Effects of L-lysine·H$_2$SO$_4$ product on the intestinal morphology and liver pathology using broiler model

Hongmin Jia$^1$, Ting He$^1$, Haitao Yu$^1$, Xiangfang Zeng$^1$, Shihai Zhang$^2$, Wenfeng Ma$^3$, Jie Zhang$^1$, Shiyan Qiao$^1*$ and Xi Ma$^{1,4*}$

Abstract

Background: Lysine is used widely in livestock production due to the shortage of feed protein resources. L-lysine·H$_2$SO$_4$ contains L-lysine sulphate as well as fermentation co-products which contain other amino acids and phosphorus. However, there are few articles about L-lysine·H$_2$SO$_4$ product regarding intestinal morphology and liver pathology of broiler chickens. In this article, we focus on the absorption and metabolism of L-lysine·H$_2$SO$_4$ revealed in the variation of intestinal morphology and liver pathology to determine the tolerance of chicks for L-lysine·H$_2$SO$_4$.

Methods: To evaluate the tolerance of broilers for L-lysine·H$_2$SO$_4$, 240 one day old broilers were allocated randomly to one of five dietary treatments which included corn-soybean diets containing 0, 1%, 4%, 7% or 10% L-lysine·H$_2$SO$_4$ (L-lysine content = 55%).

Results: Supplementation of 1% L-lysine·H$_2$SO$_4$ in the diet had no negative effects. However, 4%, 7% or 10% L-lysine·H$_2$SO$_4$ supplementation produced negative responses on broiler performance, carcass characteristics, blood biochemistry, and particularly on intestinal morphology and liver pathology compared with broilers fed the control diet.

Conclusion: Our results show that supplementation with 1% L-lysine·H$_2$SO$_4$ had no negative effects on performance, carcass characteristics, blood biochemistry, intestinal morphology and liver pathology in broilers, but supplementation with 4%, 7% or 10% L-lysine·H$_2$SO$_4$ produced a negative response, particularly with respect to intestinal morphology and liver pathology.

Keywords: Broilers, Intestinal morphology, Liver pathology, L-lysine·H$_2$SO$_4$

Background

Lysine is an indispensable amino acid that is commonly supplemented in the form of L-lysine·HCl in diets fed to pigs [1, 2], rainbow trout [3], and broiler chickens [4]. In recent years, an alternative source of lysine in the form of L-lysine·H$_2$SO$_4$ (55% lysine) has been developed with its use being highly attractive for both ecological and economic reasons. This occurs because the production of L-lysine·HCl involves a costly, multiple-stage process requiring the handling of hazardous materials such as ammonia solution (elution of the ion exchange column) and hydrochloric acid (neutralization of the L-lysine base) which generates residual solutions that are harmful to the environment [5, 6].

L-lysine·H$_2$SO$_4$ is a new source of lysine containing L-lysine-sulphate and fermentation co-products which contain other amino acids and phosphorus. This new source of lysine has been shown to be equally efficacious compared with L-lysine·HCl in diets fed to broilers [6, 7]. Furthermore, the mortality of broiler chickens fed L-lysine·H$_2$SO$_4$ was lower from d 1 to d 42 compared with birds fed L-lysine·HCl, which suggests that L-lysine·H$_2$SO$_4$ can improve the bird’s immune status.

Lysine is an indispensable amino acid for non-ruminant animals and has been widely used as a supplement in livestock diets [8, 9]. However, excessive

* Correspondence: qiaoshiyan@cau.edu.cn; maxi@cau.edu.cn
$^1$State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China
Full list of author information is available at the end of the article
concentrations of dietary lysine can produce negative effects in chickens, such as decreased weight gain, and increased incidence of severe leg problems [10–12]. Furthermore, there are no reports about the effects of high levels of L-lysine-H₂SO₄ on performance, carcass characteristics, blood biochemistry, intestinal morphology or liver pathology of broiler chickens. Therefore, we designed this experiment to determine the tolerance of broiler chickens to L-lysine-H₂SO₄ and observe the effect of this product on intestinal morphology and liver pathology when used as a source of lysine in diets.

Methods

Experimental material
L-lysine-H₂SO₄ (L-lysine content = 55%) was manufactured by the Changchun Dacheng Industrial Group (Changchun, China).

Birds and experimental design
All management and experimental procedures followed the animal care protocol approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China). This study was conducted on Arbor Acres broiler chickens (Beijing, China) for a 35-day period with a completely randomized design. One day old (n = 240) broiler chickens were housed in a standard broiler house with separate feed and drinking facilities. These broilers were weighed individually, wing banded, and allocated randomly to 1 of 5 treatments with 8 replicates per treatment and 6 birds per replicate.

