Effects of reducing dietary amino acid density and stocking density on growth performance, carcass characteristics, meat quality, and occurrence of white striping in broiler chickens

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ABSTRACT A 49-day trial was conducted to determine the impact of dietary amino acid (**AA**) density and stocking density (**SD**) on growth performance, carcass traits, meat quality, and white striping (**WS**) occurrence in broiler chickens. Two hundred eighty-eight Ross 308 male broilers consisting of 6 replicate cages with 8 broilers per replicate were used. Treatments were arranged in a 3 × 2 factorial and consisted of 3 AA densities (normal, 10, or 20% lower than normal) and 2 different SD (high 35 kg/m^2 or low 26 kg/m^2). Breasts were classified as normal, moderate, and severe for WS. Data were analyzed as a completely randomized design using the GLM procedure. Decreasing AA density decreased overall growth performance, carcass, breast yields, and fillet dimensions linearly, while leg and rib cage yields increased linearly ($P < 0.01$). High SD decreased hot carcass, breast, wings, and rib cage weights in birds fed normal AA diets ($P < 0.05$). High SD increased the length of breast fillet ($P < 0.05$). Cooking loss, breast lightness (L*), and redness (a*) at 48 h postmortem increased linearly with decreasing AA density, while ultimate breast pH (**pH$_u$**) and nitrogen content decreased linearly ($P < 0.05$). The occurrence of normal, moderate, and severe WS fillets was 45.3, 49.1, and 5.6%, respectively. As the dietary AA density decreased, the occurrence of no WS breast fillets increased linearly, whereas the occurrence of moderate WS fillets and mean WS score decreased linearly ($P < 0.05$). SD did not affect the occurrence of WS. Severe WS fillets were heavier and had higher cranial thickness, pH$_u$, and fat content and lower yellowness ($P < 0.05$), but water-holding capacity, nitrogen content, L*, and a* value did not differ among different WS scores. Taken together, WS occurrence and severity increased with higher growth rate. Growth depression created by lowering dietary AA density regardless of SD resulted in a decrease in mean WS score, but it also compromised the growth and meat quality.

Key words: amino acid density, breast fillet, broiler chicken, stocking density, white striping

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INTRODUCTION

Consumer interest to purchase meat products requiring less time to prepare is on the rise, and therefore, boneless-skinless chicken breast meat is gaining more popularity (Napolitano et al., 2013). The higher ultimate pH (**pH$_u$**) and thus better water-holding capacity (**WHC**) were the most noticeable and reported benefits of selection toward lean breast meat in broiler chickens (Berri et al., 2007; Schmidt et al., 2009). The higher WHC resulted in producing superior quality processed products, which also helped the broiler industry to improve productivity. However, these remarkable improvements on both yield and quality have not come about without some problems, with the major one being the higher tendency to exhibit muscle myopathies (Kuttappan et al., 2012a; Tijare et al., 2016). Owing to

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the increase mentioned previously in the consumption of boneless-skinless chicken breast meat along with an apparent rise in muscle-related problems in the field, the quality of breast meat has become a significant research area in the broiler industry globally.

White stripings (**WS**) appearing on breast meat is one of the major myopathies facing the broiler industry because of its high prevalence. WS is a condition characterized by the presence of white striations parallel to the muscle fibers in the pectoralis major muscle of broiler chickens (Salles et al., 2019). Prevalence of WS in broiler breast has been reported to vary from 12 to 78% in Italy and from 52 to 76% in the United States (Kuttappan et al., 2012a, 2013; Petracci et al., 2013; Russo et al., 2015). Although exact reasons behind this wide variation remain elusive, it may be due to differences in genotype used (mostly not reported), the number of birds used (from 280–28,000), and slaughtering age (from 45–57 d) in these studies. Previous studies have defined WS as one of the most common degenerative myopathies associated with increased growth rate, higher slaughter age, and weight (Kuttappan et al., 2012a, 2013; Livingston et al., 2019). Findings of Lorenzi et al. (2014) showed that reduced growth rate results in lower occurrence and severity of WS in broiler breast muscle, which also supported the hypothesis that higher metabolic rate plays a vital role in the occurrence of WS. Different studies have shown that WS had negative impacts on overall breast meat quality by worsening WHC and tenderness (Bowker and Zhuang, 2016; Brambila et al., 2016; Kato et al., 2019). On the other hand, Mudalal et al. (2015) reported that WS had a negligible or relatively low effect on meat quality. Besides reducing meat quality, the appearance of WS was shown to affect consumer acceptance negatively (Fletcher, 2002; Kato et al., 2019).

The nutrient level of the diets is known to be the most significant single environmental factor affecting muscle development and, therefore, meat quality. For example, dietary nutrient density has been reported to influence meat quality as a result of altered histological and initial energy and metabolic characteristics of the muscle (Zhao et al., 2012). In one of the few studies on the effect of dietary factors on the occurrence of WS in broilers breast, Kuttappan et al. (2012b) reported no association between dietary vitamin E quantity and WS. In another recent study, time-limited feeding has been shown to reduce WS myopathy in broiler breast fillets, and this positive effect was attributed to a slower growth rate obtained by limiting feeding time (Livingston et al., 2019). Cruz et al. (2017) reported that broilers fed diets with increased amounts of digestible lysine produced larger breast muscle that increased the occurrence and severity of WS. Besides, a recent study has demonstrated reductions in the severity of WS when broilers were fed with a diet that was reduced (10%) in both energy and amino acid (**AA**) density (Meloche et al., 2018).

Increasing stocking density (**SD**) beyond 30 kg BW/m^2 has been shown to adversely affect the growth of heavy broilers at 49 d of age (Dozier et al., 2005). In other studies examining the effect of SD on broiler performance, high SD was reported to decrease feed intake (**FI**) and BW gain (**BWG**) and to increase feed conversion ratio at 42 d of age (Houshmand et al., 2012; Tong et al., 2012). Moreover, Simitzis et al. (2012) reported that intramuscular fat was significantly decreased at higher SD. Therefore, reduced growth due to higher SD may also help reduce the occurrence of WS and lipid accumulation in breast muscle as most reports showed that WS is associated with fast growth and affected fillets had higher intramuscular fat (Mudalal et al., 2014).

Taken together, the exact reasons and mechanisms of WS occurrence in broiler breast meat remain mostly unknown. Therefore, the present study aimed to test the hypothesis that feeding broilers on reduced AA diets should decrease the occurrence/severity of WS by reducing growth. It was also hypothesized that increasing SD might decrease fat accumulation in breast meat and thereby help further reduce WS severity.

MATERIALS AND METHODS

The Adnan Menderes University Animal Care and Use Committee (Aydın, Turkey) approved all experimental protocols.

Birds, Experimental Design, Diets, SD, and Housing

In total, 288 one-day-old male broiler chicks (Ross 308) were obtained from a local hatchery (Egetav Tavukculuk San. ve Tic. A.S., Izmir, Turkey). The experimental treatments consisted of 3 × 2 factorial arrangement with 3 dietary levels of AA density (normal; **Norm**, 10% lower than normal; **10%low**, or 20% lower than normal; **20%low**) and 2 different SD (**high**; 35 kg/m^2 or **low**; 26 kg/m^2). Treatments were randomly assigned to floor pens (6 pens per treatment, and 8 birds per pen) in a completely randomized design.

