Absorption and endogenous excretion of phosphorus in growing broiler chicks, as influenced by calcium and phosphorus ratios in feed

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Absorption and endogenous excretion of P by male broiler chicks (14–29 d old) were quantitatively evaluated at different Ca:P ratios (1, 1:1; 2, 1·5:1; 3, 2:1; 4, 2·5:1) in four groups given experimental diets *ad lib*. The P content was the same in all diets. An isotope-dilution technique was used to determine endogenous faecal and renal excretion. Ca and P retentions in the whole body were estimated according to the comparative slaughter technique. P absorption was calculated from retention and endogenous excretion. Absorption and endogenous excretion of P amounted to (mg P/d per chick): 304, 270, 160 and 158; and 135, 109, 31 and 30 in groups 1, 2, 3 and 4 respectively. Widening of the Ca:P ratio in the feed limited the P absorption. Availability of feed P amounted to (%): (1) 66, (2) 57, (3) 32 and (4) 30, and the amounts of absorbed P retained were (%): (1) 56, (2) 60, (3 and 4) 81. The increasing Ca concentration in the feed showed a greater effect on P absorption than on P retention. The ratios of relative retention to relative endogenous excretion of absorbed P were: (1) 1·27, (2) 1·50, (3 and 4) 4·26.

Phosphorus absorption: Endogenous phosphorus: Broilers: Ca:P ratios

Growth performance in poultry is related to the metabolism of minerals, especially Ca and P, for bone formation. P metabolism is affected by the amount of P in the feed (Günther *et al.* 1982), the age of the animal and sources of P supply (Günther *et al.* 1978; Günther & Al-Masri, 1988), the amounts of P and Ca and the Ca:P ratio in the feed (Günther & Tekin, 1968*a*, *b*; Hermes, 1977) and phytase (*EC* 3.1.3.8) activity (Simons *et al.* 1990; Schöner *et al.* 1993) in addition to vitamin D supply.

In evaluating the metabolism of P in the animal body and its influence on chick growth, P absorption, retention of feed P, and endogenous P excretion in relation to the Ca:P ratio in the feed are important variables that affect the supply of minerals to the skeleton. A radioisotope-dilution technique may be used to measure the endogenous faecal and renal P excretion. Absorbed P in relation to feed P gives P availability. In the same way, P retention in the whole body in relation to feed P gives the relative P retention. The present study provides quantitative information on the retention and endogenous excretion of absorbed P, and evaluates the relationship between them for homeostasis of this mineral in growing chicks given diets with different Ca:P ratios. P retention, endogenous P and P absorption were estimated at intervals between 14 and 29 d of age.

MATERIALS AND METHODS

Experimental design

The experiments were done with male broiler chicks (1 d old; Lohmann). This experiment lasted for 29 d. The first 10 d were designated as a preparation period, during which 120 chicks were kept on the floor and fed on a conventional ration. The ambient temperature was

32° in the first week, and lowered by 2° for every successive week. The relative humidity was 50–60%. On day 10 the chicks were transferred to metabolism cages. On day 14 eighty chicks of similar weight were divided into four groups (G1, G2, G3, G4) and each group was allocated to one of four diets which differed only in the Ca:P ratio. On that day four birds (group N) from each group were killed for assessment of the initial contents of Ca and P in the whole body. The remaining birds (sixteen per group) were kept in metabolism cages (two chicks per cage) until the 29th day. Also on the 14th day of age, each of the sixty-four chicks in the four experimental groups was injected intramuscularly with 1 ml [^{32}P]Na_3PO_4 solution (6·037 MBq) into the right breast muscle. On days 3, 7, 11 and 15 after injection, four chicks from each experimental group were slaughtered for assessment of the contents of Ca and P in the whole body. The blood plasma and excreta were also collected for analysis of stable and radioactive P.

Statistical analyses

A factorial type with complete randomized design was used in this experiment, with two factors: (1) diet as factor 1 with four levels (G1, G2, G3 and G4); (2) time as factor 2 with four levels (3, 7, 11 and 15 d after injection). Four diets were given to chicks in thirty-two cages and the chicks (in two cages/diet) were killed at four different times. Results were subjected to ANOVA using the Statview512 on a personal computer to test the effects of diet and time, and their interactions using Fisher's protected least significant difference at the 0·05 level. Means with their pooled standard error, *P* values for diet, time, interactions and the residual degrees of freedom are presented.

