

## Dietary phosphorus restriction to half the minimum required amount slightly reduces weight gain and length of tibia, but sustains femur mineralization and prevents nephrocalcinosis in female kittens

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The effects of dietary P restriction to half the recommended minimum level on growth, bone and renal mineralization and urinary composition were studied in female kittens. In two separate experiments, 8-week-old weanling kittens were fed on purified diets containing either 4.6 or 9.2 mmol P/MJ (2.8 or 5.6 g P/kg diet). In the second experiment there was an additional low-P diet in which the Ca concentration was reduced from 9.5 to 4.8 mmol/MJ (7.5 v. 3.8 g Ca/kg diet). P restriction slightly but systematically reduced weight gain (to a maximum of 16%) and growth of the tibia (by 1–4%); the former effect was statistically significant ( $P < 0.05$ ) between the ages of 15 and 20 weeks in Expt 1 only, and the latter did not reach statistical significance at any time point ( $P \geq 0.13$ ). No adverse effect of P restriction was found on mineralization of femur at the age of 39 weeks. Kidney Ca concentrations were significantly lowered (Expt 1, 6 v. 20  $\mu\text{mol/g}$  dry weight,  $P < 0.001$ ; Expt 2, 7 v. 16  $\mu\text{mol/g}$  dry weight,  $P < 0.01$ ) in cats fed on the low-P diets, this effect not being affected by the dietary Ca:P ratio. Urinary P concentration was significantly depressed (by 50–96%) after feeding the low-P diets ( $P < 0.001$ ). P intake did not influence P, Ca and Mg retention during the period of 15 to 39 weeks of age.

Phosphorus: Mineral excretion: Bone: Nephrocalcinosis: Cat

The minimum P requirement of growing kittens has been set at 9.2 mmol P/MJ dietary metabolizable energy (5.6 g P/kg diet; 19.7 MJ/kg diet; National Research Council, 1986). This recommendation is based on a single communication that kittens thrived on daily P intakes of 150 to 200 mg (Scott, 1965). So far there are no additional reports to support or refute the current recommended P intake of young cats.

P restriction relative to the P levels in most commercial cat diets could contribute to improved health of the cat (Finco, 1983). Diets with low levels of P reduce nephrocalcinosis in cats (Lewis *et al.* 1978; Ross *et al.* 1982), which in turn counters the development and/or progression of renal insufficiency in these animals (Lewis *et al.* 1987). Renal failure is a major cause of death in cats (Cowgill, 1983). Urethral obstruction due to uroliths composed of struvite (magnesium ammonium phosphate hexahydrate) is also a common problem in cats (Lawler *et al.* 1985). Phosphate is a component of struvite, and low P intakes reduce urinary phosphate concentrations (Pastoor *et al.* 1991*b*), which may lower the risk of urolithiasis (Buffington *et al.* 1989). On the other hand, too extreme dietary P restriction may impair bone mineralization in kittens as has been shown in young rats (Schoenmakers *et al.* 1989).

We have studied the effect of reduction of the dietary P level to half the minimum requirement (National Research Council, 1986) on growth, bone and renal mineralization,

and urinary composition in female kittens. In the first experiment dietary P concentration was lowered while Ca remained at a constant level. The second experiment served to check the reproducibility of the first one and also to determine whether maintenance of a constant Ca:P ratio affects the impact of dietary P restriction.

#### MATERIALS AND METHODS

The protocols of both experiments were approved by the animal experiments committee of the Department of Laboratory Animal Science, Utrecht University.

##### *Animals*

Female, 8-week-old weanling cats (Hsd/Cpb:CaDs; Harlan Cpb, Zeist, The Netherlands) were used throughout.

##### *Expt 1. Housing and diets*

The kittens were divided into two groups of ten animals each, which were stratified for body weight and litter. The groups were each housed in a separate stall (2.1 × 2.0 × 3.0 m) provided with resting shelves and a scratching post. The stalls were located in the same room. At 1–2 d before and during the balance periods (see below) the cats were housed individually in stainless steel cages (1.16 × 0.56 × 0.67 m) all placed in one room (2.9 × 5.9 × 3.0 m). In the two rooms a controlled light cycle (light: 07.00–19.00 hours), temperature (20–23°) and humidity (50–65%) were maintained.

Within 3 d after arrival the cats were trained to eat a pelleted, purified diet, containing either 9.2 (normal) or 4.6 (low) mmol P/MJ (5.6 v. 2.8 g P/kg diet; Table 1). Except for the concentration of P in the low-P diet, the two diets were formulated according to the minimum nutrient requirements of cats (National Research Council, 1986). P was added in the form of NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O and the diets were balanced for Na using Na<sub>2</sub>CO<sub>3</sub>. The ingredients and analysed composition of the diets are given in Table 1. The cats were given free access to the diets and demineralized water. Body weights of the cats were measured weekly.

##### *Expt 1. Interventions in the course of the experiment*

After 11 weeks (age 19 weeks) most cats in both dietary groups began to develop signs characteristic of biotin deficiency (Carey & Morris, 1977). At week 15, for each dietary group half of the animals were supplemented orally with 0.5 mg biotin/d. After another 2 weeks clinical signs in the supplemented cats had become less severe (Pastoor *et al.* 1991*b*). We suggest that the pasteurized egg-white powder used as protein source (Table 1) still contained avidin, which rendered biotin unavailable. From week 17 (age 24 weeks) dietary levels of biotin were raised to 3 mg/kg (Table 1). After another 15 weeks (age 39 weeks) the clinical signs had disappeared and the condition of the cats, which was blindly evaluated by a veterinarian, was found to be similar for the low- and normal-P groups.

The temporary biotin deficiency might not have interfered with comparing the effects of the low- and normal-P diets. During the entire experiment the two groups as separate entities differed in P intake only. There were no signs of lagged growth. Body weights of our cats did not differ from those of their counterparts kept by the breeder and fed on a commercial diet. The oral biotin supplementation raised serum biotin levels measured after 16 d (Pastoor *et al.* 1991*a*), but left unchanged plasma concentrations of Ca, Mg, P, urea and creatinine and activity of alkaline phosphatase (EC 3.1.3.1) (results not shown).

