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Determination and validation of available phosphorus equivalency of *Escherichia coli*-derived phytase in broiler diets

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Primary Audience: Nutritionists, Broiler Producers

SUMMARY

The objective of this experiment was to determine and validate the available phosphorus (**AP**) equivalency of *Escherichia coli*-derived phytase (**ECP**) in broiler diets. A total of nine hundred sixty 8-d-old broiler chicks were subjected to 8 dietary treatments with 10 replicates. The positive control (**PC**) diet contained recommended concentrations of calcium (**Ca**) and AP, whereas the negative control (**NC**) diet was formulated to contain 0.76% calcium and 0.10% AP. The remaining 6 diets included varying levels of AP from monosodium phosphate (**MSP**) or ECP. Nonlinear regression analysis indicated that AP equivalency values of 500 and 1,000 FTU/kg ECP were 0.986 and 1.589 g/kg, respectively. These values were validated in a subsequent growth trial. A total of two thousand 8-d-old broiler chicks were randomly allotted to 1 of 5 dietary treatments with 8 replicates. Five treatments included negative control diet 1 (**NC1**, highly deficient in AP and Ca), negative control diet 2 (**NC2**, deficient in AP and Ca), phytase diet 1 (**P1**, NC1 + 1,000 FTU/kg ECP), phytase diet 2 (**P2**, NC2 + 500 FTU/kg ECP), and positive control diet (PC, recommended concentrations of AP and Ca). Results indicated that birds fed diets supplemented with 500 or 1,000 FTU/kg ECP had similar growth performance and tibia characteristics compared with those fed PC diets. In conclusion, AP equivalency values of 0.986 and 1.589 g/kg AP determined for 500 and 1,000 FTU/kg ECP, respectively, can be applicable in commercial broiler diets.

Key words: available phosphorus, broiler chicken, phosphorus equivalency value, phytase

2022 J. Appl. Poult. Res. 31:100298
https://doi.org/10.1016/j.japr.2022.100298

DESCRIPTION OF PROBLEM

Phosphorus (**P**) is an essential nutrient for poultry (Kumar et al., 2012). A large amount of P is present in the organic form of *myo*-inositol hexaphosphate (i.e., phytate) in plants (Lott, 1984), which is hardly available to poultry (Hill et al., 2008). However, organic P in phytate can be hydrolyzed to inorganic P as available P (**AP**) by *myo*-inositol hexaphosphate phosphohydrolase (i.e., phytase). Thus, dietary phytase is widely used as a feed additive in poultry diets (Nelson et al., 1968; Józefiak et al., 2010; Adeola and Cowieson, 2011; Dos Santos et al., 2013). The well-known benefits of dietary phytase include increasing P utilization and mitigating antinutritional effects of phytate on calcium (**Ca**) and other nutrient utilization, which ultimately lead to an improvement in poultry productivity (Ravindran et al., 2006; Kiarie et al., 2015; Walk and Olukosi, 2019). In

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addition, improved utilization of P in diets by dietary phytase also reduces both supplementation of inorganic P sources in diets and P excretion to the environment (Simons et al., 2007; Thacker et al., 2009). Although dietary phytase is considered a promising means to improve poultry productivity and decrease environmental pollution, the accurate estimation of its efficacy is essential to optimize the application of phytase in poultry diets.

The accurate determination of AP concentrations in diets supplemented with phytase is difficult because various factors, such as supplemental levels and sources of phytase, amounts of phytate, and other nutrient components in diets, affect the AP equivalency values of phytase (Vieira et al., 2015). In particular, each phytase has specific chemical characteristics depending on its microbial origin (Vieira et al., 2015), which can influence its main acting site in the gastrointestinal tract, P-hydrolyzing mechanisms, and interaction with dietary Ca (Maenz and Classen, 1998). Therefore, the accurate estimation of phytase activity and its subsequent AP equivalency is critical for the optimal use of dietary phytase. Several previous experiments have determined AP equivalency values of various sources of phytase in broiler diets (De Freitas et al., 2019; Dersjant-Li et al., 2019). However, few experiments have validated the determined AP equivalency value of phytase in the feeding trial, which may raise questions about the actual application of dietary phytase in the commercial situation.

Therefore, the objective of the present experiment was to determine the AP equivalency values of *Escherichia coli*-derived phytase (**ECP**) in broiler diets, relative to AP from monosodium phosphate (**MSP**), and subsequently to validate the determined AP equivalency values.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the Institutional Animal Care and the Use Committee at Chung-Ang University, Republic of Korea.

