Effects of dietary lysine levels on carcass performance and biochemical characteristics of Chinese local broilers

Yuncong Yuan,1 Xiaoling Zhao,2 Qing Zhu,1 Juan Li,3 Huadong Yin,1 Elizabeth R. Gilbert,4 Yao Zhang,1 Yiping Liu,1 Yan Wang,1 Diyan Li,1 Zhiqing Yang,1 Gang Shu5
1Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Sichuan Agricultural University, Cheng’du, Sichuan Province, China
2Department of Animal Science, Sichuan Agricultural University, Cheng’du, Sichuan Province, China
3Research Institute of Raising Livestock, Chengdu Academy of Agriculture and Forestry Sciences, Cheng’du, Sichuan Province, China
4Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
5Department of Pharmacy, Sichuan Agricultural University, Ya’an, Sichuan Province, China

Abstract

Lysine is typically the second-limiting amino acid in poultry diets. The objective of this study was to evaluate the effects of dietary lysine concentration on carcass and meat quality traits, and serum parameters in two lines – SD02 and SD03 – which originated from a Chinese local breed, the Erlang Mountainous chicken. Live body weight, carcass traits, meat quality traits (myofibre diameter and density), and serum metabolic markers were measured in high and low dietary lysine groups (HL and LL, respectively) at the end of the starter (1-28 days), grower (29-49 days) and finisher (50-70 days) periods. The results showed that mortality, live weight (LW), myofibre diameter of leg muscle (LFDM) and serum cholesterol (CHO) were greater in HL than LL (P<0.05). The chickens from HL had reduced subcutaneous fat thickness and heart weight than LL (P<0.05). The chickens from line SD02 had greater leg muscle weight, myofibre diameter in breast, and LFDM than line SD03 (P<0.05). The chickens from line SD02 had more serum urea nitrogen and less total proteins than line SD03 (P<0.05). In conclusion, high lysine diets improved slaughter performance and muscle fibre diameter, and SD02 chickens had greater carcass yield and superior meat quality compared with chickens from line SD03.

Introduction

Lysine is typically the second-limiting amino acid in poultry diets and the reference amino acid for the ideal protein (Baker, 1994). A method for evaluating amino acid requirements is to delineate lysine requirements and calculate other amino acid requirements as a ratio to lysine (Baker et al., 2002). Dietary lysine levels affect growth performance and carcass quality of growing chickens (Han and Baker, 1994; Kidd et al., 1998; Sterling et al., 2006). However, most studies focused on commercial broiler breeds (Dozier et al., 2008; Kiess et al., 2013; Plumstead et al., 2007). There are few reports of the dietary amino acid requirements of diverse local breeds around the world (Minh and Ogle, 2005). Although local breeds tend to display slower growth rates and less muscle yield as compared to commercial breeds, they display superior adaption to the local environment and have greater meat quality, which caters to the meat quality preferences of the niche market. For protecting and utilizing these breeds, a local population of the Erlang Mountainous (EM) chicken from Southwest China were used in the present study, both of which were described by Zhao et al. (2012). We explore the interaction between dietary lysine levels and genetics on carcass performance and biochemical characteristics, in order to determine an appropriate concentration of dietary lysine for improving performance without sacrificing meat quality.

Materials and methods

Chicken experiments were conducted in accordance with the National Regulations for the Administration of Affairs Concerning Experimental Animals (State Scientific and Technological Commission, P. R. China, 1988).

Populations and management

Based on a preliminary dose titration study (data not shown) and the nutrient requirements of Chinese yellow-feather chickens (NY/T33-2004), we used two levels of dietary lysine (HL and LL) for the current study. A total of 960 1-d-old female (480 each of line SD02 and SD03) EM chickens were weighed (34.03 g ± 1.1 g) and half of the chicks from each line were randomly assigned to one of two dietary lysine groups: the high (HL) and low dietary lysine group (LL). There were 8 repeat pens (30 chicks / pen, 0.096 m2 / chick) for each line by lysine combination. In the starter (1-28 d), grower (29-49 d) and finisher (50-70 d) periods, the HL chickens were fed diets containing 1.15 %, 1 % and 0.85 % total lysine, while the LL group was fed 1%, 0.85 % and 0.70 % total lysine, respectively. The diets were formulated according to the nutrient requirements described in NY/T33-2004, as shown in Table 1. Feed and water were available to the chickens ad libitum.

