Calcium and phosphorus requirements of the very young turkey as determined by response surface analysis

BY ALAN M. SANDERS¹, HARDY M. EDWARDS JR¹ AND GEORGE N. ROWLAND III²

Departments of ¹ Poultry Science and ² Avian Medicine, The University of Georgia, Athens, GA 30602, USA

(Received 12 February 1991 – Accepted 5 July 1991)

The first experiment was a central composite rotatable design with calculated calcium levels of 6.2, 7.0, 9.0, 11.0, and 11.8 g/kg diet and total phosphorus levels of 5.2, 6.0, 8.0, 10.0, and 10.8 g/kg diet (2.8 g phytin-P/kg by analysis). This design involved three replicates for each rotatable point and fifteen replicates of the central point. The second experiment was a 4×4 factorial design with calculated Ca levels of 8.0, 10.0, 12.0, and 14.0 g/kg diet and calculated total P levels of 7.0, 9.0, 11.0, and 13.0 g/kg diet (2.5 g phytin-P/kg by analysis). There were four replicates for each treatment. In both 16 d experiments maize-soya-bean diets were used and each replicate consisted of one pen containing 10-d-old broad-breasted, white tom turkeys. The Ca and total P requirements for optimum growth were estimated to be 12.5 and 10.0 g/kg diet respectively. Bone ash was adequate at these levels of Ca and total P, but maximum bone ash was not achieved until much higher levels of Ca and total P were employed. At the required levels of Ca and total P for growth the incidences of Ca- and P-deficiency rickets were very low. There were no treatment effects on feed efficiency. Increasing dietary Ca decreased the incidence of the Ca-deficiency lesion. There was a quadratic response due to dietary total P on both P-deficiency rickets and plasma dialysable P; intermediate levels of dietary P resulted in low incidence of the P-deficiency lesion and high levels of plasma dialysable P. There was a strong negative correlation between the incidence of P-deficiency rickets and plasma dialysable P. Percentage retention was very low at high levels of dietary P and low levels of Ca which corresponded with slightly higher P-deficiency rickets and low plasma dialysable P. No such obvious relationships existed between Ca retention, incidence of Ca-deficiency rickets, and plasma Ca. The incidence of tibial dyschondroplasia was very low in the present study. There were pronounced dietary treatment effects on phytin-P retention; at 14 d percentage phytin-P retention treatment means ranged from 18 to 46 in Expt 1 and from 0 to 40 in Expt 2 with the highest retention of phytin-P at low levels at both Ca and total P.

Calcium: Phosphorus: Phytin-phosphorus retention: Rickets: Tibial dyschondroplasia: Turkey

There have been numerous studies concerning the calcium and phosphorus requirements in the very young turkey for over 50 years (Mussehl & Ackerson 1935). The latest study that examined both the Ca and P requirement in the turkey was conducted more than 13 years ago (Choi & Harms, 1977). Further study in this area is warranted simply on the grounds that the modern, faster-growing turkey may respond differently from the turkey of 13 years ago. Almost all studies to date have dealt only with growth, feed efficiency, and bone ash as the response criteria measured. The gross, microscopic, and radiographic lesions of both Ca- and P-deficiency-type rickets have been well documented in the broiler (Long *et al.* 1984*a*, *b*, *c*), but the effects of various levels of dietary Ca and P on the incidence of these lesions have not been investigated. Edwards & Veltmann (1983) reported that a narrow Ca:total P ratio will induce tibial dyschondroplasia in young broilers; no work to date,

A. M. SANDERS AND OTHERS

however, has been reported that examined the possible relationship between Ca, P, and tibial dyschondroplasia in the turkey. Edwards (1983) and Edwards & Veltmann (1983) have reported that the percentage retention of Ca, P, and phytin-P of the broiler and commercial layer can vary tremendously with changing levels of dietary Ca and P. Such an examination of the retention of these minerals has not been examined in the turkey.

The purpose of the present study was to determine the Ca and total P requirements of the modern broad-breasted white tom turkey for performance and bone ash. Further, the effects of graded levels of dietary Ca and total P on the incidence of Ca- and P-deficiencytype rickets, the incidence of tibial dyschondroplasia, plasma Ca, plasma dialysable P, and the retention of Ca, P, and phytin-P will be examined. In addition, the relationships between the various response criteria will be addressed, as well as how they relate to the Ca and total P requirements.

EXPERIMENTAL PROCEDURES

Both experiments were conducted identically except as noted. Nicholas Large White tom turkeys were obtained from a commercial hatchery, identified with wing bands, and placed 10 per pen in electrically heated Petersime Battery brooders equipped with wire-mesh floors. The batteries were kept in a room in which the temperature was maintained at 22° . Light was provided 24 h/d by fluorescent lights and sunlight was present during daylight hours from large windows on the east side of the room. The fluorescent lights used in the battery were General Electric, F15T8-CW, with no diffusers, providing 3.4% of the wattage in the u.v. range (260–400 nm). Feed and water were provided *ad lib*.

