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Broiler response to variable rates of inclusion of calcium and non-phytate phosphorus in feed

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Abstract

A trial was conducted to investigate the response of broilers to changing levels of calcium and non-phytate phosphorus. A total of 1344 chicks were allocated to 96 cages in a controlled-environment house. Limestone and phosphoric acid were added to a maize–soya basal diet so that six levels of calcium were fed, with four levels of non-phytate phosphorus (nPP) at each. Birds were weighed on days 0, 7, 14, and 21. Each week, two birds per pen were killed and tibial ash and breaking strength were determined. Feed intake and bird weight for each pen were measured weekly. Calcium and non-phytate phosphorus had a marked effect on body-weight gain, feed intake, and gain/feed. The poorest performance was recorded for the highest Ca:P and the best at 1.36 g Ca/g nPP (7.5 g Ca and 5.5 g nPP/kg feed). Multiple regression analysis of the weekly measurements showed linear effects on body weight gain of calcium and nPP, a quadratic effect of calcium, and an interaction between calcium and nPP. The same variables influenced tibial breaking strength and ash at 14 and 21 d. Feed intake was influenced by nPP. The negative effects of high calcium on the availability of phosphorus affected bird growth. A digestible phosphorus model that takes account of calcium and nPP in the complete feed is a better predictor of performance than nPP alone. This approach is recommended if calcium and phosphorus in broilers is to be modelled and birds are to be fed more precisely.

Keywords: body weight gain, feed intake, minerals, tibial ash, tibial breaking strength

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Introduction

Calcium (Ca) and phosphorus (P) are required by broilers for skeletal development and for vital metabolic functions in the body. The cost of P sources and the pollution potential of P in excreta have encouraged producers to seek more precise specifications for this nutrient in broiler feeds. The interactions between Ca and P in digestion and in the skeleton require nutritionists and researchers to consider both minerals together. However, this also makes understanding how contents in the diet affect feed intake (FI) and growth more challenging. A large proportion of these elements, particularly Ca, is found in bone (van Krimpen *et al*., 2013), hence measurements of the effects of Ca and P on bone mineralisation and strength may provide an indication of the effectiveness of nutrition in meeting the animal's needs.

The theory of growth and FI proposed by Emmans (1981) states that an animal will strive to grow at a genetically-determined potential rate. It will moderate its FI to achieve this and to satisfy any maintenance requirements. The first-limiting nutrient in any feed is that which will require the animal to eat the largest amount of feed to satisfy requirements and reach its potential. This desired daily FI may be calculated from the concentration of the first-limiting nutrient in the feed and the daily maintenance and growth requirements (Ferguson *et al*., 1994). This nutrient is normally an amino acid, and it has

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been comprehensively demonstrated that broilers do adjust FI in accordance with this theory (Burnham *et al*., 1992; Lemme *et al*., 2006). However, it does not seem that this applies to Ca and P, even though an appetite for Ca has been demonstrated when it is fed separately (Wilkinson *et al*., 2014; Abdollahi *et al*., 2015). To the contrary, Cozannet *et al*. (2021) fed diets in which total Ca (tCa) and available P (aP) were described as first-limiting and found that lower levels of these minerals were associated with reduced FI. Similarly, increases in the non-phytate P (nPP) content in feed have been associated with increased FI in some response studies (Bradbury *et al*., 2014; Hamdi *et al*., 2015). Increased Ca and nPP levels were both associated with increased FI in a recent meta-analysis (Kermani *et al*., 2023). It would appear that the association between the level of the first-limiting nutrient and FI may be reversed in the case of P at least.

The objectives of this trial were to determine the response of broilers in FI and body weight gain (BWG) to a range of Ca and nPP contents and the subsequent effects on bone ash and bone-breaking strength. A wider range of both dietary Ca and P levels was used than has generally been applied in past experiments, to ensure that the response to these minerals was better defined than is the case when a narrow range of contents is applied. Particularly low Ca contents were included in some treatments to minimise the effect on P in the digestive tract.

Materials and Methods

This study was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/017/019M) and took place at the University of KwaZulu-Natal research unit, Ukulinga Research Farm, Pietermaritzburg, South Africa.

