A novel consensus bacterial 6-phytase variant completely replaced inorganic phosphate in broiler diets, maintaining growth performance and bone quality: data from two independent trials

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ABSTRACT Total replacement of dietary inorganic phosphate (**Pi**) by a novel consensus bacterial 6-phytase variant (**PhyG**) in phytate-rich diets (>0.3% phytate-P) was investigated in 2 trials using growth performance and bone quality as outcome measures. Both trials utilized a completely randomized design with 5 dietary treatments across 4 phases: starter (0–10 d), grower (10–21 d), finisher 1 (21–35 d), and finisher 2 (35–42 d). Treatments comprised a nutritionally adequate positive control (**PC**) diet containing monocalcium phosphate and 4 experimental diets (IPF1, IPF2, IPF3, and IPF4), all containing no added Pi and reduced in Ca by 0.2 to 0.3% units vs. PC. IPF1contained PhyG at 1,000 FTU/kg (all phases); IPF2 contained PhyG at 1,000 FTU/kg (all phases) and was additionally reduced in digestible AA, ME, and sodium (−0.2 to −0.4% points, −74 kcal/kg, –0.04% points, respectively, vs. PC); IPF3 contained PhyG at 3,000 FTU/kg in starter, 2,000 FTU/kg in grower, and 1,000 FTU/kg in finisher phases; and IPF4 contained xylanase (2,000 U/kg) and PhyG (2,000 FTU/kg in starter, 1,500 FTU/kg in grower, and 1,000 FTU/kg in finisher phases) and was additionally reduced in ME (–71 kcal/kg vs. PC). Ross 308 broilers were used (trial 1: n = 1,200 mixed sex; 24 birds per pen × 10 replicates; trial 2: n = 1,300 males; 26 birds × 10 replicates). During all phases in both trials, all IPF treatments maintained or improved BW, ADG, ADFI, FCR and BW-corrected FCRc and bone quality parameters vs. PC. vs. PC, treatment IPF3 increased ADG during starter phase (+10.8%) and reduced overall FCRc (−12 points, $P < 0.05$) in Trial 1, and increased overall ADG (+4.4%), day 35 and day 42 BW (+3.5%, +4.9%), and reduced overall FCRc (−11 points) in Trial 2 ($P < 0.05$). IPF4 produced equivalent performance to IPF3 (both trials). These are the first data to demonstrate total replacement of Pi by microbial phytase during an entire growth cycle in broiler diets.

Key words: broiler, inorganic phosphate, phytase, phytate

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INTRODUCTION

Phosphorus (**P**) is essential for broiler growth and bone formation, as well as supporting the development of the nervous system, energy conversion, and egg production (Li et al., 2017, 2020). Availability of P in plant-based broiler diets is low because the majority (70–80%) is in the form of phytate (the salt of phytic acid, myoinositol hexaphosphate; IP_6) which is largely inaccessible due to a lack of endogenous enzyme activity in birds fed commercial diets containing limestone (Selle and Ravindran, 2007). Inorganic sources, typically monocalcium phosphate (**MCP**) or dicalcium phosphate are routinely added to feed to meet the P requirement of the birds, but this confers a high cost on producers because feed phosphate ingredients are one of the most costly animal feed components; the global feed phosphate market was estimated at USD 2.25 billion in 2018 (Markets and Markets, 2018). Any undigested P is excreted which is a wasteful use of a finite resource and a potential environmental pollutant (Sharply et al., 1994). Based on current usage levels, it is estimated that total removal of inorganic phosphate from broiler production could reduce usage of MCP and its equivalent in broiler feeds by 1 million tons per year. This would reduce phosphorus excretion and improve

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the environmental sustainability of broiler reduction substantially. Thus, nutritional approaches that maximize the utilization of dietary P and reduce the need to add inorganic P (**Pi**) are sought.

Microbial phytases are well established as the primary current nutritional approach for increasing phytate degradation and P (and Ca) availability in broiler diets (Lei et al., 2013; Dersjant-Li et al., 2015). Their efficacy has been clearly demonstrated (see, e.g., reviews by Selle and Ravindran, 2007; Dersjant-Li et al., 2015; Abd El-Hack et al., 2018) and they are commonly used in animal diets. Studies have revealed complex physical and chemical relationships occur in the gastrointestinal tract (**GIT**) between phytate, phytase, minerals, and other nutrients (Ravindran et al., 2008; Amerah et al., 2014; Li et al., 2016; Sommerfeld et al., 2018; Bello et al., 2019). These must be taken into account when formulating diets with phytase to achieve maximum efficacy while maintaining nutritional balance. The current knowledge base has enabled the use of phytase-supplemented diets with down specification of P (and Ca) content, and a consequent reduction in Pi addition to diets which has reduced P excretion into the environment. However, total Pi replacement by commercial dose-levels of a phytase throughout the entire growth cycle has yet to be demonstrated in broilers.

A recent study has shown that a high dose (4,000 FTU/kg) of a phytase derived from *Citrobacter braakii* was able to ameliorate the negative effect of total removal of Pi from a Ca-reduced diet on broiler growth performance and bone conformation in grower and finisher phases (Ribeiro et al., 2019). In pigs, whose digestible-P requirement is lower than that of young broilers (NRC 1994, 2012), a novel consensus bacterial 6-phytase with high capacity to degrade phytate was recently shown to be effective as a total replacement for inorganic-P during 40 to 70 d of age when applied at a dose-level of 1,000 FTU/kg to a corn-soybean meal–based diet containing a commercially relevant phytate-P content (Dersjant-Li et al., 2020a). It was estimated that the same phytase at the same dose level in broilers could replace 2.07 g MCP-P per kilogram of diet based on digestible-P improvement in a comparable diet with similar phytate-P content, maintaining performance and tibia ash during all growth phases equivalent to a nutritionally adequate control diet (Dersjant-Li et al., 2020b). These studies raise the question as to whether, if combined with other nutritional approaches to maximize substrate availability and feed efficiency, the novel consensus bacterial phytase has the potential to totally replace added Pi throughout an entire broiler growth cycle (0–42 d of age) in diets that do not contain P-rich animal-derived ingredients such as meat and bone meal. Achieving total Pi-replacement is especially challenging in young birds (day 0–10) where the P requirement is high, bones are not yet mature, but the rate of overall growth is high, so that there is a risk of P-deficiency (Rath et al., 2000; Dibner et al., 2007). Increasing the phytate content of the diet is one approach to increasing substrate availability so that exogenous phytase can release sufficient available P to meet the P requirement of young birds, but this has to be balanced against the potential antinutritional effects of increased dietary phytate (Selle and Ravindran, 2007; Humer et al., 2015). Other strategies could include the addition of nonstarch polysaccharide (**NSP**)-degrading enzyme(s), such as xylanase, to support the digestion of nutritionally complex commercial diets.

