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The interplay of dietary nutrient specification and varying calcium to total phosphorus ratio on efficacy of a bacterial phytase: 1. Growth performance and tibia mineralization

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4 The interplay of dietary nutrient level and varying Ca to phosphorus ratio on efficacy of a bacterial
5 phytase: 1. growth performance and tibia mineralization

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7 Abbreviated title: DIET FACTORS AND PHYTASE EFFECT ON GROWTH

8

9 **ABSTRACT** A 14-d experiment was conducted to study the effects of two dietary variables
10 on efficacy of a 6-phytase from *Citrobacter braakii* on broiler growth performance and tibia
11 mineralization. Diets were formulated with or without nutrient matrix values for phytase as
12 negative or positive control (NC or PC, respectively) and with two Ca:total P, tP (2:1 or
13 2.5:1). The diets were supplemented with 0, 1,000 or 2,000 FYT/kg of phytase thus
14 producing a 2×2×3 factorial arrangement. Birds and feed were weighed on days 7 and 21 and
15 tibia bones were collected from all the birds on day 21. The main effects of nutrient matrix,
16 Ca:tP and phytase supplementation were significant ($P < 0.05$) for all the growth performance
17 responses (except for gain:feed for which there was no effect of matrix). Ca:tP × phytase and
18 matrix × phytase interactions were significant ($P < 0.05$) for weight gain. In the PC diets,
19 phytase increased weight gain ($P < 0.05$) relative to the control only in diets with 2,000
20 FYT/kg phytase, whereas in NC diets weight gain increased ($P < 0.01$) only from 0 to 1,000
21 FYT/kg phytase levels. Broilers consuming diets with 2.5:1 Ca:tP had lower ($P < 0.05$) tibia
22 ash whereas phytase increased ($P < 0.01$) tibia ash, Ca, P, and Zn but decreased ($P < 0.01$)
23 tibia K. Phytase supplementation of diets with 2:1 Ca:tP increased ($P < 0.05$) tibia P in birds
24 receiving 1,000 FYT/kg relative to the control with no further increase at 2,000 FYT/kg
25 whereas each level of phytase supplementation increased ($P < 0.05$) tibia P in the diets with
26 2.5:1 Ca:tP. It was concluded that the best response to lower phytase supplementation (1,000
27 FYT/kg) was in NC diets with narrow Ca:tP whereas the best response to higher level of
28 phytase supplementation (2,000 FYT/kg) was achieved in diets in PC diets with wide Ca:tP.

29 Key words: broiler, calcium:phosphorus, growth, phytase matrix,

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INTRODUCTION

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The beneficial effects of phytase in diets marginally deficient in P and Ca and some amino acids have been widely reported in the literature (Olukosi et al., 2007; Selle and Ravindran, 2007; Adeola and Cowieson, 2011). Although phytase is supplemented primarily to liberate P from phytate and hence is a necessary additive in diets that are deficient in P and Ca, phytase have been beneficial as well in diets that are formulated to be adequate in P (Watson et al., 2006; Adeola and Cowieson, 2011). In addition, phytase supplementation at levels higher than 1,000 FTU/kg has been shown to be beneficial to birds in terms of growth performance response (Shirley and Edwards, 2003; Cowieson et al., 2006).

The negative effect of wide Ca:P on phytase efficacy is well known (Tamim et al., 2004; Adeola et al., 2006) and this is related to the formation of recalcitrant Ca-phytate (Taylor, 1965; Nelson and Kirby, 1987) or Ca-phosphate complexes (Long et al., 1984). Phytase is usually supplemented to diets that are deficient in P and hence most of the investigations of the effects of Ca: total P (**tP**) on phytase efficacy are done in P-deficient diets. Because there is increasing interest in phytase supplementation of nutrient-adequate diets (Walk et al., 2013), it is also vital to investigate the effect of widening Ca:tP on phytase efficacy in such diets.

