Effect of supplementation of phytase to diets low in inorganic phosphorus on growth performance and mineralization of broilers

D. V. Scholey*[1], N. K. Morgan*, A. Riemensperger[†], R. Hardy[†], and E. J. Burton*

*School of Animal, Rural and Environmental Science, Nottingham Trent University, Southwell, Nottinghamshire, England NG25 0QF

Telephone +44 115 8485327

Email dawn.scholey@ntu.ac.uk

Email nat.morgan@ntu.ac.uk

Email emily.burton@ntu.ac.uk

[†]Danisco (UK) Ltd, Marlborough, Wiltshire, England SN8 1XN

Telephone +44 00 353 2530668

Email angela.riemensperger@gmx.com

Email rachael.hardy@Dupont.com

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[1]Corresponding author: dawn.scholey@ntu.ac.uk

ABSTRACT

There has been discussion regarding microbial phytase replacing inorganic phosphorus (**P**) supplementation in broiler diets. Therefore, an experiment was conducted to examine the effect of phytase supplementation on diets low in inorganic P. Ross 308 broilers (n=288) were fed one of six experimental diets in four phases. The control diet had 16.20, 10.90, 9.40 and 6.10g/kg inorganic P in the Starter, Grower 1, Grower 2 and Finisher phase respectively. The remaining diets had 10.50g/kg inorganic P in the Starter phase. Two of the diets had graded reductions in inorganic P of 5.10, 3.60 and 0.60g/kg or 2.00, 0.50 and 0.60g/kg for the Grower 1, Grower 2 and Finisher phase respectively, plus 500 FTU phytase. Three of the diets had inorganic P levels of 0.40, 0.50 and 0.60g/kg for the Grower 1, Grower 2 and Finisher diets respectively and either 500, 750 or 1000 FTU phytase. Broiler performance was analyzed at d10, 20, 26 and 35. On d35 ileal calcium (**Ca**) and P digestibility and tibia bone strength, mineralization and mineral content were analyzed. There were no significant differences between the control diet and diet containing 1000 FTU phytase and low inorganic P in the grower or finisher diets based on bird performance, tibia strength and Ca and P digestibility. Birds fed the control diet had significantly higher BWG ($P = 0.001$), bone strength ($P<0.001$) and ash content ($P<0.001$) compared to birds fed the diets with 500 FTU or 750 FTU phytase and low inorganic P in the grower and finisher stages. This may be due to incomplete dephosphorylation of the inositol ring of phytate with these doses of phytase, but with 1000 FTU phytase there was almost complete phosphate hydrolysis of each phytate. This study showed that relying on phytase alone to ensure full supply of P in broiler diets is viable in finisher diets but is not recommended in grower diets unless phytase is supplied at doses of 1000 FTU or greater.

Key words: broiler, phytase, phosphorus, bone mineralization

INTRODUCTION

The genetic selection of broilers for rapid growth has led to a substantial incidence of bone abnormalities and lameness, with great economic cost to the industry. Phosphorus (**P**) is an essential mineral for skeletal integrity but is limited and an expensive component in poultry feed where margins are small. The phosphorus in cereals and oilseeds is bound as phytate which is largely unavailable to poultry due to their limited endogenous phytase (Morgan et al. 2015). Microbial phytases are routinely used in poultry feeds as a means of combating the anti-nutritional effects of phytate, improving dietary P availability and reducing P excretion in manure. It has been well documented that dietary additions of microbial phytase to poultry diets allows lower inclusion of inorganic P to be used in diet formulations. The new generation of improved phytases may have the potential to release sufficient phosphorus from phytate to provide for the needs of the bird, reducing further, or even eliminating, the need for supplemental inorganic P. There is however a lot of variability in improvements in response to phytase due to factors such as substrate concentration, phytase level and the intrinsic properties and source of the phytase, as well as formation of Ca-phytate complexes that are not susceptible to phytase degradation (Amerah et al., 2014). This has resulted in a lack of consistency among studies with regards to the extent to which phytase could replace inorganic P supplementation.

The safety margins for P requirements in modern broiler diets have reduced (Świątkiewicz et al., 2014) which means there is little margin in diets for the effect of varying P and phytate-P content of feed ingredients, diet interactions and the poor calculation of digestible P content. As a result, relying on phytase as the sole phosphate source has the potential to under-supplement the essential P to meet the P requirements of the bird. Additionally, P digestibility and phytase inclusion levels do not follow a linear relationship which means that if phytase is not correctly applied and calculated, the phosphate equivalent values can lead to lower P supply than expected (Dilger and Adeola, 2006).