Here, we present data from 2 trials that aimed to test whether the novel bacterial consensus 6-phytase, with or without supplemental xylanase, could maintain (vs. a positive control diet and breeders' performance objectives) normal growth, bone characteristics, and carcass quality in broilers throughout an entire growth cycle, when added to Pi-free diets containing a high substrate content (>0.3% phytate-P) and without meat and bone meal.

MATERIALS AND METHODS

Birds, Housing, and Experimental Design

All experimental protocols were approved by the Animal Care and Use Committee of Texas A&M University, USA, where both studies (trial 1 and trial 2) were conducted.

Trial 1: A total of 1,200 Ross 308 broilers were obtained from a commercial hatchery on day of hatch. Hatchlings were weighed, sexed, and randomly assigned to floor pens (24 birds per pen; stocking density 0.07–0.09 m^2/bird) containing clean litter. Ten pens were randomly assigned to each of 5 treatments in a completely randomized design. Each pen contained equal numbers of males and females. Pens were ventilated with mechanical ventilation in which the temperature was maintained initially at 35°C and gradually reduced to 27°C by week 4. The lighting regimen was 18L:6D. Birds were offered ad libitum access to water and to the experimental diets for the duration of the study (42 d). There were 24 birds per pen at each of starter (day 0–10) and grower (day 10–21) phases and 20 birds per pen at each of finisher 1 (day 21–35) and 2 (day 35–42) phases.

Trial 2: A total of 1,300 Ross 308 male broilers were obtained on day of hatch and assigned to floor pens as described above but with 26 birds per pen at each of starter and grower phases and 20 birds per pen at each of finisher 1 and finisher 2 phases. Litter, environmental conditions and stocking density were identical to Trial 1. In both trials, birds were offered *ad libitum* access to water and to experimental diets for the duration of the study (42 d).

Treatments, Diets, and Enzymes

In both trials, birds were fed phased diets in crumble form during starter and in pelleted form during grower and finisher 1 and 2 phases. Five treatments were used, as follows: 1) a positive control diet containing Pi in the form of MCP, formulated to meet (but not exceed) the nutritional requirements of the birds as set by the

Table 1. Details of the experimental treatments.

Treatment	PhyG, FTU/kg				Xylanase, XU/kg	Nutrient down specification *vs.* PC
Phase	day 0–10	day 10–21	day 21–35	day 35–42	All phases	All phases
PC	None				None	None
IPF1	1,000				None	Pi-free, Ca −0.2 to −0.3 p.p.
IPF2	1,000				None	Pi-free, Ca −0.2 to −0.3 p.p., ME −74 kcal/kg, digAA −0.2 to −0.4 p.p., Na −0.04 p.p.
IPF3	3,000	2,000	1,000	1,000	None	Pi-free, Ca −0.2 to −0.3 p.p.
IPF4	2,000	1,500	1,000	1,000	2,000	Pi-free, Ca −0.2 to −0.3 p.p, ME −71 kcal/kg

Abbreviations: Pi, inorganic phosphate; p.p, percentage points.

breeder (Aviagen, 2014a) (PC); 2) a basal diet (**BD**) without added Pi and with a reduction in Ca (of approximately 0.2–0.3% points) relative to PC, supplemented with a novel bacterial consensus 6-phytase variant expressed in *Trichoderma reesei* (PhyG, DuPont Nutrition and Biosciences) at a dose-level of 1,000 FTU/kg in all phases (IPF1); 3) the BD down specified in digestible amino acids (dig AA), ME, and Na, supplemented with PhyG at 1,000 FTU/kg in all phases (IPF2); 4) the BD supplemented with PhyG at 3,000 FTU/kg in starter phase, 2,000 FTU/kg in grower phase, and 1,000 FTU/kg in finisher 1 and 2 phases (IPF3); 5) the BD reformulated with a reduction of 71 kcal/kg ME, supplemented with 2,000 XU/kg of a commercial xylanase produced in *T. reesei* (Danisco xylanase, DuPont Nutrition, and Biosciences) and with PhyG at 2,000 FTU/kg in starter, 1,500 FTU/kg in grower, and 1,000 FTU/kg in finisher 1 and 2 phases, respectively (IPF4). Details of the treatments and nutrient down specifications are presented in Table 1.

The negative control BD was not administered as a stand-alone diet because the total removal of Pi would have been insufficient to support growth and therefore unethical as a treatment.

The ingredient and nutrient content of the phased diets are presented in Table 2 (trial 1) and Table 3 (trial 2). The BD were based on corn, wheat, soybean meal, rapeseed, rice, and wheat bran, with high phytate content (>0.3% phytate-P). Diets were essentially the same for the 2 trials except that in trial 2 oat hulls were maintained at 1% and soy hulls were included as well. Oat hulls were included to stimulate the gizzard development and soy hulls were used as filler materials.

Sampling and Measurements

In both trials, body weight and feed intake were measured on day 0, 10, 21, 35, and 42, on a per pen basis, and used to calculate average BW, expressed on a per bird basis. Pens were checked daily for bird mortality which was recorded and used to calculate the mortality corrected average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**). For the overall period (day 0–42) body weight corrected feed conversion ratio (**FCRc**) was also calculated, by correction of FCR values by 3 points per 100g of BW difference from the PC.

On day 21 and day 42, 4 birds per pen (2 males and 2 females, trial 1; all males, trial 2) were selected at random, euthanized by CO_2 gas and their left tibias collected and frozen at −20°C for later determination of defatted tibia ash (individual birds in trial 1 and pooled per pen in trial 2) and breaking strength (tibias measured individually). On day 42, 6 birds per pen (3 males and 3 females in trial 1, all males in trial 2) were weighed, euthanized by CO_2 gas and carcass component yields were measured using certified standard commercial processing procedures.

Samples of the PC, treatments IPF1, IPF2, IPF3, and IPF4 were analyzed for total P and Ca by Texas A&M University, USA. Phytase and xylanase activities in the final diets were analyzed by DuPont Research Centre, Brabrand, Denmark.