##### *Expt 1. Collection of samples*

At the ages of 11, 15, 21, 31 and 39 weeks balance studies were performed. During periods of 6 d each the cats were housed individually. Twice daily they were allowed to leave their cages for 1 h. Feed intake was recorded and 24 h samples of urine and faeces were collected

Table 1. *Composition of the experimental diets*\*†

Ingredient (g/kg)	Expt 1		Expt 2		
	Normal P Normal Ca	Low P Normal Ca	Normal P Normal Ca	Low P Normal Ca	Low P Low Ca
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	21·700	7·500	21·790	7·719	7·719
Na <sub>2</sub> CO <sub>3</sub>	19·280	24·080	19·233	23·998	23·998
CaCO <sub>3</sub>	15·200	15·200	16·118	16·118	6·718
Dextrin	339·040	348·440	337·899	347·205	356·605
Constant components‡	604·780	604·780	604·960	604·960	604·960
Chemical analysis (mmol/kg)§					
P	174	92	174	90	90
Ca	179	177	182	182	90
Mg	15	15	14	14	14

\* The metabolizable energy density of the diets was calculated to be 19·7 MJ/kg, using values of 16·8, 16·8 and 37·8 kJ/g for metabolizable energy contents of protein, carbohydrate and fat respectively.

† Calculated dietary P and Ca concentrations: Normal P 9·2 mmol P/MJ (5·6 g P/kg diet); Low P 4·6 mmol P/MJ (2·8 g P/kg diet); Normal Ca 9·5 mmol Ca/MJ (7·5 g Ca/kg diet); Low Ca 4·8 mmol Ca/MJ (3·8 g Ca/kg diet).

‡ The constant components consisted of the following (g): egg-white 186·5, herring meal 56·2, beef tallow 197·2, maize oil 8·5, glucose 56·2, cooked maize starch 56·2, cellulose 11·2, MgCO<sub>3</sub> 0·40 (Expt 1) or 0·58 (Expt 2), taurine 0·38, vitamin premix 12, mineral premix 20. The diets were formulated taking into account analysed P, Ca and Mg concentrations in the egg-white and herring-meal preparations. These concentrations in Expts 1 and 2 were as follows (mmol/kg product): egg-white; P 32·29 and 24·22, Ca 9·98 and 14·97, Mg 32·91 and 24·68; herring meal; P 645·79 and 645·79, Ca 626·25 and 426·65, Mg 81·45 and 74·04. The vitamin premix consisted of (mg/12 g): retinyl acetate and retinyl palmitate (150 µg/mg) 6·3, cholecalciferol (12·5 µg/mg) 0·94, DL- $\alpha$ -tocopheryl acetate (0·5 mg/mg) 56·6, menadione 0·094, thiamin 4·7, riboflavin 3·78, pyridoxine 3·78, nicotinamide 37·8, DL-calcium pantothenic acid (0·45 mg/mg) 10·48, pteroylmonoglutamic acid 0·755, cyanocobalamin (1 µg/mg) 18·9, choline chloride (0·5 mg/mg) 5228·46, myo-inositol 200, biotin 0·066 (Expt 1, up until week 16) or 3·0 (Expt 1, as from week 17 and Expt 2) and cooked maize starch 6427·345 or 6424·411. The mineral premix consisted of (mg/20 g): KCl 7191, FeSO<sub>4</sub>·7H<sub>2</sub>O 375·6, CuSO<sub>4</sub>·5H<sub>2</sub>O 18·5, MnO<sub>2</sub> 7·4, ZnCl<sub>2</sub> 98·3, KI 0·45, Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O 0·31, cooked maize starch 12308·44.

§ Results are mean values for the analysis of four batches of food within each experiment.

each day. The method used to collect excreta has been published previously (Pastoor *et al.* 1990).

At 7 d after the start of the experiment and at the end of each balance period the cats were anaesthetized (0·2 mg atropine and 20 mg ketamine/kg administered intramuscularly, and 0·5 mg xylazine/kg administered subcutaneously). Blood was taken from the jugular vein and samples were collected in heparinized tubes. An X-ray photograph (MCD 125; Philips, Eindhoven, The Netherlands) of the tibia of each cat was made. A leaden ruler was photographed simultaneously to determine bone length on the X-ray photographs.

Immediately after blood sampling at the age of 39 weeks the anaesthetized cats were killed by an overdose of sodium pentobarbital (0·4–0·8 g/animal administered intravenously). Kidneys, heart, liver and left femur and tibia were removed. Kidney capsules were discarded. The organs were weighed and frozen at –20° until chemical analysis, except for the right kidney which was fixed in 100 ml/l neutral phosphate-buffered formalin for histological examination.

#### *Expt 2. Housing and diets*

Three groups of eight kittens each, which were stratified for body weight and litter, were housed in separate stalls (2·2 × 2·6 × 3·0 m) in the same room (8 × 6 × 3 m). Each stall had four open stainless steel cages (1·16 × 0·56 × 0·67 m). During the balance period (see below)

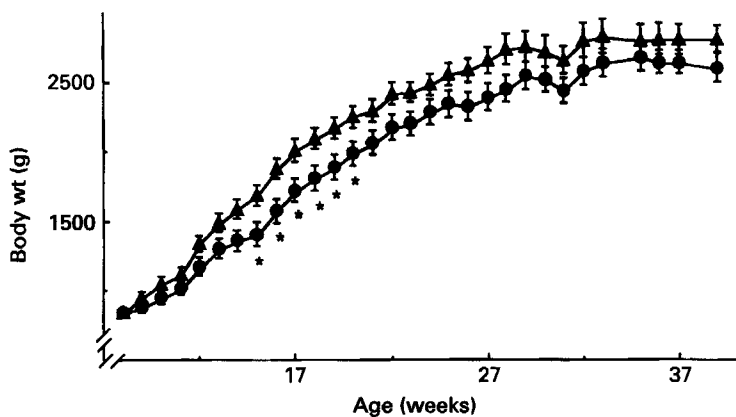


Fig. 1. Expt 1. Time course of body-weight gain of female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ. Values are means for ten cats, with their standard errors indicated by vertical bars. ( $\blacktriangle$ ), Normal-phosphorus diet; ( $\bullet$ ), low-phosphorus diet. Student's *t* test was performed to compare the two dietary groups at the same age: \*  $P < 0.05$ .

four extra cages were placed in each stall. For the last 5 weeks of the experiment the cats were kept in another room (8 × 6 × 3 m) in which each group again had its own stall (1.6 × 2.6 × 3.0 m). In the rooms, lighting (light: 07.00–19.00 hours), temperature (18–23°) and humidity (50–70%) were controlled.