Body-weight was measured by pen on days 7 and 16. Feed consumption was recorded for determination of feed efficiency on day 16. Each diet contained 1 g chromic oxide/kg (Table 1) in order to determine the retention of Ca, P, and phytin-P. Dried feed and excreta samples (collected on days 7 and 14) were analysed for Cr₂O₃ (Brisson, 1956), Ca (Hill, 1955), P (O'Neil & Webb, 1970) and phytin-P (Common, 1940), and the percentage retention of Ca, P and phytin-P was calculated. In Expt 2, but not Expt 1, blood samples were taken by cardiac puncture with heparinized syringes from one randomly chosen bird from each pen. The plasma samples were then analysed for Ca (Technicon Instruments Corporation, 1969) and dialysable P (Technicon Instruments Corporation, 1970). On day 16, after the birds were weighed, they were all killed, mixed to obscure dietary treatment, and scored grossly for the incidence of Ca-deficiency-type rickets (Long et al. 1984b), Pdeficiency-type rickets (Long et al. 1984a) and tibial dyschondroplasia (Edwards & Veltmann, 1983) by making a sagittal cut at the proximal end of the right tibia (Plate 1). Birds in which the growth plate band proliferating prehypertrophied zone was noticeably lengthened were classified as having Ca-deficiency rickets. Birds which had a normal proliferating prehypertrophied zone and a lengthened metaphyseal primary spongiosa were classified as having P-deficiency rickets. Birds in which the avascular zone of hypertrophied cells was focally lengthened were classified as having tibial dyschondroplasia. The left tibias were removed for bone ash determination on the fat-free dry bone (Association of Official Agricultural Chemists, 1955).

The basal diets for both experiments were practical maize-soya-bean-type diets (Table 1) formulated to meet or exceed all National Research Council (1984) nutrient requirements with the exception of Ca and P. The various levels of Ca and P used in both experiments were achieved by the substitution of limestone and dicalcium phosphate into the diet at the expense of maize. Expt 1 was designed according to Box & Wilson's (1951) central composite rotatable design. Dietary levels of Ca and P were the two independent variables. The dietary levels of Ca in Expt 1 were 6.2, 7.0, 9.0, 11.0, and 11.8 g/kg. The

 Ingredients	Expt 1	Expt 2	
 Maize*	444	437	-
Sovabean meal, dehulled	500	500	
Poultry fat	40	40	
Dicalcium phosphate		5.0	
Vitamin mixture [†]	2.5	2.5	
NaH _a PO ₄ .H _a O	4.2	7.7	
DL-methionine	2.0	2.0	
L-lysine hydrochloride	1.0	1.0	
Selenium concentrate [‡]	0.5	0.5	
Sodium chloride	3.5	2.0	
Trace mineral mixture§	1.0	1.0	
Chromic oxide	1.0	1.0	

Table 1. Composition of basal diets (g/kg)

* Various levels of calcium and phosphorus were achieved by substitution of dicalcium phosphate and limestone at the expense of maize.

[†] Vitamin mixture provides (mg/kg diet): vitamin A (as all-*trans*-retinyl acetate) 1892, vitamin E (all DL- α -tocopheryl acetate) 11, menadione (as menadione sodium bisulphite) 11, cholecalciferol 27.5 μ g, riboflavin 44, calcium pantothenate 12, nicotinic acid 44, choline chloride 220, vitamin B₁₂ 9 μ g, vitamin B₆ 30, thiamin (as thiamin mononitrate) 22, folic acid 3, biotin 03, and ethoxyquin 125.

‡ 0.2 g Se from sodium selenite/kg in a calcium carbonate carrier.

§ Trace mineral mixture provides mg/kg diet: manganese 120, zinc 100, iron 60, copper 10, iodine 2·10, Ca 150 (min), 180 (max).

dietary levels of total P were 5.2, 6.0, 8.0, 10.0, and 10.8 g/kg (phytin-P was 2.8 g/kg by analysis). There were three replicates for each rotatable point and fifteen replicates for the central point, with each pen constituting a replicate for a total of thirty-nine pens and 390 birds. Expt 2 was an incomplete 4×4 factorial design. The Ca levels were 8.0, 10.0, 12.0, and 14.0 g/kg diet. The total P levels were 7.0, 9.0, 11.0 and 13.0 g/kg diet (phytin-P was 2.5 g/kg by analysis). An incomplete factorial design was utilized because the diet with 8.0 g Ca and 13.0 g P/kg was impossible to formulate with the ingredients used; this treatment was, therefore, omitted. There were a total of fifteen treatments with four replicates per treatment, each pen constituting a replicate, for a total of sixty pens and 600 birds.

The data were analysed by multiple-regression analysis and regression equations were derived. Response surfaces were generated from these equations for the various responses measured. Also, correlation coefficients between several of the response criteria were determined within each experiment.