Chemical Analyses

All samples were analyzed in duplicate. Thawed tibias were defatted and tibia ash determined in accordance with the method described in the study by Dersjant-Li et al. (2020b). The breaking strength of the tibias was measured by the 3-point bending test using an Instron Universal Testing Instrument, as described by Bello et al. (2019). Phosphorus and Ca in feed were analyzed by microwave digestion and inductively coupled plasma–optical emission spectrometry in accordance with method AOAC 2011.14 (AOAC, 2011). Phytase activities were determined using an internally validated method adapted from ISO 30024:2009, where one FTU (phytase unit) was defined as the quantity of enzyme that released 1 μmol of Pi per minute from 5.0 mmol/L sodium phytate substrate at pH5.5 at 37°C. Xylanase activities were determined according to the method described by Romero et al. (2014), where one xylanase unit was defined as the amount of enzyme that released 0.48 μmol of reducing sugar as xylose from wheat arabinoxylan per minute at pH4.2 and 50°C.

Statistical Analysis

Pen was the experimental unit in all analyses except for tibia ash in trial 1 and bone strength, where individual bird was the experimental unit. For each outcome measure, analysis of variance was performed to determine differences between treatments in a randomized design,

Table 2. Ingredient and calculated nutrient content (%, as fed basis) of the 5 treatment diets, by phase; trial 1.

	Starter (day 0–10)				Grower (day 10–21)			
Treatment	PC	IPF1, 3	IPF2[2]	IPF4	PC	IPF1, 3	IPF2[2]	IPF4
Ingredient (%, as fed basis)								
Corn	34.94	34.94	32.30	32.12	32.43	32.43	30.91	30.06
Soybean meal, 48% CP	27.64	27.64	25.62	27.64	26.53	26.53	24.78	26.53
Wheat	20.44	20.44	20.44	20.44	22.89	22.89	22.89	22.89
Rapeseed meal	5.00	5.00	5.51	5.00	5.50	5.50	6.23	5.50
Rice bran	5.00	5.00	6.00	5.00	4.50	4.50	5.00	4.50
Wheat bran	1.00	1.00	2.15	2.53	1.50	1.50	2.84	2.65
Oat hulls	1.00	2.65	4.92	4.17	1.00	2.22	3.53	3.73
Soy oil	0.74	0.74	0.62	0.51	2.25	2.25	1.76	1.96
Limestone	1.33	1.17	1.17	1.16	1.11	0.98	1.05	0.97
Monocalcium phosphate	1.49	-	-	-	1.10	-	-	-
DL-methionine	0.31	0.31	0.36	0.31	0.24	0.24	0.22	0.24
L-lysine HCL	0.37	0.37	0.29	0.36	0.24	0.24	0.22	0.23
L-threonine	0.10	0.10	0.09	0.10	0.08	0.08	0.06	0.08
NaCl	0.35	0.35	0.24	0.35	0.35	0.35	0.24	0.35
Vitamin and mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Phytase, FTU/kg	-	1,000, 3,000	1,000	2,000	-	1,000, 2,000	1,000	1,500
Xylanase, U/kg	-	-		2,000	-	-	-	2,000
Nutrient composition, %								
ME, kcal/kg	2,950	2,950	2,876	2,879	3,050	3,050	2,978	2,978
Crude protein	22.01	22.09	21.50	22.18	21.64	21.70	21.30	21.76
Calcium	0.92	0.62	0.62	0.62	0.77	0.54	0.54	0.54
Total phosphorus	0.84	0.50	0.53	0.52	0.74	0.50	0.52	0.51
Available phosphorus[3]	0.43	0.15	0.15	0.15	0.36	0.15	0.15	0.15
Sodium	0.17	0.17	0.13	0.17	0.17	0.17	0.13	0.17
Dig. lysine	1.22	1.22	1.18	1.22	1.10	1.10	1.06	1.10
Dig. methionine and cysteine	0.91	0.91	0.87	0.91	0.84	0.84	0.81	0.84
Dig. Threonine	0.76	0.76	0.73	0.76	0.73	0.73	0.70	0.73
Dig. Tryptophan	0.22	0.22	0.22	0.22	0.22	0.22	0.21	0.22
Phytate-P	0.35	0.36	0.38	0.37	0.35	0.35	0.37	0.36

	Finisher 1 (day 21–35)				Finisher 2 (day 35–42)			
Treatment	PC	IPF1, 3	IPF2[2]	IPF4	PC	IPF1, 3	IPF2[2]	IPF4
Ingredient (%, as fed basis)								
Corn	31.89	31.89	29.51	31.28	31.19	31.18	28.38	30.61
Soybean meal, 48%	21.28	21.28	19.65	21.30	18.26	18.26	17.25	18.26
Wheat	28.21	28.21	28.21	28.21	31.32	31.32	31.32	31.32
Rapeseed meal	6.00	6.00	7.47	6.00	6.30	6.30	6.82	6.30
Rice bran	4.30	4.30	4.85	4.30	4.30	4.30	4.42	4.30
Wheat bran	2.00	2.00	4.00	2.05	2.20	2.20	4.00	2.54
Oat hulls	1.00	1.90	2.87	3.06	1.00	1.99	3.76	2.93
Soy oil	2.50	2.50	1.85	1.89	2.67	2.67	2.50	1.97
Limestone	0.87	0.79	0.67	0.78	0.88	0.71	0.70	0.71
Monocalcium phosphate	0.82	-	-	-	0.82	-	-	-
DL-methionine	0.19	0.19	0.21	0.19	0.15	0.15	0.19	0.15
L-lysine HCL	0.25	0.25	0.15	0.25	0.22	0.22	0.12	0.22
L-threonine	0.06	0.06	0.03	0.06	0.05	0.05	0.02	0.05
NaCl	0.34	0.34	0.23	0.34	0.34	0.34	0.23	0.33
Vitamin and mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Phytase, FTU/kg	-	1,000, 1,000	1,000	1,000	-	1,000	1,000	1,000
Xylanase, U/kg	-	-	-	2,000	-	-	-	2,000
Nutrient composition, %								
ME, kcal/kg	3,100	3,100	3,023	3,027	3,120	3,120	3,047.00	3,048
Crude protein	20.06	20.10	20.02	20.13	19.10	19.14	18.94	19.20
Calcium	0.62	0.42	0.42	0.42	0.62	0.42	0.42	0.42
Total phosphorus	0.67	0.48	0.52	0.48	0.66	0.47	0.50	0.48
Available phosphorus	0.30	0.14	0.15	0.14	0.30	0.14	0.15	0.14
Sodium	0.17	0.17	0.13	0.17	0.17	0.17	0.13	0.17
Dig. lysine	1.00	1.00	0.96	1.00	0.92	0.92	0.88	0.92
Dig. Methionine and cysteine	0.76	0.76	0.73	0.76	0.70	0.70	0.67	0.70
Dig. threonine	0.66	0.66	0.63	0.66	0.61	0.61	0.58	0.61
Dig. tryptophan	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
Phytate-P	0.34	0.35	0.38	0.35	0.34	0.34	0.36	0.35

[1]Vitamin premix added at this rate of 8,818 IU vitamin A, 3,086 IU vitamin D_3, 37 IU vitamin E, 0.0132 mg B_{12}, 4.676 mg riboflavin, 36.74 mg niacin, 16.17 mg d-pantothenic acid, 382.14 mg choline, 1.18 mg menadione, 1.4 mg folic acid, 5.74 mg pyridoxine, 2.35 mg thiamine, 0.44 mg biotin per kg diet and trace mineral premix added at this rate of 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contain less than 1% mineral oil.