Sexed, day-old Ross 308 chicks (National Chicks, Umlaas Rd), vaccinated at the hatchery for Newcastle Disease and Infectious Bronchitis, were used in a 21-day feeding trial. The 1344 chicks were randomly assigned to 96 pens (14 birds/pen), with the sexes kept separate. The pens measured 0.8 m × 0.5 m and the stocking density was a maximum of 4.6 kg/m^2 from days 0–7, 10 kg/m^2 from days 7–14, and 13.5 kg/m^2 from days 14–21.

Chicks were subjected to 12L: 12D fluorescent lighting throughout the trial, and the temperature was reduced from 32 °C on day one by 1 °C every second day throughout the 21-d experimental period. Feed and water (nipple drinkers) were available *ad libitum* throughout the trial.

The experiment was designed to measure the response to dietary Ca and nPP in male and female broiler chicks and to determine the extent of the interaction between the two minerals. Six levels of Ca were fed, with four levels of nPP at each. The 96 available pens provided two replications of each treatment in the 6 × 4 × 2 design, each pen being an experimental unit.

A basal feed was formulated to conform to the energy and amino acid recommendations of Aviagen (2019), but with minimal amounts of Ca and nPP (Table 1). This feed was mixed at a commercial feed mill. To achieve the 24 experimental diets, appropriate amounts of Ca and nPP in the form of limestone and feed-grade phosphoric acid (PA) were added to the basal diet at the research farm. PA was assumed to contain 268 g nPP/kg, and limestone to contain 350 g/kg Ca. The concentrations of Ca and nPP applied, and the resultant Ca:nPP ratios, are in Table 2.

The measured responses were BWG, FI, feed conversion efficiency (FCE, g gain/kg feed), tibial ash, and tibial breaking strength. On sampling days 7, 14, and 21, two birds per pen were randomly selected to be weighed and then killed using cervical dislocation. Both tibiae were removed from the carcases, and these were stored at -16 °C. Total pen broiler body weights and feed intakes were recorded at every sampling date. Mortalities and culls were recorded daily.

After thawing, the tibiae were stripped of muscle and weighed. Breaking strength (kg) was determined on the left tibiae using a three-point loading test, with the supports set 40 mm apart and a vertical hydraulic force applied at the midpoint of the bone shaft; the peak force required to break the bone was recorded on a tensiometer (loadcell – Loadtech, South Africa; digital display – Red Lion Controls, USA). Bones were pooled per treatment at each age before chemical analysis. Consequently, there were no replications for calculation of the SEM for the ash measurements, but the SEM of the two factors (Ca and nPP) and their interaction was calculated for tibial breaking strength.

Table 1 Composition of the basal diet

Ingredient	Concentration (g/kg)
Maize	568
Soybean meal 48	368
L-lysine HCl	2.6
DL-methionine	3.9
L-threonine 99% powder	0.6
Vit+min premix[1]	2.0
Limestone	0.9
Monocalcium phosphate	1.0
Salt	1.6
Sodium bicarbonate	3.5
Oil - soya	47.3
Nutrient content	
AMEn (MJ/kg)	13.0
Crude protein	230
Lysine[2]	12.7
Methionine	6.7
Methionine + cystine	9.4
Threonine	8.0
Tryptophan	2.2
Arginine	14.1
Isoleucine	9.0
Valine	9.5
Calcium	1.6
Non-phytate phosphorous	1.5
Phytate phosphorus	2.4

[1] supplied per kilogram of feed: Fe, 50 mg; Cu, 17.5 mg; Mn, 120 mg; Zn, 100mg; I, 2 mg; Se, 0.25 mg; vitamin A (retinyl acetate), 13500 IU; cholecalciferol, 0.125 mg; vitamin E (DL-ɑ-tocopheryl acetate), 50 mg; vitamin K3 (menadione), 5 mg; thiamine, 4 mg; riboflavin, 8 mg; pantothenic acid, 25 mg; niacin, 75 mg; pyridoxine, 7 mg; folic acid, 3 mg; biotin, 0.3 mg; vitamin B12, 0.03 mg, and choline, 300 mg
[2] digestible amino acid contents, g/kg

Table 2 Calcium and non-phytate phosphorus (nPP) contents (g/kg) and ratios in the trial feeds

	Dietary Ca content (g/kg feed)					
	1.9	3.8	5.7	7.5	9.5	11.3
Series	**Dietary nPP content (g/kg feed)**					
1	1.4	1.4	1.4	1.4	1.4	1.4
2	2.8	2.8	2.8	2.8	2.8	2.8
3	4.1	4.1	4.1	4.1	4.1	4.1
4	5.5	5.5	5.5	5.5	5.5	5.5
	Ca/nPP ratio in feeds					
1	1.36	2.71	4.07	5.36	6.79	8.07
2	0.67	1.34	2.01	2.64	3.35	3.98
3	0.46	0.93	1.39	1.83	2.32	2.76
4	0.34	0.69	1.03	1.36	1.72	2.05

