrence of WS and WB is shown in Figure 1. In Exp. 1, WS occurrence ranged from 18.8 to 56.3% among treatments, averaging 32.3%. Score 1 occurrence increased when broilers were fed diets with 1.01% dLys and then moderately decreased, whereas score 2 tended to linearly increase as the level of dLys increased in Exp. 1 and 2. White striping occurrence in Exp. 2 ranged from 58.1 to 100%, averaging 87.1% of the fillets having scores 1 and 2. Score 1 was consistent among increasing levels of dLys, except at 0.92% dLys, where the lowest value was observed. Furthermore, a WS score of 2 increased when broilers were fed diets with 0.92% dLys and then the score tended to decrease (Figure 1).

The occurrence of WB in Exp. 1 ranged from 65.6 to 100% among treatments, averaging 85.9% (Figure 1).

Table 3. Ingredient and nutrient composition of the common diets.[1]

Item	Diets		
	Starter	Grower	Finisher
Ingredients, %			
Corn	47.48	54.73	59.39
Soybean meal	44.45	36.98	33.07
Soybean oil	4.04	5.33	5.11
Sodium bicarbonate	0.08	0.01	0.02
Dicalcium phosphate	1.33	0.74	0.40
Limestone	1.35	1.03	0.85
Salt	0.50	0.45	0.42
Vitamin and mineral mix[2]	0.15	0.15	0.15
DL-Methionine, 99%	0.40	0.34	0.31
L-Lysine HCl, 78%	0.14	0.16	0.18
L-Threonine, 98.5%	0.05	0.04	0.04
Choline chloride, 60%	0.03	0.04	0.06
Energy and nutrients, % or unless noted			
AME_n, kcal/kg	2,960	3,150	3,200
CP	24.20	21.40	20.00
Ca	1.05	0.80	0.66
Av. P	0.52	0.40	0.33
Choline, mg/kg	1,600	1,500	1,500
dLys	1.34	1.18	1.10
dMet + dCys	1.03	0.91	0.85
dThr	0.87	0.77	0.72
dVal	1.03	0.91	0.85

[1]Exp. 1: Starter and finisher provided before and after the experimental phase (12 to 28 d), respectively; Exp. 2: starter and grower provided before the experimental phase (28 to 42 d).

[2]Composition per kg of feed: vit. A, 8,000 UI; vit. D_3, 2,000 UI; vit. E, 30 UI; vit. K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamine, 0.012 mg, panthothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg; phytase, 100 mg, monensin sodium, 100 mg.

Score 1 had a consistent occurrence throughout all dLys levels, whereas scores 2 and 3 tended to increase. Wooden breast occurrence in Exp. 2 ranged from 51.6 to 100% among treatments, averaging 89.2%. Score 1 occurrence of WB was fairly constant among dLys levels, except when 0.76 and 0.92% dLys were tested; these levels of dLys had the highest and lowest occurrences, respectively. Score 2 was prone to increase as the level of dLys increased, whereas score 3 increased as dLys increase to 0.92% and then gradually decreased as the level of dLys approached 1.08%.

The average WS and WB scores in broilers evaluated at 35 and 42 d are shown in Figures 2 and 3. The severity of WS and WB was lower ($P < 0.01$) when broilers were fed diet without supplemental L-Lysine HCl. Compared to broilers fed diets with 1.01% dLys in Exp. 1. In Exp. 2, means of WS and WB scores were higher ($P < 0.01$) in all dLys levels compared to the basal diet with 0.68% of dLys.

Regression Analysis of White Striping and Wooden Breast Scores

White striping and WB scores had a positive relationship with dLys levels and performance variables in the grower and finisher phases (Table 5). A linear response ($P < 0.001$) of WS and WB scores was observed in BW, breast weight, and breast yield of broilers at 35 d in Exp. 1. One exception was the relationship between WB and dLys, which was quadratic and the score was estimated to be the highest at 1.10% dLys. In Exp. 2, WS had quadratic responses ($P < 0.001$) for dLys (0.96%), BW (3,400 g), breast weight (842 g), and breast yield (30.1%). A quadratic response ($P < 0.001$) of WB score was also observed for dLys (0.98%), BW (2,598 g), breast weight (884 g), and breast yield (32.1%).