[2]With down specification of dig AA, ME and Na in addition of reduction of Ca and total removal of inorganic P.

[3]Available P excluding contribution from phytases, for example, from basal diets.

Table 3. Ingredient and calculated nutrient content (%, as fed basis) of the 5 treatment by phase; trial 2.

	Starter (day 0–10)				Grower (day 10–21)			
Treatment	PC	IPF1, 3	IPF2[2]	IPF4	PC	IPF1, 3	IPF2[2]	IPF4
Ingredient, % of diet (as fed basis)								
Corn	34.94	34.94	32.30	32.12	32.43	32.43	30.91	30.06
Soybean meal, 48%	27.64	27.64	25.62	27.64	26.62	26.53	24.78	26.53
Wheat	20.44	20.44	20.44	20.44	22.45	22.89	22.89	22.89
Rapeseed meal	5.00	5.00	6.00	5.00	5.50	5.50	6.23	5.50
Rice bran	5.00	5.00	5.00	5.00	4.50	4.50	5.00	4.50
Wheat bran	1.00	1.00	3.00	2.54	1.50	1.50	2.84	2.65
Oat hulls	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soy hulls	0.05	1.41	3.41	2.94	0.10	1.00	2.42	2.53
Soy oil	0.75	0.75	0.61	0.53	2.38	2.25	1.73	1.97
Limestone	1.30	1.39	1.35	1.36	1.27	1.18	1.16	1.15
Monocalcium phosphate	1.44	-		-	1.04	-		-
DL-methionine	0.31	0.31	0.35	0.31	0.24	0.24	0.22	0.24
L-lysine HCL	0.37	0.37	0.28	0.36	0.23	0.24	0.22	0.23
L-threonine	0.10	0.10	0.08	0.10	0.08	0.08	0.06	0.08
NaCl	0.37	0.37	0.26	0.37	0.37	0.37	0.26	0.36
Vitamin and mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.35	0.30
Phytase, FTU/kg	-	1,000, 3,000	1,000	2,000	-	1,000, 2,000	1,000	1,500
Xylanase, U/kg	-	-	-	2,000	-	-	-	2,000
Nutrient composition, %								
ME, kcal/kg	2,950	2,950	2,876	2,879	3,050	3,050	2,977.71	2,978
Crude protein	21.38	21.54	21.27	21.74	20.99	21.11	20.85	21.29
Calcium	0.92	0.72	0.72	0.72	0.84	0.64	0.64	0.64
Total phosphorus	0.87	0.55	0.57	0.57	0.78	0.54	0.57	0.56
Available phosphorus	0.43	0.16	0.16	0.16	0.36	0.16	0.16	0.16
Sodium	0.17	0.17	0.13	0.17	0.17	0.17	0.13	0.17
Dig. lysine	1.22	1.22	1.18	1.22	1.10	1.10	1.06	1.10
Dig. methionine and cysteine	0.91	0.91	0.87	0.91	0.84	0.84	0.81	0.84
Dig. threonine	0.76	0.76	0.73	0.76	0.73	0.73	0.70	0.73
Dig. tryptophan	0.22	0.22	0.22	0.22	0.22	0.22	0.21	0.22
Phytate-P	0.34	0.34	0.36	0.35	0.34	0.34	0.36	0.35

	Finisher 1 (day 21–35)				Finisher 2 (day 35–42)			
Treatment	PC	IPF1, 3	IPF2[2]	IPF4	PC	IPF1, 3	IPF2[2]	IPF4
Ingredient, % of diet (as fed basis)								
Corn	31.89	31.89	29.47	31.28	31.19	31.18	28.38	30.61
Soybean meal, 48%	21.28	21.28	19.55	21.30	18.26	18.26	17.25	18.26
Wheat	27.45	28.21	28.21	28.21	30.76	31.32	31.32	31.32
Rapeseed meal	6.00	6.00	7.72	6.00	6.30	6.30	6.82	6.30
Rice bran	4.30	4.30	4.64	4.30	4.30	4.31	4.42	4.30
Wheat bran	2.00	2.00	4.00	2.24	2.20	2.20	4.00	2.54
Oat hulls	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soy hulls	0.15	0.67	1.67	1.71	0.20	0.80	2.61	1.75
Soy oil	2.76	2.50	1.85	1.85	2.87	2.67	2.49	1.98
Limestone	1.15	0.99	0.95	0.97	1.08	0.88	0.84	0.86
Monocalcium phosphate	0.87	-		-	0.76	-		-
DL-methionine	0.19	0.19	0.21	0.19	0.15	0.15	0.19	0.15
L-lysine HCL	0.25	0.25	0.15	0.25	0.23	0.22	0.12	0.22
L-threonine	0.07	0.06	0.03	0.06	0.05	0.05	0.02	0.05
NaCl	0.36	0.36	0.25	0.36	0.36	0.35	0.25	0.35
Vitamin and mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Phytase, FTU/kg	-	1,000, 1,000	1,000	1,000	-	1,000, 1,000	1,000	1,000
Xylanase, U/kg	-	-	-	2,000	-	-		2,000
Nutrient composition, %								
ME, kcal/kg	3,100	3,100	3,023	3,028	3,120	3,120	3,047	3,048
Crude protein	19.31	19.47	19.50	19.58	18.36	18.50	18.45	18.62
Calcium	0.75	0.55	0.55	0.55	0.70	0.50	0.50	0.50
Total phosphorus	0.73	0.53	0.57	0.54	0.70	0.53	0.55	0.53
Available phosphorus[3]	0.32	0.16	0.16	0.16	0.30	0.15	0.16	0.15
Sodium	0.17	0.17	0.13	0.17	0.17	0.17	0.13	0.17
Dig. lysine	1.00	1.00	0.96	1.00	0.92	0.92	0.88	0.92
Dig. methionine and cysteine	0.76	0.76	0.73	0.76	0.70	0.70	0.67	0.70
Dig. threonine	0.66	0.66	0.63	0.66	0.61	0.61	0.58	0.61
Dig. tryptophan	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
Phytate-P	0.33	0.33	0.36	0.33	0.33	0.33	0.34	0.33

[1]Vitamin premix added at this rate of 8,818 IU vitamin A, 3,086 IU vitamin D_3, 37 IU vitamin E, 0.0132 mg B_{12}, 4.676 mg riboflavin, 36.74 mg niacin, 16.17 mg d-pantothenic acid, 382.14 mg choline, 1.18 mg menadione, 1.4 mg folic acid, 5.74 mg pyridoxine, 2.35 mg thiamine, 0.44 mg biotin per kg diet and trace mineral premix added at this rate of 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contain less than 1% mineral oil.

