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Akmed S. Hussein
University of Kentucky

Austin H. Cantor
University of Kentucky, acantor@uky.edu

Thomas H. Johnson
University of Kentucky

Robert A. Yokel
University of Kentucky, ryokel@email.uky.edu

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Relationship of Dietary Aluminum, Phosphorus, and Calcium to Phosphorus and Calcium Metabolism and Growth Performance of Broiler Chicks[1]

AHMED S. HUSSEIN,[2,3] AUSTIN H. CANTOR,[2,4] THOMAS H. JOHNSON,[2] and ROBERT A. YOKEL[5]

Department of Animal Sciences and Division of Pharmacology and Toxicology, University of Kentucky, Lexington, Kentucky 40546-0215

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ABSTRACT Dietary treatments providing three levels of added Al (0, .196, or .392%) as aluminum sulfate and of available phosphorus (P_{av}) (.45, .68, or .78%) in a factorial arrangement were administered to day-old chicks in Experiment 1. Plasma inorganic phosphorus (P_i) was significantly ($P<.05$) elevated by increasing P_{av} and was decreased by Al. Body weight gain, feed intake, and the gain:feed ratio at Day 21 were significantly decreased by increased concentrations of Al, but were unaffected by the P_{av} concentrations. Decreases of 39 and 73% in weight gain and of 34 and 66% in feed intake resulted from feeding .196 and .392% Al, respectively.

In Experiment 2, day-old chicks were fed diets supplemented with 0 or .392% Al in combination with .9% Ca plus .45% P_{av}, .9% Ca plus .78% P_{av}, 1.8% Ca plus .45% P_{av}, or 1.8% Ca plus .9% P_{av}. After 21 days, the supplemental Al resulted in: 1) significantly poorer growth performance; 2) decreased plasma P_i, total Ca, Zn, and Mg; and 3) decreased tibia weight and breaking strength. Elevating P_{av} improved growth performance, plasma P_i, and tibia weight and strength, and decreased plasma total Ca. Increasing dietary Ca significantly decreased plasma P_i and increased plasma total Ca without affecting other parameters. Increasing P_{av} alleviated the negative effect of Al on plasma P_i without correcting the negative effect of Al on growth performance.

(*Key words*: aluminum, phosphorus, calcium, broiler chicks)

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INTRODUCTION

The element aluminum, when given at high levels in the diet, negatively affects growth, feed intake, mineral metabolism, and the egg production of birds. Deobald and Elvehjem (1935) observed rickets as well as reduced growth, bone ash, and blood P in response to feeding high levels of soluble aluminum salts to Leghorn chicks. Additional dietary phosphate corrected the depression in growth and bone formation.

Storer and Nelson (1968) found that feeding Single Comb White Leghorn (SCWL) chicks .5% soluble Al as the acetate, chloride, nitrate, or sulfate salts caused high mortality. Lower concentrations of Al (.1 to .4% as the sulfate) decreased growth, bone ash, and feed efficiency. Similar results were obtained when Al was supplied as the chloride, but not when Al was provided in the form of insoluble salts.

Depressed performance of White Rock chicks was observed by Lipstein and Hurwitz (1981) when feeding algae that had been flocculated with alum (aluminum potassium sulfate). The algae, providing 1.325% Al in the diet, decreased plasma inorganic phosphate (P_i), body weight gain, and feed efficiency. In another study, Lipstein and Hurwitz (1982) observed that feeding diets with .5% Al, provided by 10% algae meal, depressed the tibia ash, tibia P, and plasma P_i of White Rock chicks. These effects were alleviated by providing supplemental dietary phosphate. The authors indicated that .76 g of supplemental P was required to counteract the effect resulting from each gram of Al.

The effect of Al on P metabolism is thought to be due to an interaction in the gastrointestinal tract, resulting in the formation of insoluble aluminum phosphate (Cox *et al.*, 1931; Street, 1942; Storer and Nelson, 1968; Lipstein and Hurwitz, 1982). This concept is supported

[1]Journal Paper Number 89-5-83, Kentucky Agricultural Experiment Station, Department of Animal Sciences.

[2]Department of Animal Sciences.

[3]Present address: Nutrition Laboratory, Department of Human Ecology, University of Maryland Eastern Shore, Princess Anne, MD 21853.

[4]Corresponding author.

[5]Division of Pharmacology and Toxicology.