Pens were equipped with feeders and tube drinkers. Chicks were housed in floor pens covered in bedding (chaff 50% per m2, wood shaving 50% per m2) with 23 h of lighting and 1h of dark. House temperature was approximately 89.6 °F from d 1 to 7, 84.2 °F from d 8 to 14, 78.8 °F from d 15 to 21, and thereafter 68°F until the end of the experiment. Chicks were vaccinated against Marek’s, Newcastle, and Infectious Bronchitis virus at hatch, as
described by Zhao et al. (2012). Mortality was recorded daily and those individuals necropsied and eliminated (body weight and age) from the population calculations throughout the experiment.

Carcass traits
At the end of the starter (1-28 d), grower (29-49 d) and finisher (50-70 d) periods, eight chickens with 12 h fasted body weights that ranged from 110% to 90% of the group average were removed from each lysine and line combination group (Zhao et al., 2012). Blood was collected from the brachial vein and chickens were then slaughtered and organs dissected within 10 min. Subcutaneous fat thickness (SFT) and weights of heart (HW), liver (LIW), breast muscle (left pectoralis major, BMW), leg muscle (boneless left drum plus thigh, LMW) and abdominal fat (AW) were determined as Zhao et al. described (2012).

Muscle fibre characteristics
Pectoralis major and thigh muscles were collected at three time points (d 28, 49 and 70) for histology analysis. Tissue specimens were fixed in neutral buffered formalin for 24 h at 4°C and routinely processed for paraffin embedding and serial sectioning into 5 μm thick sections for subsequent hematoxylin-eosin staining and nuclei evaluation. For each bird, muscle fibre diameter and density were estimated via a motic microscope with three sections per slide. The muscle fibre diameter was measured from the fibre area (fibres/mm²) as previously described (Li et al., 2013a).

Serum parameters
After storage at 4°C for 24 h, blood samples were centrifuged at 4,000 g for 15 min at 4 °C, and 1 mL of serum was stored at -80 °C. Serum parameters including total protein (TP), serum urea nitrogen (SUN), cholesterol (CHO), triglycerides (TG), and glucose (GLU) were measured with commercial kits, following the manufacturer’s instructions (Beijing Stac Medical Science & Technology Co. Ltd., Beijing, China).

Statistical analysis
Data were analyzed with the following model with JMP Pro v. 10 (SAS Institute):

\[ Y_{ijk} = \mu + A_i + L_j + D_k + A_iL_j + A_iD_k + L_jD_k + A_iL_jD_k + e_{ijk} \]

where \( Y_{ij} \) is the performance of chickens at age i from Line j fed Diet treatment k; \( \mu \) is overall mean; \( A_i \) is fixed effect of Age i (i = d 28, 49 and 70); \( L_j \) is fixed effect of Line group j (j = SD02 and SD03); \( D_k \) is fixed effect of diet k (k = LL and LL group); \( A_iL_j \) is interaction of Age i by Line j; \( L_jD_k \) is interaction of Line j by Diet k; \( A_iD_k \) is interaction of Age i by Diet k; \( A_iL_jD_k \) is interaction of Age i by Line j by Diet k; \( e_{ijk} \) is random residual effect. Tukey’s test was used as a post-hoc test for pairwise comparisons. Results are shown as least squares means ± standard errors, and significance assigned at P < 0.05.