Therefore the objective of the current experiment was to investigate how the use of a nutrient matrix values for a phytase, from *Citrobacter braakii*, affects the efficacy of the phytase on growth performance and tibia bone mineralization and especially within the context of variable dietary Ca:tP. The companion article considers how these dietary factors influence nutrient utilization in broilers.

54

MATERIALS AND METHODS

55 All the animal experimentation procedures used in the current study were approved by
56 the Scotland's Rural College's Animal Experimentation Committee.

57 *Diets and experimental design*

58 A total of 576 day-old birds were used for the 14-d experiment to study the influence
59 of nutrient specifications (use of nutrient matrix values for phytase) and Ca:tP on efficacy of
60 phytase on growth performance and tibia mineralization. The birds were brooded together in
61 a floor pen for the first 7 days during which they received a standard diet that meets NRC
62 (1994) nutrient requirement for broilers. On day 7, the birds were weighed and allocated to
63 12 dietary treatments in a randomized complete block design and a 2×2×3 factorial
64 arrangement of treatments. Each treatment had 8 replicate cages and 6 birds per replicate
65 cage. The factors were two levels of nutrient specifications (explained below), two levels of
66 Ca:tP (narrow, 2.1 and wide, 2.5:1) and three levels of phytase supplementation (0, 1,000
67 and 2,000 FYT/kg). Birds and feed were weighed on day 21 after which the birds were
68 euthanized and the left tibia were collected from each of the birds.

69 The composition of the experimental diets is presented in Table 1. Nutrient
70 specification was used to define the diets that were formulated to meet all the nutrient
71 requirements for broilers (full nutrient specification without nutrient matrix phytase or
72 positive control, **PC**) and another set of diets with reduced nutrient specification formulated
73 to be deficient in P, Ca, CP, amino acids, and energy (down specification or negative control,
74 **NC**). The nutrients and energy levels in the NC diets were reduced relative to the PC diets on
75 the basis of the amount of nutrients and energy that the phytase was expected to release
76 (nutrient matrix values for phytase). The matrix values used per kg feed for 1,000 FYT were
77 approximately, 75 kcal ME, 1.5 g for available P, 1.8 g for Ca, 0.26 g for crude protein, 0.11,

78 0.07, 0.04, and 0.07 g for digestible Lys, total sulphur amino acids, Met, and Thr,
79 respectively. One phytase (**FYT**) unit is defined as the activity that releases 1 μmol inorganic
80 phosphate from 5.0 mM phytate per minute at pH 5.5 and 37°C.

81 *Analyses*

82 **Chemical analysis.** The tibia bones were first extracted with ethyl ether prior to being
83 ashed in a muffle furnace and the ash analyzed for minerals. Bones were ashed for 24 hours
84 at 600°C in a muffle furnace (Method 942.05; AOAC, 2006). Diets were analyzed for dry
85 matter, N, gross energy, and minerals. Dry matter was determined by drying the samples in a
86 drying oven (Uniterm, Russel-Lindsey Engineering Ltd., Birmingham, England, UK) at 100°C
87 for 24 hours (Method 934.01; AOAC, 2006). Total N content was determined by the
88 combustion method (Method 968.06; AOAC, 2006). Gross energy value was determined in
89 an adiabatic oxygen bomb calorimeter (Model 6200, Parr Instruments, Moline, IL) using
90 benzoic acid as standard. Mineral content was determined using Inductively Coupled Plasma
91 – Optical Emission Spectroscopy (Method 990.08; AOAC, 2006) following digestion, in
92 turn, in concentrated HNO_3 and HCl.

93 **Statistical analysis.** The data on growth performance and tibia bone mineralization
94 were analyzed using the GLM procedure of SAS as appropriate for a factorial arrangement of
95 treatments. When interactions were not significant, the interaction term was removed and the
96 data re-analyzed to examine the main effects. Multiple regression analyses were performed
97 using REG procedure of SAS to examine the effects of Ca:tP or nutrient matrix on response
98 of birds to phytase supplementation. In all cases, significance was declared at $P \leq 0.05$.

