STUDIES ON POTASSIUM-LYSINE INTERRELATIONSHIPS IN BROILER CHICKS

2. EFFECT OF POTASSIUM-LYSINE INTERRELATIONSHIPS ON BLOOD PARAMETER, SERUM AND BONE COMPOSITION

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Summary

To determine the effect of dietary potassium and lysine levels on blood parameters, serum and bone composition, 360 male broiler chicks of 3 days of age were used in a completely randomized 3×3 factorial experiment for 6 weeks. Experimental diets contained three supplemented levels of dietary potassium (0.3, 0.6 and 1.2%) and three supplemented levels of dietary lysine (0.6, 1.2 and 2.4%). Dietary levels of potassium and lysine did not influence blood pH, pCO_2 , pO_2 , HCO_3 and total CO_2 and interaction between potassium and lysine was not shown ($p > 0.05$). Serum lysine and arginine contents were significantly different by the levels of dietary lysine ($p < 0.05$). Lysine-arginine antagonism was observed in high lysine diet. But increasing dietary potassium did not alleviate the lysine-arginine antagonism. Serum sodium, potassium and chloride were not affected by dietary potassium and lysine levels ($p > 0.05$). Femur weight, length and P contents were affected by the levels of dietary lysine ($p < 0.01$). But no difference was observed in femur ash and Ca contents ($p > 0.05$). Interaction between potassium and lysine was shown in ash and P contents ($p < 0.01$).

(Key Words: Potassium, Lysine, Interrelationship, Broiler)

Introduction

It is now generally recognized that the addition of excess lysine to the young chick increases the requirement for arginine (Jones, 1964; O'Dell et al., 1966). This increased requirement is manifested by decreased growth rate, symptoms of arginine deficiency, and decreased plasma arginine level (Jones, 1964). Several investigators demonstrated that the toxicity was due to a lysine-arginine antagonism and the cation-anion balance in the diet affected this antagonism in the chick (O'Dell et al., 1966; Stutz et al., 1971). When casein is the chief source of amino acids, supplementation with sodium and potassium salt produces marked growth stimulation. Electrolyte, especially potassium is the principal cation of the cell. This is replaced in part by sodium ions and is accompanied by an increase in the concentration of basic free amino acids in tissue (Eckel et al., 1958; Brandt et al., 1960). It is evident

that dietary cations tend to counteract the detrimental effect of excess lysine, and there appears to be a relationship at the cellular level between fixed cations and such amino acids as arginine and lysine.

Materials and Methods

Experimental designs and procedure used in the present studies were same as used in experimental 1 (effect of potassium-lysine interrelationship on growth performance and nutrient utilization) except those for blood and bone analysis.

Blood and bone collection were made after feeding trials. Four birds per treatment were randomly sacrificed for blood and bone collections. Blood samples were collected from the carotid artery with 23-gauge needle attached to 1 ml heparized syringe. Within 15 minutes following blood collection, blood samples were analyzed for pH, pCO_2 , pO_2 , base excess, bicarbonate and total carbon dioxide using an Automated Blood Gas Analyzer (Instrumental Laboratory, Model IL 813) calibrated for chicken blood. A portion of blood sample was centrifuged at 1200 g for 15 minutes. The supernatants were separated, sealed and preserved in the refrigerator until

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laboratory assays were conducted. The serum proteins were precipitated with a ten percent trichloroacetic acid solution. And the serum proteins were precipitated with a sulfosalicylic acid solution for serum amino acid analysis. Serum lysine and arginine levels were analysed by automatic amino acid analyser (LKB, Model 1450-alpha). An aliquot of clear supernatant obtained by centrifugation was used to determine the serum sodium, potassium and chloride. Serum sodium, potassium levels were measured by using atomic absorption spectrophotometer (Shimadzu, Model AA625) and chloride levels were determined by titration method (Schaes and Schaes, 1941). Bone samples were removed from the right femur per chick. The removed bone was soaked in boiling water for 4 minutes to facilitate scaling process of adhered muscle and tendons, and dried in an air-forced drying oven for 72 hours at 60°C. After measuring weight and length of femur, fat was extracted for 12 hours with petroleum ether in a Soxhlet apparatus. The femur were the ashed individually in a muffle furnace at 550°C for 2 hours, and the crude ash, Ca and

P contents were expressed as a percent of moisture-free, fat-free bone. Proximal analyses and minerals of experimental diets and bones were conducted according to the methods of AOAC (1984).