Within 3 d after arrival the cats ate one of the three purified, experimental diets (Table 1). Two diets were almost identical to those used in Expt 1; they contained either 4.6 (low) or 9.2 (normal) mmol P/MJ (2.8 v. 5.6 g P/kg diet) and the minimum required (National Research Council, 1986) Ca level of 9.5 (normal) mmol/MJ (7.5 g Ca/kg diet). In the third diet both P and Ca levels were reduced (4.6 mmol P and 4.8 mmol Ca/MJ; 2.8 g P and 3.8 g Ca/kg diet) so that the Ca:P ratio was the same as that in the normal-P, normal-Ca diet. The ingredients and analysed composition of the diets are given in Table 1. The cats were given free access to the diets and demineralized water. Body weights of the cats were recorded weekly.

#### *Expt 2. Interventions in the course of the experiment*

From the start of the experiment the cats did not grow well. After 3 weeks they were found to have coccidiosis. They were then treated for 9 d with sulphamethoxypridazin (50 mg/kg on days 1 and 7; 25 mg/kg on days 2, 3, 8 and 9). After this treatment the kittens were free from coccidiosis. From 2 to 6 weeks after the start of the experiment, six cats (one fed on the low-P, normal-Ca diet; five fed on the low-P, low-Ca diet) were found to be in shock. The cats showed hypersalivation, a fall in body temperature and finally lost consciousness. They were each given saline (9 g NaCl/l; 10 ml/animal, subcutaneously), prednisolone (0.4 mg/animal, intramuscularly) and 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluor-1,4-dihydro-4-oxo-3-chinoline carbonate (5 mg/animal, subcutaneously) after which they recovered within a few hours, except for a cat of the low-P, low-Ca group, which died. Affected cats were clinically evaluated, but no abnormalities could be detected. Autopsy of the dead cat did not give any clues to the cause of the shock. After week 5 of the experiment all cats grew well and by week 8 (age 15 weeks) group means of body weights had reached normal values, i.e. body-weight values for kittens of the same age but fed on a commercial diet, as based on data provided by the breeder. One cat which was fed on the normal-P, normal-Ca diet had to be removed from the experiment in week 8, because it did not thrive on the purified diet. After transfer to a commercial cat diet this animal rapidly attained a good condition.

Table 2. Expt 1. Feed intake, urinary volume and retention of calcium, phosphorus and magnesium in kittens fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ†  
(Mean values with their standard errors for ten kittens)

	Age (weeks)												Significant‡
	11		15		21		31		39		SE		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Feed intake (g/d)													
Normal P	51.1	2.1	66.6	3.2	57.3	1.7	51.4	2.9	52.9	3.3			P, A, P × A
Low P	41.5**	2.4	49.1**	3.5	59.6	1.9	44.4	3.9	49.0	4.1			
Urinary volume (ml/d)													
Normal P	60.5	5.5	93.0	6.8	71.2	4.3	74.9	7.6	91.7	12.7			P, A, P × A
Low P	49.9	2.8	59.8**	3.7	73.4	5.9	56.0	7.5	67.9	13.2			
Ca retention (mmol/MJ)													
Normal P	5.1	0.3	4.1	0.2	2.3	0.3	0.9	0.6	0.14	0.5			A
Low P	5.2	0.1	4.8	0.3	2.7	0.3	1.4	0.5	0.01	0.5			
Mg retention (mmol/MJ)													
Normal P	0.37	0.02	0.29	0.01	0.09	0.02	-0.15	0.04	-0.30	0.06			A
Low P	0.37	0.02	0.29	0.01	0.16*	0.02	-0.12	0.08	-0.24	0.06			
P retention (mmol/MJ)													
Normal P	4.6	0.4	3.4	0.1	2.2	0.1	0.8	0.3	1.5	0.2			A, P × A
Low P	4.1	0.1	4.0***	0.1	2.4	0.2	0.6	0.3	1.1	0.3			

Mean values were significantly different from those for Normal P at the same age. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  (Student's  $t$  test).

† For details of diets and procedures, see Table 1 and pp. 86-90.

‡ For data for which variances were evenly divided over the matrix of variance, multivariate ANOVA, repeated measures, was used ( $P < 0.05$ ). For data for which variances were not evenly divided over the matrix of variance, the effect of age was evaluated by Friedman's test ( $P < 0.025$ ) and the effect of phosphorus by Student's  $t$  test ( $P < 0.025$ ). P = phosphorus effect; A = age effect; P × A = phosphorus-age interaction.

*Expt 2. Collection of samples*

Blood samples and X-ray photographs were taken at the ages of 9, 23, 31 and 39 weeks. A balance study was performed when the cats were 31 weeks old. At the end of the study the cats, aged 39 weeks, were killed and organs and bones were removed as described above.

*Preparation of samples*

Samples of faeces, urine, plasma, organs and bones were prepared for analysis as described previously (Pastoor *et al.* 1994*a, b*).

For histological examination, 5- $\mu$ m-thick longitudinal sections of each kidney were stained with Von Kossa's solution for detection of phosphate-containing deposits (Mallory, 1961). The severity of nephrocalcinosis was scored blind and in random order on a scale from 0 (no deposits) to 3.

*Chemical analyses*

Ca, Mg and P in feed, faeces, urine, plasma, organs and femurs, plasma activity of alkaline phosphatase, and levels of creatinine and urea in plasma and urine were analysed as described previously (Pastoor *et al.* 1994*a*). Hydroxyproline in non-acidified urine was determined using a Hypronosticon test-combination kit (Organon Teknika Ltd, Boxtel, The Netherlands). Urinary pH was measured with an electrode (Phm 83 autocal pH meter; Radiometer, Copenhagen, Denmark). We had found earlier that the pH of freshly voided urine increased while in the litter box during the day, and thus we corrected the pH of the 24 h samples using a regression line,  $Y = 2.222 + 0.647X$  ( $r = 0.87$ ,  $P < 0.001$ ,  $n = 18$ ), established with urine samples of which the pH was measured immediately after micturition ( $Y$ ) and 16–24 h later ( $X$ ). The range of the  $X$  values was 8.0–9.6, which corresponded with that for the urinary pH values measured in the present studies.