The right tibiae underwent ash analysis at the University of KwaZulu-Natal, using Official Method of Analysis 932.16 (AOAC, 2005). Cartilage heads from the tops of the tibial bones were removed prior to the start of the analysis. All samples were then defatted using Soxhlet fat extraction, oven-dried, and ashed in a muffle furnace at 400 °C for 2 h followed by 4 h at 600 °C. The furnace was allowed to cool for 60 min, after which the samples were placed in a desiccator to cool down and were then re-weighed.

Statistical analyses were conducted using Genstat (VSN International, 2017). Main effects and interactions of all variables measured were subjected to ANOVA, whilst responses to Ca, nPP, their squared terms, and interactions were fitted using multiple regression analysis with groups. Polynomial surface functions were fitted to the effects of Ca and nPP on FI and BWG (R Core Team, 2024). When plotting the responses in feed intake and body weight gain, the regions of greatest interest were those around the peaks, so the lowest Ca and nPP points were excluded from the polynomial fitting to improve the correspondence to the measured data around the peaks.

Results and Discussion

The effects of varying Ca and nPP levels on BWG, FI, and FCE are presented in Table 3. The main effects of both Ca and nPP were significant (P <0.001) for all three variables but that for sex was significant only for BWG (P <0.001). The lowest gains recorded were on Ca:nPP of 6.79 (12.8 and 15.5 g/d for females and males, respectively) and 8.07 (14.9 and 14.0 g/d for females and males, respectively) and the highest on a Ca:nPP of 1.36 at the higher Ca and nPP contents (31.2 and 33.0 g/d, respectively). Fitted responses to Ca and nPP content are given in Table 4. Although the main effect of sex on BWG was significant, when fitting a multiple regression model with groups there was no difference in the constant term or regression coefficients between sexes. Body weight gain responded to the Ca content of the feed, its squared term, and to Ca:nPP, but not to the nPP content. Feed intake was influenced by both Ca and nPP, as well as their squared terms, and the ratio between them, although Ca had a substantially greater effect than nPP. As with BWG, FCE was a function of Ca, Ca^2, and Ca:nPP, but not nPP directly.

The mean breaking strength and mean ash contents (g/kg dry, fat-free bone) of the tibiae of broiler males aged 7, 14, and 21 d are shown in Table 5. Dietary Ca and its squared term had a marked impact on breaking strength at all three ages, whereas nPP content on its own had no effect. The interaction between Ca and nPP influenced breaking strength at 14 and 21 d only. Feed nPP content influenced tibia ash only at 7 d (P <0.05), with Ca having no effect, whereas at 14 and 21 d, tibial ash responded to changes in Ca and Ca^2 but not to nPP. The interaction between Ca and nPP was significant at both 14 and 21 d (P <0.01) but not at 7 d. This study demonstrates the importance of considering the interactions between Ca and P, as well as their concentrations in the diet. In the gastro-intestinal tract, Ca may bind both phytate P (PP) and nPP, reducing the availability of both minerals for absorption and hence for bone development (Driver *et al*., 2005). Both Ca and P must be present for the mineralisation of bone, in which they are found mainly in hydroxyapatite, which has a Ca:P mass ratio of approximately 2.

The constant terms and regression coefficients for tibial breaking strength and ash are presented in Table 6. The wide range of Ca concentrations provided some insight into two possible constraints on growth: at low Ca and P concentrations, bone mineralisation was likely reduced by deficiency of Ca supply in the feed, whereas at high Ca concentrations, it is likely that P availability from the feed was compromised (P absorption from the intestine was reduced). The interaction (P <0.001) between Ca and nPP for FI was due to the low FI on high Ca levels when nPP content was low, and the high FI on the same Ca levels when nPP was high. At low Ca levels, FI was high on all levels of P. This suggests that the lowest level of nPP fed in this trial may have been sufficient for growth in the absence of an inhibiting effect of Ca on P availability. The response surfaces of FI and BWG to Ca and nPP are shown in Figures 1 and 2. At the lowest level of Ca, there was no marked response in performance to P. BWG and FI appeared to be constrained when bone mineralisation was severely limited. The negative effect of higher Ca levels was particularly apparent, with the highest FI and BWG achieved at 7.5 g Ca/kg feed and 5.5 g nPP/kg feed.