Diets were formulated by using total AA contents of ingredients and keeping similar total AA ratios to lysine in all experimental diets for each growing phase (Table 1). The normal AA diet was formulated based on nutrient recommendations by the breeder company (Aviagen, 2019). The 10%low AA and 20%low AA diets were formulated to be equivalent to approximately 90 and 80% of the recommended essential AA by the breeder company, respectively. Diets were isocaloric and formulated to contain similar levels of Ca, aP, and Ca to aP ratio. Experimental diets with low AA were made by adjusting the levels of corn, soybean meal, vegetable oil, and crystal AA. All diets were fed in mash form, and birds were provided ad libitum access to feed and water throughout the study. Starter, grower, and finisher diets were provided from 0 to 15 d, 16 to 30 d, and 31 to 49 d of age, respectively.

The 2 levels of SD were calculated as 9 birds/m^2 for low SD and 12 birds/m^2 for the high SD treatments and corresponded to a floor space of 0.11 m^2/bird for low SD and 0.08 m^2/bird for high SD treatments. The final production (live bird weight before slaughter) BW per area for low and high SD was calculated to be

Table 1. Composition and analysis of experimental diets (g/kg, as-fed basis).

	Starter			Grower			Finisher		
	AA density			AA density			AA density		
Ingredients	Norm[1]	10%Low[2]	20%Low[3]	Norm	10%Low	20%Low	Norm	10%Low	20%Low
Corn	552.37	607.46	673.75	581.85	636.55	696.20	634.28	671.15	731.62
Soybean meal (47% CP)	380.00	335.00	280.00	350.00	305.00	256.00	300.00	270.00	220.00
Vegetable oil	24.50	15.00	3.50	30.50	21.00	10.70	32.00	25.80	15.00
Dicalcium phosphate	17.23	17.50	17.82	14.85	15.10	15.40	13.02	13.20	13.50
Limestone (38% Ca)	12.80	12.94	13.13	11.90	12.05	12.20	10.80	10.91	11.08
Salt	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin-mineral premix[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine	3.60	3.10	2.50	3.00	2.50	2.00	2.60	2.10	1.70
L-Lysine HCl	2.50	2.20	2.50	1.30	1.30	1.20	1.30	0.84	1.10
Threonine	1.00	0.80	0.80	0.60	0.50	0.30	0.00	0.00	0.00
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Calculated composition									
ME, kcal/kg	3,082	3,082	3,082	3,150	3,150	3,150	3,217	3,217	3,217
Ca	9.6	9.6	9.6	8.7	8.7	8.7	7.8	7.8	7.8
aP	4.9	4.9	4.9	4.4	4.4	4.4	3.9	3.9	3.9
Ca/aP	1.96	1.96	1.96	1.98	1.98	1.98	2.0	2.0	2.0
Total amino acids									
Arg	15.3	14.0	12.3	14.4	13.0	11.6	12.9	11.9	10.4
His	6.1	5.7	5.1	5.8	5.4	4.9	5.3	5.0	4.5
Ile	9.7	8.9	7.9	9.1	8.3	7.4	8.2	7.7	6.8
Leu	19.7	18.6	17.2	18.9	17.8	16.5	17.6	16.8	15.5
Lys	14.6	13.2	12.0	12.9	11.7	10.3	11.5	10.4	9.3
Met	7.1	6.4	5.6	6.4	5.7	5.0	5.7	5.1	4.5
Met + Cys	10.9	9.9	8.8	10.0	9.0	8.1	9.1	8.3	7.4
Phe	11.0	10.1	9.1	10.4	9.6	8.6	9.4	8.9	7.9
Phe + Tyr	20.1	18.5	16.6	19.0	17.4	15.7	17.2	16.1	14.4
Thr	9.7	8.8	8.0	8.8	8.0	7.1	7.4	7.0	6.2
Trp	3.1	2.8	2.5	2.9	2.6	2.3	2.6	2.4	2.1
Val	10.6	9.9	8.9	10.1	9.3	8.5	9.2	8.7	7.8
Analyzed composition									
DM	894.0	897.3	896.7	894.1	896.8	891.1	906.0	898.6	904.6
CP	212.8	193.3	188.8	208.5	186.8	174.6	203.4	173.2	158.5
Ether extract	44.3	36.4	26.8	51.8	40.8	34.8	51.3	43.4	36.4
Crude ash	69.1	68.8	60.5	61.1	56.5	54.9	60.8	46.3	51.5
Total P	7.8	7.5	7.2	6.3	6.3	6.3	6.0	5.7	5.8

[1]NORM = diet with a recommended amino acid (AA) density.
[2]10%Low = diet with a 10% lower amino acid density than Norm diet.
[3]20%Low = diet with a 20% lower amino acid density than Norm diet.
[4]Supplied the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 1,500 IU; vitamin E, 30 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; niacin, 40 mg; calcium D-pantothenate, 10 mg; folic acid, 0.75 mg; D-Biotin, 0.075 mg; choline chloride, 375 mg; Mn, 80 mg; Fe, 40 mg; Zn, 60 mg; Cu, 5 mg; I, 0.4 mg; Co, 0.1 mg; Se, 0.15 mg; and antioxidant, 10 mg.

26 and 35 kg/m^2, respectively, at 49 d of age. The number of birds in each pen were the same (8 birds), and pen dimensions were changed to produce the target SD of 26 and 35 kg/m^2. The width and length of pens used to create low and high SD were 85 × 110 cm and 65 × 110 cm, respectively, and this change was made possible by moving the front part of the pen.

The room temperature was maintained at 32°C from day 0 to 7, 28°C from day 8 to 15, and 22°C from day 16 to 49. A continuous lighting schedule was provided for the first week, and a 20L:4D cycle was applied thereafter. The birds were housed in pens equipped with nipple drinkers and floor feeders from day 0 to 15 and plastic tube feeders for the 16 to 49 d period with using pine wood shavings at a depth of approximately 10 cm as litter in each pen.

Growth Performance and Carcass Characteristics

Broilers and feeds were weighed at 15, 30, and 49 d of age for the calculation of growth rate, FI, and G:F. The mortality was recorded daily, and FI was adjusted by using bird-days.

Three birds from each pen were randomly selected for processing to determine carcass weights, part weights, and yield information. After arrival at the processing unit at day 49 of the study, birds were placed in a slaughter funnel upside down to prevent wing flapping. Then, they were manually bled by severing the left carotid artery and jugular vein, bled out (∼2 min), soft-scalded by immersion in water (62°C, ∼2 min), and feathers were removed with the use of batch defeathering equipment (Cimuka, Ankara, Turkey). Eviscerating and rinsing were subsequently performed manually. Carcasses were separated into the breast (with skin and bone), wings, leg (thigh and drumstick together with skin), and rib cage. After separation of the parts, they were weighed individually and added together to find the hot carcass weight for determining the dressing percentage. After cooling the breast with skin in ice water for 2 h, breast muscle was separated to determine WS score. Then, breast muscles were stored at 4°C before meat quality analyses were conducted.

Meat Quality Analyses

The cranial portion of left fillets (from the ventral part) was used for determining pH, color measurements (L*, a*, b*), fillet dimensions, and chemical composition. In contrast, the cranial section of right fillets (ventral part) was used for cook loss and WHC determinations (Kuttappan et al., 2012a).

