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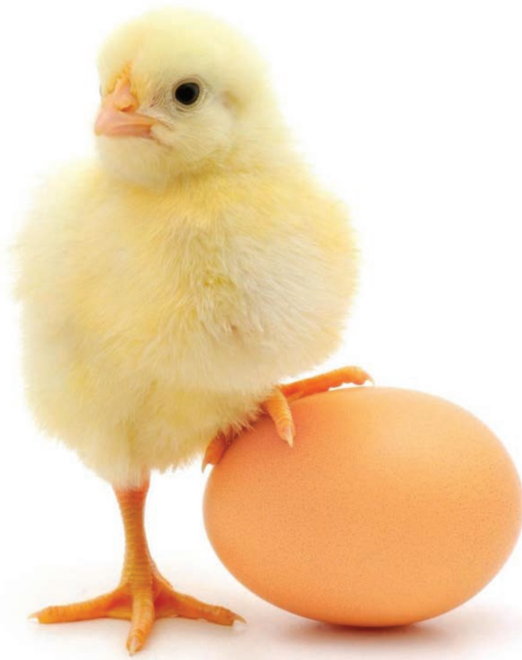

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Research Article
Corn-Expressed Phytase Influence on Broiler Growth Performance

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Abstract
Background and Objective: Formulating a balanced diet that would provide the required nutrients is very important for achieving optimal broiler growth. This study was conducted to determine the optimal calcium to available phosphorus ratio (Ca:AvP$_i$) for feed with or without corn-expressed phytase (CEP) addition. **Materials and Methods:** A 35d trial was conducted to determine growth performance, effects of either decreasing Ca:AvP$_i$ ratio as bird ages, without CEP, or increasing Ca matrix credit when feeding CEP. A total of 1152 one-day-old male broilers were assigned to 6 dietary treatments (Diets A to F) with 16 birds per pen and 12 replicate pens per treatment. Diets A and B didn't contain CEP and had 0.5 and 0.45% AvP$_i$ in the starter (0-15 day) and grower (16-35 day) feeds, respectively. Diet A had a decreasing Ca:AvP$_i$ from 2:1 in starter to 1.85:1 in grower, whereas Ca:AvP$_i$ of diet B was constant at 2:1 for both phases. Remaining diets contained 3000 FTU kg^{-1} of CEP. Diet C contained CEP added on top. Diets D to F had 0.12% lower AvP$_i$ while Ca was reduced by 0.11, 0.15 or 0.17%, respectively. **Results:** No differences in growth performance (p>0.05) were observed between birds fed diets A or B. In general, birds fed CEP had higher early (0-15 and 16-28 day) and overall body weight (BW), body weight gain (BWG) and feed intake (FI) compared to those fed diet A or B (p<0.05). Overall (0-35 day) feed conversion ratio (FCR) was improved for all CEP-supplemented diets compared to Diet A. Assigning different Ca matrix values among CEP treatments had no effect (p>0.05) on growth performance of broilers. Overall FCRs (0-35 day) with all CEP-supplemented diets were better than that of the unsupplemented diet A. **Conclusion:** Results demonstrated that adding CEP improved growth performance and reducing Ca:AvP$_i$ ratio as the bird aged did not affect performance for diets without CEP. In addition, with a 0.12% AvP$_i$ matrix value, adjusting Ca matrix values between 0.11 and 0.17% did not have a large effect on animal performance when diets included 3000 FTU kg^{-1} CEP.

Key words: Broiler, calcium, phosphorus, nutrition, corn-expressed phytase

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

When formulating broiler diets, the essential minerals calcium (Ca) and inorganic phosphorus (P_i), cannot be considered independently as they play a major role in each other's homeostasis and both are involved in bone formation[1]. Feed ingredients from plant sources are not adequate to meet requirements for these minerals. Therefore, poultry diets are usually supplemented with inorganic sources of Ca and P_i[2]. However, a significant amount of P stored in plants is present as phytate, which broiler chickens cannot readily digest[3]. To remedy this problem, exogenous phytase enzyme, which is usually microbial-derived, is included in poultry diets. The enzyme digests phytate in corn and soybean resulting in the release of P_i[4], Ca and other nutrients[5-7]. Therefore, supplementation of poultry diets with phytase reduces the need to provide mineral supplements to fulfill the P_i requirement. Moreover, it also provides other benefits; it has been shown that supplementing poultry diets with phytase decreased fecal P_i content[8] and therefore decreased the addition of Pi from fecal matter applied to soil. This helps in preventing eutrophication of surface waters, a major environmental concern associated with poultry production[9]. Dietary supplementation with phytase will also aid in cost reduction of feed compounding, because mineral supplements commonly used to provide P_i, such as dicalcium phosphate (DCP), are expensive.