Results and Discussion

Blood parameters obtained in this experiment are shown in table 1. Blood pH, pCO₂, pO₂, HCO₃⁻ and total CO₂ was not affected by increasing dietary potassium or lysine levels. This result does not agree with other reports that potassium has an alkalogenic effect (Cohen et al., 1972; Hamilton and Thompson, 1980; Johnson and Karunajedwa, 1985; Leach and Nesheim, 1972). Several investigators have shown significant changes in blood pH and bicarbonate concentration when the electrolyte balance of the diet was manipulated. Cohen et al. (1972) found a significant decrease in pH, pCO₂ and bicarbonated concentration with low Na+K/Cl diet. Cohen and Hurwiz (1974) observed an increase in blood pH and bicarbonate concentration with Na or

TABLE 1. BLOOD VALUES OF BROILER FED DIETS WITH DIFFERENT LEVELS OF LYSINE AND POTASSIUM

Treatment	pH ²	pCO ₂	pO ₂	HCO ₃	Total CO ₂
		 mm Hg			
LL-0.3	7.31 ± .08 ^{NS1}	38.57 ± 8.3 ^{NS}	60.50 ± 14.5 ^{NS}	18.42 ± .92 ^{NS}	19.57 ± 1.12 ^{NS}
LL-0.6	7.27 ± .10 ^{NS}	42.87 ± 14.1 ^{NS}	58.37 ± 14.3 ^{NS}	18.45 ± 3.22 ^{NS}	19.73 ± 3.54 ^{NS}
LL-1.2	7.29 ± .02 ^{NS}	37.70 ± 5.1 ^{NS}	65.67 ± 18.0 ^{NS}	17.72 ± 2.64 ^{NS}	18.85 ± 2.77 ^{NS}
OL-0.3	7.26 ± .07 ^{NS}	41.00 ± 6.9 ^{NS}	46.20 ± 20.0 ^{NS}	17.75 ± 1.75 ^{NS}	18.98 ± 1.85 ^{NS}
OL-0.6	7.33 ± .10 ^{NS}	34.07 ± 10.5 ^{NS}	63.43 ± 13.8 ^{NS}	17.01 ± 1.72 ^{NS}	18.03 ± 1.96 ^{NS}
OL-1.2	7.28 ± .09 ^{NS}	43.63 ± 4.4 ^{NS}	43.93 ± 5.2 ^{NS}	19.66 ± 2.28 ^{NS}	20.97 ± 2.17 ^{NS}
HL-0.3	7.27 ± .09 ^{NS}	44.80 ± 10.9 ^{NS}	65.70 ± 12.6 ^{NS}	19.41 ± .16 ^{NS}	20.76 ± .45 ^{NS}
HL-0.6	7.25 ± .03 ^{NS}	43.70 ± 8.3 ^{NS}	63.60 ± 4.9 ^{NS}	18.58 ± 3.76 ^{NS}	19.89 ± 4.00 ^{NS}
HL-1.2	7.30 ± .07 ^{NS}	38.33 ± 2.2 ^{NS}	60.33 ± 20.7 ^{NS}	18.36 ± 3.10 ^{NS}	19.50 ± 3.13 ^{NS}
Among lysine levels					
LL	7.29 ± .07 ^{NS}	39.71 ± 8.9 ^{NS}	61.51 ± 14.0 ^{NS}	18.20 ± 2.06 ^{NS}	19.39 ± 2.35 ^{NS}
OL	7.29 ± .08 ^{NS}	39.70 ± 7.8 ^{NS}	50.69 ± 15.9 ^{NS}	18.10 ± 2.02 ^{NS}	19.29 ± 2.13 ^{NS}
HL	7.28 ± .06 ^{NS}	41.56 ± 6.4 ^{NS}	62.61 ± 13.9 ^{NS}	18.66 ± 2.71 ^{NS}	19.91 ± 2.82 ^{NS}
Among K (KHCO ₃) supplemented levels					
0.3	7.29 ± .07 ^{NS}	41.02 ± 7.3 ^{NS}	55.30 ± 17.1 ^{NS}	18.34 ± 1.35 ^{NS}	19.57 ± 1.47 ^{NS}
0.6	7.28 ± .08 ^{NS}	41.20 ± 10.7 ^{NS}	61.80 ± 10.6 ^{NS}	18.01 ± 2.73 ^{NS}	19.21 ± 2.98 ^{NS}
1.2	7.29 ± .06 ^{NS}	39.73 ± 4.4 ^{NS}	57.01 ± 17.5 ^{NS}	18.56 ± 2.56 ^{NS}	19.75 ± 2.61 ^{NS}