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RESULTS

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101 The analyzed compositions of experimental diets are presented in Table 2. Overall the
102 nutrient, energy and phytase contents were within target except in diet 3 where the phytase
103 recovery was at 2,977 instead of the expected 2,000 FYT/kg. The levels of Ca were higher
104 than expected but the levels of total and phytate P were within the expected levels. The
105 effects of the dietary treatments on growth responses are shown in Table 3. The main effects
106 of nutrient matrix (i.e. NC vs. PC), Ca:tP and phytase supplementation were significant ($P <$
107 0.05) for all the growth performance responses except for gain:feed for which there were no
108 significant matrix and Ca:tP effects. Weight gain and feed intake were lower ($P < 0.05$) in
109 NC compared with PC diets as well as in the diets with wide Ca:tP compared with the diets
110 with narrow Ca:tP. There was no matrix \times Ca:tP interaction for any of the growth
111 performance responses; matrix \times phytase and Ca:tP \times phytase interactions were only
112 significant ($P < 0.05$) for weight gain. In PC diets, phytase increased weight gain relative to
113 the control only at 2,000 FYT/kg whereas in NC diets weight gain increased ($P < 0.01$)
114 dramatically in diets with 1,000 FYT/kg compared to the control and only marginally
115 increased (numerically only) further with 2,000 FYT/kg. In addition, in the diets with 2:1
116 Ca:tP, phytase supplementation at 1,000 FYT/kg increased weight gain ($P < 0.05$) relative to
117 the control but no further increase was observed at 2,000 FYT/kg whereas phytase
118 supplementation produced a stepwise increase ($P < 0.05$) in weight gain in the diets with
119 2.5:1 Ca:tP.

120 The tibia bone mineralization responses to the dietary treatments are shown in Table
121 4. Tibia ash, Ca and P were lower ($P < 0.01$) in NC relative to PC diets. Birds consuming
122 diets with 2.5:1 Ca:tP had lower tibia ash and Mg whereas phytase supplementation increased
123 ($P < 0.01$) tibia ash, Ca and Mg. The two-way interactions were largely not significant for
124 most of the tibia mineralization responses except for Ca:tP \times phytase effect ($P < 0.05$) for

125 tibia P and matrix \times phytase interaction ($P < 0.05$) for tibia K and Zn. Phytase
126 supplementation of diets with 2:1 Ca:tP increased ($P < 0.05$) tibia P in diets with 1,000
127 FYT/kg relative to the control with no further response at 2,000 FYT/kg whereas each level
128 of phytase supplementation increased ($P < 0.05$) tibia P in the diets with 2.5:1 Ca:tP. In
129 addition, phytase supplementation had no effect on tibia K in the PC diets but tibia K was
130 lower ($P < 0.05$) in NC diets supplemented with phytase.

131

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DISCUSSION

133 The objective of the current study was to investigate the interactivity of Ca:tP and
134 dietary nutrient content or matrix (PC vs. NC) on phytase supplementation effects on growth
135 performance and tibia mineralization. In view of the importance of P for bone development,
136 and ultimately, bird growth, the diets were formulated to be either limiting or sufficient in P
137 and hence the birds were expected to respond rapidly to phytase supplementation.

138 As expected growth performance responses were greater in the un-supplemented PC
139 compared with the un-supplemented NC and this can be directly related to nutrient content of
140 the diets. The PC diets were formulated to meet nutritional requirements of the birds whereas
141 the NC diets were not. Response to dietary manipulation ultimately depends on the supply of
142 the nutrients limiting for growth (Olukosi et al., 2007). In the PC diets, the limiting nutrient
143 (i.e. P in this experiment) was supplied in the diets as inorganic P whereas in the NC diets, P
144 was expected to be released from hydrolysis of phytic acid by the supplemented phytase.
145 Consequently when phytase improves growth performance of birds in diets not limiting in P,
146 this effect can only be attributed to other factors apart from P (Cabahug et al., 1999; Walk et
147 al., 2013). Such factors could be the release of other nutrients or minerals that may otherwise