It has been shown that through genetic engineering, corn can express phytase genes derived from microbial origin[10]. This development promises advancement in the animal nutrition industry, because transgenic corn can supply phytase and partially replace a fraction of the corn commonly added to feed making the addition of microbial phytase unnecessary. The mechanism and efficacy of an enzyme's activity in making various nutrients available is critical and many studies have been conducted to reveal the efficacy of nutrient release from phytate. Studies have shown that the residual activity of phytase from transgenic corn is higher in the crop, proventriculus, gizzard, jejunum and ileum sections of the gastrointestinal (GI) tract of birds[11] than that of microbial derived phytase. A study on feeding transgenic-corn phytase also showed that the transgenic phytase DNA is degraded in the GI tract and was not transferred to tissues of broilers[10] is counting a potential concern with transgenic products. The information derived from phytase studies allows nutritionists to define compounded diets with greater precision to meet an animal's requirements as well as to prevent nutrient over-formulation and thereby reduce dietary costs. Calcium can form an insoluble complex with phytate, making it more difficult for the phytase enzyme to degrade it[12]. Thus, when using feed formulation software in which a higher matrix values of Ca are attributed to phytase enzyme, then less Ca should be supplemented in diets, which might increase the enzyme efficacy and growth performance. Furthermore, assuming that a higher matrix value of Ca can be assigned to CEP indicates that less inorganic source of Ca is needed for feed manufacturing thus providing the benefit of saving on feed cost. The purpose of this research was to compare growth performance of broilers consuming diets with decreasing Ca:AvP_i ratio or constant Ca:AvP_i as broilers grew to market age, as well as testing multiple assigned matrix values of Ca to CEP enzyme effect on growth performance of broilers raised to 35 day.

MATERIALS AND METHODS

Animals and husbandry: All animal usage conformed to The Guide for Care and Use of Agricultural Animals in Research and Teaching[13] and North Carolina State University approved Institutional Animal Care and Use Committee protocol. Broiler chickens were hatched from eggs produced by 62 week old Ross YPM×708 broiler breeders maintained at the site. A total of 1,152 males were feather-sexed and placed randomly into 72 floor pens in a curtain-sided house with 16 birds per pen. Birds were exposed to 23 h light and 1 h dark for the first 2 weeks, reduced to 20 h light at 14 day and to 18 h light from 15-21 day. After 22 day, only natural light was provided. Initial brooding temperature was 35°C, then gradually reduced to 27°C by 15 day of age and then held at 27°C until 21 day of age. From 22 day of age until the end of the experiment, temperature was maintained at approximately 24°C. Feed and water were available for *ad libitum* consumption.

Treatments design: A total of 6 treatments (Table 1) were tested with 12 replicate pens per treatment. All diets were formulated to meet or exceed National Research Council[14] suggested requirements. Corn-expressed phytase (CEP; Grainzyme®, Agrivida) was included in phytase-supplemented diets C, D, E and F at the amount of 2 kg of transgenic corn/MT feed (4 lbs/ton), which amounts to 3000 FTU CEP kg^{-1} diet. To include the 2 kg of transgenic corn/MT feed, a simple removal of an equal amount of standard corn was done, because the transgenic corn that produces CEP has same nutritional specifications as standard corn. Consequently, the feed compounder can use the transgenic corn as a source of enzyme supplement as well as a basic ingredient in poultry diets. Treatments A and B were controls having no CEP. Treatment A had a decreasing Ca to available P_i ratio (Ca:AvP_i) when going from starter (2:1) to grower (1.85:1), while treatment B had a fixed Ca:AvP_i ratio at both feeding phases (2:1). Treatment C had the same stepped down Ca:AvP_i content between phases as A but was supplemented with CEP