Breast muscle pH at 15 min (initial, $\mathbf{pH_i}$) and 24 h (pH_u) postmortem was taken using a portable pH meter (Testo 205; Testo Inc, Lenzkirch, Germany). Measurements were taken with an electrode in triplicate on the ventral side of the cranial part of the left pectoralis major muscle. The L* (lightness), a* (redness), and b* (yellowness) values were measured on breast muscles at 24 and 48 h postmortem in triplicate using a Minolta Chroma Meter (model CR400; Minolta Camera Co, Osaka, Japan) with illuminant D65 as the light source, 8-mm aperture size, and 2° observation angle. Areas were chosen that were free of any visible blood-related defects such as bruises or hemorrhages to avoid discolorations of breast surface.

To determine cook loss (%), breast meat samples (approximately 25 g) were placed in a plastic bag and cooked in a water bath at 85°C until the core temperature reached 75°C (Meek et al., 2000). The shape of the samples and orientation of fibers were constant among the samples. Cooked breast meat samples were cooled in running tap water for 1 h, and then cooked samples were reweighed. Cook loss (%) was estimated by means of the percentage of weight loss of the cooked meat sample to initial meat sample weight. WHC was measured in pectoralis major muscle according to the filter press method of Hamm (1961). In brief, approximately 5 g of meat sample was minced to 5 pieces and placed between 2 filter papers (15 × 15 cm) that were weighed and kept under pressure of 2,250 g weight for 5 min. Then, after removal of meat from filter papers, filter papers were weighed again, and released water was expressed with as a percentage with respect to the initial weight of breast meat placed in between filter papers.

Breast fillet dimensions were measured according to the study by Mehaffey et al. (2006). In brief, the length and width of breast fillets were described as the longest horizontal distance between cranial and caudal parts and widest distance from one side to the other side of fillets, respectively. Measurements of thickness were taken at the cranial and caudal thickest part of each breast fillet. Breast fillets with no visual white striations on the surface were classified as normal and given a WS score of 0; fillets showing white striations smaller than 1 mm running parallel to muscle fibers on the surface were considered to be moderate WS and given a WS score of 1; and fillets with >1-mm-thick white striations covering a visually noticeable area were given a 2 WS score and classified as severely affected by WS (Kuttappan et al., 2012c).

Nutrient Composition of Diets and Breast Meat

Samples were ground to pass through a 0.5-mm screen using an ultracentrifugal rotor mill grinder (Retsch ZM 200; Retsch GmbH Co, KG, Haan, Germany). All nutrients were determined in triplicate. The dry matter content of samples was determined by drying samples in an oven (Memmert 500; Memmert GmbH, Schwabach, Germany) at 105°C for 18 h (method 930.15; AOAC, 2006). The crude ash content of samples was determined by ashing in a muffle furnace (Model MF 110/30, Protherm Furnaces, Batikent, Ankara, 06,370, Turkey) overnight at 600°C (method 942.05; AOAC, 2006). Ether extract content of the samples was determined by using a gravimetric extraction procedure using petroleum benzene in a Soxhlet apparatus (Model Soxtherm 416; Gerhardt Laboratory Systems GmbH, Koenigswinter, Germany) for approximately 2 h and 15 min (method 920.39; AOAC, 2006). Nitrogen was determined by the Kjeldahl method using a Kjeltec analyzer (method 984.13; AOAC, 2006). Briefly, samples were digested in a digester (Gerhardt Kjeldatherm KB, Bonn, Germany) using sulfuric acid, and then resulting ammonium sulfate was distilled using NaOH in a distillation unit (Gerhardt Vapodest 50 Carrousel, Germany). Crude protein values were derived from multiplying the nitrogen values by a factor of 6.25. Samples for the determination of total P contents were prepared using nitric-perchloric acid wet digesting. Acid molybdate and Fiske-Subbarow reducer solutions were used to measure the concentration of P through the formation of a phospho-molybdenum complex. Then, P concentrations in the digested samples were determined by spectrophotometry with measuring the absorbance at 620 nm (method 946.06; AOAC, 2006) using a plate reader (Biotek Synergy Neo2; Biotek Instruments, Winooski, VT).

Statistical Analysis

Data were analyzed as a 3 × 2 factorial arrangement with 3 levels of dietary AA density and 2 different SD levels, in a completely randomized design using the GLM procedure of IBM SPSS statistics 22 for windows software (version 22.0; Armonk, NY). Treatments were replicated 6 times, with 8 birds per replicate. Comparisons of means for the significant effects were made by Tukey's significant test. The orthogonal polynomial contrasts test was used to determine the linear and quadratic effects of dietary AA density on the measured dependent variables. Differences were considered to be significant at $P \leq 0.05$. Pen mean was the experimental unit for statistical analysis. Mean WS score data were analyzed by the nonparametric test of the Kruskal-Wallis/Mann-Whitney U test. To evaluate the effects of WS category on different parameters, first, a Levene's test was used to verify the equality/unequality of variances (homogeneity of variance) in the samples (n = 49, n = 53, and n = 6 for normal, moderate, and severe WS categories, respectively). Unequally sized groups with equal variances were analyzed by an ANOVA (one-way) followed by parametric post hoc test of Hochberg's Gt2. Unequally sized groups with unequal variances were analyzed by an ANOVA (one-way)

Table 2. Growth performance of broiler chickens fed diets with different amino acid (AA) densities under different stocking densities from 0 to 49 d of age[1].

AA density[2]	Stocking[3] density	Day 0–15			Day 16–30			Day 31–49			Day 0–49		
		BWG g/bird	FI g/bird	G:F g/kg	BWG g/bird	FI g/bird	G:F g/kg	BWG g/bird	FI g/bird	G:F g/kg	BWG g/bird	FI g/bird	G:F g/kg
Norm	High	339	680	501	948	1,500	633	1,560^{a,b}	2,948^{a,b}	528	2,847	5,127	555
Norm	Low	354	654	546	975	1,507	648	1,678^{a}	3,062^{a}	548	3,007	5,213	577
10%Low	High	327	678	499	875	1,451	603	1,518^{a,b}	2,944^{a,b}	516	2,720	5,064	537
10%Low	Low	302	684	455	877	1,425	616	1,552^{a,b}	2,973^{a,b}	522	2,731	5,066	539
20%Low	High	323	584	564	804	1,433	561	1,404^{b,c}	2,898^{a,b}	484	2,531	4,915	515
20%Low	Low	315	587	546	780	1,391	561	1,316^{c}	2,752^{b}	478	2,410	4,729	510
SEM		13.1	40.2	37.3	20.2	31.9	7.6	39.6	51.5	7.7	58.7	76.4	7.9
AA density													
Norm		346^{a}	667	524	962^{a}	1,503^{a}	640^{a}	1,619^{a}	3,005^{a}	538^{a}	2,927^{a}	5,170^{a}	566^{a}
10%Low		315^{b}	681	477	876^{b}	1,438^{a,b}	609^{b}	1,534^{a}	2,959^{a}	519^{b}	2,726^{b}	5,065^{a}	538^{b}
20%Low		319^{b}	585	555	792^{c}	1,412^{b}	561^{c}	1,360^{b}	2,825^{b}	481^{c}	2,471^{c}	4,822^{b}	512^{c}
SEM		9.3	28.4	26.4	14.3	22.6	5.4	28.0	36.4	5.4	41.5	54.0	5.6
Stocking density													
High		330	647	522	876	1,461	599	1,494	2,930	509	2,699	5,036	536
Low		324	641	516	877	1,441	608	1,515	2,929	516	2,716	5,003	542
SEM		7.6	23.2	21.6	11.7	18.4	4.4	22.8	29.8	4.4	33.9	44.1	4.6
P values													
AA density		0.046	0.051	0.127	<0.001	0.022	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
Stocking density		0.567	0.862	0.856	0.918	0.441	0.158	0.515	0.980	0.292	0.728	0.604	0.325
AA density × stocking density		0.325	0.906	0.482	0.456	0.734	0.555	0.045	0.050	0.247	0.072	0.209	0.221
AA density linear		0.045	0.052	0.405	<0.001	0.007	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
AA density quadratic		0.129	0.127	0.064	0.966	0.488	0.192	0.197	0.336	0.177	0.603	0.306	0.893

[a-c]Means with different superscripts within a trait in a column differ significantly ($P \leq 0.05$).
[1]Means represent 6 replicates with 8 birds per replicate for each treatment.
[2]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.
[3]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

Table 3. Carcass and yield traits of broiler chickens fed diets with different amino acid (AA) densities under different stocking densities from 0 to 49 d of age[1].

