Research Note: Formulating broiler diets using digestible calcium significantly improved growth performance but reduced apparent ileal digestibility of calcium and phosphorus

C. L. Walk,[*,1] P. Jenn,[†] J. O. B. Sorbara,[‡] I. Gaytan-Perez,[†] and R. Aureli[†]

[*]DSM Nutritional Products, Heanor, Derbyshire, DE75 7SG, United Kingdom; [†]DSM Nutritional Products, 68128 Village-Neuf, France; and [‡]DSM Nutritional Products, Kaiseraugst, 4303, Switzerland

ABSTRACT An experiment was conducted to compare the use of a total and digestible Ca formulation system in broilers from hatch to d 37 post-hatch. Ross 308 (n = 288) male broilers were obtained and allocated to one of 2 dietary treatments in floor pens. There were 18 birds per pen and 8 replicate pens per diet. One diet was formulated using ingredient and nutrient recommendations for total Ca and the second diet was formulated using ingredient and nutrient recommendations for standardized ileal digestible (**SID**) Ca. All diets were formulated to be nutrient adequate using a 2-phase feeding program and including 0.19% available P (**avP**) and 0.209% total Ca or 0.073% digestible Ca from 1,000 FYT/kg of phytase. On d 17 and 37, tibias and ileal contents were obtained. From hatch to d 37, birds fed diets formulated using digestible Ca gained ($P <$ 0.05) more and were more efficient ($P < 0.05$) compared with birds fed diets formulated using total Ca. There was no impact of formulation system on tibia ash or minerals. Litter pH ($P < 0.05$) was greater and litter dry matter ($P < 0.05$) was lower in birds fed the diets formulated using digestible Ca compared with those fed diets formulated using total Ca. Apparent ileal digestibility (**AID**) of Ca ($P < 0.05$), AID of P ($P < 0.05$) and digestible P intake ($P < 0.05$) were lower in birds fed diets formulated using digestible Ca compared with those formulated using total Ca at d 17 or 37. However, apparent ileal digested Ca and digestible Ca intake were not different between the experimental diets on d 17 or 37. In conclusion, formulating diets using digestible Ca improved weight gain and feed conversion ratio, but reduced the AID of Ca and P compared with birds fed diets formulated using total Ca. These findings might be reflective of the higher total Ca concentration in the diets formulated using digestible Ca compared with those formulated using total Ca.

Key words: calcium, digestibility, growth performance, litter, tibia ash

2022 Poultry Science 101:102069
https://doi.org/10.1016/j.psj.2022.102069

INTRODUCTION

Formulating broiler diets using digestible compared to total formulation systems has led to improvements in growth performance, efficiency, prudent use of resources, and reductions in nutrient excretion into the environment. This is currently standard practice for essential amino acids and P in some regions. Whereas other nutrients, such as Ca are still formulated using total values. Moving to a digestible Ca formulation system may also have benefits for broilers, such as: improved growth performance and nutrient utilization, enable users to understand and monitor the Ca contribution of their ingredients, and reduce excretion of P into the environment due to close and sometimes antagonistic relationships between dietary Ca and P. The standardized ileal digestible (**SID**) Ca requirements for broilers from hatch to d 10 (David et al., 2021; Walk et al., 2021b) and d 11 to 24 post-hatch (Walk et al., 2022) were recently published. Therefore, the objective of this work was to compare the use of a total and a digestible Ca formulation system in broilers from hatch to d 37 using growth performance, tibia ash, nutrient digestibility, and markers of welfare such as foot pad and litter scores.

MATERIALS AND METHODS

The animal protocol for this research was approved by the Ethical Committee of DSM Animal Nutrition Research Center and complied with the guidelines in the European Union council directive 2010/63/EU for animal experiments. This experiment was conducted at

Received April 26, 2022.
Accepted July 6, 2022.

[1]Corresponding author: carrie.walk@dsm.com

DSM Animal Nutrition Research Center in Village-Neuf, France.