Table 1: Experimental diets showing the theoretical Ca:AvP_i of diets, formulated percentages of Ca and AvP_i and corn-expressed phytase (CEP) matrix value for Ca and P_i in starter and grower feed

			Starter				Grower				
			Diet formulation (%)		CEP matrix value[3] (%)			Diet formulation (%)		CEP matrix value[3] (%)	
Treatment code[1]	Phytase, FTU kg^{-1}	Theoretical Ca:AvP_i	Ca	AvP_i[2]	Ca	P_i	Theoretical Ca:AvPi	Ca	AvP_i[2]	Ca	P_i
A	0	2:1	1.0	0.5	NA	NA	1.85:1	0.835	0.45	NA	NA
B	0	2:1	1.0	0.5	NA	NA	2:1	0.90	0.45	NA	NA
C	3000	2:1	1.0	0.5	NA	NA	2:1	0.90	0.45	NA	NA
D	3000	2:1	0.89	0.38	0.11	0.12	2:1	0.79	0.33	0.11	0.12
E	3000	2:1	0.85	0.38	0.15	0.12	2:1	0.75	0.33	0.15	0.12
F	3000	2:1	0.83	0.38	0.17	0.12	2:1	0.73	0.33	0.17	0.12

[1]A: Descending Ca:AvP_i (No CEP), B: Fixed 2:1 Ca:AvP_i (No CEP), C: Theoretical 2:1 Ca:AvP_i with CEP added on top, D to F: Theoretical 2:1 Ca:AvP_i with CEP given matrix value for Ca and P_i. P_i matrix value is assumed to be fixed but Ca matrix value increasing from diet D to F. [2]The basal numbers shown above are the actual formulation targets without CEP matrix values added

Table 2: Experimental diet composition (starter)

	Treatment code[1]					
Ingredient (%)	A	B	C	D	E	F
Corn	55.76	55.76	55.56	57.00	57.04	57.14
Soybean meal 48%	34.59	34.59	34.59	34.21	34.36	34.35
Poultry byproduct meal	3.00	3.00	3.00	3.00	3.00	3.00
Poultry fat	2.45	2.45	2.45	1.98	1.90	1.86
Dicalcium phosphate	2.23	2.23	2.23	1.48	1.48	1.48
Limestone	0.64	0.64	0.64	0.79	0.68	0.63
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.26	0.26	0.26	0.26	0.26	0.26
L-Threonine	0.01	0.01	0.01	0.01	0.01	0.01
Choline chloride (60%)	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix[3]	0.05	0.05	0.05	0.05	0.05	0.05
L-lysine	0.01	0.01	0.01	0.02	0.02	0.02
Coban[4]	0.05	0.05	0.05	0.05	0.05	0.05
CEP	0.00	0.00	0.20	0.20	0.20	0.20
Calculated nutrient content (%)						
Metabolizable energy (kcal kg^{-1})	2,950	2,950	2,950	2,950	2,950	2,950
Crude protein	22.50	22.50	22.50	22.50	22.50	22.50
Calcium	1.00	1.00	1.00	0.89	0.85	0.83
Total phosphorus	0.84	0.84	0.84	0.71	0.71	0.71
Available phosphorus	0.50	0.50	0.50	0.38	0.38	0.38
Total lysine	1.24	1.24	1.24	1.24	1.24	1.24
Total methionine	0.59	0.59	0.59	0.59	0.59	0.59
Analyzed nutrient content as fed (%)						
Crude fat	5.18	5.18	5.22	4.79	4.94	4.79
Crude protein	23.03	23.03	23.31	24.55	23.81	22.89
Crude fiber	1.40	1.40	1.60	1.40	1.50	1.60
Total digestible nutrients	67.88	67.88	66.97	67.44	69.10	67.51
Ash	6.05	6.05	6.27	5.94	5.76	5.47
Calcium	0.93	0.93	1.08	1.03	0.84	0.85
Total phosphorus	0.85	0.85	0.93	0.83	0.76	0.79