Diets

The composition of the experimental diets is given in Table 1. Dicalcium phosphate ($CaHPO_4.2H_2O$) and $CaCO_3$ were added as supplemental P and Ca in experimental diets (G1, G2, G3, G4). During the period 14–29 d the chicks were fed *ad lib.* on the experimental diets. The diets were not pelleted. The chicks were offered distilled water. The dry matter intake was measured quantitatively for the experimental days and the P and Ca consumed were assessed to calculate relative retention or availability values.

Analytical methods

The levels of radioactive P in plasma and excreta were determined on days 3, 7, 11 and 15 after the injection with ^{32}P by liquid scintillation counting and with the help of Cerenkov-radiation according to Vemmer & Gütte (1964). The stable P in the ash of samples was determined according to the method of Lantzsch (1961), and the P in plasma was estimated with the help of a test kit (Test-Combination. Phosphorus, Phospholipids. Colorimetric method. Cat. no. 124974; Boehringer, Mannheim, Germany). The Ca concentration was determined by atomic absorption spectrometry. The endogenous P in the excreta was estimated according to a radioisotope-dilution technique (Hevesey, 1948, 1962). Specific radioactivity in excreta (Bq/mg P) to specific radioactivity in blood plasma (Bq/mg P) multiplied by the amount of P in excreta (mg P/d per chick) gives the endogenous P in excreta (mg P/d per chick). Ca and P retentions in the whole body (mg/d per chick) were assessed according to the comparative slaughter technique from the difference between the Ca and P retentions at 17, 21, 25 and 29 d of age (mg P or Ca/d per chick) and those at 14 d of age (mg P or Ca/d per chick) (group N). P retained in the body (mg P/d per chick) and endogenous P in excreta (mg P/d per chick) constitute P absorbed (mg P/d per chick).

Table 1. *Composition of the experimental diets*

Diet...	G1	G2	G3	G4
Ingredient (g/kg)				
Yellow maize	195·0	200·0	190·0	190·0
Sorghum	195·0	195·0	190·0	190·0
Soyabean meal	300·0	290·0	293·0	290·0
Wheat	100·0	95·0	100·0	95·0
Maize starch	100·0	100·0	100·0	100·0
Lucerne meal	42·5	43·4	41·2	40·1
Casein	32·0	32·0	32·0	32·0
Skimmed milk	18·0	18·0	18·0	18·0
NaCl	2·0	2·0	2·0	2·0
Dicalcium phosphate	12·3	12·3	12·3	12·3
Calcium carbonate	0·2	9·3	18·5	27·6
Minerals*	1·0	1·0	1·0	1·0
Vitamins†	2·0	2·0	2·0	2·0
Composition (g/kg DM)				
DM (g/kg)	924·3	924·3	929·9	927·7
Crude ash	59·6	59·3	67·3	72·5
Crude protein	235·0	233·4	235·6	233·1
Crude fat	30·5	31·9	28·9	29·8
Crude fibre	35·8	35·5	35·4	36·4
Ca	6·6	9·6	12·6	15·8
Total P	6·5	6·4	6·4	6·3
Organic P	2·4	2·4	2·4	2·4
Ca:P ratio	1·01:1	1·50:1	1·97:1	2·51:1
Metabolizable energy (MJ/kg)	12·23	12·36	12·25	12·27

* Supplied (/kg diet): MnO_2 35·0 mg, ZnO 25·0 mg, $FeSO_4$ 25·0 mg, $CuSO_4$ 2·5 mg, CaI_2 0·25 mg, $CoCO_3$ 0·10 mg, Se 0·05 mg, available P 0·10 g, Ca 0·23 g.

† Supplied (/kg diet): retinol 3 mg, cholecalciferol 0·05 mg, α-tocopherol 5 mg, menadione 2·0 mg, thiamin 1·0 mg, riboflavin 2·5 mg, pyridoxine 2·0 mg, cyanocobalamin 0·01 mg, pteroylmonoglutamic acid 0·4 mg, calcium pantothenate 5·0 mg, nicotinamide 20·0 mg.

