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Metabolism and requirements for calcium and phosphorus in the fast-growing chicken as affected by age

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Three series of experiments were conducted with fast-growing chickens in order: to evaluate the effects of dietary Ca and P on cholecalciferol metabolism and expression; to determine dietary Ca requirements; to determine dietary P requirements. The results of the first series confirmed previous results on the effects of dietary Ca and P on some variables of vitamin D metabolism and expression, Ca homeostasis and P metabolism in the young chicken (1- to 21-d-old), and extended them to older birds (22- to 43-d-old). The bone formation rate and the duodenal calbindin content were maintained at high levels until the age of 43 d. Dietary Ca or P restriction increased duodenal calbindin and decreased bone ash in both 22- and 43-d-old chickens, but the effect on bone ash was less pronounced in the 43-d-old birds than in the younger ones. These results suggest that: (a) the capabilities for adaptation to dietary Ca and P restriction remain high during the whole growing period; (b) the growing broilers express a high adaptive capability even when the diet contains the recommended Ca and P contents. The results of the second and third series of experiments suggest that: (c) unlike the Ca requirements of the 1- to 22-d-old chick, P requirements for growth and bone ash are similar, and are as high in the older chicks as in the younger ones (7·4–8·3 g P/kg or 4·8–5·7 g non-phytate P/kg diet); (d) although growth and bone ash in the 29- to 43-d-old chickens appear to be less sensitive to dietary Ca content, within a range close to the calculated P requirement, 10 g Ca/kg diet appears to be required for best tibia mineralization, and to a lesser extent for better growth at this age.

Bone: Growth: Calbindin: Cholecalciferol: 1-Hydroxylase

Chicken growth has been accelerated enormously during recent decades (Havenstein *et al.* 1994), and there has been a matching increase in bone mass. Thus, the Ca regulatory system has become stressed to accommodate the increased Ca and P needs. Insufficient supplies of these two minerals reduce growth rate and bone calcification, and excess Ca intake also induces similar, but moderate reductions in growth and bone ash (Shafey *et al.* 1990; Hurwitz *et al.* 1995). Because of feedback relationships, various components of the regulatory system, such as kidney 25 hydroxycholecalciferol-1-hydroxylase (1-hydroxylase), intestinal calbindin, and plasma Ca, P or 1,25 dihydroxycholecalciferol ($1,25(OH)_2D_3$), are markedly influenced by the rate of growth (Bar & Hurwitz, 1981; Hurwitz *et al.* 1995). Modulation of growth, by energy intake, genetic selection, sex, ambient temperature changes, or age, alters the response of the fast-growing chicken to dietary Ca (Bar & Hurwitz, 1981; Hurwitz *et al.* 1995) or P (Orban & Roland, 1990; Shafey *et al.* 1990) content. Some of these responses tend to be affected similarly by dietary Ca and P contents; others are affected differently by the respective minerals. For instance, intestinal absorption of Ca and P is induced by $1,25(OH)_2D_3$. Ca or P depletion increases plasma $1,25(OH)_2D_3$, but only Ca depletion induces kidney 1-hydroxylase activity in birds (Friedlander *et al.* 1977; Montecuccoli *et al.* 1977; Bar *et al.* 1982). Plasma Ca modulates parathyroid hormone secretion, which, in turn, affects renal $1,25(OH)_2D_3$ formation; the latter and parathyroid hormone cause the bones to release Ca and P into the circulation. However, whereas the renal reabsorption of both minerals is affected similarly by $1,25(OH)_2D_3$, parathyroid hormone has contrary effects on the two minerals (for reviews, see Fitzpatrick & Bilezikian, 1996; Kumar, 1997; Wasserman, 1997). As a result of the above differences, the fast-growing chicken may exhibit anti-homeostatic responses, such

Abbreviations: BW, body weight; 1-hydroxylase, 25 hydoxycholecalciferol-1-hydroxylase; NRC, National Research Council; $1,25(OH)_2D_3$, 1,25 dihydroxycholecalciferol.

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as increased Ca absorption associated with P deficiency, which may aggravate the hypercalcaemia and may damage the kidney (Page *et al.* 1979).

Our previous study (Hurwitz *et al.* 1995) focused on the interactions between growth, and dietary P and Ca homeostasis and requirements in the young (1- to 21-d-old) growing chick. Less attention was given to the older chicken, although its Ca and P consumption and consequently its P excretion to the environment are greater. Furthermore, leg disorders associated with cholecalciferol, Ca and P have become more widespread and severe in the older chicken (Riddle, 1991; Sorensen *et al.* 2000). Thus, in addition to the economic implications, Ca and P imbalance may also impair chicken welfare.

Previous publications have suggested that ageing decreases the basal metabolism and expression of cholecalciferol (as indicated by the changes in calbindin synthesis) in chickens (Bar & Hurwitz, 1981), and diminishes the adaptive capability of cholecalciferol metabolism and expression in mammals (Armbrecht *et al.* 1998, 1999) and in laying hens (Bar & Hurwitz, 1987). However, it is not yet clear whether the adaptive capability of a chicken is also impaired within the rearing period of 6 to 7 weeks.