¹ NS. Means non-significant.

² Values are mean ± SD; n=5.

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K supplementation and a decrease in the same parameters with excess dietary chloride. The reason why the dietary potassium did not influence acid-base balance, may be due that the difference of electrolyte level between treatments was too narrow the change the acid-base balance of blood. The highest pH value was shown in optimum lysine and 0.6% potassium supplemented group (OL-0.6) and the lowest in optimum lysine and 0.3% potassium supplemented group (OL-0.3).

The concentration of serum lysine, arginine and electrolyte influenced by levels of lysine and dietary potassium are summarized in table 2. As shown in table 2, except serum amino acids, electrolyte parameters were not affected by the experimental diets ($p > 0.05$). Interaction expected between dietary lysine and potassium was found to be significant in serum lysine and arginine concentration ($p < 0.01$). The concentration of serum lysine increased by addition of lysine while

arginine decreased. The highest serum lysine content was obtained at high lysine and 1.2% potassium (HL-1.2) and the lowest at low lysine and 1.2% potassium (LL-1.2). This result was in good agreement with that of Stutz et al. (1971). Using crystalline amino acid, Zimmerman and Scott (1965) also found that levels of plasma lysine in chicks decreased inversely with the levels of dietary arginine. Shao and Hill (1961) have shown that α -aminoisobutyric acid, a presumably nonmetabolizable amino acid, has a similar effect on the concentrations of plasma arginine, lysine and ornithine of chicks fed a casein based diet. In general, serum and kidney arginine concentrations and growth rates were inversely related to lysine concentration. Excess dietary lysine interferes with arginine utilization in the chicks, at least in part, by increasing renal arginase activity which thereby increases arginine degradation (Austic and Nesheim, 1972). One purpose of this study was to test the possibility that

TABLE 2. EFFECTS OF LYSINE AND POTASSIUM ON THE SERUM LYSINE, ARGININE, SODIUM, POTASSIUM, CHLORIDE AND SODIUM PLUS POTASSIUM TO CHLORIDE RATIO IN BROILER CHICKS