Experiment 1: Determination of AP Equivalency of ECP in Broiler Diets

Animals, Experimental Design, and Diets. A total of one thousand 1-d-old male and female Ross 308 broiler chicks were obtained from a local hatchery (Dongsan hatchery, Cheonan, Republic of Korea) and raised according to the general guidelines of Ross 308 broiler management until 7 d of age (Aviagen, 2019). All chicks were fed a crumbled commercial diet containing adequate amounts of all nutrients (Aviagen, 2019) before the start of the experiment. On 8 d of age, all chicks were weighed and 480 chicks with a narrow body weight (**BW**) of 203.7 ± 8.2 g were finally selected to obtain the homogeneous BW at the start of the experiment. The selected chicks were allotted to 1 of 8 treatments with 5 replicates in a completely randomized design. Each replicate consisted of 6 male and 6 female birds per battery cage. This trial was repeated to produce a total of 10 replicates.

The phytase used in the present experiment was an ECP (CJ bio, Seoul, Republic of Korea). Eight diets were formulated based on the expected AP equivalency value of ECP. A negative control (**NC**) diet was formulated to contain 0.76% Ca and 0.10% AP but adequate amounts of all other nutrients according to the recommendations of the Ross manual (Table 1; Aviagen, 2019). The concentrations of Ca and AP in the NC diet were determined based on the previous experiment (Vieira et al., 2015). A positive control (**PC**) diet was also formulated to contain adequate amounts of all nutrients (Aviagen, 2019). The remaining 6 diets included the NC diet plus 0.5, 1.0, or 2.0 g/kg AP from MSP and the NC diet plus 250, 500, or 1,000 phytase unit (**FTU**)/kg of ECP. All diets were provided in a mash form, and the powder form of ECP was added to the diets in place of celite. The diets and water were provided on an ad libitum basis for 17 d from 8 to 24 d of age. The room temperature was maintained at 30°C at the start of the experiment and then gradually decreased to 24°C. A lighting schedule of 23:1 h of light to dark cycle was maintained during the experiment.

Sample Collection and Chemical Analysis. The BW, BW gain (**BWG**), and feed intake (**FI**) were measured at the conclusion of the experiment. The mortality was recorded daily. The feed efficiency (**FE**) was calculated by dividing BWG by FI after correcting for mortality. At the end of the experiment, one male and one female birds per replicate with a BW close

Table 1. Composition and nutrient concentration of the experimental diets from experiment 1 (8−24 d).

	Dietary treatments							
Items	NC diet	0.05 AP	0.10 AP	0.20 AP	250 FTU	500 FTU	1,000 FTU	PC diet
Ingredients (%)								
Corn	50.60	50.60	50.60	50.60	50.60	50.60	50.60	50.60
SBM (45% CP)	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Corn gluten meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Tallow	6.46	6.46	6.46	6.46	6.46	6.46	6.46	6.46
Limestone	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.94
MSP[1]	0.00	0.20	0.40	0.80	0.00	0.00	0.00	1.34
Phytase[2]	0.00	0.00	0.00	0.00	0.00417	0.00830	0.01670	0.00
Celite	2.00	1.80	1.60	1.20	1.99583	1.99170	1.98330	0.32
DL-Met (98%)	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Lys-HCl (50%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Thr (98%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
NaCl	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
$NaHCO_3$	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Premix	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrients								
AME_n (kcal/kg)	3,100	3,100	3,100	3,100	3,100	3,100	3,100	3,100
CP (%)	21.6	21.6	21.6	21.6	21.6	21.6	21.6	21.6
Total Lys (%)	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Total Met+Cys (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total Met (%)	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Total Thr (%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Total Trp (%)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Digestible Lys (%)	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Digestible Met + Cys (%)	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Digestible Met (%)	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Digestible Thr (%)	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Digestible Trp (%)	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Total Ca (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.88
Available P (%)	0.10	0.15	0.20	0.30	0.15	0.20	0.31	0.44
Total P (%)	0.36	0.41	0.46	0.56	0.42	0.48	0.61	0.69
Phytate (%)	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Analyzed nutrients								
Total Ca (%)	0.76	0.77	0.78	0.75	0.76	0.78	0.77	0.87
Total P (%)	0.35	0.41	0.48	0.55	0.38	0.37	0.36	0.71

[1]Monosodium phosphate (25% total P).

[2]Phytase activity = 6,000,000 FTU/kg.

to the replicate mean BW were selected. The selected birds were euthanized by CO_2 asphyxiation to collect tissue samples for further analysis.

The right tibia was collected from both euthanized male and female birds to analyze bone characteristics. The concentrations and total amounts of ash in the right tibia were analyzed. Average values from male and female birds per replicate were used for the final data analysis. The detailed procedure used in this experiment was reported by Choi et al. (2021). These analyses were performed at the BT research facility center, Chung-Ang University, Republic of Korea.

