Summary

The dietary calcium and phosphorus content and their mutual ratio affect bone development in broilers. The dietary concentration of these minerals should be attuned to the bird's requirement to ensure adequate bone development on the one hand and minimize phosphorus excretion on the other. In this experiment the optimal calcium/available phosphorus ratio was determined in broiler diets, containing two levels of phytate phosphorus (basal diets). These diets were fed with and without added microbial phytase and five levels of calcium.
The optimal calcium/available phosphorus ratio in four week old broiler was around 1.28 (as absorbed from the ileum). At this optimum, both calcium and phosphorus retention (as a percentage of intake) were maximal. The corresponding calcium level was 0.59% at the low phytate phosphorus diet with no added phytase. The optimal calcium content was about 8% higher in the high phytate-phosphorus diet (relative to the low phytate-phosphorus diet). Phytase addition reduced the calcium level at which the optimum was reached. These calcium levels were sufficient for adequate bone development (maximal tibia breaking strength, and tibia bone volume), while bone weight and ash, calcium and phosphorus contents were not affected by the dietary calcium level in four week old broilers.

Introduction

The retention of calcium and phosphorus in broilers is affected by the daily intake of both minerals, the dietary calcium/phosphorus ratio and the bird's requirements at a specific age. A suboptimal calcium/phosphorus ratio might result in complexation in the intestinal tract (Shafey et al, 1991), a reduced absorptive capacity, and a reduced bone mineralization (Nelson et al, 1990, Shafey et al, 1990, Wilson et al, 1991). So, this ratio can affect the absorbability and retention of either one of these minerals (Morrissey and Wasserman, 1971), which will result in excretion in faeces and/or in urine.
Environmental pollution problems in the Netherlands have urged to minimize phosphorus excretion in livestock production. The availability of phytate phosphorus (which is about two third of the plant phosphorus) for monogastric animals is very low and non absorbable dietary phosphorus will be excreted in the faeces. Dietary addition of microbial phytase liberates part of the phosphorus from phytate and thereby increases its absorbability. Furthermore, the absorbability of complexed cations (eg. calcium) might also be positively affected by microbial phytase. So far the net effect of phytase on the optimal calcium/(available) phosphorus ratio has not been established.
In the present experiment some of these topics have been studied. The effect op the calcium/phosphorus ratio on the performance of the broilers, on the absorption of both minerals from the small intestine, on their retention and on some bone parameters (bone breaking strength, bone volume, ash, calcium and phosphorus contents) has been determined. Two basal diets (containing 0.20 or 0.30 % phytate phosphorus) were fed with increasing calcium/available phosphorus ratios (from 1.6 to 2.8 in five steps) without and with added microbial phytase (Natuphos®, produced by Gist Brocades, Delft, The Netherlands). From 0

to 2 weeks of age 500 FTU/kg were added , and from 2 to 4 weeks 250 FTU/kg were exchanged for monocalcium phosphate (assumed equivalence was 250 FTU with 0.5 g MCP-P) (Simons et al, 1992).

Materials and methods

Animals and housing

1920 Ross male broilers were housed in three-tier battery cages (24 birds per 0.45 m^2 cage). At one day of age these broilers were vaccinated against New Castle Disease and at 17 days of age against Gumboro. The poultry house was lit continuously and the temperature was decreased from 33°C at one days to 21°C at 32 days of age. Seven birds per cage were killed after the first faecal collection period (13 days of age) for tibia collection. Subsequently, the number of birds per cage was standardized at 14. Feed and water were continuously available.

Experimental diets

The feedstuff composition of the basal diets is given in Appendix 1. Limestone, monocalcium phosphate, and/or microbial phytase were used to realize the experimental treatments (shown in Appendix 2). The dietary content of available phosphorus was calculated based on a dataset with in vivo determined values for single feedstuffs (van der Klis and Versteegh, 1993). The basal diet compositions in phase I (0-13 days of age) contained 0.35 % available phosphorus (based on calculation). During the second phase (14-28 days of age) the dietary phosphorus levels were reduced. The calcium/available phosphorus ratio was increased from 1.6 to 2.8 in both phases. The Weender composition of the four basal diets is shown in Table 1.

Table 1. The Weender composition of the basal diets (%).

Diet	dry matter	ash	crude protein	crude fat	crude fiber	N free extract
A	90.9	6.4	18.9	9.6	3.2	52.8
B	91.1	6.1	20.5	11.3	5.9	47.3
C	91.0	6.1	18.8	9.4	3.5	53.2
D	91.1	6.0	20.4	11.2	6.4	47.1

Calcium, phosphorus and phytate-phosphorus contents of the experimental diets are shown in Table 2. Weender and mineral analysis of the individual feedstuffs are given in Appendix 3. 0.15% Cr_2O_3 was added to the phase II diets as an inert marker. All diets were pelleted without steam addition (pelletϕ 3mm).

Table 2. Dietary calcium. phosphorus and phytate phosphorus contents (%).

Basal diet	A	B	C	D
Calcium level				
Ca 1	0.62		0.45	
Ca 2	0.74		0.54	
Ca 3	0.84		0.61	
Ca 4	0.96		0.70	
Ca 5	1.07		0.78	
Phosphorus level				
P 1	0.54	0.61	0.42	0.49
P 2	-	-	0.35	0.42
Phytate P	0.19	0.30	0.19	0.30

Measurements

The broilers were weighed at 13 and 28 days of age, directly following the faecal collection period. Feed and water intake were measured from 0 to 13 days of age and from 13 to 28 days of age. Water intake was measured for those experimental treatments with the highest, middle and lowest calcium/available phosphorus ratio. The retention of calcium and phosphorus were determined during two seven day balance periods from 6 to 13 and from 21 to 28 days of age. These retention data are based on quantitative faeces sampling during these balance periods, which were started and ended with a feed withdrawal period of six hours. Feed was sampled once per two days during the first balance period and once daily during the second period. Following the second balance period, the experimental diets were supplied again for ad libitum intake to ensure a steady state situation in the gastro-intestinal tract. From 31 to 34 days of age the broilers were killed for ileal chyme collection (van der Klis, 1993). These samples were used to determine the ileal calcium and phosphorus absorption. Right and left tibia were collected at 13 days of age and at the end of the experiment to measure bone characteristics (fat free tibia weight, bone volume, bone breaking strength, ash, calcium and phosphorus contents). Left and right tibia were randomly used for measurement of the bone breaking strength or bone volume mesurements and chemical analysis. Chemical analysis were done in pooled samples of seven tibia.