Despite the marked acceleration in chicken growth during the last decade, the NRC's recommendations for dietary Ca and P for chicks at the ages 1–21, 22–42 and 43–56 d (10, 9·0 and 8·0, and 4·5, 3·5 and 3·0 g Ca and non-phytate P/kg diet, respectively) have not changed (National Research Council, 1994). Since 1994 only a few relevant papers have been published focusing mainly on P requirements for the young broiler, with special attention paid to decreasing the recommended P allocation by supplementing the diet with $1,25(OH)_2D_3$, phytase, or both (for example, Mitchell & Edwards, 1996; Waldroup *et al.* 2000). In these and in other studies the NRC recommendations on Ca and P contents (National Research Council, 1994) were accepted. A few other publications (Hurwitz *et al.* 1995; Rao & Reddy, 1999) addressed the reassessment of the recommendations, especially those for Ca, although Ca appeared to be less threatening to the environment than P. Therefore, the present study aimed: (a) to extend the evaluation of the effects of age on Ca, P and cholecalciferol metabolism and expression; (b) to determine the requirements for P in the fast-growing chick during the whole rearing period. In the light of the Ca–P interrelationships, the secondary aim was: (c) to determine the requirements for Ca in the 29- to 42-d-old fast-growing chicken.

Materials and methods

Animals

Day-old Cobb chicks were obtained from a commercial hatchery. The chicks were raised in battery brooders situated in windowless constant-temperature rooms at 24°C, with continuous fluorescent lighting. At the age of 29 d the chicks were transferred to individual cages in a similar room at 20°C. Until the beginning of the experiments, all birds received standard diets, designed to satisfy the recommendations of the National Research Council (1994) except for Ca and P. At the beginning of the experiments, the chicks were divided into the experimental groups with the aid of a computer algorithm that equalized both average

Table 1. Composition of the basal experimental diets

Age (weeks)...	1–4		5 and 6	
Basal diet...	a	b*	c	d*
Ingredients (g/kg)				
Yellow maize	528·0	340·2	594·0	500·0
Sorghum		200·0		45·7
Soyabean oil meal	366·0	357·0	300·0	323·0
Soyabean oil, refined	30·0		30·0	
Soapstock		45·0		55·0
Fish meal		10·0		
NaCl	3·0	3·0	3·0	3·0
DL-methionine	1·94	2·0	2·0	2·0
Vitamin mixture†	2·5	2·5	2·5	2·5
Trace mineral mixture‡	0·3	0·3	0·3	0·3
KH_2POD_4+$NaH_2PO_4.H_2O$ (1:1)	Varied	8·0	0	8·0
$CaHPO_4.2H_2O$ (commercial grade)	Varied	7·5	Varied	7·5
Ground limestone	Varied	Varied	Varied	Varied
Calculated composition				
Metabolizable energy (mJ/kg)	12·1	12·5	12·3	12·9
Crude protein	208	211	184	190
Ca	1·2	1·3	1·0	1·1
Total P	3·9	7·2	3·6	6·8
Available P	1·3	4·5	1·2	4·3

* Experiment 5 only.

† The vitamin mixture supplied (/ kg feed): retinyl acetate 2·9 mg (8400 IU); cholecalciferol, 50 μg (2000 IU); α-tocopheryl acetate, 24 mg; menadione, 2 mg; riboflavin, 5·6 mg; Ca-pantothenate, 11·2 mg; thiamine, 0·8 mg; niacin, 29·6 mg; vitamin B_{12}, 0·008 mg; folic acid, 0·8 mg; pyridoxine, 2·4 mg; biotin, 0·1 mg, choline chloride, 200 mg; manox (antioxidant), 125 mg.

‡ The mineral mix supplied (mg/kg feed): Mn, 80; Zn, 50; Fe, 25; Cu, 2; I, 1.2; Co, 0.2; Se, 0.1.

body weight (BW) and variance. This procedure was followed within each age and sex. In experiments 5 to 7, each diet was fed for 7 or 14 d to four or six replicate groups of ten or five birds aged 8–21 or 29–43 d, respectively. The experimental diets were designed to satisfy the NRC recommendations (National Research Council, 1994). The basal ingredients of the diets are given in Table 1; the designed experimental diets varied in their Ca and P contents, through the addition of various amounts of ground limestone, $CaHPO_4.2H_2O$ and $NaH_2PO_4.H_2O+KH_2PO_4$ to the basal ingredients. When $NaH_2PO_4.H_2O+KH_2PO_4$ was used, $NaHCO_3$ and $KHCO_3$ were added to keep the Na and K contents constant. The dietary Ca and P contents presented in the text, figures. and Tables 2 to 6 are always those obtained by chemical analysis.

During the experiments, BW and feed intake were determined weekly on individual and group bases, respectively. At the end of experiments 1 to 4, blood samples were obtained by cardiac puncture, with heparin used as an anticoagulant. Birds were killed by neck dislocation and sampled for the determination of duodenal calbindin, bone ash and renal 1-hydroxylase activity. At the end of experiments 5 to 7, the eight birds with BW closest to the average were removed from each treatment group for bone analysis. They were killed by neck dislocation, and the tibiae were removed for bone ash determination. The experimental protocols were approved by the National Committee for Animal Experimentation Ethics, and were conducted according to the Israeli Law on Animal Welfare and Experimentation, 1994.

Three series of experiments were conducted in order: (a) to evaluate the effects of dietary Ca and P on cholecalciferol metabolism and expression (experiments 1 to 4); (b) to determine dietary Ca requirements in 29- to 43-d-old chickens (experiment 5); (c) to determine dietary P requirements in fast-growing chickens (experiments 6 and 7).