TABLE 1. *Composition of the experimental diets*

	Diet[1]						
	A	B	C	D	E	F	G
	(% of diet)						
Calcium	.90	.90	.90	.90	.90	1.80	1.80
Available phosphorus	.45	.68	.78	.45	.78	.45	.90
Ingredient							
Ground yellow corn	56.91	54.23	53.70	55.30	53.80	50.12	48.50
Dehulled soybean meal	34.00	34.96	35.06	34.77	35.04	35.71	35.65
Animal vegetable fat	4.61	5.83	6.02	5.45	5.99	7.31	8.00
Salt	.52	.53	.53	.52	.52	.52	.52
Ground limestone	1.08	.30	.00	1.06	.00	3.42	1.90
Dicalcium phosphate (22% Ca, 18.5% P)	1.67	2.94	3.48	1.69	3.44	1.71	4.22
Vitamin-mineral premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-methionine (99%)	.21	.21	.21	.21	.21	.21	.21

[1]Calculated nutrient composition: ME_n, 3,150 kcal per kg; CP, 21.5%; methionine, .55 to 56%; methionine + cystine, .91%; and lysine, 1.19 to 1.20%.

[2]Provided the following per kilogram of diet: vitamin A, 6,000 IU; vitamin D_3, 1,000 ICU; vitamin E, 15 IU; menadione dimethylpyrimidinol bisulfite, 4.4 mg; thiamin, 2.7 mg; riboflavin, 5.4 mg; pantothenic acid, 15 mg; niacin, 41 mg; pyridoxine, 4.5 mg; biotin, .23 mg; choline, 1,450 mg; folacin, .83 mg; vitamin B_{12}, .014 mg; ethoxyquin, 125 mg; selenium, .2 mg; copper, 6 mg; iodine, .53 mg; iron, 120 mg; manganese, 83 mg; zinc, 60 mg; and cobalt, 5 mg.

by the aforementioned results showing alleviation from the effects of Al by supplemental dietary P; also, by data showing that Al is poorly absorbed (McCollum *et al.*, 1928; Myers and Morrison, 1928; Mackenzie, 1930, 1931).

The negative effect of Al on bone mineralization involves the metabolism of both phosphorus and calcium. The objectives of the present study were to: 1) re-examine the interaction of dietary Al and P; and 2) determine the interactive effects of dietary Al, P, and Ca on growth performance, plasma mineral concentrations, and bone mineralization.

MATERIALS AND METHODS

Experiment 1

Four replicate groups of 10 male broiler chicks, 1 day of age, were randomly assigned to each of nine dietary treatments. The chicks were housed in battery cages (76 cm wide by 102 cm long by 28 cm high). The cages were equipped with a raised wire floor, two stainless-steel feeders, and a stainless-steel water trough. Feed and water were provided for *ad libitum* intake during the 21-day experiment. The treatments consisted of diets containing three levels of available P (P_{av}) and three levels of added Al in a complete factorial arrangement. The diets were formulated to contain .45, .68, and .78% P_{av} (Table 1, Diets A, B, and C). Each diet was fed with 0, .196, or .392% Al as aluminum sulfate. On a molar basis, the highest level of Al (.392%) was equal to the lowest level of P_{av} in the diet.

Experiment 2

Three groups of 10 male chicks, 1 day of age, were randomly assigned to each of eight treatments. The chicks were housed and managed the same as in Experiment 1. The treatments consisted of feeding two levels of Al (0 or .392%, as aluminum sulfate) in combination with .9% Ca plus .45% P_{av}, .9% Ca plus .78% P_{av}, 1.8% Ca plus .45% P_{av}, or 1.8% Ca plus .9% P_{av}. The diets shown in Table 1 (Diets, D, E, F, and G) were each fed with or without added Al.

Tissue Sampling

Blood samples were obtained weekly from two chicks selected at random from each replicate group, using cardiac puncture with heparin as an anticoagulant. The samples were centrifuged (10 min at 1000 × *g*); the plasma was separated, then pooled within replicates and stored at < 0 C until analysis. At the end of each

TABLE 2. *Experiment 1: Effect of dietary aluminum and available phosphorus (P_{av}) on growth performance, bone mineralization, plasma inorganic phosphorus (P_i), and plasma total calcium (Ca_t)*[1]