Treatment	Lysine ⁴ (μ g/ml)	Arginine (μ g/ml)	Sodium	Potassium	Chloride mEq/liter	Na+K-Cl
LL-0.3	2.46 $\pm$ 1.2 ^{BC1}	2.30 $\pm$ 6.8 ^{b2}	30.43 $\pm$ 1.2 ^{NS3}	15.35 $\pm$ 1.0 ^{NS}	108.20 $\pm$ 3.5 ^{NS}	-62.4 $\pm$ 3.2 ^{NS}
LL-0.6	2.51 $\pm$ 1.4 ^{BC}	2.57 $\pm$ 7.0 ^b	44.93 $\pm$ 9.1 ^{NS}	35.81 $\pm$ 1.2 ^{NS}	77.53 $\pm$ 7.9 ^{NS}	3.2 $\pm$ 5.9 ^{NS}
LL-1.2	1.04 $\pm$ 3.6 ^C	2.86 $\pm$ 1.0 ^a	44.93 $\pm$ 1.8 ^{NS}	43.48 $\pm$ 2.0 ^{NS}	101.87 $\pm$ 2.9 ^{NS}	-39.5 $\pm$ 3.8 ^{NS}
OL-0.3	1.97 $\pm$ 1.1 ^C	1.20 $\pm$ 4.2 ^{cd}	41.31 $\pm$ 9.2 ^{NS}	42.20 $\pm$ 2.7 ^{NS}	144.20 $\pm$ 2.8 ^{NS}	-60.7 $\pm$ 3.9 ^{NS}
OL-0.6	2.67 $\pm$ 2.6 ^{BC}	2.05 $\pm$ 7.8 ^b	49.27 $\pm$ 1.3 ^{NS}	38.36 $\pm$ 1.3 ^{NS}	120.53 $\pm$ 1.1 ^{NS}	-32.9 $\pm$ 2.1 ^{NS}
OL-1.2	1.87 $\pm$ 4.7 ^C	1.12 $\pm$ 2.0 ^d	50.73 $\pm$ 1.3 ^{NS}	37.51 $\pm$ 2.0 ^{NS}	114.87 $\pm$ 1.6 ^{NS}	-57.9 $\pm$ 2.5 ^{NS}
HL-0.3	4.14 $\pm$ 1.3 ^{BC}	1.50 $\pm$ 6.3 ^c	44.93 $\pm$ 1.4 ^{NS}	29.84 $\pm$ 1.5 ^{NS}	128.87 $\pm$ 2.4 ^{NS}	-54.1 $\pm$ 4.8 ^{NS}
HL-0.6	2.91 $\pm$ 6.9 ^{BC}	0.96 $\pm$ 1.1 ^d	58.70 $\pm$ 1.5 ^{NS}	71.62 $\pm$ 3.6 ^{NS}	116.20 $\pm$ 4.2 ^{NS}	14.0 $\pm$ 2.1 ^{NS}
HL-1.2	6.38 $\pm$ 1.2 ^A	1.40 $\pm$ 3.8 ^c	47.83 $\pm$ 8.7 ^{NS}	45.18 $\pm$ 5.9 ^{NS}	150.87 $\pm$ 1.6 ^{NS}	-57.9 $\pm$ 2.5 ^{NS}
Among lysine levels						
LL	1.88 $\pm$ 1.1 ^B	2.25 $\pm$.54 ^a	42.85 $\pm$ 12.9 ^{NS}	36.17 $\pm$ 17 ^{NS}	92.34 $\pm$ 47 ^{NS}	-13.31 $\pm$ 47 ^{NS}
OL	2.19 $\pm$ 0.5 ^B	1.46 $\pm$.63 ^b	47.83 $\pm$ 11.4 ^{NS}	39.00 $\pm$ 16 ^{NS}	124.33 $\pm$ 16 ^{NS}	-37.49 $\pm$ 27 ^{NS}
HL	4.67 $\pm$ 1.8 ^B	1.29 $\pm$.45 ^a	49.46 $\pm$ 12.1 ^{NS}	46.04 $\pm$ 24 ^{NS}	133.95 $\pm$ 26 ^{NS}	-38.49 $\pm$ 44 ^{NS}
Among K (KHCO ₃) supplemented levels						
0.3	3.14 $\pm$ 1.4 ^{NS}	1.67 $\pm$.43 ^{NS}	41.30 $\pm$ 11.3 ^{NS}	31.55 $\pm$ 18 ^{NS}	130.53 $\pm$ 20 ^{NS}	-57.69 $\pm$ 35 ^{NS}
0.6	2.67 $\pm$ 0.8 ^{NS}	1.86 $\pm$.72 ^{NS}	50.00 $\pm$ 11.9 ^{NS}	45.72 $\pm$ 12 ^{NS}	103.33 $\pm$ 50 ^{NS}	- 7.64 $\pm$ 41 ^{NS}
1.2	3.10 $\pm$ 2.6 ^{NS}	1.79 $\pm$.32 ^{NS}	47.83 $\pm$ 12.3 ^{NS}	42.06 $\pm$ 15 ^{NS}	122.53 $\pm$ 25 ^{NS}	-32.64 $\pm$ 33 ^{NS}

¹ A,B,C: Mean values with different superscript within the same column are significantly different ($p < .01$).