RESULTS

Table 2 gives the endogenous P and P absorption values used to test the effects of diet, time and their interactions. Endogenous P in excreta decreased as Ca concentration in the feed was increased. The mean levels of endogenous P in excreta over the experimental period were (mg P/d per chick): (G1) 135, (G2) 109, (G3) 31 and (G4) 30. P absorption is the sum of P retention and endogenous P in excreta. P absorption decreased with increasing Ca concentration in the feed. Table 3 gives the comparison between group 1 (= 100) and the other experimental groups for endogenous P excretion, retained P and P absorbed. The effect of the concentration of Ca in feed (with the constant concentration of P) on P absorption was higher than that of P retention. The mean levels of retained P in the whole body during the experiment were (mg P/d per chick): (G1) 169, (G2) 161, (G3) 129 and (G4) 128. Fig. 1 shows the changes in P retained in the whole body as affected by the Ca:P ratio in the feed.

The average feed intakes over the experimental period were (g/d per chick): (G1) 70, (G2) 73, (G3) 75 and (G4) 81. Table 4 indicates the amounts of P and Ca consumed (mg/d per chick) on the experimental days. P absorbed : P consumed from feed (mg P/d per chick) × 100 gives P availability (%). P or Ca retention in the body (mg P or Ca/d per chick) : P or Ca consumed from feed (mg P or Ca/d per chick) × 100 gives the relative P or Ca retention (%) (Table 5). The experiments indicated a decrease in relative Ca retention

Table 2. *Influence of the calcium:phosphorus ratio of the diet* on daily excretion of endogenous phosphorus in faeces and phosphorus absorption into the body of growing chicks (mg P/d per chick) from 3 to 15 d after injection with* ^{32}P†

(Mean values with their pooled standard error)

Time after injection (d)	Endogenous P‡ G1	G2	G3	G4	Pooled SE (df 16)	P absorption‡ G1	G2	G3	G4	Pooled SE (df 16)
3	76	74	21	19	10·6	181	169	72	69	20·0
7	113	87	33	37	12·9	277	236	152	165	19·7
11	145	122	31	32	19·7	332	304	185	185	25·7
15	208	153	40	31	28·4	427	371	233	213	34·2
Pooled SE (df 16)	18·3	12·0	2·8	2·7		33·6	28·7	22·5	20·6	

* Ca:P ratios of the diets were: G1, 1·01:1; G2, 1·50:1; G3, 1·97:1; G4, 2·50:1.
† For details of diets and procedures, see Table 1 and pp. 407–408.
‡ Statistical significance of effect of diet (D), $P < 0.0001$; time (T), $P < 0.0001$; D × T interaction, $P < 0.0001$. For endogenous P, s (square root of residual mean square) = 7·1; for P absorption s = 10·3.

Table 3. *Excretion of endogenous phosphorus, phosphorus retention and phosphorus absorption by chicks fed on diets with different calcium:phosphorus ratios*, from 3 to 15 d after injection with* ^{32}P†

(Values are expressed as a proportion of those obtained with a calcium:phosphorus ratio of 1·01:1)

Time after injection (d)	Endogenous P G1	G2	G3	G4	P retention G2	G3	G4	P absorption G2	G3	G4
3	100	97	28	25	90	48	48	93	40	38
7	100	77	29	33	91	72	79	85	55	60
11	100	84	21	22	99	82	82	100	61	61
15	100	73	19	14	99	88	83	87	55	50
Mean	100	83	24	23	95	72	73	91	53	52

* Ca:P ratios of the diets were: G1, 1·01:1; G2, 1·50:1; G3, 1·97:1; G4, 2·50:1.
† For details of diets and procedures, see Table 1 and pp. 407–408.