[1]A: Descending Ca: AvP_i (No CEP), B: Fixed 2:1 Ca: AvP_i (No CEP), C: Theoretical 2:1 Ca: AvP_i with CEP added on top, D to F: Theoretical 2:1 Ca: AvP_i with CEP given matrix value for Ca and P_i. P_i matrix value is assumed to be fixed but Ca matrix value increasing from diet D to F. [2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin and 1.1 mg folic acid. [3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3). [4]Coban supplied monensin sodium at 90 mg kg^{-1} of feed

(no matrix value assignment). Treatments D to F had fixed Ca:AvP_i ratios (2:1) and were supplemented with CEP at both feeding phases. However, each treatment was assigned a different Ca matrix value for CEP with Ca and AvP_i content in the diet adjusted accordingly. The CEP matrix value for P_i was fixed at 0.12% for all treatments, whereas Ca matrix values were either 0.11, 0.15, or 0.17% at both feeding phases for treatments D, E and F, respectively. Starter (1-14 day) feed (Table 2) was crumbled and grower (15-35 day) feed (Table 3) was pelleted. Feed proximate analysis was determined using

Table 3: Experimental diet composition (grower)

	Treatment code[1]					
Ingredient (%)	A	B	C	D	E	F
Corn	62.48	62.12	62.00	63.18	63.41	63.51
Soybean meal 48%	30.02	30.08	30.00	29.88	29.84	29.83
Poultry byproduct meal	1.50	1.50	1.50	1.50	1.50	1.50
Poultry fat	2.10	2.23	2.23	1.77	1.68	1.64
Dicalcium phosphate	2.18	2.18	2.18	1.44	1.44	1.44
Limestone	0.41	0.58	0.58	0.72	0.62	0.57
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.22	0.22	0.22	0.22	0.22	0.22
L-Threonine	0.01	0.01	0.01	0.01	0.01	0.01
Choline chloride, 60%	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix[3]	0.05	0.05	0.05	0.05	0.05	0.05
L-lysine	0.03	0.03	0.03	0.03	0.03	0.03
Coban[4]	0.05	0.05	0.05	0.05	0.05	0.05
CEP	0.00	0.00	0.20	0.20	0.20	0.20
Calculated nutrient content (%)						
Metabolizable energy (kcal kg^{-1})	3,000	3,000	3,000	3,000	3,000	3,000
Crude protein	20.00	20.00	20.00	20.00	20.00	20.00
Calcium	0.84	0.90	0.90	0.79	0.75	0.73
Total phosphorus	0.78	0.78	0.78	0.64	0.64	0.64
Available phosphorus	0.45	0.45	0.45	0.33	0.33	0.33
Total lysine	1.10	1.10	1.10	1.10	1.10	1.10
Total methionine	0.52	0.52	0.52	0.52	0.52	0.52
Analyzed nutrient content as fed (%)						
Crude fat	4.94	5.13	4.88	4.83	4.73	4.81
Crude protein	20.18	20.02	20.60	19.67	19.76	20.23
Crude fiber	1.60	1.30	1.50	1.70	1.80	1.80
Total digestible nutrients	67.15	67.51	67.35	67.92	67.98	68.42
Ash	5.52	5.86	5.99	5.59	5.25	5.22
Calcium	0.86	1.23	1.00	0.83	0.80	0.86
Total phosphorus	0.83	1.06	0.92	0.74	0.75	0.76

[1]A: Descending Ca:AvP_i (No CEP), B: Fixed 2:1 Ca:AvP_i (No CEP), C: Theoretical 2:1 Ca:AvP_i with CEP added on top; D to F: Theoretical 2:1 Ca:AvP_i with CEP given matrix value for Ca and P_i. P_i matrix value is assumed to be fixed but Ca matrix value increasing from diet D to F. [2]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 1,984 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin and 1.1 mg folic acid. [3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3). [4]Coban supplied monensin sodium at 90 mg kg^{-1} of feed

AOAC official methods; crude fat was determined using ether extraction, AOAC official method 920.39 (A)[15], crude protein was determined using AOAC Official Method 990.03[16]. Crude fiber was determined using AOAC official method 978.10[17]. Total digestible nutrients (TDN) was calculated as the sum of digestible protein, nitrogen free extract, crude fiber and fat. Ash content was determined following AOAC official method 942.05[18], while Ca and P were determined following AOAC official method 968.08[19]. Feed particle size distribution was analyzed at both feeding phases for each treatment using ASAE Standard S319[20]. The average geometric diameters for the starter diets A, B, C, D, E and F were 876, 876, 876, 807, 867 and 850 µm respectively and 577, 546, 548, 460, 383 and 445 µm for grower treatments A, B, C, D, E and F, respectively. For the treatments that contained CEP, analyzed phytase activity averaged 3309 FTU kg^{-1} for crumbled starter and 3043 FTU kg^{-1} for pelleted grower.

