

Influence of Dietary Arginine Concentration on Lymphoid Organ Growth in Chickens^{1,2}

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ABSTRACT *In vivo* effects of graded dietary levels of arginine on the body and lymphoid organs were investigated using Cornell K strain chickens of the B¹⁵/B¹⁵ haplotype. Two-week-old birds were fed an arginine-deficient basal diet (0.53% arginine) supplemented with additional arginine (up to 1.0% L-arginine to the diet). At four weeks of age, body weight, lymphoid organ weight, and concentrations of amino acids in plasma were measured. Arginine supplementation produced significant increases in plasma arginine (from 200 nM in chicks fed the basal diet

to 2,000 nM in chicks receiving the 1.5% arginine diet) and ornithine concentrations (from 17 nM in chicks fed the basal diet to 500 nM in chicks receiving the 1.5% arginine diet). The arginine-deficient diet reduced body weight gain ($P < 0.0001$) and thymus, spleen, and bursa of Fabricius weights ($P < 0.05$). In contrast to the bursa weight, the thymus and spleen weights, as percentages of body weight, were also decreased ($P < 0.05$). This study suggests that arginine markedly influences lymphoid organ development, with a more pronounced effect on the thymus and spleen than on the bursa of Fabricius.

(Key words: arginine, chicken, lymphoid organs, plasma amino acid concentration)

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INTRODUCTION

The amino acid arginine is essential for optimal growth and nitrogen balance in growing animals (Borman *et al.*, 1946; Milner *et al.*, 1974). Whereas most mature mammals can synthesize arginine to meet their requirements, chickens cannot synthesize arginine *de novo* and, therefore, are completely dependent on dietary arginine to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). Chickens can vary, based on genotype, in the dietary levels of arginine that they require for growth. Two strains of chickens, the high arginine-requiring strain (HA) and low arginine-requiring strain (LA) chickens, were developed by genetic selection from a common ancestral population based on their ability to survive and grow on an arginine-deficient diet (Hutt and Nesheim, 1966). The HA and LA strains require high and low dietary concentrations of arginine for their maximum growth, respectively (Nesheim and Hutt, 1962; Hutt and Nesheim, 1966).

In addition to the requirement for growth, arginine has been shown recently in several studies to have beneficial

effects on the immune status of animals. Supplementation of the diet with L-arginine has been reported to improve wound healing significantly (Barbul *et al.*, 1980, 1990; Nirgiotis *et al.*, 1991; Daly *et al.*, 1992;) and improve survival in tumor models in rodents and humans (Moriguchi *et al.*, 1987; Reynolds *et al.*, 1988a, 1990; Taylor *et al.*, 1992; Brittenden *et al.*, 1994), indicating that arginine can influence disease resistance. Many studies have been performed to investigate how arginine affects immunity, but the exact mechanism of arginine effects needs more investigation. To understand the effect of arginine, one of the basic kinds of information needed is its effect on each lymphoid organ. In addition, the effect of arginine on lymphoid organs is of great interest because effective development of these organs is crucial for optimal immune responses. However, little is known about the arginine requirement for lymphoid organ development in either mammals or chickens. Furthermore, the sensitivity of each lymphoid organ to arginine concentration is not clear.

Thus, the effects of an arginine deficiency on each lymphoid organ development were investigated in this study. Chickens were used as an animal model for the study. Because chickens do not have the enzyme profile that would allow them to synthesize arginine (Tamir and Ratner, 1963) and are completely dependent on the diet as their source of arginine (National Research Council,

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Abbreviation Key: HA = high arginine requiring; LA = low arginine requiring.

TABLE 1. Composition of basal diet

Ingredients	(% of diet)
Corn gluten meal (60% protein)	25.00
Cerelose (glucose-H ₂ O)	60.09
Cellulose	3.00
Corn oil	3.00
L-Lysine-HCl	0.8
L-Tryptophan	0.1
L-Threonine	0.16
Vitamins ¹	1.25
Minerals ²	6.6

¹Vitamin mix components per kilogram of diet: thiamine-HCl, 15.0 mg; riboflavin, 15.0 mg; nicotinic acid, 50.0 mg; folic acid, 6.0 mg; pyridoxine, 6.0 mg; biotin, 0.6 mg; vitamin B₁₂, 0.02 mg; choline-Cl, 2,500 mg; d-calcium pantothenate, 20.0 mg; menadione sodium bisulfite, 1.5 mg; vitamin E, 50 IU (dl- α -tocopheryl acetate); cholecalciferol, 4,500 IU; vitamin A, 4,500 IU (retinyl acetate); antioxidant (butylated hydroxytoluene), 100 mg; and glucose to make 12.5 g.