Birds that accidently died during the experiment were examined post mortem.

Statistical analysis

Weight gain, feed and water intake, feed to gain and water to feed ratio, calcium and phosphorus retention and absorption and bone parameters were analysed per age period according to the following statistical model:

$$Y = \mu + Block_i + Phytate\ P_j + Phytase_k + Ca_l + interactions + e_{ijkl}$$

Where:			
	Y	=	response parameter
	block	=	effect block (i=4)
	phytate P	=	effect basal diet (j=2)
	phytase	=	effect phytase addition(k=2)
	Ca	=	effect calcium level (l=5)
			(l=3 for water intake measurements)
	interactions	=	two- and three way interactions
	e	=	errorterm

Results and discussion

During phase I, phytase was added to the experimental diets (500 FTU/kg diet), while phytase was exchanged for monocalcium phosphate during phase II. This exchange was based on the assumption that 250 FTU phytase/kg diet is equivalent with 0.5 g MCP-P/kg diet (Simons et al, 1992). Based on this difference in dietary phytase supplementation, results are reported per age period.

Phase I (0-13 days of age)

Weight gain, feed and water intake and feed to gain and water to feed ratio are given in Table 3.

Table 3 Weight gain, feed and water intake (g/animal/period), feed to gain and water to feed ratios (g/g) of broilers from 0 to 2 weeks of age, at different dietary concentrations of phytate-P (phP), phytase (phy) and calcium (Ca).

	age 0-2 weeks				
	weight gain	feed intake	water intake	feed to gain	water to feed
phP 2.0	277	404	896	1.45	2.25
phP 3.0	264	379	906	1.43	2.38
LSD ($P \leq 0.05$)	*4*	*5*	-	*0.013*	*0.054*
phy 0	265	387	902	1.46	2.33
phy 500	276	396	899	1.42	2.30
LSD ($P \leq 0.05$)	*4*	*5*	-	*0.013*	-
Ca 0.63	269	397	909	1.48	2.29
Ca 0.74	272	394	nd	1.44	nd
Ca 0.85	270	393	886	1.43	2.28
Ca 0.96	272	388	nd	1.43	nd
Ca 1.07	269	385	907	1.42	2.38
LSD ($P \leq 0.05$)	-	*8*	-	*0.021*	*0.066*

nd not determined
LSD Least significant difference, not indicated in absense of significant effects ($P > 0.05$)

Feed intake and weight gain were lower in broilers fed the high phytate P diet, with a slightly better feed to gain ratio compared to the low phytate P diet. Dietary supplementation with 500 FTU phytase/kg improved all performance data. The effect of increasing calcium/available phosphorus ratios was dependent on the phytate P content of the basal diet (phP x Ca interaction, Appendix 4). Weight gain was slightly increased by the calcium/available phosphorus ratio at the high phytate P diet, while it was not affected or even decreased at the low phytate P diet. Water intake was not affected by either one of the experimental treatments (no main effects). The significant phP*phy interaction (Appendix 4) was caused by a decreased water intake due to phytase addition to the low phytate P diet, while water intake was increased by phytase addition to the high phytate P diet. Furthermore, the phP*Ca interaction indicated a higher water intake at the widest calcium/available phosphorus ratio in the high phytate P diet, compared to the low phytate P diet at the corresponding calcium level, while water intake was not significantly different on the other treatments (Appendix 4). Water to feed ratio was higher than in previous experiments (e.g. van der Klis, 1993). It was higher in high phytate P diets, than in the low phytate P diets, which may be caused by differences in feedstuff composition. Part of the tapioca from the low phytate P diet was exchanged for sunflower seed meal in the high phytate P diet (Appendix 1). The water to feed ratio was increased at the highest calcium/available phosphorus ratio compared to both lower ratios.

The retention of calcium and phosphorus (mg/animal) from 6 to 13 days of age is given in Table 4.

Table 4 Phosphorus and calcium retention (% of intake and mg/period) and their mutual ratio in broilers from 1 to 2 weeks of age, at different dietary concentrations of phytate-P (phP), phytase (phy) and calcium (Ca).

	age 1-2 weeks				
	P (%)	Ca (%)	P (mg)	Ca (mg)	Ca/P
phP 2.0	51.3	43.5	19.9	26.2	1.30
phP 3.0	47.1	43.8	19.1	24.1	1.26
LSD (P≤0.05)	*1.0*	-	*0.6*	*0.7*	*0.02*
phy 0	47.3	41.3	18.3	23.3	1.26
phy 500	51.2	45.9	20.7	26.9	1.30
LSD (P≤0.05)	*1.0*	*1.2*	*0.6*	*0.7*	*0.02*
Ca 0.63	35.9	48.3	18.4	20.9	1.14
Ca 0.74	50.2	47.6	20.1	24.4	1.22
Ca 0.85	50.0	43.2	19.6	24.4	1.24
Ca 0.96	50.1	40.7	19.7	26.9	1.36
Ca 1.07	50.0	38.5	19.7	29.0	1.45
LSD (P≤0.05)	*1.5*	*1.8*	*0.9*	*1.2*	*0.03*

LSD Least significant difference, not indicated in absense of significant differences (P>0.05).

The retention of phosphorus (as % and as mg) was lower in the high phytate P diet compared to the low phytate P diet. The retention of phosphorus was improved considerably by dietary supplementation with phytase. At the lowest calcium levels, less phosphorus was retained compared to the diets with higher calcium levels. The retention of calcium (%) was not affected by the phytate P content of the diet, while absolute calcium retention (mg) was decreased at the high phytate P diet. Calcium retention was improved by dietary supplementation with 500 FTU phytase. Although calcium retention improved from 20.9 to 29.0 mg/animal at higher calcium intakes, retention as a percentage of intake was decreased. Phytase addition was more beneficial in the high phytate P diets, compared to the low phytate P diets (phP*phy interaction, Appendix 5). The Ca*phP interaction on the absolute calcium retention was caused by the stronger effect of increasing dietary calcium levels in the phytase containing diets, compared to those with no additional phytase. The calcium/phosphorus ratio (as retained) was lower at the high phytate P diet and at the diets with no supplemental phytase, compared to the high phytate P diet and the phytase supplemented diets respectively. Increasing the calcium/available phosphorus ratio resulted in more calcium retained per g phosphorus.

Tibia parameters at two weeks of age age shown in Table 5.