Experiment 1

The purpose of experiment 1 was to evaluate the effects of restriction or excess of dietary Ca, P or both, on plasma Ca and inorganic P, on bone ash, and on the metabolism and expression of cholecalciferol, in young fast-growing chicks. The experimental diets were fed for 11 d to ten 1-d-old chicks. At the end of the experiment, six to eight birds were bled and sampled for the determination of bone ash, and for calbindin and 1-hydroxylase analyses.

Experiments 2 and 3

The purpose of these experiments was to determine the effects of age on plasma Ca, inorganic P, duodenal calbindin, and bone ash. Day-old chicks were fed standard commercial diets, designed to satisfy the NRC recommendations (National Research Council, 1994). Seven birds were bled and sampled for duodenal calbindin and bone ash at each of the ages of 8, 15, 22, 29 and 43 d. Experiments 2 and 3 were conducted in 1991 and 2001, respectively.

Experiment 4

The purpose of experiment 4 was to compare the effects of dietary Ca or P restriction on plasma Ca and inorganic P, duodenal calbindin and bone ash, in 22- or 43-d-old chicks. The experimental diets were fed to ten chicks for 7 d. The diets supplied the chicks with 10·5 or 5·5 g Ca/kg diet and 7·5 or 4·4 g total P/kg diet. At the end of the experiment, eight birds were bled and sampled.

Experiment 5

The purpose of experiment 5 was to evaluate the effects of age on the requirements for Ca. The experimental diets were fed for 7 d to 8- or 15-d-old chicks, and for 14 d to 29-d-old chicks. The diets for the 8- to 22-d-old and the 29- to 43-d-old chicks contained 7·2 and 6·9 g P/kg diet, respectively. The basal diets used were b and d (Table 1). Bone ash was determined at the ages of 15, 22 and 43 d.

Experiment 6

The purpose of experiment 6 was to evaluate the effects of age and sex on the requirements for P. The experimental diets were fed for 14 d to 8- or 29-d-old chicks, and contained 10·5 g Ca/kg diet.

Experiment 7

The purpose of experiment 7 was to evaluate the effects of dietary Ca on the P requirements of 29- to 43-d-old fast-growing chickens.

Chemical analysis

Bones were cleaned of any adhering tissues, dried at 105°C and ashed at 650°C for 4 h. Blood samples were centrifuged and plasma was harvested. Plasma Ca was determined in HNO_3-digested samples by means of an Atomic Emission Spectrometer (Spectro, Germany), or directly by automatic titration (Bar & Hurwitz, 1981; experiment 1). Inorganic P was determined in supernatant fractions of TCA-treated plasma, with an Atomic Emission Spectrometer or colorimetrically according to Gomori (1942) (experiment 1).

Renal 1-hydroxylase activity was determined as described previously (Montecuccoli *et al.* 1977). Duodenal calbindin was determined with a radioimmunoassay (Bar & Hurwitz, 1979) (experiments 1 and 2) or by means of a newly developed specific ELISA (S Yosefi and A Bar, unpublished results; experiments 3 and 4).

Numerical analysis

The results were analysed routinely by ANOVA (Snedecor & Cochran, 1967), and differences between means were tested by Duncan's multiple range test (Duncan, 1955). Statistical tests were performed on replicate averages for growth and feed efficiency data, to account for the effects of location, whereas individual values were used for the chemical assays.

For calculation of the Ca or P requirements, the results of BW gain and tibia ash determinations were fitted to a quadratic equation (Hurwitz *et al.* 1995). The coefficients of determination (R^2) served as measures of goodness of fit. Values given in Tables are based on the analysed dietary contents, together with values based on the calculated dietary content.

Results

Experiment 1

The results of experiment 1 (Table 2) show that dietary Ca restriction caused slight hypocalcaemia, increased the kidney 1-hydroxylase and duodenal calbindin and decreased bone ash. Dietary P restriction caused hypercalcaemia and hypophosphataemia, increased duodenal calbindin but not kidney 1-hydroxylase, and markedly reduced bone ash. The hypercalcaemia and hypophosphataemia observed in the dietary P-restricted birds were ameliorated by reducing the dietary Ca level from 10·4 to 1·9 g/kg diet; however, the duodenal calbindin remained as high and the bone ash as low as in the P-restricted birds fed 10·3 g Ca/kg diet. Hypercalcaemia and hypophosphataemia were observed also when the dietary Ca was increased from 10·4 to 21·6 g/kg diet. The reductions in duodenal calbindin and in bone ash in chicks fed the high dietary Ca were ameliorated by raising the dietary P level from 7·2 to 10·7 g/kg diet. The dietary combination of high Ca and high P resulted in the highest bone ash content.

Experiments 2 and 3

Duodenal calbindin (Fig. 1) decreased progressively in experiment 2 (conducted in 1991), but in experiment 3 (conducted in 2001) it increased slightly up to the 29th day, and then decreased slightly. The effects on duodenal calbindin in both experiments were significant ($P<0·05$). Bone ash increased progressively in both experiments, but its values were higher in experiment 3 (conducted in 2001). In experiment 3 the tibia ash:BW ratio tended to increase with age.