²Mineral mix components per kilogram of diet: CaCO₃, 13.8 g; CaCl₂, 5.5 g; CaHPO₄-2H₂O, 18.0 g; KH₂PO₄, 14.0 g; NaHCO₃, 11.0 g; MnSO₄-H₂O, 0.33 g; FeSO₄-7H₂O, 0.33 g; MgSO₄, 3.0 g; KI, 0.0026 g; CuSO₄-5H₂O, 0.0167 g; ZnO, 0.10 g; CoCl₂-6H₂O, 0.0017 g; NaMoO₄-2H₂O, 0.0083 g; and Na₂SeO₃, 0.0001 g.

1994), these animals provide a good model to study the relative effects of arginine on lymphoid organs.

MATERIALS AND METHODS

Animals

Female Cornell K-strain (B¹⁵/B¹⁵) White Leghorn chickens (Cole and Hutt, 1973) were grown to 2 weeks of age with free access to a practical starter diet.⁴ Then, they were divided into experimental groups and were fed with either the basal diet or one of the arginine-supplemented diets to investigate the effects on lymphoid organ growth. Each group had 3 birds, and triplicate groups were randomly assigned to either side of a chick brooder battery. Experimental diets were administered from 2 to 4 wk of age. Birds were housed in thermostatically controlled battery cages with raised wire floors and were given free access to feed and water under conditions providing constant room temperature and 16 h of light daily throughout the experiment. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Cornell University.

Basal Diet

Corn gluten meal has a relatively low arginine content (National Research Council, 1982) and was, therefore, used as the source of protein in the basal diet (Table 1).

⁴A nutritionally adequate diet containing corn, soybean oil, meat and bone meal, corn oil, dehydrated alfalfa meal, DL-methionine, distillers dried grains, minerals, and vitamin supplements.

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The amount of each amino acid provided by corn gluten meal and the amount of each amino acid needed for supplementation to meet the requirement of each amino acid were calculated. L-lysine, L-tryptophan, and L-threonine were supplemented to provide a margin of safety of 5% above the requirement (National Research Council, 1994) for all indispensable amino acids except arginine. The basal diet provided 0.53% arginine, which was inadequate based on the requirement (about 1%). Four arginine levels were tested for the evaluation of plasma amino acid concentrations: 0.53, 0.73, 1.03, and 1.53% dietary arginine. Three arginine levels out of four tested were used in studies of lymphoid organ development: 0.53, 0.73, and 1.53% arginine in diet (deficient, optimal, and surplus arginine level, respectively). All arginine additions to the basal diet were in the form of L-arginine (free base). Arginine and lysine concentrations in each diet were analyzed by HPLC analysis.⁵

Body Weights and Organ Weights

Chickens were weighed individually before they were provided experimental diets at 2 wk of age, and were weighed again after 2 weeks on the diets. Feed consumption was measured during the experimental period. After weighing, birds were killed by CO₂ asphyxiation. The spleen, bursa of Fabricius, and all thymic lobes were removed from the body and collected in sterile PBS (pH 7.2). Each organ was stripped of adhering tissue and then weighed individually. Relative organ weights were calculated as percentages of body weight = [(organ weight/body weight) × 100].

Amino Acid Concentrations

Four-week-old chickens were bled by brachial vein puncture, and the blood was collected into heparin-treated syringes.⁶ The samples were centrifuged at 600 × g for 20 min, and the plasma was collected. Plasma was deproteinized with 7% sulfosalicylic acid containing norleucine as an internal standard, and amino acid concentrations were determined by Beckman HPLC⁷ ion exchange chromatography with fluorescence detection and peak area integration system.⁸

Statistical Analysis

The effects of dietary arginine concentration were analyzed by one-way ANOVA followed by Fisher's Least Significant Difference Test. The Number Crunching Statistical System⁹ was used to analyze data. Percentage data were transformed using arc sine transformation before statistical analysis. A *P* value of < 0.05 was used to determine significant differences among treatment means.

