

Effect of digestible amino acids to energy ratios on performance and yield of two broiler lines housed in different grow-out environmental temperatures

Pramir Maharjan,^{*} Garret Mullenix,^{*} Katie Hilton,^{*} Justina Caldas,[†] Antonio Beitia,^{*} Jordan Weil,^{*} Nawin Suesuttajit,^{*} Antonio Kalinowski,[‡] Nadia Yacoubi,[§] Victor Naranjo,[#] Judy England,^{*} and Craig Coon^{*,1}

^{*}Department of Poultry Science, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville 72701, USA; [†]Cobb Vantress, Inc., Siloam Springs 72761, AR, USA; [‡]Cargill Protein Latin America, Heredia, Costa Rica; [§]Evonik Operations GmbH, Essen 45128, Germany; and [#]Evonik Guatemala S.A., Edificio Punto Diez, Oficina 3D, Cidade da Guatemala, Guatemala

ABSTRACT Two broiler lines, Line A and Line B, were fed experimental diets from 22 to 42 d with objectives to determine effects of digestible amino acids (**AA**) to metabolizable energy ratios on feed intake (**FI**), performance, and processing yield. Experimental diets were formulated to 3,150 kcal/kg with 5 levels of digestible lysine (**dLys**)—80, 90, 100, 110, and 120% of recommended AA level giving g dLys/Mcal values of 2.53, 2.85, 3.17, 3.48, and 3.80, respectively. All other AA were formulated to a fixed ratio to dLys. A total of 4,050 chicks were utilized in each trial (9 replicate pens for each AA level and each line; 45 chicks/pen) conducted twice: one in hot environmental temperature (**HT**) (24 h mean ~85.3 °F; 80.9% RH) and another in cool environmental temperature (**CT**) (24 h mean ~71.6 °F; 61.7% RH). Results showed that FI was not impacted by dietary AA levels in HT for both lines. Higher FI ($P < 0.05$) was observed in CT for lower dietary AA levels (<100% AA level) for both lines, with overall higher FI occurring in Line B. Higher FI for Line B was also accompanied by higher body weight in HT and CT. Treatment diets had quadratic effects on average daily gain (**ADG**), feed

conversion ratio (**FCR**), and processing yields (breasts and tenders) in both HT and CT, with broilers in CT performing better ($P < 0.05$). The optimal response values for ADG in HT and CT were 89.72 g and 113.44 g occurring at 120 and 109.5% AA level, respectively. The optimal response values for FCR in HT and CT were 1.79 and 1.58 occurring at 120 and 117.5% AA level, respectively. The optimal response values for breast meat yield in HT and CT were 575.9 g and 776.5 g occurring at 112.6 and 114.5% AA level, respectively. The optimal response values for tender meat yield in HT and CT were 119.8 g and 154.9 g occurring at 120 and 115% AA level, respectively. Line A had a higher breast and tender yield % (of live weight) for both environmental temperatures which correlated to body composition data with higher % protein mass and % digestible AA retention. In this study, findings indicated that effects of increased digestible AA density on FI, performance, and processing yield are specific to strain and grow-out temperature, but the optimum response was attained for both lines with diets containing 110 to 120% AA levels (3.48–3.80 g dLys/Mcal) during the 22 to 42 d finisher period.

Key words: broiler lines, amino acids to energy ratio, performance, yield, environmental temperature

2020 Poultry Science 99:6884–6898

<https://doi.org/10.1016/j.psj.2020.09.019>

INTRODUCTION

Productive traits of meat broilers such as rate of gain and breast meat yield have been exponentially

improving because of genetic progress (Fancher, 2014; Aftab, 2019). The selection for increased gain rate has sustained the improved efficiency seen with broilers (Carre et al., 2014). Increased feed consumption has fueled increased rate of gain, as broilers show the capacity to process increasing amount of nutrients on a daily basis. In the past, the feed intake (**FI**) of broilers was thought to be regulated by the energy concentration of the diet (Fisher and Wilson, 1974), a concept that is strongly retained by nutritionists (Leeson, 2013). There

© 2020 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received February 5, 2020.

Accepted September 1, 2020.

¹Corresponding author: ccoona@uark.edu

are ongoing research to understand factors that govern FI regulation of current broiler genetics such as physiological ability of broilers to digest feed and dietary energy content. Lemme (2005) showed that FI was regulated not only by dietary energy level but also by the concentration of amino acids (AA) in the diet (balanced protein). More recently, Classen (2013) conducted a series of trials where special attention was given to control the confounding factors when evaluating the impact of dietary energy and AA levels on performance such as in vivo energy measurements, constant energy sources, and pellet quality. Classen (2013) reported that energy level did not affect FI and the energy level of diet needed to be determined based on the expected protein accretion of the bird.

It is generally known that hot environmental temperature (HT) decreases FI and broiler performance. There is some dispute in literature as to the benefits of lower or higher CP and AA levels during heat stress period (Ojano-Dirain and Waldroup, 2002; Furlan et al., 2004; Awad et al., 2019). A review by Dozier et al. (2008) noted that changes in the body weight (BW), FI, feed conversion ratio (FCR), and increased breast meat yield of the modern boiler compared with past decades indicates an increase in digestible AA needs of the current broiler lines.

As the industry continues to make advances toward attaining higher BW at market, improved FCR, and increases in breast and tender yields, the requirement for digestible AA should continue to increase. Increased digestible AA requirement has to be supplied by increased FI or increased dietary AA levels.

The objectives of present study were to evaluate the effects of increasing digestible AA to metabolizable energy ratio on FI, weight gain, FCR, and processing yield of 2 modern high yielding broiler lines during grow-out finisher period from 22 to 42 d. Two repeated trials were conducted—1 in HT and another in cool environmental temperature (CT).

MATERIALS AND METHODS

Birds and Husbandry

Two thousand twenty-five male chicks from Line A and 2,025 male chicks from Line B were placed in 90 floor pens (House 1 and House 2, 45 pens each house), 45 chicks per pen. Both the lines were fast-growing current meat broiler lines. Pens were in 2 adjacent tunnel ventilated houses and (measured 1.524 m × 3.048 m) provided 4.645 m² total space or 1,032 cm² per bird. Each

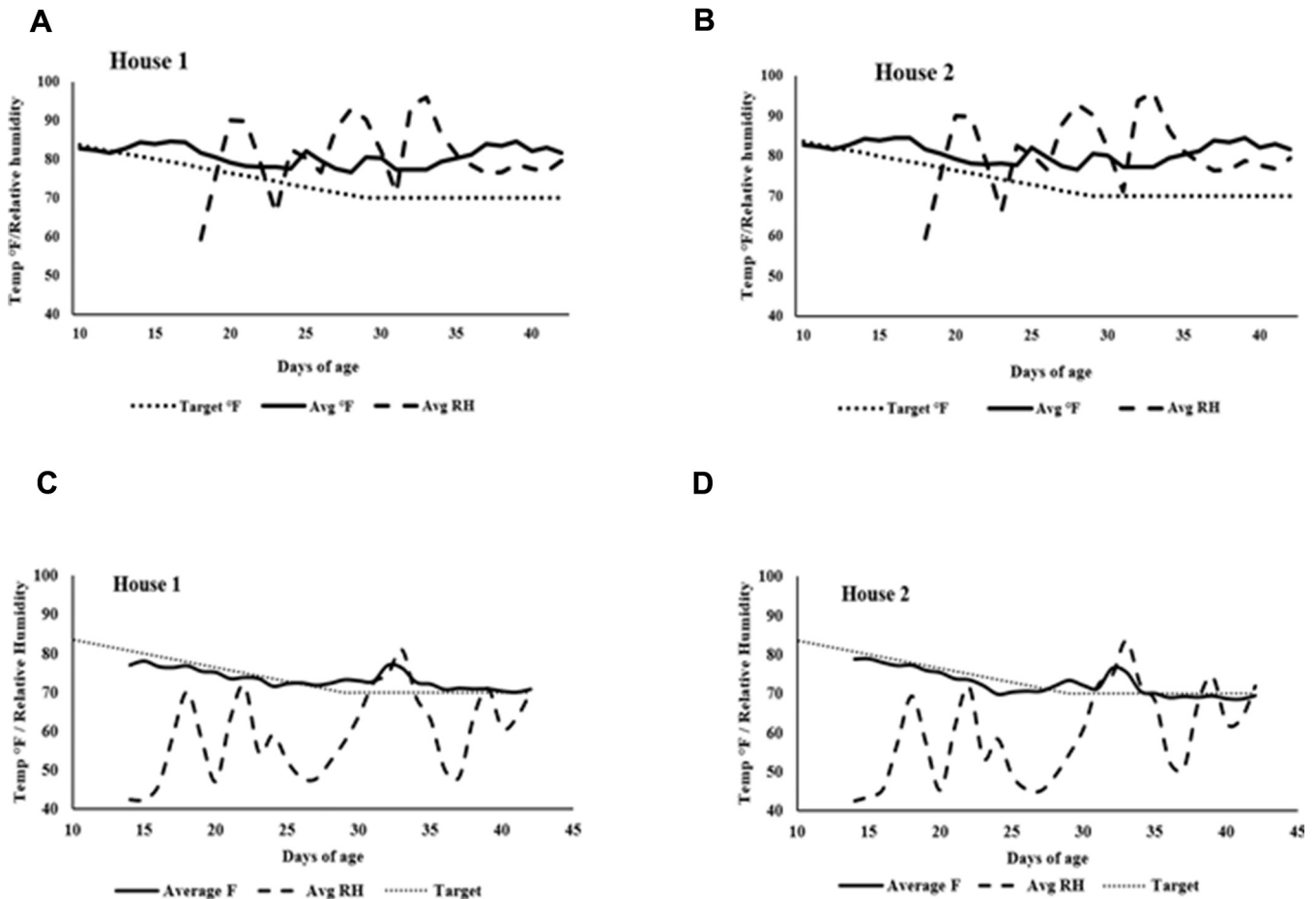


Figure 1. Average temperature and relative humidity (RH) for 2 grow-out houses at hot environmental temperature (HT) (A, B) and cool environmental temperature (CT) (C, D). Average daily temperature values (21–42 d) recorded for HT and CT for House 1 and House 2 were 85.3°F and 85.1°F and 70.9°F and 72.6°F, respectively. Average daily % RH (21–42 d) recorded for HT and CT for House 1 and House 2 were 80.97 and 81.03 and 61.55 and 61.92, respectively.

Table 1. Ingredient and nutrient composition of experimental finisher diets used in hot environmental temperature.