Table 5 Tibia weight (g dry fat free), ash, phosphorus and calcium content (% on dry matter basis), bone volume (cm^3) and bone breaking strength (kg) in broilers at 2 weeks of age, at different dietary concentrations of phytate P (phP), phytase (phy) and calcium (Ca).

	age 2 weeks					
	weight	ash	P	Ca	volume	breaking strength
phP 2,0	0.75	39.0	7.8	14.4	1.82	5.26
phP 3.0	0.72	37.4	7.4	13.8	1.78	4.61
LSD (P≤0.05)	*0.02*	*0.5*	*0.2*	*0.3*	-	*0.20*
phy 0	0.70	37.2	7.4	13.7	1.74	4.47
phy 500	0.77	39.1	7.8	14.5	1.86	5.40
LSD (P≤0.05)	*0.02*	*0.5*	*0.2*	*0.3*	*0.05*	*0.20*
Ca 0.63	0.72	35.7	7.0	12.8	1.78	4.38
Ca 0.74	0.75	37.8	7.8	14.1	1.87	4.82
Ca 0.85	0.74	39.1	7.6	14.5	1.76	5.10
Ca 0.96	0.74	39.2	8.0	14.7	1.80	5.02
Ca 1.07	0.75	39.1	7.6	14.5	1.80	5.34
LSD (P≤0.05)	-	*0.8*	*0.4*	*0.4*	-	*0.32*

LSD Least significant difference, not indicated in absense of significant effects (P>0.05)

Bone development and mineral deposition in the tibia was lower in the high phytate P diet, compared to the low phytate P diet. Dietary supplementation with phytase was beneficial for all parameters measured. The tibia ash, calcium and phosphorus content were lower at the lowest calcium/available phosphorus ratio compared to the higher calcium levels. The beneficial effect of phytase addition on bone breaking strength was higher at the high phytate-P diets (increased by 1.20 kg) compared to the low phytate P diets (0.65 kg). Such a phP*phy interaction was also evident for the ash and calcium content in the tibia (Appendix 6). The effect of phytase on bone breaking strength was also dependent on the dietary calcium concentration. 500 FTU phytase/kg diet improved bone breaking strength by respectively 0.46, 0.40, 1.18, 1.19 and 1.42 kg at the five increasing calcium concentrations.

Phase II (14 to 34 days of age)

The dietary available phosphorus level from 13 days of age onwards was decreased by 0.1% compared to the phase I diets. Furthermore, in the phase II diets 250 FTU phytase/kg diet was exchanged for monocalcium phosphate based on an assumed equivalence of 250 FTU/kg with 0.5 g P from monocalcium phosphate.

Weight gain, feed and water intake and feed to gain and water to gain ratios in broilers from 14 to 28 days of age are given in Table 6.

Table 6 Weight gain, feed and water intake (g/animal/period) and feed to gain and water to feed ratios (g/g) of broilers from 13 to 28 days of age, at different dietary concentrations of phytate P (phP), phytase (phy) and calcium (Ca).

	age 2-4 weeks				
	weight gain	feed intake	water intake	feed to gain	water to feed
phP 2.0	751	1283	2760	1.71	2.19
phP 3.0	596	1025	2470	1.73	2.37
LSD ($P \leq 0.05$)	*18*	*24*	*67*	-	*0.053*
phy 0	673	1152	2526	1.72	2.22
phy 250	674	1156	2708	1.72	2.33
LSD ($P \leq 0.05$)	-	-	*67*	-	*0.053*
Ca 0.63	642	1121	2528	1.75	2.24
Ca 0.74	673	1160	nd	1.73	nd
Ca 0.85	693	1178	2612	1.70	2.24
Ca 0.96	676	1165	nd	1.73	nd
Ca 1.07	683	1147	2710	1.70	2.36
LSD ($P \leq 0.05$)	*28*	-	*88*	*0.034*	*0.065*

nd not determined
LSD Least significant difference, not indicated in absense of significant effects ($P>0.05$)

Weight gain, feed and water intake was lower at the high phytate P basal diet, compared to the low phytate P diet. Water to feed ratio was higher at the high phytate P diet, compared to the low phytate P diet, which was also shown in phase I. The water to feed ratio was increased by the exchange of phosphorus from monocalcium phosphate by microbial phytase, while the other performance parameters were not affected by this exchange. The latter was expected based on the exchange of anorganic phosphorus by phytase (which makes organic phosphorus available for absorption) on a previously determined equivalence (Simons et al, 1992). Weight gain of birds fed the diet with the lowest calcium/available phosphorus was significantly lower than all other birds. This was also shown in the higher feed to gain ratio at those specific diets. Water intake and water to feed ratio were significantly increased at the highest calcium/available phosphorus ratio. The phP*phy interaction on weight gain, feed and water intake was caused by a positive response by phytase supplementation at the high phytate P diet, compared to a negative response or no response at the low phytate P diet.

Calcium and phosphorus retention during the fourth week of life are given in Table 7.

Table 7 Phosphorus and calcium retention(% of intake and mg/period) and their mutual ratio in broilers from 3 to 4 weeks of age, at differents dietary concentrations of phytate P (phP), phytase (phy) and calcium (Ca).

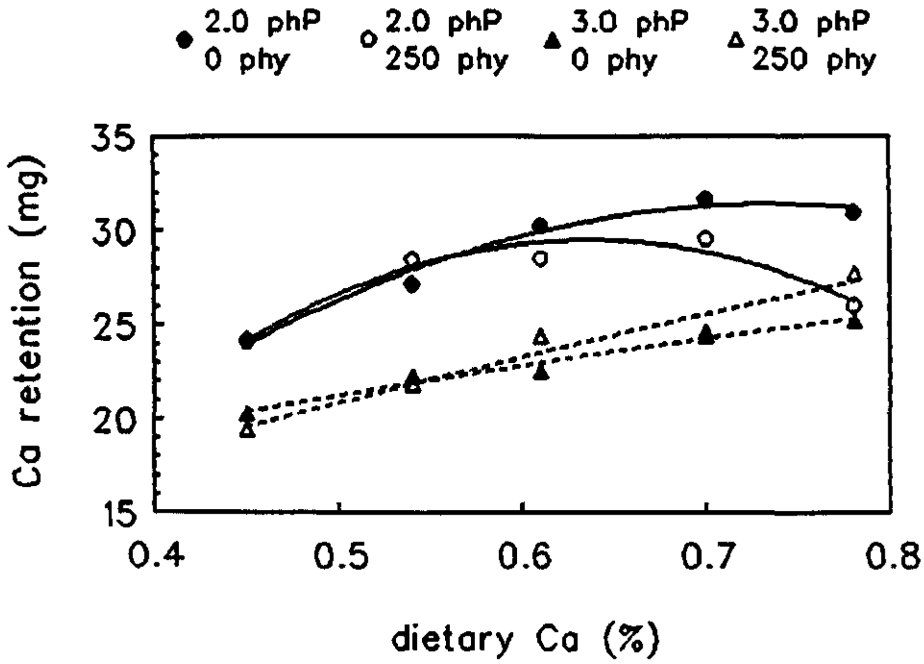
	age 3-4 weeks				
	P (%)	Ca (%)	P (mg)	Ca (mg)	Ca/P
phP 2.0	56.2	46.6	21.5	28.0	1.30
phP 3.0	49.4	48.0	17.9	23.3	1.29
LSD ($P \leq 0.05$)	*0.9*	*1.0*	*0.6*	*1.0*	-
phy 0	49.9	47.7	20.2	25.9	1.28
phy 250	55.7	47.0	19.2	25.4	1.31
LSD ($P \leq 0.05$)	*0.9*	-	*0.6*	-	*0.02*
Ca 0.45	53.3	55.8	19.2	22.0	1.14
Ca 0.53	53.8	51.8	19.9	24.9	1.25
Ca 0.61	53.2	45.9	20.8	26.4	1.25
Ca 0.69	52.8	43.8	19.7	27.5	1.41
Ca 0.77	51.0	39.3	19.0	27.5	1.42
LSD ($P \leq 0.05$)	*1.3*	*1.6*	*1.0*	*1.6*	*0.03*