The corn-soybean meal diets were formulated as shown in Table 1. The experimental diets were comprised of a control diet with no supplemental lysine and 4 diets supplemented with 1%, 4%, 7% or 10% L-lysine-H₂SO₄ in two periods (first period: d 1 to 21, second period: d 22 to 35). According to the “European Food Safety Authority” [13], “Guidelines for the Evaluation of Tolerance of Feed and Feed Additives on Livestock and Poultry Target Animals” (2011) and “Feeding Standard for Poultry” (2004), the multidose group is generally 10 times as the effective dose for evaluating new feed products. As prescribed, the general addition of lysine in feed was 1%. Thus, 1% was selected as the initial concentration, and increased by 3% points in turn, reaching the highest concentration of 10%. Meanwhile, 10% as the highest dose for lysine was also consistent with the standard. The control diet provided 3.04 Mcal/kg according to the nutrient requirements for chickens.

Experimental management
The broiler chickens were kept in zinc-plated cages (90 cm × 70 cm × 50 cm) with plastic slatted flooring. The chicks were housed in cages with 24 h constant-lighting and had free access to feed and water. All broilers were vaccinated with live, combined Newcastle disease and infectious bronchitis vaccine on d 7 and d 21 and inactivated infectious bursal disease vaccine on d 14 and d 28.

Dietary analysis
Diets were analyzed for dry matter, crude protein, calcium, total phosphorus and amino acids according to AOAC [14] procedures. Lysine and threonine were determined by Ion-Exchange Chromatography using an AA Analyzer (Hitachi L-8800, Tokyo, Japan) after acid hydrolysis with 6 mol/L HCl (refluxed for 24 h at 110°C). Methionine and cystine were determined after oxidation with performic acid and subsequent hydrolysis with 6 mol/L HCl at 120°C for 16 h and separated by Reversed-Phase HPLC (Agilent 1200, Santa Clara, CA). Tryptophan was measured after LiOH hydrolysis for 22 h at 110°C using High-Performance Liquid Chromatography (Agilent 1200 Series, Santa Clara, CA).

Growth performance
Broilers were weighed on d 1, 21, and 35. Feed consumption on a pen was measured every week. Average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR, ADFI: ADG) were calculated from d 1 to 21, d 22 to 35 and d 1 to 35, respectively.

Carcass characteristics
On the d 35 of the trial, one broiler chicken was selected randomly from each cage after an overnight fast. The broilers were slaughtered by exsanguination and eviscerated manually. Carcass weight, semi-eviscerated weight, eviscerated weight, liver weight, abdominal fat weight and pH₄₅ min of the breast and thigh muscle were measured. Dressing percentage, semi-eviscerated percentage, eviscerated percentage, abdominal fat percentage and drip loss were calculated according to the Performance Terms and Measurement for Poultry (NY/T 823–2004, China). Drip loss was calculated by hanging a section (breast and thigh muscle sample) in an inflated and closed plastic bag for 24 h at 4°C.

Intestinal morphology and liver pathology
Samples of the duodenum, jejunum, ileum, and liver were dissected from the slaughtered broilers and immediately fixed in 4% (v/v) paraformaldehyde solution for histological examination. The fixed tissues were processed routinely, embedded in paraffin and cut into 5 μm serial sections, and sections from each tissue sample were selected and stained with hematoxylin–eosin for identification [15]. Five well-oriented villi (determined as the distance between the crypt openings and the end of the villi) and their associated crypt (measured from the crypt villous junction to the base of the crypt) per
section were selected and measured under a light microscope (CK-40, Olympus, Tokyo, Japan) at 40 × magnification and analyzed with an Image Analyzer (Lucia Software, Lucia, Za Drahou, Czechoslovakia). The sections of liver were prepared for microscopic examination and representative samples were photographed.

**Blood biochemistry**

Blood was collected in uncoated serum tubes and EDTA-coated tubes at 35 days of age from 8 birds in each treatment by puncturing the brachial vein. Serum was collected and stored at −20 °C. Serum amino acid concentrations were determined with an Amino Acid Analyzer (S-433D AA Analyzer, Sykam, Germany). Individual serum samples were analyzed for albumin, total protein, globulin, total bilirubin, creatinine and uric acid. The activities of alanine amino transferase, aspartate amino transferase and lactic dehydrogenase were analyzed at the Beijing Sino-UK Institute of Biological Technology Company (Beijing, China). Hematology parameters such as white blood cell count, red blood cell count, hemoglobin, hematocrit, corpuscular volume, corpuscular hemoglobin concentration, red cell distribution width, platelet distribution width, platelet volume and proportion of large platelets were measured by corresponding commercial kits (BioSino Bio-Technology and Science Company, Beijing, China) using an Automatic Biochemical Analyzer (Hitachi 7160, Hitachi High-Technologies Corporation, Tokyo, Japan).