Two-hundred and eighty-eight Ross 308 male broilers were obtained on the d of hatch and randomly allocated to 16 floor pens with 18 birds per pen. There were 2 dietary treatments and 8 replicate pens per diet. Birds were fed a 2-phase feeding program, including a starter diet from hatch to d 17 and a grower diet from d 18 to 37. One treatment was formulated using total Ca ingredient and nutrient recommendations. The other treatment was formulated using SID Ca ingredient and nutrient recommendations (Walk et al., 2021a,b, 2022). In the diet formulated using digestible Ca, the limestone digestible Ca coefficient was estimated at 0.567 (without phytase) or 0.640 (with phytase) by using vitro solubility measured at 15 (96.4%) and 30 (98.7%) minutes and mean particle size (128 um) according to the methods of Kim et al. (2019).

The ingredients (corn, soybean meal, limestone, dicalcium phosphate, and the vitamin and mineral premix) were analyzed for nutrient composition, including total Ca and P. The analyzed concentrations were used to formulate the experimental diets. The total Ca diet was formulated to meet Ross 308 recommended nutrient requirements (Ross Nutrition Specifications, 2019, Huntsville, AL), including 0.19% available P (**avP**) and 0.209% total Ca provided from 1,000 FYT/kg of phytase (HiPhorius, DSM Nutritional Products, Kaiseraugst, Switzerland). The digestible Ca diet was formulated using previously estimated SID Ca requirements for broilers from hatch to d 24 (Walk et al., 2021b, 2022), including 0.19% avP, the limestone digestible Ca coefficient for phytase estimated using an in vitro assay (Kim et al., 2019), and 1,000 FYT/kg of phytase (HiPhorius, DSM Nutritional Products, Kaiseraugst, Switzerland).

All birds were reared on clean shavings in an environmentally controlled room with a lighting program of 12L:12D for the duration of the trial. Birds were allowed ad libitum access to feed and water. At placement (d 0), on d 17 and d 37, all birds were weighed by pen to determine mean BW and calculate mean BW gain (**BWG**). Feed added and feed left over were weighed at d 0, 17, and 37 to calculate feed intake (**FI**). Body weight gain and FI were used to calculate feed conversion ratio (**FCR**). Mortality was recorded daily, and any culled or dead birds were weighed. Feed intake and subsequently FCR were adjusted for mortality according to the number of bird days per pen.

On d 17 and 37, after weighing, 5 birds per pen were stunned and then euthanized by cervical dislocation. Ileal digesta (defined as the Meckel's diverticulum to 40 mm proximal to the ileocecal junction) was collected from the distal two-thirds of tract by flushing with distilled water, pooled within pen, and immediately frozen. Left tibias were obtained from 2 birds, close to the average BW per pen, and pooled within cage to determine tibia ash, tibia Ca, and tibia P.

Digesta were freeze dried to a constant weight. Diets and digesta were ground to pass a 0.5-mm screen and then analyzed for Ca, P, and Ti using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Optima TM 8000, Perkin Elmer, Shelton, CT) after sulfuric acid mineralization (based on method 985.01; AOAC International, 2006). Calcium, P, and Ti were then used to determine apparent ileal digestibility (**AID**). Tibias were stripped of adhering tissues, dried at 105°C for 24 h, and then ashed at 550°C for 48 h for determination of tibia ash percent. Tibia P and Ca were determined using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Optima TM 8000, Perkin Elmer, Shelton, CT) after a mineralization with 18.5% HCl. On d 37, litter samples were obtained from three points in each pen, avoiding the areas near and below the drinkers and feeders. Litter quality and foot pad dermatitis were assessed by visual scoring classification based on the methods of Welfare Quality (2009). Litter samples were weighed, dried at 105°C for 48 h, and weighed again to determine moisture content. A 10 g subsample of fresh litter was solubilized in 100 mL of distilled water and pH measurements were performed using a pH meter according to methods of Brauer-Vigoderis et al. (2014). Xylanase and protease activities in the diets were analyzed using methods based on dye-labeled substrates (Azo-Xylan and Suc-Ala-Ala-Pro-Phe-pNA, respectively). Phytase activity was measured by Method PHY-102/06E DSM and one phytase unit was defined as the amount of enzyme that releases 1 μmol of inorganic phosphate from 50 mM phytate per minute at 37°C and pH 5.5.