RESULTS

Dietary Ca level had very little effect on growth in either experiment (Tables 2 and 3; Figs 1 and 2(A and B)). There was, however, a consistent quadratic growth response due to dietary total P in both experiments at both 7 and 16 d; total P levels above 10.6 g/kg diet resulted in depressed growth. The regression analysis indicates that 16.7 g Ca/kg and 10.6 g total P/kg are the requirements for optimum 16 d growth in Expt 1 and 12.4 g Ca/kg and 10.2 g total P/kg are the requirements for optimum 16 d growth in Expt 2. There were no significant treatment effects in either experiment on feed efficiency (Tables 2 and 3; Figs 1 and 2(F)).

There was a significant, although not readily obvious, quadratic bone ash response due to Ca in both experiments (Tables 2 and 3; Figs 1 and 2(E)). There was also a quadratic

Dietary trea	tments (g/kg)	Body-	wt (g)		Rickets incidence (%)			
Ca	P‡	7 d	16 d	Gain:feed	Ca§	P	Bone ash (g/kg)	
9.0	5.2	113	312	0.706	0	94	270	
7.0	6.0	143	389	0.784	42	10	350	
11.0	6.0	141	375	0.799	25	56	320	
6.2	8.0	142	385	0.744	64	13	370	
9.0	8.0	150	416	0.789	9	1	410	
11.8	8.0	150	419	0.796	15	4	420	
7.0	10.0	148	404	0.768	43	0	390	
11.0	10.0	145	427	0.821	3	0	420	
9.0	10.8	139	407	0.793	15	8	410	
Mean		146	400	0.781	19	15	380	
SEM		4	11	0.021	7	7	10	
Regression as	nalysis model							
Intercept		24.0	16.0	0.619*	327.7*	233.3*	-17.6*	
Ca		63.7	-40.2	-0.064	-723.3*	236.0	3.6	
Р		231.6*	878·4*	0.316	104.3	-752.3*	116.0*	
Ca × Ca		-30.5	-62.3	-0.050	422·7*	8.0	-18.3*	
$Ca \times P$		- 6.3	235.4	0.235	-145.8	-286.9*	41.7*	
$\mathbf{P} \times \mathbf{P}$		-136.8*	-602.2*	-0.279	17.1	557.7*	-82.5*	
R^2		0.35	0.71	0.28	0.72	0.82	0.94	
Maximum	Ca response¶	9.6	16.7	-11.6	12.5	7.4	13.0	
Maximum	P response¶	8.2	10.6	0.8	22.6	8.6	10.2	
Predicted r	naximum	150	446	0.668†	-5	- 5†	44	

 Table 2. Expt 1. The effects of various dietary calcium and phosphorus levels on body-weight, gain: feed, rickets, and bone ash of very young turkeys

† The solution was a saddle point; all others were a maximum or minimum.

‡ Phytin-P was analysed to be 2.8 g/kg diet.

§ Incidence of Ca-deficiency-type rickets.

Incidence of P-deficiency-type rickets.

¶ g/kg diet.

bone ash response due to total P; this quadratic response due to dietary P was very pronounced in both experiments. In addition there was a Ca \times total P interaction in both experiments on bone ash; at low levels of either element high levels of the other element resulted in decreased bone ash. At high levels of both elements, however, no such detrimental effects were observed. For maximum bone ash in Expt 1 the regression analysis indicates that 13.0 g Ca/kg and 10.2 g total P/kg were required; in Expt 2, 13.8 g Ca/kg and 11.7 g total P/kg were required.

There was clearly a linear relationship between dietary Ca and Ca-deficiency rickets in both experiments with increasing levels of Ca decreasing the incidence of the Ca-deficiency lesion (Tables 2 and 3; Figs 1 and 2(C)). There was a small but significant quadratic Ca-deficiency rickets response due to dietary Ca in Expt 1, but not in Expt 2. For minimum Ca-deficiency rickets in Expt 1 a dietary level of 12.5 g Ca/kg was required while in Expt 2 a dietary Ca level of 14.7 g/kg was required.

There was a quadratic response due to total dietary P on the incidence of P-deficiency rickets in both experiments with the incidence of P-deficiency rickets decreasing to a point, then increasing with the highest levels of dietary total P (Tables 2 and 3); Figs. 1 and 2(D)). There was a Ca \times total P interaction on the P deficiency response; high levels of dietary Ca increased the incidence of P-deficiency rickets at low levels of total P. In Expt 1 the levels