| Treatment: | 80AA | | 90AA | | 100AA | | 110AA | | 120AA | |
|-----------------------------------|------------|-------------------|------------|-------------------|------------|-------------------|------------|-------------------|------------|-------------------|
| Ingredient | % | | % | | % | | % | | % | |
| Corn | 75.03 | | 73.33 | | 69.82 | | 67.73 | | 64.34 | |
| Soybean meal, 48% CP | 14.71 | | 15.09 | | 18.72 | | 23.05 | | 26.49 | |
| Corn gluten meal, 60% CP | 5.29 | | 6.31 | | 5.34 | | 1.88 | | 1.01 | |
| Dicalcium phosphate 19 | 1.81 | | 1.81 | | 1.81 | | 1.82 | | 1.82 | |
| Soybean oil | 1.00 | | 1.00 | | 1.68 | | 2.56 | | 3.19 | |
| Limestone | 0.85 | | 0.85 | | 0.82 | | 0.78 | | 0.76 | |
| Sodium bicarbonate | 0.35 | | 0.40 | | 0.41 | | 0.42 | | 0.43 | |
| Salt NaCl | 0.23 | | 0.19 | | 0.18 | | 0.18 | | 0.17 | |
| L-lys-HCl | 0.24 | | 0.35 | | 0.38 | | 0.40 | | 0.43 | |
| MetAmino® | 0.07 | | 0.13 | | 0.19 | | 0.31 | | 0.37 | |
| Choline Chloride 60% | 0.15 | | 0.15 | | 0.14 | | 0.12 | | 0.10 | |
| ThreAmino® | 0.01 | | 0.06 | | 0.09 | | 0.15 | | 0.18 | |
| ValAmino | | | | | 0.04 | | 0.13 | | 0.17 | |
| L-isoleucine | | | 0.01 | | 0.04 | | 0.11 | | 0.15 | |
| L-arginine | | | 0.07 | | 0.08 | | 0.11 | | 0.13 | |
| Vitamin premix ¹ | 0.10 | | 0.10 | | 0.10 | | 0.10 | | 0.10 | |
| Trace mineral premix ² | 0.10 | | 0.10 | | 0.10 | | 0.10 | | 0.10 | |
| Selenium premix 60% | 0.02 | | 0.02 | | 0.02 | | 0.02 | | 0.02 | |
| Ethoxyquin ³ | 0.02 | | 0.02 | | 0.02 | | 0.02 | | 0.02 | |
| molCurb ⁴ | 0.05 | | 0.05 | | 0.05 | | 0.05 | | 0.05 | |
| Nutrient Composition | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ |
| Dry matter, % | 89.24 | 87.96 | 89.31 | 88.03 | 89.39 | 88.45 | 89.42 | 88.35 | 89.49 | 88.31 |
| Crude protein, % | 17.00 | 17.03 | 18.00 | 18.02 | 19.00 | 19.15 | 19.00 | 19.37 | 20.00 | 20.58 |
| Crude fiber, % | 2.24 | | 2.24 | | 2.33 | | 2.46 | | 2.54 | |
| Ether extract, % | 4.05 | | 4.05 | | 4.60 | | 5.29 | | 5.81 | |
| Ash, % | 5.45 | | 5.48 | | 5.64 | | 5.84 | | 5.99 | |
| Starch, % | 48.44 | | 47.57 | | 45.39 | | 43.71 | | 41.62 | |
| AMEn, kcal/kg | 3,150 | 3,306 | 3,150 | 3,353 | 3,150 | 3,324 | 3,150 | 3,362 | 3,150 | 3,355 |
| Ca, % | 0.85 | | 0.85 | | 0.85 | | 0.85 | | 0.85 | |
| P, % | 0.65 | | 0.65 | | 0.66 | | 0.66 | | 0.67 | |
| avP, % | 0.42 | | 0.42 | | 0.42 | | 0.42 | | 0.42 | |
| Na, % | 0.20 | | 0.20 | | 0.20 | | 0.20 | | 0.20 | |
| Cl, % | 0.23 | | 0.23 | | 0.23 | | 0.23 | | 0.23 | |
| K, % | 0.52 | | 0.53 | | 0.58 | | 0.65 | | 0.70 | |
| Electrolyte balance | 156 | | 157 | | 171 | | 189 | | 202 | |
| Lys, % | 0.87 | 0.891 | 0.98 | 0.987 | 1.08 | 1.119 | 1.19 | 1.232 | 1.29 | 1.342 |
| Met, % | 0.39 | 0.372 | 0.46 | 0.444 | 0.53 | 0.505 | 0.61 | 0.589 | 0.68 | 0.634 |
| Met + Cys % | 0.69 | 0.664 | 0.77 | 0.754 | 0.84 | 0.823 | 0.91 | 0.897 | 0.99 | 0.948 |
| Thr, % | 0.62 | 0.627 | 0.69 | 0.701 | 0.76 | 0.770 | 0.82 | 0.833 | 0.89 | 0.895 |
| Trp, % | 0.17 | 0.165 | 0.17 | 0.174 | 0.19 | 0.195 | 0.20 | 0.211 | 0.22 | 0.228 |
| Arg, % | 0.93 | 0.962 | 1.02 | 1.046 | 1.13 | 1.177 | 1.24 | 1.314 | 1.35 | 1.430 |
| Ile, % | 0.67 | 0.668 | 0.71 | 0.706 | 0.78 | 0.796 | 0.85 | 0.869 | 0.93 | 0.946 |
| Val, % | 0.78 | 0.797 | 0.81 | 0.826 | 0.90 | 0.917 | 0.97 | 0.999 | 1.06 | 1.077 |
| SID Lys, % | 0.80 | 0.84 | 0.90 | 0.96 | 1.00 | 1.05 | 1.10 | 1.18 | 1.20 | 1.27 |
| SID Met, % | 0.36 | 0.35 | 0.43 | 0.41 | 0.50 | 0.45 | 0.58 | 0.55 | 0.65 | 0.61 |
| SID Thr, % | 0.52 | 0.58 | 0.59 | 0.64 | 0.65 | 0.69 | 0.72 | 0.76 | 0.78 | 0.83 |
| SID Trp, % | 0.14 | 0.16 | 0.14 | 0.19 | 0.16 | 0.23 | 0.18 | 0.21 | 0.19 | 0.21 |
| SID Arg, % | 0.86 | 0.85 | 0.95 | 0.93 | 1.05 | 1.03 | 1.16 | 1.18 | 1.26 | 1.28 |
| SID Val, % | 0.70 | 0.66 | 0.72 | 0.79 | 0.80 | 0.87 | 0.88 | 0.97 | 0.96 | 1.05 |
| SID Ile, % | 0.60 | 0.66 | 0.64 | 0.72 | 0.71 | 0.78 | 0.78 | 0.87 | 0.85 | 0.90 |

Abbreviations: AA, amino acid; SID, standardized ileal digestible.

¹Vitamin premix: Vit A, 13,227 IU/kg; Vit D3, 3,968 IU/kg; Vit E, 66 IU/kg; Vit B12, .040 mg/kg; Biotin, .254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg.

²Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).

³Ethoxyquin: Monsanto Santoquin.

⁴Kemin, Des Moines, IA.

⁵Analysis on as is basis.

pen was equipped with 2 hanging type feeders and a nipple-type drinker line (10 nipples per line). Lighting program was 23L: 1D from day 0 to day 7 and 18L: 6D from day 8 to day 42. Broilers were reared under recommended typical husbandry settings and welfare guidelines (under the approval of IACUC #15048). Two trials were conducted; 1 in HT and then repeated in CT. Therefore, a total of 8,100 broilers were utilized in the study. Ambient temperature and relative humidity

for each house were recorded during experimental period for both trials (Figures 1A–1D).

Experimental Diets and Design

All ingredients to be used were analyzed before feed formulation. Diets were formulated based on standardized ileal digestible (SID) AA and nitrogen corrected apparent metabolizable energy (AMEn), according to

Evonik AminoChick recommendations. The analysis of AMEn involved analysis of gross energy (GE), dry matter, and nitrogen in feed and excreta. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL). Dry matter was analyzed by method 934.01 (AOAC, 1990) and nitrogen determined by the method 990.03 (AOAC, 1995). The titanium in feed and excreta was measured utilizing the method described in Myers et al. (2004). The AMEn was calculated as follows: $\text{AMEn kcal/kg} = (\text{GE}_{\text{diet}} - \text{GE}_{\text{excreta}} \times \text{TiO}_2_{\text{diet}} / \text{TiO}_2_{\text{excreta}}) - 8.22 \times (\text{N}_{\text{diet}} - \text{N}_{\text{excreta}} \times \text{TiO}_2_{\text{diet}} / \text{TiO}_2_{\text{excreta}})$. The SID AA calculations were discussed further below in the methods.

The broilers were fed a common starter feed from 0 to 10 d formulated to 3,030 kcal/kg and 1.27% digestible lysine (dLys) (Supplementary Table 1). A common grower feed, fed from 11 to 21 d, was formulated to 3,080 kcal/kg and 1.09% dLys (Supplementary Table 2). Broiler pens were randomly reallocated to treatment pens using completely randomized block design post 21 d BW to start the experimental study (22–42 d). A 2 × 5 factorial experiment was created with both broiler lines placed on the 5 experimental diets (9 replicate pens for each diet and each line) formulated to 3,150 kcal/kg with 5 increasing levels of dLys as 2.53 g, 2.85 g, 3.17 g, 3.48 g, and 3.80 g (Table 1 and Table 2). Experimental diets contained 80, 90, 100, 110, and 120% AA levels using Evonik AminoChick recommendations. All other AA were formulated in relationship to the dLys level (Supplementary Table 3). The experimental diets and design were kept similar for both the trials conducted at HT and CT.

Performance Parameters and Yield Evaluation

For obtaining performance data, broilers and feed were weighed at 0, 10, 21, and 42 d to determine BW, ADG, mortality corrected FCR, and dLys intake. At 42 d of age, 10 broilers per pen ($n = 90$ broilers/treatment; 900 broilers/trial) were selected within 1 SD of the average BW for each treatment for processing yield determination. Initial live weight (LW) and ready-to-cook parts—breast, tender, wing, and thigh were measured in terms of % LW.

Body Composition

Body composition (BC) was determined by utilizing dual energy X-ray absorptiometry (DEXA) (GE, Madison, WI) equipped with Lunar Prodigy small animal software (version 12.2). Broilers were euthanized with CO₂ gas and were scanned (feathers-on). Scanned broilers were used in previously determined equations (Caldas et al., 2018) to calculate the total lean, protein, fat mass, and energy content of scanned broilers. Two broilers per pen were selected at 22 and 42 d of age for

scanning (18 broilers per treatment—180 broilers at each age; 360 broilers each trial). Broilers were selected to be within 1 SD of the average BW for the treatment.

Percent digestible AA retention and % AMEn retention values at day 42 were also determined utilizing DEXA values. Initially, the cumulative digestible AA intake for each treatment from 22 to 42 d was calculated and expressed as g digestible AA consumed as;

$(\text{PI}_{22-42}) = (\text{FI}_f \times \% \text{CP}_a)$. Where PI_{22-42} = digestible AA intake (g) at 22–42 d; FI_f = cumulative feed intake (g) at 22–42d; f = finisher; $\% \text{CP}_a$ = analyzed digestible AA in feed.

The % digestible AA retention, 22 to 42 d was then calculated by the equation as follows:

$$\% \text{ digestible AA} = (\text{PC}_{42} - \text{PC}_{22}) / \text{PI}_{22-42}$$

where, PC_{42} and PC_{22} is whole bird protein content at day 42 and day 22, respectively.

Percent energy retention (% AMEn_{22–42 d}) values were similarly determined by calculating the energy content of broilers at 42 d and 22 d (EC_{42} and EC_{22}) utilizing DEXA values for protein and fat and determining the cumulative AMEn energy feed intake for 22 to 42 d broilers for each treatment.

Standardized Ileal Digestible Amino Acids

At 42 d, 6 broilers from Line A and Line B were selected from each dietary treatment diet to be within 1 SD of the mean BW for that treatment to determine the AA digestibility of the finisher diets. Broilers were acclimated to the digestibility cages for 2 d. The 5 experimental finisher diets with 0.2% TiO₂ added were fed ad libitum. Feed was removed at 2,400 h for 8-h period on the evening of day 44. The broilers were fed *ad libitum* from 0,800 to 1,000 h (2 h period) on day 45 and immediately euthanized by CO₂ inhalation. The digesta was removed by squeezing from the terminal ileum and immediately frozen in liquid nitrogen. The digesta from each broiler was then freeze-dried and analyzed for AA content by reverse phase HPLC utilizing AOAC 982.30 and AOAC 985.28 methods (AOAC, 1990) as discussed in Caldas et al. (2018). The experimental diets with 0.2% TiO₂ were also analyzed for AA content. The titanium in feed and digesta was measured utilizing the method described in Myers et al. (2004). The apparent AA digestibility (AID) was calculated using the expression (Maharjan et al., 2019a);

$$\text{AID} = 1 - [(\text{Ci} / \text{Co}) \times (\text{Xo} / \text{Xi})]$$

where, Ci is the concentration of TiO₂ present in diet, Co is the concentration of TiO₂ present in digesta, Xo is the AA content in digesta, and Xi is the AA content present in diet. All values for Ci, Co, Xo, and Xi were expressed on % DM basis. Digestibility coefficient (DC) values for each individual AA were determined.