| Table 1 | Ingredient composition and nutrient levels of the experimental diets (% as fed) |
| --- | --- |
| **Items** | **Dietary L-lysine·H₂SO₄ levels (%) fed from d 1 to 21** | **Dietary L-lysine·H₂SO₄ levels (%) fed from d 22 to 35** |
| **Ingredient, %** | **0** | **1** | **4** | **7** | **10** | **0** | **1** | **4** | **7** | **10** |
| Corn | 59.12 | 59.75 | 62.41 | 65.21 | 68.85 | 60.67 | 61.27 | 63.67 | 67.77 | 71.58 |
| Soybean meal | 31.00 | 29.40 | 24.00 | 19.95 | 14.65 | 31.75 | 30.10 | 24.45 | 18.34 | 12.43 |
| Fish meal | 3.25 | 3.15 | 2.95 | 1.55 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Soybean meal | 3.00 | 2.97 | 2.80 | 2.00 | 1.00 | 4.00 | 4.00 | 3.95 | 2.70 | 1.70 |
| Limestone | 1.40 | 1.46 | 1.35 | 1.30 | 1.25 | 1.10 | 1.15 | 1.20 | 1.25 | 1.20 |
| Dicalcium phosphate | 1.10 | 1.12 | 1.20 | 1.50 | 1.60 | 1.40 | 1.40 | 1.52 | 1.55 | 1.55 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mineral and vitamin premix¹ | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| L-lysine·H₂SO₄ | 0.00 | 1.00 | 4.00 | 7.00 | 10.00 | 0.00 | 1.00 | 4.00 | 7.00 | 10.00 |
| DL-methionine | 0.15 | 0.16 | 0.20 | 0.25 | 0.28 | 0.10 | 0.10 | 0.15 | 0.20 | 0.21 |
| L-tryptophan | 0.00 | 0.00 | 0.04 | 0.07 | 0.07 | 0.00 | 0.00 | 0.00 | 0.02 | 0.06 |
| L-threonine | 0.00 | 0.11 | 0.22 | 0.32 | 0.00 | 0.00 | 0.08 | 0.19 | 0.29 |
| Choline chloride | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
| Antioxidant | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| **Calculated nutritional value** | | | | | | | | | | |
| **ME, Mcal/kg** | 3.04 | 3.05 | 3.10 | 3.10 | 3.10 | 3.10 | 3.11 | 3.16 | 3.15 | 3.16 |
| **Analyzed nutritional value, %²** | | | | | | | | | | |
| **Dry matter** | 88.24 | 88.33 | 88.62 | 88.85 | 88.82 | 89.10 | 89.11 | 89.28 | 89.47 | 90.03 |
| **Crude protein** | 21.70 | 22.82 | 22.59 | 23.13 | 22.71 | 20.02 | 21.20 | 21.58 | 20.40 | 20.31 |
| **Total calcium** | 1.05 | 1.14 | 0.73 | 0.71 | 1.01 | 0.96 | 1.20 | 0.97 | 0.82 | 1.10 |
| **Total phosphorus** | 0.49 | 0.55 | 0.49 | 0.61 | 0.57 | 0.57 | 0.55 | 0.54 | 0.55 | 0.58 |
| **Lysine** | 1.30 | 1.89 | 3.18 | 4.19 | 5.78 | 1.25 | 1.69 | 3.57 | 4.61 | 6.47 |
| **Tryptophan** | 0.23 | 0.23 | 0.20 | 0.21 | 0.19 | 0.23 | 0.22 | 0.19 | 0.18 | 0.20 |
| **Methionine** | 0.49 | 0.49 | 0.49 | 0.48 | 0.43 | 0.36 | 0.38 | 0.42 | 0.42 | 0.39 |
| **Cystine** | 0.30 | 0.29 | 0.28 | 0.25 | 0.21 | 0.28 | 0.29 | 0.28 | 0.25 | 0.21 |
| **Threonine** | 0.81 | 0.80 | 0.83 | 0.83 | 0.80 | 0.78 | 0.75 | 0.73 | 0.75 | 0.77 |

¹The mineral and vitamin premix provided the following per kg of feed: zinc, 75 mg; iron, 200 mg; copper, 15 mg; iodine, 0.88 mg; selenium, 0.35 mg; manganese, 100 mg; vitamin A, 9000 IU; vitamin D, 3750 IU; vitamin E, 25 IU; vitamin K, 2 mg; thiamine, 1.5 mg; vitamin B₁₂, 120 μg; riboflavin, 6 mg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; biotin, 0.1 mg

²Values are the means of a chemical analysis conducted in duplicate, n = 6/diet
Statistical analysis
Data were analyzed by the GLM procedure of SAS (Statistical Analysis System, Version 9.2) using a randomized design. Means are expressed as the least squares means with pen as the experimental unit. An alpha level of $P < 0.05$ was the criterion for statistical significance. Polynomial contrasts were performed to determine linear and quadratic relationships of lysine levels.

Results
Growth performance
Increasing dietary $L$-lysine·$\text{H}_2\text{SO}_4$ linearly and quadratically decreased both ADG and ADFI and increased FCR from d 1 to 21, d 22 to 35, and d 1 to 35 ($P < 0.05$) (Table 2). No significant difference in performance was observed between broiler chicks fed the control and 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets during any phase of the experiment (Table 2). When the $L$-lysine·$\text{H}_2\text{SO}_4$ level in the diet increased to 4%, 7% or 10%, the performance of broilers was negatively affected compared with broiler chicks fed the control and 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets during all phases of the experiment ($P < 0.05$) (Table 2).