Dietary treatment Al	Dietary treatment P_{av}	Weight gain	Feed intake	Gain: feed ratio	Tibia weight[2]	Breaking strength[2]	Tibia ash[2]	Plasma P_i[3]	Plasma Ca_t[3]
(%)		(g)		(g/g)	(g)	(kg)	(%)	(mg/dL)	
0	.45	599	827	.72	1.61	3.86	49.7	7.4	9.2
0	.68	592	873	.69	1.61	4.83	48.2	8.9	8.5
0	.78	564	805	.70	1.54	4.33	51.5	10.0	8.9
.196	.45	328	517	.63	1.03	3.99	48.8	4.8	9.9
.196	.68	369	568	.64	1.16	3.20	48.8	8.0	9.7
.196	.78	381	569	.67	1.00	3.12	50.0	7.5	9.0
.392	.45	141	256	.55	.40	1.61	44.0	4.7	9.1
.392	.68	164	291	.56	.50	2.56	49.5	6.3	8.6
.392	.78	175	311	.56	.57	2.44	50.2	9.0	9.1
SEM		18	9	.02	.07	.30	.9	.7	.3
Significant effects[4]		Al	Al	Al	Al	Al, Al × P_{av}	Al, P_{av}, Al × P_{av}	Al, P_{av}	Al[5]

[1]Each diet was fed to four groups of 10 chicks for 21 days.

[2]The tibiae were fat-extracted and were dried before weighing, breaking, and ashing. Each value represents the mean of four samples.

[3]Each value represents the mean of four blood samples, each pooled from two birds per replicate at Day 7.

[4]$P<.05$.

[5]Quadratic effect of Al ($P<.05$).

experiment, two chicks per replicate group were sacrificed by cervical dislocation in order to obtain tibia samples.

Plasma P_i was analyzed using the procedure reported by Hussein *et al.* (1988). Plasma total Ca (Ca_t) as well as Zn and Mg were determined by using methods provided by the Perkin-Elmer Corporation (1982). Plasma Al was measured using the method described by Oster (1981). Bone parameters were determined according to methods described by Cantor et al. (1980).

Statistical Analysis

The data were subjected to an ANOVA based on a factorial design (Experiment 1) or to a one-way ANOVA using the general linear model procedures (SAS Institute, 1982). The test of least significant difference was applied to separate means only when a significant value for F was found in the ANOVA (Snedecor and Cochran, 1980). A probability of <.05 was required for significance in all statistical analyses.

RESULTS

Experiment 1

Weight gain, feed intake, gain:feed ratio, tibia weight, tibia breaking strength, and tibia ash were all significantly decreased by the addition of dietary Al (Table 2). Added Al produced a significant linear effect on all of these parameters. The negative effect of Al on breaking strength was partially counteracted by increasing the level of dietary P_{av}, resulting in a significant Al-by-P_{av} interaction. Higher Pav also corrected the depression in the percentage of bone ash caused by .392% Al.

Plasma P_i was significantly reduced by Al and was increased by P_{av} as early as Day 7 (Table 2). Similar effects were noted at Days 14 and 21. Thus, higher concentrations of P_{av} alleviated the depression in that parameter due to Al. A significant interaction between Al and P_{av} on plasma P_i was observed at 21 days of age. The addition of Al to the diet containing .45% available P significantly depressed plasma P_i, but had little or no effect on the chicks fed diets with higher levels of P_{av}. Plasma Ca_{at} was higher for the birds receiving .196% Al than for those given 0% Al or .392% Al, resulting in a significant quadratic effect for Al at Day 7. There were no significant treatment effects on Ca_{at} measured at Day 14 or at Day 21.

Experiment 2

Dietary Al decreased the weight gain, feed intake, gain:feed ratio, tibia weight, tibia breaking strength, and tibia ash (Table 3). Increasing

TABLE 3. *Experiment 2: The effect of dietary aluminum, calcium, and available phosphorus (P_{av}) on growth performance and bone mineralization*[1]

Dietary treatment			Weight gain	Feed intake	Gain:feed ratio	Tibia weight	Tibia strength	Tibia ash
Al	Ca	P_{av}						
(%)			(g)		(g/g)	(g)	(kg)	(%)
0	.90	.45	593^a	822^a	$.724^a$	1.93^a	5.58^{ab}	47.4^{ab}
0	.90	.78	560^a	758^b	$.739^a$	1.96^a	6.92^a	45.9^b
0	1.80	.45	510^b	684^c	$.745^a$	1.60^b	5.84^{ab}	43.6^b
0	1.80	.90	587^a	811^{ab}	$.725^a$	2.10^a	6.71^{ab}	50.5^a
.392	.90	.45	101^{de}	203^e	$.487^c$	$.38^d$	1.78^c	32.1^c
.392	.90	.78	120^d	227^e	$.487^c$	$.41^d$	2.46^c	39.6^c
.392	1.80	.45	76^e	200^e	$.382^d$	$.37^d$	1.83^c	27.4^c
.392	1.80	.90	178^c	288^d	$.618^b$	$.69^c$	4.83^b	46.3^{ab}
SEM			15	18	.026	.08	.68	1.6

[a–e]Means within a measurement with no common superscript are significantly different (P<.05).