For all chemical analyses accuracy was verified to be within 5% deviation from the targets with the use of reference samples (reference serum, Roche N; Roche Diagnostica, Basel, Switzerland and in-house reference pools of feed, faeces and urine).

*Statistical analyses*

All statistical analyses were carried out according to Steel & Torrie (1981) and using a SPSS/PC+ computer program (SPSS Inc., 1988*a, b*). The two-sided level of statistical significance was pre-set at  $P < 0.05$ . Multivariate analysis of variance (MANOVA), repeated measures, was used to evaluate effects of age, diet and their interaction. When in Expt 1 the variances were not evenly divided over the matrix of variance, even after logarithmic transformation of the data, the effect of age was evaluated by Friedman's test ( $P < 0.025$ ) and the effect of diet by Student's  $t$  test ( $P < 0.025$ ). Group means for the same age were compared by Student's  $t$  test or, when data were not normally distributed, by the Mann-Whitney U test. Group means in Expt 2 were compared by one-way ANOVA followed by the Tukey test or, for non-normally distributed data, by the Kruskal-Wallis test followed by Mann-Whitney U tests. To take into account the greater probability of a type 1 error due to multiple comparisons, the level of statistical significance was reduced to  $P < 0.017$  (Bonferroni's adaptation).

## RESULTS

The numerical values of data mentioned but not shown here can be found elsewhere (Pastoor, 1993).

*Expt 1. Body weight and feed intake*

Fig. 1 shows that mean body weights of kittens fed on the low-P diet were systematically lower than those of cats fed on the normal-P diet; between the ages of 15 and 20 weeks the



difference was statistically significant. During the balance periods at 11 and 15 weeks of age the cats fed on the low-P diet ate significantly less (Table 2).

#### *Expt 1. Mineral retention and absorption*

In order to facilitate direct comparisons of data from periods or diets with different feed intakes, mineral retention was expressed as mmol/MJ metabolizable dietary energy (Table 2). Retention was calculated as intake minus urinary-plus-faecal excretion. Ca retention fell during the course of the experiment, and did not differ significantly from zero at the age of 31 to 39 weeks. The low-P diet did not influence retention of Ca. Retention of Mg dropped with age and at the ages of 31–39 weeks group means were negative. The amount of P in the diet did not systematically affect Mg retention. MANOVA yielded significant effects of age and diet–age interaction on retention of P. Except for the first balance period during which feed intake and absolute retention of P (mmol/d) were significantly depressed in the low-P group, absolute P retention was not influenced by dietary P level (results not shown).

Fig. 2 shows the time course of percentages of apparent absorption of Ca, Mg and P. Apparent absorption was calculated as intake minus faecal excretion and expressed as a percentage of intake. MANOVA revealed a significant lowering effect of age on absorption of Ca and Mg. Absorption of Ca and Mg tended to be somewhat higher in the low-P group. The percentage apparent P absorption was affected by age and dietary P level; it was consistently lower in the low-P group and dropped with age.

#### *Expt 1. Urinary composition*

Urinary volume expressed as ml/d was lower in the low-P group (Table 2), but when expressed as ml/g feed it was similar for both dietary groups (results not shown). Urinary pH was slightly higher in the low-P group (Fig. 3). Low P intake was initially associated with a higher urinary concentration of Ca, which disappeared when the cats grew older. Urinary concentrations of Mg were systematically higher in the low-P group. Urinary P levels were significantly depressed when P intake was reduced. In the low-P group urinary concentration of P rose with age up to 31 weeks.

#### *Expt 1. Plasma minerals*

Plasma Ca levels were not influenced by dietary P concentration, but dropped slightly with age (results not shown). MANOVA yielded a significant effect of age and diet–age interaction on plasma concentration of Mg: at the ages of 9 and 11 weeks plasma Mg concentrations were slightly lower in the low-P group, but at the ages of 15 and 31 weeks the opposite was seen (results not shown). Plasma levels of P were significantly affected by dietary P level, age and diet–age interaction. At the ages of 11 and 15 weeks, plasma levels of P were significantly reduced in the low-P group (1.8 (SE 0.2) and 2.2 (SE 0.1) mmol/l v. 2.6 (SE 0.1) and 2.5 (SE 0.1) mmol/l,  $n$  10). Plasma levels of P dropped with ageing.

#### *Expt 1. Urea and creatinine levels*

Plasma levels of urea and creatinine were not affected by dietary P level (results not shown), but rose with age. Urinary excretion of urea and creatinine clearance dropped with age, and were not systematically influenced by P intake (results not shown).

#### *Expt 1. Bone development*

There was a strong correlation ( $r$  0.99,  $P$  < 0.001,  $n$  20) between the length of the tibia as estimated from X-ray photographs taken just before necropsy and that measured directly thereafter. During the course of the experiment tibias were systematically, but not