Positive effects of feeding diets containing low inorganic P and phytase have however been observed and studies have been conducted into the possibility of replacing inorganic P with phytase. A number of years ago Broz et al. (1994) stated that inclusion of phytase at a level of 500 U/kg allowed the omission of additional dietary inorganic P in birds fed low P diets. Since this study, new phytases have developed and substantially improved. Catalá-Gregori et al. (2006) found that broilers fed wheat-soybean based diets with very low total P (0.56 and 0.49% for starter and finisher diets respectively) supplemented with 600 U/kg of phytase had similar BW, tibiotarsus mineralization and mineral metabolism compared to those fed diets with much higher total P levels (0.96 and 0.79% for starter and finisher diets respectively) and no supplemental phytase. Also, Rutherfurd et al. (2012) observed that total P retention and phytate-P absorption was significantly higher in birds fed low P diets (0.57%) with 2000 FTU/kg phytase compared to those fed a control diet with higher dietary P content (0.65%), and Mondal et al. (2007) found there was no significant difference between a control diet containing 0.65 % total P and 0.46% available P (**AvP**) and a diet with 0.50% total P and 0.30% AvP and 500 FTU/kg phytase with regards to plasma Ca and P levels and bone ash and P content. If successful, replacing inorganic P supplementation with phytase could have significant positive economic and environmental impacts.

The aim of this study was to quantify the effect of varying phytase concentrations in broiler diets with low inorganic P on performance measures, bone strength and mineral content and ileal mineral digestibility. The objective of this study was to determine if phytase can replace all inorganic P in grower and finisher broiler diets.

MATERIALS AND METHODS

Birds and Husbandry

Male, Ross 308 broilers (n = 288) from a 42-week-old breeder flock were obtained from a commercial hatchery at day of hatch. Chicks were randomized by weight and placed in 0.64 m^2 floor pens in groups of 6, bedded on clean wood shavings. Birds were allowed ad libitum access to the treatment diets and water for the duration of the trial. The room was thermostatically controlled to produce an initial temperature of 32°C on d1 and reduced in steps of 0·5°C per d, reaching 21°C by d21. The lighting regimen used was 24 hours light on d1, with darkness increasing by 1 hour a day until 6 hours of darkness was reached, which was maintained throughout the remainder of the study. All birds sampled were euthanized by cervical dislocation. This occurred at the same time each sampling day; after at least 6 hours of light, to ensure maximal gut fill. Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the University's College of Science ethical review committee.

Dietary Treatments

Diets were formulated in four phases; Starter (d0-10), Grower 1 (d10-21), Grower 2 (d21-28) and Finisher (d28-35), with diet formulations shown for each phase in Tables 1 (Starter and Grower 1) and 2 (Grower2 and Finisher). The control diet (Diet A) had 16.20, 10.90, 9.40 and 6.10g/kg inorganic P in the Starter, Grower 1, Grower 2 and Finisher phase respectively. The remaining 5 diets all had 10.50g/kg inorganic P in the Starter phase. Two of the diets had graded reductions in inorganic P of 5.10, 3.60 and 0.60g/kg (Diet B) or 2.00, 0.50 and 0.60g/kg (Diet C) for the Grower 1, Grower 2 and Finisher phase respectively, plus addition of 500 FTU phytase. Three of the diets had inorganic P levels of 0.40, 0.50 and 0.60g/kg for the Grower 1, Grower 2 and Finisher diets respectively and either 500 FTU (Diet D), 750 (Diet E) or 1000 FTU (Diet F) exogenous phytase. The analyzed total P for each diet in each phase are shown in Tables 1 and 2. The total phytase levels measured were as follows:

Diet A- 415FTU/kg; Diet B-727 FTU/kg; Diet C-821 FTU/kg; Diet D-718 FTU/kg; Diet E-912 FTU/kg and Diet F-1529 FTU/kg.

Diets were fed in mash form, mixed in house, and were analyzed for gross energy by bomb calorimetry (Robbins and Firman, 2006), dry matter, extractable fat and protein content (calculated as nitrogen multiplied by 6.25) by the AOAC standard methods (930.15, 2003.05 and 990.03, respectively). Phosphorus and Ca content of the diets were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP-OES) following an aqua regia digestion step (AOAC 985.01, Leytem et al. 2006). Titanium dioxide was added at a rate of 0.5% to act as an inert marker for evaluation of ileal Ca and P digestibility and the dietary titanium dioxide content quantified by the method of Short et al. (1996). Phytase activity was analyzed in the finisher diets according to the method of Engelen et al. (2001) (ISO 30024). Briefly, diluted diet samples were incubated at 37^{o}C before adding a phytate substrate. A color reagent (molybdate/vanadate/nitric acid) was added to all samples and the samples centrifuged before the optical density was measured at 415nm. Calculated and analyzed values for each diet are shown in Tables 1 and 2.