[1]Each diet was fed to three groups of 10 chicks for 21 days. Tibiae (two per replicate group) were fat-extracted and dried before weighing, breaking and ashing.

dietary P_{av} increased weight gain, feed intake, tibia weight, and tibia ash in the presence of 1.8% Ca. When the diet contained 1.8% Ca plus .392% Al, those four parameters plus the gain: feed ratio and tibia breaking strength were increased by elevating dietary P_{av}. As observed in Experiment 1, increasing P_{av} alleviated the depression in tibia ash and breaking strength due to dietary Al. After 1 wk of treatment, chicks given diets containing the combination of .392% Al, .45% P_{av}, and 1.8% Ca had a high incidence of rickets, which was not observed when the lower level of dietary Ca was used.

Plasma P_i was significantly decreased by Al in the presence of the lower level of dietary P_{av} at Days 7, 14, and 21 when the diet contained .90% Ca and at Days 14 and 21 when the diet contained 1.8% Ca (Table 4). In diets containing .45% P_{av}, feeding 1.8% Ca decreased plasma P_i at Days 7, 14, and 21. Significant improvements in plasma P_i were obtained by elevating the dietary P_{av}. The lowest value for this parameter was observed in chicks given the diet with .392% Al, .45% P_{av}, and 1.8% Ca. The depression in plasma P_i obtained by feeding the diet containing 1.8% Ca plus .45% P_{av} was equivalent to that due to adding Al to the diet containing .90% Ca plus .45% P_{av}. In general, plasma Ca_t was significantly increased by Al at Days 7 and 14. This parameter, on average, was increased by the high level of Ca and was decreased by the high level of P_{av} at Days 7, 14, and 21, respectively. Dietary Al decreased plasma zinc with all combinations of Ca and P_{av} and decreased plasma magnesium in chicks given .9% Ca with .45% P_{av} and 1.8% Ca with .90% P_{av}. Plasma Al was unaffected by the dietary treatments.

DISCUSSION

The negative effect of Al on plasma P_i in these experiments was similar to results obtained by Storer and Nelson (1968), by Lipstein and Hurwitz (1981, 1982), and by miles and Rossi (1985). This negative effect was alleviated by increasing dietary P_{av}, which agrees with observations made by Deobald and Elvehjem (1935) and by Lipstein and Hurwitz (1981, 1982). Increasing the concentration of dietary Ca had a negative effect on plasma P_i when fed in combination with the lower level of P_{av}, similar to the effect of dietary Al. The depression in plasma P_i was most severe when Al was also in the diet.

In the present study, dietary Al and Ca both increased plasma Ca_t (Table 4). However, increasing the concentration of dietary P_{av} tended to decrease that parameter. Alterations in Ca and P metabolism have also been observed in humans ingesting Al-containing antacids for long periods of time. These changes, including hypophosphatemia, hypophosphaturia, and hypercalciuria, were observed by Lotz *et al.* (1968), Pronove *et al.* (1961), and Spencer *et al.* (1982). They suggested that the hypercalciuria resulted both from increased intestinal Ca absorption and from bone demineralization.

Hulan *et al.* (1985) reported that tibia dry weight, tibia breaking strength, and tibia ash were maximized by feeding diets with 1.45% Ca and .67% available P to broiler chicks. These levels are considerably higher than the requirements (.9% Ca and .45% P, respectively) stated by the National Research Council (1984). In Experiment 2, the highest values for

TABLE 4. *Experiment 2: The effect of dietary treatments on plasma inorganic phosphorus (P_i), total calcium (Ca_t), zinc, magnesium, and aluminum*[1]