Results
Interaction effects on carcass traits
Three-way interactions of diet by age by line were significant for liver weight (LIW) and cholesterol content in serum (CHO). During the starter period (d 1-28), LIW of chickens from line SD03 fed HL diets (21.00 g) were heavier than line SD03 fed LL diets (16.55 g), whereas there were no differences between HL (15.63 g) and LL (13.78 g) groups within line SD02 (P > 0.05). In line SD02, chickens from the HL group had greater CHO concentrations (3.93 mmol/L) than the LL group (2.53 mmol/L) (P < 0.05). In the grower period (d 29-49), chickens from line SD03 fed the LL diet (23.83 g) had heavier LIW than those fed the HL diet (23.50 g) (P < 0.05). During the finisher stage (50-70 d), there were no differences among dietary lysine groups or between lines for LIW and CHO.

There were two-way interactions of diet by age on live weight (LW), heart weight (HW), breast muscle weight (BMW), leg muscle weight (LMW), and cholesterol (CHO) content in serum (P < 0.05; Table 2). The LW, LMW and BMW of the HL chickens were greater than the LL chickens during the finisher period (P < 0.05). The HW of the LL chickens was greater than the HL chickens during the grower period (P < 0.05). The CHO of the HL chickens was greater than the LL chickens during the starter period (P < 0.05).

There was an interaction of diet and line on myofibre diameter of leg muscle (LFD) (P < 0.05). The chickens of line SD03 fed HL diets had greater LFD than other diet and line

Table 1. Ingredient and chemical composition of the starter, grower and finisher diets.

|                     | Starter (1-28 d) | Grower (29-49 d) | Finisher (50-70 d) |
|---------------------|---------------|----------------|-------------------|
| LL                  |               |               |                   |
| Corn                | 58.35         | 58.35         | 63.31             |
| Wheat bran          | 6.00          | 6.00          | 5.50              |
| Extruded soybean    | 0.00          | 0.00          | 5.00              |
| Soybean meal        | 19.20         | 19.20         | 10.00             |
| Rapeseed meal       | 3.00          | 3.00          | 4.10              |
| Fermentation protein| 8.00          | 8.00          | 7.00              |
| CaHPO<sub>4</sub>   | 1.67          | 1.67          | 1.43              |
| CaCO<sub>3</sub>    | 0.95          | 0.95          | 0.87              |
| DL-Methionine       | 0.17          | 0.17          | 0.14              |
| L-Lysine            | 0.04          | 0.23          | 0.03              |
| Multi-vitamin       | 0.03          | 0.03          | 0.03              |
| Premix<sup>+</sup>  | 0.50          | 0.50          | 0.05              |
| Choline chloride    | 0.10          | 0.10          | 0.10              |
| Mixed oil           | 1.00          | 1.00          | 1.00              |
| Nac                 | 0.40          | 0.40          | 0.40              |
| Bentonite           | 3.00          | 3.00          | 0.59              |
| Total               | 1.00          | 1.00          | 1.00              |
| Nutrient levels     |               |               |                   |
| ME, MJ/kg           | 12.13         | 12.13         | 12.55             |
| CP                  | 19.00         | 19.00         | 17.00             |
| Lys                 | 1.00          | 1.15          | 0.85              |
| Met                 | 0.45          | 0.45          | 0.40              |
| Met+Cys             | 0.74          | 0.74          | 0.68              |
| Ca                  | 1.00          | 1.00          | 0.90              |
| AP                  | 0.45          | 0.45          | 0.40              |

LL, low dietary lysine group; HL, high dietary lysine group; ME, metabolizable energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cysteine; Ca, calcium; AP, aminopyridine. The HL diets in starter, grower and finisher periods contained 1.15%, 1.00% and 0.85% lysine, respectively. The LL diets in starter, grower and finisher periods contained 1.00%, 0.85% and 0.70% lysine, respectively. The digestibility of lysine in the present study was 92%. Each kilogram of premix contained the following: vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 2,000 IU; vitamin E, 30 IU; vitamin K<sub>3</sub>, 3.0 mg; vitamin B<sub>6</sub>, 4.0 mg; vitamin B<sub>12</sub>, 8.0 mg; vitamin B<sub>2</sub>, 4.0 mg; vitamin B<sub>1</sub>, 0.02 mg; nicotinic acid, 50 mg; calcium pantothenate, 13 mg; folic acid, 1.0 mg; Cu, 20 mg; Fe, 30 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.4 mg.
combinations (P<0.05). The LFDM of chickens from SD02 that consumed HL and LL diets were 31.32 and 30.29 µm, respectively, while the LFDM of chickens from SD03 that consumed HL and LL diets were 36.42 and 30.44 µm, respectively.

