Effects of arginine on the growth performance, hormones, digestive organ development and intestinal morphology in the early growth stage of layer chickens

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ABSTRACT
The objective of this study was to investigate the effects of arginine on the growth performance, hormones, digestive organ development and intestinal morphology of chicks of laying hens. A total of three hundred 1-d-old male Lohmann Brown chicks were randomly assigned to five groups, each with six replicate sets of 10 birds. The five groups were fed different diets containing 1.19, 1.44, 1.69, 1.94 or 2.19% arginine from 1 d to 42 d of age. The results showed that dietary levels of arginine had a significant effect on body weight at 14, 28 and 42 d (p < .05). The insulin-like growth factor-I (IGF-I) concentration in serum was significantly increased with increasing levels of dietary arginine (p < .05). With the increase in dietary arginine, chicks had a higher relative liver weight (p < .05). Levels of 1.94 and 2.19% dietary arginine had a more positive effect on the length (p < .05) and relative weight of the small intestine (p < .05), respectively. The morphology of the duodenal mucosa and the villus height of the ileum in chicks were significantly affected by the dietary arginine levels (p < .05). This study suggested that 1.44% dietary arginine provided the maximum body weight of layer chickens during their early development by increasing the villus height in both the duodenum and ileum, whereas 2.19% dietary arginine inhibited the growth of the chicks via a shorter villus height in the intestinal mucosa and excessive serum IGF-I.

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Introduction
Arginine, a nutritional additive, has a positive effect on the growth of chickens when included in their diet (Fernandes et al. 2009; Jahanian 2009; Youssef et al. 2016). In contrast to mammals, chickens cannot synthesise arginine de novo because they lack a functional urea cycle; thus, they have an absolute requirement for arginine to meet their needs for protein synthesis and other functions (Tamir and Ratner 1963). The arginine requirement of the chicken has been of considerable interest because the magnitude of the requirement is highly variable under different conditions. The requirement is influenced by the age and genotype of the animals (Kwak et al. 1999), the source of dietary protein (Khajali and Wideman 2010) and ambient temperature (Brake et al. 1998; Chamruspollert et al. 2004).

The starting period is a very important basic stage of hen production. The growth and development of chickens at this stage are directly related to growth during the rearing period and the egg performance of the laying period. Thus, the proper addition of arginine in the starter diet of layer chicks is of great importance to layer and egg production. Weight is an important indicator of the early growth of layer chickens. To a certain extent, the body weight of chicks depends on their capacity to digest and absorb nutrients, and this capacity is associated with the growth and development of digestive organs. In addition, arginine has a secretagogue activity by which it stimulates the release of pituitary and gastrointestinal hormones, including glucagon, insulin, ghrelin and growth hormone (GH). This induced-hormone production could increase protein synthesis and feed consumption (Kwak et al. 1999).

Several studies have shown that dietary arginine levels higher than National Research Council (NRC) recommendations (1994) can promote the growth of broilers (Fernandes et al. 2009; Jahanian 2009) and improve the performance of laying hens (Youssef et al. 2015). Thus, it has been speculated that levels of dietary arginine above the NRC recommended levels (1994) can promote the growth of chicks via promoting the secretion...
of certain hormones and the development of digestive organs. The objective of this study was to investigate the effects of arginine on the growth performance of layer chicks, including an analysis of serum hormones, digestive organ development and intestinal morphology.

Materials and methods

Birds and experimental design

Three hundred 1-d-old male Lohmann Brown chicks were obtained from a local commercial hatchery and randomly divided into five treatments with six replicates of 10 birds each (60 chicks per group). All birds were provided with a corn-soybean meal basal diet formulated to meet or exceed the NRC standards (1994) (Table 1). Dietary treatments were formulated by supplementing the basal diet with 0.00, 0.25, 0.50, 0.75 or 1.00% arginine (99%, Xi’an Partnership Biological Engineering Co., Ltd, Xi’an, China). Therefore, the total amounts of arginine in the five treatments were 1.19, 1.44, 1.69, 1.94 and 2.19%.