Statistical Analysis. All data were analyzed using ANOVA in a completely randomized design via the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). All data were checked for outliers and normal distribution using the UNIVARIATE procedure of SAS. The replicate was used as an experimental unit for all measurements. The preplanned contrast test was performed with PC vs. NC and MSP treatments vs. ECP treatments. The orthogonal polynomial contrasts test was also

performed to determine the linear and quadratic effect of increasing MSP or ECP supplementation. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $P < 0.05$.

Linear and nonlinear regression analysis were conducted to calculate AP equivalency values of ECP based on concentrations and total amounts of tibia ash as the response criteria using the JMP statistical package from SAS. The detailed procedure of linear and nonlinear regression analysis used in the current experiment was reported by Jendza et al. (2006).

Experiment 2: Validation of AP Equivalency of ECP in Broiler Diets

Animals, Experimental Design, and Diets. A total of twenty eight hundred 1-d-old mixed-sex Ross 308 broiler chicks were obtained from a local hatchery (Dongsan hatchery, Cheonan, Republic of Korea) and were raised according to the general guidelines of Ross 308 broiler management until 7 d of age (Aviagen, 2019). All chicks were fed a crumbled commercial diet containing adequate amounts of all nutrients (Aviagen, 2019) before the start of the experiment. On 8 d of age, all chicks were weighed and 2,000 chicks with a narrow BW of 188.2 ± 2.7 g were finally selected. The selected chicks were allotted to 1 of 5 treatments with 8 replicates in a completely randomized design. Each replicate consisted of 50 birds per floor pen.

The phytase used in this experiment was identical to that used in experiment 1. The growth trial was divided into 2 periods, a growing phase (from 8 to 21 d of age; Table 2) and a finishing phase (from 22 to 35 d of age; Table 3). Five diets for each phase were formulated based on the determined AP equivalency values of 500 FTU/kg and 1,000 FTU/kg ECP in experiment 1. Although the Ca equivalency value of ECP was not directly determined in experiment 1, the value was assumed to be identical to the AP equivalency value of ECP (Adeola and Cowieson, 2011). Dietary treatments included negative control diet 1 (**NC1**, highly deficient in AP and Ca), negative control diet 2 (**NC2**, deficient in AP and Ca), phytase diet 1 (**P1**, NC1 + 1,000 FTU/kg ECP), phytase diet 2 (**P2**, NC2 + 500 FTU/kg ECP), and positive control diet (**PC**, recommended concentrations of AP and Ca). The calculated concentrations of AP and Ca in P1 and P2 diets were equalized to those of AP and Ca in the PC diet based on the equivalency values of AP and Ca for ECP determined in experiment 1. All diets were prepared to contain similar amounts of all nutrients except AP and Ca in both phases according to the recommendations of the Ross manual (Aviagen, 2019). All diets were fed in a mash form, and the powder form of ECP used in experiment 1 was added to diets in place of celite. The diets and water were provided on an ad libitum basis for 28 d from 8 to 35 d of age. All experimental pens were placed in an environmentally controlled facility. The room temperature was maintained at 30˚C at the start of the experiment and then gradually decreased to 21˚C. The experiment was performed under a 24-h lighting condition.

Sample Collection, Chemical Analysis, and Calculation. The BW, BWG, and FI were measured at the end of the experiment. The mortality was recorded daily. The FE was calculated by dividing BWG by FI after correcting for mortality. At the end of the experiment, one male and one female birds per replicate with a BW close to the replicate mean BW were selected. The selected birds per replicate were euthanized by CO_2 asphyxiation to collect tissue samples for further analysis.

The breast muscle was excised to measure meat quality and stored at 4˚C until further analysis. The right portion of the breast muscle was used for pH and meat color assays, whereas the left portion was used to determine water holding capacity. The detailed procedure of meat quality analysis used in this experiment was reported by Goo et al. (2019).

Both right and left tibias were collected to analyze bone characteristics. The concentrations and total amounts of Ca, P, and ash in the right tibia were analyzed, whereas the left tibia was used to measure the breaking strength. The detailed procedure of the analysis of tibia characteristics was reported by Choi et al. (2021). These analyses were performed at the BT research facility center, Chung-Ang University, Republic of Korea.

Table 2. Composition and nutrient concentration of the experimental diets in experiment 2 (8–21 d).