148 have been deficient (Keshavarz, 2000) or by means of modified digesta viscosity (Watson et
149 al., 2006)

150 The improvement in weight gain was 68% greater in NC diets containing 2,000
151 FYT/kg phytase compared with the same level of phytase supplementation in PC diets.
152 Phytase at 1,000 FYT/kg was sufficient to completely reverse the negative effects on weight
153 gain produced by the low level of non-phytate P in the NC diets and produced weight gain
154 that was numerically greater than that observed in PC diet without phytase. Supplementation
155 of additional 1,000 FYT/kg phytase produced only marginal improvement in weight gain in
156 the NC diet and hence in the present study 1,000 FYT/kg was sufficient to produce optimum
157 weight gain response in the NC diet.

158 Unlike weight gain, the increase in bone mineralization in response to phytase
159 supplementation was independent of nutrient matrix. The interaction of nutrient matrix (i.e.
160 PC or NC) and phytase supplemental level on weight gain can therefore be disassociated
161 from the effect of these factors on bone mineralization. The rates of weight gain and bone
162 development in birds are directly related to some extent (Leterrier and Nys, 1992; Williams et
163 al., 2004) and several studies with phytase have simultaneously investigated its effects on
164 weight gain and bone mineralization (Onyango et al., 2004; Olukosi et al., 2013). However as
165 shown in the current study, weight gain and bone mineralization do not respond the same way
166 to dietary manipulations likely because bone mineralization depends to a greater extent on P
167 and Ca supply than weight gain. This likely account for why phosphorus equivalency is
168 different when calculated using weight gain or bone ash (Yi et al., 1996; Adedokun et al.,
169 2004)

170 Three inferences are possible from the observation of the effects of phytase on weight
171 gain in PC and NC diets. First, because the weight gain in PC diet without phytase was 17%

172 greater than the weight gain in NC diet without phytase, the PC diet provided “less room” or
173 “opportunity” for phytase effect on weight gain. In a situation where P is presumably no
174 longer the limiting nutrient for growth, it would require higher levels of phytase
175 supplementation to produce an effect. This may be one of the reasons why Walk et al. (2013)
176 did not observe a weight gain response to phytase supplementation of the P-adequate diet in
177 their study. Secondly, because the PC diet was formulated to meet all nutrients requirement
178 and the NC diets were formulated to be limiting in P, the improvement in growth
179 performance observed at 2,000 FYT/kg in the PC diet could be due to other beneficial effects
180 of phytase beyond P release (Cabahug et al., 1999; Walk et al., 2013).

181 The third inference relates to the magnitude of response to phytase supplementation
182 produced by the additional 1,000 FYT/kg phytase in PC and NC diets (i.e. the 2,000 FYT/kg
183 diets). Beyond the 1,000 FYT/kg phytase level, every additional 100 FYT/kg produced 6.5 g
184 of weight gain in PC diet but only 2 g of weight gain in the NC diet. Because the PC diet met
185 all the nutrient requirements and NC did not, and also because weight gain produced by 1,000
186 FYT/kg phytase was the same for both NC and PC, the comparatively lower weight gain
187 response to phytase supplementation in the NC diet beyond 1,000 FYT is possibly an
188 indication that higher rate of phytase supplementation to NC diet may be hampered by factors
189 not yet understood. For example, it could offset Ca:P ratio or produce other nutrient
190 imbalances which may negatively influence performance. In contrast this effect was not
191 observed in PC diet, in which case it is possible that the totality of the available nutrient
192 levels in the diets and nutrients released by phytase were in performance-conforming balance.
193 In fact, the ratio of ileal digestible Ca to digestible tP was consistently wider in NC compared
194 with PC diets irrespective of phytase supplementation (reporting in companion article).