of feed Ca with an increase in the concentration of feed Ca, and relative P retention of feed P also decreased when comparing groups 1 and 2 with 3 and 4. This is attributed to the low rate of P absorption. Moreover, Ca:P retention in the body increased with increasing Ca concentration in the feed. Ca:P retention reached an average for the whole experimental period of (G1) 1·21, (G2) 1·25, (G3) 1·70 and (G4) 1·71. Relative P retention is clearly connected to the relative Ca retention (R^2 0·815). An increase in the Ca concentration of feed limits P retention in the body. The comparison between G1 (100) and the other experimental groups shows that relative P retention values during the whole experiment were: (G2) 92, (G3) 70 and (G4) 65. Fig. 2 gives the P availability of feed P as influenced by the Ca:P ratio of the feed. Availability of feed P amounted on average to (%): (G1) 66, (G2) 57, (G3) 32 and (G4) 30. The comparison between G1 (100) and the other experimental groups shows that the P availability values were: (G2) 86, (G3) 48 and (G4)

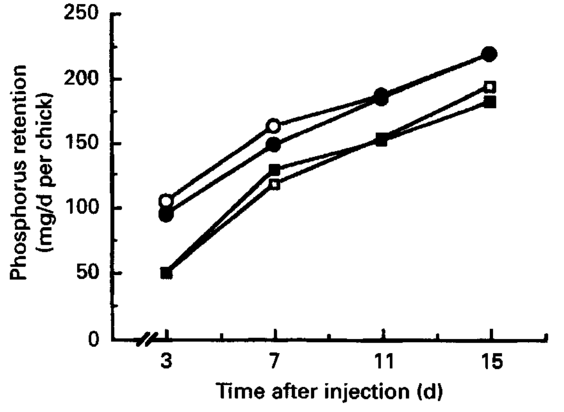

Fig. 1. Influence of the calcium:phosphorus ratio of the diet on daily retention of phosphorus (mg P/d per chick) in the whole body of growing chicks, from 3 to 15 d after injection of ^{32}P. Ca:P ratios of the diets were: (○), 1·01:1; (●), 1·50:1; (□), 1·97:1 and (■) 2·50:1. For details of diets and procedures, see Table 1 and pp. 407–408.

Table 4. *Amounts of calcium and phosphorus (mg/d per chick) consumed by growing chicks fed on diets with different calcium:phosphorus ratios* from 3 to 15 d after injection of* ^{32}P†

(Mean values with their pooled standard errors)

	P consumed‡					Ca consumed§				
Time after injection (d)	G1	G2	G3	G4	Pooled SE (df 16)	G1	G2	G3	G4	Pooled SE (df 16)
3	287	318	302	308	5·7	282	463	567	697	58·2
7	421	405	431	457	7·8	414	598	806	1116	98·7
11	516	565	572	588	13·2	509	830	1068	1416	127·1
15	608	591	663	734	22·8	643	873	1257	1771	162·0
Pooled SE (df 16)	45·0	43·7	52·5	59·7		50·0	65·2	100·9	149·4	

* Ca:P ratios of the diets were: G1, 1·01:1; G2, 1·50:1; G3, 1·97:1; G4, 2·50:1.

† For details of diets and procedures, see Table 1 and pp. 407–408.

‡ Statistical significance of effect of diet (D), $P < 0.0004$; time (T), $P < 0.0001$ and D×T, $P = 0.0284$.

§ Statistical significance of effect of D, $P < 0.0001$, T, $P < 0.0001$ and D×T, $P < 0.0001$. For P consumed, s (square root of residual mean square) = 24·3; for Ca consumed, s = 50·9.

45. Table 6 indicates the relationship of P absorption and P retention to endogenous P excretion, and shows decreases in P absorption with increasing Ca:P ratio in the diet. This is attributed to the decreases in P retention and endogenous P excretion. The latter has a higher effect than P retention in this case. Since the endogenous P excretion : P absorption ratio decreases more than the P retention : P absorption ratio, the ratio of relative retention to relative endogenous P excretion is clearly increased by a wide ratio of Ca:P in the diet.

The values of Ca retention in the whole body were similar among the experimental groups despite the differences of Ca:P ratio in the feeds. These values were on average (mg Ca/d per chick): (G1) 201, (G2) 198, (G3) 191 and (G4) 195, whereas a difference in the relative Ca retention of feed Ca was found among the experimental groups. The comparison between G1 (100) and the other experimental groups shows that the values of relative Ca retention during the experiment were: (G2) 66, (G3) 48 and (G4) 37.