AA density[3]	Stocking density[4]	BW g	Hot carcass		Carcass part weight, g				Carcass part yield, %[2]			
			Weight g[5]	Yield, %[6]	Breast[7]	Wings[8]	Leg quarters[9]	Rib cage[10]	Breast	Wings	Leg quarters	Rib cage
Norm	High	2,859	2,098^{b,c}	73.3	655^{b}	185^{b,c}	835	423^{b,c}	31.1^{a,b}	8.9	39.8	20.2^{b}
Norm	Low	3,062	2,325^{a}	76.3	748^{a}	206^{a}	903	468^{a}	32.1^{a}	8.9	38.9	20.1^{b}
10%Low	High	2,902	2,137^{a,b}	73.6	628^{b,c}	197^{a,b}	866	446^{a,b}	29.2^{b,c}	9.2	40.6	20.9^{a,b}
10%Low	Low	2,822	2,061^{b,c}	73.0	610^{b,c}	185^{b,c}	846	421^{b,c}	29.6^{b,c}	9.0	41.0	20.4^{b}
20%Low	High	2,610	1,897^{c}	72.6	541^{c,d}	173^{c}	789	394^{c}	28.5^{c,d}	9.1	41.6	20.8^{a,b}
20%Low	Low	2,635	1,899^{c}	72.0	515^{d}	171^{c}	806	407^{b,c}	27.0^{d}	9.0	42.4	21.5^{a}
SEM		64.5	52.7	0.84	22.4	4.0	21.4	10.7	0.52	0.12	0.39	0.22
AA density												
Norm		2,961^{a}	2,212^{a}	74.8^{a}	702^{a}	195^{a}	869^{a}	446^{a}	31.6^{a}	8.9	39.4^{c}	20.2^{b}
10%Low		2,862^{a}	2,099^{a}	73.3^{a,b}	619^{b}	191^{a}	856^{a}	433^{a}	29.4^{b}	9.1	40.8^{b}	20.7^{a,b}
20%Low		2,622^{b}	1,898^{b}	72.3^{b}	528^{c}	172^{b}	797^{b}	401^{b}	27.8^{c}	9.1	42.0^{a}	21.1^{a}
SEM		45.6	37.3	0.59	15.9	2.8	15.1	7.6	0.36	0.09	0.27	0.16
Stocking density												
High		2,791	2,044	73.2	608	185	830	421	29.6	9.1	40.7	20.6
Low		2,840	2,095	73.7	625	187	852	432	29.6	9.0	40.8	20.7
SEM		37.2	30.5	0.48	13.0	2.3	12.4	6.2	0.30	0.07	0.22	0.13
P values												
AA density		<0.001	<0.001	0.014	<0.001	<0.001	0.003	<0.001	<0.001	0.092	<0.001	<0.001
Stocking density		0.354	0.228	0.403	0.378	0.534	0.218	0.217	0.888	0.237	0.722	0.711
AA density × stocking density		0.091	0.012	0.052	0.015	<0.001	0.119	0.005	0.050	0.578	0.054	0.032
AA density linear		<0.001	<0.001	0.004	<0.001	<0.001	0.001	<0.001	<0.001	0.063	<0.001	<0.001
AA density quadratic		0.210	0.333	0.731	0.832	0.041	0.222	0.269	0.499	0.246	0.661	0.877

[a-d]Means with different superscripts in a column for a trait differ significantly ($P \leq 0.05$).
[1]Means represent 6 replicates with 3 randomly selected birds per replicate and 18 birds per treatment.
[2]Parts yield relative to hot carcass weight.
[3]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.
[4]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.
[5]Hot carcass weight taken just before chill tank.
[6]Hot carcass as a percentage of BW.
[7]Breast weight includes skin and bone.
[8]Wings include humerus, radius-ulna, and metacarpals with skin (including wing tip).
[9]Leg quarters defined as thigh and drumstick with skin plus a portion of the back.
[10]Rib cage includes kidneys.

followed by parametric post hoc test of Dunnet T3 by SPSS software.

RESULTS

The results of growth performance are shown in Table 2. Mortality throughout the experiment was low and unrelated to any treatments (2 dead out of 288 birds from 0 to 49 d of age). BWG of broilers decreased linearly ($P < 0.05$) with the decreasing dietary AA density while FI and G:F were not affected during the starter phase (0–15 d). There was no significant effect of SD on growth performance throughout the trial ($P > 0.05$). No interaction was observed between dietary AA density and SD during the starter and grower phases ($P > 0.05$). BWG, FI, and G:F linearly decreased ($P < 0.01$) with the decreasing levels of dietary AA density during the grower phase and overall period (0–49 d). Dietary AA density × SD interaction effects ($P \leq 0.05$) were observed on BWG and FI during the finisher phase (31–49 d), indicating that regardless of SD, decreasing dietary AA density resulted in a linear decrease in BWG and FI. Gain to feed ratio decreased linearly ($P < 0.001$) with the reduction of dietary AA density during the finisher period.

Significant interactions ($P < 0.05$) between AA density and SD were observed. Broilers reared at high SD had decreased weights of hot carcass, breast, wing, and rib cage when fed on norm AA density diets (Table 3). On the other hand, increasing SD did not have any effect on birds fed on low AA density diets. As dietary AA density decreased, there were linear decreases ($P < 0.01$) in BW at slaughter, hot carcass weight, part weights, and hot carcass yield. On the other hand, decreasing dietary AA density increased leg quarters yield linearly ($P < 0.001$). Breast yield decreased and rib cage yield increased linearly with decreasing dietary AA density, regardless of SD ($P < 0.05$).

Breast fillet length, width, and cranial and caudal thickness linearly decreased ($P < 0.01$), as the dietary AA density decreased (Table 4). High SD increased the length of breast fillet ($P < 0.05$). There was no significant dietary AA density × SD interaction on breast fillet dimensions.

Table 4. Breas fillet dimensions from broilers fed diets with different amino acid (AA) densities under different stocking densities from 0 to 49 d of age[1].