Response Variables

On arrival birds were individually weighed and allocated to a pen. Pen allocation was randomized across the room. Total pen weight and mean chick body weight (BW) were calculated, and diet allocation was arranged to ensure there was no significant difference in BW by pen across diets. Total pen weight and feed intake (FI) were determined on d 10, 20, 26 and 35 post-hatch and was used to calculate feed conversion ratio (FCR). The pen weight and intake was divided by the number of birds in the pen to determine individual bird BW and FI. Mortality was recorded daily, and any birds culled or dead were weighed. FCR was corrected by mortality.

On d35 two birds per pen were euthanized. Ileum digesta contents from the two birds was collected by gentle digital pressure into one pot pen and stored at -20°C prior to freeze drying. Once freeze dried the samples were finely ground with a pestle and mortar. The ground digesta samples were analyzed for titanium dioxide content by the method of Short et al. (1996). Digesta was also analyzed for Ca and P content using ICP-OES after aqua regia digestion. Apparent ileal Ca and P digestibility coefficients were obtained using the following equation: $[(\text{nutrient}/TiO_2\ (\text{g/kg DM}))_{\text{diet}} - (\text{nutrient}/TiO_2\ (\text{g/kg DM}))_{\text{ileum digesta}}]/(\text{nutrient}/TiO_2\ (\text{g/kg DM}))_{\text{diet}}$

Tibias were removed between the tibial-tarsal joint and the tibial-femoral joint. Bone strength of both the tibia and femur was analyzed using a TA.XT plus texture analyzer (Stable Microsystems, Guildford, UK) set up with a 50kg load cell and 3 point-bend fixture (Shaw et al., 2010). Firstly, the bones were defleshed of muscle and tissue by hand using a scalpel. The texture analyzer was set to measure force in compression; test speed was set at 1mm/sec, and trigger force was set at 7g (0.069N). The defleshed bone was placed on the fixtures, a test was run and the peak force in Newtons was recorded. Bone strength per kilogram bird bodyweight was also calculated.

The tibias were then autoclaved at 121^{o}C for 15 minutes and any remaining flesh and cartilage caps removed carefully by hand. All remaining fat was then removed by extracting the bones with petroleum ether for 4 hours using a Soxhlet apparatus, followed by drying at 105^{o}C until constant weight. The bones were then ashed at 650^{o}C for 13 hours and percentage ash was calculated by the ash weight divided by the dried bone weight. The bone ash for each tibia was then digested with aqua regia and analyzed for Ca and P content by ICP-OES.

Data Analysis

All data were analyzed using JMP (v.10.0). After Kolmogorov–Smirnov testing to confirm normality, statistical analysis was carried out using either one way ANOVA or Krustal

Wallis independent sample tests to compare the performance parameters, bone measures and ileal mineral digestibility of the dietary treatments. Bonferroni post hoc tests were used where appropriate to elucidate differences between sources. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Interestingly, there were no significant difference between the control diet (Diet A) and diet containing 1000 FTU phytase and low inorganic P in the grower 1, grower 2 or finisher diet (Diet F) based on bird performance (Table 3), tibia strength (Table 4) and Ca and P digestibility (Table 5). This suggests that at 1000 FTU/kg the enzyme was present at a sufficiently high concentration to nearly complete phosphate hydrolysis of each phytate molecule thereby producing enough inorganic P moieties (Selle and Ravindran, 2007) to meet the needs of the bird throughout the post-starter growth phases without use of dietary inorganic P. There was also no significant difference between the control diet (Diet A) and diet contatining 500 FTU phytase and low inorganic P in just the finisher diets (Diet B) for bird performance (Table 3), bone strength and ash content (Table 4) and Ca and P digestibility (Table 5). This is in agreement with previous studies that show low levels of P can be fed during the finisher stage without having any detrimental effects on bird performance (Driver et al., 2006) and bone parameters (Skinner et al., 1992). This finding suggests a phytase dose of 500 FTU/kg feed is sufficient enough to provide adequate P for birds in the finisher stage, potentially meaning the use of excessive quantities of P in finisher diets to provide safety margin could be minimized, having significant economic and environmental implications (Dhandu and Angel, 2003). However, this study uses mash diets resulting in poorer overall bird performance compared to industry standards for pellet-fed birds, so a direct econmoic comparision is difficult from this data.