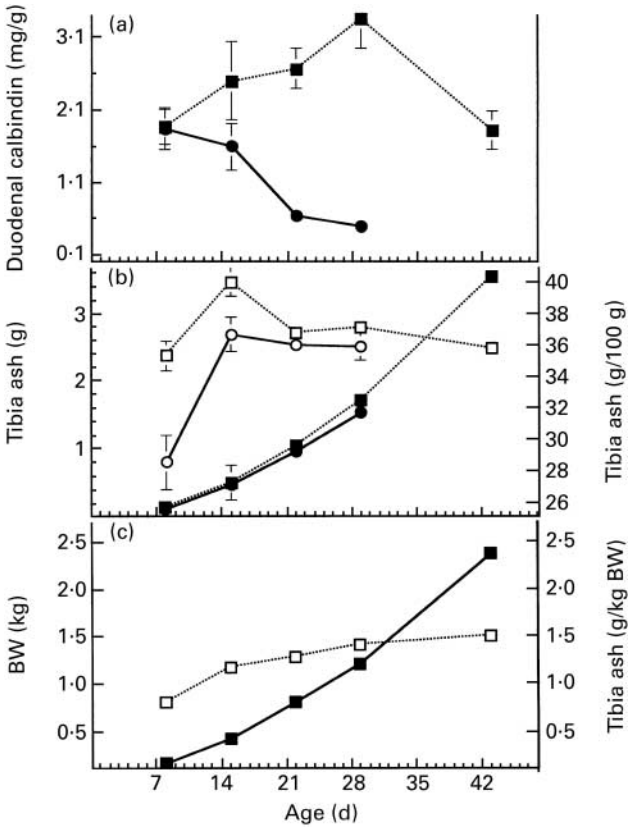

Fig. 1. (a), Duodenal calbindin; (b), tibia ash (○ and □, g/100 g; ● and ■, g); (c), body weight (BW) (■) and tibia ash/kg BW (□) in fast-growing chickens, as affected by age and year of experiment (○ and ●, 1991 (experiment 2); □ and ■, 2001 (experiment 3). Mean values are shown, with standard errors of difference represented by vertical bars.

Experiment 4

Results of experiment 4 (Table 3) indicate that with increasing age, bone ash weight increased significantly (not given in the Table), but its percentage (g/100 g) decreased significantly. Dietary Ca or P restriction

Table 2. The effects of dietary calcium and phosphorus on plasma concentrations of total calcium and inorganic phosphorus (P_i), duodenal calbindin, renal 25 hydoxycholecalciferol-1-hydroxylase (1-hydroxylase), and tibia ash of 12-d-old chicks fed the experimental diets for 11 d (experiment 1)*

(Means values with their standard errors of the difference for six to eight birds)

Dietary level (g/kg)		Plasma concentration (nmole/l)				Duodenal calbindin (mg/g)		Renal 1-hydroxylase (pmole/g per min)		Tibia ash (g/100 g)	
		Ca		P_i							
Ca	P	Mean	SED	Mean	SED	Mean	SED	Mean	SED	Mean	SED
1·9	3·9	$2·13^{c}$	0·10	$2·07^{ab}$	0·23	$2·03^{a}$	0·10	$1·77^{b}$	0·19	$21·6^{d}$	1·2
1·9	7·2	$2·35^{bc}$	0·10	$2·50^{a}$	0·17	$1·90^{a}$	0·11	$3·25^{a}$	0·51	$27·6^{c}$	0·5
10·3	3·9	$3·30^{a}$	0·18	$0·53^{c}$	0·10	$1·59^{b}$	0·22	$1·55^{bc}$	0·25	$18·4^{d}$	1·0
10·4	7·2	$2·73^{b}$	0·05	$2·10^{ab}$	0·13	$0·71^{cd}$	0·06	$0·75^{bc}$	0·11	$37·9^{ab}$	0·9
21·6	7·1	$3·55^{a}$	0·23	$0·90^{c}$	0·17	$0·48^{d}$	0·10	$0·71^{bc}$	0·13	$31·3^{b}$	0·9
10·1	11·1	$2·68^{b}$	0·13	$1·87^{b}$	0·13	$1·04^{c}$	0·06	$1·53^{bc}$	0·28	$37·5^{ab}$	0·7
21·8	10·7	$2·73^{b}$	0·10	$2·30^{ab}$	0·13	$0·71^{cd}$	0·04	$0·58^{c}$	0·07	$40·2^{a}$	1·0

^{a,b,c,d}Mean values within a column with unlike superscript letters were significantly different ($P<0·05$) by Duncan's test.
* For details of diets and procedures, see Table 1 and p. 53.