AA density[2]	Stocking density[3]	Breast fillet			
		Length cm	Width cm	Cranial thickness cm	Caudal thickness cm
Norm	High	19.49	10.21	2.88	0.97
Norm	Low	19.46	10.22	2.88	0.83
10%Low	High	18.85	9.78	2.79	0.72
10%Low	Low	18.26	9.61	2.84	0.74
20%Low	High	17.86	9.00	2.43	0.65
20%Low	Low	17.48	9.03	2.72	0.79
SEM		0.205	0.151	0.111	0.061
AA density					
Norm		19.48[a]	10.21[a]	2.88[a]	0.90[a]
10%Low		18.55[b]	9.70[b]	2.82[a,b]	0.73[b]
20%Low		17.67[c]	9.02[c]	2.58[b]	0.72[b]
SEM		0.145	0.107	0.079	0.043
Stocking density					
High		18.74[a]	9.67	2.70	0.78
Low		18.40[b]	9.62	2.81	0.79
SEM		0.112	0.087	0.064	0.035
P values					
AA density		<0.001	<0.001	0.017	0.006
Stocking density		0.047	0.719	0.232	0.853
AA density × stocking density		0.388	0.772	0.391	0.080
AA density linear		<0.001	<0.001	0.007	0.004
AA density quadratic		0.907	0.522	0.344	0.131

[a-c]Means with different superscripts in a column differ significantly ($P \leq 0.05$).
[1]Means represent 6 replicates with 3 randomly selected birds per replicate and 18 birds per treatment.
[2]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.
[3]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

Initial breast muscle pH was not affected by either AA density or SD ($P > 0.05$). Ultimate breast muscle pH decreased linearly ($P < 0.001$) with decreasing levels of dietary AA density (Table 5). Breast muscle L* at 24 h (linear and quadratic, $P < 0.05$) and 48 h (linear, $P < 0.05$) postmortem increased with decreasing dietary AA levels. At 24 h postmortem, there was an interaction ($P \leq 0.05$) between dietary AA density and SD, in which birds fed norm AA diet at high SD had higher breast muscle b* value than birds fed on norm AA diet at low SD. Breast muscle a* value at 48 h postmortem linearly increased ($P < 0.05$) with decreasing dietary AA levels.

Breast muscle cook loss at 48 h postmortem increased linearly (interaction between AA and SD, $P < 0.05$) with decreasing dietary AA density regardless of SD level (Table 6). Decreasing dietary AA density increased WHC of breast muscle at 24 h postmortem (linear and quadratic, $P < 0.05$), but not at 48 h ($P > 0.05$). Decreasing dietary AA density resulted in a linear decrease ($P < 0.05$) in N content of breast muscle. Ether extract content of breast muscle was not affected by dietary AA density or SD ($P > 0.05$).

No WS (normal), moderate WS, and severe WS occurrence frequencies were 45.3, 49.1, and 5.6%, respectively (Table 7). Decreasing dietary AA density resulted in a linear increase ($P < 0.05$) in the percentage of breast fillets scored normal, while it resulted in a linear decrease ($P < 0.05$) in the percentage of breast fillets scored moderate for WS. However, the percentage of severely WS affected breast fillets was not affected ($P > 0.05$) by dietary AA density and SD. There was no interaction between dietary AA density and SD on the frequency of occurrence of WS ($P > 0.05$). Mean breast fillet WS score linearly decreased ($P = 0.05$) with decreasing dietary AA density but was not affected by SD (Table 8).

Bodyweight, hot carcass weight, leg weight, breast fillet weight, and breast fillet cranial thickness of birds with severe WS were higher ($P < 0.05$) than those of birds with normal or moderate WS breast fillets (Table 9). In contrast, the yield of wings, legs, and rib cage was lower in birds having severe WS breast fillets than that of birds with normal breast fillets ($P < 0.05$). Breast yield and breast fillet pH_u were higher in birds having severe WS breast fillets than in those having normal breast fillets ($P < 0.001$). Severe WS fillets had higher ether extract content than normal and moderate WS fillets ($P = 0.01$). Breast fillets with severe WS exhibited less yellowness (b*) than normal breast fillets at 48 h postmortem ($P < 0.05$). The degree of WS did not significantly affect length, width, caudal thickness, pH_i, N content, L*, a*, cook loss, and WHC of breast fillets.

Table 5. Breast meat pH and color properties of broiler chickens fed diets with different amino acid (AA) densities under different stocking densities from 0 to 49 d of age[1].

AA density[2]	Stocking density[3]	pH		24 h			48 h		
		15 min	24 h	L*	a*	b*	L*	a*	b*
Norm	High	6.59	5.86	59.5	2.0	9.4^{a}	59.2	1.9	9.3^{a,b}
Norm	Low	6.56	5.81	58.4	2.2	8.1^{b}	59.0	2.2	7.7^{b}
10%Low	High	6.52	5.77	59.2	2.4	9.5^{a}	59.9	2.4	9.5^{a}
10%Low	Low	6.53	5.78	58.2	2.9	9.4^{a}	58.8	2.5	9.4^{a}
20%Low	High	6.53	5.74	60.4	2.5	8.8^{a,b}	60.3	2.7	9.1^{a,b}
20%Low	Low	6.61	5.74	61.3	2.5	9.7^{a}	60.6	2.5	9.3^{a,b}
SEM		0.029	0.020	0.625	0.263	0.447	0.595	0.209	0.405
AA density									
NORM		6.57	5.83^{a}	58.9^{b}	2.1	8.7	59.1	2.0^{b}	8.5
10%Low		6.53	5.77^{b}	58.7^{b}	2.6	9.5	59.4	2.5^{a,b}	9.4
20%Low		6.57	5.74^{b}	60.9^{a}	2.5	9.2	60.4	2.6^{a}	9.2
SEM		0.020	0.014	0.442	0.186	0.316	0.421	0.148	0.286
Stocking density									
High		6.55	5.79	59.7	2.3	9.2	59.8	2.3	9.3
Low		6.57	5.78	59.3	2.5	9.1	59.5	2.4	8.8
SEM		0.017	0.012	0.361	0.152	0.258	0.344	0.121	0.234
P values									
AA density		0.228	<0.001	0.001	0.122	0.243	0.062	0.029	0.057
Stocking density		0.357	0.427	0.446	0.345	0.675	0.479	0.691	0.137
AA density × stocking density		0.118	0.304	0.247	0.722	0.043	0.542	0.622	0.050
AA density linear		0.841	<0.001	0.003	0.160	0.272	0.026	0.011	0.081
AA density quadratic		0.089	0.450	0.031	0.133	0.202	0.433	0.435	0.097

[a,b]Means with different superscripts in a column differ significantly ($P \leq 0.05$).
[1]Means represent 6 replicates with 8 birds per replicate for each treatment.
[2]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.
[3]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

DISCUSSION

The present study was undertaken to study how reducing growth through changing dietary AA density and SD would affect meat quality and to measure the extent or degree to which WS occurrence in breast fillet could be affected by these changes in broiler chickens. As expected, decreasing dietary AA density by 10 or 20% significantly reduced the growth performance of broilers. BWG was linearly decreased with reduced AA density diets during starter, grower, finisher, and overall growth period of 49 d. FI and G:F of broilers also severely decreased (linear) by decreased AA density except for the starter period. These results on growth performance were in good agreement with studies of Kidd et al. (2004) and Corzo et al. (2005), who demonstrated that decreasing dietary AA density resulted in reduced BW and impaired feed conversion.