195 The impact of Ca:P on phytase efficacy using growth performance is well known
196 (Qian et al., 1997; Delezie et al., 2012). As shown in the companion article, the negative

197 effect of increasing Ca:tP was more severe in the NC diets. However, there were no
198 interaction between nutrient matrix and Ca:tP on growth and bone mineralization responses
199 in the current study. On the other hand, efficacy of phytase in improving body weight gain
200 and tibia mineralization (tibia content of P and Ca) depended on Ca:tP. In the diets with
201 narrow Ca:tP, phytase efficacy was only apparent between the 0 and 1,000 FYT/kg. On the
202 other hand, in the diets with wider Ca:tP there was a step-wise increase in weight gain. The
203 improvement in weight gain produced by 2,000 FYT/kg phytase (relative to 0 FYT/kg) in the
204 diets with wide Ca:tP was 250% of that produced in the diet with narrow Ca:tP. There was no
205 difference in weight gain at 2,000 FYT/kg phytase level in the diets with wide or narrow
206 Ca:tP but in the diets without phytase, weight gain in the diets with narrow Ca:tP was 14%
207 greater than the weight gain in diets with wide Ca:tP. Consequently, phytase effect was
208 muted in the diets with narrow Ca:tP than in the diets with wide Ca:tP. This is presumably
209 because the diets with narrow Ca:tP had more readily available P (the limiting mineral for
210 growth in the current study) and hence the phytase effect was less apparent in these diets.

211 Further calculations of Ca and P intake as well as retained Ca and P in relation to
212 dietary Ca:tP can help explain some of the effects of Ca:tP observed on growth performance.
213 The ratios of intakes of Ca and P followed dietary Ca to tP ratios. However, the ratio of
214 retained Ca:tP (1.4:1 for diets with Ca:tP of 2:1 and 1.1:1 for diets with 2.5:1, respectively)
215 was narrower and reversed compared with the dietary and intake ratios of Ca:tP. These
216 reversals were because the retained Ca and P were greater for diets with narrow Ca:tP which
217 is likely an indication of greater availability of these minerals (possibly due to less Ca-
218 phosphate complex formation) in the diets with narrow Ca:tP.

219 When the observations of the weight gain responses to low and high phytase
220 supplemental levels are taken together, two conclusions are apparent. One conclusion is that
221 1,000 FYT/kg is sufficient to offset the initial depressing effect on weight gain of the low

222 dietary non-phytate P in the current study. The second conclusion is that higher level of
223 phytase supplementation is needed in the diets containing wide Ca:tP to more fully reverse
224 the negative impact of wide Ca:tP. Because the diets with wide Ca:tP had higher
225 concentration of Ca relative to P, it can be reasoned that the use of phytase up to 1,000
226 FYT/kg released more P and hence possibly helped to offset the negative consequence of the
227 imbalance in Ca:tP. Phytase supplementation is also expected to increase P release in diets
228 with narrow Ca:tP, but in this case because the Ca:tP ratio has always been more favorable,
229 increased P release may not produce a very noticeable effect. Or it could be that increased P
230 concentration relative to Ca, increases the chances for formation of calcium-phosphate (Long
231 et al., 1984) thus reducing both the absorption of Ca and weight gain (Masuyama et al.,
232 2003).

233 Phytase is routinely added at the rates of between 500 and 1,000 phytase units per
234 kilogram however the use of higher levels of phytase supplementation has been reported to
235 provide greater response than observed at the lower supplementation level and some of these
236 positive effects are not necessarily due to increased P release (Shirley and Edwards, 2003;
237 Cowieson et al., 2006; Cowieson et al., 2011). The current study shows that phytase
238 supplementation up to 2,000 FYT/kg is more advantageous in the PC diets and in the diets
239 with wide Ca:tP. The observation that the high phytase level was not more effective than
240 1,000 FYT/kg in the NC, in contrast to PC, diets is an indication that the additional benefit
241 observed beyond 1,000 FYT/kg level was not due to increased P supply as also suggested by
242 Walk et al. (2013). It is reasonable to assume that there is a limit to phosphate uptake from
243 the lumen of birds and hence greater P availability will not necessarily produce greater
244 performance response once the requirement for P is met (Yan et al., 2003). The observation
245 therefore that high phytase supplemental level was more efficacious in diets already meeting
246 P requirement is a demonstration of the extra-phosphoric effect of phytase. On the other

247 hand, in the diets with narrow Ca:tP it might be necessary to understand the nutrient
248 imbalances that could results from the use of phytase to optimize growth.