Birds were housed in thermostatically controlled battery cages with raised wire floors and were given free access to feed and water. Incandescent lighting was provided, and the photoperiod consisted of 3 d of 24-h light followed by 4 d of 20 h of light. From the second week onward, the illumination time was gradually decreased by 1.5 h each day until 11 h per day was reached. The temperature in the house was maintained at 32–34°C for the first week and was decreased by 2°C each week until the house temperature was 25°C. At 14, 28 and 42 d of age, all birds were weighed, and the average weight was calculated for each replicate set. The experimental procedures were approved by The Yangzhou University Animal Care and Use Committee.

Serum hormones

At the end of the 42-d feeding period, blood samples (2 mL) were collected from the wing veins of 60 birds (two birds per replicate, 12 birds per treatment) and centrifuged at 3500×g for 10 min at 4°C. The serum concentrations of GH and insulin-like growth factor-I (IGF-I) were measured by radioimmunoassay as described by Darras et al. (1992) and Tong et al. (2012). The assay kits were purchased from the Beijing North Institute of Biotechnology (Beijing, China). These hormones were detected by using a gamma radioimmunoassay counter GC-911γ (USTC Chuangxin Corporation Limited Zonkia Branch, Hefei, China).

Digestive organ development

After the collection of blood samples, all birds were killed by exsanguination. The following organs were removed from the body, stripped of adhering tissue, and weighed: liver, proventriculus, gizzard, duodenum, jejunum and ileum. Additionally, the lengths of the intestines were recorded. Relative organ weights were calculated as percentages of body weight = [(organ weight/body weight) × 100].

Intestinal morphology

After the intestinal lengths and weights of the chicks were recorded, segments of approximately 2 cm were taken from the midpoint of the duodenum, jejunum and ileum. Before segments were fixed in 10% neutral buffered formalin solution and embedded in paraffin, they were gently flushed twice with phosphate-buffered saline to remove the intestinal contents. Each fragment was cut into 5-μm semi-serial sections and stained with haematoxylin and eosin. For histological morphometric observations, the slides were analysed by light microscopy and digital images were captured (LY-WN-HP SUPER CCD, Chengdu, China). The height of villi and depth of crypts were measured at 20 different points in the duodenum, jejunum and ileum of each bird.

Table 1. Composition and nutrient levels of the diet (air-dried basis) (%).

| Item                  | Content |
|-----------------------|---------|
| Ingredients           |         |
| Corn                  | 67.62   |
| Soybean meal          | 27.30   |
| Wheat bran            | 1.50    |
| Calcium hydrogen phosphate | 1.50  |
| Limestone             | 0.70    |
| NaCl                  | 0.35    |
| s- Met                | 0.03    |
| Premix*               | 1.00    |
| Total                 | 100.00  |
| Calculated composition|         |
| ME, MJ/kg             | 11.93   |
| Analysed composition  |         |
| Crude protein         | 18.05   |
| Calcium               | 0.80    |
| Total phosphorus      | 0.71    |
| Arginine              | 1.19    |
| Lysine                | 0.90    |
| Methionine            | 0.30    |

*The premix provided the following per kg of the diet: vitamin A, 1500 U; vitamin D₃, 200 U; vitamin E, 10 U; riboflavin, 3.6 mg; pantothenic acid, 10 mg; niacin, 27 mg; vitamin B₁₂, 9 mg; choline chloride, 1300 mg; biotin, 0.15 mg; folic acid, 0.52 mg; thiamine, 1.8 mg; pyridoxine, 3 mg; Fe, 80 mg; Zn, 40 mg; Mn, 60 mg; I, 0.35 mg; Cu, 8 mg; Se, 0.15 mg.
Statistical analysis

The effects of dietary arginine concentration were analysed by one-way ANOVA followed by Duncan’s multiple comparison test using SPSS statistical software (Ver. 17.0 for windows, SPSS, Inc., Chicago, IL). Differences were considered statistically significant when \( p < .05 \).

Results

Growth performance

The weights of chicks at 14, 28 and 42 d of age are shown in Table 2. The body weight of chicks responded significantly by increasing as the arginine concentration increased towards 1.44% dietary arginine (0.25% in addition to the basal diet) and decreasing as it approached 2.19% dietary arginine (1% in addition to the basal diet) \( (p < .05) \).

Hormone measurements

GH and IGF-I levels in the serum are shown in Table 3. The IGF-I levels of chicks significantly increased as the dietary arginine level increased \( (p < .05) \). However, no effects were observed on GH due to dietary arginine supplementation \( (p > .05) \).