Dietary treatment			Plasma P_i			Plasma Ca_t			Plasma Mg	Plasma Zn	Plasma Al
Al	Ca	P_{av}	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	Day 21	Day 21	Day 21
(%)			(mg/dL)							(µg/dL)	
0	.90	.45	5.8[a]	5.9a	4.6[a]	6.9[cd]	9.5[bc]	8.1[c]	2.99[ab]	72.0[cd]	7.6
0	.90	.78	5.5[a]	5.5[ab]	4.7[a]	6.3[d]	7.8[cd]	7.9[c]	2.92[ab]	88.4[ab]	9.6
0	1.80	.45	2.6[cd]	3.5[c]	2.4[b]	10.1[b]	12.8[a]	10.5[ab]	2.79[bc]	96.0[a]	10.3
0	1.80	.90	6.1[a]	6.1[a]	4.7[a]	7.1[cd]	7.1[cd]	8.6[c]	3.13[a]	79.7[bc]	5.9
.392	.90	.45	2.6[cd]	3.2[c]	2.7[b]	9.1[bc]	10.0[b]	9.5[abc]	2.51[c]	56.5[e]	10.2
.392	.90	.78	3.6[bc]	5.0[ab]	3.9[a]	8.9[bc]	9.0[bc]	8.0[c]	2.69[bc]	57.8[e]	8.7
.392	1.80	.45	1.0[d]	.8[d]	1.2[c]	13.0[a]	13.2[a]	10.8[a]	2.75[bc]	54.8[e]	8.5
.392	1.80	.90	5.3[ab]	4.2[bc]	4.5[a]	6.9[cd]	8.8[bc]	9.0[bc]	2.78[bc]	60.6[de]	5.0
Pooled SEM			.6	.5	.4	.8	.6	.6	.11	4.0	1.7

[a–e]Means within a column with no common superscript are significantly different ($P<.05$).

[1]Each diet was fed to three groups of 10 chicks for 21 days. Each value represents the mean of three samples, each pooled from two birds per replicate.

tibia weight and tibia ash were noted in the chicks given the diet with 1.8% Ca and 9% P_{av} without Al.

In the present study, increasing P_{av} in the presence of dietary Al had a greater relative effect on correcting the percentage of bone ash than on correcting tibia breaking strength and tibia weight, as would be expected. The latter two parameters are functions of body weight, which was not substantially improved by raising the P_{av} concentration.

Cox *et al.* (1931) found that bone ash was reduced when dietary Al was equal to the P content of a guinea pig's diet. The addition of more monosodium phosphate, equivalent to the Al content, corrected the depression in bone ash. Alsmeyer *et al.* (1963) found that .74% Al as the sulfate significantly reduced bone ash in rats. Lipstein and Hurwitz (1982) reported that .76 g of P intake was required per gram of Al intake to correct the negative effects on tibia P, tibia ash, and plasma P_i, when the dietary Al was provided by alum-flocculated algae. The present study was not designed to obtain a similar value for use with aluminum sulfate. However, when .392% Al was fed in Experiment 1, raising the concentration of dietary P_{av} from .45 to .68% corrected the values for tibia ash. Chicks fed the diet with .68% P_{av} consumed approximately .85 g more of P_{av} than the chicks given .45% P_{av}, and consumed a total of 1.11 g of Al. Thus, approximately .77 g of additional P intake (or less) was needed per gram of Al intake to correct the value for tibia ash.

The main effects of feeding high levels of Al to broiler chicks in the current study were reductions in growth, feed intake, efficiency of feed utilization, plasma P_i, bone weight, bone breaking strength, and bone ash. Some of these effects were observed previously in studies with Japanese quail and laying hens (Hussein *et al.*, 1988, 1989a,b). Storer and Nelson (1968) reported that high levels of soluble salts of Al in the diet depressed weight gain, efficiency of feed utilization, and the bone ash of chicks. Based on calculations from their data, one can conclude that dietary Al also depressed feed intake severely. In contrast to those findings, Lipstein and Hurwitz (1981) observed a relatively small reduction in the feed:gain ratio and the weight gain of White Rock chicks due to feeding *ca.* .48% Al supplied by alum-flocculated algae. Significant reductions in the same parameters were observed in the present study when feeding less than .2% Al as the sulfate salt. In a subsequent study by Lipstein and Hurwitz (1982), decreased tibia ash, tibia P, and plasma P_i without any effects on weight gain or on the feed:gain ratio were obtained by feeding the Al provided by algae. Storer and Nelson (1968) observed no negative effects from feeding the insoluble salts of Al to chicks.

The various findings indicate that there are greatly differing responses to high dietary levels of Al, depending on what source is used. In the present study, Al caused a major reduction in feeding intake and growth as well as affecting P and Ca metabolism. These effects were aggravated by high concentrations of dietary Ca. Increasing dietary P_{av} alleviated the depression in plasma P_i and percentage of tibia ash, but had little effect on correcting the reduction in growth performance.

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