Statistical Analysis and Calculations

Data were subjected to an analysis of variance using JMP Pro v. 16.0 (SAS Institute, Cary, NC). Pen served as the experimental unit. Prior to statistical analyses, the distribution platform was used to verify normality. Any outliers, determined as 3 times the root mean square error plus or minus the mean of the response, were removed from the statistical analysis. Growth performance, mortality, tibia ash, AID, litter parameters, and the calculated digested Ca or P were analyzed as a one-way ANOVA using the fit Y by X platform. Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Experimental Diets

Phytase activity recovered in the experimental diets was approximately 25% higher than expected, most likely due to analytical variation and 10% overage included in the calculated amount to add to the diets (Table 1). Xylanase and protease recoveries were variable but indicate the products were included in the diets as expected. Analyzed total Ca and total P were within the expected concentrations formulated in the diets.

Growth Performance and Tibia Ash

Initial BW was 40.5 ± 0.5 g. There was no effect of diet on initial BW ($P = 0.48$). Final BW was 2,833 g

Table 1. Calculated and analyzed ingredient and nutrient content of the experimental diets, as fed.

	Starter diets (hatch to d 17)		Grower diets (d 18–37)	
Ingredients (%)	Total Ca[1]	Digestible Ca[2]	Total Ca[1]	Digestible Ca[2]
Corn	57.96	56.92	60.78	59.72
Soybean meal	36.41	36.60	32.93	33.13
Soy oil	2.42	2.73	3.51	3.83
Salt	0.28	0.28	0.29	0.29
Limestone	0.74	1.29	0.72	1.22
Dicalcium phosphate	1.02	1.00	0.73	0.77
Sodium bicarbonate	0.20	0.20	0.20	0.20
Lysine HCl	0.11	0.11	0.06	0.06
DL-Methionine	0.29	0.29	0.24	0.24
Threonine	0.06	0.06	0.02	0.02
Premix[3]	0.40	0.40	0.40	0.40
Phytase[4]	0.01	0.01	0.01	0.01
Titanium	0.10	0.10	0.10	0.10
Xylanase[5]	0.005	0.005	0.005	0.005
Protease[6]	0.0045	0.0045	0.0045	0.0045
Calculated nutrient contents				
Crude protein, %	23.00	23.00	21.50	21.50
ME, kcal/kg	3,000.00	3,000.00	3,100.00	3,100.00
Formulated total Ca[1], %	0.69	0.90	0.59	0.80
Formulated SID Ca[2], %	0.43	0.50	0.32	0.45
Total P, %	0.55	0.54	0.48	0.49
Non-phytate P, %	0.48	0.48	0.42	0.43
Phytate P, %	0.25	0.25	0.24	0.24
Digestible Met + Cys, %	0.95	0.95	0.87	0.87
Digestible Lys, %	1.28	1.28	1.15	1.15
Digestible Thr, %	0.86	0.86	0.77	0.77
Digestible P, %	0.44	0.44	0.39	0.40
Available P, %	0.45	0.45	0.40	0.41
Analyzed nutrients and recovered enzyme activities				
Phytase, FYT/kg	1,316	1,315	1,219	1,143
Xylanase, FXU/kg	219	122	164	218
Glucanase, IU/kg	46	28	33	32
Protease, NFP/kg	30,090	25,680	33,780	24,390
Dry matter, %	87.88	88.45	88.87	88.89
Crude protein, %	20.93	20.85	18.72	21.58
Total Ca, %	0.69	0.93	0.58	0.80
Total P, %	0.58	0.59	0.50	0.52

[1]The phytase matrix applied to the total Ca diets was 0.19% avP and 0.209% total Ca.