Birds fed the control diet (Diet A) had signficantly higher BWG (Table 3), bone strength and ash content (Table 4) compared to birds fed the diets with 500 FTU phytase and either low inorganic P in the grower 2 and finisher stage (Diet C) or in the grower 1, grower 2 and finisher stage (Diet D). This observed effect on birds fed Diet C and D is likely to be because these birds were fed the P deficient diets for a longer duration and supports the earlier findings of Zyla et al. (2001). This suggests that although 500 FTU phytase is able to provide sufficient P in finisher diets it does release enough P to meet the demands of birds in the grower phase. A possible explanation is that at 500 FTU the phytate was only partially degraded so there was incomplete dephosphorylation of the inositol ring so only partial release of phosphates with some residual myoinositol esters (Zeller et al., 2015). The amount of phosphates released was unable to compensate for the reduction of approximately 0.2% total P in these diets, suggesting a higher dose was required to provide adequate inorganic phosphorus, in keeping with the conclusion of Walk et al. (2013) that supplying phytases at levels in excess of 500FTU illicits further degradation of myoinositol esters. In addition, the lower total P in the starter phase of birds fed these diets compared to those fed the control diet, may have led these birds to have lower P reserves. This reduction in P was not sufficiently compensated for in the later phases in birds fed the diets with 500 FTU, hence the observed P deficiency and reduced performance and bone health. Also, increased production of inert Ca-phytate complexes, particularly due to non-parallel release of Ca and P from phytate, may have promoted free phosphate or phytate precipitation of Ca (Maenz et al., 1999). It may be that in birds fed the 500 FTU phytase dose more Ca may have been released than P, but when the higher doses of phytase were fed more P than Ca may have been released, as there was near complete phytate destruction. This may have resulted in a balanced digestible Ca to P ratio, as the amount of P released from phytate was then balanced with the amount of Ca available for absorption (Angel et al., 2002). This may partly explain the observed reduced bone strength and ash content and significantly lower

213 Ca digestibility in birds fed the diets with 500 FTU phytase and low inorganic P in the finisher
214 stage (Diet B) compared to those fed the diets with 750 and 1000 FTU phytase (Diet E and F
215 respectively). Feed intake was lower in birds fed the diet with 500 FTU phytase compared to
216 control and low inorganic P in the grower 1 and 2 and the finisher stage (Diet D) possibly
217 because the dietary P level was lower; as illustrated by Perney et al. (1993) and Persia and
218 Saylor (2006), increasing available dietary P level increases feed intake.

219 BWG (Table 3) and tibia strength and ash (Table 4) were significantly lower in birds
220 fed the diets with low inorganic P in the grower 1, grower 2 and finisher diets with 750 FTU
221 phytase (Diet E) compared to the control diet (Diet A). Phytases tend to have a 1:1 relationship
222 between Ca and P (Qain et al., 1997) but it may be that when 500 or 750 FTU phytase was
223 supplemented the Ca to P ratio was closer to 2:1 than 1:1. The mechanism of continuing
224 improvement in P digestibility with inclusion of high doses of phytase, as illustrated primarily
225 by the diets containing 1000 FTU phytase, may be because the phytate can be degraded at a
226 faster rate or to a greater extent, the phytase is able to find the phytate substrate more quickly
227 or because the active phytase continues working in the small intestine after leaving the gizzard
228 (Kies et al., 2006; Zeng et al., 2014). Further investigation is needed to determine the impact
229 of feeding diets with even higher doses of phytase and low inorganic P in birds from d10 and
230 older.

231 The impact of the reduction in inorganic P content appears to be greatest in the grower
232 2 phase, as shown by significant reductions in tibia stength and ash (Table 4) and numerical
233 reduction in BWG (Table 3) observed between birds fed diets with 500 FTU phytase and just
234 low inorganic P in the finisher stage (Diet B) compared to low inorganic P in the grower 2 and
235 finisher stage (Diet C). This is illustrated by Skinner et al. (1992) in which it was found that
236 feeding grower diets containing 0.25% P from d21 to finisher caused increased incidences of
237 blood-splashed breast meat due to bone failure.