Digestive organs development

As shown in Table 4, only the liver relative weight increased significantly with increasing arginine levels \( (p < .05) \). There were no significant effects of dietary arginine supplementation on the proventriculus or gizzard relative weights \( (p > .05) \).

The effects of dietary arginine on the growth of the intestine in chicks from 1 to 42 d are shown in Table 5. The length \( (p < .05) \) and relative weight \( (p < .05) \) of the small intestine showed significant effects due to the changes in dietary arginine, mainly manifesting in the jejunum length and duodenum relative weight \( (p < .05) \). There were no differences among treatments in duodenum length, ileum length, jejunum relative weight or ileum relative weight \( (p > .05) \). The arginine requirements for maximum length and maximum relative weight of chicks were 1.94 and 2.19% of the diet, respectively.

Intestinal morphology

The villus heights, crypt depths and villus:crypt ratios of the small intestinal mucosa (duodenum, jejunum and ileum) of chicks fed diets supplemented with different levels of arginine are listed in Table 6. The morphology of the duodenal mucosa was significantly affected by the dietary arginine levels \( (p < .05) \), and 1.69% arginine resulted in a greater villus height and a higher villus:crypt ratio. There was a significant difference in the villus height of the ileum \( (p < .05) \), and 1.44% arginine supplementation resulted in a higher villus. When dietary arginine reached 2.19%, a shorter villus and shallower crypts were found in the small intestinal mucosa. No effect was observed for arginine supplementation on the jejunum and crypt depth or the villus:crypt ratio of the ileum \( (p > .05) \).

Discussion

Chickens fed practical diets based on corn and soybean meal and containing the crude protein and dietary lysine levels recommended by the NRC (1994) would not be expected to be arginine deficient (Lihalan et al. 2001). However, some studies have shown that better
growth performance can be obtained by adding a small amount of arginine to the corn–soybean meal-based diet (Fernandes et al. 2009; Jahanian 2009; Youssef et al. 2016). In the present study, a small dietary supplement of arginine (0.25% in addition to the basal diet) increased body weight to the maximum, suggesting that the 1.44% arginine diet had better effects than the NRC (1994) recommendations (1% dietary arginine for growing Leghorn-type chickens) for the growth of Lohmann Brown chicks. These results were similar to those of Youssef et al. (2016), who reported that chickens that received a diet supplemented with 2 or 4% arginine above the NRC (1994) requirements had a significantly higher body weight than controls during the starter period. The growth-regulating properties of arginine involve its function as a primary component of body protein and creatine, as a precursor of connective tissue forming proline and hydroxy-proline (Khajali and Wideman 2010) and as a precursor of growth-promoting polyamines, encouraging cell division, protein synthesis and tissue growth (Pegg and McCann 1982).

Nevertheless, Keshavarz and Fuller (1971) reported that the addition of 2.0% arginine to a maize–soybean-meal diet decreased the growth rate of chicks. In the present study, birds fed the 1.94 and 2.19% arginine diets, as expected, exhibited reduced growth. The possible cause of this result is that a high arginine:lysine ratio (1.94:0.90 or 2.19:0.90) in the diet reduced the utilisation rate of lysine and led to poor growth performance because lysine and arginine compete for intestinal and renal transporters (Closs et al. 2004).

The growth of animals is mediated mainly by the growth axis, and the main component of the growth axis is the GH/IGF-I axis. GH regulates the metabolism of carbohydrates and lipids directly through the activation of specific GH receptors (Giustina et al. 2008) or indirectly through IGF-I (Laviola et al. 2007), which is produced mainly in the liver in response to GH stimulation. IGF-1 is a powerful mitogen, with roles in regulating the growth of various tissues, including muscle and brain (Stuart and Page 2010). One of the reasons that arginine can affect growth may be that it is a powerful secretagogue, increasing the release of insulin, GH and IGF-I into the blood stream (Silva et al. 2012). In the present study, even though serum GH did not change as the dietary arginine level increased, the IGF-I levels of the chicks significantly increased. This change occurred because serum IGF-I concentrations are also controlled by insulin and nutritional factors except GH (Thissen et al. 1994). In vitro, levels of IGF-1 increased in a dose-dependent manner with arginine (0–7500 μmol/L) by hepatocytes (Ma 2003). In