It has been reported that chickens fed severely deficient AA diets had a reduction in FI (Summers and Leeson, 1985; Carew et al., 1997, 2003). Animals reject diets that lead to indispensable AA deficiency as adaptive behavior, and long-term indispensable AA depletion is considered to be incompatible with the maintenance of protein synthesis and survival (Gietzen et al., 2007). Factors other than just dietary crude protein level and probably one or several AA were shown to be responsible for FI regulation in broilers (Sklan and Plavnik, 2002). However, as AA ratios were kept similar and all essentials AA levels were reduced at the same time, it is not possible to determine whether a specific AA was responsible for the observed decrease in the FI, in the present study.

Significant interactions between AA density and SD showed that high SD resulted in poorer growth and reduced hot carcass and carcass part weights, including breast in birds fed on norm AA diet. Therefore, the results implied that a higher SD level (35 kg/m^2 vs. 26 kg/m^2) used in the present study was successful in creating a stressful condition on birds fed norm AA diet which were heavier than birds on lower AA density diets. However, it also indicated that high SD level used appeared not to be enough to produce growth depression when birds fed decreased AA density diets because their lower BW allowed them to have more space. In agreement with previous studies, breast and leg yields were not significantly affected by SD (Bilgili and Hess, 1995; Feddes et al., 2002; Zuowei et al., 2011). Simitzis et al. (2012) used 12.6 or 27.2 kg/m^2 SD and Tong et al. (2012) used 14 or 24 kg/m^2 SD, and they both reported no difference in carcass yield, but they also found a depression in BWG and FI with higher SD level. Breast fillet dimension data reflected the lower AA density-induced fillet weight decrease, and therefore, fillet length, width, and height decreased as dietary AA density decreased. The longer breast fillet obtained with

Table 6. Cook loss properties and ether extract and nitrogen content of breast meat of broiler chickens fed diets with different amino acid (AA) densities under different stocking densities from 0 to 49 d of age[1].

AA density[2]	Stocking density[3]	Cook loss (%)		WHC (%)		Ether extract	N
		24 h	48 h	24 h	48 h	(g/kg, as-is)	(g/kg, as-is)
Norm	High	26.4	31.1^{d}	11.4	11.3	10.4	37.5
Norm	Low	27.9	30.0^{d}	12.3	11.4	12.9	36.3
10%Low	High	30.2	32.6^{b,c}	13.9	12.7	14.0	37.3
10%Low	Low	28.1	31.3^{c,d}	13.9	12.3	18.3	34.2
20%Low	High	29.3	33.0^{a,b}	14.1	12.2	12.0	34.7
20%Low	Low	30.8	34.1^{a}	12.7	11.5	10.1	33.1
SEM		1.71	0.48	0.58	0.53	2.7	1.1
AA density							
Norm		27.3	30.6^{c}	11.9^{b}	11.3	11.9	36.8
10%Low		28.4	31.9^{b}	13.9^{a}	12.5	15.7	36.0
20%Low		29.5	33.6^{a}	13.4^{a}	11.9	11.1	33.9
SEM		0.94	0.34	0.41	0.37	2.3	0.80
Stocking density							
High		29.6	32.3	13.1	12.1	12.4	36.4
Low		28.2	31.8	13.0	11.7	13.2	34.6
SEM		0.69	0.28	0.34	0.30	2.1	0.64
P values							
AA density		0.257	<0.001	0.003	0.097	0.429	0.052
Stocking density		0.851	0.258	0.748	0.415	0.635	0.055
AA density × stocking density		0.493	0.023	0.141	0.749	0.730	0.689
AA density linear		0.133	<0.001	0.012	0.316	0.885	0.019
AA density quadratic		0.721	0.760	0.016	0.056	0.210	0.750

[a–d]Means with different superscripts within a trait differ significantly ($P \leq 0.05$).

Abbreviation: WHC, water-holding capacity.

[1]Means represent 6 replicates with 3 randomly selected birds per replicate and 18 birds per treatment.

[2]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.

[3]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

high SD than with low SD was surprising, and we are at a loss to explain it. In agreement with the study by Kidd et al. (2004), yields of carcass and breast as a percentage of live weight decreased linearly with decreasing dietary AA density; however, leg quarter and rib cage yield increased simultaneously. Fast-growing birds were mostly selected for superior breast yield. Therefore, breast muscle is much more sensitive to a dietary AA deficiency than leg muscle, which is in line with the results of the present study. Birds fed on 20%low AA diet had a nearly 25% decrease in breast weight when compared with norm AA diet; however, the reduction on the leg weight was only 8%. Therefore, this lower decrease in leg weight by AA decrease compared with breast weight resulted in a proportional increase in leg yield in the present study.

Early postmortem pH is well controlled and could not be easily altered by preslaughter conditions, which confirms the absence of a significant difference in pH_i by different treatments in the present study (Zhao et al., 2012). The L* value (lightness) is used as an indicator of paleness degree, and a higher L* value indicates paler meat (Tong et al., 2015). In the present study, paleness of breast meat increased with decreasing dietary AA density, whereas pH_u decreased at the same time. These results agree with the study of Petracci et al. (2004), who reported that paler meat was associated with lower muscle pH_u and, therefore, poor meat quality. Moreover, Le Bihan-Duval et al. (2001) examined the association between genetic parameters of meat quality and body composition in broilers and reported that pH_u of breast meat was genetically negatively correlated with L* value at 24 h postmortem. The results of the present study also indicated that as pH_u decreased by decreasing dietary AA density, L* at 24 h postmortem increased, which supports the theory of genetic correlation between these 2 parameters. Decreasing dietary AA density resulted in a linear increase in the a* value of breast meat, which is consistent with the study by Conde-Aguilera et al. (2013), who showed a rise in breast redness value in the case of a sulfur AA deficiency. Mancini and Hunt (2005) demonstrated that lower oxidized myoglobin in breast muscle results in a higher a* value (redder). However, another previous report attributed the higher redness value of muscle to its higher myoglobin content (Lindahl et al., 2001). Therefore, the higher a* value at 48 h postmortem in the present study obtained with lower dietary AA densities might have indicated a higher myoglobin content or better oxidative stability of breast meat from birds fed lower AA density diets.

Table 7. Frequency of occurrence of different scores of white striping (WS) in different treatments[1,2].

AA density[3]	Stocking density[4]	0 score		1 score		2 score	
		Total birds	%	Total birds	%	Total birds	%
Norm	High	5	27.7	12	66.8	1	5.5
Norm	Low	6	33.3	11	61.2	1	5.5
10%Low	High	9	50.0	8	44.5	1	5.5
10%Low	Low	8	44.3	9	50.2	1	5.5
20%Low	High	11	61.2	6	33.2	1	5.7
20%Low	Low	10	55.5	7	38.8	1	5.7
SEM			18.6		14.9		7.9
AA density							
Norm		11	30.5	23	64.0	2	5.5
10%Low		17	47.2	17	47.3	2	5.5
20%Low		21	58.3	13	36.0	2	5.7
SEM			12.6		12.7		5.3
Stocking density							
High		25	46.3	26	48.2	3	5.6
Low		24	44.4	27	50.1	3	5.6
SEM			7.2		8.2		3.01
P values							
AA density			0.084		0.086		0.999
Stocking density			0.863		0.864		0.999
AA density × stocking density			0.481		0.463		0.999
AA density linear			0.043		0.044		0.976
AA density quadratic			0.811		0.819		0.986

[1]Means represent 6 replicates with 3 randomly selected birds per replicate and 18 birds per treatment.
[2]Score 0 = normal (no white striping), score 1 = moderate WS (white lines < 1-mm thick), score 2 = severe WS (white lines > 1-mm thick).
[3]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20% low = 20% lower than norm AA density.
[4]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