	Dietary treatments				
Items	PC	NC1	NC2	P1	P2
Ingredients (%)					
Corn	53.07	53.07	53.07	53.07	53.07
SBM (45% CP)	32.74	32.74	32.74	32.74	32.74
Corn gluten meal	5.60	5.60	5.60	5.60	5.60
Tallow	3.82	3.82	3.82	3.82	3.82
Limestone	1.201	1.081	1.127	1.081	1.127
MDCP[1]	1.689	0.952	1.231	0.952	1.231
Phytase[2]	0.00	0.00	0.00	0.0167	0.0083
Celite	0.0050	0.8620	0.5370	0.8453	0.5287
DL-Met (98%)	0.345	0.345	0.345	0.345	0.345
L-Lys HCl (98%)	0.33	0.33	0.33	0.33	0.33
Thr (98%)	0.14	0.14	0.14	0.14	0.14
NaCl	0.40	0.40	0.40	0.40	0.40
$NaHCO_3$	0.14	0.14	0.14	0.14	0.14
Premix	0.52	0.52	0.52	0.52	0.52
Total	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrients					
AME_n (kcal/kg)	3,050	3,050	3,050	3,050	3,050
CP (%)	22.3	22.3	22.3	22.3	22.3
Total Lys (%)	1.37	1.37	1.37	1.37	1.37
Total Met + Cys (%)	1.04	1.04	1.04	1.04	1.04
Total Met (%)	0.68	0.68	0.68	0.68	0.68
Total Thr (%)	0.95	0.95	0.95	0.95	0.95
Total Trp (%)	0.24	0.24	0.24	0.24	0.24
Digestible Lys (%)	1.23	1.23	1.23	1.23	1.23
Digestible Met + Cys (%)	0.93	0.93	0.93	0.93	0.93
Digestible Met (%)	0.63	0.63	0.63	0.63	0.63
Digestible Thr (%)	0.82	0.82	0.82	0.82	0.82
Digestible Trp (%)	0.21	0.21	0.21	0.21	0.21
Total Ca (%)	0.920	0.761	0.821	0.920	0.920
Available P (%)	0.460	0.301	0.361	0.460	0.460
Total P (%)	0.714	0.556	0.616	0.714	0.714
Phytate (%)	0.25	0.25	0.25	0.25	0.25
Analyzed nutrients					
Total Ca (%)	0.918	0.768	0.816	0.761	0.822
Total P (%)	0.718	0.568	0.626	0.543	0.613

[1]Mono-dicalcium phosphate (21.5% P).

[2]Phytase activity = 6,000,000 FTU/kg; P1, 1,000 FTU/kg = 0.159% of Available P, total P, and total Ca; P2, 500 FTU/kg = 0.099% of Available P, total P, and total Ca.

Statistical Analysis. All data were analyzed using ANOVA in a completely randomized design via the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). All data were checked for outliers and normal distribution using the UNIVARIATE procedure of SAS. The replicate was used as an experimental unit for all measurements. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $P < 0.05$.

RESULTS AND DISCUSSION

Phosphorus is an essential nutrient required by poultry for normal physiological functions, such as optimal growth, reproduction, and bone development (Veum, 2010). However, approximately 60 to 80% P in plant ingredients is present as unavailable phytate-P forms for poultry (Ravindran et al., 1994). Therefore, nutritionists need to formulate diets with substantial safety margins of AP to guarantee that poultry does not experience with P deficiency (Li et al., 2016). However, increasing P concentrations in

Table 3. Composition and nutrient concentration of the experimental diets in experiment 2 (22–35 d).

	Dietary treatments				
Items	PC	NC1	NC2	P1	P2
Ingredients (%)					
Corn	58.08	58.08	58.08	58.08	58.08
SBM (45% CP)	26.96	26.96	26.96	26.96	26.96
Corn gluten meal	4.91	4.91	4.91	4.91	4.91
Tallow	5.64	5.64	5.64	5.64	5.64
Limestone	1.017	0.897	0.943	0.897	0.943
MDCP[1]	1.433	0.694	0.973	0.694	0.973
Phytase[2]	0.00	0.00	0.00	0.0167	0.0083
Celite	0.2400	1.0990	0.7740	1.0823	0.7657
DL-Met (98%)	0.29	0.29	0.29	0.29	0.29
L-Ly HCl (98%)	0.27	0.27	0.27	0.27	0.27
Thr (98%)	0.10	0.10	0.10	0.10	0.10
NaCl	0.40	0.40	0.40	0.40	0.40
$NaHCO_3$	0.14	0.14	0.14	0.14	0.14
Premix	0.52	0.52	0.52	0.52	0.52
Total	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrients					
AME_n (kcal/kg)	3,200	3,200	3,200	3,200	3,200
CP (%)	19.5	19.5	19.5	19.5	19.5
Total Lys (%)	1.16	1.16	1.16	1.16	1.16
Total Met + Cys (%)	0.91	0.91	0.91	0.91	0.91
Total Met (%)	0.59	0.59	0.59	0.59	0.59
Total Thr (%)	0.81	0.81	0.81	0.81	0.81
Total Trp (%)	0.21	0.21	0.21	0.21	0.21
Digestible Lys (%)	1.04	1.04	1.04	1.04	1.04
Digestible Met+Cys (%)	0.81	0.81	0.81	0.81	0.81
Digestible Met (%)	0.55	0.55	0.55	0.55	0.55
Digestible Thr (%)	0.69	0.69	0.69	0.69	0.69
Digestible Trp (%)	0.18	0.18	0.18	0.18	0.18
Total Ca (%)	0.790	0.631	0.691	0.790	0.790
Available P (%)	0.395	0.236	0.296	0.395	0.395
Total P (%)	0.635	0.476	0.536	0.635	0.635
Phytate (%)	0.25	0.25	0.25	0.25	0.25
Analyzed nutrients					
Total Ca (%)	0.791	0.631	0.685	0.643	0.696
Total P (%)	0.637	0.485	0.533	0.469	0.521

[1]Mono-dicalcium phosphate (21.5% P).