David *et al*. (2021) showed that FI decreased as digestible Ca (dCa) in the feed increased when digestible P (dP) was low (4 g/kg), but this effect was much less marked at 5 g/kg dP and not discernible at 6 g/kg dP. In contrast, some studies have shown that increasing the content of Ca in the feed resulted in increased FI when aP was greater than 4 g/kg feed (Huyghebaert, 1997), with a constant aP level of 3.2 g/kg (Hu *et al.*, 2020), or by maintaining a constant ratio between Ca (3.6–9.6 g/kg) and nPP (1.8–4.8 g/kg) and increasing both minerals (Kiani & Taheri, 2020). However, a quadratic effect was noted over the period 14–21 d (Hu *et al*., 2020), and with similar Ca and P levels fed to broilers from 2–23 d of age (Gautier *et al*., 2018) and from 1–42 d of age (Han *et al*., 2016). Hu *et al*. (2020) also measured P and Ca digestibility at 21 d of age, and these decreased in response to increasing Ca concentrations in the feed.

Table 3 Mean daily weight gain, feed intake, and feed conversion efficiency (FCE) in male and female broilers to 21 d fed differing concentrations of calcium (Ca) and non-phytate phosphorus (nPP). Standard errors of the means (SEM) are given for main effects and interactions

TRT	**Ca**	**nPP**	**Ca:nPP**	**Weight gain g/d**		**Feed intake g/d**		**FCE g gain/kg feed**	
	g/kg	**g/kg**		**F**	**M**	**F**	**M**	**F**	**M**
1	1.9	1.4	1.36	22.1	20.9	35.3	34.0	627	614
2	3.8	1.4	2.71	27.1	25.3	39.1	35.7	692	707
3	5.7	1.4	4.07	19.8	22.3	31.9	36.1	620	620
4	7.5	1.4	5.36	18.2	17.3	27.7	26.0	659	667
5	9.5	1.4	6.79	12.8	15.5	23.0	24.5	557	632
6	11.3	1.4	8.07	14.9	14.0	22.8	23.0	655	609
7	1.9	2.8	0.67	19.4	21.5	31.6	32.8	614	657
8	3.8	2.8	1.34	25.8	26.7	37.8	37.9	684	706
9	5.7	2.8	2.01	27.4	27.4	38.8	38.9	706	704
10	7.5	2.8	2.64	24.5	27.5	35.9	38.3	683	717
11	9.5	2.8	3.35	21.6	21.9	31.8	32.6	679	673
12	11.3	2.8	3.98	21.4	20.2	32.4	30.5	662	662
13	1.9	4.1	0.46	20.4	22.2	32.0	34.3	638	648
14	3.8	4.1	0.93	27.6	29.5	40.4	43.4	684	681
15	5.7	4.1	1.39	29.2	29.5	41.7	41.0	700	722
16	7.5	4.1	1.83	30.5	31.6	40.5	43.6	754	725
17	9.5	4.1	2.32	26.2	27.5	38.7	39.6	677	694
18	11.3	4.1	2.76	27.8	27.9	41.5	39.5	671	704
19	1.9	5.5	0.34	18.6	20.8	29.3	34.8	636	602
20	3.8	5.5	0.69	27.2	29.3	40.9	40.6	665	722
21	5.7	5.5	1.03	29.3	31.9	42.5	45.0	690	707
22	7.5	5.5	1.36	31.2	33.0	45.6	46.8	685	705
23	9.5	5.5	1.72	28.1	28.7	41.4	40.1	679	715
24	11.3	5.5	2.05	24.8	25.7	37.2	50.8	667	549

		Weight gain g/d	Feed intake g/d	FCE g gain/kg feed
SEM	Ca	0.39**	0.88**	11.4**
	nPP	0.32**	0.72**	9.29**
	Sex	0.22**	0.51	6.57
	Ca × nPP	0.78	1.76**	22.7
	Ca × nPP × Sex	1.10**	2.48	32.2

** denotes significance (*P* <0.001) of main effect or interaction

Table 4. Constant terms and regression coefficients (± SE) describing daily weight gain and feed intake to, and feed conversion efficiency (FCE) at, 21 d in broilers given feeds differing in dietary calcium (Ca) and non-phytate phosphorus (nPP)