249 Taken together, the data from the current study showed that the best response to
250 lower phytase supplementation is in diets with narrow Ca:tP combined with reduced nutrient
251 specification (NC) whereas the best response to high level of phytase supplementation is
252 achieved in diets with full nutrient specification (PC) and having wider Ca:tP.

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325

326 **Table 1.** Ingredient composition of the experimental basal diets

Basal diets	1	2	3	4
Ca:tP	2:1		2.5:1	
Control (phytase matrix)	Positive	Negative	Positive	Negative
Corn	482.6	477.4	466.6	499.4
Wheat	-	50.0	-	-
Soybean meal	397.5	382.5	400.5	394.5
Soybean oil	58.0	40.0	60.0	45.0
Corn starch	15.0	15.0	15.0	15.0
Dicalcium phosphate	17.5	9.0	17.5	10.0
Limestone	17.0	15.5	28.0	24.0
Titanium dioxide	0.5	0.5	0.5	0.5
L-Lysine·HCl	1.0	0.4	1.0	0.7
DL-Methionine	2.8	1.9	2.8	2.8
Threonine	0.6	0.3	0.6	0.6
Vitamin-mineral premix ¹	2.5	2.5	2.5	2.5
Salt	5.0	5.0	5.0	5.0
Phytase premix ²	To 1000	To 1000	To 1000	To 1000
Total	1000	1000	1000	1000

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328

329 ¹Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU;
 330 vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic
 331 acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2
 332 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I,
 333 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

334 ²Phytase premix containing 200 phytase units (FYT)/g replaced corn starch to provide
 335 1,000 or 2,000 FYT/kg.

Table 2. Analyzed nutrient composition (% , dry matter basis) and phytase activity in the experimental diets

Diets	1	2	3	4	5	6	7	8	9	10	11	12
Ca:tP	2:1						2.5:1					
Matrix	Positive Control			Negative Control			Positive Control			Negative Control		
GE, kcal/kg	4,452	4,681	4,652	4,672	4,619	4,603	5,720	5,782	4,610	4,641	4,572	4,580
Ether extract	10.2	10.3	8.8	7.11	7.83	6.84	11.4	11.3	8.94	7.67	8.26	7.95
Acid hydrolysed fat	11.1	9.49	9.80	8.03	7.96	8.03	12.1	12.9	9.70	8.30	9.37	8.37
N	3.99	3.86	4.13	3.84	3.90	3.80	5.11	4.70	4.15	4.04	3.73	3.88
Ca	1.37	1.63	1.40	1.31	1.18	1.17	2.23	2.24	1.87	1.06	1.47	1.38
P	0.73	0.81	0.71	0.65	0.61	0.57	0.93	0.91	0.74	0.48	0.60	0.62
Phytate P	0.26	0.24	0.23	0.25	0.23	0.24	0.28	0.28	0.29	0.24	0.23	0.29
Non-phytate P ¹	0.47	0.57	0.48	0.40	0.38	0.33	0.65	0.63	0.45	0.24	0.37	0.33
Na	0.23	0.26	0.24	0.26	0.25	0.23	0.24	0.28	0.23	0.17	0.23	0.19
Mg	0.18	0.18	0.16	0.18	0.18	0.16	0.23	0.22	0.18	0.15	0.18	0.19
Cu, mg/kg	11.3	15.8	14.7	12.5	10.2	10.2	28.2	18.2	12.4	10.2	13.6	15.8
Fe, mg/kg	82.4	87.0	79.2	90.7	78.5	71.3	111.3	106.5	83.3	56.6	81.4	79.0
Mn, mg/kg	81.3	94.9	87.1	100.9	84.2	83.8	107.1	105.1	83.3	61.1	83.7	86.9
Zn, mg/kg	79.1	96	99.5	92.9	84.2	81.5	101.4	99.5	83.3	58.9	75.7	79
K	1.16	1.14	1.00	1.16	1.19	0.96	1.47	1.43	1.14	0.97	1.15	1.28
Phytase, FTY/kg	BD ²	1121	2977	BD	1406	2400	BD	1,717	2,537	BD	1011	2640