The AID values were converted to SID values using basal endogenous AA losses (**BEL**) for SID calculations (Blok and Dekker, 2017) and using the following expression;

$$\text{SID coefficient (\%)} = \text{AID (\%)} + (\text{BEL of AA (g/kg DM)} / \text{AA content of diet (g/kg)}) \times 100.$$

The SID coefficients were then used to calculate the % SID AA in experimental diets for each individual AA using the expression:

$$\% \text{ SID AA} = (\text{SID coefficient} * \% \text{ AA in diet ("as is")})$$

Data Analysis

The data obtained for variables measured were analyzed by one-way ANOVA using JMP Pro 14 (SAS Institute, Inc., Cary, NC). Mean values were obtained for variables measured (BW, FCR, ADG, processing yields). One-way ANOVA was performed for differentiating significant means for treatment diet effects using Student *t* test or HSD test where appropriate within each line. Means were considered significant with a *P*-value ≤ 0.05 . For understanding if there was any dietary AA level (factor A) and line (factor B) interaction on response variable, two-way ANOVA model was utilized as follows:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{ijk}$$

where, μ = mean, T_i = effect of i^{th} level of factor A, B_j = effect of j^{th} level of factor B, $(TB)_{ij}$ = effect of interaction between the i^{th} level of factor A and the j^{th} level of factor B, e_{ijk} = random error associated with the k^{th} replicate.

Nonlinear second degree polynomial regression analyses were applied for ADG, FCR, and yield data to determine the relationship of dietary AA level (combined lines) on these response variables. The following equation was utilized:

$$y = a + bx + cx^2$$

where, y = response variable x = AA level, a = intercept, b = slope, and c = quadratic.

Prediction profiler was created for the curve obtained for each response variable to understand the optimal response areas for given AA level.

RESULTS

There was no diet and line interaction observed for the parameters measured in HT or CT in the study.

Feed and Nutrient Intake

Hot environmental temperature: Cumulative FI and thus nutrient intake was higher for Line B in both feeding phases (0–21 d and 22–42 d) (Table 3). Feed intake was not different for both lines when compared between AA levels. Cumulative CP intake was higher

for Line B. When compared between dietary treatments, there was a linear increment ($R^2 = 0.96$) in dLys intake from 27.99 to 41.30 g per broiler (g/b) for Line A and from 30.39 to 44.66 g/b for Line B. Again, cumulative

AMEn intake was higher ($P < 0.05$) for Line B; however, it was not different within lines between dietary treatments.

Cool environmental temperature: Cumulative FI remained higher for Line B than Line A for feeding phases (0–21 d and 22–42 d) (Table 3). Feed intake was higher ($P < 0.05$) for both lines (than in HT), and FI decreased as dietary AA levels increased ($P < 0.05$). Line B had higher cumulative dLys intake ($P < 0.05$) for both feeding phases. The dLys intake (22–42 d) was highest at 110 to 120% AA levels for both lines. Again, cumulative AMEn was higher for Line B. Apparent metabolizable energy intake decreased as dietary AA levels increased for both lines.

The nutrient intake was higher ($P < 0.05$) in CT season than in HT for both lines.

Body Weight, Average Daily Gain, and Feed Conversion Ratio

Hot environmental temperature: The BW differences between lines were observed from day 36 onward with Line B having a higher BW than Line A (Table 4). Cumulative ADG was higher for Line B than Line A. Cumulative FCR was not different between lines during experimental period (22–42 d); however, the FCR values improved with the increased AA level in treatment diets.

Cool environmental temperature: The BW difference was observed between lines from day 10 until day 42, with Line B having higher BW (Table 4). Cumulative ADG was higher for Line B than Line A. Cumulative FCR was higher ($P < 0.05$) for Line B during experimental period (22–42 d). The FCR values improved with increasing AA levels for both lines.

The quadratic effects of AA level on ADG and FCR were observed on 22 to 42 d. The second degree polynomial fit showed the ADG values of 78.05, 82.43, 85.74, 87.96, and 89.10 g and 105.99, 110.37, 112.97, 113.78, and 112.82 g for 80 to 120% AA levels in HT and CT, respectively ($R^2 = 0.91$ and 0.92, respectively) (Figure 2). The second degree polynomial fit showed the 22 to 42 d FCR values of 2.13, 2.03, 1.94, 1.86, 1.79; and 1.81, 1.71, 1.63, 1.59, and 1.58 for 80 to 120% AA levels in HT and CT, respectively ($R^2 = 0.98$ and 0.97, respectively) (Figure 3).

The BW, ADG, or FCR responses were higher in CT ($P < 0.05$) than in HT when compared at each AA level.

Processing Yield

The % breast and tender meat yield increased ($P < 0.05$) with the increasing dietary AA levels. The

Table 2. Ingredient and nutrient composition of experimental finisher diets used in cool environment temperature.

| Treatment: | 80AA | 90AA | 100AA | 110AA | 120AA | | | | | |
|--------------------------------|------------|-------------------|------------|-------------------|------------|-------------------|------------|-------------------|------------|-------------------|
| Ingredient | % | % | % | % | % | | | | | |
| Corn | 71.93 | 68.05 | 62.61 | 60.52 | 58.00 | | | | | |
| Soybean Meal, 48% | 14.26 | 18.69 | 26.10 | 30.36 | 32.35 | | | | | |
| Corn gluten meal, 60% | 8.00 | 6.63 | 3.32 | | | | | | | |
| Dicalcium phosphate 19 | 1.80 | 1.80 | 1.80 | 1.82 | 1.81 | | | | | |
| Soybean Oil | 1.43 | 2.21 | 3.60 | 4.44 | 4.70 | | | | | |
| Limestone (CaCO ₃) | 0.86 | 0.82 | 0.77 | 0.73 | 0.72 | | | | | |
| Sodium bicarbonate | 0.35 | 0.36 | 0.32 | 0.34 | 0.37 | | | | | |
| L-lysine-HCl | 0.27 | 0.27 | 0.20 | 0.23 | 0.30 | | | | | |
| MetAmino | 0.06 | 0.13 | 0.21 | 0.32 | 0.38 | | | | | |
| Salt | 0.22 | 0.22 | 0.24 | 0.24 | 0.22 | | | | | |
| Choline chloride 60% | 0.16 | 0.14 | 0.11 | 0.09 | 0.08 | | | | | |
| ThreAmino | 0.01 | 0.04 | 0.05 | 0.11 | 0.15 | | | | | |
| ValAmino | | | 0.01 | 0.09 | 0.14 | | | | | |
| L-isoleucine | | | | 0.07 | 0.10 | | | | | |
| L-arginine | | | | | 0.03 | | | | | |
| Vitamin premix ¹ | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | | | | | |
| Trace min premix ² | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | | | | | |
| Ethoxyquin ³ | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | | | | | |
| mycoCurb ⁴ | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | | | | | |
| Ameri-Bond 2x | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | | | | | |
| Nutrient Composition | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ | Calculated | Anal ⁵ |
| Dry matter, % | 88.16 | 87.57 | 88.26 | 87.71 | 88.35 | 87.52 | 88.37 | 87.91 | 88.46 | 88.01 |
| Crude protein, % | 17.00 | 17.82 | 18.00 | 18.44 | 19.00 | 19.75 | 19.00 | 19.37 | 20.00 | 20.56 |
| Crude fiber, % | 2.01 | | 2.05 | | 2.12 | | 2.15 | | 2.17 | |
| Ether extract, % | 4.95 | | 5.58 | | 6.69 | | 7.31 | | 7.52 | |
| Ash, % | 5.82 | | 6.00 | | 6.29 | | 6.46 | | 6.55 | |
| Starch, % | 48.78 | | 46.31 | | 42.72 | | 41.16 | | 39.62 | |
| AMEn, kcal/kg | 3,150 | 3,097 | 3,150 | 3,094 | 3,150 | 3,187 | 3,150 | 3,091 | 3,150 | 3,136 |
| Ca, % | 0.85 | | 0.85 | | 0.85 | | 0.85 | | 0.85 | |
| P, % | 0.65 | | 0.66 | | 0.67 | | 0.68 | | 0.68 | |
| avP, % | 0.42 | | 0.42 | | 0.42 | | 0.42 | | 0.42 | |
| Na, % | 0.20 | | 0.20 | | 0.20 | | 0.20 | | 0.20 | |
| Cl, % | 0.23 | | 0.23 | | 0.23 | | 0.23 | | 0.23 | |
| K, % | 0.51 | | 0.58 | | 0.69 | | 0.76 | | 0.79 | |
| Electrolyte balance | 152 | | 170 | | 200 | | 218 | | 225 | |
| Lys, % | 0.87 | 0.921 | 0.98 | 1.009 | 1.10 | 1.130 | 1.20 | 1.230 | 1.30 | 1.345 |
| Met, % | 0.38 | 0.395 | 0.45 | 0.467 | 0.52 | 0.521 | 0.60 | 0.593 | 0.67 | 0.633 |
| Met + Cys, % | 0.69 | 0.712 | 0.77 | 0.796 | 0.84 | 0.855 | 0.91 | 0.911 | 0.99 | 0.966 |
| Thr, % | 0.62 | 0.649 | 0.69 | 0.714 | 0.76 | 0.781 | 0.82 | 0.823 | 0.89 | 0.900 |
| Trp, % | 0.16 | 0.172 | 0.18 | 0.189 | 0.22 | 0.224 | 0.23 | 0.234 | 0.24 | 0.250 |
| Arg, % | 0.92 | 0.944 | 1.03 | 1.038 | 1.20 | 1.218 | 1.27 | 1.268 | 1.35 | 1.374 |
| Ile, % | 0.67 | 0.703 | 0.73 | 0.737 | 0.79 | 0.801 | 0.86 | 0.867 | 0.94 | 0.940 |
| Val, % | 0.79 | 0.818 | 0.84 | 0.847 | 0.90 | 0.916 | 0.98 | 0.972 | 1.06 | 1.065 |
| SID Lys, % | 0.80 | 0.95 | 0.90 | 1.05 | 1.00 | 1.13 | 1.10 | 1.23 | 1.20 | 1.33 |
| SID Met, % | 0.35 | 0.38 | 0.42 | 0.42 | 0.49 | 0.49 | 0.58 | 0.55 | 0.65 | 0.61 |
| SID Thr, % | 0.52 | 0.66 | 0.59 | 0.71 | 0.65 | 0.78 | 0.72 | 0.81 | 0.78 | 0.88 |
| SID Trp, % | 0.13 | 0.14 | 0.15 | 0.17 | 0.19 | 0.19 | 0.20 | 0.19 | 0.21 | 0.22 |
| SID Arg, % | 0.84 | 0.90 | 0.95 | 0.99 | 1.11 | 1.15 | 1.18 | 1.20 | 1.26 | 1.29 |
| SID Val, % | 0.70 | 0.82 | 0.74 | 0.85 | 0.80 | 0.91 | 0.88 | 0.94 | 0.96 | 1.05 |
| SID Ile, % | 0.60 | 0.71 | 0.65 | 0.75 | 0.71 | 0.82 | 0.78 | 0.87 | 0.85 | 0.94 |

Abbreviations: AA, amino acid; AMEn, apparent metabolizable energy; SID, standardized ileal digestible.

¹Vitamin premix: Vit A, 13,227 IU/kg; Vit D3, 3,968 IU/kg; Vit E, 66 IU/kg; Vit B12, 0.040 mg/kg; Biotin, 0.254 mg/kg; Menadione, 3.968 mg/kg; Thiamine, 3.968 mg/kg; Riboflavin, 13.228 mg/kg; Vit B6, 7.937 mg/kg; Niacin, 110.229 mg/kg; Folic acid, 2.205 mg/kg.

²Trace mineral premix: Mn, 60 mg/kg (manganese sulfate); Zn, 60 mg/kg (zinc sulfate); Fe, 40 mg/kg (ferrous sulfate); Cu, 5 mg/kg (copper sulfate); I, 1.25 mg/kg (calcium iodide); Co, 0.5 mg/kg (cobalt sulfate).

³Ethoxyquin: Monsanto Santoquin.

⁴Kemin, Des Moines, IA.

⁵Analysis on as is basis.

% wing and leg quarter yields did not change (Table 5) with increasing digestible AA levels in treatment diets. The increasing dietary digestible AA levels increased % breast and tender yield of LW for both HT and CT, but broilers housed in CT showed greater increase in yield with higher AA levels ($P < 0.05$).

The effects of AA level on 42 d breast yield and tender yield were predicted for combined lines utilizing second degree polynomial regression (Figure 4) in HT

and CT. The breast yield values were 505.36, 541.9, 565.30, 575.56, and 572.68 g and 693.24, 734.3, 761.5, 774.84, and 774.32 g for 80–120% AA levels in HT and CT, respectively ($R^2 = 0.93$ and 0.98 , respectively). The tender yields were 106.32, 111.90, 115.90, 118.32, and 119.16 g and 139.15, 146.73, 151.74, 154.16, and 154.00 g for 80 to 120% AA levels in HT and CT, respectively ($R^2 = 0.95$ and 0.99 , respectively).