Carcass characteristics
No significant difference in carcass characteristics was observed between broilers fed the control and 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets (Table 3). Increased dietary $L$-lysine·$\text{H}_2\text{SO}_4$ linearly and quadratically decreased eviscerated percentage and abdominal fat percentage of broilers ($P < 0.05$), and increased liver index and drip loss (breast and thigh muscle) of broiler chickens ($P < 0.05$). There were no notably changes in pH value among different levels of $L$-lysine·$\text{H}_2\text{SO}_4$ treatments.

Intestinal morphology
Intestinal morphology was influenced by high levels of dietary $L$-lysine·$\text{H}_2\text{SO}_4$ (Fig. 1), but no significant differences were observed between broilers fed control and 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets. Villous height and crypt depth in broilers fed the 7% and 10% $L$-lysine·$\text{H}_2\text{SO}_4$ diets were lower than those of the broilers fed 0, 1% and 4% $L$-lysine·$\text{H}_2\text{SO}_4$ diets ($P < 0.05$). Villous height, crypt depth and villous height to crypt depth ratio in the duodenum, jejunum, and ileum were not affected when comparing to broilers fed control with those fed 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets ($P > 0.05$).

Liver pathology
No differences pathology of livers were observed between broilers fed the control and 1% $L$-lysine·$\text{H}_2\text{SO}_4$ diets (Fig. 2). Hepatocytes were arranged compactly and orderly. There were a few lipid droplets in hepatocytes of broilers fed the 4% $L$-lysine·$\text{H}_2\text{SO}_4$ diets. When broilers were fed 7% $L$-lysine·$\text{H}_2\text{SO}_4$, obvious pathological changes were present in those livers. Liver cells were dissociated from each other in hepatic cords, cytoplasmic fat vacuoles. Pushed hepatocyte nuclei to the cell periphery in some places and reduction in size of a few hepatocytes was also seen. There was diffuse infiltration of fat vacuoles indicating vacuolar degeneration in livers of broilers fed the 10% $L$-lysine·$\text{H}_2\text{SO}_4$ diet. Furthermore, liver cells were dissociated from each other and cytoplasmic fat vacuoles pushed hepatocyte nuclei to the cell periphery in many cells. Tissue edema was obvious in livers.

Serum amino acid concentrations
No significant differences were observed among broilers fed control, 1%, 4%, 7% and 10%

Table 2 Effect of dietary $L$-lysine·$\text{H}_2\text{SO}_4$ levels on performance of broiler chickens

| Items                        | Dietary $L$-lysine·$\text{H}_2\text{SO}_4$ levels, % | SEM | $P$-value |
|------------------------------|------------------------------------------------------|-----|-----------|
|                              | 0  | 1  | 4  | 7  | 10          | ANOVA | Linear | Quadratic |
| D 1 to 21                    |    |    |    |    |             |       |        |           |
| Average daily feed intake, g | 43.0$^a$ | 42.7$^a$ | 32.9$^b$ | 13.5$^c$ | 6.5$^d$ | 0.66 | < 0.01 | < 0.01 | < 0.01 |
| Average daily gain, g        | 32.7$^a$ | 32.7$^a$ | 22.6$^b$ | 6.0$^c$  | 1.5$^d$ | 0.77 | < 0.01 | < 0.01 | < 0.01 |
| Feed conversion ratio        | 1.3$^d$ | 1.3$^d$ | 1.5$^c$ | 2.3$^b$  | 4.4$^a$ | 0.06 | < 0.01 | < 0.01 | < 0.01 |
| D 22 to 35                   |    |    |    |    |             |       |        |           |
| Average daily feed intake, g | 111.3$^a$ | 109.2$^a$ | 56.5$^b$ | 24.7$^c$ | 10.8$^d$ | 2.00 | < 0.01 | < 0.01 | < 0.01 |
| Average daily gain, g        | 67.0$^a$ | 65.6$^a$ | 19.5$^b$ | 6.3$^c$  | 1.9$^d$ | 1.52 | < 0.01 | < 0.01 | < 0.01 |
| Feed conversion ratio        | 1.7$^d$ | 1.7$^d$ | 3.0$^c$ | 4.1$^b$  | 6.0$^a$ | 0.26 | < 0.01 | < 0.01 | < 0.01 |
| D 1 to 35                    |    |    |    |    |             |       |        |           |
| Average daily feed intake, g | 70.3$^a$ | 69.3$^a$ | 42.4$^b$ | 18.0$^c$ | 8.2$^d$ | 1.00 | < 0.01 | < 0.01 | < 0.01 |
| Average daily gain, g        | 46.4$^a$ | 45.9$^a$ | 21.4$^b$ | 6.1$^c$  | 1.6$^d$ | 0.83 | < 0.01 | < 0.01 | < 0.01 |
| Feed conversion ratio        | 1.5$^d$ | 1.5$^d$ | 2.1$^c$ | 3.0$^b$  | 5.1$^a$ | 0.12 | < 0.01 | < 0.01 | < 0.01 |