	Weight gain, g/d	**Feed intake, g/d**	**FCE, g gain/kg feed**
Constant	16.5 ± 1.11**	35.4 ± 3.32**	588 ± 16.3**
Ca	4.29 ± 0.389**	4.69 ± 0.507**	36.1 ± 5.73**
Ca^2	-0.261 ± 0.029**	-0.263 ± 0.035**	-2.27 ± 0.419**
nPP		-3.70 ± 168*	
nPP^2		0.450 ± 0.204*	
Ca × nPP	-2.56 ± 0.165**	-3.88 ± 0.461**	-13.5 ± 2.43**
R^2	77.2	76.3	36.2

[1] showing only significant (* *P* <0.05; ** *P* <0.001) coefficients

Table 5 Breaking strength and ash content[1] of bones of male broilers at 7, 14, and 21 d fed differing concentrations of calcium (Ca) and non-phytate phosphorus (nPP). Standard errors of the means (SEM) are given for main effects and interactions

TRT	**Ca**	**nPP**	**Tibial breaking strength (kg)**			**Tibial ash content[1] (% dry, fat-free bone)**		
	g/kg	**g/kg**	**7 d**	**14 d**	**21 d[1]**	**7 d**	**14 d**	**21 d**
1	1.9	1.4	0.72	4.78	8.26	32.1	25.7	33.9
2	3.8	1.4	1.75	5.76	12.2	25.7	36.0	37.9
3	5.7	1.4	2.42	4.71	7.05	30.1	33.1	42.8
4	7.5	1.4	2.59	5.39	9.19	25.5	32.5	35.2
5	9.5	1.4	1.86	4.03	7.58	28.4	31.5	35.8
6	11.3	1.4	1.74	3.57	8.04	31.0	31.5	34.1
7	1.9	2.8	0.72	4.33	8.86	29.2	36.8	34.0
8	3.8	2.8	1.36	6.00	11.2	43.7	39.7	41.5
9	5.7	2.8	1.20	7.92	19.6	34.8	40.3	41.4
10	7.5	2.8	2.02	4.76	12.5	32.7	35.6	37.6
11	9.5	2.8	3.05	8.40	14.3	28.3	42.9	41.7
12	11.3	2.8	1.60	5.17	13.3	31.7	31.0	37.9
13	1.9	4.1	1.83	4.91	13.8	35.4	34.6	37.2
14	3.8	4.1	1.72	6.64	13.9	39.6	40.1	39.2
15	5.7	4.1	2.49	10.2	16.1	34.9	44.6	40.3
16	7.5	4.1	1.76	8.30	18.4	40.3	42.1	44.7
17	9.5	4.1	1.81	7.45	17.2	39.7	41.2	45.4
18	11.3	4.1	1.72	9.41	13.5	33.7	44.1	40.2
19	1.9	5.5	1.60	4.60	12.3	36.3	35.3	37.4
20	3.8	5.5	2.25	8.65	14.8	38.5	45.9	39.3
21	5.7	5.5	2.25	6.84	24.9	40.5	48.2	43.1
22	7.5	5.5	1.31	11.4	16.0	33.1	45.4	43.8
23	9.5	5.5	1.96	6.22	21.7	32.6	40.4	45.2
24	11.3	5.5	1.74	6.04	18.7	35.9	50.9	42.6
SEM[2]								
Ca			0.118**	0.439**	0.655**			
nPP			0.096**	0.358**	0.535**			
Ca × nPP			0.025**	0.877**	1.311**			