Table 3. Feed intake (FI) and nutrient consumption per bird of 2 broiler lines fed 5 levels of digestible amino acid (AA). Two repeated trials were conducted—one in hot environmental temperature (HT) and another in cool environmental temperature (CT).¹

| Line | Diet | Hot environmental temperature | | | | | Cool environmental temperature | | | | |
|------------------------------|------|-------------------------------|--------------------|--------------------|----------------------|---------------------|--------------------------------|--------------------|--------------------|--------------------|-----------------------|
| | | 0–21 d | | 22–42 d | | | 0–21 d | | 22–42 d | | |
| | | FI | dLys | FI | dLys | AMEn | FI | dLys | FI | dLys | AMEn |
| | g/b | g/b | g/b | g/b | kcal/b | g/b | g/b | g/b | g/b | kcal/b | |
| A | C | 1,343 ^a | 14.63 ^a | 3,293 ^b | 34.889 ^b | 10,999 ^b | 1,210 ^b | 13.18 ^b | 3,760 ^b | 41.88 ^b | 11,714 ^b |
| B | C | 1,319 ^b | 14.37 ^b | 3,590 ^a | 38.00 ^a | 11,991 ^a | 1,260 ^a | 13.73 ^a | 3,930 ^a | 43.91 ^a | 12,292 ^a |
| <i>P</i> -value | | 0.0182 | 0.0126 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| A | A-1 | | | 3,333 | 27.99 ^d | 11,019 | | | 3,940 ^d | 36.24 ^b | 12,202 ^a |
| | A-2 | | | 3,259 | 31.28 ^{c,d} | 10,927 | | | 3,830 ^a | 39.06 ^b | 11,850 ^{a,b} |
| | A-3 | | | 3,304 | 34.69 ^{b,c} | 10,982 | | | 3,720 ^b | 41.66 ^a | 11,855 ^{b,c} |
| | A-4 | | | 3,318 | 39.15 ^{a,b} | 11,155 | | | 3,660 ^c | 44.65 ^b | 11,313 ^{b,c} |
| | A-5 | | | 3,252 | 41.30 ^a | 10,910 | | | 3,620 ^c | 47.78 ^a | 11,352 ^c |
| B | B-1 | | | 3,619 | 30.39 ^d | 11,964 | | | 4,020 ^a | 36.38 ^c | 12,450 ^{a,b} |
| | B-2 | | | 3,673 | 35.26 ^{c,d} | 12,315 | | | 4,050 ^a | 41.38 ^b | 12,531 ^a |
| | B-3 | | | 3,528 | 37.04 ^{b,c} | 11,727 | | | 3,930 ^b | 44.01 ^a | 12,525 ^{a,b} |
| | B-4 | | | 3,613 | 42.63 ^{a,b} | 12,146 | | | 3,840 ^c | 46.84 ^a | 11,869 ^{b,c} |
| | B-5 | | | 3,517 | 44.66 ^a | 11,800 | | | 3,820 ^c | 50.42 ^a | 12,082 ^{b,c} |
| SEM | | | | 68.31 | 1.75 | 174 | | | 30 | 1.48 | 139 |
| ² <i>P</i> -value | | | | 0.6157 | <0.0001 | 0.6150 | | | 0.0354 | 0.0189 | 0.0318 |

Abbreviations: AMEn, apparent metabolizable energy; dLys, digestible lysine; FI, feed intake.

¹Diets A1–A5 or B1–B5 for Line A or Line B represent 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. C represents combined analysis of all AA levels. Different letters in superscripts represent significantly different means between dietary AA levels within line in each column.

²*P*-values presented are for combined lines for dietary AA levels. No diet by line interaction was observed.

Body Composition

Lean mass and protein mass increased as the level dietary AA level increased from 80 to 120% AA, whereas the fat mass and energy (per g of tissue) decreased in HT and CT (Table 6). The % lean mass and protein mass of total body mass was higher in CT than in HT for the same dietary AA level ($P < 0.05$).

The digestible AA retention % was highest for Line A with 120% AA (54.86%) and at 110% AA level (56.58%) for Line B in HT (Figure 5). The digestible AA retention % was highest at 90 and 100% level, respectively, for Line A and Line B in CT. Cool environmental temperature had the higher digestible AA retention % ($P < 0.05$) than HT for both the lines. The % AMEn increased with increasing AA level in HT, whereas it was not different in CT ($P > 0.05$). The % AMEn was lower numerically ($P = 0.31$) for Line A in HT, whereas it was higher for Line A in CT ($P < 0.05$).

Standardized Ileal Digestible Amino Acids

There was no difference between lines in standardized ileal DC values ($P > 0.05$) between treatment diets within HT or CT. Amino acid SID coefficient values were higher for CT than in HT ($P < 0.05$). The standardized ileal digestible AA (“as is” basis) increased ($P < 0.05$) for essential and nonessential AA for both lines with increasing dietary AA level (Table 7).

DISCUSSION

The present study investigated the effects of dietary AA levels on performance parameters and processing yield. Experimental diets consisted of a balanced

digestible AA formulation with the dLys ratio to other AA adjusted to a constant value. Synthetic AA as well as the dietary SBM inclusions were increased across experimental diets from 80 to 120% AA to increase the dietary AA density, whereas same energy level (isocaloric) was maintained between diets. CP and dLys intake were greater for broilers with the higher FI. Leeson et al. (1996) studied effects of dietary energy levels with isonitrogenous diets and found that broilers tend to regulate their energy intake by consuming more feed with the lower energy level diets. Dietary energy helped regulate FI in the current study in HT because FI and energy intake were not different ($P > 0.05$) for treatment diets for both broiler lines. Broilers reared in CT had higher FI for lower AA level diets and higher energy intake was observed. The higher FI in CT for lower AA level treatment diets (<100% AA level) could be driven by requirement to meet the digestible AA needs. Research findings showed that a deficiency of essential AA would increase FI in broilers (Steinrueck et al., 1990; Picard et al., 1993; Carew et al., 2003; Jahanian and Khalifeh-Gholi, 2018).

Past studies conducted with broiler strains fed various dietary AA or CP levels showed that feed consumption and feed efficiency are strain specific (Morris et al., 1987; Leclercq and Guy, 1991; Alleman et al., 2000). Smith and Pesti (1998) compared a high yielding and a fast-growing line fed 3 levels of CP and found line, protein level, and line x protein level differences in performance. High yielding line responded better (than fast-growing strain) to the increased dietary CP in BW and FCR. Smith and Pesti (1998) reported decreased abdominal fat in broilers fed diets containing increasing levels of CP, which was consistent to current study findings where reduced fat mass for broilers fed increasing AA levels were observed. Both lines in the current trial

Table 4. Body weight (BW) and FCR per bird of 2 broiler lines fed 5 levels of digestible amino acid (AA). Two repeated trials were conducted—one in hot environmental temperature (HT) and another in cool environmental temperature (CT).¹

| Line | Diets | Hot environmental temperature | | | | | | | | Cool environmental temperature | | | | | | | |
|------------------------------|-------|-------------------------------|--------|--------------------|----------------------|---------------------|-------------------|-----------|----------|--------------------------------|------------------|--------------------|--------------------|------------------|-------------------|-------------------|----------|
| | | BW | | | | ADG | FCR | | | BW | | | | ADG | FCR | | |
| | | Day 10 | Day 21 | Day 36 | Day 42 | Day 22–42 | Day 0–21 | Day 22–42 | Day 0–42 | Day 10 | Day 21 | Day 35 | Day 42 | Day 21–42 | Day 0–21 | Day 22–42 | Day 0–42 |
| | g | g | g | G | G | g:g | g:g | g/b | g | g | g | g | g | g:g | g:g | g/b | |
| A | C | 290 | 1,040 | 2,340 ^b | 2,740 ^b | 81 ^b | 1.39 ^a | 1.96 | 1.94 | 230 ^b | 900 ^b | 2,320 ^b | 3,180 ^b | 109 ^b | 1.42 ^a | 1.65 ^b | 1.59 |
| B | C | 290 | 1,030 | 2,400 ^a | 2,890 ^a | 90 ^a | 1.34 ^b | 1.95 | 1.92 | 250 ^a | 980 ^a | 2,440 ^a | 3,350 ^a | 113 ^a | 1.36 ^b | 1.69 ^a | 1.59 |
| <i>P</i> value | | 0.2022 | 0.5928 | 0.0032 | 0.0022 | <0.0001 | <0.0001 | 0.5603 | 0.6799 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.8712 |
| A | A-1 | 300 | 1,030 | 2,250 ^b | 2,620 ^c | 75 ^c | 1.39 | 2.13 | 2.08 | 230 | 900 | 2,260 ^c | 3,130 | 106 ^b | 1.42 | 1.79 | 1.68 |
| | A-2 | 290 | 1,010 | 2,240 ^b | 2,670 ^{b,c} | 78 ^{b,c} | 1.4 | 2.05 | 1.99 | 230 | 890 | 2,310 ^b | 3,160 | 108 ^b | 1.42 | 1.7 | 1.62 |
| | A-3 | 290 | 1,050 | 2,410 ^a | 2,750 ^b | 81 ^{a,b,c} | 1.38 | 1.98 | 1.99 | 230 | 910 | 2,360 ^a | 3,230 | 111 ^a | 1.42 | 1.61 | 1.56 |
| | A-4 | 290 | 1,030 | 2,370 ^a | 2,830 ^a | 86 ^a | 1.38 | 1.84 | 1.83 | 230 | 910 | 2,350 ^a | 3,190 | 109 ^b | 1.41 | 1.6 | 1.54 |
| | A-5 | 290 | 1,050 | 2,420 ^a | 2,850 ^a | 84 ^a | 1.39 | 1.79 | 1.8 | 220 | 890 | 2,340 ^a | 3,200 | 110 ^b | 1.41 | 1.57 | 1.53 |
| B | B-1 | 290 | 1,060 | 2,320 ^b | 2,780 ^c | 82 ^c | 1.33 | 2.13 | 2.06 | 250 | 970 | 2,310 ^b | 3,180 ^b | 105 ^b | 1.35 | 1.85 | 1.69 |
| | B-2 | 290 | 1,020 | 2,400 ^b | 2,820 ^{b,c} | 88 ^{b,c} | 1.36 | 2.03 | 1.96 | 250 | 970 | 2,430 ^a | 3,340 ^a | 113 ^a | 1.36 | 1.72 | 1.61 |
| | B-3 | 290 | 1,040 | 2,420 ^a | 2,870 ^b | 88 ^{b,c} | 1.31 | 1.93 | 1.94 | 260 | 980 | 2,500 ^a | 3,410 ^a | 116 ^a | 1.35 | 1.63 | 1.55 |
| | B-4 | 280 | 1,020 | 2,440 ^a | 3,000 ^a | 95 ^a | 1.35 | 1.84 | 1.83 | 260 | 980 | 2,480 ^a | 3,390 ^a | 115 ^a | 1.36 | 1.64 | 1.55 |
| | B-5 | 290 | 1,010 | 2,430 ^a | 3,000 ^a | 94 ^a | 1.34 | 1.83 | 1.83 | 250 | 980 | 2,500 ^a | 3,410 ^a | 116 ^a | 1.36 | 1.59 | 1.52 |
| SEM | | 5 | 16 | 29 | 64 | 2.7 | 0.01 | 0.03 | 0.05 | 3.5 | 9.361 | 16.7 | 22.9 | 0.9 | 0.0058 | 0.008 | 0.0063 |
| ² <i>P</i> -value | | 0.2817 | 0.3450 | <0.0001 | 0.0011 | 0.0002 | 0.1430 | <0.0001 | <0.0001 | 0.8754 | 0.4635 | 0.0096 | 0.0150 | 0.0022 | 0.3909 | <0.0001 | <0.0001 |

Abbreviations: ADG, average daily gain; BW, body weight; dLys, digestible lysine; FCR, feed conversion ratio.

¹Diets A1–A5 or B1–B5 for Line A or Line B represent 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. C represents combined analysis of all AA levels. Different letters in superscripts represent significantly different dietary AA levels within line in each column.²*P*-values presented are for combined lines for dietary AA levels. No diet by line interaction was observed.