High SD caused an increase in breast muscle b* value only in birds fed norm AA diet. This finding is contrary to that of Simitzis et al. (2012) and Tong et al. (2012), who both showed increasing SD did not affect meat b* value. Different broiler genotypes have been shown to have different meat pH, and meat pH is known to be correlated to meat color (Fletcher, 1999). One reason for the discrepancy observed between studies on meat b* value might be the different genotypes used in these studies (Cobb 700, Suqin yellow, and Ross 308, in Simitzis et al. (2012), Tong et al. (2012), and the present study, respectively). As AA density decreased, corn level was subsequently increased in the diet in the present study. Therefore, the numeric increase in breast muscle yellowness (b*) (P = 0.057 for 48 h postmortem b* value), with decreasing dietary AA density, might be related to an accumulation of corn carotenoids in the muscle (Sandeski et al., 2014). Broilers with higher BW and breast yield resulting from feeding on norm AA density diets had greater pH_u and lower cook loss at 48 h postmortem than broilers with reduced BW and breast yield in the present study. It was in agreement with previous studies that showed that birds with higher growth and breast yield exhibit lower muscle glycogen levels, resulting in greater pH_u and lower drip loss (Berri et al., 2001, 2005). On the other hand, decreasing dietary AA density resulted in a linear decrease in pH_u and an increase in cooking loss at 48 h postmortem, which might have resulted from a possible increase in glycogen content of muscle through a reduction of growth and breast yield in the present study. Except for interaction between AA density and SD on cook loss at 48 h postmortem, there was no significant effect of SD on cook loss or WHC, which agreed well with a previous report by Simitzis et al. (2012). Taken together, these results indicated that broiler breast meat from birds fed decreased AA density diets had poor meat-processing quality relative to decreased pH_u and increased cook loss, but SD did not have any effect on meat quality.

Decreasing AA density resulted in a linear decrease in the nitrogen content of breast meat without affecting fat content. Lower tissue protein synthesis through AA deficiency could have resulted in the reduction of the protein accretion by a decrease in the ribosomal capacity in the present study (Zhang et al., 2012). Previous reports showed a decline in protein content in breast muscle of birds fed lysine or total sulfur AA–deficient diets, and authors explained this result with a change in protein turnover (Tesseraud et al., 1996; Conde-Aguilera et al., 2013). Similarly, Summers and Leeson (1985) reported that total carcass protein was reduced with lower dietary protein levels. However, they also reported an increased carcass fat content with a decreased dietary protein level. This difference between studies may be due to the parts used for analysis, the cranial portion of the

Table 8. Mean white striping (WS) scores of broilers at 49 d of age[1,2].

AA density[3]	Stocking density[4]	WS score
Norm	High	0.78 ± 0.55
Norm	Low	0.72 ± 0.57
10%Low	High	0.56 ± 0.62
10%Low	Low	0.61 ± 0.61
20%Low	High	0.44 ± 0.62
20%Low	Low	0.50 ± 0.62
P		0.414
AA density		
Norm		0.75 ± 0.59
10%Low		0.58 ± 0.60
20%Low		0.47 ± 0.61
P		0.094
Linear		0.050
Quadratic		0.820
Stocking density		
High		0.59 ± 0.60
Low		0.61 ± 0.60
P		0.860

[1]Means (mean ± SD) represent 6 replicates with 3 randomly selected birds per replicate and 18 birds per treatment.
[2]Mean WS score data were analyzed by Kruskal Wallis/Mann Whitney U test.
[3]Norm = recommended amino acid (AA) density; 10%low = 10% lower than norm AA density; 20%low = 20% lower than norm AA density.
[4]High = 35 kg of body weight/m^2; low = 26 kg of body weight/m^2 of stocking density.

breast fillets used for nutrient analysis in the present study, although Summers and Leeson (1985) analyzed the whole carcass. Moreover, Moran and Bilgili (1990) showed that fat percentage in breast did not change with different dietary lysine levels ranging from deficient to adequate, although fat in skin and thigh altered, implying a different response by various parts in fat content. SD did not lead to any difference in breast muscle nitrogen and fat content in the present study. On the other hand, Simitzis et al. (2012) showed lower intramuscular fat levels in the breast muscle of broilers reared at high SD (27.2 kg/m^2 vs. 12.6 kg/m^2). They attributed this response to higher lipolysis under increased stress levels. The same authors explained the lower intramuscular fat levels with decreased liver weight and enzyme activities found in birds under high SD. They proposed that the birds under high SD compensated their energy need by lipolyzing their body lipids. However, data are scarce on the effects of different SD on breast meat nutrient content, and therefore, it is difficult to discuss the current results thoroughly.

Under the circumstances of the present study, 49.15% of the investigated breast fillets showed moderate WS, which was very similar to the reported average of 46.9% moderate WS occurrence in medium-sized birds by Lorenzi et al. (2014). It is also in good agreement with the study of Russo et al. (2015), who reported 56.9 and 56.8% moderately WS affected breast fillets for medium- and heavy-weight broiler flocks, respectively. Moreover, the occurrence of moderate WS fillets in the present study was also very close to 47.5% moderate WS presence shown by Kuttappan et al. (2013), despite the large differences in bird material used (288 vs. 739 birds, male birds vs. mixed-sex, 49 d of age vs. 59 to 63 d of age, in the present study and Kuttappan et al. (2013), respectively). On the other hand, Petracci et al. (2013) assessed the occurrence of WS in a commercial plant using a total of 28,000 breast fillets processed at 45 to 54 d of age and reported that the incidence of moderate WS was 8.9%. Although it is not clear what was the exact reason for this discrepancy, the differences on the sample size and study setting (experimental vs. commercial) might have had a notable impact on the extremely large difference noted in the occurrence of moderate WS in breast fillets between studies. Moderately WS affected fillets can be marketed without further processing and, therefore, should not have a significant economic impact in the broiler industry, according to Trocino et al. (2015). However, economic data regarding WS are still lacking.

The occurrence of severely WS affected breast fillet was 5.6% in the present study. It was in good agreement with 8.3, 3.1, and 2.5% severe WS occurrences reported by Kuttappan et al. (2013), Petracci et al. (2013), and Ferreira et al. (2014), respectively. In commercial settings, Lorenzi et al. (2014) and Russo et al. (2015) reported severe WS prevalence of 5.0 and 13.3% for medium weight broilers and 7.2 and 25.7% for heavy weight broilers, respectively. Mean WS score was 0.60 in the present study, which was lower than 1.00 mean WS score reported by Russo et al. (2015). The mean WS score decreased linearly (0.75–0.47) with decreasing dietary AA density. Moreover, moderate WS occurrence decreased and normal breast occurrence increased as the dietary AA density decreased, respectively; however, severe WS occurrence did not differ among birds fed diets with different AA densities in the present study. Together, these data implied that the decreased growth rate obtained by decreasing dietary AA density resulted in smaller breast fillets with decreased thickness. Therefore, in turn, it could have prevented hypoxia, which was postulated as the most likely initiator of WS in broilers, and lead to an increase in the integrity of muscle fibers (Ferreira et al., 2014; Boerboom et al., 2018). Similarly, Livingston et al. (2019) was also able to reduce WS occurrence by slowing the growth rate with time-limited feeding for the broiler chickens. On the other hand, the current data, especially on severe WS occurrence, also suggested that the magnitude of the dietary AA density effect depends not only on differences in growth rate but also on differences in genetic background of broilers. The hypothesis of the present study was to decrease breast muscle thickness and also help reduce intramuscular fat through increasing SD; therefore, it was expected to lower the mean occurrence of WS. However, increasing SD was only partially efficacious in birds fed norm AA diet, but not in birds fed lower AA density diets. Therefore, the lack of SD effect on mean WS occurrence is logical, although more research is needed in this area by solely focusing on different SD levels.