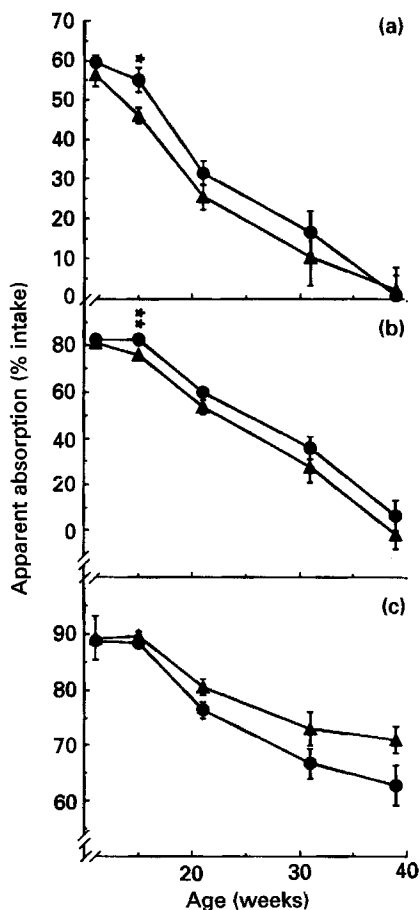


Fig. 2. Expt 1. Time course of change in percentage apparent absorption of (a) calcium, (b) magnesium and (c) phosphorus in female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ. Values are means for ten cats with their standard errors indicated by vertical bars. ( $\blacktriangle$ ), Normal-phosphorus diet; ( $\bullet$ ), low-phosphorus diet. Multivariate ANOVA, repeated measures ( $P < 0.05$ ), revealed significant effects of age on the percentage apparent absorption of calcium, magnesium and phosphorus. The percentage apparent absorption of phosphorus was also affected by dietary phosphorus level. Student's *t* test was performed to compare the two dietary groups at the same age: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

significantly, shorter in the cats fed on the low-P diet (Fig. 4). Plasma activity of alkaline phosphatase, which is an indicator of bone formation, and urinary excretion of hydroxyproline, which is an indicator of bone resorption, were not influenced by P intake (results not shown) but dropped from 2.9 (SE 0.2)  $\mu\text{kat/l}$  and 62 (SE 4) mmol/d per kg body weight (BW) at the age of 15 weeks to 1.0 (SE 0.1)  $\mu\text{kat/l}$  and 17 (SE 1) mmol/d per kg BW ( $n$  20) at the age of 39 weeks.

Dietary P level did not significantly affect final (age 39 weeks) length, circumference, weight, volume and density of the femur (results not shown); average values were 9.7 (SE 0.1) cm, 2.6 (SE 0.03) cm, 6.4 (SE 0.2) g, 5.1 (SE 0.1)  $\text{cm}^3$  and 1.27 (SE 0.01)  $\text{g/cm}^3$  ( $n$  20) respectively. Femur ash, expressed on a dry weight basis, was slightly, but significantly, elevated in cats fed on the low-P diet (642 (SE 4) v. 630 (SE 2) mg/g,  $n$  10,  $P < 0.05$ ). Femur Ca content was significantly raised in the low-P group when expressed on a dry weight basis (233 (SE 1) v. 228 (SE 1) mg/g,  $n$  10,  $P < 0.05$ ), but not when expressed as mmol/ $\text{cm}^3$  (results



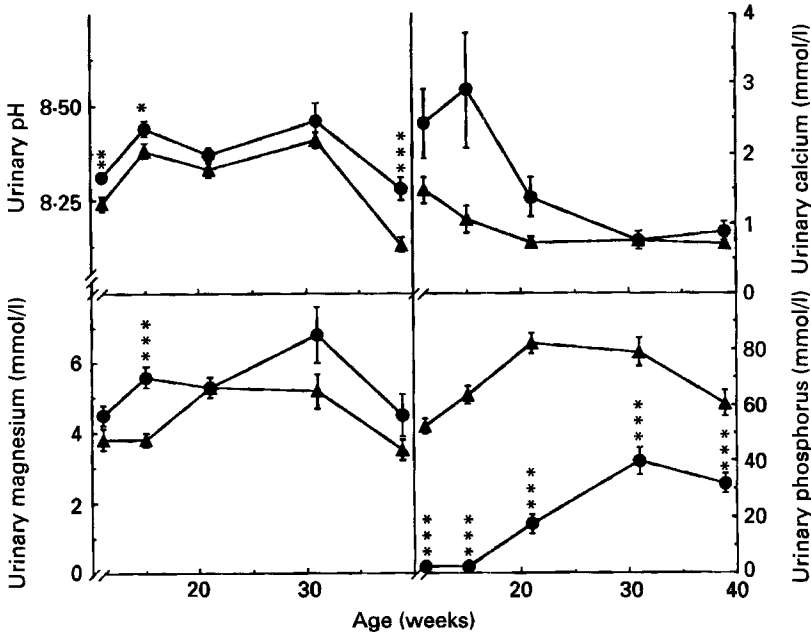


Fig. 3. Expt 1. Time course of changes in urinary pH and urinary concentrations of calcium, magnesium and phosphorus in female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ. Values are means for ten cats with their standard errors indicated by vertical bars. (▲), Normal-phosphorus diet; (●), low-phosphorus diet. Multivariate ANOVA, repeated measures ( $P < 0.05$ ), revealed significant effects of age and dietary phosphorus level on urinary pH and urinary concentrations of calcium, magnesium and phosphorus. Urinary concentrations of magnesium and phosphorus were also affected by diet-age interaction. Student's  $t$  test was performed to compare the two dietary groups at the same age: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

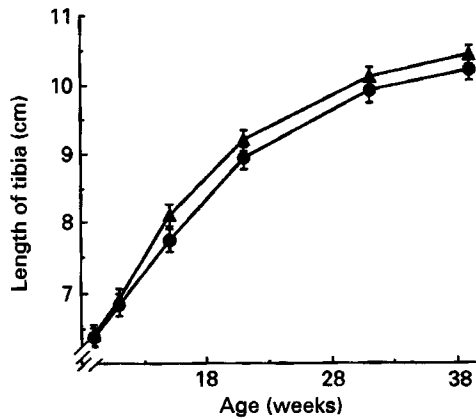


Fig. 4. Expt 1. Time course of growth of the tibia of female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ. Values are means for ten cats with their standard errors indicated by vertical bars. (▲), Normal-phosphorus diet; (●), low-phosphorus diet. Multivariate ANOVA, repeated measures ( $P < 0.05$ ), revealed significant effects of age and diet-age interaction on the length of the tibia. Student's  $t$  test was performed to compare the two dietary groups at the same age:  $P = 0.13-0.85$ .