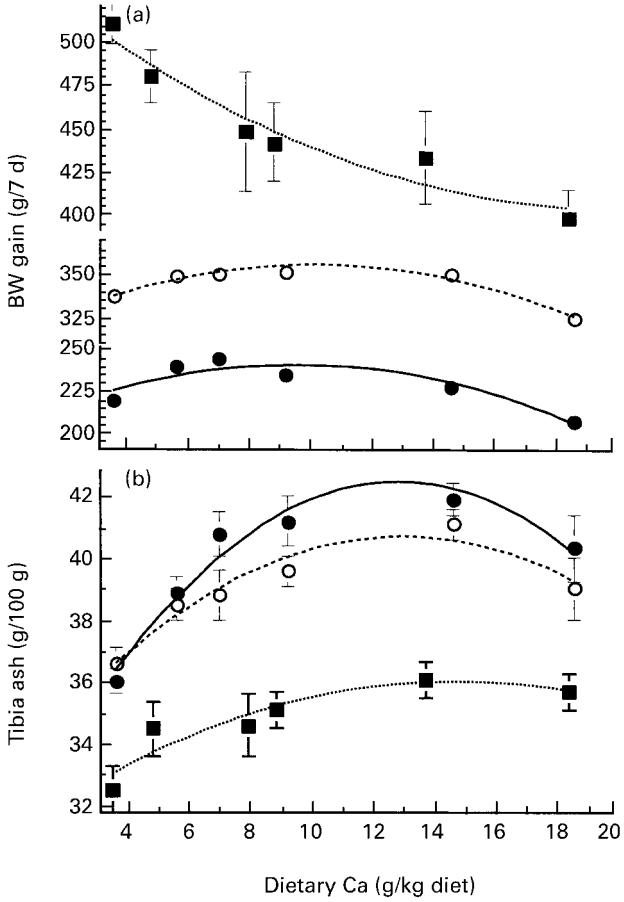

Fig. 2. (a), Body weight (BW) gain (during the last 7 d); (b), tibia ash in fast-growing chickens as affected by age and dietary calcium (*n* 4 or 6 replicates and *n* 8 individuals for weight gain and tibia ash, respectively) in experiment 5. (●), 15 d old; (○), 22 d old; (■), 43 d old. Mean values are shown, with standard errors of difference represented by vertical bars.

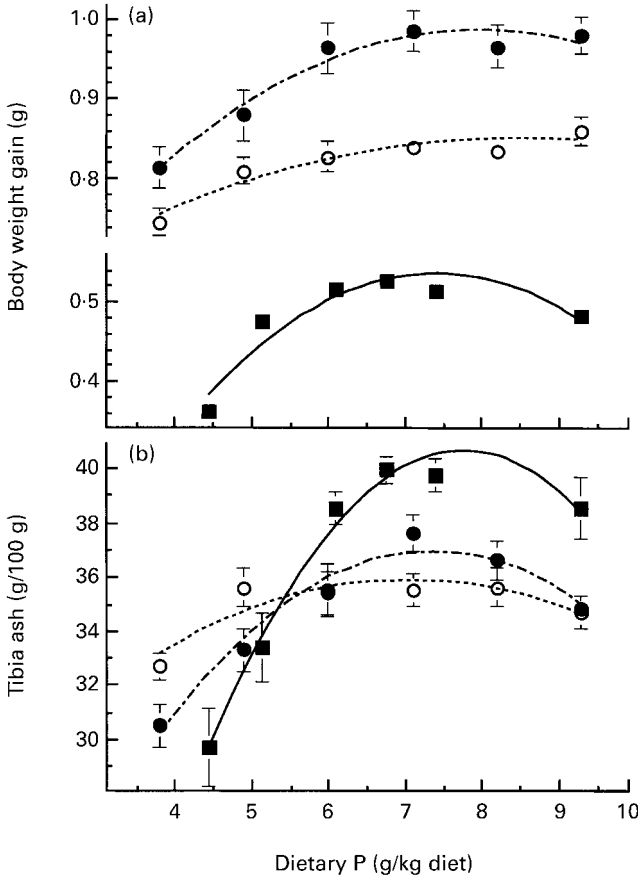

Fig. 3. (a), Body weight gain; (b), tibia ash in 8- to 22-d-old fast-growing chickens, as affected by age and sex, and dietary total phosphorus (*n* 6 replicates and *n* 8 individuals for weight gain and tibia ash, respectively) in experiment 6. (■), 22-d-old males; (●), 43-d-old males; (○), 43-d-old females. Mean values are shown, with standard errors of difference represented by vertical bars.

significantly induced the formation of duodenal calbindin and decreased bone ash; the effect of dietary P restriction was more pronounced. Dietary P restriction significantly increased plasma Ca and decreased plasma inorganic P; the effect on plasma Ca was more pronounced in the 22-d-old chicks (significant interaction). BW (not given in Table 3) was higher in the 43-d-old chicks but was not affected significantly by dietary Ca or P.

Experiment 5

When the responses of Ca requirements to age were studied, the effects of dietary Ca and age were significant ($P<0{\cdot}05$) for all response criteria. A bell-shaped (Fig. 2) response of tibia ash to dietary Ca level was observed. A similar response of BW gain to dietary Ca level was also observed in 8- to 15-, and 15- to 22-d-old chickens (Fig. 2). In contrast, weight gain in 36- to 43-d-old chickens was progressively reduced as dietary Ca increased, indicating a significant Ca × age interaction. The results of experiment 5 (Table 4) show that the optimal Ca requirements for bone calcification were higher than those for BW gain in 8- to 22-d-old chickens. A similarly high calculated Ca requirement for bone ash was observed in 29- to 43-d-old chickens, although the ash content and its sensitivity to dietary Ca were less.

Experiment 6

In experiment 6 the P requirements of 8- to 22-d-old male and 29- to 43-d-old male and female fast-growing chicks were determined. The results are shown in Fig. 3. The effects of P, age and sex on BW gain, and the effects of P and age on bone ash were significant (not given in detail). Bell-shaped responses of BW gain and tibia ash to dietary P level were observed in male chickens. A similar bell-shaped response of bone ash was observed in the female chicks, whereas the response of the BW gain of the female chickens tended to reach a plateau, and not a clear maximum. Quadratic equations were used to calculate the optimal P requirements. The results of experiment 6 (Table 5) show that, except for BW gain in the 22- to 43-d-old female chicks, P requirements for optimal bone calcification and BW gain at both ages were quite similar.