² a,b,c,d: Mean values with different superscript within the same column are significantly different ($p < .05$).

³ NS: Means non-significant.

⁴ Values are mean $\pm$ SD; n=4.

dietary levels of potassium may alleviate the lysine-arginine antagonism for chick growth. Some report demonstrated that potassium and sodium were the most effective metal cations in alleviating the lysine-arginine antagonism if added to the diet in conjunction with metabolite such as acetate or bicarbonate. Increasing dietary chloride exacerbates the lysine-arginine antagonism but has no effect on plasma lysine or arginine concentration (Calvert et al., 1981). In the role of dietary cations on lysine-arginine antagonism, high levels of dietary potassium decrease kidney arginase activity, increase muscle protein synthesis and decrease bacterial urease activity in chicks (Stutz et al., 1972). But the results showed that supplemented potassium did not affect the lysine and arginine levels in serum ($p > 0.05$). The reason why the supplemented potassium did not alleviate lysine-arginine antagonism may be due that the supplemented potassium levels were too small to affect the phenomena.

Among the potassium levels, serum sodium, potassium and chloride were not affected by the supplemented potassium levels ($p > 0.05$). Dietary lysine also did not affect serum sodium, potassium and chloride level ($p > 0.05$). Potassium $\times$ lysine interaction was not found in serum sodium, potassium and chloride ($p > 0.05$).

Femur bone formation and calcification as influenced by level of dietary potassium and lysine are summarized in table 3 and shown in figure 1. Generally, birds having long tibia and femur were heavier than those having short ones.

Optimum lysine and 1.2% potassium supplemented group (OL-1.2) showed the highest bone weight, length ($p < 0.01$) and crude ash contents ($p < 0.05$) with significant difference. The lowest bone weight and length were shown in high lysine and 0.6% potassium supplemented group (HL-0.6). Bone calcium in femur was not influenced by treatment. Dietary potassium levels did not affect femur weight, and crude ash, Ca and P

TABLE 3. EFFECTS OF LYSINE AND POTASSIUM ON THE FEMUR BONE IN BROILER CHICKS (DEFATTED DRY MATTER BASIS)