[2]With down specification of dig AA, ME, and Na in addition of reduction of Ca and total removal of inorganic P.

[3]Available P excluding contribution from phytases, for example, from basal diets.

Table 4. Analyzed nutrient content (% as is) and enzyme activities of the treatment experimental diets, by phase; trial 1.

	PC	IPF1	IPF2	IPF3	IPF4
Total phosphorus, %					
Starter	0.81	0.53	0.52	0.53	0.55
Grower	0.74	0.49	0.53	0.49	0.53
Finisher 1	0.71	0.54	0.55	0.54	0.53
Finisher 2	0.74	0.51	0.52	0.51	0.53
Calcium, %					
Starter	0.92	0.69	0.64	0.69	0.63
Grower	0.78	0.58	0.65	0.58	0.56
Finisher 1	0.78	0.57	0.50	0.57	0.59
Finisher 2	0.74	0.48	0.49	0.48	0.51
Phytase activity, FTU/kg					
Starter	351	1,206	1,349	3,116	2,122
Grower	396	1,476	1,558	2,655	1,769
Finisher 1	385	1,540	1,334	1,344	1,443
Finisher 2	380	1,419	1,451	1,227	1,252
Xylanase activity, XU/kg					
Starter	-	-	-	-	1,999
Grower	-	-	-	-	2,244
Finisher 1	-	-	-	-	2,352
Finisher 2	-	-	-	-	1,836

using the fit model platform of JMP 14.0 (JMP, version 14.0, SAS Institute Inc., Cary, NC). Tukey's Honest Significant Difference test was used for post hoc separation of means. Significance was determined at $P < 0.05$.

RESULTS

Analyzed Ca, total P, and xylanase and phytase activities in each treatment diet at each growth phase are presented in Table 4 (trial 1) and 5 (trial 2). Compared with formulated levels, analyzed Ca among diets/phases ranged from 100 to 140% in trial 1, and from 83 to 125% in trial 2, whereas analyzed total P ranged from 96 to 113% in trial 1, and from 88 to 100% in trial 2. The analyzed xylanase activities in treatment IPF4 and phytase activities in treatments IPF1 to IPF4, after accounting for endogenous activity in the PC, were generally in line with targeted dose levels (Tables 4 and 5).

Growth Performance

The effects of treatment on growth performance measures per growth phase and cumulatively are shown in Table 6 (trial 1) and Table 7 (trial 2).

In both trials, during all growth phases, the growth performance of birds fed Pi-free diets supplemented with PhyG (at all evaluated dose-levels, with or without xylanase) was equivalent to, or improved, compared with that of birds fed the nutritionally adequate control diet (PC).

In trial 1 with mixed-sex birds, birds received treatment IPF3 exhibited increased ADG during day 0 to 10 and increased ADFI during day 0 to 10, compared with the PC diet (+10.8%, and +8.0% vs. PC, respectively, $P < 0.05$, Table 6). Overall (day 0–42), FCRc was reduced in the IPF3 group compared with PC (by −12 points, $P < 0.05$). During day 0 to 10, the phase-specific dosing regimen of PhyG in treatment IPF3 (PhyG added at 3,000, 2,000, 1,000, and 1,000 FTU/kg during starter, grower, finisher 1 and 2 phases, respectively) produced a greater ADG than the lower dosing regimen in IPF1 (1,000 FTU/kg in all phases) ($P < 0.05$). Compared with treatment IPF3, treatment IPF2 (PhyG at 1,000 FTU/kg plus full nutrient matrix applied) produced lower ADG (−6.4%) during day 0 to 10, and higher overall FCR (−7 points) ($P < 0.05$) but maintained ADG and FCRc compared with PC. Birds fed treatment IPF4 (that included xylanase in combination with a lower dosing regimen of PhyG than treatment IPF3), exhibited overall (day 0–42) performance characteristics (day 42 BW, ADG, ADFI, FCR, and FCRc) that were equivalent to IPF3.

In trial 2 with male broilers, improvements in growth performance among PhyG supplemented birds (compared to PC) were evident in a greater number of individual measures and growth phases than in trial 1, with the greatest improvements seen in the IPF3 treatment. In this group, FCR was improved during each of day 10 to 21, day 35 to 42, and overall (by −10, −7, and −6 points, respectively, vs. PC, $P < 0.05$, Table 7). Overall ADG, day 35 and day 42 BW and overall FCRc were also improved in IPF3 vs. PC (by +4.4, 3.5, 4.9% and −11 points, respectively, in IPF3 *vs.* PC, $P < 0.05$). Treatment IPF1 (equivalent in composition to IPF3 but with lower PhyG dosing regimen), also exhibited improvements in day 10 to 21, day −35 to 42, and overall FCR and FCRc compared with PC ($P < 0.05$; Table 7), but the magnitude of effects was lower than for treatment IPF3. Compared with IPF1, IPF3 improved day 10 to 21 ADG ($P < 0.05$). Treatment IPF2 produced improved day 10 to 21 FCR (−7 points) and day 21 to 35 ADFI (+5.5%) compared with PC ($P < 0.05$) but was otherwise equivalent to PC. Compared with the PC, treatment IPF4 (containing xylanase and PhyG) improved FCR during day 10 to 21 (−8 points) and overall FCRc (−9 points), and improved day 35 BW (+4.1%) and overall (+4.0%)

Table 5. Analyzed nutrient content (% as is) and enzyme activities of the treatment diets, by phase; trial 2.