Table 3. The effects of dietary calcium or phosphorus restriction and age on plasma concentrations of total Ca and inorganic phosphorus (P_i), duodenal calbindin and tibia ash of chicks fed the experimental diets for 7 d (experiment 4)*

(Means values and standard errors of the means for six to eight birds in each experimental group)

	Dietary level (g/kg)		Plasma concentration (nmole/l)		Duodenal calbindin	Tibia ash	
Age (d)	Ca	P	Ca	P_i	(mg/g)	g	g/100 g
22			3·28	1·73	2·95	0·913[a]	36·5[a]
43			3·11	1·82	2·72	3·183[b]	33·8[b]
SEM			0·08	0·11	0·23	0·045	0·4
	10·5	7·5	2·96[b]	2·09[a]	1·64[b]	2·336[a]	37·9[a]
	5·5	7·5	2·78[b]	2·17[a]	3·18[a]	2·025[b]	36·4[a]
	10·5	4·4	3·86[a]	1·08[b]	3·83[a]	1·742[c]	31·2[b]
SEM			0·10	0·13	0·29	0·055	0·5
22	10·5	7·5	2·98[c]				40·0[a]
22	5·5	7·5	2·69[c]				38·4[a]
22	10·5	4·4	4·18[a]				31·2[c]
42	10·5	7·5	2·94[c]				35·8[b]
42	5·5	7·5	2·86[c]				34·4[b]
42	10·5	4·4	3·53[b]				31·2[c]
SEM			0·14				0·7
Probability							
Age			0·141	0·249	0·688	<0·001	<0·001
Diet			<0·001	<0·001	<0·001	<0·001	<0·001
Age x diet			0·020	0·592	0·472	0·314	0·009

[a,b,c]Mean values within a column for each variable with unlike superscript letters were significantly different ($P<0·05$) by Duncan's test.
* For details of diets and procedures, see Table 1 and p. 53.

Experiment 7

When the influence of dietary Ca on P requirements was studied in 29- to 43-d-old chickens, the effects of dietary P (Table 6), but not those of Ca, were significant for all response criteria. Ca × P interactions were significant for BW gain and tibia ash (Fig. 4). These interactions most probably resulted from the slightly higher BW of chickens fed 10·4 g Ca/kg diet and the two higher dietary P levels, and from the remarkable effect of dietary Ca on bone ash of chicks fed 6·2 to 8·5 g total dietary P/kg diet, whereas at the lowest P intake the higher dietary Ca content markedly reduced the BW and bone ash. Quadratic equations were used to calculate the optimal P requirements for BW gain and tibia ash. The values given in Table 5 indicate that the optimal P levels for BW gain and for bone ash are quite similar.

Discussion

As previously shown (Hurwitz *et al.* 1995) and confirmed later (Williams *et al.* 2000), the Ca requirement for growth of the young (7- to 21-d-old) fast-growing chicken fed the recommended dietary P level is similar to, or even slightly higher than, the National Research Council (1994) recommendation. However, the Ca requirement for optimal bone ash is markedly higher than the recommended level (Table 4). Unlike Ca, we found the P requirements for growth and bone ash of the young fast-growing chicken to be similar (Table 5). Our estimated P requirement is slightly higher (7·4–7·8 g P/kg or 4·8–5·2 g non-phytate P/kg diet) than the NRC recommendation or than the estimates of Rao & Reddy (1999) or Waldroup *et al.* (2000), but are quite similar to the findings of Mitchell & Edwards (1996).

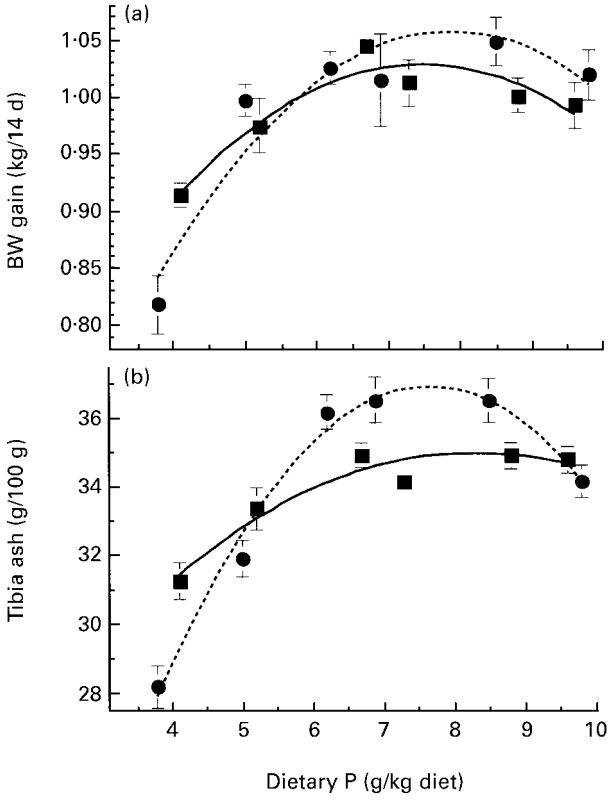

Fig. 4. (a), Body weight (BW) gain; (b), tibia ash in fast-growing Cobb chickens as affected by dietary calcium and phosphorus (*n* 6 replicates and *n* 8 individuals for weight gain and tibia ash, respectively) in experiment 7. (■), 8·1 g Ca/kg diet; (●), 10·4 g Ca/kg diet. Mean values are shown, with standard errors of difference represented by vertical bars.