DISCUSSION

The best responses for broiler BW in Exp. 1 and 2 were estimated at 1.08% and 0.99% of dLys, respectively. Estimations obtained in the present study are in agreement with those presented by Dozier et al. (2009, 2010) for BW gain, which were 1.07% dLys from 14 to 28 d and 0.99% dLys from 28 to 42 d. Values are higher than those observed with birds used in research from previous decades, which is likely related to less feed intake per unit of BW and a higher rate of meat accretion by the modern broiler (Havenstein et al., 2003a; b). However, the objective of this study was not to reassess dLys requirements, but to evaluate the effect of increasing the dietary level of dLys on WS and WB occurrence and severity.

Lysine is well known as an important amino acid for broiler growth performance and proper muscle development. It has been reported to increase carcass yield and alter its composition by increasing meat yield and reducing carcass fat (Leclercq, 1998; Sterling et al., 2006). Dietary lysine plays an important role in breast muscle protein turnover by modulating protein synthesis and

Table 4. Body, carcass, and breast fillet (*Pectoralis major*) weights of broilers fed increased dLys from 12 to 28 d and 28 to 42 d and processed at 35 (Exp. 1) and 42 d (Exp. 2), respectively.[1]

dLys, %[2]		Body weight, g		Carcass weight[3], g		Breast fillets[4]			
Exp. 1	Exp. 2	35 d	42 d	35 d	42 d	g		%	
(12 to 28 d)	(28 to 42 d)					35 d	42 d	35 d	42 d
0.77	0.68	2,159[d]	3,084[c]	1,656[d]	2,424[c]	353[c]	524[c]	21.3[c]	21.7[d]
0.85	0.76	2,282[c]	3,285[b]	1,778[c]	2,592[b]	405[b]	597[b]	22.8[b,c]	23.0[c]
0.93	0.84	2,323[b,c]	3,452[a]	1,813[b,c]	2,764[a]	429[a,b]	677[a]	23.7[a,b]	24.5[b]
1.01	0.92	2,415[a]	3,517[a]	1,896[a]	2,837[a]	463[a]	728[a]	24.4[a]	25.7[a]
1.09	1.00	2,389[a,b]	3,468[a]	1,873[a,b]	2,793[a]	454[a]	698[a]	24.2[a]	25.0[a,b]
1.17	1.08	2,393[a,b]	3,513[a]	1,866[a,b]	2,804[a]	450[a]	698[a]	24.1[a]	24.9[a,b]
SEM		14.9	27.0	13.3	24.4	4.1	11.8	0.21	0.23
P-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Item		Regression equations[5]	*P*-value	r^2	Maximum response %
Body weight, g	35 d	$Y = -2.4787x^2 + 5.3709x - 0.5030$	<0.001	0.720	1.08
	42 d	$Y = -4.5875x^2 + 9.0593x - 0.9471$	<0.001	0.685	0.99
Breast weight, g	35 d	$Y = -1.1822x^2 + 2.5290x - 0.8925$	<0.001	0.734	1.07
	42 d	$Y = -2.2425x^2 + 4.3833x - 1.4251$	<0.001	0.746	0.98
Carcass weight, g	35 d	$Y = -2.4991x^2 + 5.3519x - 0.9789$	<0.001	0.765	1.07
	42 d	$Y = -4.6018x^2 + 9.0202x - 1.5857$	<0.001	0.760	0.98
Breast yield, %	35 d	$Y = -31.43x^2 + 67.8x - 12.29$	<0.001	0.335	1.08
	42 d	$Y = -34.98x^2 + 70.5x - 10.20$	<0.001	0.417	1.01