Dietary treatments (g/kg)		Body-wt (g)			Rickets in	cidence (%)	
Са	P‡	7 d	16 d	Gain:feed	Ca§	Р	Bone ash (g/kg)
8	7	142	371	0.757	35	22	.180
10	7	144	386	0.758	11	37	380
12	7	150	403	0.780	13	67	370
14	7	140	375	0.788	0	98	350
8	9	154	410	0.747	41	11	410
10	9	152	409	0.769	21	5	410
12	9	152	389	0.735	5	3	420
14	9	151	418	0.797	8	8	420
8	11	140	367	0.722	50	8	390
10	11	149	408	0.762	38	3	410
12	11	151	409	0.758	30	3	430
14	11	150	406	0.755	15	5	430
10	13	147	397	0.755	41	9	410
12	13	146	381	0.753	11	8	420
14	13	153	400	0.774	24	8	420
Mean		148	395	0.760	23	19	400
SEM		4	14	0.018	9	7	10
Regression analy	sis model						
Intercept		83.4*	107.1	0.816*	118.9	255-3*	7.7
Ca		50.5	205.7	0.039	-244.8*	49.8	6.5
Р		71.4	347.2*	-0.192	102.2	-531.2*	53.0*
Ca × Ca		- 39.1	-114-1	0.016	94-6	106.2*	-11.3*
$Ca \times P$		44.8*	76.1	-0.051	-20.9	-226.5*	21.1*
$\mathbf{P} \times \mathbf{P}$		-60.2*	-216.4*	0.096	-22.0	382.1*	-35.0*
R^2		0.22	0.15	0.12	0.45	0.81	0.83
Maximum Ca	response	12.5	12.4	-6.2	14.7	11.3	13.8
Maximum P r	esponse	10.6	10.2	9.6	16.3	10.9	11.7
Predicted max	imum	153	412	0.709	22†	-6	43.4

 Table 3. Expt 2. The effects of various dietary calcium and phosphorus levels on body weight, gain: feed, rickets, and bone ash of very young turkeys

[†] The solution was a saddle point; all others were a maximum or minimum.

‡ Phytin-P was analysed to be 2.5 g/kg diet.

§ Incidence of Ca-deficiency-type rickets.

|| Incidence of P-deficiency-type rickets.

¶ g/kg diet.

of Ca and total P required for minimum P-deficiency rickets were 7.4 and 8.6 g/kg respectively, while in Expt 2 they were 11.3 and 10.9 g/kg respectively.

Only one bird was found to have tibial dyschondroplasia in Expt 1. In Expt 2, however, the incidence of tibial dyschondroplasia was somewhat higher, although it was still very low. Since the incidence of tibial dyschondroplasia was so low, this information was omitted from the tables. However, it was noticed that the birds with tibial dyschondroplasia tended to be in the treatments with the best growth.

None of the regression coefficients was significant in the plasma Ca surface, but the quadratic total P term approached significance (P < 0.07) (Table 4; Fig. 2(G)). Further, the total P main effect was significant. There was no indication that dietary Ca had any effect on plasma Ca. There was a quadratic plasma dialysable P response due to dietary total P with the highest levels of plasma dialysable P at intermediate levels of dietary total P (Table 4; Fig. 2(H)). There was a Ca × total P interaction on plasma dialysable P indicating

425



ΰ

B

E

1.20

1.05





A. M. SANDERS AND OTHERS

Dietary treatments (g/kg)) Plasma mi	nerals (mg/l)	
 Ca P†	Ca	P <u>+</u>	
 	04	6.4	
10 7	100	69	
12 7	106	67	
14 7	100	44	
8 0	102	44 92	
10 9	103	85	
12 0	105	02	
14 9	96	75 85	
8 11	90	03	
10 11	105	100	
	103	85	
14 11	04	00	
14 11	02	99 77	
10 13	93	07	
12 13	00	92	
I4 L3	99	60 81	
NICALL SEM	99	01 7	
SEM	0	/	
Regression analysis model			
Intercept	1.2	-17.1*	
Ca	8.6	6.8	
Р	10.2	42.3*	
Ca×Ca	-3.8	-7.1	
$Ca \times P$	0.0	9.0*	
$\mathbf{P} \times \mathbf{P}$	- 5.9	-24.4*	
R^2	0.17	0.61	
Maximum Ca response§	11.4	11.7	
Maximum P response§	8.5	10.8	
Predicted maximum	10.4	9.8	

 Table 4. Expt 2. The effects of various dietary calcium and phosphorus levels on plasma

 Ca and plasma P of very young turkeys

* The coefficient was significantly less than zero ($P \le 0.05$).

† Phytin-P was analysed to be 2.5 g/kg diet.

‡ Plasma dialysable phosphorus.

§ g/kg diet.

that increasing Ca levels decrease plasma dialysable P at low levels of dietary total P. For maximum plasma dialysable P a Ca level of 11.7 g/kg diet and a total P level of 10.8 g/kg diet are required.

There was a quadratic percentage Ca retention response due to dietary total P at both 7 and 14 d in Expt 1 and at 7 d in Expt 2, with the highest percentage retention at intermediate levels of total P (Tables 5 and 6; Figs 3 and 4 (A and D)). At 7 d in Expt 1, but at no other time in either experiment, there was a quadratic Ca retention response due to dietary Ca. Also at 7 d in Expt 1 there was $Ca \times total P$ interaction indicating that, at low levels of total P, increasing Ca levels decrease percentage Ca retention.