[2]Phytase activity = 6,000,000 FTU/kg; P1, 1,000 FTU/kg = 0.159% of Available P, total P, and total Ca; P2, 500 FTU/kg = 0.099% of Available P, total P, and total Ca.

poultry diets increase both the cost of poultry production and P excretion into manure (Li et al., 2016). Thus, dietary phytase is introduced to improve P utilization in diets and is currently considered an essential feed supplement (Jongbloed et al., 1992; Radcliffe and Kornegay, 1998; Simons et al., 2007). Many studies have evaluated the extent of P utilization by dietary phytase, which is often referred to as the AP equivalency of phytase, for its efficient use in diet formulation (Jendza et al., 2006; Vieira et al., 2015).

Poultry diets frequently include monocalcium phosphate (**MCP**) or dicalcium phosphate (**DCP**) as the source of supplemental inorganic P (Jendza et al., 2006; Trairatapiwan et al., 2018; Marchal et al., 2021). However, the NRC (1994) represents that P in DCP has 95 to 100% bioavailability as compared to P in MSP or MCP. Therefore, in the current experiment, the MSP was used as a reference P source, assuming that P in MSP is 100% available for poultry (Jendza et al., 2006), moreover to minimize the confounding effects of Ca from MCP or DCP

(Adedokun et al., 2004; Jendza et al., 2006; Wang et al., 2021).

Experiment 1: Determination of AP Equivalency of ECP in Broiler Diets

Birds fed PC diets had greater ($P < 0.01$) BW, BWG, FI, and FE than those fed NC diets (Table 4). Birds fed diets supplemented with P from MSP had greater ($P < 0.05$) for BW, BWG, and FI than those fed diets supplemented with ECP. Increasing supplementation of MSP in diets increased BW, BWG, FI (linear and quadratic, $P < 0.01$), and FE (linear, $P < 0.05$). Similarly, increasing supplementation of ECP in diets increased BW, BWG, and FI of broiler chickens (linear and quadratic, $P < 0.01$).

Birds fed PC diets had greater ($P < 0.01$) concentrations and total amounts of tibia ash than those fed NC diets (Table 4). No differences in tibia bone characteristics were found between birds fed diets supplemented with MSP or ECP. Increasing supplementation of MSP in diets increased the concentrations and total amounts of tibia ash (linear and quadratic, $P < 0.01$). Likewise, increasing supplementation of ECP in diets increased the concentrations and total amounts of tibia ash of broiler chickens (linear and quadratic, $P < 0.01$).

These results indicated that increasing concentrations of AP from MSP in diets improved growth performance and tibia characteristics of broiler chickens, and these effects were similar to the effects of increasing supplementation of ECP in diets, suggesting that ECP used in this experiment is capable of improving P utilization in broiler diets. Improvement in growth performance and tibia characteristics of broiler chickens by feeding diets supplemented with ECP was consistent with the previous observations (Kriseldi et al., 2021; Hakami et al., 2022). However, quadratic improvements in growth performance and tibia characteristics of broiler chickens by increasing supplementation of ECP were identified, indicating that the efficacy of the ECP used in this experiment could reach to the peak or plateau around 1,000 FTU/kg. As a consequence, the nonlinear regression model was adopted to determine AP equivalency values of ECP based on the concentrations and total amounts of tibia ash.

The concentrations and total amounts of tibia ash were better fit by the nonlinear equation for AP equivalency based on probabilities of greater than 99% (Table 5). Assuming that P in MSP is 100% available, AP equivalency of ECP relative to AP in MSP was calculated using the nonlinear equation with the concentrations and total amounts of tibia ash as the response criteria (Table 6). These final nonlinear regression models were taken from the procedure of Harper et al. (1997), Jendza et al. (2006). Denbow et al. (1995), and Ravindran et al. (2001) used R^2 as the determinant for selecting either the linear or nonlinear regression model to describe the responses upon increasing supplementation of phytase. However, Neter et al. (1996) indicated that R^2 values may not be a meaningful statistic for the nonlinear regression model because the product of the error sum of squares and the regression sum of squares may differ from the total sum of squares. Motulsky and Christopoulos (2003) suggested that the 2 best methods for comparing 2 regression equations for the goodness of fit with the same database should be based on either an *F*-test if the 2 equations are nested or AIC_c if they are not. Because it is difficult to obtain the linear equation with parameters of the mechanistic growth model ($Y = 1 - \theta_1 \times \left(1 - \theta_2 \times e^{(-\theta_3 \times X)}\right)$), to 0 or 1, the AIC_c was used to determine the final nonlinear regression models in the current experiment (Jendza et al., 2006).