[2]The phytase matrix applied to the standardized ileal digestible (SID) Ca diets was 0.19% avP and the estimated digestible Ca coefficient for limestone (0.640%) with phytase (Kim et al., 2019).

[3]Vitamin and mineral premix provided (per kilogram of diet): vitamin A 10,000 IU; vitamin D3 3,000 IU; vitamin E 40 mg; vitamin K3 3 mg; vitamin B1 2.5 mg; vitamin B2 8 mg; vitamin B6 5 mg; vitamin B12 0.025 mg; biotin 0.15 mg; folic acid 1.5 mg; niacinamide 50 mg; D-pantothenic acid 12 mg; Fe (as FeSO4) 60 mg; Cu (as CuSO4) 15 mg; Mn (as MnO) 80 mg; Zn (as ZnO) 54 mg; I (as Ca(IO3)2) 1.2 mg; Se (as Na2SeO3) 0.297 mg; calcium as calcium carbonate (carrier) 26.4 mg.

[4]HiPhorius (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 10,047 FYT/g.

[5]Ronozyme Multigrain (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 3,490 U/g was added over the top with no energy contribution considered in the formulation.

[6]ProAct 360 (DSM Nutritional Products, Kaiseraugst, Switzerland) with an analyzed activity of 785,470 NFP/g, contributed 0.67% crude protein, 0.04% digestible methionine + cysteine, 0.05% digestible lysine, and 0.05% digestible threonine.

and this was 9% greater than breed expectations, whereas FI was 9% below breed guidelines and this resulted in mortality corrected FCR approx. 86% of the breed guidelines. Average overall (hatch to d 37) mortality was 5.9% and not influenced by diet (Table 2). Birds fed diets formulated using digestible Ca gained ($P < 0.05$) more (+5%) and FCR ($P < 0.05$) was improved compared with birds fed diets formulated using total Ca from d 18 to 37 and overall (Table 2). Walk et al. (2021b, 2022) reported birds fed diets formulated to meet the SID Ca from hatch to d 10 were significantly heavier (+6%) compared with birds fed diets formulated using total Ca. In the previous studies, the diets formulated using SID Ca also contained 3,000 FYT/kg of phytase, which the authors attributed to some of the improvements in BWG compared with birds fed the total Ca diets. In the current study, both treatments contained 1,000 FYT/kg of phytase and there may be other beneficial factors associated with formulating diets using SID Ca that are unknown or not tested in the current study. Unfortunately, additional data to compare, confirm, or refute the use of SID Ca vs. total Ca formulations are not available in peer-reviewed publications and this limits the inferences that can be made at this time. There was no impact of diet on tibia ash percent, tibia ash weight, or tibia Ca or P (Table 2). The current results suggest formulating broiler diets using SID Ca had no negative impact on skeletal development and significantly improved growth performance.

Table 2. Growth performance, tibia ash, litter quality and apparent ileal digestibility (AID) of calcium and phosphorus of broilers fed diets formulated using total or digestible calcium from hatch to d 37 post-hatch.