*Means in the same row with different superscripts significantly differ from one another ($P < 0.05$). SEM standard error of the mean; $n = 8$/diet
Table 3 Effect of dietary L-lysine-H₂SO₄ levels on carcass characteristics of broiler chickens<sup>1</sup>

| Items                          | Dietary L-lysine-H₂SO₄ levels, % | SEM       | P-value |
|-------------------------------|----------------------------------|-----------|---------|
|                               | 0  | 1   | 4   | 7   |       | ANOVA | Linear | Quadratic |
| Dressing percentage, %        |    |     |     |     | 0.83 |         |         |         |
| 91.5                         | 90.2 | 90.9 | 91.4 |     |       | 0.66  | 0.97   | 0.54     |
| Hemoglobin concentration, %   |    |     |     |     | 1.11 |         |         |         |
| 73.4<sup>a</sup>             | 71.9<sup>a</sup> | 67.5<sup>b</sup> | 62.7<sup>c</sup> |     |       | <0.01 | <0.01 | <0.01     |
| Abdominal fat percentage, %   |    |     |     |     | 1.13 |         |         |         |
| 1.1<sup>ab</sup>             | 1.2<sup>b</sup> | 0.8<sup>b</sup> | 0.08<sup>b</sup> |     |       | <0.01 | <0.01 | <0.01     |
| Liver index, %                |    |     |     |     | 0.19 |         |         |         |
| 2.1<sup>b</sup>              | 2.1<sup>b</sup> | 2.8<sup>b</sup> | 4.3<sup>b</sup> |     |       | <0.01 | <0.01 | <0.01     |
| Breast muscle                 |    |     |     |     |       |         |         |         |
| pH<sub>45 min</sub>           |    |     |     |     | 0.09 |         |         |         |
| 6.2                          | 6.2 | 6.5 | 6.4 |     |       | 0.11  | 0.05   | 0.11     |
| Drip loss, %                  |    |     |     |     | 0.43 |         |         |         |
| 2.1<sup>b</sup>              | 2.2<sup>b</sup> | 3.3<sup>b</sup> | 6.7<sup>b</sup> |     |       | <0.01 | <0.01 | <0.01     |
| Thigh muscle                  |    |     |     |     |       |         |         |         |
| pH<sub>45 min</sub>           |    |     |     |     | 0.06 |         |         |         |
| 6.5                          | 6.5 | 6.6 | 6.7 |     |       | 0.05  | 0.01   | 0.02     |
| Drip loss, %                  |    |     |     |     | 0.91 |         |         |         |
| 3.3<sup>b</sup>              | 4.4<sup>b</sup> | 4.2<sup>b</sup> | 12.7<sup>b</sup> |     |       | <0.01 | <0.01 | <0.01     |

<sup>1</sup>Broiler chickens fed with 10% L-lysine-H₂SO₄ supplemented diet were too small to collect carcass characteristics in this experiment. Means in the same row with different superscripts significantly differ from one another (P < 0.05). SEM standard error of the mean; n = 6/diet

L-lysine-H₂SO₄ diets for serum concentrations of arginine, histidine, methionine, cysteine, serine and tyrosine (Table 4). Serum lysine and threonine concentrations of broilers were linearly and quadratically increased with increasing dietary L-lysine-H₂SO₄ level (P < 0.05). Serum glycine concentration of broilers fed 1% L-lysine-H₂SO₄ diet was higher than that of broilers fed control diets (P < 0.05), while serum prolactin concentration of the broilers fed 1% L-lysine-H₂SO₄ diet was higher than that of broilers fed the 4% L-lysine-H₂SO₄ diet (P < 0.05). Serum isoleucine, leucine and valine concentrations of the broilers fed the 7% L-lysine-H₂SO₄ diet was higher than that of broilers fed the control diet (P < 0.05).

**Blood chemistry and hematology**

No differences in blood chemistry were observed between broilers fed the control and 1% L-lysine-H₂SO₄ diets (P > 0.05) (Table 5). Both albumin and total protein of broilers fed the 7% and 10% L-lysine-H₂SO₄ diets were higher than those of the broilers fed the control diet (P < 0.05), meanwhile increased dietary L-lysine-H₂SO₄ linearly and quadratically increased albumin and total protein of broiler chickens (P < 0.05). Total bilirubin of the broilers fed the 7% L-lysine-H₂SO₄ diet was higher than that of broilers fed 0, 1%, 4% and 10% L-lysine-H₂SO₄ diets (P < 0.05).