Based on the nonlinear regression models, 250 FTU/kg ECP was determined to be equivalent to 0.514 and 0.553 g/kg AP from MSP based on the concentrations and total amounts of tibia ash, respectively. Likewise, 500 FTU/kg ECP was determined to be equivalent to 0.924 and 1.047 g/kg AP from MSP based on the concentrations and total amounts of tibia ash, respectively. Finally, 1,000 FTU/kg ECP was determined to be equivalent to 1.406 and 1.771 g/kg AP from MSP based on the concentrations and total amounts of tibia ash, respectively. However, AP equivalency values from the concentrations and total amounts of tibia ash for each supplemental level of ECP were averaged to determine the final AP equivalency values of ECP (i.e., 0.534, 0.986, and 1.589 g/kg AP for 250, 500, and 1,000 FTU/kg

Table 4. Effect of different amounts of available phosphorus (AP) from monosodium phosphate (MSP) and phytase in diets on growth performance and tibia characteristics of broiler chickens (8–24 d).[1]

			MSP (%)			Phytase (FTU/kg)				*P*-value					
												MSP[5]		Phytase[6]	
Treatments	PC	NC	0.2	0.4	0.8	250	500	1,000							
AP (%)	0.44	0.10	0.15	0.20	0.30	0.15	0.20	0.31	SEM	PC vs. NC[3]	MSP vs. phytase[4]	L	Q	L	Q
Growth performance[2]															
BW (g)	1,141	952	1,061	1,138	1,131	1,071	1,085	1,088	12.7	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
BWG (g)	937	748	857	935	927	867	881	884	14.1	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01
FI (g)	1,232	1,039	1,180	1,246	1,231	1,178	1,202	1,200	14.0	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01
FE (g/kg)	760	721	727	750	753	736	733	736	8.4	<0.01	0.23	<0.05	0.41	0.26	0.42
Tibia characteristics															
Tibia ash (%)	48.8	37.4	42.1	46.4	48.1	42.7	45.4	48.0	0.69	<0.01	0.81	<0.01	<0.01	<0.01	<0.01
Tibia ash (mg)	1,077	660	803	996	1,047	819	935	1,030	23.0	<0.01	0.27	<0.01	<0.01	<0.01	<0.01

[1]Data are least squares means of 10 observations per treatment. AP (%) in phytase treatments were calculated from expected AP equivalency of phytase provider (CJ bio, Seoul, Republic of Korea).

[2]BW, body weight; BWG, BW gain; FI, feed intake; FE, feed efficiency.

[3]Contrast test for PC vs. NC.

[4]Contrast test for MSP treatments (0.2, 0.4, and 0.8% MSP) vs. phytase treatments (250, 500, and 1,000 FTU/kg ECP).

[5]Linear (L) or quadratic (Q) effects of increasing supplementation of MSP (NC, 0.2, 0.4, and 0.8% MSP).

[6]Linear (L) or quadratic (Q) effects of increasing supplementation of phytase (NC, 250, 500, and 1,000 FTU/kg ECP).

Table 5. Regressions of the concentrations and total amounts of tibia ash on available phosphorus (AP) from monosodium phosphate (MSP) and phytase supplementation in broiler diets (8–24 d).

	Regression equation		
Supplement	Nonlinear	Linear	Probability[3]
AP from MSP			
Tibia ash[1] (%)	$Y = 49.51 \times (1 - 0.2482 \times e^{(-1.17 \times X_{AP})})$, $R^2 = 0.8347$	$Y = 38.90 + 5.23 \times X_{AP}$, $R^2 = 0.7323$	0.9998
Tibia ash[2] (g)	$Y = 1.12 \times (1 - 0.4185 \times e^{(-0.85 \times X_{AP})})$, $R^2 = 0.8550$	$Y = 0.7064 + 0.1582 \times X_{AP}$, $R^2 = 0.7671$	0.9997
Phytase			
Tibia ash[1] (%)	$Y = 47.66 \times (1 - 0.2161 \times e^{(-0.00298 \times X_{phy})})$, $R^2 = 0.8285$	$Y = 39.14 + 0.00923 \times X_{phy}$, $R^2 = 0.6891$	1.0000
Tibia ash[2] (g)	$Y = 1.06 \times (1 - 0.3776 \times e^{(-0.00184 \times X_{phy})})$, $R^2 = 0.8258$	$Y = 0.7080 + 0.00028 \times X_{phy}$, $R^2 = 0.7397$	0.9986

[1] Y = Predicted performance for a given criteria; X_{AP} = g of supplemental AP from MSP per kg; X_{phy} = FTU of phytase per kg.
[2] Y = Predicted performance for a given criteria; X_{AP} = g of AP intake from MSP; X_{phy} = FTU intake from phytase.
[3] Probability that the nonlinear regression equation fits the data better than the linear regression equation.