There was a quadratic total P retention response due to dietary total P at both 7 and 14 d in Expt 1 and at 7 d in Expt 2; this resulted in the highest percentage retention of total P varying between low and intermediate levels of dietary total P (Tables 5 and 6; Figs 3 and 4 (B and E)). There was also a Ca \times total P interaction on percentage total P retention at both 7 and 14 d in Expt 1, but not in Expt 2, indicating that increasing dietary total P decreases the retention of total P more dramatically at low levels of dietary Ca. There was

Dietary treatments (g/kg)		Ca retention (%)		P retention (%)		Phytin-P retention (%)	
Са	P‡	7 d	14 d	7 d	14 d	7 d	14 d
9.0	5.2	29	45	43	49	17	33
7.0	6.0	52	62	52	57	29	45
11.0	6.0	37	47	48	53	14	28
6.2	8.0	60	67	45	46	27	46
9.0	8.0	64	63	54	51	23	32
11.8	8.0	57	56	58	51	16	18
7.0	10.0	63	67	41	41	24	31
11.0	10-0	63	59	50	47	16	20
9.0	10.8	65	60	42	41	18	25
Mean		57	60	50	50	21	31
SEM		2	2	1	2	2	3
Regression an	alysis model						
Intercept		88.4*	8.2	-9.6	68.6*	41.1	87.6*
Ca		24.5	-36.4	4.4	-28.1	-50.8	-66.8
Р		311.1*	183.0*	144.6*	0.6	39.8	5.0
Ca × Ca		-60-4*	-14.0	-29.5*	-8.7	-6.3	-2.3
$Ca \times P$		90.8*	45·7	79·3 *	61.7*	47.1	36.0
$\mathbf{P} \times \mathbf{P}$		-210.9*	-124.8*	-139.2*	47·9*	- 52.3*	-36.2
R^2		0.93	0.82	0.84	0.57	0.62	0.68
Maximum	Ca response§	9.0	-1.5	12.5	12.5	37.9	48.3
Maximum	P response§	9.3	7.1	8.8	8.1	20.9	24.7
Predicted n	naximum	68	56	56	51†	-14†	-67^{+}

 Table 5. Expt 1. The effects of various dietary calcium and phosphorus levels on the retention of Ca, P, and phytin-P

† The solution was a saddle point; all others were a maximum or minimum.

‡ Phytin-P was analysed to be 2.8 g/kg diet.

§ g/kg diet.

a quadratic effect on percentage total P retention due to Ca at 7 d in Expt 1, but at no other time in either experiment. In Expt 1 there was a quadratic percentage phytin-P retention response due to dietary total P at 7 d but not at 14 d. In Expt 2, however, no such quadratic response was found (Tables 5 and 6; Figs 3 and 4 (C and F)). None of the other coefficients in the regression equations for percentage retention of phytin-P was significant. However, the main effects of both dietary Ca and total P were significant at both 7 and 14 d in both experiments, and the R^2 terms were high in Expt 1. There were clearly Ca and total P effects on percentage phytin-P retention with the highest retention of phytin-P at the lowest levels of Ca and P.

DISCUSSION

The present study indicates that the Ca and total P requirements for optimum growth in the very young turkey are 12.5 and 10.0 g/kg diet respectively. This conclusion is based primarily on the findings in Expt 2. The predicted optima in Expt 1 for growth are not as reliable since they were beyond the actual levels of Ca and total P employed in the experiment. For optimum bone ash, Ca and total P must be somewhat higher. However, when the above levels of Ca and total P are fed to optimize growth, the predicted bone ash value would be, according to the prediction equation, greater than 410 g/kg which is approaching the predicted maximum of 440 and 430 g/kg respectively in Expts 1 and 2. Almost all, if not all, Ca and P requirement studies in poultry have used bone ash as one

429

Dietary treatments (g/kg)		Ca retention (%)		P retention (%)		Phytin-P retention (%)	
Ca	P‡	7 d	14 d	7 d	14 d	7 d	14 d
8	7	38	51	57	48	28	23
10	7	27	49	48	49	17	29
12	7	28	39	54	50	16	40
14	7	20	38	55	50	14	18
8	9	46	46	47	42	24	25
10	9	45	48	56	44	24	25
12	9	37	41	54	44	17	14
14	9	34	42	54	45	5	9
8	11	47	50	41	34	22	25
10	11	43	44	44	38	17	8
12	11	39	42	45	37	13	3
14	11	40	36	50	40	11	3
10	13	45	50	36	34	12	12
12	13	40	43	36	33	5	8
14	13	36	43	40	36	9	-9
Mean		38	44	48	42	15	16
SEM		3	3	3	2	4	8
Regression an	nalvsis model						
Intercept		-14.3	94-3*	4 4·7	79.6*	63-8	8.9
Ca		-44.0	-27.1	-21.8	1.4	- 69-4	116-1
Р		160.7*	-56.0	52.9	- 59.8*	17.0	- 50.9
Ca×Ca		2.3	0.8	3.4	-2.0	14-1	-32.8
$Ca \times P$		20.4	7.4	24.1	9.0	17.2	-72.3
$\mathbf{P} \times \mathbf{P}$		-81.2*	25.6	- 54·8*	11.5	-24.4	48.6
R^2		0.67	0.34	0.65	0.74	0.39	0.32
Maximum	Ca response§	33.0	336.0	8.5	33-2	18-5	6.6
Maximum	P response§	14.0	-37.0	6.7	13.0	10.0	10.1
Predicted maximum		25.4†	-256.8	53-1†	43.0†	8-1†	21.3†

 Table 6. Expt 2. The effects of various dietary calcium and phosphorus levels on the retention of Ca, P, and phytin-P

[†] The solution was a saddle point; all others were a maximum or minimum.