[a–d]Means followed by different letters in the same column differ by Tukey test ($P \leq 0.05$).
[1]35 and 42 d refer to data obtained at the end of experiments 1 and 2, respectively.
[2]Digestible Lys in Exp. 1 and 2, respectively.
[3]Eviscerated carcass without neck and feet.
[4]*Pectoralis major* weight or as a proportion of the eviscerated carcass.
[5]Quadratic polynomial: $Y = \beta3 \times X^2 + \beta2 \times X + \beta1$; where Y is the dependent variable, X is the dietary level of dLys, $\beta1$ is the intercept, $\beta2$ and $\beta3$ are the linear and quadratic coefficients, respectively; maximum response were obtained by calculating: - $\beta2 \div (2 \times \beta3)$.

breakdown rates (Tesseraud et al., 2001; Urdaneta-Rincon and Leeson, 2004). Furthermore, Lys deficiency results in reduced protein synthesis, especially in the *Pectoralis major*, which is more sensitive to Lys than wings and thigh muscles (Tesseraud et al., 1996). Conversely to leg muscles, breast muscles are a direct product of genetic selection, have minor functional purpose (McDonald and Swick, 1981), and represent a considerable protein store during periods of nutrient deficiency (Tesseraud et al., 1996).

In this study, the mean occurrence of WS was 31.3% in Exp. 1 and 89.0% in Exp. 2 (Figure 1). Findings are in agreement with Russo et al. (2015), who observed 82.5% occurrence of WS in 55 d-of-age broilers with 3.6 kg mean BW and with Kuttappan et al. (2012a), who reported WS prevalence of 74.6% in birds with 3.0 kg average BW. Conversely, Petracci et al. (2013) reported WS occurrence as low as 12% in broiler chickens from 45 to 54 d of age reared under commercial conditions with average live weight of 2.75 kg. These differences may be influenced by BW (Kuttappan et al., 2012a; Kuttappan et al., 2013a; Petracci et al., 2013), as well as growth rate (Kuttappan et al., 2012a) and strain (Kuttappan et al., 2013a). Kuttappan et al. (2013b) reported that WS is associated with increased occurrence of muscle damage, which may be a result of muscles outgrowing their physiological support systems (i.e., vascular system, O_2 carrying capacity, nutrient-metabolite exchange) (Wilson et al., 1990). Reduced capillary density in heavier birds with higher percentage of breast meat could result in decreased supply of nutrients and oxygen and slower removal of lactic acid from breast muscle, which ultimately may lead to muscle damage (Hoving-Bolink et al., 2000).

Birds slaughtered with higher BW had higher severity of WS and WB lesions. The difference in the severity of myopathies between Exp. 1 and 2 could be explained by the different BW of broilers in both experiments (2.23 vs. 3.38 kg). In the present study, average scores of WS occurrence in broilers with 2.4 kg and 3.5 kg were 0.62 and 1.67, respectively. These results are in agreement with findings by Russo et al. (2015), who compared WS score in medium (2.59 kg) and heavy (3.64 kg) broilers and observed 0.84 and 1.09 average scores, respectively. Occurrences of severe WS score was 9.4% in Exp. 1 and 40.9% in Exp. 2, which is considerably higher than reported by Kuttappan et al. (2012a), Kuttappan et al. (2013a), Petracci et al. (2013), Ferreira et al. (2014), who observed severe score prevalence of 8.7%, 8.3%, 3.1%, and 2.5%, respectively. In both trials, increasing level of dLys increased the occurrence and severity of WS lesions, possibly because the higher levels of dLys maximized the genetic potential of the broiler, but overwhelmed the support systems that allow for normal tissue turnover.