ECP, respectively), as suggested by Adedokun et al. (2004) and Jendza et al. (2006) who averaged the values for all response criteria to determine the AP equivalency of ECP. The AP equivalency values determined for each supplemental level of ECP in this experiment were close to those reported by Jendza et al. (2006) who used the similar source of phytase and nonlinear regression model, but were less than the values ranging from 1.250 to 1.310 g/kg AP for the supplemental level of 500 FTU/kg phytase in other previous experiments (Augspurger et al., 2003; Ribeiro et al., 2016). The possible reason for this variation may be the differences in diet compositions, supplemental levels and sources of phytase, experimental design, and statistical analysis among experiments (Adedokun et al., 2004; Jendza et al., 2006; Wang et al., 2021).

Experiment 2: Validation of AP Equivalency of ECP in Broiler Diets

Birds fed P1 or P2 diets had greater ($P < 0.05$) BW and BWG than those fed NC1 or NC2 diets, but showed similar growth performance to those fed PC diets (Table 7). However, the FE and mortality were not influenced by dietary treatments. Similarly, breast meat quality including meat colors including lightness (L*), redness (a*), and yellowness (b*), water holding capacity, and pH values was not affected by dietary treatments (Table 7).

Birds fed P1 or P2 diets had greater ($P < 0.05$) breaking strength, concentrations of Ca and P, and total amounts of ash, Ca, and P in the tibia than those fed NC1 or NC2 diets, but showed similar tibia characteristics to those fed PC diets (Table 8). However, the concentrations of ash were not influenced by dietary treatments.

The AP equivalency values of 0.986 and 1.589 g/kg determined for 500 and 1,000 FTU/kg ECP, respectively, were validated in a conventional feeding trial. In addition, the Ca equivalency values of ECP at each supplemental level were also adopted in diet formulation, assuming that the extent of improvement in Ca utilization by dietary phytase is similar to that in P utilization (Adeola and Cowieson, 2011). The reason for adopting Ca equivalency is that dietary phytase liberates

Table 6. Available phosphorus (AP) equivalency of phytase relative to AP in monosodium phosphate (MSP) for the concentrations and total amounts of tibia ash in broiler chickens (8–24 d).

Item	Equivalency equation	Model
Tibia ash[1] (%)	$X_{AP} = Ln(0.1505 + 0.838e^{(-0.00298 \times X_{phy})}) / -1.17$	Nonlinear
Tibia ash[2] (g)	$X_{AP} = Ln(0.1281 + 0.854e^{(-0.00184 \times X_{phy})}) / -0.85$	Nonlinear

[1] Equivalency equation created by setting the regression equations for AP and phytase supplementation equal to each other and solving for X_{AP}, with X_{AP} = g of supplemental AP from MSP per kg, and X_{phy} = FTU of phytase per kg.
[2] Equivalency equation created by setting the regression equations for AP and phytase intake equal to each other and solving for X_{AP}, with X_{AP} = g of AP intake from MSP, and X_{phy} = FTU intake from phytase.

Table 7. Effect of dietary phytase supplementation on growth performance and breast meat quality of broiler chickens (8–35 d).[1]

	Dietary treatments[2]						
Item[3]	PC	NC1	NC2	P1	P2	SEM	*P*-value
Growth performance							
BW (g)	2,220[a]	2,108[b]	2,140[b]	2,221[a]	2,224[a]	24.3	<0.01
BWG (g)	2,032[a]	1,920[b]	1,951[b]	2,032[a]	2,036[a]	23.9	<0.01
FI (g)	3,145[a]	3,042[b]	3,075[ab]	3,131[a]	3,120[a]	24.9	<0.05
FE (g/kg)	646	631	635	649	652	6.0	0.08
Mortality (%)	2.5	2.5	2.8	2.0	1.5	0.70	0.73
Breast meat quality							
Yield (%)	18.30	19.55	17.02	18.62	18.50	0.695	0.17
pH (1h)	6.57	6.51	6.52	6.54	6.56	0.052	0.93
pH (24h)	6.07	6.12	6.10	6.12	6.11	0.026	0.48
WHC (%)	71.50	70.47	71.98	72.07	70.89	2.195	0.98
L*	45.48	44.21	44.45	43.98	43.21	0.539	0.08
a*	3.09	3.11	3.11	3.93	3.79	0.480	0.57
b*	13.57	12.46	12.64	13.68	13.24	0.517	0.37

[a-b]Means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]Dietary treatments: PC, grower diet (Ca = 0.920%, AP = 0.460%) and finisher diet (Ca = 790%, AP = 0.395%); NC1, grower diet (Ca = 0.761%, AP = 0.301%) and finisher diet (Ca = 0.631%, AP = 0.236%); NC2, grower diet (Ca = 0.821%, AP = 0.361%), and finisher diet (Ca = 0.691%, AP = 0.296%); P1, NC1 + 1,000 FTU/kg; P2, NC2 + 500 FTU/kg.