¹Non-phytate phosphorus level was determined by subtracting phytate P from total P

²BD = below detection limit

Table 3. Growth performance response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase¹

Ca:tP	2:1						2.5:1						P-values for main effects and interactions						
	Positive Control			Negative Control			Positive Control			Negative Control			Main effects			Interactions ³			
Matrix (M) ²	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	SEM	M	Ca:tP	Ph	1	2	3
WG ⁴ , g	807	836	838	690	786	809	709	744	873	605	799	816	26.1	0.001	0.017	0.001	0.34	0.011	0.013
FI ⁴ , kg	1.07	1.13	1.13	0.99	1.08	1.08	0.98	1.01	1.12	0.90	1.06	1.09	0.003	0.010	0.001	0.001	0.21	0.051	0.051
G:F, g/kg	757	742	743	696	727	747	721	741	781	678	752	751	20.5	0.065	0.87	0.015	0.88	0.20	0.22

¹Means were obtained from 8 replicate cages of 6 birds per replicate cage.

²Matrix = nutrient matrix for phytase, positive control (full nutrient specification) and negative control (reduced nutrient specification).

³Interactions: 1 (matrix × Ca:tP); 2 (matrix × phytase); 3 (Ca:tP × phytase); the three-way interaction was not significant for any of the responses.

⁴WG = weight gain; FI = feed intake.

Table 4. Tibia bone mineralization (%) response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase¹

Ca:tP	2:1						2.5:1						P-values for main effects and interactions						
	Positive Control			Negative Control			Positive Control			Negative Control			Main effects			Interactions ³			
Matrix (M) ²													M	Ca:tP	Ph	1	2	3	
Phytase (Ph)	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	SEM						
Ash	50.1	50.7	52.0	47.8	49.4	51.1	47.6	49.5	50.8	44.9	48.9	50.8	0.78	0.005	0.002	0.001	0.65	0.18	0.16
P	8.11	8.28	8.51	7.55	8.08	8.10	7.51	7.98	8.41	6.91	7.75	8.21	0.16	0.001	0.001	0.001	0.80	0.26	0.024
Ca	17.1	17.5	17.8	16.4	17.1	17.1	16.4	17.0	17.9	15.4	16.6	17.6	0.33	0.003	0.081	0.001	0.80	0.60	0.058
Mg	0.32	0.34	0.34	0.30	0.32	0.34	0.28	0.31	0.33	0.27	0.30	0.31	0.01	0.008	0.001	0.001	0.79	0.88	0.29
K	0.38	0.38	0.37	0.41	0.38	0.38	0.38	0.37	0.38	0.41	0.38	0.37	0.01	0.032	0.98	0.001	0.94	0.035	0.91
Zn ⁴	147	174	185	162	175	191	138	174	191	159	178	186	4.30	0.006	0.58	0.001	0.75	0.007	0.46

¹Means were obtained from 8 replicate cages of 6 birds per replicate cage.

²Matrix = nutrient matrix for phytase, positive control (full nutrient specification) and negative control (reduced nutrient specification).

³Interactions: 1 (matrix × Ca:tP); 2 (matrix × phytase); 3 (Ca:tP × phytase); the three-way interaction was not significant for any of the responses.

^4Zn = unit is mg/kg