No significant differences in hematology were observed between broilers fed the control and 1% L-lysine-H₂SO₄ diets (Table 6). Hemoglobin and corpuscular hemoglobin concentration of the broilers fed the 10% L-lysine-H₂SO₄ diet were lower than those of the broilers fed the control and 1% L-lysine-H₂SO₄ diets (P < 0.05). Increasing dietary L-lysine-H₂SO₄ linearly and quadratically decreased the corpuscular hemoglobin content of broilers (P < 0.05).

**Discussion**

L-lysine-H₂SO₄, as a new source of lysine, contains L-lysine-sulphate and fermentation co-products which contain other amino acids and phosphorus [7]. We evaluated effects of L-lysine-H₂SO₄ on intestinal morphology and liver pathology when used as a source of lysine in broilers diets. We showed that excessive L-lysine-H₂SO₄ addition in corn-soybean meal diets have negative effects in broilers.

The high proportion of L-lysine-H₂SO₄ (4%, 7% and 10%) in diets had different effects on performance and carcass characteristics when compared to the control and 1% L-lysine-H₂SO₄ supplementation diets. These results are consistent with previous studies that reported excessive lysine supplementation affected feed intake, weight gain, carcass traits, and feed conversion ratio [16–18]. The content and ratio of dietary amino acids affect the efficiency of amino acid utilization for animal growth [19–21]. For example, 1.95% lysine decreased feed intake and growth rate through reducing arginine efficacy in chicks [22]. In young pigs, three to four times the recommended lysine level (1.15% for young pigs) also decreased feed intake and weight gain through aggravating an arginine deficiency [23]. These results indicated that high levels of L-lysine-H₂SO₄ that reduced growth performance in broilers may down so by lowering the utilization ratio of other amino acids. In addition, excess L-lysine-H₂SO₄ caused a high level of dietary sulphate which can influence broiler performance via alterations in acid-base balance.

It is generally known that blood plasma proteins and amino acids play key roles in the maintenance of
colloid osmotic pressure, by keeping the balance of amino acid through a rapid substitute for indispensable amino acids, assuring steady glucose levels through gluconeogenesis, forming various functional enzymes and enhancing the immune system in the organism [24–26]. Therefore, blood plasma proteins and amino acids have an exceptional significance in maintenance of homeostasis. Changes in serum levels of lysine, threonine, phenylalanine, valine, isoleucine, and leucine in the present experiment were possibly mediated through a change in dietary amino acid levels in the experimental diets.

Blood chemistry is a common parameter utilized to estimate avian body condition. Blood albumin serves as the most favorable source of amino acids for synthesis of tissue proteins in the period of quick somatic growth of birds, especially under feed restricted conditions [27, 28]. Experiments on chickens revealed a relatively constant level of albumin in blood over a period from the 2 to 42 days of age [28]. In our study, content of total protein and albumin in serum were higher with supplementation of 4%, 7% or 10% L-lysine·H2SO4 than the control group, which is probably a direct consequence of a high demand for amino acids for somatic growth in birds fed excess L-lysine diets.

The chemistry profile and hematological count in blood of animals fed different diets can indicate the chemical and nutritional value of the diets [29, 30]. Addition of lysine in poultry diets improved immunity of birds and resulted in immunological regulatory action [31, 32]. However, the number of white blood cells in blood was higher for broilers fed the diet with 7% L-lysine-H2SO4 compared with broiler chicks fed the 1% L-lysine-H2SO4 diets, which could lead to the resulting in the secretion of much more cytokine and led to inflammatory reactions [33, 34]. Therefore, the growth performance of broilers supplemented with 4%, 7% or 10% L-lysine-H2SO4 was negatively affected compared with broiler chicks fed control diets during all phases of the experiment. In addition, the corpuscular hemoglobin concentration was also markedly decreased when the diet was supplemented with 4%, 7% or 10% L-lysine-H2SO4. This response suggests that there were toxic effects on the hematological system particularly for red blood cells [35, 36].

The folds in the intestine called villi are an important exchange area for digestion and absorption [37]. Villous height and crypt depth are critical factors affecting the animal’s ability to absorb nutrients [38]. In the current study, there was a significant reduction of villous height and a decline of crypt depth in the duodenum, jejunum and ileum in broilers supplemented with 7% or 10% L-lysine-H2SO4. Similar findings were
Fig. 2 Effect of dietary L-lysine·H₂SO₄ levels on liver morphology of broiler chickens. a, Broilers fed on basal diets; b, Broilers supplemented with 1% L-lysine·H₂SO₄; c, Broilers supplemented with 4% L-lysine·H₂SO₄; d, Broilers supplemented with 7% L-lysine·H₂SO₄; e, Broilers supplemented with 10% L-lysine·H₂SO₄.