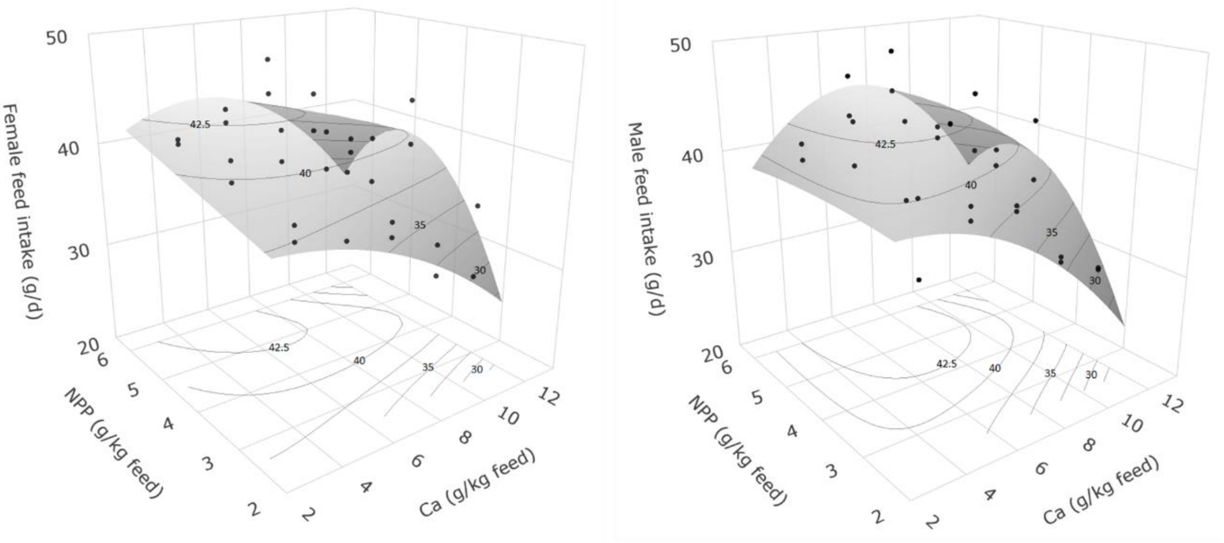
[1] samples were pooled per treatment, hence no SEM available

[2] ** denotes significance (*P* <0.001) of factor or interaction

Table 6 Constant terms and regression coefficients[1] (with SE's) describing the bone-breaking strength (kg) of male broilers at 7, 14, and 21 d in terms of dietary calcium (Ca) and non-phytate phosphorus (nPP) content

	Age, d		
	7	**14**	**21**
Tibial breaking strength (kg)			
Constant	0.563 ± 0.277*	2.63 ± 0.938**	7.03 ± 1.58**
Ca	0.410 ± 0.096**	1.67 ± 0.329**	3.15 ± 0.555**
Ca^2	-0.027 ± 0.007**	-0.097 ± 0.024**	-0.156 ± 0.041**
Ca × nPP		-0.792 ± 0.139**	-2.23 ± 0.235**
R^2	17.2	33.3	52.8
Tibial ash (% dry, fat-free bone)			
Constant	27.4 ± 1.98**	19.2 ± 3.77**	31.0 ± 1.79**
Ca		2.98 ± 1.16**	3.45 ± 0.628**
Ca^2		-0.197 ± 0.086*	-0.195 ± 0.046**
nPP	1.90 ± 0.525**		
Ca × nPP		-3.04 ± 0.498**	-1.49 ± 0.265**
R^2	34.3	64.6	69.8

[1] showing only significant (*, *P* <0.05; **, *P* <0.001) coefficients



Female $FI = 36.90 - 0.43(Ca)^2 + 0.07(Ca \times (NPP)^2) + 0.20((Ca)^2 \times NPP) - 0.03((Ca)^2 \times (NPP)^{\ 2})$

Male $FI = 39.98 - 0.43(Ca)^2 - 0.30(NPP)^2 + 0.13(Ca \times (NPP)^2) + 0.18((Ca)^2 \times NPP) - 0.03((Ca)^2 \times (NPP)^{\ 2})$

Figure 1 Average feed intake (g/bird d) of broilers from days 1–21 in response to different concentrations of total calcium and non-phytate phosphorus (g/kg feed)

Models of digestion and growth allow these complex and contradictory results to be integrated into guidance for nutritionists. Fixed digestibility values for P sources in feed formulation will be useful only if dietary Ca and P levels are also fixed. Available P should be increased or decreased when Ca is adjusted downwards or upwards, even though formulated digestibility and retention values remain static.

A quadratic model was fitted to predict BWG (g/bird 0–21 d) from nPP (g/kg feed) (Equation 1), excluding the lowest level of Ca (1.4 g/kg feed). This predicted BWG poorly over different Ca values ($R^2 = 0.643$).

$$\text{BWG} = 198 + 158 \times \text{nPP} - 15.0 \times \text{nPP}^2 \tag{1}$$

A simple empirical model modifying the available P according to the nPP and Ca in the diet calculated available P (Equation 2):

$$\text{Proportion of total P absorbed} = 0.796 - 0.020 * \text{Ca} - 0.013 * \text{nPP} \tag{2}$$

where total Ca (Ca) and nPP are measured in g/kg feed (modelled from Dieckmann, 2004).