Wooden breast occurrence was similar in both experiments (85.9 and 89.2% in Exp. 1 and 2, respectively). According to Mutryn et al. (2015) some degree of WB has been anecdotally reported to affect up to 50% of a flock. Conversely, Trocino et al. (2015)

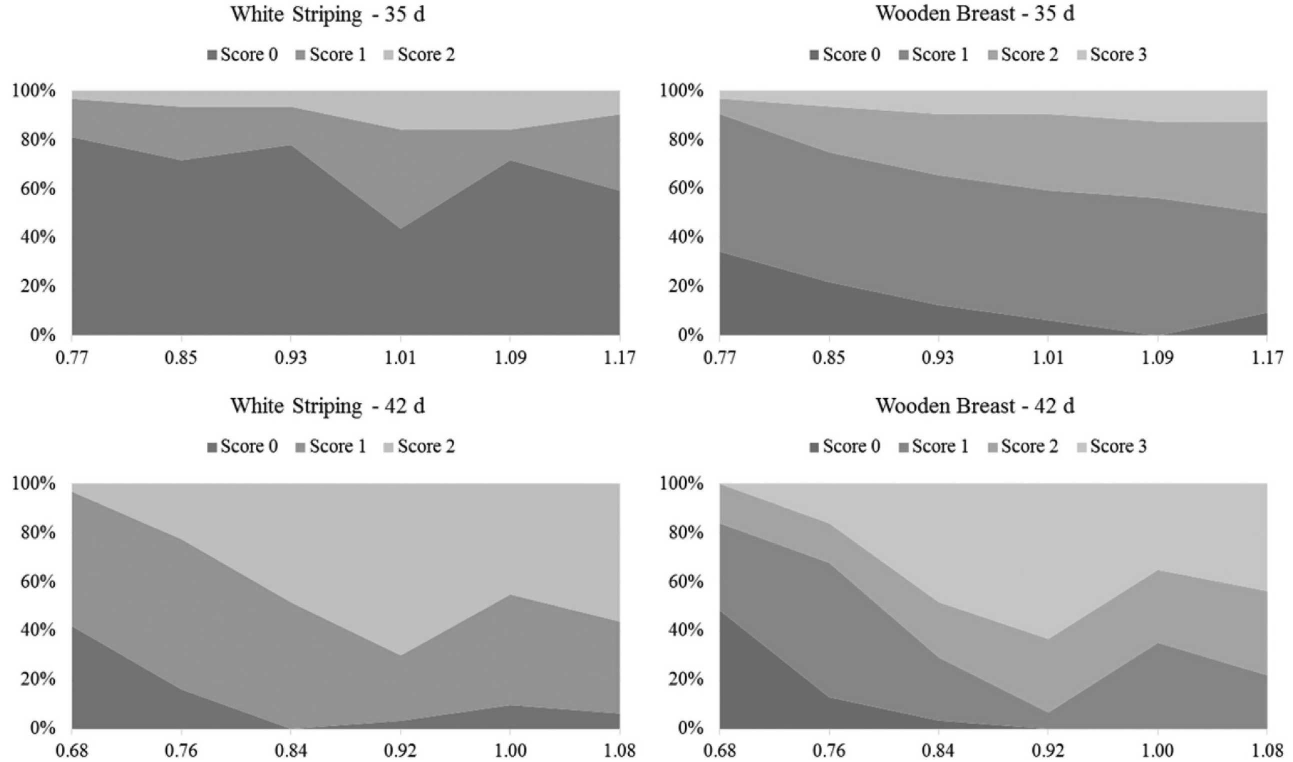

Figure 1. Occurrence of breast fillets presenting white striping[1] and wooden breast[2] from broilers fed increasing dLys levels from 12 to 28 d and processed at 35 d (Exp. 1); and from 28 to 42 d and processed at 42 d (Exp. 2), %. [1]White striping scores were evaluated according to Kuttappan et al. (2013b) as: 0 (normal, without white lines in parallel to muscle fibers), 1 (moderate, with white lines <1 mm thick, and 2 (severe, with white lines >1 mm thick). [2]Wooden breast scores were: 0 (normal, without any hardness or paleness areas), 1 (moderate light, mildly affected at the cranial and/or caudal areas), 2 (moderate severe, affected throughout the fillet), and 3 (severe, with surface hemorrhaging and exudate on the surface).