It can be concluded that relying on phytase alone to ensure full supply of P in broiler diets is viable in finisher diets but may not currently be recommended in grower diets unless phytase is supplied in high doses (1000 FTU/kg feed or greater). Findings from this study suggest that the use of high phytase doses to replace inorganic P supplementation has the potential to reduce feed costs. Further investigation is required into the impact of replacing inorganic P with phytase doses greater than 1000 FTU/kg feed and in pelletted diets.

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318 **Table 1.** Analysed and calculated composition and nutrient content of Starter and Grower 1

319 experimental diets (% inclusion of raw materials)

Ingredients	Starter diets				Early Grower Diets			
	Diet A	Diet B	Diet C	Diets D/E/F	Diet A	Diet B	Diet C	Diets D/E/F
Maize	545	545	545	545	567	567	567	567
Rapeseed meal	40	40	40	40	30	30	30	30
Full fat soya	100	100	100	100	100	100	100	100
Soybean meal[1]	240	240	240	240	230	230	230	230
Soybean oil	30	30	30	30	36	36	36	36
DL-methionine	2	2	2	2	1.9	1.9	1.9	1.9
L-Lysine HCl	1	1	1	1	1.2	1.2	1.2	1.2
L-Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	16	12	12	12	13.0	9.7	11.2	12
Monocalcium Phosphate	16	11	11	11	10.9	5.1	2.0	0
Salt	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	2.5	3.0	3.0	3.0	2.0	2.0	2.5	3.0
Vit/Min Premix[2]	5	5	5	5	5	5	5	5
Sand	0	9	9	9	0	9.1	10.7	11.9
Phytase[3]	0	500	500	500 /750 /1000	0	500	500	500 /750 /1000
Nutrient specification Calculated values (with measured values in parentheses)								
Crude protein	21.1 (21.1)	21.1 (21.9)	21.1 (22.4)	21.1 (22.3)	20.5 (21.4)	20.5 (21.4)	20.5 (21.6)	20.5 (21.9)
ME kcal/kg	3092	3092	3092	3092	3179	3179	3179	3179
Calcium (%)	1.04 (1.04)	0.81 (0.83)	0.81 (0.87)	0.81 (0.89)	0.84 (0.91)	0.62 (0.88)	0.62 (0.73)	0.63 (0.71)
Total phosphorus (%)	0.76 (0.57)	0.65 (0.42)	0.65 (0.41)	0.65 (0.47)	0.63 (0.57)	0.5 (0.50)	0.43 (0.33)	0.40 (0.31)
Available Phosphorus	0.49	0.38	0.38	0.38	0.37	0.24	0.17	0.13
Phytate Phosphorus	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26

320 [1]48% crude protein

321 [2]Premix content (volume/kg diet): Mn 100mg, Zn 88mg, Fe 20mg, Cu 10mg, I mg,
322 Mb 0.48mg, Se 0.2mg, Retinol 13.5mg, Cholecalciferol 3mg, Tocopherol 25mg,
323 Menadione 5.0mg, Thiamine 3mg, Riboflavin 10.0mg, Pantothenic acid 15mg,
324 Pyroxidine 3.0mg, Niacin 60mg, Cobalamin 30µg, Folic acid 1.5mg, Biotin 125mg.

325 [3]Phyzyme XP(FTU /kg phytase)

Table 2. Analysed and calculated composition and nutrient content of Grower 2 and Finisher experimental diets (% inclusion of raw materials)

Ingredients	Late Grower Diets				Finisher Diets			
	Diet A	Diet B	Diet C	Diets D/E/F	Diet A	Diet B	Diet C	Diets D/E/F
Maize	577	577	577	577	591	591	591	591
Rapeseed meal	25	25	25	25	20	20	20	20
Full fat soya	100	100	100	100	100	100	100	100
Soybean meal[1]	225	225	225	225	220	220	220	220
Soybean oil	39	39	39	39	39	39	39	39
DL-methionine	2	2	2	2	1.9	1.9	1.9	1.9
L-Lysine HCl	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
L-Threonine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Limestone	12.4	9.2	10.7	10.7	11.3	8	8	8
Monocalcium Phosphate	9.4	3.6	0	0	6.1	0	0	0
Salt	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Sodium bicarbonate	2.2	2.2	2.2	2.2	2	2	2	2
Vit/Min Premix[2]	5	5	5	5	5	5	5	5
Sand	0	8.9	11.1	11.1	0	9.3	9.3	9.3
Phytase[3]	0	500	500	500 /750 /1000	0	500	500	500 /750 /1000
Nutrient specification Calculated values (with measured values in parentheses)								
Crude protein	20.2 (21.3)	20.2 (21.1)	20.2 (21.8)	20.2 (21.9)	19.9 (21.0)	19.9 (21.1)	19.9 (21.6)	19.9 (21.3)
ME kcal/kg	3212	3212	3212	3212	3120	3120	3120	3120
Calcium (%)	0.78 (0.90)	0.57 (0.92)	0.58 (0.88)	0.58 (0.89)	0.68 (0.73)	0.47 (0.71)	0.47 (0.64)	0.47 (0.65)
Total phosphorus (%)	0.59 (0.41)	0.46 (0.49)	0.39 (0.45)	0.39 (0.34)	0.52 (0.50)	0.39 (0.37)	0.39 (0.33)	0.39 (0.30)
Available Phosphorus	0.33	0.2	0.13	0.13	0.26	0.13	0.13	0.13
Phytate Phosphorus	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