[‡] Phytin-P was analysed to be 2.5 g/kg diet.

§ g/kg diet.

of the major response criteria. Certainly, bone ash is a very sensitive, predictable, and repeatable response (R^2 0.94 and 0.83 in Expts 1 and 2 respectively). But what does optimum bone ash mean in terms of healthy poult development? Further work is necessary to determine the true meaning of early-age bone ash values and how they relate to later performance and leg abnormalities. There was a highly significant correlation between the bone ash response and the 16 d weight response in Expts 1 and 2 (r 0.87, P < 0.001; r 0.38, P < 0.003 respectively). In addition, the levels of Ca and total P at which optimum growth was observed resulted in very low incidence of both the Ca- and P-deficiency lesions. These findings, coupled with the fact that there were no treatment effects on feed efficiency in either experiment, indicate that growth is probably the single best response criterion in determining the P requirements in the young turkey. Since significant responses were obtained to dietary Ca levels only where Ca-deficiency rickets and bone ash were the criteria, these criteria should be studied with the growth at 14 d (Edwards & Veltmann, 1983) and





Fig. 3. Expt. 1. Response surfaces plotting criteria for each surface v. dietary calcium and total phosphorus (g/kg): (A), CART7. Ca retention (%) at 7 d; (B), PRT7, total P retention (%) at 7 d; (D), CART14, Ca retention (%) at 14 d; (E), PRT14, total P retention (%) at 14 d; (E), PRT14, total P retention (%) at 14 d; (F) PPRT14, phytin-P retention (%) at 14 d; (F), CART14, Ca see pp. 422-423 and for details of diets, see Table 1.

431

NUT 67





leghorn and broiler chick growth at 21 d (Yoshida & Hoshii, 1982a, b) were reported in experiments designed for response surface analysis. The use of response surface analysis does not solve the problem of lack of response of specific criteria to levels of a nutrient. It is apparent that the maximum response for a particular criterion has little meaning when none of the coefficients is significant.

Due to the fact that there was only one bird with tibial dyschondroplasia in Expt 1 and a very low incidence in Expt 2, it is very difficult to make any firm conclusions regarding the roles of Ca and P in the development of tibial dyschondroplasia in the young turkey. However, it was noted that the treatments with the best growth tended to be treatments where tibial dyschondroplasia was found. This observation supports the hypothesis that tibial dyschondroplasia is closely linked with rapid growth (Wise & Jennings, 1970; Riddell, 1975; Wise & Nott, 1975; Freedman *et al.* 1985). Edwards & Veltmann (1983) found that a narrow Ca: total P ratio would induce the tibial dyschondroplasia in the broiler. We found no such relationship between Ca, P and tibial dyschondroplasia in the turkey in the present study. However, it is well established that the tibial dyschondroplasia in the turkey than in the broiler (Poulos, 1978 *a*, *b*). To determine whether there is any relationship between dietary Ca, dietary P and tibial dyschondroplasia in the turkey, further research using older birds is necessary.

The accuracy of the differential diagnosis of Ca- and P-deficiency rickets by visual examination is proved by the results and the size of the terms that are significant in the equations for the surface for the different rickets, the Ca terms being very large and significant for the Ca-deficiency-rickets equation and the P terms being large and significant for the P-deficiency-rickets equation. The statistical analysis also indicated that Ca level of the diet significantly influenced the incidence of P-deficiency rickets. In these two experiments only ten birds were diagnosed as having both Ca- and P-deficiency rickets.

We found an unexpected quadratic response due to dietary total P in both experiments; increasing the P level decreased the P-deficiency lesion up to a point beyond which increases in the P level actually increased the incidence of the lesion. In addition, we found a similar quadratic response on plasma dialysable P but, over the whole range of dietary P fed, there was a high negative correlation between plasma dialysable P and the incidence of P-deficiency rickets (r-0.68, P < 0.001). Why would very high levels of dietary P increase the incidence of the P-deficiency lesion and decrease plasma dialysable P? High levels of dietary P are thought to inhibit Ca absorption by forming insoluble Ca-phosphorus complexes in the gut (Guyton, 1986). It may be that this increased difficulty in Ca absorption may result in increased parathyroid hormone secretion in an effort to increase Ca absorption from the gut to make up for the Ca deficit. If this is indeed the situation, then the parathyroid hormone would also cause increased renal excretion of P which may exceed the extra P that is being absorbed. The P retention findings support this hypothesis. Further work seems warranted to determine whether parathyroid hormone is responsible for this phenomenon.