### Table 5. The effect of arginine on the growth of small intestines in chicks.

| Item               | 1.19 | 1.44 | 1.69 | 1.94 | 2.19 | Pooled SEM | p value |
|--------------------|------|------|------|------|------|------------|---------|
| Length, cm         |      |      |      |      |      |            |         |
| Small intestine    | 110.27<sup>a</sup>,<sup>b</sup> | 109.03<sup>a</sup> | 114.29<sup>a</sup>,<sup>c</sup> | 118.09<sup>c</sup> | 112.01<sup>a</sup>,<sup>b</sup> | 0.83 | .003     |
| Duodenum           | 19.66 | 19.22 | 20.19 | 20.89 | 19.76 | 0.19 | .053     |
| Jejunum            | 45.58<sup>c</sup> | 44.78<sup>a</sup> | 48.18<sup>a</sup> | 48.96<sup>b</sup> | 46.73<sup>a</sup>,<sup>b</sup> | 0.40 | .002     |
| Ileum              | 45.03 | 45.03 | 45.93 | 48.24 | 45.53 | 0.42 | .088     |
| Relative organ weight<sup>d</sup>, % |      |      |      |      |      |            |         |
| Small intestine    | 2.82<sup>a</sup>,<sup>b</sup> | 2.57<sup>a</sup> | 2.75<sup>a</sup>,<sup>b</sup> | 2.78<sup>a</sup>,<sup>b</sup> | 2.94<sup>b</sup> | 0.04 | .033     |
| Duodenum           | 0.69<sup>a</sup> | 0.58<sup>a</sup> | 0.68<sup>a</sup> | 0.67<sup>a</sup>,<sup>b</sup> | 0.75<sup>b</sup> | 0.02 | .010     |
| Jejunum            | 1.27 | 1.14 | 1.22 | 1.21 | 1.29 | 0.02 | .169     |
| Ileum              | 0.86 | 0.85 | 0.84 | 0.90 | 0.90 | 0.01 | .123     |

<sup>a</sup>Values within a row with no common superscript differ significantly (p < .05).

<sup>b</sup>Relative organ weights were calculated as percentages of body weight = ([organ weight/body weight] × 100).

### Table 6. The effect of arginine on the small intestinal mucosa in chicks.

| Item               | 1.19 | 1.44 | 1.69 | 1.94 | 2.19 | Pooled SEM | p value |
|--------------------|------|------|------|------|------|------------|---------|
| Duodenum           |      |      |      |      |      |            |         |
| Villus height, μm   | 1420.36<sup>a</sup>,<sup>b</sup>,<sup>c</sup> | 1574.84<sup>b</sup>,<sup>c</sup> | 1637.56<sup>c</sup> | 1358.35<sup>a</sup>,<sup>b</sup> | 1321.72<sup>a</sup> | 39.50 | .033     |
| Crypt depth, μm     | 139.51<sup>b</sup> | 143.34<sup>a</sup> | 140.33<sup>a</sup> | 145.47<sup>b</sup> | 103.12<sup>a</sup> | 4.48 | .014     |
| Villus:Crypt ratio  | 10.31<sup>a</sup>,<sup>b</sup> | 11.19<sup>a</sup>,<sup>b</sup>,<sup>c</sup> | 11.71<sup>a</sup>,<sup>b</sup>,<sup>c</sup> | 9.47<sup>a</sup> | 12.92<sup>a</sup> | 0.36 | .020     |
| Jejunum             |      |      |      |      |      |            |         |
| Villus height, μm   | 1361.30 | 1398.18 | 1352.29 | 1243.97 | 1199.39 | 28.82 | .131     |
| Crypt depth, μm     | 141.26 | 138.82 | 143.42 | 145.38 | 114.62 | 4.14 | .105     |
| Villus:Crypt ratio  | 9.88 | 10.27 | 9.65 | 8.70 | 10.52 | 0.31 | .419     |
| Ileum               |      |      |      |      |      |            |         |
| Villus height, μm   | 880.69<sup>a</sup>,<sup>b</sup> | 1048.56<sup>b</sup> | 891.46<sup>a</sup>,<sup>b</sup> | 914.64<sup>a</sup>,<sup>b</sup> | 763.77<sup>a</sup> | 29.06 | .025     |
| Crypt depth, μm     | 133.80 | 150.09 | 144.10 | 139.49 | 116.06 | 4.98 | .247     |
| Villus:Crypt ratio  | 6.73 | 7.12 | 6.29 | 6.80 | 6.64 | 0.21 | .815     |