	PC	IPF1	IPF2	IPF3	IPF4
Total phosphorus, %					
Starter	0.82	0.53	0.57	0.53	0.50
Grower	0.71	0.54	0.54	0.54	0.50
Finisher 1	0.66	0.49	0.54	0.49	0.50
Finisher 2	0.62	0.50	0.52	0.50	0.50
Calcium, %					
Starter	1.02	0.77	0.80	0.77	0.66
Grower	1.03	0.77	0.72	0.77	0.72
Finisher 1	0.84	0.55	0.56	0.55	0.69
Finisher 2	0.73	0.59	0.54	0.59	0.61
Phytase activity, FTU/kg					
Starter	476	1,182	1,287	2,754	1,957
Grower	365	1,012	1,012	1,415	1,293
Finisher 1	391	1,056	991	1,179	1,097
Finisher 2	400	1,096	1,134	1,171	1,386
Xylanase activity, XU/kg					
Starter	-	-	-	-	-
Grower	-	-	-	-	2,061
Finisher 1	-	-	-	-	1,917
Finisher 2	-	-	-	-	1,382

ADG. Birds in treatment IPF4 exhibited overall performance characteristics (final BW, ADG, ADFI, FCR, and FCRc) that were equivalent to treatment IPF3.

No significant differences in mortality levels among treatments were observed during any growth phase during either trial.

Tibia and Carcass Characteristics

Effects of treatment on tibia ash, bone strength and carcass characteristics are shown in Table 8 (trial 1) and Table 9 (trial 2). In both trials, all evaluated outcome measures in PhyG-supplemented birds (with or without xylanase) at both time points (day 21 and day 42) were equivalent to or improved compared with the PC.

In trial 1, tibia breaking strength and ash content at day 21 were equivalent among all treatments, whereas at day 42 tibia breaking strength of birds in treatments IPF3 and IPF4 exceeded those of the PC (by +14.1% and +19.2%, respectively, $P < 0.05$). Carcass characteristics of treatments IPF1, IPF2, and IPF4 were equivalent to PC, but birds in treatment IPF3 exhibited increased live weight (8.0%), carcass weight (+7.8%), breast weight (+13.8%), tender weight (+10.3%), and leg weight (+5.3%), compared with PC at day 42 ($P < 0.05$, Table 8). Carcass yields (%) were maintained equivalent with the PC across all treatments, whereas breast yield was increased in treatment IPF3 ($P < 0.05$). Fat yield was marginally but significantly reduced in treatments IPF3 and IPF4 vs. PC ($P < 0.05$).

In trial 2, no differences among treatments in tibia breaking strength or ash content at either day 21 or day 42 were observed (Table 9). Breast weight at day 42 was greater among birds in the IPF3 group compared with the PC (by +5.2%, $P < 0.05$) but other carcass characteristics were equivalent to PC. Birds in treatment IPF1 exhibited increased live weight (+4.8%), carcass weight (+4.5%), breast weight (+8.4%), and tender weight (6.5%) compared with PC, whereas birds in treatment IPF2 exhibited increased live weight only (+3.2% vs. PC, $P < 0.05$). In addition, birds in treatment IPF4 exhibited increased live weight (+3.5%), carcass weight (+3.8%), breast weight (+9.3%), and tender weight (+5.8%) compared with PC ($P < 0.05$), but not compared with any of the other PhyG treatment groups (IPF1 to IPF3). Carcass yields were maintained equivalent to PC across all treatments, whereas breast yield was increased in treatments IPF1 and IPF4 ($P < 0.05$).

DISCUSSION

The analyzed Ca and P levels in the diets were well within acceptable limits and the level of variation observed is not expected to have had any influence on treatment outcomes. After accounting for analyzed endogenous phytase activity in the respective PC diets, phytase recoveries from diets IPF1 to IPF4 were generally slightly lower than targeted dose levels, particularly in trial 2. However, with the exception of IPF2 grower and finisher 1 diets in trial 2, the shortfalls were relatively modest and consistent across both treatments and growth phases such that in treatments IPF3 and IPF4, where tiered dosing regimens by phase were used, there was no overlap and good spacing between each intended dose level in terms of phytase recoveries. It is therefore concluded that the recorded variation in analyzed phytase activities in comparison with targeted levels is unlikely to have influenced the treatment outcomes and that both phytase and xylanase were not overdosed.

Effects on Growth Performance

The equivalence of all PhyG treatments with the PC during all growth phases, in all growth measures, in both trials, suggests that in the tested dietary setting of a mixed diet containing high substrate levels and total absence of Pi, PhyG at 1,000 FTU/kg or higher was able

Table 6. Effect of total replacement of inorganic P by PhyG with or without xylanase, on growth performance of broilers fed high phytate (>0.3% phytate P), Ca-reduced diets; trial 1 (mixed males and females).

	PC1	IPF1	IPF2	IPF3	IPF4	SEM	*P*-value[1]
Starter, day 0–10							
Day 10 BW, g/bird	283.9	285.4	283.5	291.9	290.6	2.72	0.101
ADG, g/bird	24.57^{b}	25.25^{b}	25.49^{b}	27.22^{a}	26.03^{a,b}	0.421	0.001
ADFI, g/bird	27.87^{b}	28.21^{a,b}	28.82^{a,b}	30.09^{a}	28.91^{a,b}	0.496	0.032
FCR, g:g	1.134	1.117	1.130	1.106	1.110	0.007	0.041
Grower, day 10–21							
ADG, g/bird	62.42	61.75	61.72	64.49	63.56	0.847	0.111
ADFI, g/bird	87.61	88.90	88.41	90.47	91.54	0.988	0.049
FCR, g:g	1.404	1.440	1.433	1.404	1.442	0.010	0.015
Cumulative, day 0–21							
Day 21 BW, g/bird	966.3^{a,b}	959.8^{a,b}	956.1^{b}	999.8^{a}	987.1^{a,b}	10.75	0.027
ADG, g/bird	44.02	43.04	43.05	44.67	43.62	0.556	0.217
ADFI, g/bird	58.71	58.28	58.24	59.17	59.02	0.695	0.834
FCR, g:g	1.334	1.354	1.353	1.325	1.353	0.007	0.017
Finisher 1, day 21–35							
ADG, g/bird	88.88	90.81	89.78	92.69	89.51	1.975	0.688
ADFI, g/bird	152.2	153.6	155.7	155.3	149.3	2.37	0.314
FCR, g:g	1.717^{a,b}	1.694^{a,b}	1.737^{a}	1.677^{a,b}	1.669^{b}	0.017	0.034
Cumulative, day 0–35							
Day 35 BW, g/bird	2,220	2,239	2,227	2,306	2,248	32.8	0.371
ADG, g/bird	55.78	54.72	54.40	56.46	54.15	0.827	0.249
ADFI, g/bird	85.49	83.83	84.52	85.12	82.08	1.129	0.245
FCR, g:g	1.534^{a,b}	1.532^{a,b}	1.554^{a}	1.508^{b}	1.516^{b}	0.009	0.006
FCRc, g:g[2]	1.534^{a,b}	1.527^{a,b}	1.552^{a}	1.482^{b}	1.508^{a,b}	0.015	0.025
Day 35 BW, % breeders' performance objective[3]	103.5	104.4	103.9	107.6	104.9		
Day 35 FCR, % breeders' performance objective[3]	100.9	101.0	99.6	102.6	102.1		
Finisher 2, day 35–42							
ADG, g/bird	74.18	89.17	82.05	93.71	88.53	5.4	0.119
ADFI, g/bird	185.2	201.9	199.3	207.6	195.9	5.46	0.072
FCR, g:g[2]	2.591	2.282	2.513	2.263	2.247	0.102	0.059
Overall, day 0–42							
Day 42 BW, g/bird	2,770	2,872	2,836	2,984	2,886	60.9	0.182
ADG, g/bird	61.63	63.70	62.15	65.46	63.71	1.255	0.237
ADFI, g/bird	104.9	107.0	106.8	108.2	105.5	1.45	0.525
FCR, g:g	1.705^{a,b}	1.681^{a,b}	1.721^{a}	1.654^{b}	1.658^{b}	0.016	0.014
FCRc[2], g:g	1.705^{a}	1.650^{a,b}	1.701^{a,b}	1.590^{b}	1.623^{a,b}	0.028	0.025
Day 42 BW, % breeders' performance objective[3]	98.6	102.2	101.0	106.2	104.9		
Day 42 FCR, % breeders' performance objective[3]	98.9	100.4	98.0	101.9	101.7		
Feed cost, USD/kg BWG	0.507	0.479	0.476	0.475	0.474		