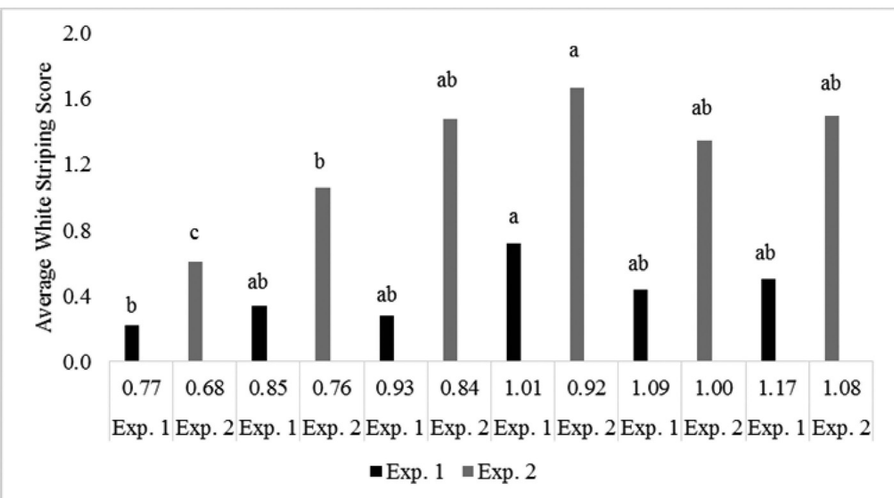

Figure 2. Average white striping scores in breast fillets from broilers fed increasing dLys from 12 to 28 d (Exp. 1) and from 28 to 42 d (Exp. 2)[1]. [a-c]Means followed by different letters in the same experiment differ using the Bonferroni test ($P \leq 0.05$), SEM for Exp. 1 and 2 were 0.057 and 0.07, respectively. [1]White striping score means in breast fillets from broilers fed increasing dLys from 12 to 28 d and processed at 35 d, and from 28 to 42 d and processed at 42 d.

observed 12.2% average WB occurrence in broilers; however, 97% of breasts submitted to histological analysis presented damaged muscle fibers, which have been attributed to WB (Sihvo et al. 2014; Soglia et al., 2015).

Wooden breast severity was remarkably different between both experiments conducted. In Exp. 1, average occurrence of severe score was 8.9% and in Exp. 2, 34.4%. Moreover, a higher occurrence of low scores was observed in Exp. 1 than in Exp. 2 (52.1% vs. 30.1%). It is important to note that this score can easily be interpreted as normal breast in commercial slaughterhouses. Furthermore, WB mean score was 1.29 in