RESULTS

Body Weights and Feed Utilization

Body weight gain of chicks fed the arginine-deficient basal diet was lower than that of chicks that received

TABLE 2. Weight gain and feed utilization of K-strain chickens provided with various dietary levels of arginine

Dietary arginine (%)	n	Weight gain ¹ (g/chick)	Feed consumption (g)	Feed efficiency (g gain/g feed intake)
0.53	9	45.4 ^b	194.0 ^c	0.23 ^b
0.73	9	102.8 ^a	262.6 ^b	0.39 ^a
1.53	9	108.9 ^a	305.8 ^a	0.36 ^a
Pooled SEM		2.32	2.71	0.006

^{a-c}Means within a column with no common superscript differ ($P < 0.05$) by Fisher's least significant difference test.

¹Mean body weight gain of 4-wk-old chickens fed the basal diet supplemented with various levels of arginine from 2 to 4 wk of age.

the arginine-supplemented diets ($P < 0.05$) (Table 2). Chickens fed the 0.53% arginine diet had less than one-half the weight gain of birds that received the 0.73% arginine diet and the 1.53% arginine diet. Feed consumption was lowest for chicks fed the arginine-deficient diet and was significantly increased by each level of arginine supplementation. Feed efficiency (grams of body weight gain/grams of feed consumed) was significantly lower for birds fed the arginine-deficient diet compared with that of birds fed the arginine-supplemented diets. No mortality was observed in any of the groups of birds during the 2-wk experimental period.

Lymphoid Organ Weights

The thymus, spleen, and bursa weights of birds that received the 0.53% arginine diet were significantly lower by approximately 75, 50, and 37% than the respective organ weights of birds that received the 0.73% arginine diet and the 1.53% arginine diet ($P < 0.05$) (Figure 1). The relative thymus weight (thymus weight as percentage of body weight) was decreased in chicks fed the arginine-deficient basal diet compared with that of chicks fed the arginine-supplemented diets ($P < 0.05$) (Figure 2). The relative spleen weight was significantly decreased in the chicks fed the arginine-deficient diet compared with the diet containing 0.73% arginine, but was not significantly different from the relative spleen weight of chicks fed diet containing 1.53% arginine (Figure 2). The relative bursa weight did not differ among treatments.

Amino Acid Concentrations in Plasma

The concentration of arginine (Figure 3) in plasma increased from 0.2 to 2.0 mM ($P < 0.01$), and the concentration of ornithine increased from 0.017 to 0.5 mM, as the dietary arginine concentration increased from 0.53 to 1.53% in the diet ($P < 0.05$). Arginine supplementation did not affect plasma lysine concentration.

DISCUSSION

The status of a nutritional factor can have profound effects on immunity; it can either enhance or depress immune response depending on the nutrient and its level

of intake. Nutritional deficiency impairs immunity and the resistance to infection. For example, protein-energy malnutrition and the deficiency of dietary lipids and micronutrients have been shown to have a substantial impact on immune responses in animals and humans (Lee and Woodward, 1996; Chandra, 1997; Scrimshaw and SanGiovanni, 1997). Deficiency of a single amino acid has been associated with impaired immunity (Barbul, 1993; Kuhlman *et al.*, 1994).

Amino acid requirements for chickens can vary greatly, depending on the age and genotype of the animals. Based on data from the National Research Council (1994) that primarily set nutrient requirements based on body weight, growing Leghorn-type chickens (0 to 6 wk) require about 1% arginine and 0.85% lysine in their diets. In our experiments, the basal diet was formulated to contain 0.53% arginine and 0.9% lysine. Therefore, the arginine content in the basal diet was lower than the requirement, and, as expected, birds fed the 0.53% arginine diet exhibited reduced growth rate. In this study, reduced lymphoid organ growth was also observed because of the low arginine intake in the basal diet. In addition, the resultant high lysine:arginine ratio (0.9:0.53) of the basal 0.53% arginine diet could contribute to reduced arginine availability and aggravate the poor growth performance because of a lysine-arginine antagonism that causes the arginine requirement to be increased when lysine is excessive (Jones, 1964; Lewis *et al.*, 1969; Austic and Nesheim, 1970; Stutz *et al.*, 1972; Wang *et al.*, 1973).

The requirement of amino acids for growth and efficiency of feed utilization of chickens has been studied.