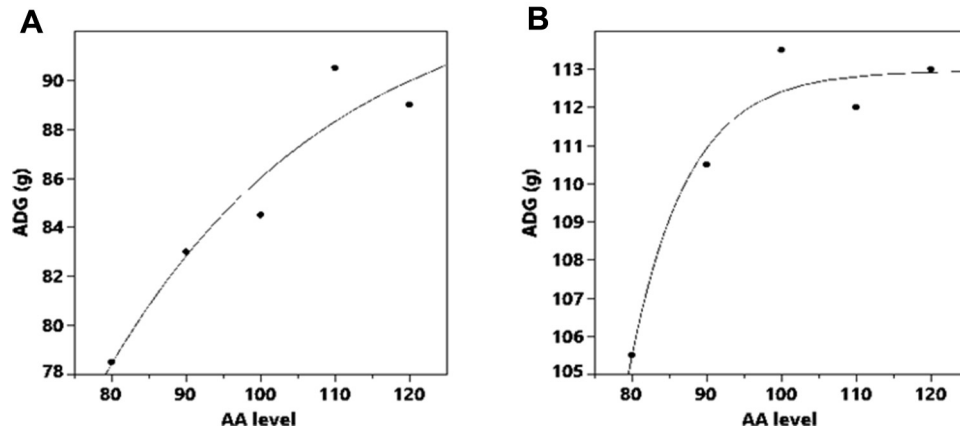


Figure 2. Effects of digestible amino acid (AA) levels on average daily gain (ADG) for trials conducted in hot environmental temperature (HT) and cool environmental temperature (CT). Analysis was performed for combined lines and were fit using second degree polynomial regression. (A) HT, $y = -0.0054x^2 + 1.3564x + 4.1$; $R^2 = 0.91$. (B) CT, $y = -0.0089x^2 + 1.9507x + 6.9$; $R^2 = 0.92$. *80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. Abbreviation: dLys, digestible lysine.

were fast growing (Table 5); this might explain why there were no line \times diet interaction effects. FI was higher in Line B than Line A in HT and CT which indicated the feed consumption could be strain specific.

The effects of AA level on ADG for combined lines followed quadratic fit where maximum ADG differences between dietary treatments were 11.05 g in HT and 7.79 g in CT observed at 120% AA level and 110% AA level, respectively. There was a continual improvement with FCR points for fitted curve from 80 to 120% AA levels in both HT and CT. The optimal response values for ADG in HT and CT were 89.72 g and 113.44 g occurring at 120 and 109.5% AA level, respectively. The optimal response values for FCR in HT and CT were 1.79 and 1.58 occurring at 120 and 117.5% AA level, respectively. The breast and tender meat yields were higher with increasing AA level and followed quadratic fit. The optimal response values for breast yield in HT and CT were 575.9 g and 776.5 g occurring at 112.6 and

114.5% AA level, respectively. The optimal response values for tender yield in HT and CT were 119.8 g and 154.9 g occurring at 120 and 115% AA level, respectively. Protein accretion increased as dietary AA level increased as indicated by the increased 42 d breast and tenders meat yields and BC results (Figure 4, Table 5, and Table 6). Protein turnover measurements in *Pectoralis major* were also conducted in the current trial where the results are reported in Maharjan et al. (2020b). The optimal turnover of mixed muscle protein was occurring at AA level between 100 and 110% for both lines in both environmental temperatures. Reduced fractional synthesis and degradation rates of mixed muscle were observed in broilers housed in HT compared with fractional synthesis and degradation rates of broilers housed in CT. Line A had a higher % yield of LW for breasts and tenders for both environmental temperatures which were correlated with BC data with higher % protein mass of total body mass or relatively higher protein retention

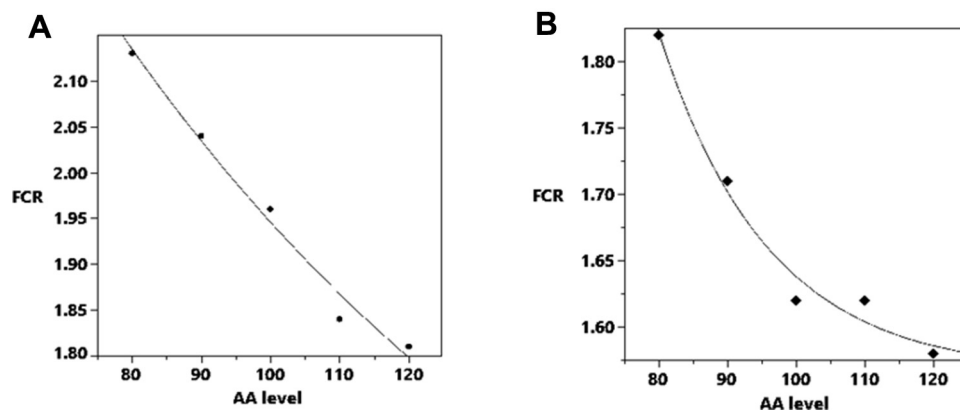


Figure 3. Effects of digestible amino acid (AA) levels on 22 to 42 d FCR for trials conducted in hot environmental temperature (HT) and cool environmental temperature (CT). Analysis was performed for combined lines and were fit using second degree polynomial regression. (A) HT: $0.000057x^2 - 0.0198x + 3.356$; $R^2 = 0.98$. (B) CT: $0.00016x^2 - 0.038x + 3.8$; $R^2 = 0.97$. *80, 90, 100, 110 and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48 and 3.80 g dLys/Mcal. Abbreviation: dLys, digestible lysine.

Table 5. Forty-two days ready-to-cook and fat pad yield in percent of live weight (LW) of 2 broiler lines fed 5 digestible amino acids (AA) levels. Two repeated trials were conducted—1 in hot environmental temperature (HT) and another in cool environmental temperature (CT).¹

| Line | Diet | Hot environmental temperature | | | | | | Cool environmental temperature | | | | | |
|------------------------------|------|-------------------------------|-------------------|--------------------|---------------------|--------------------|---------------------|--------------------------------|------------|--------------------|--------------------|--------------------|------------------|
| | | LW g | Wings % | Breast % | Tenders % | Leg quarters % | Fat pad % | LW G | Wings % | Breast % | Tenders % | Leg quarters % | Fat pad % |
| A | C | 2,771 ^b | 7.71 ^a | 19.61 ^a | 4.04 ^a | 23.44 ^b | 1.82 | 3,451 ^b | 7.62 | 21.27 ^a | 4.25 | 21.6 ^b | 1.7 ^a |
| B | C | 2,952 ^a | 7.56 ^b | 18.89 ^b | 3.97 ^b | 23.84 ^a | 1.85 | 3,647 ^a | 7.62 | 20.87 ^b | 4.18 | 22.35 ^a | 1.5 ^b |
| <i>P</i> -value | | <0.0001 | 0.0013 | <0.0001 | 0.0084 | 0.0002 | 0.4905 | <0.0001 | 0.9834 | 0.0024 | 0.0205 | <0.0001 | <0.0001 |
| A | A-1 | 2,665 ^d | 7.8 | 18.66 ^b | 3.93 ^b | 23.35 | 2.17 ^a | 3,384 ^c | 7.61 | 20.44 ^b | 4.12 ^b | 21.53 | 2.2 |
| | A-2 | 2,729 ^c | 7.82 | 19.71 ^a | 4.01 ^{a,b} | 23.34 | 1.93 ^{a,b} | 3,444 ^{b,c} | 7.55 | 20.98 ^b | 4.2 ^{a,b} | 21.67 | 1.9 |
| | A-3 | 2,750 ^{b,c} | 7.7 | 19.93 ^a | 4.1 ^a | 23.18 | 1.9 ^b | 3,494 ^b | 7.63 | 21.52 ^a | 4.27 ^a | 21.46 | 1.7 |
| | A-4 | 2,866 ^a | 7.59 | 19.88 ^a | 4.03 ^{a,b} | 23.73 | 1.57 ^c | 3,442 ^{b,c} | 7.72 | 21.68 ^a | 4.33 ^a | 21.54 | 1.5 |
| | A-5 | 2,845 ^{a,b} | 7.65 | 19.89 ^a | 4.12 ^a | 23.59 | 1.53 ^c | 3,492 ^b | 7.59 | 21.7 ^a | 4.32 ^a | 21.8 | 1.4 |
| B | B-1 | 2,821 ^d | 7.67 | 17.93 ^b | 3.85 ^b | 23.84 | 2.11 ^a | 3,474 ^b | 7.66 | 19.99 ^b | 4 ^b | 22.22 | 2 |
| | B-2 | 2,953 ^c | 7.54 | 19.03 ^a | 3.99 ^a | 23.72 | 1.99 ^{a,b} | 3,627 ^a | 7.66 | 20.5 ^b | 4.19 ^a | 22.63 | 1.7 |
| | B-3 | 2,931 ^{b,c} | 7.53 | 19.01 ^a | 3.97 ^a | 23.88 | 1.88 ^b | 3,698 ^a | 7.55 | 21.19 ^a | 4.16 ^a | 22.07 | 1.5 |
| | B-4 | 3,046 ^a | 7.51 | 19.45 ^a | 4.04 ^a | 23.66 | 1.69 ^c | 3,728 ^a | 7.56 | 21.21 ^a | 4.24 ^a | 22.55 | 1.3 |
| | B-5 | 3,008 ^{a,b} | 7.55 | 19.05 ^a | 3.99 ^a | 24.13 | 1.56 ^c | 3,709 ^a | 7.67 | 21.46 ^a | 4.31 ^a | 22.29 | 1.2 |
| | SEM | 30.8 | 0.07 | 0.21 | 0.04 | 0.17 | 0.07 | 18.23 | 0.05 | 0.19 | 0.05 | 0.16 | 0.05 |
| ² <i>P</i> -value | | <0.0001 | 0.0807 | <0.0001 | 0.0019 | 0.2534 | <0.0001 | <0.0001 | 0.0560 | <0.0001 | <0.0001 | 0.1328 | <0.0001 |

Abbreviation: dLys, digestible lysine.

¹Diets A1–A5 or B1–B5 for Line A or Line B represent 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. C represents combined analysis of all AA levels. Different letters in superscripts represent significantly different means between dietary AA levels within line in each column.²*P*-values presented are for combined lines for dietary AA levels. No diet by line interaction was observed.

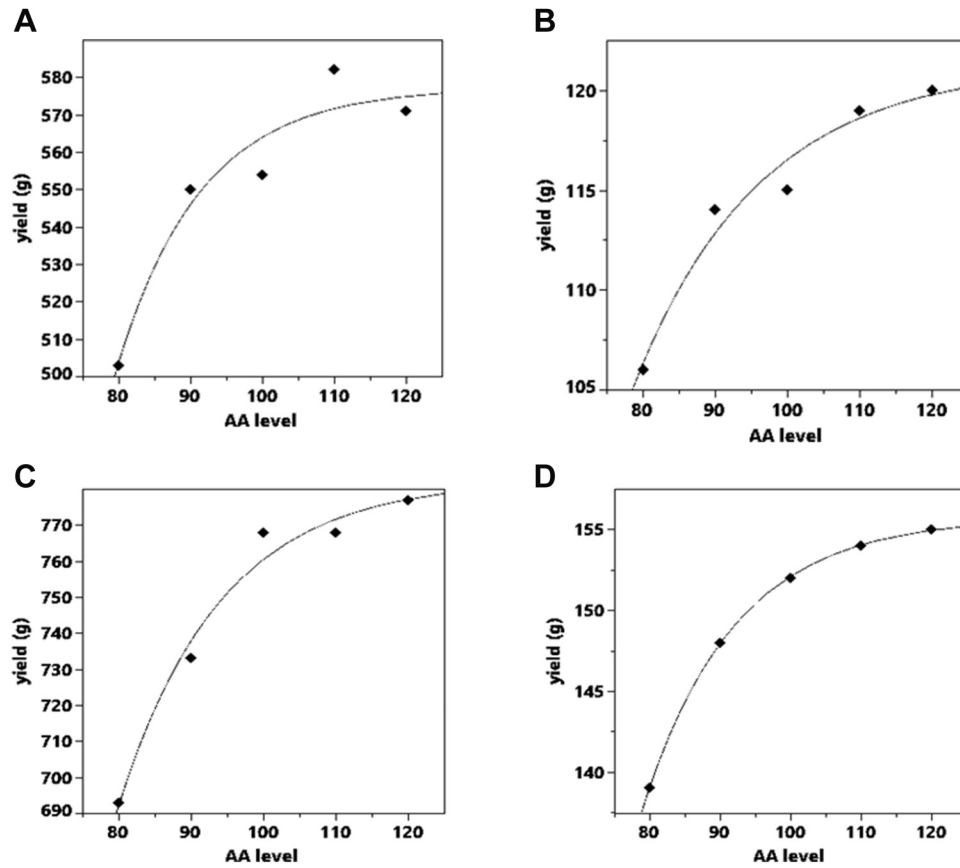


Figure 4. Effects of digestible amino acid (AA) levels on breast yield and tender yield for trials conducted in hot environmental temperature (HT) and cool environmental temperature (CT) were predicted for combined lines using second degree polynomial regression. (A) HT: $y(\text{breast}) = -0.0657x^2 + 14.823x - 260$; $R^2 = 0.93$ and (B) HT: $y(\text{tender}) = -0.0079x^2 + 1.9014x + 4.8$; $R^2 = 0.96$. (C) CT: $y(\text{breast}) = -0.0693x^2 + 15.887x - 134.2$; $R^2 = 0.98$ and (D) CT: $y(\text{tender}) = -0.0129x^2 + 2.9514x - 14.4$; $R^2 = 0.99$. *80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. Abbreviation: dLys, digestible lysine.