Table 3. *Expts 1 and 2. Mineral composition of kidneys in kittens fed on diets containing various amounts of phosphorus and calcium†‡*

(Mean values with their standard errors for ten kittens (Expt 1) or seven to eight kittens (Expt 2))

	Expt 1				Expt 2					
	Normal P Normal Ca		Low P Normal Ca		Normal P Normal Ca		Low P Normal Ca		Low P Low Ca	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Kidney mineral content ( $\mu\text{mol/g}$ dry wt)										
Ca	20	9	6***	0.2	16 <sup>a</sup>	5	7 <sup>b</sup>	0.2	7 <sup>b</sup>	0.2
Mg	23	0.9	22	0.4	25	0.4	25	0.1	26	0.6
P	320	9	306	6	370	6	369	6	366	4

<sup>a, b</sup> Mean values within Expt 2 not sharing a common superscript letter were significantly different,  $P < 0.017$  (Mann-Whitney U test).

\*\*\* Mean value was significantly different from Normal P, Normal Ca,  $P < 0.001$  (Mann-Whitney U test).

† In Expt 2 the Kruskal-Wallis test was performed to compare the three dietary groups: Kidney calcium content,  $P < 0.01$ . For details of diets and procedures, see Table 1 and pp. 86-90.

‡ Calculated dietary P and Ca concentrations were: Normal P 9.2 mmol P/MJ (5.6 g P/kg diet); Low P 4.6 mmol P/MJ (2.8 g P/kg diet); Normal Ca 9.5 mmol Ca/MJ (7.5 g Ca/kg diet); Low Ca 4.8 mmol Ca/MJ (3.8 g Ca/kg diet).

not shown). Dietary P level did not influence femur Mg and P concentrations (results not shown); average values were 3.8 (SE 0.04) and 99 (SE 1) mg/g dry weight ( $n$  20).

#### *Expt 1. Mineral concentrations of organs*

Ca, Mg and P concentrations of heart and liver were similar for both dietary groups (results not shown). Kidney Ca was significantly depressed in the low-P group, but concentrations of Mg and P were unchanged (Table 3). Histological examination of kidneys revealed calcified foci, which were mainly located in the corticomedullary junction and the inner stripe of the outer medulla. Deposits were probably located in the lumen of Henle's loop and/or collecting tubules, but due to the distortion of the cells the exact location could not be determined. Histological scores for nephrocalcinosis in the cats fed on the low-P diet were significantly ( $P < 0.001$ ) lower than in those fed on the normal-P diet (medians were 0.25 and 1 respectively). Histological scores were strongly correlated with chemically analysed renal Ca contents (Spearman rank-order correlation coefficient  $r$  0.92,  $P < 0.001$ ,  $n$  19) (Fig. 5).

#### *Expt 2. Body weight*

During the course of the experiment, body weights of the three dietary groups were not significantly different, but at 20 weeks of age cats fed on the low-P, low-Ca diet were systematically lighter than those fed on the other diets (Fig. 6). From 9 to 20 weeks of age the kittens given the low-P, normal-Ca diet had somewhat lower group mean body weights than those fed on the normal-P, normal-Ca diet.

#### *Expt 2. Mineral retention and absorption*

In the 31-week-old cats, feed intake of the three dietary groups was similar (Table 4). Ca, Mg and P retention were not affected by dietary composition. In all three dietary groups group mean retention of Mg was negative.

Percentages of apparent absorption of Ca and Mg were not significantly affected by

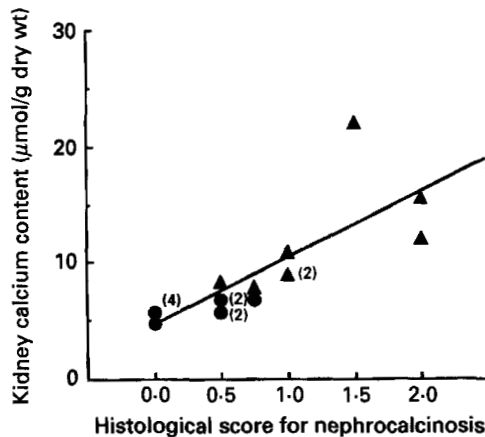


Fig. 5. Expt 1. Relation between histological scores for nephrocalcinosis and chemically analysed renal calcium contents in 39-week-old female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus/MJ. (▲), Normal-phosphorus diet; (●), low-phosphorus diet. When two or more points coincide, the number is indicated. The Spearman rank-order correlation coefficient was 0.92 ( $P < 0.001$ ,  $n$  19) and including an outlier not shown (score, 2; kidney calcium, 97  $\mu\text{mol/g}$  dry weight) it was  $R$  0.93 ( $P < 0.001$ ,  $n$  20). Although nephrocalcinosis score is a discrete variable, Pearson's line of regression is shown:  $Y = 4.67 + 5.43X$  ( $r$  0.80,  $P < 0.001$ ,  $n$  19).

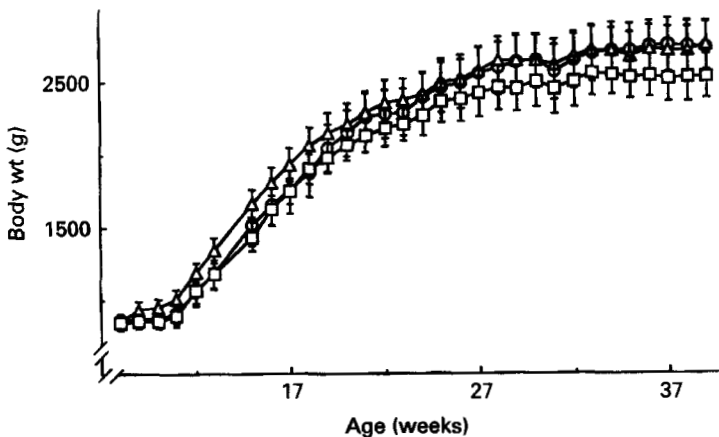


Fig. 6. Expt 2. Time course of body-weight gain of female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus and 4.8 or 9.5 mmol calcium/MJ. Values are means for seven or eight cats with their standard errors indicated by vertical bars. (▲), Normal-phosphorus, normal-calcium diet; (○), low-phosphorus, normal-calcium diet; (□), low-phosphorus, low-calcium diet. One-way ANOVA was performed to compare the three dietary groups at the same age:  $P = 0.54$ – $0.99$ .

dietary composition but group means were highest in cats fed on the low-P, low-Ca diet (Table 4). The percentage P absorption in cats fed on the low-P, low-Ca diet was significantly higher than in cats fed on the low-P, normal-Ca diet. An intermediate value was seen in the cats fed on the normal-P, normal-Ca diet.