Data collection: Chickens and feed were weighed by pen on day 1, 15, 28 and 35. Pen temperature and mortality were checked twice daily and feeders were shaken once per day to 14 day and twice per day thereafter.

Data analysis: Data were analyzed by one way ANOVA using the general linear model procedure of SAS[21] with pen as the experimental unit. Means were separated using the Least Significant Difference Procedure. Differences between means were separated using the LS means method and significance was reported at a probability level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Generally, birds fed diets with CEP had improved growth performance. The body weight (BW), cumulative feed intake (FI) and body weight gain (BWG) at 15, 28 and 35 day were higher ($p<0.05$) for birds consuming a diet supplemented with CEP (diets C, D, E and F) compared to those consuming either unsupplemented (control) diets (A and B) (Table 4). The one exception was with diet E for which FI during 0-28 day was equivalent to that with diet B and the overall 35 day FCR which was lower with diet E compared with diets A and B. There were no significant difference ($p>0.05$) among the diets supplemented with CEP in terms of FI, BW, BWG, or FCR. Birds fed diet F, which had the highest assumed matrix value for Ca (0.17%), had 1% higher mortality ($p<0.05$) during the first 14 days when compared to the other treatments but that effect disappeared after switching to the grower diet. In general, the overall mortality rate in this experiment was less than 3%. Furthermore, there were no differences in FI, BW, BWG, FCR, or mortality as broilers grew to market age between birds consuming a diet without CEP but with decreasing Ca:AvP$_i$ ratio (starter to grower) and those consuming a diet without CEP but with constant Ca:AvP$_i$ratio (diets A and B).

One of the main functions of supplementing with phytase enzyme is to degrade phytate to make P$_i$ more available. However, as can be seen from the formulation tables (Table 2 and 3), birds consuming diet C were provided adequate P$_i$ (AvP$_i$ was formulated to be same as diet B, not including matrix value), whereas AvP$_i$ supplementation was reduced in the other diets with CEP inclusion to account for the AvP$_i$ that is assumed to be available due to CEP. In previous studies, it has been shown that supplementing P$_i$-deficient diets with phytase improved bird growth performance[22-26]. Improved growth performance with phytase addition was attributed to increasing P$_i$ availability thereby fulfilling the birds' requirement. However, little is known about benefits of

Table 4: Least-square means for feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion ratio (FCR) and mortality of broiler males raised to 35 day