Treatment	Weight ¹ (g)	Length (cm)	Ash content (%)	Ca (%)	P (%)
LL-0.3	4.16 $\pm$.5 ^{BC1}	6.60 $\pm$.17 ^{ABC}	50.47 $\pm$ 1.7 ^{abc2}	25.83 $\pm$ 13.9 ^{NS3}	10.25 $\pm$.43 ^C
LL-0.6	3.96 $\pm$.9 ^{BC}	6.17 $\pm$.12 ^C	47.67 $\pm$ 1.8 ^{cd}	26.58 $\pm$ 18.4 ^{NS}	9.34 $\pm$.33 ^D
LL-1.2	3.56 $\pm$ 1.2 ^{CD}	6.22 $\pm$.47 ^C	48.86 $\pm$ 2.8 ^{bcd}	15.33 $\pm$ 2.5 ^{NS}	10.27 $\pm$.34 ^C
OL-0.3	4.67 $\pm$.47 ^{ABC}	6.75 $\pm$.05 ^{AB}	46.30 $\pm$ 3.2 ^d	16.01 $\pm$.9 ^{NS}	9.42 $\pm$.59 ^D
OL-0.6	5.06 $\pm$.45 ^{AB}	6.50 $\pm$.26 ^{BC}	52.71 $\pm$ 1.5 ^{ab}	15.33 $\pm$.6 ^{NS}	8.95 $\pm$.28 ^D
OL-1.2	5.61 $\pm$.85 ^A	7.03 $\pm$.32 ^A	53.18 $\pm$ 1.1 ^a	16.74 $\pm$ 1.1 ^{NS}	8.98 $\pm$.15 ^D
HL-0.3	2.29 $\pm$.46 ^E	5.37 $\pm$.31 ^{DE}	51.96 $\pm$ 2.8 ^{ab}	18.52 $\pm$ 1.1 ^{NS}	10.41 $\pm$.47 ^{BC}
HL-0.6	1.89 $\pm$.22 ^E	5.10 $\pm$.22 ^E	49.24 $\pm$ 2.8 ^{abcd}	20.15 $\pm$ 2.1 ^{NS}	11.25 $\pm$.35 ^A
HL-1.2	2.57 $\pm$.19 ^{DE}	5.70 $\pm$.00 ^D	51.85 $\pm$.7 ^{ab}	19.62 $\pm$ 2.4 ^{NS}	11.03 $\pm$.15 ^{AB}
Among lysine levels					
LL	3.89 $\pm$.85 ^B	6.33 $\pm$.25 ^B	49.00 $\pm$ 2.1 ^{NS}	22.58 $\pm$ 11.6 ^{NS}	10.03 $\pm$.37 ^B
OL	5.12 $\pm$.59 ^A	6.76 $\pm$.21 ^A	50.73 $\pm$ 1.9 ^{NS}	16.03 $\pm$.87 ^{NS}	9.12 $\pm$.34 ^C
HL	6.75 $\pm$.29 ^C	5.39 $\pm$.18 ^C	51.02 $\pm$ 2.1 ^{NS}	19.43 $\pm$ 1.9 ^{NS}	10.90 $\pm$.32 ^A
Among K (KHCO ₃) supplemented levels					
0.3	3.71 $\pm$.47 ^{NS}	6.24 $\pm$.18 ^A	49.58 $\pm$ 2.57 ^{NS}	20.12 $\pm$ 5.30 ^{NS}	10.03 $\pm$.50 ^{NS}
0.6	3.64 $\pm$.52 ^{NS}	5.92 $\pm$.19 ^B	49.87 $\pm$ 2.03 ^{NS}	21.35 $\pm$ 7.03 ^{NS}	9.85 $\pm$.32 ^{NS}
1.2	3.91 $\pm$.75 ^{NS}	6.32 $\pm$.26 ^A	51.30 $\pm$ 1.53 ^{NS}	17.23 $\pm$ 2.00 ^{NS}	10.09 $\pm$.21 ^{NS}

¹ A,B,C,D,E: Mean values with different superscript within the same column are significantly different ($p < .01$).

² a,b,c,d: Mean values with different superscript within the same column are significantly different ($p < .05$).

³ NS: Means non-significant.

⁴ Values are mean $\pm$ SD; n=4.

Figure 1. Effect of lysine and potassium levels on femur growth in Broiler chicks
(A: LL-0.3, B: LL-0.6, C: LL-1.2,
D: OL-0.3, E: OL-0.6, F: OL-1.2,
G: HL-0.3, H: HL-0.6, I: HL-1.2).

contents. But femur weight was influenced by dietary potassium levels ($p < 0.01$). These results are accordance with those of Pepper et al. (1952). Dietary lysine levels affected femur weight, femur length and P content with significant difference ($p < 0.01$). But like dietary potassium, the lysine levels did not influence the crude ash and Ca contents. Interactions between lysine and potassium were significantly shown in crude ash and P contents ($p < 0.01$), but in femur weight, length and Ca contents, there were no significant differences. Increasing potassium supplemented levels caused a gradual increase in bone ash, even though there was no significant difference. This result was in agreement with those of Hardy (1984), Pepper et al. (1952) and Gardiner (1962). Gillis (1948) reported a reduction of bone ash contents in potassium-deficient chicks. Later, Gillis (1952) suggested that the reduction in calcification was due to that a potassium deficiency was mediated by phosphorus rather than calcium.

In conclusion, it would be suggested that dietary potassium levels between 183.90 and 275.97 mEq/kg influenced bone calcification in the growing broiler chicks but dietary potassium levels did not affect blood parameters. Dietary lysine levels between 0.6 and 2.4% influenced serum amino acids and bone calcification but did not affect blood parameters.

There was interaction between potassium and lysine on crude ash and P contents in femur, but blood parameters were not affected by potassium $\times$ lysine interaction.

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