#### Expt 2. Urinary composition

Urinary volume, pH and urinary concentrations of Ca and Mg were not significantly affected by dietary composition (Table 4). Urinary concentrations of P were significantly reduced in cats fed on the low-P diets.

Table 4. Expt 2. Feed intake, urinary volume, pH and retention, absorption percentages and urinary concentrations of calcium, magnesium and phosphorus in 31-week-old, female cats fed on diets containing various amounts of calcium and phosphorus\*

(Mean values with their standard errors for eight (Low P, Normal Ca) or seven (Normal P, Normal Ca; Low P, Low Ca) kittens)

Dietary treatment† ...	Normal P, Normal Ca		Low P, Normal Ca		Low P, Low Ca	
	Mean	SE	Mean	SE	Mean	SE
Feed intake (g/d)	36.1	2.8	35.2	3.9	35.8	4.1
Urinary volume (ml/d)	52.6	4.0	55.7	5.9	54.8	7.0
Urinary pH	8.2	0.04	8.2	0.02	8.2	0.05
Retention (mmol/MJ)						
Ca	0.9	0.7	0.5	0.8	0.9	0.3
Mg	-0.16	0.06	-0.22	0.06	-0.17	0.07
P	0.6	0.4	0.1	0.4	0.7	0.3
Absorption (% intake)						
Ca	10.5	7.3	5.8	7.3	21.7	6.2
Mg	25.8	7.4	25.4	5.6	40.8	6.7
P	65.2 <sup>a, b</sup>	2.4	53.2 <sup>b</sup>	5.1	76.8 <sup>a</sup>	4.0
Urinary mineral concentration (mmol/l)						
Ca	0.75	0.08	0.93	0.10	1.01	0.14
Mg	4.7	0.3	5.0	0.3	6.0	0.6
P	69.0 <sup>a</sup>	3.9	28.1 <sup>b</sup>	3.4	37.1 <sup>b</sup>	3.2

<sup>a, b</sup> Mean values within a row not sharing a common superscript letter were significantly different,  $P < 0.05$  (Tukey's test).

\* One-way ANOVA was performed to compare the three dietary groups: P absorption,  $P < 0.01$ ; urinary P concentration,  $P < 0.001$ . For details of diets and procedures, see Table 1 and pp. 86-90.

† Calculated dietary P and Ca concentrations: Normal P 9.2 mmol P/MJ (5.6 g P/kg diet); Low P 4.6 mmol P/MJ (2.8 g P/kg diet); Normal Ca 9.5 mmol Ca/MJ (7.5 g Ca/kg diet); Low Ca 4.8 mmol Ca/MJ (3.8 g Ca/kg diet).

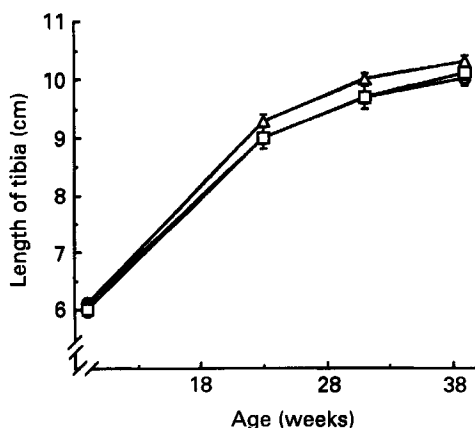


Fig. 7. Expt 2. Time course of growth of the tibia of female cats fed on diets containing either 4.6 or 9.2 mmol phosphorus and 4.8 or 9.5 mmol calcium/MJ. Values are means for seven or eight cats with their standard errors indicated by vertical bars. ( $\Delta$ ), Normal-phosphorus, normal-calcium diet; ( $\circ$ ), low-phosphorus, normal-calcium diet; ( $\square$ ), low-phosphorus, low-calcium diet. Multivariate ANOVA, repeated measures ( $P < 0.05$ ), revealed a significant effect of age on the length of the tibia. One-way ANOVA was performed to compare the three dietary groups at the same age:  $P = 0.21-0.84$ .

*Expt 2. Plasma minerals*

Plasma levels of Ca, Mg and P were not affected by dietary composition (results not shown). In general, plasma mineral concentrations dropped with age.

*Expt 2. Plasma urea and creatinine*

Plasma levels of urea and creatinine rose significantly with age, but were not influenced by dietary treatment (results not shown).

*Expt 2. Bone development*

Tibia length was somewhat lower in the low-P groups relative to the normal-P, normal-Ca group, but the difference was not statistically significant (Fig. 7). There was no significant effect of diet on plasma activity of alkaline phosphatase (results not shown). Length, circumference, weight, volume, density and Ca, Mg and P concentrations of femurs at 39 weeks of age were similar for all three dietary groups; average values were 9.4 (SE 0.1) cm, 2.4 (SE 0.04) cm, 6.2 (SE 0.2) g, 4.4 (SE 0.1) cm<sup>3</sup>, 1.42 (SE 0.01) g/cm<sup>3</sup>, and 221 (SE 1), 3.5 (SE 0.03) and 103 (SE 1) mg/g femur dry weight (*n* 22) respectively.

*Expt 2. Mineral concentrations of organs*

Dietary groups did not differ with regard to concentrations of Ca, Mg or P in heart and liver (results not shown). Kidney concentrations of Ca were significantly depressed in cats fed on the low-P diets (Table 3). Kidney levels of Mg and P were not affected by dietary composition.

## DISCUSSION

The major objective of this study was to evaluate whether P restriction to half the recommended minimum level would sustain growth and mineralization of bone in female kittens. In Expt 1 the body weights of the cats fed on the low-P diet were significantly reduced at the age of 15 to 20 weeks. In Expt 2, body weights in the low-P, normal-Ca group also tended to be reduced during the first 10 weeks of the experiment. Apparently, a dietary level of 4.6 mmol P/MJ (2.8 g P/kg diet) does not allow body-weight gain similar to that sustained by 9.2 mmol P/MJ (5.6 g P/kg diet). Since maximum growth in rats is associated with reduced lifespan and higher incidences of tumours and kidney disease in later life (Ross *et al.* 1976; Pariza, 1987), maximum growth may not be a proper criterion by which to set nutrient requirements. Thus, it is uncertain whether the small reduction in body weight seen after P restriction should be considered as a disadvantageous effect.