We also found a similar Ca deficiency response due to dietary Ca in Expt 1, but in Expt 2 the quadratic term only approached significance. There was no such significant plasma Ca response due to dietary Ca, however.

Apparently, many scientists feel that the availability of P from phytate in plants can be ignored on the assumption that it is completely unavailable (Scott *et al.* 1982). The National Research Council's (1984) *Nutrient Requirements of Poultry* has non-phytin-P values in its table of feedstuffs while it discusses the requirements of the animal in terms of 'available' P. Apparently, the committee felt that the non-phytin-P and available P are interchangeable terms. The Agricultural Research Council (1975), which presents more values from individual research workers than the National Research Council (1984), gives

A. M. SANDERS AND OTHERS

the individual worker's calculation of available phytate-P as an 'available P' calculation. The present study clearly indicates that phytin-P is indeed retained by the turkey, and the degree to which it is retained varies dramatically with different levels of dietary Ca and P. Edwards & Veltmann (1983) and Edwards (1983) found similar responses in the broiler and commercial layer. Thus, there is very strong evidence that phytin-P should not be completely ignored when formulating poultry rations. Further, non-phytin-P and 'available' P are not interchangeable terms; this is our rationale for using total P in the present study as opposed to 'available' or non-phytin-P. Since 15% phytin-P is retained at the Ca and total P levels previously defined as the requirements, one could assume that 15% of the 2.8 g dietary phytin-P/kg is available to the poult, at these specified requirements. This would mean that the non-phytin-P requirement in the present study is 7.2 g/kg diet while the available P requirement is 7.6 g/kg diet if available P is defined as non-phytin-P plus retained phytin-P. It is conceivable that if the P requirement is determined with one type of ration that is high in phytin-P and the same inorganic P level was used in a different ration with low phytin-P, a marginal P deficiency could result due to the lack of P from phytic acid.

A negative phytin-P retention value was found in Expt 2 when the diet contained 14 g Ca and 13 g P/kg. Our laboratory has previously reported such negative value for phytin-P retention (Elliot & Edwards, 1991). The most reasonable explanation is that the extraction procedure does not get 100% of the phytin-P from the diet but closer to 100% is obtained from the extraction of the faecal material.

The present study indicates a requirement (g/kg diet) for Ca of 12.5, total P 10.0, and available P (defined as non-phytin-P plus retained phytin-P) 7.6. The National Research Council (1971, 1977, 1984) has estimated the minimum Ca and P levels for young poults over the last 20 years (expressed as g/kg diet) as: 1971: 12, 8, —; 1977: 12, 8, —; 1984: 12.5, —, 6, respectively for total Ca, total P and available P, where — indicates no value was given.

The National Research Council (1984) considers phytate-P to be unavailable, so their available P value is non-phytin-P. Therefore, the results of the present study do not indicate any change in Ca requirement but do indicate that the P requirement has increased by as much as 25%. During the last part of this 20-year period (from 1983 to 1991), the average weight of the 18-week-old male and female turkey in the USA increased by 23 and 22% respectively (Sell, 1991). Perhaps this rapidly growing bird really has a higher P requirement.

There have been problems in previous studies in which there have been very large discrepancies between calculated and analysed values for Ca and P in experimental diets (Stephenson *et al.* 1962). The question could be raised as to whether calculated or analysed values should be used in the analysis of the data. Our calculated and analysed values were relatively close so we decided to use calculated values. For example, the analysed values of the diets used in Expt 1 showed the diets to contain 0.7 g more Ca/kg and 0.5 g more total P/kg than they contained by calculated values. These would both indicate errors in making the requirement estimate of approximately 5% of the estimated values, which is reasonable for a requirement to be used under an infinite number of variable conditions.

The authors acknowledge support by State and Hatch funds allocated to the Georgia Agricultural Experiment Stations of the University of Georgia.

REFERENCES

Agricultural Research Council (1975). The Nutrient Requirements of Farm Livestock. No. 1, Poultry Technical Reviews and Summaries, pp. 61-62. London: Agricultural Research Council.