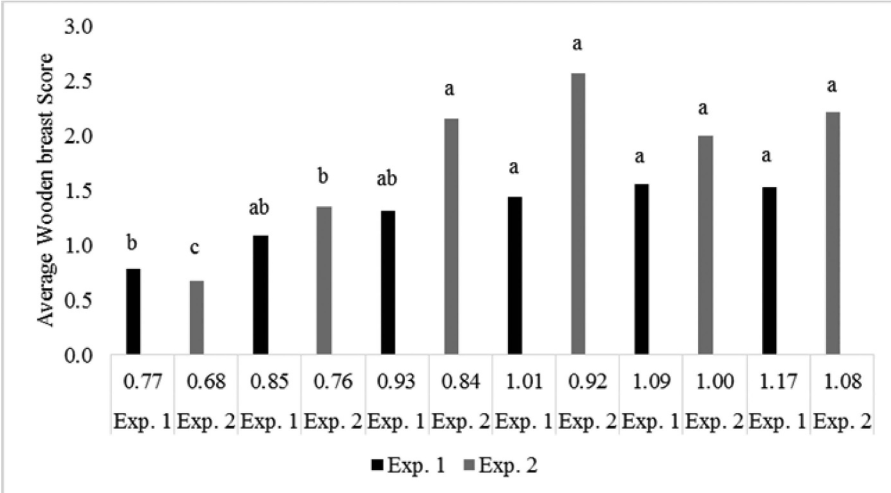

Figure 3. Average wooden breast scores in breast fillets from broilers fed increasing dLys from 12 to 28 d (Exp. 1) and from 28 to 42 d (Exp. 2)[1]. [a-c]Means followed by different letters in the same experiment differ using the Bonferroni test ($P \leq 0.05$), SEM for Exp. 1 and 2 were 0.064 and 0.109, respectively. [1]Wooden breast scoresmeans in breast fillets from broilers fed increasing dLys from 12 to 28 d and processed at 35 d, and from 28 to 42 d and processed at 42 d.

Table 5. Regression analysis estimating white striping and wooden breast occurrence from broilers fed increased dLys from 12 to 28 d (Exp. 1) and 28 to 42 d (Exp. 2) and processed at 35 and 42 d, respectively.[1]

Item	Regression equations[1]	*P*-value	r^2	Maximum score at
	dLys from 12 to 28 d[2]			
White striping				
dLys, %	$Y = 0.615x - 0.235$	0.029	0.025	–
Body weight, g	$Y = 0.001x - 2.068$	<0.001	0.053	–
Breast weight, g	$Y = 0.0029x - 0.881$	<0.001	0.081	–
Breast yield, %	$Y = 0.081x - 1.55$	<0.001	0.080	–
Wooden breast				
dLys, %	$Y = -4.37x^2 + 9.6500x - 4.14$	0.033	0.132	1.10
Body weight, g	$Y = 0.0015x - 2.534$	<0.001	0.141	–
Breast weight, g	$Y = 0.0055x - 1.303$	<0.001	0.364	–
Breast yield, %	$Y = 0.1474x - 2.409$	<0.001	0.335	–
	dLys from 28 to 42 d[3]			
White striping				
dLys, %	$Y = -7.49x^2 + 14.44x - 5.74$	<0.001	0.204	0.96
Body weight, g	$Y = -0.000001x^2 + 0.0068x - 9.42$	<0.001	0.197	3,400
Breast weight, g	$Y = -0.000005x^2 + 0.0084x - 2.48$	<0.001	0.284	842
Breast yield, %	$Y = -0.0087x^2 + 0.524x - 6.5$	<0.001	0.277	30.1
Wooden breast				
dLys, %	$Y = -10.6100x^2 + 20.6900x - 8.57$	<0.001	0.352	0.98
Body weight, g	$Y = -0.000002x^2 + 0.0104x - 14.87$	<0.001	0.370	2,598
Breast weight, g	$Y = -0.000008x^2 + 0.0142x - 4.52$	<0.001	0.467	884
Breast yield, %	$Y = -0.0095x^2 + 0.6100x - 7.89$	<0.001	0.379	32.1

[1]Linear: $Y = \beta 2 \times X + \beta 1$; where Y is the square root of the score, X is the independent variable, $\beta 1$ is the intercept, $\beta 2$ and is the linear coefficients; quadratic polynomial: $Y = \beta 3 \times X^2 + \beta 2 \times X + \beta 1$; where Y is the square root of the score, X is the independent variable, $\beta 1$ is the intercept, $\beta 2$ and $\beta 3$ are the linear and quadratic coefficients, respectively; maximum response obtained by calculating: $- \beta 2 \div (2 \times \beta 3)$.
[2]Broilers processed at 35 d.
[3]Broilers processed at 42 d.

Exp. 1 and 1.83 in Exp. 2. Based on these observations, the pronounced contrast in WB occurrence and severity between experiments seems to be related to BW and growth rate, similarly to WS. Trocino et al. (2015) observed that the occurrence of WB was doubled in males with 3.49 kg average BW compared with females with 2.85 kg average BW. There is evidence of gene expression of intracellular calcium, possible fiber-type switching, hypoxia, and oxidative stress in lesions related to the WB disease (Mutryn et al., 2015). Both myopathies have been reported to have low heritabilities and a marked non-genetic component (Bailey et al., 2015), which indicates the major role played by environmental, management, and nutritional factors in their incidence.