Table 6. Body composition as percent of total body mass of 2 broiler lines reared from 22 to 42 d of age on 5 digestible amino acid (AA) levels. Two repeated trials were conducted—one in hot environmental temperature (HT) and another in cool environmental temperature (CT).¹

| Line | Diet | Hot environmental temperature | | | | | Cool environmental temperature | | | | |
|------|------------------------------|-------------------------------|---------------------|---------------------|---------------------|----------------------|--------------------------------|--------------------|--------------------|--------------------|-------------------|
| | | Total body mass | Lean mass | Protein mass | Fat Mass | Energy | Total body mass | Lean mass | Protein mass | Fat mass | Energy |
| | | g | % | % | % | kcal/g | g | % | % | % | kcal/g |
| A | C | 940 ^a | 83.4 | 15.2 ^a | 8.9 ^a | 1.854 | 904 ^b | 87.78 ^a | 16.39 ^a | 8.76 ^b | 1.69 |
| B | C | 906 ^b | 83.7 | 15.1 ^b | 8.6 ^b | 1.853 | 987 ^a | 86.91 ^b | 16.32 ^b | 9.43 ^a | 1.73 |
| | <i>P</i> -value | <0.0001 | 0.7810 | 0.0362 | 0.0098 | 0.7274 | <0.0001 | 0.0019 | 0.0002 | 0.0015 | 0.0047 |
| A | A-1 | 2,564 ^c | 81.6 ^c | 16.7 ^d | 13.6 ^a | 1.868 ^a | 3,260 ^b | 80.83 ^c | 16.49 ^c | 12.77 ^a | 2 ^a |
| | A-2 | 2,626 ^b | 83.1 ^b | 17 ^d | 12.8 ^b | 1.856 ^b | 3,353 ^a | 82.52 ^b | 16.89 ^b | 11.86 ^b | 1.96 ^b |
| | A-3 | 2,718 ^a | 83.5 ^{a,b} | 17.1 ^b | 12.4 ^c | 1.853 ^{b,c} | 3,380 ^a | 83.23 ^b | 17.06 ^b | 11.48 ^b | 1.94 ^b |
| | A-4 | 2,768 ^a | 84.2 ^a | 17.4 ^{a,b} | 11.6 ^{c,d} | 1.848 ^c | 3,298 ^a | 85.17 ^a | 17.46 ^a | 10.44 ^c | 1.89 ^c |
| | A-5 | 2,805 ^a | 85 ^a | 17.6 ^a | 11.2 ^d | 1.844 ^c | 3,370 ^a | 84.91 ^a | 17.43 ^a | 10.58 ^c | 1.9 ^c |
| B | B-1 | 2,752 ^c | 81.5 ^c | 16.8 ^c | 13.3 ^a | 1.868 ^a | 3,339 ^b | 81.04 ^c | 16.56 ^c | 12.64 ^a | 2 ^a |
| | B-2 | 2,849 ^b | 82.6 ^b | 17.1 ^b | 12.5 ^b | 1.859 ^b | 3,484 ^a | 83.96 ^b | 17.26 ^b | 11.08 ^b | 1.93 ^b |
| | B-3 | 2,832 ^b | 84.5 ^a | 17.5 ^a | 11.4 ^c | 1.845 ^c | 3,485 ^a | 84.52 ^b | 17.38 ^b | 10.78 ^b | 1.91 ^b |
| | B-4 | 2,978 ^a | 84 ^a | 17.4 ^{a,b} | 11.4 ^c | 1.848 ^c | 3,512 ^a | 86.07 ^a | 17.73 ^a | 9.94 ^c | 1.87 ^c |
| | B-5 | 2,958 ^a | 84.4 ^a | 17.6 ^a | 11.1 ^c | 1.846 ^c | 3,517 ^a | 86.4 ^a | 17.81 ^a | 9.76 ^c | 1.86 ^c |
| | SEM | 26.193 | 0.44 | 0.08 | 0.22 | 0.0033 | 31.6 | 0.53 | 0.12 | 0.29 | 0.0138 |
| | ² <i>P</i> -value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Abbreviation: dLys, digestible lysine.

¹Diets A1–A5 or B1–B5 for Line A or Line B represent 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. C represents combined analysis of all AA levels. Different letters in superscripts represent significantly different means between dietary AA levels within line in each column.

²*P*-values presented are for combined lines for dietary AA levels. No diet by line interaction was observed.

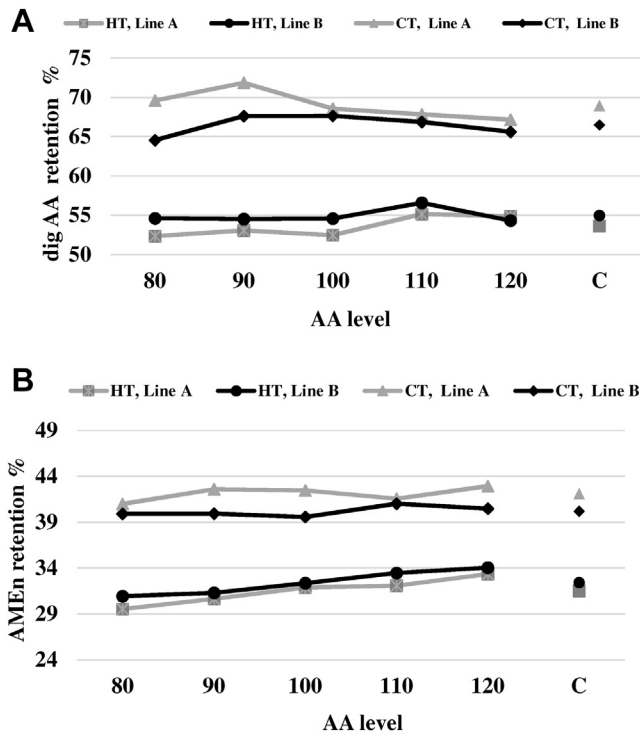


Figure 5. Effects of digestible amino acid (AA) levels on (A) digestible AA retention % and (B) metabolizable (AMEn) % for trials conducted in hot environmental temperature (HT) and cool environmental temperature (CT). Letter 'C' in the X-axis represents effects of combined AA levels. Higher digestible AA retention % was observed for CT compared with HT ($P < 0.05$) for both lines. Higher digestible AA retention % and AMEn % for Line A in CT ($P < 0.05$) compared with Line B was observed. 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. Abbreviation: dLys, digestible lysine.

(PR) %. Fat pad % yield of LW decreased linearly to ~ 0.15 for every 10% increase in dietary AA level. The performance and processing yield results were coherent with past research. Oliveira et al. (2013) reported a linear increase for BW, feed conversion, and protein accretion, and decrease of fat accretion as dietary lysine levels increased in broilers. Vieira and Angel (2012) stated that the modern high yielding broiler was especially responsive to AA density, particularly lysine. Kidd et al. (2004) observed improved live performances and higher carcass yield in broilers fed with higher AA density diets.

A broilers' response to high ambient temperatures during grow-out could be affected by factors such as age, BW, BC, humidity, genetics, and dietary ingredients. There is disagreement with regard to the negative effects of feeding additional CP or digestible AA to compensate for reduced feed intake for broilers housed in hot temperatures. (Gonzalez-Esquerria and Leeson, 2006). There are reports that recommend feeding low protein diets in hot weather with the objective of reducing further increments in heat production (Waldroup, 1982; Teeter and Belay, 1996; Cheng et al., 1997). Reports have also shown increased weight gain, feed efficiency, and processing yield in finishing broilers fed high protein diets when ambient grow-out temperatures were high ($>86^\circ\text{F}$) (Fuller and Mora, 1973; Dale and Fuller, 1979; Smith and Teeter, 1987; Beghal and

Pradhan, 1989; Cahaner et al., 1995; Alleman and Leclerq, 1997); and Temim et al., 1999. Performance results could be affected by dynamics of grow-out ambient temperature, chronic consistent, or chronic cyclic (present study) type of heat exposure. Results from the current study support feeding increasing levels of digestible AA to improve performance of broilers housed in hot temperatures. Both lines (Line A and Line B) improved BW gain, feed conversion, and carcass yield with increasing AA level in HT. Awad et al. (2019) reported that modern broilers housed in hot temperatures fed with reduced CP or AA diet had reduced gain and poorer FCR. Precise etiology for impaired growth performance in birds fed low CP diets in chronic heat stress is unclear. Lowered FI in higher grow-out environmental temperature accompanied by lowered protein content in diet could lead to physiological AA deficiency affecting performance (Furlan et al., 2004). Ojano-Dirain and Waldroup (2002) suggested feeding balanced protein with AA supplementation for improving performance of broilers housed at warm temperature. However, more investigations are sought to understand impact of feeding more AA and dietary CP to broilers housed in HT taking into consideration factors such as humidity, ventilation rate, bird behavior, weight of birds, and constant or cycling grow-out temperatures.

There is reduced protein synthesis (Maharjan et al., 2020b) in heat-stressed broilers, because of downregulation of IGF1-mTOR pathway and changes in mRNA expression of AA transporters in intestine (Habashy et al., 2017; Ma et al., 2018). Glucocorticoids such as corticosterone act as mediator of muscle protein catabolism, which tends to be elevated in heat-stressed broilers (Soleimani et al., 2011). Breast yield and AA DC values ($P < 0.05$) were lower for both broiler lines in HT (than in CT) in current study. Line A and Line B accumulated more fat in HT than in CT in current study. The increase of 0.8 and 1.6% in total carcass fat and abdominal fat, respectively, was reported for each degree increment in temperature from ~ 70 to 84°F (Howlider and Rose, 1987). Broilers housed in higher environmental temperatures have been reported to have an altered lipid metabolism with an increased expression of genes such as sterol-regulatory element-binding protein-1 (De Antonio et al., 2017). The mechanism regulating the insulin secretion during heat stress remains obscure; however, many studies have reported increased insulin level in blood of rodents, pigs, sheep, and broilers during chronic heat stress environment (Achmadi et al., 1993; Yuan et al., 2008; Morera et al., 2012; Pearce et al., 2012). Increased basal insulin level mediates decreased circulating glucose availability in blood plasma as body fuel. Heat-stressed animals could enter into a negative energy balance state coupled with reduced lipolytic activity and increased lipogenesis of adipose tissue (Morera et al., 2012).