	Treatment code[1]							
Age period (day)	A	B	C	D	E	F	SE[2]	p-value
FI (g bird^{-1})								
Day 0-15	613^{B}	625^{B}	663^{A}	659^{A}	652^{A}	653^{A}	8	0.0001
Day 0-28	2268^{C}	2306^{BC}	2428^{A}	2390^{A}	2372^{AB}	2399^{A}	24	0.0001
Day 0-35	3544^{B}	3587^{B}	3740^{A}	3705^{A}	3685^{A}	3721^{A}	25	0.0001
Day 16-28	1655^{C}	1681^{BC}	1766^{A}	1731^{AB}	1720^{AB}	1747^{A}	19	0.0010
Day 29-35	1276^{B}	1281^{B}	1312^{A}	1315^{A}	1313^{A}	1322^{A}	10	0.0010
BW (g bird^{-1})								
Day 0	47	47	47	47	47	47	0.3	0.7430
Day 15	498^{B}	505^{B}	542^{A}	539^{A}	535^{A}	538^{A}	6	0.0001
Day 28	1652^{B}	1663^{B}	1755^{A}	1738^{A}	1730^{A}	1751^{A}	16	0.0001
Day 35	2420^{B}	2445^{B}	2592^{A}	2561^{A}	2569^{A}	2565^{A}	16	0.0001
BWG (g bird^{-1})								
Day 0-15	451^{B}	459^{B}	495^{A}	493^{A}	488^{A}	491^{A}	6	0.0001
Day 0-28	1605^{B}	1616^{B}	1708^{A}	1692^{A}	1683^{A}	1703^{A}	16	0.0001
Day 0-35	2373^{B}	2398^{B}	2545^{A}	2514^{A}	2522^{A}	2518^{A}	15	0.0001
Day 16-28	1154^{B}	1157^{B}	1213^{A}	1199^{A}	1195^{A}	1213^{A}	12	0.0010
Day 29-35	768^{B}	782^{B}	837^{A}	822^{A}	839^{A}	815^{A}	11	0.0001
FCR (g:g)								
Day 0-15	1.3592	1.3651	1.3400	1.3393	1.3361	1.3338	0.012	0.2860
Day 0-28	1.4136	1.4278	1.4215	1.4133	1.4076	1.4109	0.009	0.5960
Day 0-35	1.4925^{ab}	1.4983^{a}	1.4775^{bc}	1.4758^{bc}	1.4667^{c}	1.4758^{bc}	0.007	0.0300
Day 16-28	1.4350	1.4517	1.4558	1.4425	1.4367	1.4400	0.010	0.6580
Day 29-35	1.6542^{A}	1.6425^{AB}	1.5967^{C}	1.6100^{BC}	1.5933^{C}	1.6192^{ABC}	0.013	0.0100
Mortality (%)								
Day 0-15	0.00^{b}	0.00^{b}	0.50^{ab}	0.00^{b}	0.00^{b}	1.50^{a}	0.4	0.0300
Day 0-28	1.00	0.00	1.00	0.00	1.50	0.50	0.5	0.3040
Day 0-35	1.50	0.50	2.00	0.50	2.50	2.60	0.8	0.2960
Day 16-28	1.00	0.00	1.00	0.00	1.60	0.50	0.5	0.3040
Day 29-35	0.50	0.50	0.50	1.00	0.50	0.50	0.6	0.9780

$^{a-c}$Means in a row within each variable that lack common superscript differ significantly ($p\leq 0.05$). $^{A-C}$Means in a row within each variable that lack common superscript differ significantly ($p\leq 0.01$). [1]A: Descending Ca:AvP$_i$ (No CEP), B: Fixed 2:1 Ca:AvP$_i$ (No CEP), C: Theoretical 2:1 Ca:AvP$_i$ with CEP added on top, D to F: Theoretical 2:1 Ca:AvP$_i$ with CEP given matrix value for Ca and P$_i$. P$_i$ matrix value is assumed to be fixed but Ca matrix value increasing from diet D to F. [2]SE: Standard error for n = 12 pens adding the enzyme to a P_i adequate diet. Nelson *et al.*[24] found that BWG of birds fed a P_i-deficient diet supplemented with phytase tended to be greater than that of birds fed a phytase-supplemented diet with adequate Pi. However, Nelson *et al.*[24] and dos Santos *et al.*[27] demonstrated that birds fed positive control diets supplemented with phytase exhibited better FCR than positive control without addition of the enzyme. In the current trial, CEP supplementation to an adequate P_i diet resulted in greater BW and FI compared to the same diet without CEP. Within the broiler industry, adoption of 'superdosing' (1500 FTU kg^{-1} or higher) of phytase is increasing because of the improved bird performance observed as compared to standard dosing (500-1000 FTU kg^{-1})[28,29]. In this case, the addition of phytase in adequate diet would be considered as superdosing of phytase.

Phytate is considered an anti-nutritional factor that forms insoluble complexes with various minerals and amino acids making them unavailable[30,31] in addition to reducing P_i availability[32]. Phytate also binds with enzymes and increases mucin secretion which increase exogenous losses[33,34]. Increased mucin secretion indicates that phytate may be an irritant in the gut causing reduced appetite. Phytate has also been known to upregulate the expression of somatostatin and down regulate expression of ghrelin[35] resulting in reduced appetite. One of the possible reasons, in this study, for greater FI by the birds consuming diets supplemented with phytase may be attributed to lower phytate in the gut. The FCRs among treatments were largely the same being better than controls, which is in agreement with other reports[36,37], who demonstrated a phytase effect on BW and FI but not on FCR. Simons *et al.*[26] reported improved feed efficiency with 1500 FTU kg^{-1} phytase supplementation. However, utilizing multifactorial analyses of phytase feeding trial data, Rosen[38] argued that feed efficiency response to phytase had been declining over recent years due to concurrent improvement in broiler strains, feeds and management techniques.