In conclusion, increasing dLys levels resulted in improved broiler performance to a maximum; however, birds with higher BW also presented higher proportions of myopathies, which were of an increased severity. These results are in agreement with other studies, demonstrating the influence of growth rate and slaughter weight in WS and WB (Kuttappan et al., 2012a; Kuttappan et al., 2013a; Petracci et al., 2013; Trocino et al., 2015). Since BW and growth rate are direct results of increasing dLys levels, myopathies do not seem to be associated with lysine itself but with gains in performance.

ACKNOWLEDGMENTS

Authors wish to thank the Conselho Nacional de Pesquisa (CNPq) for scholarship grants awarded to researchers and students participating in this project.

REFERENCES

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870–2879.

Dozier, W. A., III, A. Corzo, M. T. Kidd, P. B. Tillman, and S. L. Branton. 2009. Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. Poult. Sci. 88:1676–1682.

Dozier, W. A., III, A. Corzo, M. T. Kidd, P. B. Tillman, J. P. McMurtry, and S. L. Branton. 2010. Digestible lysine requirements of male broilers from 28 to 42 days of age. Poult. Sci. 89:2173–2182.

Eits, R. M., R. P. Kwakkel, M. W. A. Verstegen, and G. C. Emmans. 2003. Responses of broiler chickens to dietary protein: Effects of early life protein nutrition on later responses. Br. Poult. Sci. 44:398–409.

Ferreira, T. Z., R. A. Casagrande, S. L. Vieira, D. Driemeier, and L. Kindlein. 2014. An investigation of a reported case of white striping in broilers. J. Appl. Poult. Res. 23:1–6.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003a. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509–1518.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003b. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1500–1508.

Holsheimer, J. P., and C. H. Veerkamp. 1992. Effect of dietary energy, protein, and lysine content on performance and yields of two strains of male broiler chicks. Poult. Sci. 71:872–879.

Hoving-Bolink, A. H., R. W. Kranen, R. E. Klont, C. L. M. Gerritsen, and K. H. de Greef. 2000. Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites. Meat Sci. 56:397–402.

Huyghebaert, G., M. Pack, and G. Degroote. 1994. Influence of protein-concentration on the response of broilers to supplemental Dl-methionine. Arch. Geflugelkd. 58:23–29.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012a. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677–2685.

Kuttappan, V. A., V. B. Brewer, A. Mauromoustakos, S. R. McKee, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2013a. Estimation of factors associated with the occurrence of white striping in broiler breast fillets. Poult. Sci. 92:811–819.

Kuttappan, V. A., S. D. Goodgame, C. D. Bradley, A. Mauromoustakos, B. M. Hargis, P. W. Waldroup, and C. M. Owens. 2012b. Effect of different levels of dietary vitamin E (dl-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. Poult. Sci. 91:3230–3235.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013b. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.

Leclercq, B. 1998. Lysine: specific effects of lysine on broiler production: comparison with threonine and valine. Poult. Sci. 77:118–123.

McDonald, M. L., and R. W. Swick. 1981. The effect of protein depletion and repletion on muscle-protein turnover in the chick. Biochem. J. 194:811–819.

Mehri, M., A. A. Davarpanah, and H. R. Mirzaei. 2012. Estimation of ideal ratios of methionine and threonine to lysine in starting broiler chicks using response surface methodology. Poult. Sci. 91:771–777.

Moran, E. T., and S. F. Bilgili. 1990. Processing losses, carcass quality, and meat yields of broiler-chickens receiving diets marginally deficient to adequate in lysine prior to marketing. Poult. Sci. 69:702–710.

Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2015. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal. 9:728–734.