Significant interactions of line by age were detected for serum urea nitrogen (SUN) and total protein (TP) (P<0.05; Table 3). The SUN of the line SD02 chickens and the TP of the line SD03 during the starter phase were greater than for their respective counterparts in the other line at any of the other growth stages.

Effect of genetic line
Line affected liver weight (LW), leg muscle weight (LMW), myofibre diameter of breast muscle (BFDM) and leg muscle (LFDM), serum urea nitrogen (SUN) and total protein (TP) (P<0.05; Table 3). The LMW and SUN were greater in line SD02 than SD03 (P<0.05), while the LW, BFDM, LFDM and TP were greater in line SD03 than SD02 (P<0.05).

Effect of dietary lysine levels
The mortality, subcutaneous fat thickness (SFT), live weight (LW), heart weight (HW), myofibre diameter of leg muscle (LFDM) and cholesterol content in serum (CHO) were affected by dietary lysine concentration (Table 4). Mortality was greater in HL than LL chickens (P<0.05). The LW was greater (P<0.05) and HW and SFT reduced (P<0.05) in HL compared to LL chickens (P<0.05). The LFDM and CHO were greater in HL than LL (P<0.05).

Table 2. Interaction effects of diet by age on live weight, heart, breast and leg muscle weights, and cholesterol content (means ± standard errors).

| Measurements         | Age, d | N   | HL  | Lysine |
|----------------------|--------|-----|-----|--------|
| LW, g                | 28     | 16  | 495.20±30.69<sup>a</sup> | 513.20±30.69<sup>b</sup> |
| 49                   | 16     | 16  | 1099.81±30.69<sup>a</sup> | 1133.86±30.69<sup>b</sup> |
| 70                   | 16     | 16  | 1948.88±30.69<sup>a</sup> | 1680.25±30.69<sup>b</sup> |
| HW, g                | 28     | 16  | 1.65±0.17<sup>a</sup> | 2.10±0.17<sup>b</sup> |
| 49                   | 16     | 16  | 4.51±0.17<sup>a</sup> | 5.75±0.17<sup>b</sup> |
| 70                   | 16     | 16  | 9.25±0.17<sup>a</sup> | 9.17±0.17<sup>b</sup> |
| BMG, mg              | 28     | 16  | 18.98±2.15<sup>a</sup> | 21.86±2.15<sup>b</sup> |
| 49                   | 16     | 16  | 56.61±2.15<sup>a</sup> | 57.80±2.15<sup>b</sup> |
| 70                   | 16     | 16  | 107.12±2.15<sup>a</sup> | 99.89±2.15<sup>b</sup> |
| LMW, g               | 28     | 16  | 23.05±2.22<sup>a</sup> | 29.18±2.22<sup>b</sup> |
| 49                   | 16     | 16  | 63.02±2.22<sup>a</sup> | 69.70±2.22<sup>b</sup> |
| 70                   | 16     | 16  | 134.53±2.22<sup>a</sup> | 122.52±2.22<sup>b</sup> |
| CHO, mmol/L          | 28     | 16  | 3.76±0.13<sup>a</sup> | 2.87±0.13<sup>b</sup> |
| 49                   | 16     | 16  | 2.58±0.13<sup>a</sup> | 2.54±0.13<sup>b</sup> |
| 70                   | 16     | 16  | 3.46±0.13<sup>a</sup> | 3.22±0.13<sup>b</sup> |

<sup>a,b</sup>Means for a trait in the same column without a common superscript differ significantly (P<0.05).