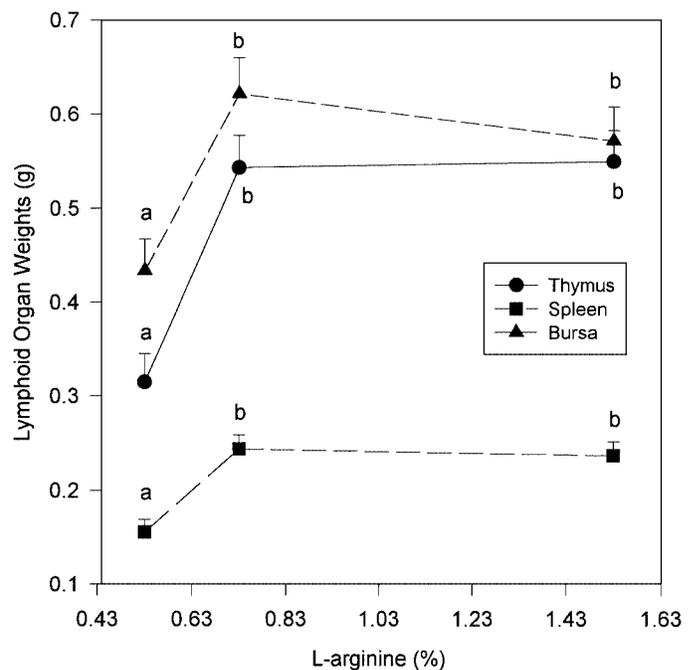


FIGURE 1. Lymphoid organ weights of 4-wk-old chickens that had been fed arginine-deficient (0.53% arginine) and arginine-supplemented diets for 2 wk. ^{a,b}Means without a common letter are different within organ ($P < 0.05$). Error bars represent standard errors. Data are from three experiments with three separate hatches ($n = 9$).

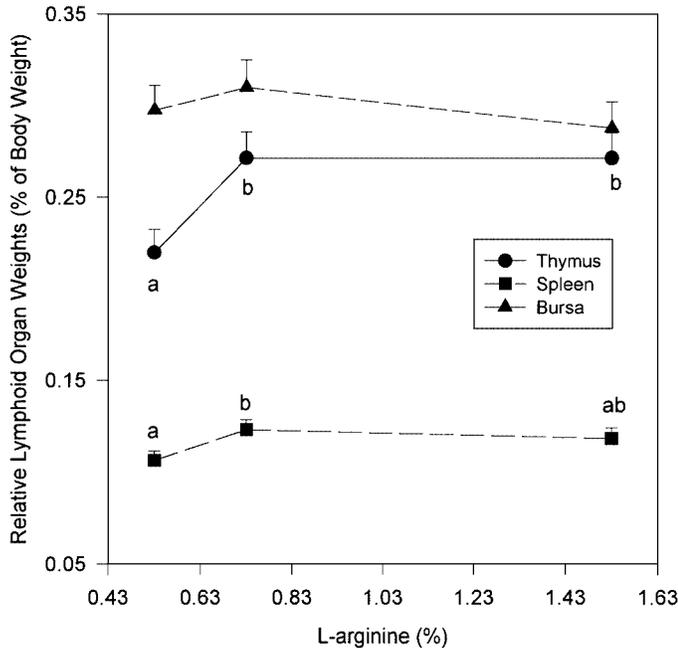


FIGURE 2. Lymphoid organ weight relative to body weight. Relative lymphoid organ weights were calculated from the body weight and thymus, spleen, and bursa weights of 4-wk-old chickens as a percentage of body weight = [(lymphoid organ weight/body weight) × 100]. ^{a,b}Means without a common letter differ within organ ($P < 0.05$), and error bars represent standard errors. Data are from three different experiments performed on three hatches of K-strain chickens ($n = 9$).

In broiler chickens, an arginine-deficient diet reduced weight gain and feed intake (Carew *et al.*, 1997). This study also demonstrated that the selected amino acid deficits result in changes in the plasma thyroid hormone concentrations. However, the requirement of specific amino acids for optimal development of each organ, including the lymphoid organs, has not been well defined.

In this study, an arginine-deficient diet led to poor development of lymphoid organs, especially reflected in both absolute and relative thymus and spleen weights. A small dietary supplement of arginine (0.2% in addition to the basal diet) increased body weight and lymphoid organ weights to their maximum size and weight relative to body weight, suggesting that the 0.73% arginine diet was sufficient for the development of body and lymphoid organs of K-strain chickens. The influence of arginine on the thymus is compatible with the findings in mammals, including humans, in which arginine supplementation has been reported to result in increased thymus weight, cellularity, T-cell blastogenic responsiveness, and expression of interleukin-2 receptor on activated T cells (Barbul *et al.*, 1981a,b; Reynolds *et al.*, 1988b; Daly *et al.*, 1990; Kirk *et al.*, 1992). However, the present study in the chicken offered the opportunity to examine the effects of dietary arginine on all three lymphoid organs. Thymus size is known to be a sensitive indicator of health and of acute and chronic stress response (Morale *et al.*, 1995; Shelat *et al.*, 1997). The growth of the thymus was affected to the greatest extent by arginine deficiency. Relative spleen weight was moderately reduced, but relative bursa