LSD Least significant difference, not indicated in absense of significant differences ($P>0.05$).

Phosphorus retention (% and mg/period) was lower at the high phytate P diet compared to the low phytate P diet. This effect was also evident during phase I (Table 4). Despite a slight improvement of the calcium retention (%), less calcium (mg) was retained at the high phytate P diet, compared to the low phytate P diet. Exchange of 0.5 g phosphorus from monocalcium phosphate for 250 units phytase resulted in a higher phosphorus retention (%), but a slight decreased retention as mg/period. The magnitude of the effect of phytase supplementation on the phosphorus retention (%) was dependent on the dietary phytate P content (phP*phy interaction, Appendix 5). This phosphorus retention was increased by 6.5% at the low phytate P diet and with 15% at the high phytate P diet. The absolute retention of phosphorus (mg/period) was decreased at the low phytate P diet, due to the exchange of monocalcium phosphate by microbial phytase, while this exchange did not affect phosphorus retention at the high phytate P diet (phP*phy interaction). This indicates that, unlike at the high phytate P diet, the assumed equivalence was not completely realized at the low phytate P diet.
The ratio between retained calcium and phosphorus was slightly increased in birds fed the diets supplemented with phytase. The phosphorus retention (%) was lower at the highest dietary calcium/available phosphorus ratio, while the maximal phosphorus retention (mg) was achieved at a dietary calcium concentration of 0.61%. The calcium retention (%) was reduced

when diets with higher calcium levels were fed. The retention in mg/period was (like the phosphorus retention) maximal at a dietary calcium content of 0.61%. Calcium retention was somewhat improved at higher calcium levels, but this improvement failed to reach significance. The ratio in ratained calcium and phosphorus was increased from 1.14 to 1.42. The phP*phy*Ca interaction on the calcium retention (Appendix 5) is shown in Figure 1.

Figure 1. The relationship between the dietary calcium concentration and the calcium retention (mg/period)in 4 week old broilers, at two phytate phosphorus (phP) and phytase (phy) levels.



All dose-response curves are based on quadratic responses of the calcium retention on increasing dietary calcium levels. It is obvious that the calcium retention reached a maximum value at the low phytate P diet, within the range of dietary calcium levels from 0.45 to 0.78%. This was not the case at the high phytate P diet. It is also clear the the calcium retention at the latter diets were at a lower level (also indicated in Table 7) compared to the low phytate P diet. This might indicate that the high phytate P diet would require a much higher dietary calcium level to obtain a maximum retention, but this does not seem to be caused by differences in calcium absorption from the intestinal tract (Table 8).

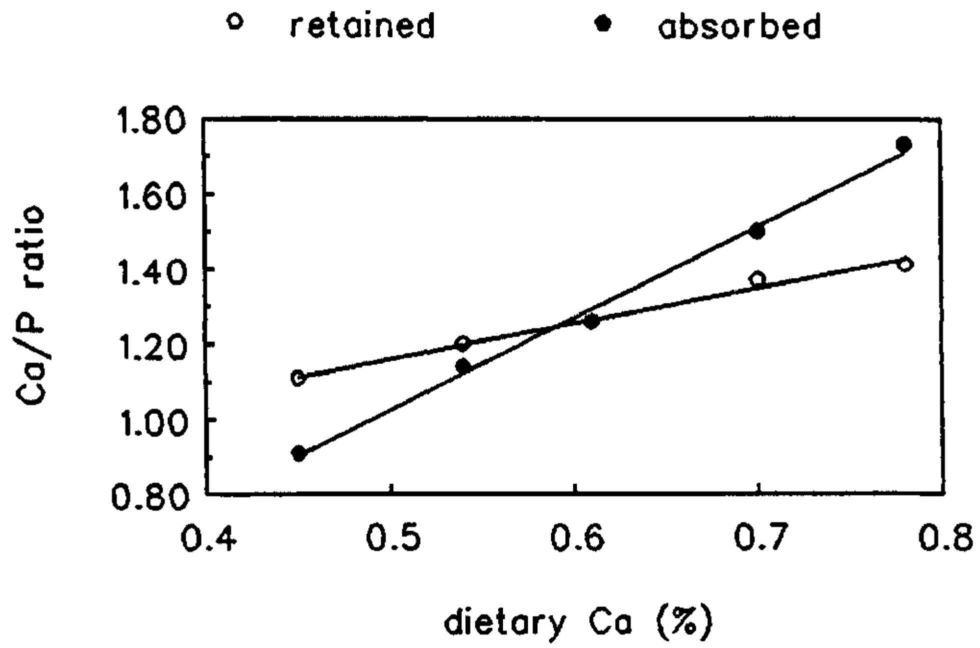
Table 8 The ileal dry matter (dm), phosphorus (P) and calcium (Ca) absorption (in %) measured in broilers of 4.5 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium.

	age 3-4 weeks		
	dm	P	Ca
phP 2.0	76.2	64.9	56.7
phP 3.0	72.4	59.6	58.1
LSD (P≤0.05)	*0.4*	*1.3*	-
phy 0	74.3	61.0	57.0
phy 250	74.3	63.5	57.8
LSD (P≤0.05)	-	*1.3*	-
Ca 0.45	74.0	72.2	60.7
Ca 0.53	74.4	63.8	58.4
Ca 0.61	73.6	59.7	55.8
Ca 0.69	74.8	57.2	56.0
Ca 0.77	74.7	58.4	55.9
LSD (P≤0.05)	*0.6*	*2.0*	*2.7*

LSD Least significant difference, not indicated in absense of significant differences (P>0.05).