In rats, P restriction to one quarter of the recommended level, which is based on attainment of maximum growth (National Research Council, 1978), significantly reduced Ca, Mg and P contents of the femur (Schoenmakers *et al.* 1989). In the cats a reduction of dietary P level to half the minimum requirement produced a reduction of tibia length, this effect not being statistically significant. Restricted P intake slightly raised the Ca content of the femur at the age of 39 weeks in Expt 1, but this was not seen in Expt 2. At the end of the experiments length, volume, density and Mg and P contents of femur were not significantly influenced by P restriction nor by the Ca:P ratio. Thus, it appears that P restriction had no major impact on bone development as assessed at the age of 39 weeks. In addition, P restriction did not affect plasma alkaline phosphatase activity and urinary hydroxyproline excretion during the course of the experiment.

In both experiments the reduction of P intake prevented renal calcification as based on chemical and histological analysis. The anti-nephrocalcinogenic effect of P restriction was independent of the Ca:P ratio. In rats, P-induced nephrocalcinosis is antagonized by urinary acidification (Kootstra *et al.* 1991). Thus, the observed renal calcification in the cats

may have been enhanced by the relatively high urinary pH values. P-induced nephrocalcinosis may impair kidney function in rats (Schaafsma & Visser, 1980; Ritskes-Hoitinga *et al.* 1989). However, renal function in the cats fed on the normal-P diets was not impaired as based on plasma levels of urea and creatinine and creatinine clearance, which were within the normal range for cats. This can be explained by the fact that the degree of kidney calcification in the cats was less severe than that seen by others in rats given high-P diets. The nephrocalcinogenic effect of the normal- v. low-P diets in the young cats corroborates that of high P intake observed by Lewis *et al.* (1978) in healthy adult cats, and by Ross *et al.* (1982) in cats with reduced renal mass. Renal disease is a major cause of death in the domestic cat (Cowgill, 1983), and Lucke & Hunt (1967) observed a high incidence of nephrocalcinosis. Generally, the P content of commercial cat foods amply exceeds the recommended minimum level (Graser *et al.* 1981; Sauer *et al.* 1985). Thus, lowering of the P level in cat diets may reduce the risk of renal disease.

P intake did not significantly affect retention of Ca and Mg. At the age of 31–39 weeks, Mg retention was negative but plasma Mg concentration and alkaline phosphatase activity were unaffected. During the balance periods at the age of 31 and/or 39 weeks most cats lost weight. In Expt 2 there was a significant, positive correlation ( $r\ 0.77$ ,  $P < 0.01$ ,  $n\ 22$ ) between body-weight gain and retention of Mg. Thus, the observed whole-body Mg loss may be explained by a temporary reduction of body weight during the balance periods.

Restricted P intake resulted in depressed amounts of absorbed P and reduced urinary excretions of P, which balanced each other so that P retention was unaffected during the period 15 to 39 weeks of age. Likewise, Ca and Mg retention values were not influenced by P restriction. Percentages of apparent absorption of Ca and Mg were highest in the cats fed on the low-P, low-Ca diet. Percentages of apparent absorption of P were lowered by reduced P intake and raised by restricted Ca intake. The reduction of apparent P absorption in the cats fed on the low-P diets is probably due to a greater portion of endogenous P in the faeces. The lowering effects of a higher dietary P level on Ca and Mg absorption values and that of a higher Ca intake on Mg absorption may be explained by the formation of an insoluble Ca–Mg–phosphate complex in the intestine (Brink *et al.* 1992).

Precipitation of struvite crystals in urine depends on the urinary concentrations of its components: when the activity product  $[Mg^{2+}] \times [NH_4^+] \times [PO_4^{3-}]$  exceeds the formation product, crystals will develop. A low urinary pH can prevent struvite urolithiasis because the activity product is diminished (Buffington *et al.* 1989). In Expt 1, urinary pH and Mg concentrations were slightly higher in the cats fed on the low-P diet, but urinary concentrations of P were markedly reduced. Urinary struvite saturation can be predicted using a nomogram (Buffington *et al.* 1989), and assuming that urinary concentrations of ammonium remained at a constant level it follows that urines of the cats fed on the low-P diet were less supersaturated than those of the cats fed on the normal-P diet. The difference between the two dietary groups was greatest until the age of 21 weeks. This is in agreement with our perception that precipitates were invariably present in urines from the cats fed on the normal-P diet, whereas in the cats fed on the low-P diet they were only found from the age of 21 weeks. The common presence of precipitates undoubtedly relates to the relatively high urinary pH values, which in turn were caused by the liberal use of carbonates to prepare the purified diets. The urinary pH values in cats generally range between 6.5 and 8.5 (Buffington *et al.* 1989).

In conclusion, dietary P restriction to half the recommended minimum amount slightly reduced body-weight gain and tibia growth in female kittens. However, in order to sustain normal growth and bone mineralization while fed on the low-P diets the cats probably had to draw heavily on their compensatory mechanisms. This is indicated by reduced plasma



levels of P and the extremely low urinary P concentrations from the age of 11 to 15 weeks. Therefore, a reduction of the dietary P concentration to 4.6 mmol P/MJ (2.8 g P/kg diet) may be too drastic, at least until the age of 15 to 20 weeks. This certainly holds for diets based on natural ingredients which generally contain P sources that are not as readily available as those in our purified diets. On the other hand, P restriction may have had a positive impact on the health of the cats. P restriction markedly lowered urinary P concentrations, which could lower the risk of struvite urolithiasis, and also prevented renal calcification, which may contribute to lowering the risk for renal disease. Thus, although P restriction to 4.6 mmol P/MJ (2.8 g P/kg diet) is discouraged, at least for weanling kittens, it seems reasonable to suggest that commercial cat foods with levels of available P higher than 9.2 mmol/MJ (5.6 g P/kg diet) should be avoided.

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