Munks, B., A. Robinson, E. F. Beach, and H. H. Williams. 1945. Amino acids in the production of chicken egg and muscle. Poult. Sci. 24:459–464.

Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics. 16:1–19.

Petracci, M., and C. Cavani. 2012. Muscle growth and poultry meat quality issues. Nutrients. 4:1–12.

Petracci, M., S. Mudalal, A. Bonfiglio, and C. Cavani. 2013. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670–1675.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, S. L. T. Barreto, and P. F. Euclides. 2011. Tabelas brasileiras para aves e suínos. in Composição De Alimentos e Exigências Nutricionais. 3rd ed. UFV, Viçosa, MG, Brazil.

Roy, B. C., I. Oshima, H. Miyachi, N. Shiba, S. Nishimura, S. Tabata, and H. Iwamoto. 2006. Effects of nutritional level on muscle development, histochemical properties of myofibre and collagen architecture in the pectoralis muscle of male broilers. Br. Poult. Sci. 47:433–442.

Russo, E., M. Drigo, C. Longoni, R. Pezzotti, P. Fasoli, and C. Recordati. 2015. Evaluation of white striping prevalence and predisposing factors in broilers at slaughter. Poult. Sci. 94:1843–1848.

Sakomura, N. K., R. D. Ekmay, S. J. Mei, and C. N. Coon. 2015. Lysine, methionine, phenylalanine, arginine, valine, isoleucine, leucine, and threonine maintenance requirements of broiler breeders. Poult. Sci. 94:2715–2721.

SAS Institute. 2009. *The SAS/STAT User's Guide*. SAS Inst. Inc., Cary, NC.

Schutte, J. B., and M. Pack. 1995. Effects of dietary sulfur-containing amino-acids on performance and breast meat deposition of broiler chicks during the growing and finishing phases. Br. Poult. Sci. 36:747–762.

Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Sklan, D., and Y. Noy. 2003. Crude protein and essential amino acid requirements in chicks during the first week posthatch. Br. Poult. Sci. 44:266–274.

Soglia, F., S. Mudalal, E. Babini, M. Di Nunzio, M. Mazzoni, F. Sirri, C. Cavani, and M. Petracci. 2015. Histology, composition, and quality traits of chicken *Pectoralis major* muscle affected by wooden breast abnormality. Poult. Sci. 95:651–659.

Sterling, K. G., G. M. Pesti, and R. I. Bakalli. 2006. Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. Poult. Sci. 85:1045–1054.

Tesseraud, S., N. Maaa, R. Peresson, and A. M. Chagneau. 1996. Relative responses of protein turnover in three different skeletal muscles to dietary lysine deficiency in chicks. Br. Poult. Sci. 37:641–650.

Tesseraud, S., S. Temin, E. Le Bihan-Duval, and A. M. Chagneau. 2001. Increased responsiveness to dietary lysine deficiency of pectoralis major muscle protein turnover in broilers selected on breast development. J. Anim. Sci. 79:927–933.

Trocino, A., A. Piccirillo, M. Birolo, G. Radaelli, D. Bertotto, E. Filiou, M. Petracci, and G. Xiccato. 2015. Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. Poult. Sci. 94:2996–3004.

Urdaneta-Rincon, M., and S. Leeson. 2004. Muscle (*Pectoralis major*) protein turnover in young broiler chickens fed graded levels of lysine and crude protein. Poult. Sci. 83:1897–1903.

Wilson, B. W., P. S. Nieberg, and R. J. Buhr. 1990. Turkey muscle growth and focal myopathy. Poult. Sci. 69:1553–1562.

Zheng, Q., Y. Zhang, Y. Chen, N. Yang, X. J. Wang, and D. Zhu. 2009. Systematic identification of genes involved in divergent skeletal muscle growth rates of broiler and layer chickens. BMC Genomics. 10:87–100.

Zuidhof, M. J., B. L. Schneider, V. L. Carney, D. R. Korver, and F. E. Robinson. 2014. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. Poult. Sci. 93:2970–2982.