Table 4. Coefficient of determination (R^2) for fitting quadratic equation, analysed and estimated calcium requirements as influenced by age and dietary total phosphorus in fast-growing chickens (experiment 5)*

		Weight gain (kg/7 d)			Tibia ash (g/100 g)		
			Ca requirement (g/kg diet)			Ca requirement (g/kg diet)	
Age (d)†	Dietary P (g/kg)‡	R^2	Analysed‡	Estimated§	R^2	Analysed‡	Estimated§
8–14	7·2	0·824	9·6	9·9	0·955	12·8	13·9
15–21	7·2	0·951	10·9	10·4	0·937	13·0	14·0
36–43	6·9	0·932	NC		0·867	14·7	15·7

NC, not calculated (because the plot was convex rather than concave).
* For details of diets and procedures, see Table 1 and p. 53.
† Experimental diets were fed from the age of 29 until 43 d.
‡ By chemical analysis. The calculated P contents are given in Table 1.
§ The estimated Ca requirements are based on the calculated dietary Ca content.

Table 5. Coefficient of determination (R^2) for fitting quadratic equation, analysed and estimated phosphorus requirements and source of variation in fast-growing chickens as influenced by age, breed, sex and dietary calcium*

			Weight gain (kg)			Tibia ash (g/100 g)		
				P requirement (g/kg diet)			P requirement (g/kg diet)	
Sex	Age (d)	Dietary Ca (g/kg)†	R^2	Analysed†	Estimated‡	R^2	Analysed†	Estimated‡
Experiment 6								
Male	8–21	10·4	0·900	7·4	8·0	0·984	7·8	8·9
Male	29–43	10·5	0·959	8·0	8·0	0·970	7·4	7·4
Female	29–43	10·5	0·814	8·5	8·6	0·788	7·0	7·2
Experiment 7								
Male	29–43	8·1	0·990	7·4	7·1	0·929	7·9	7·9
Male	29–43	10·2	0·955	7·9	7·5	0·984	7·7	7·4

* For details of diets and procedures, see Table 1 and p. 53.
† By chemical analyses. Calculated Ca contents of the diets were 11 and 10 or 8 g Ca/kg for the 8–21- and 29–43-d-old chicks.
‡ The estimated requirements are based on the calculated dietary P content.

Table 6. Major effects of dietary total phosphorus and calcium on body weight (BW), BW gain, feed efficiency and tibia ash in 29- to 43-d-old fast-growing Cobb chickens (experiment 6)*

(Means values and standard errors of the means)

Dietary Ca (g/kg)	Dietary P (g/kg)	BW (kg)†	BW gain (kg/2 weeks)†	Feed efficiency†	Tibia ash (g/100 g)‡
8·1		2·165	0·990	0·468	33·9
1·04		2·173	0·985	0·462	33·9
SEM		0·009	0·008	0·005	0·4
	3·9	2·054^{b}	0·883^{c}	0·423^{b}	29·7^{c}
	5·1	2·165^{a}	0·985^{b}	0·466^{a}	32·6^{b}
	6·4	2·211^{a}	1·035^{ab}	0·482^{a}	35·5^{a}
	7·1	2·185^{a}	1·011^{ab}	0·473^{a}	35·3^{a}
	8·6	2·201^{a}	1·024^{ab}	0·482^{a}	35·7^{a}
	9·7	2·182^{a}	1·006^{ab}	0·476^{a}	34·4^{a}
SEM		0·017	0·013	0·009	0·4
Probability					
Ca		0·882	0·602	0·518	0·989
P		$<$0·001	$<$0·001	$<$0·001	$<$0·001
Ca × P		0·123	0·025	0·383	$<$0·001

^{a,b,c}Mean values within a column for each variable with unlike superscript letters were significantly different ($P<0·05$) by Duncan's test.
* For details of diets and procedures, see Table 1 and p. 53.
† Means of six replicates of five birds.
‡ Means of eight birds.

The fast-growing young chicken adapts itself to dietary Ca or P restriction by increasing intestinal Ca and P absorption (Morrissey & Wasserman, 1971; Montecuccoli *et al.* 1977), most probably as a result of the elevation in plasma and intestinal concentrations of $1,25(OH)_2D_3$ (Bar *et al.* 1982; Hunziker *et al.* 1982), and duodenal calbindin (Tables 2 and 3; Morrissey & Wasserman, 1971; Montecuccoli *et al.* 1977), although the kidney 1-hydroxylase concentration has been shown to be increased only by dietary Ca restriction (Table 2; Friedlander *et al.* 1977; Montecuccoli *et al.* 1977). However, the increased absorption cannot compensate for the low intake under severe restriction (Bar *et al.* 1979); therefore, bone ash markedly declines. Table 2 presents, in addition to the well-known responses to dietary Ca or P restriction, the responses of the young chick to excess of dietary Ca or P and to restriction or excess of both of these two minerals in combination. Such a combined restriction of Ca and P may prevent the hypophosphataemia and hypercalcaemia caused by dietary P restriction, but cannot prevent the decline in bone mineralization. Intake of excess Ca induced similar effects to those induced by dietary P restriction, but at the same time led to a reduction, rather than to an elevation, in duodenal calbindin (Table 2; Bar *et al.* 1990). Excessive Ca and P intake may prevent these symptoms in birds fed the high-Ca diet and will markedly increase bone ash. The excess of P alone has no specific effects under normal Ca intake, except to induce small declines in BW gain and in bone ash (Fig. 4; Hurwitz *et al.* 1995).