	Formulation system			
Feeding phase	Total calcium	Digestible calcium	SEM	*P*-value
Hatch to d 17 post-hatch[1]				
Feed intake, g	820	798	9	0.1174
Body weight gain, g	706	683	8	0.0718
Feed conversion ratio, g:g	1.163	1.169	0.008	0.6031
Mortality, %	4.17	2.08	1.4	0.3186
Tibia ash, %	50.69	51.20	0.42	0.4073
Tibia ash weight, mg/bone	725	713	21	0.6966
Tibia Ca, mg/bone	254	249	7.3	0.6358
Tibia P, mg/bone	130	127	3.7	0.5035
AID P, % DM	78.00	65.75	1.5	< 0.0001
Apparent digested P, %	0.311	0.262	0.006	< 0.0001
Digestible P intake, mg/b/d	150	123	3.1	< 0.0001
AID Ca, %	63.45	49.66	1.6	< 0.0001
Apparent digested Ca, %	0.301	0.312	0.008	0.3625
Digestible Ca intake, mg/b/d	145	147	4.1	0.8488
Apparent digested Ca:P	0.967	1.189	0.016	< 0.0001
D 18 to 37 post-hatch[2]				
Feed intake, g	2,763	2,773	50	0.8962
Body weight gain, g	2,017	2,180	36	0.0067
Feed conversion ratio, g:g	1.370	1.273	0.014	0.0002
Mortality, %	4.17	1.92	1.4	0.2846
Tibia ash, %	43.27	44.48	0.53	0.1286
Tibia ash weight, mg/bone	3,009	3,059	70	0.6269
Tibia Ca, mg/bone	1,063	1,078	25	0.6805
Tibia P, mg/bone	539	546	12	0.7110
AID P, % DM	69.75	60.30	1.9	0.0038
Apparent digested P, %	0.251	0.224	0.008	0.0291
Digestible P intake, mg/b/d	347	311	12	0.0511
AID Ca, %	46.94	34.34	2.1	0.0009
Apparent digested Ca, %	0.196	0.197	0.01	0.9601
Digestible Ca intake, mg/b/d	271	273	17	0.9260
Apparent digested Ca:P	0.781	0.870	0.03	0.0717
Hatch to d 37 post-hatch				
Feed intake, g	3,583	3,571	55	0.8767
Body weight gain, g	2,722	2,863	40	0.0251
Feed conversion ratio, g:g	1.317	1.248	0.012	0.0009
Mortality, %	8.33	4.01	2.1	0.1630

[1]Data are the means of 18 birds per pen and 8 replicate pens per treatment for growth performance. Tibia ash parameters were determined from 2 birds per pen and 8 replicate pens per treatment. Apparent ileal digestibility (AID) of nutrients was determined from 5 birds per pen and 8 replicate pens per treatment.

[2]Data are the means of 13 birds per pen and 8 replicate pens per treatment for growth performance. Tibia ash parameters were determined from 2 birds per pen and 8 replicate pens per treatment. Apparent ileal digestibility (AID) of nutrients was determined from 5 birds per pen and 8 replicate pens per treatment.

Litter Parameters and Foot Pad Lesions

Litter pH (9.08 vs. 8.89; $P < 0.05$) was greater and litter dry matter (56.3 vs. 67.0%; $P < 0.05$) was lower in birds fed the diets formulated using digestible Ca compared with those fed diets formulated using total Ca. The greater litter pH and moisture could be linked to the higher total Ca level in the SID Ca diet, which has been reported to significantly increase gastrointestinal pH, reduce crude protein digestibility, and increase pathogenic bacteria (Zanu et al., 2020). In the current study, both treatments resulted in litter dry matter was lower than the recommended 75 to 80%. Many factors, such as litter-type, ventilation, feed and water intake, drinker management, and the external environmental conditions can influence litter moisture and subsequently ammonia emissions (Ritz et al., 2017). Average litter scores were 0.94 and 1.06 and were not different between the total or SID Ca diets, respectively. A score of 1 indicates the litter is dry but not easy to move with your foot (Welfare Quality, 2009). Foot pad lesions were scored by 5 different people on 2 different birds randomly chosen per pen. Only 1 bird fed the total Ca diet had a lesion score of 1, the rest of the birds had a lesion score of 0 (data not shown). It is also worth also noting the birds fed SID Ca diets were heavier and mortality was numerically lower (4 vs. 8%) compared with birds fed diets formulated using total Ca, which resulted in a greater number of birds per pen by the conclusion of the study. These factors, and possibly the higher total Ca, may have contributed to the increase in litter moisture in the absence of any difference in litter scores or foot pad lesions. Further research is warranted to determine if the change in litter moisture is a factor associated with formulating diets using SID Ca.