Table 3. Interaction effect of line by age on serum urea nitrogen and total protein content (means ± standard errors).

| Traits                | Line    | N   | Age, d |
|-----------------------|---------|-----|--------|
| SUN, mmol/L           | SD02    | 24  | 0.82±0.05<sup>a</sup> | 0.42±0.05<sup>b</sup> |
| 49                    | 0.54±0.05<sup>b</sup> | 0.53±0.05<sup>a</sup> | 0.40±0.05<sup>b</sup> |
| TP, g/L               | SD02    | 24  | 43.36±2.36<sup>a</sup> | 42.61±2.36<sup>b</sup> |
| 49                    | 55.73±2.36<sup>a</sup> | 43.73±2.36<sup>b</sup> | 39.96±2.36<sup>b</sup> |

<sup>a,b</sup>SUN, serum urea nitrogen; TP, total proteins. Means for a trait within the interaction of age and dietary lysine without a common superscript differ significantly (P<0.05).

Table 4. Main effects of line, diet and age on carcass traits, muscle fibres and serum parameters (means ± standard errors).

| Factor                  | Mortality, % (n=96) | Carcass trait (n=96) | Muscle fibre (n=96) | Serum parameters (n=96) |
|-------------------------|---------------------|----------------------|---------------------|-------------------------|
| Line                    |                     | SFM, mm g            | LW, g               | BFDM, µm                  |
| SD02                    | 8.88                | 6.10 1167.07<sup>a</sup> | 24.96              | 5.46 66.12<sup>a</sup> |
| SD03                    | 7.83                | 5.72 1123.33<sup>b</sup> | 27.16              | 5.35 59.37<sup>a</sup> |
| SEM                     | 0.37                | 0.18 20.46            | 0.59               | 0.10 1.24 1.28 |
| Diet                    |                     | HW, g                | BMW, µm            | BFD, µm/mm<sup>2</sup>   |
| HL                      | 12.18<sup>a</sup>   | 5.58 1181.29<sup>a</sup> | 26.43              | 5.14 60.90 73.55 |
| LL                      | 8.85<sup>b</sup>    | 6.24 1109.10<sup>b</sup> | 25.69              | 5.67 59.58 73.80 |
| SEM                     | 0.41                | 0.18 20.46            | 0.59               | 0.10 1.24 1.28 |
| Age, d                  |                     | LIW, g               | LMW, g             | LFD, µm/µm<sup>2</sup>   |
| 28                      | 7.92                | 3.92 504.20<sup>a</sup> | 16.74              | 1.87 20.42 26.13 |
| 49                      | 8.37                | 5.59 1116.83<sup>b</sup> | 25.24              | 5.13 57.21 66.36 |
| 70                      | 9.84                | 8.27 1814.56<sup>b</sup> | 36.20              | 9.21 103.10 128.52 |
| SEM                     | 0.42                | 0.22 25.05            | 0.72               | 0.12 1.52 1.57 |

<sup>a,b</sup>SFM, subcutaneous fat thickness; LW, live weight; LIW, liver weight; HW, heart weight; BMW, breast muscle weight; LMW, leg muscle weight; LIW, abdominal fat weight; BFDM, myofibre diameter of breast muscle; BFD, breast muscle density; LFDM, leg muscle fibre diameter; SUN, serum urea nitrogen; CHO, cholesterol; GLU, glucose; TG, triglyceride; TP, total proteins. Means for a trait in the same column without a common superscript differ significantly (P<0.05).
Effect of age

There were significant differences among the three periods (starter, grower and finisher) for carcass traits, muscle fibre characteristics and serum metabolic parameters (P<0.05; Table 4). As expected, carcass traits and muscle fibre diameters increased with age, while the muscle fibre density and SUN decreased (Table 5). The CHO, GLU, TG, and TP were greater during the grower than starter period, but were less during the finisher than grower period (Table 4).

Discussion

Optimizing dietary lysine is important for ensuring the maximal growth efficiency without wasting nutrients. The current version of recommended poultry nutrition published by the National Research Council of the United States (1994) does not include the nutrition requirements of local populations that are bred for high meat quality. Moreover, growth, body composition and digestive physiology differ among poultry strains. Hence, the current study was designed to assess the effects of dietary lysine supplementation over an extended stage in two pure lines (Line SD02 and SD03) of a Chinese local broiler.