Table 4 Effect of dietary L-lysine·H₂SO₄ levels on serum amino acid concentrations of broilers.

| Items                  | Dietary L-lysine·H₂SO₄ levels, % | SEM  | P-value | ANOVA | Linear | Quadratic |
|------------------------|-----------------------------------|------|---------|--------|--------|-----------|
|                        | 0       | 1    | 4        | 7      | 10     |           |
| Essential AA, nmol/mL  |         |      |          |        |        |           |
| Lysine                 | 402.0c  | 576.5c | 1031.4bc | 1395.3a | 1521.8a | 105.18    | < 0.01 | < 0.01 | < 0.01 |
| Arginine               | 374.9   | 310.3 | 281.7    | 319.4  | 284.4  | 43.55     | 0.58   | 0.21   | 0.37   |
| Histidine              | 114.9   | 145.6 | 137.3    | 144.7  | 157.7  | 14.91     | 0.36   | 0.07   | 0.20   |
| Isoleucine             | 112.9a  | 170.9ab | 189.0ab  | 231.9a | 156.4ab | 21.39     | < 0.01 | 0.10   | < 0.01 |
| Leucine                | 175.8b  | 258.0ab | 229.0ab  | 283.0a | 228.3ab | 24.59     | 0.04   | 0.12   | 0.04   |
| Methionine             | 68.9    | 80.8  | 79.4     | 81.5   | 76.7   | 6.93      | 0.69   | 0.44   | 0.37   |
| Phenylalanine          | 121.5b  | 166.7a | 178.8a   | 176.5a | 138.7ab | 11.96     | < 0.01 | 0.30   | < 0.01 |
| Threonine              | 623.5b  | 667.7b | 776.7b   | 1070.5b | 1453.0b | 117.63    | < 0.01 | < 0.01 | < 0.01 |
| Valine                 | 169.4c  | 260.9b | 312.9b   | 408.6a | 310.1b  | 27.71     | < 0.01 | < 0.01 | < 0.01 |
| Non-essential AA, nmol/mL |       |      |          |        |        |           |
| Alanine                | 1043.5b | 1797.6ab | 806.1b   | 2167.4a | 848.9b | 258.68    | < 0.01 | 0.92   | 0.23   |
| Asparagine             | 79.9b   | 182.1ab | 118.8ab  | 221.3a | 143.4bc | 30.28     | 0.02   | 0.11   | 0.07   |
| Glycine                | 764.9a  | 1058.2a | 918.5ab  | 911.0bc | 845.5ab | 66.07     | 0.05   | 0.95   | 0.09   |
| Cysteine               | 304.9   | 403.0 | 296.5    | 415.1  | 234.6  | 8.26      | 0.12   | 0.57   | 0.22   |
| Proline                | 1926.8ab | 2622.0a | 1464.6b  | 1881.1ab | 2415.6b | 204.41    | < 0.01 | 0.80   | 0.50   |
| Serine                 | 647.1   | 764.4 | 601.3    | 656.5  | 751.3  | 58.40     | 0.27   | 0.63   | 0.71   |
| Tyrosine               | 109.6   | 153.1 | 137.4    | 137.9  | 143.2  | 12.26     | 0.17   | 0.21   | 0.21   |

*Means in the same row with different superscripts significantly differ from one another (P < 0.05). AA amino acid, SEM standard error of the mean; n = 6/diet
reported for weanling pigs fed a lysine-deficient diet [39]. The reduction in villus height would reduce the surface area available for absorption of nutrients and this may partially explain the reduced growth performance and reduced feed efficiency in pigs fed the excessive L-lysine·H2SO4 diet.

The liver is the main site for protein synthesis, and liver histology is important in understanding pathological alterations in response to nutritional sources. Studies have shown that the addition of excessive protein in feed can reduce physiological function of the liver, so the lipoprotein in the liver cells cannot be transported in time, resulting in the accumulation of lipoprotein in the liver and liver lesions [40, 41]. In the present study, we found that high levels of L-lysine·H2SO4 might induce hepatic steatosis in broilers. The total protein content of the serum can reflect the ability of the liver to synthesize protein. The present study showed serum total protein content increased with the addition of L-lysine-H2SO4 in poultry diets, and more than 4% L-lysine-H2SO4 groups were significantly higher than the control group. Hence, excessive L-lysine-H2SO4 could cause a certain degree of damage to the liver cells, which is consistent with the results of liver histology in this study.

**Conclusions**

Our results show that supplementation with 1% L-lysine-H2SO4 had no negative effects on broilers, while supplementation with 4%, 7% or 10% L-lysine-H2SO4 negatively influenced growth performance, intestinal morphology and liver pathology. Our data indicates that doses higher than 1% dietary L-lysine-H2SO4 resulted in a negative response for broilers.