435

- Association of Official Agricultural Chemists (1955). Official Methods of Analysis, 8th ed. Washington, DC: Association of Official Agricultural Chemists.
- Box, G. E. P. & Wilson, K. B. (1951). On the experimental attainment of optimum conditions. *Journal-Royal Statistical Society* B13, 1–45.
- Brisson, G. J. (1956). On the routine determination of chromic oxide in feces. *Canadian Journal of Agricultural Science* **36**, 210-211.
- Choi, J. H. & Harms, R. H. (1977). Importance of calcium: inorganic phosphorus ratio in diets of starting poults. *Nutrition Reports International* 15, 437-442.
- Common, R. H. (1940). The phytic acid content of some poultry feeding stuffs. Analyst 65, 79-83.
- Edwards, H. M. Jr (1983). Phosphorus. 1. Effect of breed and strain on the utilization of suboptimal levels of phosphorus in the ration. *Poultry Science* 62, 77-84.
- Edwards, H. M. Jr & Veltmann, J. R. Jr (1983). The role of calcium and phosphorus in the etiology of tibial dyschondroplasia. *Journal of Nutrition* 113, 1568-1575.
- Elliot, M. A. & Edwards, H. M. Jr (1991). Some effects of dietary aluminum and silicon on broiler chickens. *Poultry Science* **70**, 1390–1402.
- Freedman, B. D., Gay, C. V. & Leach, R. M. Jr. (1985). Avian tibial dyschondroplasia. II. Biochemical changes. American Journal of Pathology 119, 191–198.
- Guyton, A. C. (1986). Medical Physiology, 7th ed., p. 937. Philadelphia, PA: W. B. Saunders and Company.
- Hill, J. B. (1955). Automated fluorometric method for determination of serum calcium. *Clinical Chemistry* 2, 122–130.
- Long, P. H., Lee, S. R., Rowland, G. N. & Britton, W. M. (1984a). Experimental rickets in broilers: gross, microscopic, and radiographic lesions. I. Phosphorus deficiency and calcium excess. Avian Diseases 28, 460–474.
- Long, P. L., Lee, S. R., Rowland, G. N. & Britton, W. M. (1984b). Experimental rickets in broilers: gross, microscopic, and radiographic lesions. II. Calcium deficiency. Avian Diseases 28, 921–932.
- Long, P. L., Lee, S. R., Rowland, G. N. & Britton, W. M. (1984c). Experimental rickets in broilers: gross, microscopic, and radiographic lesions. III. Vitamin D deficiency. Avian Diseases 28, 933–943.
- Mussehl, F. E. & Ackerson, C. W. (1935). Calcium and phosphorus requirements of growing turkeys. *Poultry Science* 14, 147-151.
- National Research Council (1971). Nutrient Requirements of Poultry, 6th ed. Washington, DC: National Academy of Sciences.
- National Research Council (1977). Nutrient Requirements of Poultry, 7th ed. Washington, DC: National Academy of Sciences.
- National Research Council (1984). Nutrient Requirements of Poultry, 8th ed. Washington, DC: National Academy of Sciences.
- O'Neil, J. V. & Webb, R. A. (1970). Simultaneous determination of nitrogen, phosphorus and potassium in plant materials by automated methods. *Journal of the Science of Food and Agriculture* 21, 217–219.
- Poulos, P. W. Jr (1978a). Tibial dyschondroplasia in the turkey. A morphologic investigation. Acta Radiologica 358, Suppl., 197–227.
- Poulos, P. W. Jr (1978b). Skeletal lesions in the broiler, with special reference to dyschondroplasia (osteochondrosis). Acta Radiologica 358, Suppl., 229–275.
- Riddell, C. (1975). Studies on the pathogenesis of tibial dyschondroplasia in chickens. III. Effect of body weight. *Avian Diseases* 19, 497-505.
- Scott, M. L., Nesheim, M. C. & Young, R. J. (1982). Nutrition of the Chicken, 3rd ed., p. 301. Ithaca, N.Y.: M. L. Scott and Associates.
- Sell, J. L. (1991). Continued improvements in turkey performance in 1990. Turkey World, pp. 12–16. Mount Morris, IL: Watt Publishing Co.
- Stephenson, E. L., Rinehart, K. B. & Bragg, D. B. (1962). Calcium-phosphorus requirements for day-old poults. *Poultry Science* 41, 1686.
- Technicon Instruments Corporation (1969). Technicon AutoAnalyzer Methodology N-3I. Tarrytown, N.Y.: Technicon Instruments Corporation.
- Technicon Instruments Corporation (1970). Technicon AutoAnalyzer Methodology N-4C. Tarrytown, N.Y.: Technicon Instruments Corporation.
- Wise, D. R. & Jennings, A. R. (1970). Dyschondroplasia in domestic poultry. Veterinary Record 86, 285-286.
- Wise, D. R. & Nott, H. (1975). Studies on tibial dyschondroplasia in ducks. *Research in Veterinary Science* 18, 193–197.
- Yoshida, M. & Hoshi, H. (1982*a*). Re-evaluation of requirement of calcium and available phosphorus for starting cgg-type chicks. *Japanese Poultry Science* 19, 93-100.
- Yoshida, M. & Hoshi, H. (1982b). Re-evaluation of requirement of calcium and available phosphorus for starting meat-type chicks. Japanese Poultry Science 19, 101–109.

Printed in Great Britain

Plate 1

British Journal of Nutrition, Vol. 67, No. 3



Plate 1. Gross comparison of sagittal sections of the proximal tibiotarsus from 16-d-old poults: (A) control, (B) calcium deficiency, and (C) phosphorus deficiency. The proliferating prehypertrophied zone (*) is increased in size in B. The metaphysis (m) is dark and increased in relative length in C.