weight was not affected by arginine deficiency. Plasma amino acid profiles reflected the dietary levels of arginine. Arginine supplementation resulted in large increases in plasma arginine concentration and smaller increases in plasma ornithine concentrations. Ornithine is one of the major products of arginine metabolism in birds (Tamir and Ratner, 1963; Austic and Nesheim, 1970), and, although ornithine can be further metabolized to glutamic acid and proline (Austic and Nesheim, 1971), the increase in plasma ornithine is not unexpected.

Arginine could influence growth in several ways. For example, arginine is a primary component of proteins. Because it is only derived from the diet in birds, a dietary deficiency of arginine can have a direct effect on protein synthesis at the level of translation. Second, arginine has a secretagogue activity by which it stimulates the release of pituitary and pancreatic hormones, including glucagon, insulin, and growth hormone. This induced-hormone production could increase protein synthesis and feed consumption (Floyd *et al.*, 1966; Rocha *et al.*, 1972; Franchimont and Burger, 1975; Palmer *et al.*, 1975; Davila *et al.*, 1987). Third, an arginine effect might occur through the formation of ornithine, a polyamine precursor by which ornithine can lead to increased DNA synthesis and cell proliferation (Pegg and McCann, 1982). Because the ornithine concentration in plasma was lowest in chicks fed the arginine-deficient basal diet, some of the observed biological effects could involve ornithine.

Although this study indicates a marked effect of arginine on lymphoid organ development as well as body growth in chickens, it remains unclear whether the beneficial effects of arginine intake on the immune organs of

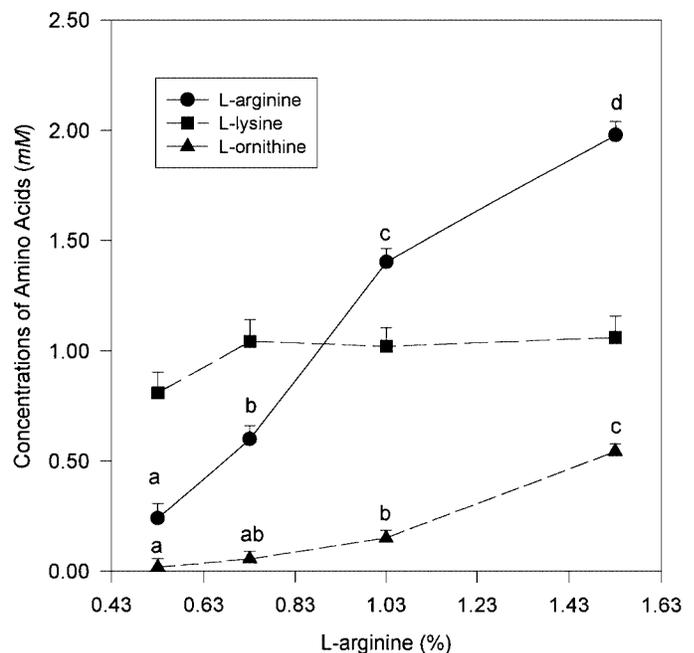


FIGURE 3. Amino acid concentrations in blood plasma. ^{a-d}Means without a common letter within amino acid are different within amino acid ($P < 0.05$). Error bars represent standard errors. Data are from two experiments performed on two hatches of K-strain chickens ($n = 8$).

chickens occur through a direct arginine-lymphoid organ interaction or through indirect effects caused by changes in neuroendocrine status. The results suggest that lymphoid compartments differ in their responses to arginine deficiency in chicken. The thymus is more sensitive to different arginine concentrations than is the bursa of Fabricius, and an arginine effect on immunity might be primarily through a thymic-dependent process of the immune response.

The current study was performed on a single genetic strain (Cornell K Strain). It is likely that genetic background would determine the precise relationships among dietary arginine requirements, availability of dietary arginine to physiological systems (including the neurological, endocrine, and immune systems), and optimized immune function. Therefore, some strain variation would be expected. A comparative examination of additional genetic backgrounds, including different B complex haplotypes, could be very informative. Additionally, a more complete understanding of the cellular mechanisms involved in arginine-immune interactions will increase the eventual utility and application of dietary approaches to achieve optimal immune responses.

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