[a,b]Means in the same row with no common superscripts are significantly different at a probability level of $P < 0.05$.

All parameters are corrected for mortality.

Abbreviation: IPF, inorganic-P-free.

[1]Displayed *P* values < 0.05 without superscripts next to associated means indicates a statistically significant ANOVA result but nonsignificance from means separation using Tukey's honest significant difference test, at $P < 0.05$.

[2]FCRc: body weight corrected FCR, calculated by correction of FCR values by 3 points per 100g of BW difference from the PC.

[3]Breeder's objective: Ross 308/Ross 308 FF broiler: performance objectives; Aviagen 2014b.

to hydrolyze enough phytate to meet P requirements to maintain bone mineralization and growth in all growth phases. To the author's knowledge, this is the first study in broilers to demonstrate normal growth performance during all growth phases in phytase-supplemented diets without added Pi (and without added meat and bone meal). Of the very few previous studies that have tested total removal of Pi in broilers, the focus has been on finisher phase (Skinner et al., 1992; Ribeiro et al., 2019) in which the P requirement is comparatively low (dietary nPP requirements for broilers set by the NRC are 0.45% during day 0 to 21, 0.35% during day 21 to 42 and 0.30% during day 42 to 56 (NRC, 1994)), or grower-finisher phases (Scholey et al., 2018). The P requirement during starter phase is considerably higher due to the rapid rate of growth and development in young birds. Therefore, the challenge of ensuring that enough digestible P is released by the phytase to support normal growth and development during starter phase, in the total absence of Pi, remains considerable, and no previous studies have attempted to totally remove Pi from broiler diets from day 1; a phytase would need to provide >0.25% digestible P to meet the P requirement in starter phase, in diets with total removal of Pi and without meat and bone meal. The present study used a highly efficient phytase, together with other approaches including formulating diets with phytate rich commercially relevant ingredients such as rapeseed meal, wheat, and rice bran, with addition of oat hulls to stimulate gizzard development, and consideration of optimal Ca to P ratio in the diet. It is suggested that all of these approaches contributed to maintaining normal growth and bone

Table 7. Effect of total replacement of inorganic P by PhyG with/without xylanase, on growth performance of broilers fed high phytate (>0.3% phytate P), Ca-reduced diets; trial 2 (males only).

	PC	IPF1	IPF2	IPF3	IPF4	SEM	*P*-value[1]
Starter, day 0–10							
Day 10 BW, g/bird	263.6	265.7	257.7	269.1	268.9	3.33	0.114
ADG, g/bird	22.57^{a,b}	23.49^{a,b}	21.94^{b}	23.16^{a,b}	23.75^{a}	0.408	0.023
ADFI, g/bird	26.73	27.72	26.69	27.37	27.71	0.386	0.172
FCR, g:g	1.187	1.181	1.218	1.183	1.167	0.013	0.097
Grower, day 10–21							
ADG, g/bird	60.57^{c}	63.88^{b}	63.59^{b}	66.28^{a}	65.15^{a,b}	0.571	<0.001
ADFI, g/bird	88.67	88.74	88.92	90.75	90.40	0.804	0.213
FCR, g:g	1.465^{a}	1.390^{b}	1.399^{b}	1.370^{b}	1.388^{b}	0.014	<0.001
Starter-grower, day 0–21							
Day 21 BW, g/bird	930.5^{c}	973.8^{a,b}	962.8^{b,c}	999.0^{a}	989.2^{a,b}	8.03	<0.001
ADG, g/bird	42.03^{b}	43.49^{a,b}	43.15^{b}	45.05^{a}	43.57^{a,b}	0.384	<0.001
ADFI, g/bird	58.59	58.21	58.49	59.68	58.11	0.542	0.274
FCR, g:g	1.394^{a}	1.339^{b}	1.356^{a,b}	1.325^{b}	1.334^{b}	0.010	<0.001
Finisher 1, day 21–35							
ADG, g/bird	94.61	95.68	97.07	94.29	97.26	1.165	0.259
ADFI, g/bird	156.2^{b}	160.0^{a,b}	164.9^{a}	157.8^{b}	161.7^{a,b}	1.70	0.008
FCR, g:g	1.652	1.674	1.700	1.675	1.662	0.017	0.375
Starter-grower-finisher 1, day 0–35							
Day 35 BW, g/bird	2,259^{b}	2,333^{a}	2,324^{a,b}	2,338^{a}	2,353^{a}	16.8	0.003
ADG, g/bird	56.96	57.51	57.71	58.13	57.15	0.582	0.644
ADFI, g/bird	87.54	87.39	89.17	87.82	86.41	0.909	0.327
FCR, g:g	1.537	1.520	1.545	1.511	1.512	0.011	0.106
FCRc, g:g[2]	1.537	1.498	1.526	1.488	1.484	0.014	0.027
Day 35 BW, % breeders' performance objective[3]	98.9	102.2	101.8	102.4	103.1		
Day 35 FCR, % breeders' performance objective[3]	100.0	101.1	99.5	101.7	101.6		
Finisher 2, day 35–42							
ADG, g/bird	103.4^{b}	117.5^{a}	108.9^{a,b}	113.5^{a,b}	112.2^{a,b}	3.25	0.032
ADFI, g/bird	229.85	236.34	237.20	229.75	236.29	2.785	0.148
FCR, g:g	2.242^{a}	2.016^{b}	2.185^{a,b}	2.033^{b}	2.122^{a,b}	0.051	0.015
Overall, day 0–42							
Day 42 BW, g/bird	2,994^{b}	3,159^{a}	3,090^{a,b}	3,140^{a}	3,138^{a}	30.0	0.002
ADG, g/bird	66.85^{b}	69.57^{a}	68.96^{a,b}	69.80^{a}	69.53^{a}	0.648	0.015
ADFI, g/bird	113.3	114.1	116.8	114.0	114.9	0.90	0.091
FCR, g:g	1.696^{a}	1.641^{b,c}	1.694^{a,b}	1.634^{c}	1.654^{a,b,c}	0.013	<0.001
FCRc[2], g:g	1.696^{a}	1.590^{b,c}	1.665^{a,b}	1.590^{c}	1.611^{b,c}	0.018	<0.001
Day 42 BW, % breeders' performance objective[3]	99.0	104.5	102.2	103.9	103.8		
Day 42 FCR, % breeders' performance objective[3]	98.4	101.7	98.6	102.2	101.0		
Feed cost, USD/kg BWG	0.490	0.461	0.458	0.462	0.461		