The ileal absorption of dry matter, calcium and phosphorus are shown in Table 8. The digestibility of dry matter in the high phytate P diet was somewhat lower than at the low phytate P diet, which was probably caused by the differences in dietary feedstuff composition (Appendix 1). As phytate P is hardly available for absorption in monogastric animals, less phosphorus was absorbed at the ileal level of birds fed the high phytate P diet. Dietary supplementation with microbial phytase improved ileal phosphorus absorption. Phosphorus absorption was negatively affected by higher dietary calcium concentrations, and was minimal when calcium concentrations exceeded 0.69%. Calcium absorption itself was negatively affected up to a dietary calcium level of 0.61%. The calcium/phosphorus ratio as absorbed and as retained was calculated, as well as the corresponding dietary calcium levels. These data are given in Figure 2 for the low phytate P diet with no added phytase.

Figure 2. The calcium/phosphorus ratio as absorbed from the intestinal tract and as retained in 4 week old broilers fed a diet with 2.0 g phytate phosphorus/kg.



The calcium/phosphorus ratio (as absorbed) was lower than this ratio (as retained) at the low calcium levels. This implicates a relative excess of absorbed phosphorus, which has to be excreted in the urine. At the high calcium levels the opposite situation is present. The optimal calcium/phosphorus ratio was defined as the point of intersection between both lines. Results of this calculation with and without added phytase and the low and high phytate P basal diets are given in Table 9.

Table 9. The optimal Ca/P ratio and calcium content in broiler diets at two dietary phytate levels, with and without phytase supplementation.

Diet		Optimal values		
Phytate P (%)	Phytase (units/kg)	Ca (%)	aCa/aP[1]	Ca/aP[2]
2.0	0	0.59	1.25	2.18
	250	0.52	1.28	2.23
3.0	0	0.64	1.33	2.32
	250	0.57	1.25	2.18

[1] available Ca/ available P
[2] total Ca/available P

The optimal available calcium/available phosphorus is around 1.25-1.30. The corresponding calcium content was 0.59% at the low phytate P diet, with no added phytase. This optimal calcium content was 0.06% higher at the high phytate P diet. In both basal diets, the optimal dietary calcium concentration was decreased by adding phytase to the diets. The optimal total calcium/available phosphorus ratio was 2.2-2.3, based on the measured calcium availability of 57%.

Bone parameters in broilers of 4.5 weeks of age are shown in Table 10.

Table 10 Tibia weight (g dry fat free), ash, phosphorus and calcium content (% on dry matter basis), bone volume (cm^3) and bone breaking strength (kg) in broilers at 4.5 weeks of age, at different dietary concentrations of phytate P (phP), phytase (phy) and calcium (Ca).

	age 4.5 weeks					
	weight	ash	P	Ca	volume	breaking strength
phP 2.0	4.83	31.2	5.9	12.4	10.77	19.77
phP 3.0	4.25	29.1	5.6	12.0	9.62	15.64
LSD ($P \leq 0.05$)	*0.34*	*1.75*	*0.28*	-	*0.28*	*1.40*
phy 0	4.45	30.4	5.8	12.3	10.05	17.95
phy 250	4.63	29.9	5.7	12.1	10.34	17.47
LSD ($P \leq 0.05$)	-	-	-	-	*0.28*	-
Ca 0.45	4.32	29.6	5.8	11.9	9.78	14.99
Ca 0.53	4.39	30.9	5.9	12.4	10.23	16.70
Ca 0.61	4.94	28.8	5.5	11.9	10.14	18.73
Ca 0.69	4.34	31.9	6.0	12.8	10.50	19.90
Ca 0.77	4.70	29.5	5.6	12.1	10.32	18.21
LSD ($P \leq 0.05$)	-	-	-	-	*0.46*	*2.20*

LSD Least significant difference, not indicated in absense of significant effects ($P<0.05$)

All parameters, except the tibia calcium content, were depending on the basal diet composition -being lower at the high phytate P diet-. Bone volume was increased by the exchange of monocalcium phosphate by microbial phytase at the high phytate P diet, while this exchange had no effect on the low phytate P diet (phP*phy interaction, Appendix 7). Bone volume and bone breaking strength were significantly reduced at the lowest dietary calcium levels compared to the higher calcium concentrations. Maximal values for both parameters were achieved at a dietary calcium level of 0.69%. The optimal calcium levels (Table 9) seem to be adequate for bone formation (Table 10).

Although it can not be excluded that the dietary treatments from 0 to 2 weeks of age might have affected the results during phase II, it is very likely that this effect was only marginal. Phosphorus and calcium retention from 1 to 2 weeks of age were only 2 to 3 mg/period higher due to dietary phytase supplementation (Table 4), compared to a total phosphorus and calcium retention of 20 and 25 mg repectively in the fourth week of life. Furthermore, calcium and phosphorus contents in the tibia were somewhat increased, but phosphorus retention in the tibia was increased by a factor 4.5 in the period from 2 to 4.5 weeks of age. If present, carry-over effect therefore will only have been marginal.

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Appendix 1

Feedstuff composition and calculated nutrient concentrations of the basal diets.