Table 5. *Influence of the calcium:phosphorus ratio of the diet* on relative retention of calcium and phosphorus (%) by growing chicks, from 3 to 15 d after injection with ^{32}P†*

(Mean values with their pooled standard error)

Time after injection (d)	Relative P retention‡					Relative Ca retention§				
	G1	G2	G3	G4	Pooled SE (df 16)	G1	G2	G3	G4	Pooled SE (df 16)
3	37·5	30·5	17·0	18·0	3·4	46·5	28·0	23·5	19·0	4·0
7	39·0	37·0	28·0	28·5	1·9	48·0	33·5	23·0	17·5	4·4
11	36·5	32·5	28·5	26·5	1·5	41·5	25·5	19·5	15·5	3·8
15	36·0	37·0	30·0	25·5	1·8	40·5	28·5	19·0	13·5	3·9
Pooled SE (df 16)	0·8	1·1	2·1	1·6		1·3	1·1	1·0	0·8	

* Ca:P ratios of the diets were: G1, 1·01:1; G2, 1·50:1; G3, 1·97:1; G4, 2·50:1.

† For details of diets and procedures, see Table 1 and pp. 407–408.

‡ Statistical significance of effect of diet (D), $P < 0.0001$, time (T), $P < 0.0001$, and D × T interaction, $P = 0.0037$.

§ Statistical significance of effect of D, $P < 0.0001$, T, $P < 0.0001$, D × T, $P < 0.0914$. For relative P retention, s (square root of residual mean square) = 1·9; for Ca retention, s = 1·7.

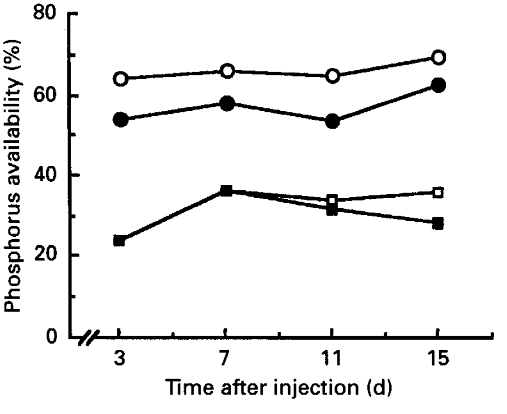

Fig. 2. Influence of the calcium:phosphorus ratio of the diet on the relative availability of feed phosphorus to growing chicks, from 3 to 15 d after injection of ^{32}P. Ca:P ratios of the diets were: (○), 1·01:1; (●), 1·50:1; (□), 1·97:1 and (■) 2·50:1. For details of diets and procedures, see Table 1 and pp. 407–408.

DISCUSSION

This study investigated the metabolic interactions between the retention and endogenous excretion of absorbed P from feed, with changes in dietary Ca:P ratio. These interactions have an important influence on the contents of minerals in the body of growing animals, especially in bones. These experiments provide important information with which to determine the P requirements of growing chicks to improve their growth performance. The growth rate and the retained minerals in bones are affected by the content of organic phytate-P and the range of Ca:P ratio in feed (Scharifi, 1978). A high Ca concentration in feed has a negative effect on phytin hydrolysis (Schultz & Oslage, 1972), and leads to a decrease in P utilization (Huyghebaert *et al.* 1981). The faecal excretion of Ca and P increases with increasing dietary levels (Damron *et al.* 1975). Hermes *et al.* (1983) reported

Table 6. *Relationship of phosphorus absorption and phosphorus retention in the whole body, to endogenous phosphorus excretion (mg P/d per chick) in growing chicks fed on diets with different calcium:phosphorus ratios*†*

(Mean values for the entire experimental period)

	G1	G2	G3	G4
Absorption (Ab)	304	270	160	158
Retention (Re)	169	161	129	128
Endogenous excretion (Ex)	135	109	31	30
Re/Ab × 100	56	60	81	81
Ex/Ab × 100	44	40	19	19
Relative Re/relative Ex	1·27	1·50	4·26	4·26