[1]48% crude protein

[2]Premix content (volume/kg diet): Mn 100mg, Zn 88mg, Fe 20mg, Cu 10mg, I mg, Mb 0.48mg, Se 0.2mg, Retinol 13.5mg, Cholecalciferol 3mg, Tocopherol 25mg, Menadione 5.0mg, Thiamine 3mg, Riboflavin 10.0mg, Pantothenic acid 15mg, Pyroxidine 3.0mg, Niacin 60mg, Cobalamin 30µg, Folic acid 1.5mg, Biotin 125mg.

[3]Phyzyme XP(FTU /kg phytase)

334 **Table 3.** Influence of diet on individual bird Bodyweight Gain (BWG) of broilers by phase
335 and overall bird performance (Feed intake, FI and Feed conversion ratio, FCR)from d0-35

Diet	BWG d0-10, g	BWG d10-20, g	BWG d20-26, g	BWG d26-35, g	BWG d0-35, g	FI d0-35, g	FCR d0-35
A	201	534[a]	465[a]	755	1754[a]	2859[a]	1.63
B	213	510[ab]	460[a]	734	1705[ab]	2842[a]	1.67
C	207	465[bc]	410[b]	729	1605[b]	2751[ab]	1.72
D	202	452[c]	407[b]	727	1587[b]	2584[b]	1.63
E	196	464[bc]	411[b]	709	1584[b]	2670[ab]	1.69
F	216	499[abc]	466[ab]	764	1708[ab]	2760[ab]	1.62
SEM	5.3	10.4	10.7	19.9	27.51	38.8	0.01
P-value	0.12	<0.001	<0.001	0.479	0.001	0.001	0.185

336 [a–b] Means within the same column with no common superscript differ
337 significantly ($P \leq 0.05$). 2-way ANOVA and Bonferroni Post-Hoc test were
338 used to differentiate between means.

Table 4. Influence of diet on tibia strength, ash and Ca and P content at d35

Diet	Strength, N	Ash, %	Ca, % ash	P, % ash
A	331.08[a]	47.48[a]	34.50	11.79
B	324.26[a]	46.94[ab]	35.60	12.20
C	231.02[bc]	42.58[c]	34.99	11.98
D	192.16[c]	41.71[c]	36.02	12.33
E	232.42[bc]	42.43[c]	35.03	12.03
F	272.49[ab]	44.43[bc]	34.57	11.91
SEM	20.72	0.92	0.22	0.07
P-value	<0.001	<0.001	0.796	0.419

[a–c] Means within the same column with no common superscript differ significantly ($P \leq 0.05$). 2-way ANOVA and Bonferroni Post-Hoc test were used to differentiate between means.

343 **Table 5.** Influence of diet on apparent ileal Ca and P digestibility[1] in broilers from d 0 to 35

344

Diet	P	Ca
A	0.60[ab]	0.43[ab]
B	0.54[b]	0.34[b]
C	0.57[b]	0.39[ab]
D	0.56[b]	0.53[ab]
E	0.70[a]	0.55[a]
F	0.69[a]	0.46[a]
SEM	0.03	0.03
P-value	<0.001	0.003

345 [a–c] Means within the same column and same row with no common superscript differ
346 significantly ($P \leq 0.05$). 2-way ANOVA and Duncan Post-Hoc test were used to differentiate
347 between means.
348 [1]Digestibility coefficients obtained using the equation:
349 $[(\text{nutrient}/TiO_2)_{diet} - (\text{nutrient}/TiO_2)_{ileum}]/(\text{nutrient}/TiO_2)_{diet}$.