**Table 5** Effect of dietary L-lysine-H2SO4 levels on serum chemistry of broiler chickens

| Items                  | Dietary L-lysine-H2SO4 levels, % | SEM | P-value                |
|------------------------|----------------------------------|-----|-----------------------|
|                        | 0      | 1     | 4      | 7      | 10     | ANOVA | Linear | Quadratic |
| Albumin, g/L           | 11.1c  | 11.3c | 12.3bc | 14.5a  | 13.0b  | 0.38  | <0.01  | <0.01    | <0.01    |
| Total protein, g/L     | 23.7c  | 25.4bc| 28.2ab | 29.1a  | 27.7ab | 0.79  | <0.01  | <0.01    | <0.01    |
| Globulin, g/L          | 12.6   | 14.2  | 15.9   | 14.6   | 14.8   | 0.85  | 0.13   | 0.09     | 0.05     |
| Alanine amino transferase, U/L | 21.3ab | 22.9ab | 16.8b  | 31.6a  | 23.8ab | 2.78  | 0.02   | 0.15     | 0.36     |
| Aspartate amino transferase, U/L | 65.3  | 86.9  | 69.6   | 68.6   | 95.1   | 7.24  | 0.06   | 0.18     | 0.33     |
| Total bilirubin, μmol/L | 19.4b  | 22.2b | 22.7b  | 42.5a  | 20.6b  | 2.90  | <0.01  | 0.04     | 0.02     |
| Creatinine, μmol/L     | 24.0   | 25.3  | 26.5   | 25.2   | 21.6   | 1.18  | 0.12   | 0.36     | 0.03     |
| Uric acid, μmol/L      | 332.2  | 356.1 | 291.7  | 250.5  | 365.5  | 46.66 | 0.43   | 0.66     | 0.55     |
| Lactic dehydrogenase, U/L | 1508.0| 1443.8| 1567.5 | 1146.0 | 1792.7 | 182.12| 0.24   | 0.84     | 0.52     |

1 Means in the same row with different superscripts differ from one another (P < 0.05); SEM standard error of the mean; n = 6/diet

**Table 6** Effect of dietary L-lysine-H2SO4 levels on blood hematology of broiler chickens

| Items                  | Dietary L-lysine-H2SO4 levels, % | SEM | P-value                |
|------------------------|----------------------------------|-----|-----------------------|
|                        | 0      | 1     | 4      | 7      | 10     | ANOVA | Linear | Quadratic |
| White blood cells, 10^9/L | 49.0ab | 37.9b | 48.6ab | 55.8a  | 54.1ab | 4.20  | 0.05   | 0.07     | 0.11     |
| Red blood cells, 10^12/L | 2.6ab  | 2.6ab | 2.7ab  | 2.8a   | 2.5b   | 0.07  | 0.04   | 0.91     | 0.11     |
| Hemoglobin, g/L         | 86.5a  | 86.6a | 83.6a  | 89.1a  | 73.0b  | 3.30  | 0.02   | 0.06     | 0.04     |
| Hematocrit, %           | 33.7ab | 33.7ab| 33.0ab | 34.9a  | 30.1b  | 0.99  | 0.05   | 0.26     | 0.08     |
| Corpuscular volume, fl  | 127.7ab| 130.4a| 121.7b | 124.7ab| 122.4b | 1.81  | 0.01   | 0.01     | 0.05     |
| Corpuscular hemoglobin concentration, g/L | 261.1a | 258.4a| 252.3ab| 255.4ab| 242.2b | 4.07  | 0.03   | <0.01    | 0.01     |
| Red cell distribution width, fl | 35.7  | 37.5  | 36.3  | 37.3  | 38.5  | 1.40  | 0.66   | 0.22     | 0.47     |
| Platelet distribution width, fl | 6.8   | 6.1   | 7.1   | 6.6   | 6.5   | 0.59  | 0.80   | 0.91     | 0.99     |
| Platelet volume, fl     | 8.2    | 8.8   | 9.2   | 9.1   | 8.3   | 0.49  | 0.49   | 0.65     | 0.18     |
| Proportion of large platelets, % | 15.2  | 20.5  | 20.4  | 18.7  | 15.4  | 3.20  | 0.62   | 0.98     | 0.28     |

1 Means in the same row with different superscripts significantly differ from one another (P < 0.05); SEM standard error of the mean; n = 6/diet
Abbreviations
AA: Amino acids; ADFI: Average daily feed intake; ADG: Average day gain; AOAC: Association of Official Analytical Chemists; FCR: The ratio of average daily feed intake to average daily gain; GLM: General liner model; HCl: Hydrogen chloride; HPLC: High performance liquid chromatography; ME: Metabolic energy; SAS: Statistical Analysis System

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Availability of data and materials
All the data were presented in the main manuscript and available to readers.

Authors’ contributions
The article was mainly conceived and designed by SQ and XM. Experimental data were collected and analyzed by HJ and WM. The manuscript was mainly written by HJ, assisting the revised draft by TH, JZ and Lee Johnston; and edited by TH, SZ, JZ, HJ and SQ and XZ resourced the project. All the authors contributed to, read and approved the final manuscript.

Ethics approval and consent to participate
All procedures used in this study were performed according to the guidelines for the ethical treatment of animals by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China. 2Guangdong Provincial Key Laboratory of Animal Nutrition Control, College of Animal Science, South China Agricultural University, Guangzhou 510642, China. 3College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, Henan, China. 4College of Animal Science and Technology, Qingdao Agricultural University, Qingdao 266109, China.

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