	Basal diet[1]			
Feedstuff	A	B	C	D
corn	-	7.2	-	7.2
tapioca	41.4	25.2	41.4	25.2
soybean meal (solv.extr.)	36.5	34.2	36.5	34.2
sunflower seed meal	-	10.0	-	10.0
corn starch	10.0	10.0	10.0	10.0
soya oil	8.2	9.8	8.2	9.8
vitamin premix[2]	0.5	0.5	0.5	0.5
mineral premix[3]	1.0	1.0	1.0	1.0
lysine	0.06	-	0.06	-
methionine	0.43	0.28	0.43	0.28
threonine	0.02	-	0.02	-
arginine	0.13	-	0.13	-
limestone	-	0.10	-	0.10
monocalcium phosphate	0.50	0.30	0.20	-
cellulose	-	0.16	-	0.16
total	98.74	98.74	98.44	98.44
Calculated nutrient concentration				
AME, kcal/kg	3164	3166	3164	3166
lys, %	1.22	1.22	1.22	1.22
met+cys, %	0.91	0.91	0.91	0.91
Ca, %	0.40	0.40	0.35	0.35
total P, %	0.36	0.43	0.29	0.36
phytate P, %	0.20	0.30	0.20	0.30

[1] Basal diet A and B contain a higher level of minocalcium phosphate compared to diet C and D respectively
[2] Commercial vitamin preparation (van der Klis et al, 1993)
[3] Mineral premix contains (%): Corn starch, 22.5; $CaCO_3$, 44.0; NaCl, 25.0; $FeSO_4.7H_2O$, 4.0; $CuSO_4.5H_20$, 0.45; $MnSO_4.4H_2O$, 2.4; $ZnSO_4.7H_2O$, 0.6; KI preparation, 1.05.

Appendix 2

Experimental treatments (calcium (Ca), available phosphorus (aP), phytate phosphorus (phP) and phytase).

Diet	Basal diet	phP	aP[1]	Ca	phytase	Ca/aP
		g/kg			units/kg	
experimental diets from 0 to 2 weeks of age						
1	A	2.0	3.5	5.60	0	1.60
2		2.0	3.5	6.65	0	1.90
3		2.0	3.5	7.70	0	2.20
4		2.0	3.5	8.75	0	2.50
5		2.0	3.5	9.80	0	2.80
6		2.0	3.5	5.60	500	1.60
7		2.0	3.5	6.65	500	1.90
8		2.0	3.5	7.70	500	2.20
9		2.0	3.5	8.75	500	2.50
10		2.0	3.5	9.80	500	2.80
11	B	3.0	3.5	5.60	0	1.60
12		3.0	3.5	6.65	0	1.90
13		3.0	3.5	7.70	0	2.20
14		3.0	3.5	8.75	0	2.50
15		3.0	3.5	9.80	0	2.80
16		3.0	3.5	5.60	500	1.60
17		3.0	3.5	6.65	500	1.90
18		3.0	3.5	7.70	500	2.20
19		3.0	3.5	8.75	500	2.50
20		3.0	3.5	9.80	500	2.80
experimental diets from 2 to 4 weeks of age						
21	C	2.0	2.5	4.00	0	1.60
22		2.0	2.5	4.75	0	1.90
23		2.0	2.5	5.50	0	2.20
24		2.0	2.5	6.25	0	2.50
25		2.0	2.5	7.00	0	2.80
26		2.0	2.0	4.00	250	2.00
27		2.0	2.0	4.75	250	2.38
28		2.0	2.0	5.50	250	2.75
29		2.0	2.0	6.25	250	3.13
30		2.0	2.0	7.00	250	3.50
31	D	3.0	2.5	4.00	0	1.60
32		3.0	2.5	4.75	0	1.90
33		3.0	2.5	5.50	0	2.20
34		3.0	2.5	6.25	0	2.50
35		3.0	2.5	7.00	0	2.80
36		3.0	2.0	4.00	250	2.00
37		3.0	2.0	4.75	250	2.38
38		3.0	2.0	5.50	250	2.75
39		3.0	2.0	6.25	250	3.13
40		3.0	2.0	7.00	250	3.50

[1] The following phosphorus availabilities in individual feedstuffs were used to calculate the dietary available phosphorus content: corn, 29; soybean meal, 61; tapioca, 66; sunflower seed meal, 39; monocalcium phosphate, 80

Appendix 3

Results of the Weender analysis in feedstuffs (%)

	dry matter	ash	crude protein	crude fat	crude fiber	N free extract
Corn	88.6	1.1	8.8	4.2	1.7	72.8
Soybean meal	89.4	6.1	46.7	2.0	3.4	31.2
Tapioca	89.9	4.7	3.2	0.8	3.7	77.5
Sunflower seed meal	90.1	6.9	30.0	2.9	22.6	27.7

The analyzed phosphorus (P), phytate phosphorus (phP) and calcium (Ca) content in feedstuffs (%)

	P	phP	Ca
Corn	0.27	0.20	0.01
Soybean meal	0.57	0.49	0.16
Tapioca	0.10	0.04	0.20
Sunflower seed meal	1.22	1.03	0.34
Monocalciumfosfaat	22.44	-	17.45

Appendix 4

Weight gain, feed and water intake (g/animal/period), feed to gain and water to feed ratio (g/g) of broilers from 0 to 2 and 2 to 4 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium (Ca).

			age 0-2 weeks					age 2-4 weeks				
phP	phy	Ca	weight gain	feed intake	water intake	feed to gain	water to gain	weight gain	feed intake	water intake	feed to gain	water to gain
2.0	0	1	279	418	950	1.50	2.28	717	1256	2644	1.75	2.11
		2	275	402	nd	1.46	nd	775	1329	nd	1.72	nd
		3	270	402	910	1.46	2.26	773	1328	2781	1.72	2.10
		4	274	403	nd	1.48	nd	787	1332	nd	1.70	nd
		5	266	380	883	1.43	2.32	755	1257	2792	1.66	2.22
	1	1	282	407	876	1.44	2.15	700	1226	2686	1.75	2.19
		2	285	412	nd	1.44	nd	758	1290	nd	1.70	nd
		3	278	413	883	1.44	2.24	764	1278	2728	1.68	2.16
		4	285	400	nd	1.40	nd	744	1280	nd	1.72	nd
		5	276	401	870	1.40	2.25	738	1258	2943	1.70	2.34
3.0	0	1	254	380	884	1.49	2.32	558	970	2212	1.74	2.28
		2	260	378	nd	1.45	nd	590	1020	nd	1.73	nd
		3	257	367	866	1.43	2.34	606	1035	2361	1.71	2.28
		4	253	363	nd	1.44	nd	575	996	nd	1.73	nd
		5	262	372	919	1.42	2.47	590	1000	2366	1.70	2.36
	1	1	260	384	925	1.47	2.40	591	1034	2565	1.75	2.37
		2	269	383	nd	1.42	nd	570	1003	nd	1.76	nd
		3	276	389	885	1.38	2.28	630	1073	2578	1.70	2.40
		4	275	384	nd	1.40	nd	600	1050	nd	1.76	nd
		5	274	386	955	1.41	2.48	647	1072	2740	1.72	2.51