Total mortality of HL was higher than for LL chickens, in agreement with Kidd (Kidd et al., 1998) and Latshaw (Latshaw, 1993). Previous studies have shown that at higher dietary concentrations, lysine competes for transport/utilization with arginine, which is a substrate for synthesis of nitric oxide, a powerful endogenous pulmonary vasodilator, and that reduced arginine availability is associated with increased incidence of metabolic disease (Luiking and Deutz, 2007), resulting in an increased death rate due to ascites or pulmonary hypertension syndrome (Lott et al., 1997).

The present results indicate that dietary lysine affects body weight (BW) and muscle weight (BMW and LMW) but not other organ weights, at d 70. There were studies that reported that adding lysine enhances body weight gain of broilers, especially when added during the grower period, but that its effect on breast and leg muscle weights is controversial. Our result is in agreement with some studies (Kidd and Fancher, 2001; Li et al., 2013b; Tesseraud et al., 1999), but not others (Carlos et al., 2014; Tesseraud et al., 2001). The conflicting results might be explained by the contribution of sex, age, and genetic background to myofibre development (Acar et al., 1991; Bilgili et al., 1992; Han and Baker, 1991; Moran Jr and Bilgili, 1990). Meanwhile, we observed that LW, BMW and LMW were greater in Line SD02 than SD03, with Line SD02 having less abdominal fat than SD03, consistent with our previous reports (Jie et al., 2010; Li et al., 2013b). Thus, the results suggest that increasing dietary lysine had beneficial effects on body gain and muscle weight, that the meat produced from SD02 is superior to line SD03.

In the current study, leg muscle fibre diameter of HL-fed chickens was greater than chickens that consumed the LL diet. Previous studies have clearly pointed out a relationship between muscle structure (myofibre density and diameter) and meat quality. Reduced myofibre diameter was correlated with increased meat tenderness (Sifre et al., 2005). Myofibre growth is controlled by two possible mechanisms. One is the diameter increase resulting in myofibril accumulation. The other is myofibre elongation through the connection of the sarcomeres (Williams and Goldspink, 1978). Thus, one could speculate that dietary lysine affects the enlargement of muscle fibres and further studies are needed to fully elucidate the effect of dietary lysine on muscle development in broilers. We also noticed that the BFDM and LFDM line SD03 chickens were significantly greater than those in SD02, suggesting that the tenderness of line SD02 meat is superior to SD03.

There was a significant increase in serum cholesterol (CHO) in chickens fed higher lysine diets. CHO is a major blood lipid that is a precursor to all steroid hormones and bile salts. More CHO in HL chickens indicated that dietary lysine concentration may affect cholesterol metabolism indirectly. We also found that serum urea nitrogen (SUN) was greater in SD02 chickens than SD03 while total protein (TP) was greater in SD03 chickens than SD02. The SUN reflects amino acid metabolism, with greater amino acid catabolism associated with an increase in urea nitrogen (Donsbough et al., 2010; Swamy et al., 2002). The decrease in SUN may indicate that protein utilization and synthesis were enhanced in chickens fed the diet containing more lysine. Our results indicate that there were significant line effects on SUN and TP, and SD03 chickens utilized amino acids more efficiently than SD02 chickens during all growth periods.

Conclusions

In conclusion, the results demonstrate that chickens achieved faster body weight gain and muscle growth when fed relatively higher lysine concentrations, with no differences in carcass organ weights or lipid accumulation as compared to chickens fed the diet that was lower in lysine. Dietary lysine also influenced the enlargement of leg muscle fibres. Genetic background also influenced growth performance and carcass traits. Chickens from line SD02 had superior carcass traits and meat quality compared to line SD03. Overall, results showed that increased dietary lysine enhanced growth performance of Erlang Mountainous chickens, and thus can serve as a strategy to improve nutrient utilization efficiency in native chicken breeds.

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