<sup>a</sup>Values within a row with no common superscript differ significantly (p < .05).
addition, IGF-I acts on the hypothalamus and the pituitary to depress GH secretion (Nass et al. 2002). This helps to explain why arginine did not increase the serum GH levels. High levels of dietary arginine led to lower body weight in this study, probably because high levels of serum IGF-I help lower blood sugar, much like insulin (Wang and Feng 1999).

The growth and development of digestive organs directly affect the digestive and absorptive capacity of nutrients, and the weight and size of the digestive organs can be an indicator of physiological function. Although organ growth was equally directed within each organ group, the sensitivity to dietary arginine differed among organ groups as well as within them (Lieboldt et al. 2015). Individual organ sensitivities might be mediated through the organ-specific expression of arginine uptake transporters, the cationic amino acid transporters (CAT) (Humphrey et al. 2004; Humphrey and Klasing 2005). In this study, the only significant increase was detected in relative liver weight. This finding indicates that the metabolic capacity of the liver was greatly enhanced. The results for the liver relative weight completely disagree with those reported by Youssef et al. (2016). A possible reason is that the Lohmann Brown chicks studied in this experiment are a commercial variety, whereas two local strains (Fayoumi and Golden Montazah) were studied by Youssef et al. (2016). In addition, the ages of the chicks in the two trials differed (6 and 12 weeks, respectively).

One of the most important digestive organs is the small intestine because it is the main site for the digestion and absorption of nutrients. Arginine is the precursor of polyamines, and these biogenic amines are considered nutritionally important local factors for the growth and development of the small intestinal mucosa (Löser et al. 1999). When polyamines are lacking, the proliferation, migration and apoptosis of intestinal cells are inhibited (Ruemmele et al. 1999). In this study, 1.69–1.94% arginine led to longer intestinal length, while 2.19% arginine resulted in a greater relative weight of the small intestine. However, several investigations in broiler hens have found no effect of arginine supplementation on small intestine weight and length (Murakami et al. 2012; Fernandes et al. 2015). According to McBride and Kelly (1990), the maintenance of the intestinal epithelium and its supporting structures represents 20% of the total energy consumed by the animal. This might explain why better growth performance was not observed with longer or larger small intestines.

A normal structure of the small intestinal mucosa is necessary for optimal growth as well as nutrient digestion and absorption (Yao et al. 2012). Intestinal morphology, including the villus height and crypt depth, determines the effective absorption area of the intestine. As a precursor of polyamines, arginine may be considered a trophic agent in the stimulation of the development of the intestinal mucosa, accelerating the mitotic process in the villus-crypt region with a resulting increase in the number and size of villus cells (Murakami et al. 2012). Additionally, arginine and its product nitrite oxide (NO) have been reported to stimulate intestinal epithelial cell proliferation and migration via the mechanistic target of rapamycin (mTOR) or focal adhesion kinase pathways (Rhoads and Wu 2009). According to Kelly et al. (1991), the villus height may be positively related to body weight gain. In the present study, the results obtained based on intestinal villus analysis demonstrate that the addition of arginine in higher amounts than NRC requirements (1994) is required to maintain the intestinal epithelial development. Levels of 1.44% dietary arginine provided a greater villus height in the duodenum and the greatest villus height in the ileum, which improves the absorption of nutrients. However, 2.19% dietary arginine led to the shortest villus height in the intestinal mucosa, which was consistent with the lowest body weight, indicating that excessive dietary arginine may reduce growth by lowering villus height.

Conclusions

In conclusion, these findings suggest that a level of 1.44% dietary arginine above the NRC requirements provides the maximum body weight of layer chickens during the starting period (0–42 d). Levels of 1.44% dietary arginine increased the growth rate by increasing the villus height in both the duodenum and ileum. In contrast, excessive dietary arginine (2.19%) decreased the growth rate via a shorter villus height in the intestinal mucosa and excessive serum IGF-I.

Disclosure statement

No potential conflict of interest was reported by the authors.

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