[3]BW, body weight; BWG, BW gain; FI, feed intake; FE, feed efficiency; Yield, relative breast weight; WHC, water holding capacity; L*, lightness; a*, redness; b*, yellowness.

phytate-bound Ca, and thus, concurrently improve Ca utilization (Selle et al., 2009). In commercial diet formulation, both Ca and AP concentrations are adjusted together in diets supplemented with phytase (Adeola and Cowieson, 2011).

The results showed that birds fed either NC1 or NC2 diets, which were deficient in AP and Ca, had decreased growth performance and tibia characteristics than those fed PC diets. These results indicate that both NC diets were sufficient to induce AP and Ca deficiency in broiler chickens. However, interestingly, no distinguishable effects were observed between NC1 and NC2 diets although NC1 diets were more deficient in AP and Ca compared with NC2 diets. The reason is unclear; however, it may be related to the Ca:AP ratio in diets. The ideal ratio of Ca to AP

Table 8. Effect of dietary phytase supplementation on tibia characteristics of broiler chickens (8–35 d).[1]

	Dietary treatments[2]						
Item	PC	NC1	NC2	P1	P2	SEM	*P*-value
Ash (mg)	2,330[ab]	2,037[c]	2,204[b]	2,359[a]	2,343[a]	45.3	<0.01
Ash (%)	52.04	51.33	51.04	52.01	52.34	0.430	0.19
Ca (mg)	756[a]	627[b]	652[b]	762[a]	763[a]	15.0	<0.01
Ca (%)	32.44[a]	30.78[b]	29.56[c]	32.31[a]	32.52[a]	0.360	<0.01
P (mg)	405[a]	340[b]	352[b]	411[a]	408[a]	7.3	<0.01
P (%)	17.41[a]	16.69[b]	15.97[c]	17.42[a]	17.44[a]	0.247	<0.01
BS[3] (kgf)	37.1[a]	26.9[c]	26.8[c]	32.8[b]	34.2[ab]	1.38	<0.01

[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]Dietary treatments: PC, grower diet (Ca = 0.920%, AP = 0.460%) and finisher diet (Ca = 790%, AP = 0.395%); NC1, grower diet (Ca = 0.761%, AP = 0.301%) and finisher diet (Ca = 0.631%, AP = 0.236%); NC2, grower diet (Ca = 0.821%, AP = 0.361%), and finisher diet (Ca = 0.691%, AP = 0.296%); P1, NC1 + 1,000 FTU/kg; P2, NC2 + 500 FTU/kg.

[3]BS, Breaking strength.

in broiler chickens is close to 2 to 1 (Aviagen, 2019), and exceeding this ratio shows a negative effect on growth performance and bone development (Gautier et al., 2017). In the current experiment, the ratio of Ca to AP was 2.67 to 1 for NC1 diets and 2.33:1 for NC 2 diets. Therefore, it is speculated that the extent of the negative effects may be indistinguishable at very high ratio of Ca to AP used in this experiment (Gautier et al., 2017).

Birds fed P-deficient diets supplemented with 500 or 1,000 FTU/kg ECP had similar growth performance, breast meat quality, and tibia characteristics to those fed PC diets. These results indicated that AP equivalency values of ECP determined in this experiment could be highly applicable in diet formulation for broiler chickens. However, it should be noted that no fixed AP equivalency values of dietary phytase are possible in all diet formulations because many factors, including animals and dietary compositions such as phytate and Ca concentrations in diets, can exert a large impact on phytase actions (Dersjant-Li et al., 2015). Thus, AP and Ca equivalency values of dietary phytase should be cautiously determined and implemented in poultry diets based on the specific diet composition and source of phytase using appropriate statistical prediction models (Plumstead, 2007).

CONCLUSIONS AND APPLICATIONS

1. The AP equivalency values of 500 and 1,000 FTU/kg ECP are 0.986 and 1.589 g/kg for broiler diets, based on nonlinear regression models.
2. The determined AP equivalency values of ECP are validated in a feeding trial. Therefore, these values can be applicable in the commercial broiler diet.
3. It is likely that the Ca equivalency values of phytase are similar to its AP equivalency values.

ACKNOWLEDGMENTS

This research was carried out with the support of CJ Bio, Seoul, Republic of Korea.

DISCLOSURES

Authors declare that there are no competing interests.

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