Bornstein (1970) reported the dLys requirement to be ~ 3 g/Mcal for 5 to 8 wk finishing broiler. Hruby et al. (1995) determined dLys, digestible total sulfur AA, tryptophan, and threonine requirement per Mcal for 3 to 6 wk male broiler to be similar as compared with NRC values (NRC, 1994). The requirement prediction for

Table 7. % Standardized ileal digestible amino acids (“as is” basis) of experimental diets for trials conducted in hot environmental temperature (HT) and cool environmental temperature (CT).¹

| Amino acids | Lys | Met | Thr | Trp | Arg | Val | Leu | Ile | His | Phe | Cys | Gly | Ser | Pro | Ala | Asp | Glu |
|--------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|------|-------------------|-------------------|-------------------|------|-------------------|---------------------|
| Hot environmental temperature | | | | | | | | | | | | | | | | | |
| Line A | 1.06 | 0.48 | 0.70 | 0.20 | 1.05 | 0.89 | 1.76 | 0.79 | 0.43 | 0.90 | 0.26 | 0.66 | 0.78 | 1.20 | 1.00 | 1.52 | 3.20 |
| Line B | 1.07 | 0.48 | 0.71 | 0.21 | 1.06 | 0.89 | 1.76 | 0.80 | 0.43 | 0.90 | 0.26 | 0.66 | 0.78 | 1.20 | 1.00 | 1.53 | 3.21 |
| A-1 | 0.84 ^e | 0.35 ^e | 0.58 ^e | 0.17 ^d | 0.85 ^e | 0.77 ^e | 1.73 ^b | 0.67 ^c | 0.40 ^d | 0.84 ^c | 0.25 | 0.60 ^c | 0.73 ^d | 1.20 | 0.99 | 1.33 ^c | 2.99 ^c |
| A-2 | 0.96 ^d | 0.41 ^d | 0.64 ^d | 0.19 ^c | 0.93 ^d | 0.79 ^d | 1.86 ^a | 0.72 ^d | 0.41 ^c | 0.89 ^b | 0.26 | 0.62 ^c | 0.77 ^c | 1.23 | 1.05 | 1.38 ^d | 3.17 ^b |
| A-3 | 1.06 ^c | 0.45 ^c | 0.71 ^c | 0.24 ^b | 1.03 ^c | 0.88 ^c | 1.88 ^a | 0.80 ^b | 0.44 ^b | 0.94 ^a | 0.27 | 0.66 ^b | 0.80 ^b | 1.27 | 1.07 | 1.52 ^c | 3.31 ^a |
| A-4 | 1.18 ^b | 0.56 ^b | 0.76 ^b | 0.21 ^a | 1.18 ^b | 0.97 ^b | 1.66 ^c | 0.87 ^c | 0.45 ^a | 0.90 ^b | 0.27 | 0.70 ^a | 0.80 ^b | 1.16 | 0.95 | 1.63 ^b | 3.22 ^{a,b} |
| A-5 | 1.26 ^a | 0.62 ^a | 0.82 ^a | 0.21 ^a | 1.27 ^a | 1.05 ^a | 1.65 ^c | 0.90 ^a | 0.47 ^a | 0.92 ^{a,b} | 0.26 | 0.72 ^a | 0.81 ^a | 1.12 | 0.94 | 1.73 ^a | 3.29 ^a |
| B-1 | 0.84 ^e | 0.35 ^e | 0.58 ^e | 0.17 ^d | 0.84 ^e | 0.77 ^e | 1.74 ^b | 0.68 ^e | 0.40 ^d | 0.85 ^d | 0.25 | 0.60 ^c | 0.72 ^d | 1.21 | 0.99 | 1.32 ^e | 2.99 ^c |
| B-2 | 0.97 ^d | 0.42 ^d | 0.65 ^d | 0.19 ^c | 0.94 ^d | 0.79 ^d | 1.86 ^a | 0.72 ^d | 0.41 ^c | 0.90 ^c | 0.26 | 0.63 ^c | 0.77 ^c | 1.24 | 1.05 | 1.39 ^d | 3.19 ^b |
| B-3 | 1.05 ^c | 0.45 ^c | 0.70 ^c | 0.24 ^b | 1.03 ^c | 0.87 ^c | 1.87 ^a | 0.79 ^c | 0.43 ^b | 0.93 ^a | 0.26 | 0.65 ^b | 0.79 ^b | 1.26 | 1.05 | 1.50 ^c | 3.28 ^{a,b} |
| B-4 | 1.19 ^b | 0.56 ^b | 0.78 ^b | 0.21 ^a | 1.19 ^b | 0.98 ^b | 1.68 ^c | 0.88 ^b | 0.46 ^a | 0.91 ^{a,b} | 0.27 | 0.71 ^a | 0.80 ^b | 1.18 | 0.96 | 1.67 ^b | 3.27 ^{a,b} |
| B-5 | 1.28 ^a | 0.63 ^a | 0.84 ^a | 0.22 ^a | 1.28 ^a | 1.06 ^a | 1.66 ^c | 0.91 ^a | 0.47 ^a | 0.93 ^a | 0.26 | 0.72 ^a | 0.83 ^a | 1.14 | 0.95 | 1.76 ^a | 3.31 ^a |
| Cool environmental temperature | | | | | | | | | | | | | | | | | |
| Line A | 1.13 | 0.50 | 0.78 | 0.19 | 1.11 | 0.92 | 1.75 | 0.82 | 0.48 | 0.94 | 0.32 | 0.73 | 0.92 | 1.21 | 1.02 | 1.71 | 3.34 |
| Line B | 1.13 | 0.49 | 0.77 | 0.19 | 1.11 | 0.92 | 1.75 | 0.82 | 0.48 | 0.94 | 0.32 | 0.72 | 0.92 | 1.21 | 1.02 | 1.71 | 3.34 |
| A-1 | 0.94 ^e | 0.39 ^e | 0.67 ^e | 0.15 ^d | 0.91 ^e | 0.82 ^c | 1.93 ^a | 0.72 ^e | 0.43 ^d | 0.92 ^{b,c} | 0.33 | 0.65 ^d | 0.88 ^c | 1.30 ^b | 1.10 | 1.44 ^d | 3.23 ^b |
| A-2 | 1.03 ^d | 0.42 ^d | 0.72 ^d | 0.18 ^c | 0.99 ^d | 0.84 ^c | 1.86 ^b | 0.75 ^d | 0.46 ^c | 0.93 ^b | 0.32 | 0.68 ^c | 0.89 ^c | 1.25 ^a | 1.09 | 1.54 ^c | 3.29 ^b |
| A-3 | 1.12 ^c | 0.50 ^c | 0.80 ^b | 0.19 ^b | 1.16 ^c | 0.93 ^b | 1.81 ^c | 0.82 ^c | 0.50 ^a | 0.98 ^a | 0.32 | 0.75 ^b | 0.95 ^b | 1.25 ^b | 1.04 | 1.80 ^b | 3.46 ^a |
| A-4 | 1.21 ^b | 0.55 ^b | 0.82 ^c | 0.18 ^b | 1.20 ^b | 0.94 ^b | 1.56 ^e | 0.87 ^b | 0.48 ^b | 0.91 ^c | 0.31 | 0.76 ^a | 0.93 ^b | 1.11 ^d | 0.91 | 1.85 ^b | 3.27 ^b |
| A-5 | 1.33 ^a | 0.62 ^a | 0.88 ^a | 0.23 ^a | 1.29 ^a | 1.06 ^a | 1.62 ^d | 0.94 ^a | 0.51 ^a | 0.97 ^a | 0.33 | 0.79 ^a | 0.97 ^a | 1.14 ^c | 0.96 | 1.93 ^a | 3.45 ^a |
| B-1 | 0.93 ^e | 0.39 ^e | 0.67 ^e | 0.15 ^d | 0.90 ^e | 0.81 ^c | 1.91 ^a | 0.72 ^e | 0.43 ^d | 0.90 ^{b,c} | 0.32 | 0.64 ^d | 0.87 ^c | 1.29 ^a | 1.09 | 1.42 ^d | 3.21 ^b |
| B-2 | 1.03 ^d | 0.42 ^d | 0.72 ^d | 0.18 ^c | 1.00 ^d | 0.85 ^d | 1.87 ^b | 0.75 ^d | 0.46 ^c | 0.93 ^b | 0.32 | 0.68 ^c | 0.89 ^c | 1.26 ^a | 1.09 | 1.55 ^c | 3.30 ^b |
| B-3 | 1.12 ^c | 0.49 ^b | 0.78 ^c | 0.19 ^b | 1.15 ^c | 0.91 ^b | 1.80 ^c | 0.81 ^c | 0.50 ^a | 0.97 ^a | 0.32 | 0.74 ^b | 0.94 ^b | 1.23 ^b | 1.03 | 1.79 ^b | 3.44 ^a |
| B-4 | 1.22 ^b | 0.55 ^c | 0.82 ^b | 0.19 ^b | 1.20 ^b | 0.95 ^b | 1.56 ^e | 0.87 ^b | 0.48 ^b | 0.91 ^c | 0.31 | 0.76 ^a | 0.92 ^b | 1.11 ^d | 0.91 | 1.85 ^b | 3.29 ^b |
| B-5 | 1.33 ^a | 0.62 ^a | 0.89 ^a | 0.24 ^a | 1.29 ^a | 1.06 ^a | 1.63 ^d | 0.94 ^a | 0.51 ^a | 0.97 ^a | 0.33 | 0.79 ^a | 0.98 ^a | 1.14 ^c | 0.96 | 1.94 ^a | 3.46 ^a |

Abbreviation: dLys, digestible lysine.

¹Diets A1–A5 or B1–B5 for Line A or Line B represent 80, 90, 100, 110, and 120% AA levels equivalent to 2.53, 2.85, 3.17, 3.48, and 3.80 g dLys/Mcal. C represents combined analysis of all AA levels. Different letters in superscripts represent significantly different means between dietary AA levels within line in each column. The *P*-values for combined analysis between lines were not different (*P* > 0.05), whereas *P*-values between dietary treatments within line were < 0.0001 for amino acids analyzed in both seasons. No diet by line interaction was observed.

AA (g/Mcal) for broiler reared in hot grow-out environment (89.9 °F) and cool grow-out environment (69.9 °F) were not different (Hruby et al., 1995). The requirement of AA/Mcal in HT and CT was not different in the current study but higher than reported in Hruby et al. (1995). Similar results were reported with other studies where AA requirement for broiler remained constant irrespective of environmental temperature as long the protein/AA needs were fulfilled (Attia et al., 2006; Dagher, 2008). Diets with higher inclusion levels of ~1.1 to 1.2% for dLys showed optimal performance and yield in finishing broilers in the present study. Sharma et al. (2018) showed positive linear response in performance in 14 to 34 d broilers as dLys increased from 0.95 to 1.15%. Cerrate and Corzo (2019) has reported the dLys requirement in meat broilers increased by 0.009% per year. The update in requirement of digestible AA/Mcal in primary breeder has been suggested (Applegate and Angel, 2014; Aftab, 2019) because of the increased need for nutrients for the modern high yielding broiler. An increased requirement of digestible AA for the modern broiler is because primary breeders have genetically selected for increased breast meat yield (Dozier et al., 2008). Liu et al. (2019) reported the dLys requirement of 1.03% for optimal weight gain, and 1.22% for FCR at thermoneutral environment. Carre et al. (2014) modeled the dLys requirement for meat broilers as a function of lysine content of protein gain, body weight, daily gain, and total protein content. Rate of daily gain and body weight is continuously increasing

with the progress made in current broiler genetics, thus indicating the increasing need of dLys. An increased intake of digestible AA is producing mixed muscle fractional synthesis rates of ~20%/d (Maharjan et al., 2020b) in breast tissue compared with fractional synthesis rates of ~12%/d for broilers 2 decades ago (Tesseraud et al., 1996; Temim et al., 2000).

The overall findings in this study indicated that effects of increased digestible AA density on FI, performance, and processing yield are specific to strain and grow-out environmental temperature, but the optimal responses were attained for both lines with diets containing 110 to 120% AA levels (3.48–3.80 g dLys/Mcal). The findings of present study indicated an additional performance response could be obtained by increasing digestible AA to metabolizable energy ratio instead of decreasing digestible AA to metabolizable energy ratio for broilers housed in HT. It is imperative that more feeding experiments intended to understand the optimal requirement of digestible AA in broiler genotypes housed in varied environmental conditions be conducted to evaluate performance and yield characteristics while simultaneously accounting the economics involved in production inputs and output delivered.

ACKNOWLEDGMENTS

The authors acknowledge Evonik Operations GmbH for generous support by funding this project.

DISCLOSURES

No conflict of interest is reported in this study.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.09.019>.