The quadratic model fitted to predict BWG (g/bird 0–21 d) from aP (g/kg feed) calculated in this way (Equation 3) shows an improved fit ($R^2 = 0.832$)

$$\text{BWG} = -444 + 482 \times \text{aP} - 54.2 \times \text{aP}^2 \tag{3}$$

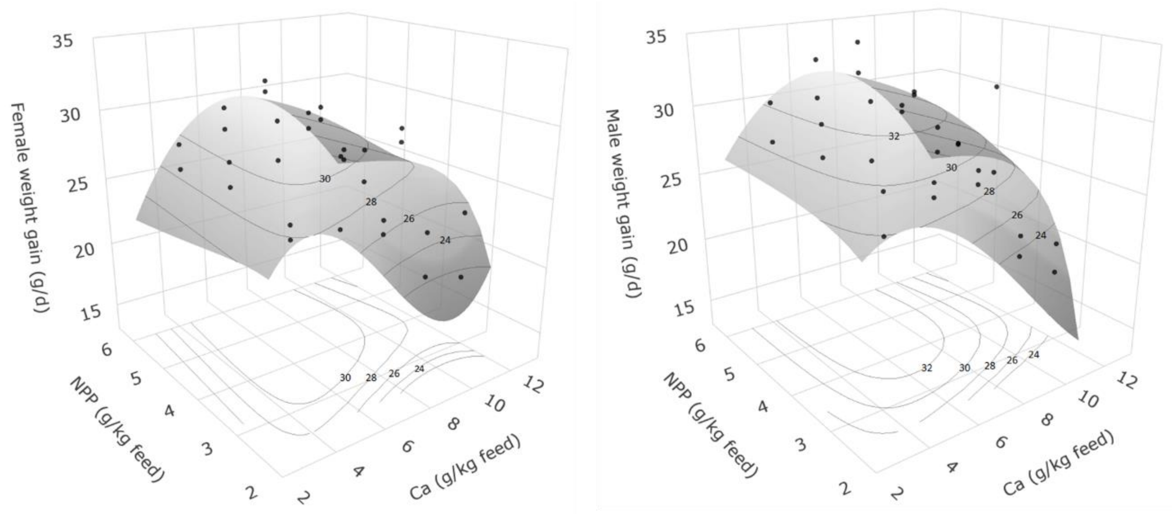
The model described in Reis *et al*. (2023) calculates dP (g/kg feed) from Ca, nPP, and PP concentrations in the feed. It includes a squared term for Ca and interactions between Ca and PP and Ca and nPP (Equation 4).

$$\text{dP} = -0.290 - 0.376 \times \text{Ca} + 1.101 \times \text{PP} + 0.917 \times \text{nPP} + 0.018 \times \text{Ca}^2 - 0.011 \times \text{Ca} \times \text{PP} - 0.017 \times \text{Ca} \times \text{nPP} \tag{4}$$

where total Ca (Ca), nPP, and PP are measured in g/kg feed (Reis *et al*., 2023).

Using dP as a predictor of BWG produces the quadratic model shown in Equation 5 ($R^2 = 0.859$).

$$\text{BWG} = 22.9 + 249\,\text{dP} - 25.8\,\text{dP}^2 \tag{5}$$



Female $BWG = 9.15 + 4.56(Ca) - 0.40(Ca)^2 + 12.26(Ca \times NPP) - 7.78(Ca \times NPP)^2 + 0.48(Ca \times (Ca \times NPP)\ ^2)$

Male $BWG = 17.53 + 4.55(Ca) - 0.49(Ca)^2 - 4.77(Ca \times (NPP)^2) - 1.36\big(Ca \times (Ca \times NPP)\big) + 0.16(Ca \times (Ca \times NPP)\ ^2)$

Figure 2 Average body weight gain (g/bird d) of broilers from days 1–21 in response to different concentrations of total calcium and non-phytate phosphorus (g/kg feed)

The current study could not determine whether the feedback mechanism that reduces growth when mineral supply is insufficient for growth acts directly on appetite (reducing FI) or inhibits growth and hence, reduces FI. As a means of addressing this issue, Reis *et al*. (2023) modified the desired FI, derived from an established broiler growth model, by applying a coefficient based on the levels of Ca, nPP, and exogenous phytase in the feed. The linear effect of Ca (g/kg feed) on FI was negative, whereas the linear effect of nPP (g/kg feed) was positive. The adjusted FI brings about a modification of bird growth in the model.