During growth, the needs of minerals for bone formation appeared to be maintained constant or even to increase slightly (Fig. 1). This conclusion is based on the progressive increase in the bone ash:BW ratio, and on the elevation in duodenal calbindin during the first 4 weeks of life (Fig. 1, experiment 3). The duodenal calbindin subsequently decreased slightly and finally reached a similar level to that observed at the age of 8 d. As intestinal calbindin concentration is considered to be a reliable indicator of adaptive capability and of cholecalciferol expression (Bar *et al.* 1990; Christakos, 1996), the changes in calbindin with age suggest that the normal modern chicken retains the typical accelerated adaptive mechanisms that it developed in its first few days, even at the ages of 29 to 43 d. This was not the case when chicks of the same breed were previously tested, 10 (Fig. 1; Experiment 2) or 20 (Bar & Hurwitz, 1981) years ago. Then, the adaptive mechanism (as indicated by the duodenal calbindin concentration) rapidly declined. This change in adaptability could be explained by the fact that since 1991 the BW of the 42-d-old male chicken has increased from 2·28 to 3·01 kg (Israeli Breed Tests, unpublished results), with consequent increases in the body Ca and P contents. Therefore, the needs for these elements appeared to have increased.

It is not surprising that the adaptive capability (as indicated by the high calbindin content) of the modern chicken breeds was also retained within the tested range of ages (Table 3). The high age-related adaptability (Fig. 1) observed in the unrestricted 29- to 43-d-old chick can account for the marked elevation in duodenal calbindin observed in the dietary Ca- or P-restricted chicks in this age range (Table 3). Nevertheless, the higher calbindin contents (and most likely the higher rates of Ca and P absorption; Bar & Hurwitz, 1979; Bar *et al.* 1979) did not prevent the hypercalcaemia, hypophosphataemia and reduction in bone ash (Table 3).

Thus, taken together, these findings indicate the need for re-evaluation of the requirements for these minerals in the modern chick, especially in the 28- to 42-d-old one. Although the effects of dietary P restriction on plasma Ca and P, and the effect of dietary Ca or P restriction on bone ash were less pronounced in the 29- to 43-d-old chickens (Table 3, Fig. 2) than in the younger ones, the curves describing bone ash as a function of dietary Ca or P still had a bell shape (Figs. 2, 3 and 4). This facilitated the estimation of the Ca or P requirements for bone formation in 8- to 22-d-old and in 29- to 43-d-old fast-growing chickens (Tables 4 and 5). As was previously shown (Hurwitz *et al.* 1995), the Ca requirement for bone ash was markedly higher than the NRC recommendations, especially in the 29- to 43-d-old chickens. On the other hand, increasing the dietary Ca in 29- to 43-d-old chickens that were fed the recommended P contents tended to reduce BW progressively (Fig. 2), or hardly to affect it, according to another experiment (A. Bar *et al.* unpublished results). Thus, a clear determination of the Ca requirements for growth in 29- to 43-d-old chicks that are fed the recommended P content was not possible. On the other hand, experiments 6 and 7 clearly demonstrated the significant effects of dietary P intake on growth, feed efficiency (Table 6) and bone ash. In contrast to our findings on Ca requirements in the 8- to 22-d-old chicken, P requirements for growth and bone ash (Table 5) were quite similar, and were as high in the older chick as in the young male chicks (7·4–8·0 g P/kg or 4·8–5·6 g non-phytate P/kg diet). The average P requirements for growth and bone ash, as determined in five (not all are presented here) trials conducted with 29- to 43-d-old male chicks fed diets containing 9·6 to 10·5 g Ca/kg, were 7·9 (SD 1·0) and 7·5 (SD 0·3) g/kg, respectively. The calculated requirement for growth of the 29- to 43-d-old female chick was higher (8·5 g/kg), but as only one trial with female chicks was conducted, this value is not necessarily the best or a reliable estimate. On the other hand, the female chick appears to be less sensitive to dietary P for both growth and bone formation, most likely because of its slower rate of growth (Bar & Hurwitz, 1981; Hurwitz *et al.* 1995).

The major effects of sex and age (not given in the Tables) or dietary Ca (Table 6) on the estimated P requirements were small or negligible. In experiment 7, which was conducted in order to determine the effect of dietary Ca on P requirements, Ca did not affect the pooled averages of growth or of tibia ash but, within the total P range of 6·2 to 8·5 g/kg diet (3·8 to 6·1 g available P/kg diet; Fig. 4, Table 6; Ca × P significant interactions) a Ca content of 10·4 g/kg diet increased bone ash. BW gain was slightly higher in chicks fed 8·6 to 10·0 g P/kg and 10·4 g Ca/kg diet. Similar results (A. Bar *et al.* unpublished results) were observed in another trial in which the dietary Ca contents were 10 and 12 g/kg diet). This finding on bone ash is supported by the data presented in Table 1, which indicate that high dietary P not only prevents the

hypercalcaemia and hypophosphataemia caused by high dietary Ca, but also ensures highly calcified bones. Therefore, although Ca appears to be less important in the 29- to 43-d-old chicken than in the younger one, in order that the effect of P be expressed (Fig. 4), diets for 29- to 43-d-old fast-growing chickens should contain 10·0 to 10·5 g Ca/kg diet.

The estimated requirements obtained in the present study should not necessarily be considered as recommendations, as the study was conducted with chicks raised in cages, and used specific sources of inorganic and organic P. However, the data obtained indicate that re-evaluation of Ca and P requirements under commercial conditions has become essential. New recommendations should take into account additional factors such as sex differences, prices, the environment and the use of phytases.

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