Appendix 4

	age 0-2 weeks					age 2-4 weeks				
	weight gain	feed intake	water intake	feed to gain	water to feed	weight gain	feed intake	water intake	feed to gain	water to feed
Significancies[1]										
phP	***	***	NS	*	***	***	***	***	t	***
phy	***	***	NS	***	NS	NS	NS	***	NS	***
Ca	NS	*	NS	***	**	*	t	***	*	***
phP*phy	NS	NS	*	NS	NS	*	**	***	NS	NS
phP*Ca	*	NS	*	t	NS	NS	NS	NS	NS	NS
phy*Ca	NS	t	NS	NS	NS	NS	NS	NS	NS	NS
phP*phy*Ca	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Least Significant Differences										
phP	3.8	5.0	27.5	0.014	0.054	18.2	24.8	71.7	0.022	0.053
phy	3.8	5.0	27.5	0.014	0.054	18.2	24.8	71.7	0.022	0.053
Ca	6.1	8.0	33.7	0.022	0.066	28.8	39.3	87.6	0.034	0.065
phP*phy	5.4	7.1	38.9	0.019	0.077	25.8	35.1	101.1	0.031	0.075
phP*Ca	8.6	11.2	47.6	0.031	0.094	40.8	55.6	123.7	0.049	0.092
phy*Ca	8.6	11.2	47.6	0.031	0.094	40.8	55.6	123.7	0.049	0.092
phP*phy*Ca	12.1	15.9	67.4	0.043	0.133	57.7	78.6	175.2	0.069	0.130

nd: not determined

[1] Significance: NS, not significant; t, $P \leq 0,10$; *, $P \leq 0,05$; **, $P \leq 0,01$; ***, $P \leq 0,001$.

Appendix 5

Phosphorus and calcium retention (% of intake and mg/period) in broilers, measured from 1 to 2 and from 3 to 4 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium (Ca).

			age 1-2 weeks					age 3-4 weeks				
phP	phy	Ca	P (%)	Ca	P (mg)	Ca (mg)	Ca/P	P (%)	Ca (%)	P (mg)	Ca (mg)	Ca/P
2.0	0	1	46.4	46.9	18.6	21.6	1.16	52.9	52.9	21.7	24.2	1.11
		2	50.9	46.4	19.3	24.0	1.25	52.4	49.2	22.6	27.1	1.20
		3	50.0	40.9	19.2	24.4	1.26	53.7	46.7	24.0	30.2	1.26
		4	50.3	39.1	19.8	27.5	1.39	53.3	44.0	23.1	31.6	1.37
		5	50.8	35.2	18.3	27.2	1.41	52.1	39.6	22.0	30.9	1.41
	1	1	49.3	49.7	19.2	22.2	1.16	59.5	56.4	19.8	24.1	1.22
		2	53.2	47.8	21.6	26.6	1.23	61.7	52.6	21.6	28.4	1.31
		3	53.4	45.2	20.9	26.3	1.26	59.8	46.7	20.9	28.5	1.30
		4	54.2	41.7	20.8	28.6	1.37	59.7	43.4	20.2	29.5	1.45
		5	55.3	42.1	21.8	33.6	1.54	56.7	34.3	19.2	26.0	1.35
3.0	0	1	42.6	46.0	17.1	18.8	1.10	48.3	58.8	18.0	20.3	1.13
		2	46.9	46.4	18.8	22.4	1.19	46.8	52.5	17.9	22.2	1.25
		3	46.1	40.5	17.5	21.0	1.20	47.1	44.5	18.9	22.5	1.19
		4	45.3	37.2	17.4	22.5	1.29	47.0	45.3	17.7	24.6	1.39
		5	43.6	34.6	17.4	24.2	1.39	45.7	43.2	16.5	25.2	1.52
	1	1	45.3	50.5	18.6	21.0	1.13	52.5	55.0	17.4	19.4	1.12
		2	49.9	49.6	20.7	24.8	1.20	54.0	52.8	17.5	21.8	1.25
		3	50.4	46.2	21.0	26.2	1.25	52.2	45.5	19.3	24.4	1.26
		4	50.5	44.6	20.9	29.0	1.39	51.3	42.7	17.6	24.4	1.42
		5	50.3	41.9	21.1	30.8	1.46	49.5	40.2	18.3	27.7	1.41

Appendix 5

	age 1-2 weeks					age 3-4 weeks				
	P (%)	Ca (%)	P (mg)	Ca (mg)	Ca/P	P (%)	Ca (%)	P (mg)	Ca (mg)	Ca/P
Significancies[1]										
phP	***	NS	**	***	***	***	**	***	***	NS
phy	***	***	***	***	**	***	NS	**	NS	**
Ca	***	***	**	***	***	**	***	**	***	***
phP*phy	NS	t	t	**	NS	*	t	***	*	**
phP*Ca	NS	NS	NS	NS	NS	NS	**	NS	t	***
phy*Ca	NS	NS	t	***	*	t	**	NS	NS	***
phP*phy*Ca	NS	NS	NS	NS	NS	NS	*	NS	*	NS
Least Significant Differences										
phP	0.98	1.16	0.56	0.73	0.021	0.85	0.99	0.63	0.99	0.020
phy	0.98	1.16	0.56	0.73	0.021	0.85	0.99	0.63	0.99	0.020
Ca	1.54	1.83	0.89	1.16	0.034	1.35	1.56	0.99	1.56	0.032
phP*phy	1.38	1.64	0.80	1.04	0.030	1.21	1.450	0.89	1.40	0.028
phP*Ca	2.19	2.59	1.26	1.04	0.048	1.90	2.21	1.40	2.21	0.045
phy*Ca	2.19	2.59	1.26	1.64	0.048	1.90	2.21	1.40	2.21	0.045
phP*phy*Ca	3.10	3.66	1.78	2.32	0.068	2.70	3.12	1.99	3.12	0.064

[1] Significance: NS, not significant; t, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Appendix 6

Tibia weight (g dry fat free), ash, phosphorus and calcium content (% on dry matter basis), measured in broilers of 2 and 4,5 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium (Ca).