Phosphorus plays a central role in many metabolic processes including glycolysis, the tricarboxylic acid cycle, and the uric acid cycle. It is also a component of cell membranes. Hence, consuming feed with extremely low P may lead to the toxic buildup of nutrients, such as amino acids and glucose, when these processes cannot proceed. A feedback mechanism to produce anorexia in such situations has been proposed by Aderibigbe *et al.* (2022). However, feed intake and bone mineralisation are both compromised at levels where there is expected to be sufficient P for soft tissue growth. In this case, the relationship may in fact be reversed: reduced potential protein growth caused by bone mineral deficiency may lower the desired FI. The response studies cited above noted a similar effect of Ca and P levels in feed on both FI and BWG, making it difficult to discern cause and effect. Nonetheless, the evolutionary advantage of lowering FI during marginal deficiency is harder to defend than a mechanism to decrease growth to accompany lower bone mineralisation and hence skeletal weakness.

The multiple regression models for tibial breaking strength and tibial ash indicate that the suppressive effects of high Ca contents are seen in bone mineralisation as well as body growth. In another study, increasing the dietary Ca level from 6 to 7 g/kg increased tibial breaking strength over a range of nPP contents between 3 and 4.5 g/kg feed, but beyond this content of Ca, increasing nPP from 4 to 4.5 g/kg feed decreased the tibial breaking strength (Rama Rao *et al*., 2003). Han *et al*. (2012) found that at a constant concentration of P (2.5 g nPP /kg feed), Ca had a quadratic effect on tibial breaking strength, with a maximum at 6 g Ca/kg feed (63 N). This indicates the importance of Ca:nPP in the feed, resulting in a low optimum Ca content at low contents of nPP. Akter *et al*. (2016) also demonstrated that higher contents of Ca (10 g/kg feed) decreased bone-breaking strength, particularly when nPP was low (3 g/kg feed). This effect was mitigated by the addition of phytase to the feed, suggesting that the availability of P from the gastro-intestinal tract is likely to play an important role in limiting bone mineralisation at a high Ca:PP.

Because both Ca and P are required for bone mineralisation, it is to be expected that even once the availability of Ca and P from the digestive tract has been accurately modelled, an interaction between the two minerals will persist. If there is insufficient Ca for the deposition of hydroxyapatite, then increasing levels of available P are unlikely to yield improved bone mineralisation. This was evident in the lack of response to nPP at the lowest level of Ca (1.4 g/kg feed). Lower levels of Ca in feed are associated with greater PP digestibility and hence, improved P absorption (Tamim *et al*., 2004).

However, P in excess of that which can be incorporated into the bone mineral will be excreted in the urine. When Ca was at a moderate level (7.5 g/kg feed), a positive, linear BWG response to dP was observed ($R^2 = 0.93$). The presence of adequate levels of Ca may have allowed bone mineralisation to improve as more P was made available. A model of this phenomenon must compare available Ca and P and only allow the assimilation of one when the other is present in sufficient quantities. Some models have fixed the ratio between Ca and P in bone at 2 g Ca/g P, although experimental data indicate variation in these proportions from 1.7–2.3 g Ca/g P (Skinner and Waldroup, 1995; Angel, 2007; Dersjant-Li *et al*., 2018; Li *et al*., 2020).

Conclusions

The complex picture of bone mineralisation responses to the contents of Ca and P in feed highlights the value of a mechanistic model that can estimate the available quantities of minerals (digestible Ca and P), the amounts required for maximum growth and bone mineralisation, and the extent to which bone mineralisation can be reduced without compromising welfare or performance.

Authors' contributions

CEB collected the data for this study, wrote the initial draft of this manuscript, and collaborated in interpreting the results; NT co-supervised the experiments and collaborated in interpreting the results; FS conducted the literature review, collaborated in interpretation of the results, and developed the manuscript; AJC contributed to the design of the experiment and collaborated in interpretation of the results; CJB analysed the data to produce the response surfaces and contributed to a model used in the interpretation of results; RMG collaborated in the design of the experiment, supervised the experiment, collaborated in the interpretation of results, and finalised the manuscript. All authors have read and approved the finalised manuscript.

Conflict of interest declaration

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