As mentioned earlier, there was no difference in growth performance among diets assigned different CEP Ca matrix values. It was expected that diets with a higher inclusion of inorganic Ca due to a CEP low matrix value would have poor performance parameters compared to other diets. Earlier phytase studies have shown phytate precipitation, caused by Ca through formation of insoluble Ca-phytate complexes, are less accessible to phytase[39]. There is possibility of direct depression of phytase enzyme activity in response to excessive Ca competition for the active sites on the enzyme[40]. Additionally, due to a more basic intestinal pH associated with elevated dietary Ca, reduced levels of soluble minerals might interfere with mineral absorption[41].

These observations are in contrast to results reported by Sebastian *et al.*[25], who suggested that low Ca availability in diet (0.60%) associated with supplemental phytase resulted in better growth performance when compared with high Ca availability (1.25%) in phytase-supplemented diets. The P_i was kept constant at 0.3% for both Ca levels. The 1.25% Ca level is greater than the requirement of broilers[14] and excessive Ca intake with a larger Ca:AvP_i ratio is known to cause kidney damage[42] and increased incidence of leg abnormalities[43]. Kidney damage can lead to calcification of soft tissues where excess Ca can precipitate[44]. High Ca:P_i ratio would have also reduced P_i availability[14]. These factors have led to poor performance due to high Ca consumption in the Sebastian *et al.*[25] trial, while low Ca with phytase have resulted in optimum Ca level for birds leading to better performance. High matrix values of CEP could cause a lower requirement for supplemental Ca. The Ca requirement according to National Research Council[14] should be 0.8-1.0% depending on age and gender but the 0.76-0.90% Ca level is recommended by the Cobb 500 broiler performance and nutrition supplement guide[45]. The Ross nutrient specification guide[46] specifies 0.68-0.96% depending on the age of bird. Based on current findings, it is possible that either phytase was releasing Ca as assumed, or it was releasing less Ca, which was not supported by the growth performance of broilers, since Ca requirement is closer to what Driver *et al.*[47] suggested it to be (0.625% Ca) to maximize BWG and FCR. Another reason for the lack of BWG difference, across different Ca matrix values observed in current trial, could have been from smaller variations in matrix values.

The goal of this experiment was to compare growth performance between decreasing Ca:AvP_i ratio and constant Ca:AvP_i as broilers grew to market age. There were no differences among decreasing or constant Ca:AvP_i ratios, which means that low ratios can be utilized without affecting broiler growth performance. This indicates that a Ca:AvP_i ratio, lower than the current standard, can be used for optimized growth performance as suggested by Driver *et al.*[47]. However, in order to observe significant differences in performance, greater variation of matrix ratio needs to be applied, which can be addressed in future trials. Moreover, measuring phytate P content in feed could allow for measuring the matrix in relation to phytate P instead of available P. As of now, the CEP has not been extensively investigated, however, it offers a sustainable option that is capable of improving nutrient utilization in monogastrics.

CONCLUSION

The results of this experiment allowed us to conclude that supplementation of the CEP increased BW and FI without a consistent effect on FCR when with or without including Ca (and P_i) matrix values. Furthermore, decreasing the Ca:AvP_i ratio in grower phase produced the same result as a fixed ratio of 2:1, which might indicate that a lower Ca:AvP_i than the standard Ca:AvP_i (2:1) can be used as broilers grow to market age. Also, assigning different matrix values to phytase did not affect performance of birds fed diets formulated according to Ross nutritional guidelines. Greater variation in matrix values might be necessary in order to achieve a significant response.

SIGNIFICANCE STATEMENT

This study explored the use of a feed additive expressed in corn. Phytase is widely used in monogastric nutrition, having the ability to incorporate the enzyme in diets by simply adding corn to the feed could simplify feed manufacturing. In addition, based on the matrix values investigated, it is possible to reduce the inclusion rate of inorganic P by assigning a higher matrix value for the CEP without negatively affecting performance.

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