			age 2 weeks				age 4.5 weeks			
phP	phy	Ca	weight	ash	P	Ca	weight	ash	P	Ca
2.0	0	1	0.73	35.0	6.8	12.4	4.56	29.4	5.7	11.2
		2	0.74	38.3	7.9	14.2	4.47	33.0	6.4	13.4
		3	0.72	39.9	8.0	14.9	5.44	30.2	5.8	12.7
		4	0.72	39.5	8.1	14.9	4.50	34.6	6.5	13.7
		5	0.72	39.2	7.8	14.7	4.84	31.2	5.9	12.0
	1	1	0.74	37.6	7.5	13.7	4.96	29.6	5.8	12.6
		2	0.77	38.8	8.0	14.5	4.66	32.5	6.1	12.5
		3	0.80	39.8	7.9	14.8	5.29	28.3	5.2	11.6
		4	0.77	41.2	8.3	15.4	4.59	32.6	6.2	13.0
		5	0.82	40.4	8.0	15.0	4.99	30.6	5.8	11.8
3.0	0	1	0.68	34.8	6.6	12.6	3.87	28.9	5.8	11.9
		2	0.72	36.1	7.6	13.3	4.29	28.0	5.5	11.8
		3	0.67	36.6	6.9	13.3	4.40	28.0	5.5	11.4
		4	0.66	36.5	7.5	13.7	3.92	30.8	5.8	12.5
		5	0.66	36.4	6.8	13.2	4.20	29.4	5.6	12.4
	1	1	0.72	35.5	6.9	12.8	3.90	30.3	6.0	12.1
		2	0.78	38.0	7.8	14.2	4.12	30.1	5.6	12.1
		3	0.78	40.0	7.7	14.9	4.64	28.7	5.5	11.8
		4	0.79	39.7	8.1	15.0	4.34	29.8	5.4	11.9
		5	0.78	40.2	7.7	15.0	4.78	26.9	5.3	12.0

Appendix 6

	age 2 weeks				age 4.5 weeks			
	weight	ash	P	Ca	weight	ash	P	Ca
Significancies[1]								
phP	**	***	***	***	***	*	*	NS
phy	***	***	***	***	NS	NS	NS	NS
Ca	NS	***	***	***	NS	NS	NS	NS
phP*phy	NS	**	NS	**	NS	NS	NS	NS
phP*Ca	NS	NS	NS	NS	NS	NS	NS	NS
phy*Ca	*	NS	NS	NS	NS	NS	NS	NS
phP*phy*Ca	NS	*	NS	*	NS	NS	NS	NS
Least Significant Differences								
phP	0.020	0.50	0.23	0.26	0.35	1.75	0.28	0.59
phy	0.020	0.50	0.23	0.26	0.35	1.75	0.28	0.59
Ca	0.032	0.80	0.36	0.42	0.55	2.77	0.44	0.94
phP*phy	0.029	0.71	0.32	0.37	0.49	2.48	0.40	0.84
phP*Ca	0.046	1.13	0.51	0.59	0.78	3.92	0.63	1.33
phy*Ca	0.046	1.13	0.51	0.59	0.78	3.92	0.63	1.33
phP*phy*Ca	0.064	1.60	0.72	0.83	1.11	5.54	0.89	1.88

[1] Significance: NS, not significant; t, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Appendix 7

Tibia bone volume (cm^3) and bone breaking strength (kg) in broilers, measured at 2 and 4 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium (Ca).

			age 2 weeks		age 4 weeks	
phP	phy	Ca	volume	breaking strength	volume	breaking strength
2.0	0	1	1.79	4.37	9.89	15.74
		2	1.84	5.12	10.82	19.01
		3	1.70	5.02	11.18	22.30
		4	1.80	4.88	11.00	21.88
		5	1.72	5.23	10.96	21.53
	1	1	1.81	4.98	10.54	17.40
		2	1.93	5.09	11.39	19.06
		3	1.91	5.83	10.22	19.33
		4	1.82	5.61	11.21	21.06
		5	1.91	6.43	10.50	20.44
3.0	0	1	1.72	3.93	9.11	14.07
		2	1.80	4.13	8.93	14.31
		3	1.61	3.99	9.54	14.67
		4	1.68	3.98	9.60	19.86
		5	1.73	4.02	9.46	16.08
	1	1	1.78	4.23	9.57	12.77
		2	1.90	4.96	9.78	14.43
		3	1.84	5.56	9.64	18.64
		4	1.89	5.63	10.18	16.78
		5	1.85	5.67	10.36	14.80

Appendix 7

	age 2 weeks		age 4 weeks	
	volume	breaking strength	volume	breaking strength
Significancies[1]				
phP	t	***	***	***
phy	***	***	*	NS
Ca	NS	***	*	***
phP*phy	NS	**	*	NS
phP*Ca	NS	NS	NS	NS
phy*Ca	NS	**	NS	NS
phP*phy*Ca	NS	NS	NS	NS
Least significant differences				
phP	0.051	0.20	0.29	1.40
phy	0.051	0.20	0.29	1.40
Ca	0.081	0.32	0.46	2.20
phP*phy	0.073	0.29	0.41	1.97
phP*Ca	0.115	0.45	0.64	3.12
phy*Ca	0.115	0.45	0.64	3.12
phP*fy*Ca	0.162	0.64	0.91	4.41

[1] Significance : NS,not significant; t, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Appendix 8

The ileal dry matter (dm), phosphorus (P) and calcium (Ca) absorption measured in broilers at 4.5 weeks of age, at different dietary concentrations of phytate phosphorus (phP), phytase (phy) and calcium (Ca).

phP	phy	Ca	dm	P	Ca
2.0	0	1	75.5	73.9	60.4
		2	76.7	66.8	59.4
		3	76.0	61.4	53.4
		4	76.4	58.9	53.4
		5	77.2	58.6	54.9
	1	1	76.1	75.8	60.8
		2	76.1	67.0	58.1
		3	75.2	61.9	55.8
		4	76.4	61.0	55.9
		5	76.1	63.6	55.0
3.0	0	1	72.4	69.0	61.3
		2	72.4	60.2	57.8
		3	71.7	56.3	56.7
		4	72.9	52.2	56.0
		5	72.1	52.8	56.3
	1	1	71.9	70.1	60.4
		2	72.5	61.3	58.4
		3	71.6	58.9	57.2
		4	73.6	56.5	58.9
		5	73.2	58.7	57.5

	dm	P	Ca
Significancies[1]			
phP	***	***	NS
phy	NS	***	NS
Ca	**	***	***
phP*phy	NS	NS	NS
phP*Ca	NS	NS	NS
phy*Ca	NS	NS	NS
phP*phy*Ca	NS	NS	NS
Least Significant Differences			
phP	0.43	1.30	1.71
phy	0.43	1.30	1.71
Ca	0.68	2.05	2.71
phP*phy	0.61	1.84	2.42
phP*Ca	0.96	2.90	3.83
phy*Ca	0.96	2.90	3.83
phP*phy*Ca	1.36	4.11	5.42

[1] Significance: NS, not significant; t, $P \leq 0.10$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.