Apparent Nutrient Utilization and Calculated Digested Nutrients

The AID of N was not influenced by diet on d 17 ($P = 0.66$) or d 37 ($P = 0.67$; data not shown). Apparent ileal digestibility of Ca ($P < 0.05$) or P ($P < 0.05$) were lower in birds fed diets formulated using SID Ca at d 17 and 37 (Table 2). These results were unexpected. However, the total Ca concentration in the diets formulated using SID Ca was greater than those formulated using total Ca and when calculated on a digested Ca or digestible Ca intake basis, there were no differences between the two formulation systems for Ca.

It is well established that increasing total Ca will result in significant decreases in P digestibility and this most likely contributed to the decrease in AID of P, apparent ileal digested P, and digestible P intake in birds fed the SID Ca diets. Therefore, a balance is needed to ensure optimal growth performance and skeletal development are achieved while minimizing nutrient excretion. The formulated SID Ca concentrations in the current study were greater than those estimated to optimize BW gain or P retention, but less than those estimated to optimize tibia ash (David et al., 2021; Walk et al., 2021b, 2022). The lack of a significant difference in the tibia ash percent, ash weight, or mineral content between the two diets could indicate the SID Ca level in the digestible Ca diets could be reduced to optimize P utilization. However, this may negate the beneficial effects of formulating diets using SID Ca on BWG and FCR. Repeating this work using different levels of digestible Ca to optimize gain or bone ash and at different feeding phases is required to provide adequate commercial recommendations and advice on formulating diets using SID Ca.

DISCLOSURES

The authors have no conflicts of interest to report.

REFERENCES

AOAC. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC, Arlington, VA.

Brauer-Vigoderis, R., I. de Fátima Ferreira-Tinôco, H. Pandorfi, M. Bastos-Cordeiro, J. Pinheiro de Souza Jr, and M. C. de Carvalho-Guimarães. 2014. Effect of heating systems in litter quality in broiler facilities in winter conditions. Dyna 81:2346–2383.

David, L. S., M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2021. Requirement of digestible calcium at different dietary concentrations of digestible phosphorus for broiler chickens. 1. Broiler starters (d 1 to 10 post-hatch). Poult. Sci. 100:101439.

Kim, S.-W., W. Li, R. Angel, and P. W. Plumstead. 2019. Modification of a limestone solubility method and potential to correlate with *in vivo* limestone calcium digestibility. Poult. Sci. 98:6837–6848.

Ritz, C. W., B. D. Fairchild, and M. P. Lacy. 2017. Litter quality and broiler performance. UGA Extension Bull 1267:1–5.

Walk, C. L., L. F. Romero, and A. J. Cowieson. 2021a. Towards a digestible calcium system for broiler chicken nutrition: a review and recommendations for the future. Anim. Feed Sci. Technol. 276:114930.

Walk, C. L., Z. Wang, S. Wang, J. O. B. Sorbara, and J. Zhang. 2022. Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from day 11 to 24 post-hatch. Poult. Sci. 101:1011836.

Walk, C. L., Z. Wang, S. Wang, J. Wu, J. O. B. Sorbara, and J. Zhang. 2021b. Determination of the standardized ileal digestible calcium requirement of male Arbor Acres Plus broilers from hatch to day 10 post-hatch. Poult. Sci. 100:101364.

Welfare Quality®. 2009. Welfare Quality® Assessment Protocol for Poultry (Broilers, Laying Hens). Welfare Quality® Consortium, Lelystad, Netherlands.

Zanu, H. K., S. K. Kheravii, N. K. Morgan, M. R. Bedford, and R. A. Swick. 2020. Interactive effect of 2 dietary calcium and phytase levels on broilers challenged with subclinical necrotic enteritis: part 1 – broiler performance, gut lesions and pH, bacterial counts, and apparent ileal digestibility. Poult. Sci. 99:4861–4873.