* Ca:P ratios of the diets were: G1, 1·01:1; G2, 1·50:1; G3, 1·97:1; G4, 2·50:1.
† For details of diets and procedures, see Table 1 and pp. 407–408.

that P absorption decreased with increasing width of Ca:P ratio in the feed of growing chicks and the amounts excreted from injected ^{32}P were 45, 31 and 9% at Ca:P ratios of 0·66:1, 1·5:1 and 2·5:1 respectively. The efficiency of utilization of feed P for retention in the body amounted to 39% (0·66:1), 62% (1·5:1 and 2·5:1). Further increasing the Ca intake above 440 mg/d progressively depressed the absorption of P (Hurwitz *et al.* 1978). This is in agreement with our results where P availability reached 66, 57, 32 and 30% when using Ca:P ratios of 1:1, 1·5:1, 2:1, and 2·5:1 respectively. Relative P retention amounted to 33% for growing chicks given feed containing 6·0 g total P/kg, of which 1·4 g was organic phytate-P, with a Ca:P ratio of 1·68:1 (Günther & Al-Masri, 1988). In the present experiments, relative P retention amounted to 34% at a Ca:P ratio of 1·5:1. Further increases in the ratio led to decreases in relative P retention.

Our results indicate that values for the specific ratio of the radioactivity of ^{32}P in excreta to the specific radioactivity of ^{32}P in plasma were: (G1) 0·678:1, (G2) 0·568:1, (G3) 0·165:1 and (G4) 0·146:1. According to Hevesey *et al.* (1939) and Hevesey (1948, 1962) the radioactivity of ^{32}P of endogenous P in faeces must be equal to the radioactivity of ^{32}P of the inorganic P in blood plasma, at the total absorption of feed P.

An increase in feed phytate-P limits endogenous P excretion and decreases P absorption (Günther *et al.* 1978) and also decreases relative Ca retention of feed Ca at constant Ca:P ratio in the feed (Günther & Al-Masri, 1988). The latter authors reported that 29 (group 1), 34 (group 2), 35 (group 3) and 64% (group 4) of the absorbed P was retained in the body and the endogenous P excretions were 249, 160, 90 and 38 mg/d per chick respectively. Group 1 contained only 6·2 g inorganic P/kg DM, group 2 contained 6·0 g total P/kg DM in which 1·4 g/kg DM was organic phytate-P. Group 3 contained 6·5 g total P/kg DM in which 3·3 g/kg DM was organic phytate-P. Group 4 contained 3·2 g only inorganic P/kg DM. In comparison, our results indicated that 56 (G1), 60 (G2) and 81% (G3 and G4) of P absorbed was retained, and the endogenous P excretion amounted to (mg P/d per chick): 135, 109, 30 and 31 respectively. Comparing the results of Günther & Al-Masri (1988) with the present results, we conclude that the effect of Ca concentration in the diet on endogenous P excretion and P absorption is higher than the effect of phytate-P and the latter is higher than the effect of total P in the diet.

The rate of relative P retention to relative endogenous P excretion increased with increasing Ca concentration in the feed. Our results indicate that, if 100 mg P were absorbed daily, then the amounts of endogenous P excreted would be 44, 40, 19 and 18 mg for groups 1, 2, 3 and 4 respectively. However, Abel *et al.* (1982) reported that total P absorbed per animal amounted to 3759 mg during a 14 d experimental period, and 2459 mg P/animal was retained. Chickens were fed *ad lib.* on a phytic acid-free diet containing 5 g P/kg DM and 7·5 g Ca/kg DM.

The results indicate that the difference between absorbed P and P retention in the body was most clearly observed at narrow Ca:P ratios in the feed, which can be accounted for by the high rates of endogenous P excretion. The difference between P availability and relative retention of feed P was 29% for G1 (Ca:P 1:1) and 6% for G4 (Ca:P 2·5:1). P availability of feed P decreased at wide Ca:P ratios in the feed due to the low amount of endogenous P excretion and the increase in Ca concentration of the feed, where Ca limits the absorption of P and decreases endogenous P in urine. It is apparent that the kidney plays an important role in the regulation of P absorption and thus in its homeostasis in the body.

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