Earlier studies demonstrated that higher slaughter age, BW, and BWG were associated with higher

Table 9. Body weight, carcass traits, pH, CIELAB color characteristics, cook loss, water-holding capacity, and nutrient content of broiler breast fillets having different white striping scores[1,2,3].

Trait	Normal	Moderate	Severe	SEM	*P*
Body weight, g	2,763[b]	2,825[b]	3,150[a]	29.8	0.013
Hot carcass weight, g	2,015[b]	2,086[b]	2,379[a]	25.4	0.004
Breast weight, g	579[b]	634[b]	769[a]	11.6	<0.001
Breast fillet length, cm	18.38	18.68	19.10	0.110	0.212
Breast fillet width, cm	9.50	9.73	10.03	0.077	0.194
Breast fillet cranial thickness, cm	2.65[b]	2.79[b]	3.33[a]	0.047	<0.01
Breast fillet caudal thickness, cm	0.70	0.84	0.92	0.026	0.087
Wings, g	185	185	204	2.0	0.094
Legs, g	831[b]	839[b]	935[a]	9.3	0.043
Rib cage, g	420	428	471	4.9	0.065
Hot carcass yield, %	72.8	73.8	75.5	0.36	0.154
Breast yield, %	28.6[b]	30.2[a,b]	32.3[a]	0.26	<0.001
Wings yield, %	9.2[a]	8.9[a,b]	8.6[b]	0.05	0.001
Leg yield, %	41.3[a]	40.3[a,b]	39.4[b]	0.19	0.010
Rib cage yield, %	20.9[a]	20.5[a,b]	19.8[b]	0.10	0.030
Breast meat traits					
Initial pH	6.57	6.55	6.53	0.011	0.721
Ultimate pH	5.74[b]	5.81[a,b]	5.82[a]	0.009	<0.001
L* at 24 h	60.03	58.86	60.8	0.269	0.050
a* at 24 h	2.45	2.41	2.04	0.108	0.704
b* at 24 h	9.46	8.92	8.33	0.186	0.216
L* at 48 h	60.1	59.12	60.4	0.246	0.115
a* at 48 h	2.38	2.38	2.12	0.086	0.792
b* at 48 h	9.47[a]	8.76[a,b]	7.88[b]	0.172	0.035
Cook loss at 24 h, %	29.16	28.62	28.16	0.369	0.459
Cook loss at 48 h, %	32.02	31.86	33.32	0.239	0.381
Water holding capacity at 24 h, %	13.41	13.02	11.26	0.256	0.161
Water holding capacity at 48 h, %	12.28	11.67	11.70	0.219	0.057
Ether extract, g/kg	9.5[b]	9.7[b]	18.0[a]	1.470	0.010
Nitrogen, g/kg	36.4	35.7	34.6	1.450	0.464

[a,b]Means within a row with no common superscript differ significantly ($P \leq 0.05$).

[1]A Levene's test was used to verify the equality/unequality of variances in the samples (homogeneity of variance).n = 49, n = 53, and n = 6 for normal, moderate and severe white striping categories, respectively.

[2]Unequally sized groups with equal variances were analyzed by an ANOVA (one way) followed by parametric post hoc test of Hochberg's Gt2.

[3]Unequally sized groups with unequal variances were analyzed by an ANOVA (one way) followed by parametric post hoc test of Dunnet T3.

incidence and severity of WS occurrence in broiler breast fillets (Lorenzi et al., 2014; Russo et al., 2015). Bodyweight, carcass weight, breast weight/yield, and cranial thickness of broilers showing severe WS were also higher than those of the birds with moderate WS or normal breast in the present study. This result was supported by the hypothesis mentioned previously that the WS severity increases with increasing growth rate and carcass/breast weight. Livingston et al. (2019) attributed the severe WS occurrence in heavier broilers to an increased metabolic rate and, therefore, consequently increased need to remove the by-products of the metabolism from fast-growing breast muscle. The pH_u of severely WS affected breasts was higher than that of the normal breasts in the present study, and this further confirms the positive genetic association between WS and breast muscle pH_u shown by Alnahhas et al. (2016), who also suggested that breast muscle fiber hypertrophy and lack of glycogen reserve in muscle could be responsible for WS occurrence. Cook loss and WHC of WS-affected breasts were not significantly different from normal breasts in the present study. Similarly, Mudalal et al. (2015) showed that breast fillets affected by WS had the highest pH_u, but WS occurrence had a negligible or relatively low effect on drip loss of both raw and cooked breast meat. Similarly, Kuttappan et al. (2013), in an experimental setting, failed to show any significant relationship between the degree of WS and meat quality parameters, including cook loss and color. Although several studies showed that WS fillets exhibited higher values of b* (Kuttappan et al., 2013; Petracci et al., 2013; Baldi et al., 2018), L* (Alnahhas et al., 2016), and a* (Petracci et al., 2013) than normal fillets, overall magnitude of the differences reported was small. The current data on color attributes were in good agreement with observations showing that the effect of WS on color traits of breast fillets was limited and not of practical importance (Bowker and Zhuang, 2016; Brambila et al., 2016).

The fat content in severe WS fillets was reported to increase through an increased lipogenesis in the liver or an accelerated uptake of the serum fat (Kuttappan et al., 2012a). Results of the present study also showed that breast meat with severe WS score had significantly higher fat content than that with moderate WS or no WS, which supports the presence of a possible tissue inflammation with lipidosis (Mudalal et al., 2014; Mazzoni et al., 2015). Increased intracellular calcium was shown to trigger programmed cell death of muscle cells in WS-affected broilers, followed by the replacement of muscle

fibers with adipocytes and interstitial tissue, which increases fat content (Marchesi et al., 2019). However, in contrast to the study by Mudalal et al. (2014), although the fat level was higher, the nitrogen content was not different in severely WS affected breasts in the present study. An increase in collagen content and a decrease in sarcoplasmic-myofibrillar protein content were reported in WS-affected breasts (Petracci et al., 2014). Thus, severely WS affected breasts in the present study might have had a shift in the source protein content instead of a total protein decrease. However, this probably was not enough to explain the lack of a proportional reduction in total nitrogen content concerning the increased fat content in severely WS affected fillets.

In conclusion, the mean occurrence of moderate and severe WS was 49.15 and 5.6%, respectively. Reduction of dietary AA density by 10 or 20% adversely affected growth performance, carcass traits, and meat quality of broilers. Dietary AA reduction reduced mean WS occurrence, and this reduction was explicitly achieved in moderate WS occurrence. Increasing SD decreased hot carcass and part weights, although SD did not affect WS occurrence. Severe WS fillets were heavier, thicker, and had high pH_u and fat content than normal breast fillets. However, WHC was not affected by WS occurrence. Overall, WS affected breast meats had minor meat quality problems. Decreasing dietary AA density reduced WS occurrence. However, SD did not affect WS occurrence and severity under the circumstances of the present study.

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