REFERENCES

- Achmadi, J., T. Yanagisawa, H. Sano, and Y. Terashima. 1993. Pancreatic insulin secretory response and insulin action in heat-exposed sheep given a concentrate or roughage diet. *Domest. Anim. Endocrinol.* 10:279–287.
- Aftab, U. 2019. Energy and amino acid requirements of broiler chickens: keeping pace with the genetic progress. *Worlds Poult. Sci. J.* 75:507–514.
- Alleman, F., and B. Leclercq. 1997. Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *Br. Poult. Sci.* 38:607–610.
- Alleman, F., J. Michel, A. Chagneau, and B. Leclercq. 2000. The effects of dietary protein independent of essential amino acids on growth and body composition in genetically lean and fat chickens. *Br. Poult. Sci.* 41:214–218.
- Association of Official Analytical Chemists International (AOAC). 1990. Official Methods of Analysis, 934.01 for Dry Matter; 982.30 and 985.28 for Amino Acids. Association of Official Analytical Chemists, Arlington, VA.
- AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC.
- Applegate, T. J., and R. Angel. 2014. Nutrient requirements of poultry publication: History and need for an update. *J. Appl. Poult. Res.* 23:567–575.
- Attia, Y. A., B. M. Böhmer, and D. A. Roth-Maier. 2006. Responses of broiler chicks raised under constant relatively high ambient temperature to enzymes, amino acid supplementations, or a high-nutrient diet. *Arch.Geflügelk.* 70:80–91.
- Awad, E. A., I. Zulkifli, A. F. Soleimani, F. L. Law, S. K. Ramiah, I. M. Mohamed-Yousif, and E. S. Khalil. 2019. Response of broilers to reduced-protein diets under heat stress conditions. *World's Poult. Sci. J.* 75:583–598.
- Baghel, R. P. S., and K. Pradhan. 1989. Energy, protein and limiting amino acid requirements of broilers at very high ambient temperature. *Br. Poult. Sci.* 30:295–304.
- Blok, M., and R. Dekker. 2017. Table 'Standardized Ileal Digestibility of Amino Acids in Feedstuffs for Poultry'. Wageningen Livestock Research, Wageningen, The Netherlands.
- Bornstein, S. 1970. The lysine requirement of broilers during their finishing period. *Br. Poult. Sci.* 11:197–207.
- Caldas, J. V., K. Hilton, N. Boonsinchai, J. A. England, A. Mauromoustakos, and C. N. Coon. 2018. Dynamics of nutrient utilization, heat production, and body composition in broiler breeder hens during egg production. *Poult. Sci.* 97:2845–2853.
- Cahaner, A., Y. Pinchasov, I. Nir, and Z. Nitsan. 1995. Effects of dietary protein under high ambient temperature on body weight, breast meat yield, and abdominal fat deposition of broiler stocks differing in growth rate and fatness. *Poult. Sci.* 74:968–975.
- Carew, L. B., J. P. McMurtry, and F. A. Alster. 2003. Effects of methionine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors-I and-II, liver and body weights, and feed intake in growing chickens. *Poult. Sci.* 82:1932–1938.
- Carré, B., B. Veda, and H. Juin. 2014. Progress in broiler selection: benefits, limitations as assessed by the digestive function and consequence on dietary lysine concentration. Pages 189 in *Proc. XIV Eur. Poult. Conf.*, Stavanger, Norway, 23–27.
- Cerrate, S., and A. Corzo. 2019. Lysine and energy trends in feeding modern commercial broilers. *Int. J. Poult. Sci.* 18:28–38.
- Cheng, T. K., M. L. Hamre, and C. N. Coon. 1997. Responses of broilers to dietary protein levels and amino acid supplementation to low protein diets at various environmental temperatures. *J. Appl. Poult. Res.* 6:18–33.
- Classen, H. L. 2013. Response of broiler chickens to dietary energy and its relationship to amino acid nutrition. Pages 107–114 in *24th Annual Australian Poultry Science Symposium*, Sydney, New South Wales, Australia, February 17–20. Poultry Research Foundation.
- Daghir, N. 2008. Nutrient requirements of poultry at high temperature. Pages 60 in *Poultry Production Hot Climate*, 133.
- Dale, N., and H. Fuller. 1979. Effects of diet composition on feed intake and growth of chicks under heat stress: I. Dietary fat levels. *Poult. Sci.* 58:1529–1534.
- De Antonio, J., M. Fernandez-Alarcon, R. Lunedo, G. Squassoni, A. Ferraz, M. Macari, R. Furlan, and L. Furlan. 2017. Chronic heat stress and feed restriction affects carcass composition and the expression of genes involved in the control of fat deposition in broilers. *J. Agric. Sci.* 155:1487–1496.
- Dozier, III, W., M. Kidd, and A. Corzo. 2008. Dietary amino acid responses of broiler chickens. *J. Appl. Poult. Res.* 17:157–167.
- Fancher, B. I. 2014. What Is the Upper Limit to Commercially Relevant Body Weight in Modern Broilers. Aviagen, Huntsville.
- Fisher, C., and B. J. Wilson. 1974. Pages 151–184 in *Energy Requirements of Poultry*. T. R. Morris and B. M. Freeman, eds. British Poultry Science Ltd., Edinburgh.
- Fuller, H., and G. Mora. 1973. Effect of diet composition on heat increment, feed intake and growth of chicks subjected to heat stress. *Poult. Sci.* 52:2029.
- Furlan, R. L., D. de Faria Filho, P. Rosa, and M. Macari. 2004. Does low-protein diet improve broiler performance under heat stress conditions? *Braz. J. Poult. Sci.* 6:71–79.
- Gonzalez-Esquerra, R., and S. Leeson. 2006. Physiological and metabolic responses of broilers to heat stress-implications for protein and amino acid nutrition. *World's Poult. Sci. J.* 62:282–295.
- Habashy, W. S., M. C. Milfort, A. L. Fuller, Y. A. Attia, R. Rekeya, and S. E. Aggrey. 2017. Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. *Int. J. Biometeorol.* 61:2111–2118.
- Howliger, M. A. R., and S. P. Rose. 1987. Temperature and the growth of broilers. *World's Poult. Sci. J.* 43:228–237.
- Hruby, M., M. L. Hamre, and C. N. Coon. 1995. Predicting amino acid requirements for broilers at 21.1 C and 32.2 C. *J. Appl. Poult. Res.* 4:395–401.
- Kidd, M., C. McDaniel, S. Branton, E. Miller, B. Boren, and B. Fancher. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. *J. Appl. Poult. Res.* 13:593–604.
- Jahanian, R., and M. Khalifeh-Gholi. 2018. Marginal deficiencies of dietary arginine and methionine could suppress growth performance and immunological responses in broiler chickens. *J. Ani. Physiol. Ani. Nutr.* 102:11–20.
- Leclercq, B., and G. Guy. 1991. Further investigations on protein requirement of genetically lean and fat chickens. *Br. Poult. Sci.* 32:789–798.
- Leeson, S. 2013. Editorial contemporary issues: future considerations in poultry nutrition. *Poult. Sci.* 91:1281–1285.
- Leeson, S., L. Caston, and J. Summers. 1996. Broiler response to energy or energy and protein dilution in the finisher diet. *Poult. Sci.* 75:522–528.
- Lemme, A. 2005. Optimum dietary amino acid level for broiler chicken. Pages 117–144 in *Simposio Internacional Sobre Exigencias Nutricionais De Aves E Suinos*, 2.
- Liu, S. Y., S. J. Rochell, C. W. Maynard, J. Caldas, and M. T. Kidd. 2019. Digestible lysine concentrations and amino acid densities influence growth performance and carcass traits in broiler chickens from 14 to 35 days post-hatch. *Anim. Feed Sci. Technol.* 255:114216.
- Ma, B., X. He, Z. Lu, L. Zhang, J. Li, Y. Jiang, G. Zhou, and F. Gao. 2018. Chronic heat stress affects muscle hypertrophy, muscle protein synthesis and uptake of amino acid in broilers via insulin like growth factor-mammalian target of rapamycin signal pathway. *Poult. Sci.* 97:4150–4158.
- Maharjan, P., M. Mayorga, K. Hilton, J. Weil, A. Beitia, J. Caldas, and C. Coon. 2019a. Non-cellulosic polysaccharide content in feed ingredients and ileal and total tract non-cellulosic polysaccharide digestibility in 21-and 42-day-old

- broilers fed diets with and without added composite enzymes. *Poult. Sci.* 98:4048–4057.
- Maharjan, P. G. Mullenix, K. Hilton, J. Caldas, V. D. Naranjo, and C. Coon. 2020b. Effects of dietary amino acid levels and ambient temperature on mixed muscle protein turnover in *Pectoralis major* during finisher feeding period in two broiler lines. *J. Ani. Physiol. Ani. Nutr.* In press.
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183.
- Morera, P., L. Basirico, K. Hosoda, and U. Bernabucci. 2012. Chronic heat stress up-regulates leptin and adiponectin secretion and expression and improves leptin, adiponectin and insulin sensitivity in mice. *J. Mol. Endocrinol.* 48:129–138.
- Morris, T., K. Al-Azzawi, R. Gous, and G. L. Simpson. 1987. Effects of protein concentration on responses to dietary lysine by chicks. *Br. Poult. Sci.* 28:185–195.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Ojano-Dirain, C., and P. Waldroup. 2002. Protein and amino acid needs of broilers in warm weather: a review. *Int. J. Poult. Sci.* 1:40–46.
- Oliveira, W. P. d., Rita Oliveira, Flávia Miranda de, J. L. Donzele, L. F. T. Albino, Campos, Paulo Henrique Reis Furtado, E. M. Balbino, Maia Ana Paula de Assis, and S. M. Pastore. 2013. Lysine levels in diets for broilers from 8 to 21 days of age. *Revista Brasileira de Zootecnia* 42:869–878.
- Pearce, S., V. Mani, R. Boddicker, J. Johnson, T. Weber, J. Ross, L. Baumgard, and N. Gabler. 2012. Heat stress reduces barrier function and alters intestinal metabolism in growing pigs. *J. Anim. Sci.* 90:257–259.
- Picard, M. L., G. Uzu, E. A. Dunnington, and P. B. Siegel. 1993. Food intake adjustments of chicks: short-term reactions to deficiencies in lysine, methionine and tryptophan. *Br. Poult. Sci.* 34:737–746.
- Sharma, N. K., M. Choct, M. Toghyani, Y. C. Laurenson, C. K. Girish, and R. A. Swick. 2018. Dietary energy, digestible lysine, and available phosphorus levels affect growth performance, carcass traits, and amino acid digestibility of broilers. *Poult. Sci.* 97:1189–1198.
- Smith, E. R., and G. M. Pesti. 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. *Poult. Sci.* 77:276–281.
- Smith, M., and R. Teeter. 1987. Influence of feed intake and ambient temperature stress on the relative yield of broiler parts. *Nutr. Rep. Int.* 35:299–306.
- Soleimani, A., I. Zulkifli, A. Omar, and A. Raha. 2011. Physiological responses of 3 chicken breeds to acute heat stress. *Poult. Sci.* 90:1435–1440.
- Steinruck, U., F. X. Roth, and M. Kirchgesner. 1990. Selective feed intake of broilers during methionine deficiency. *Archiv für Geflügelkunde* 54:173–183.
- Vieira, S., and C. Angel. 2012. Optimizing broiler performance using different amino acid density diets: what are the limits? *J. Appl. Poult. Res.* 21:149–155.
- Teeter, R. G., and T. Belay. 1996. Broiler management during acute heat stress. *Feed. Sci. Technol.* 58:127–142.
- Temim, S., A. M. Chagneau, S. Guillaumin, J. Michel, R. Peresson, P. A. Geraert, and S. Tesseraud. 1999. Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. *Reprod. Nutr. Dev.* 39:145–156.
- Temim, S., A. Chagneau, R. Peresson, and S. Tesseraud. 2000. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens fed 20 or 25% protein diets. *J. Nutr.* 130:813–819.
- Tesseraud, S., R. Peresson, and A. Chagneau. 1996. Age-related changes of protein turnover in specific tissues of the chick. *Poult. Sci.* 75:627–631.
- Waldroup, P. W. 1982. Influence of environmental temperature on protein and amino acid needs of poultry. *Fed. Proc.* 41:2821–2823.
- Yuan, L., H. Lin, K. Jiang, H. Jiao, and Z. Song. 2008. Corticosterone administration and high-energy feed results in enhanced fat accumulation and insulin resistance in broiler chickens. *Br. Poult. Sci.* 49:487–495.