[a-c]Means in the same row with no common superscripts are significantly different at a probability level of $P < 0.05$.

All parameters are corrected for mortality.

Abbreviation: IPF, inorganic-P-free.

[1]Displayed P values < 0.05 without superscripts next to associated means indicates a statistically significant ANOVA result but nonsignificance from means separation using Tukey's honest significant difference test, at $P < 0.05$.

[2]FCRc: body weight–corrected FCR, calculated by correction of FCR values by 3 points per 100g of BW difference from the PC.

[3]Breeder's objective: Ross 308/Ross 308 FF broiler: performance objectives; Aviagen, 2014b.

quality despite total removal of Pi from day 1 in wholly vegetable diets.

A dose-dependent response to supplemental microbial phytase in broilers has been described by several recent studies, including in low-phosphorus diets (Qian et al., 1997; Liu et al., 2015; Dersjant-Li et al., 2018). The magnitude of the dose-response effect can be influenced by the Ca to total P ratio (Dersjant-Li et al., 2018). The traditional dose level for microbial phytase in broiler diets has been 500 FTU/kg, but the knowledge that higher efficacy can be delivered with higher dose levels, provided Ca:P is in balance, is leading to an upward shift in the dose levels being utilized in production settings. A positive, linear, dose-response effect has previously been reported for PhyG on P-release and growth performance within a dose range of 0 to 1,000 FTU/kg, with optimal responses observed at 1,000 FTU PhyG in broilers (Dersjant-Li et al., 2020b). It is recognized that severe reduction or removal of Pi from the diet is likely to require higher dose levels of a phytase to release sufficient available P from phytate, or to breakdown large proportion of phytate to reduce its antinutritional effect. The actual, minimum, dose level that can adequately compensate for the reduction or removal of Pi is likely to be different for each individual phytase depending on its biochemical characteristics and efficacy to degrade phytate. The study by Ribeiro et al. (2019) used a combination of a high phytase dose level (4,000 FTU/kg) combined with an extreme reduction in Ca (so that dietary Ca and P remained in balance) to overcome the total removal of Pi during grower and finisher phases, and reported similar weight gain, feed conversion, and bone

Table 8. Effect of total replacement of added inorganic P by PhyG supplementation with or without xylanse on tibia ash, tibia breaking strength and carcass characteristics of broilers; trial 1 (mixed males and females).

	PC	IPF1	IPF2	IPF3	IPF4	SEM	*P*-value[1]
Day 21							
Tibia breaking strength, kgF	32.52	31.25	32.27	33.66	31.99	0.913	0.469
Tibia ash content, % DM	51.35	50.58	50.70	50.70	51.13	0.223	0.073
Day 42							
Tibia breaking strength, kgF	39.39^{b}	44.49^{a,b}	41.68^{a,b}	44.92^{a}	46.95^{a}	1.398	0.001
Tibia ash content, % DM	46.59	46.60	45.68	45.82	45.77	0.405	0.289
Live weight, g	2,740.6^{b}	2,850.0^{a,b}	2,815.0^{b}	2,958.3^{a}	2,850.6^{a,b}	34.12	<0.001
Carcass weight, g	2,107.4^{b}	2,191.6^{a,b}	2,141.0^{b}	2,272.5^{a}	2,194.5^{a,b}	25.19	<0.001
Breast weight, g	519.5^{c}	560.6^{a,b}	539.5^{b,c}	591.1^{a}	552.0^{b,c}	9.47	<0.001
Tender weight, g	99.93^{c}	106.1^{a,b}	104.41^{b,c}	110.3^{a}	107.1^{a,b}	1.51	<0.001
Leg weight, g	706.3^{b}	716.1^{a,b}	705.1^{b}	743.8^{a}	723.9^{a,b}	8.76	0.012
Fat pad weight, g	29.85	27.15	26.97	28.00	26.40	1.081	0.189
Carcass yield, %	76.9	77.0	76.2	76.8	77.0	0.38	0.491
Breast yield, %	24.5^{c}	25.5^{a,b}	25.2^{a,b,c}	26.0^{a}	25.1^{b,c}	0.23	<0.001
Tender yield, %	4.74	4.84	4.88	4.86	4.88	0.04	0.119
Leg yield%	33.6	32.7	32.9	32.7	33.0	0.24	0.063
Fat yield, %	1.42^{a}	1.24^{a,b}	1.26^{a,b}	1.23^{b}	1.20^{b}	0.049	0.013

[a-c]Means in the same row with no common superscripts are significantly different at a probability level of $P < 0.05$.
Abbreviation: IPF: inorganic-P-free.
[1]Displayed P values < 0.05 without superscripts next to associated means indicates a statistically significant ANOVA result but non-significance from means separation using Tukey's honest significant difference test, at $P < 0.05$.

quality indices to those of birds fed a nutritionally adequate diet. Scholey et al. (2018) tested the effect of graded reductions in Pi (and Ca) during grower and finisher phases with phytase supplementation at 500, 750, or 1,000 FTU, and concluded that the phytase could adequately replace Pi during finisher phase but not in grower diets unless dosed at 1,000 FTU or higher. In both trials reported herein, PhyG at a dose level of 1,000 FTU/kg in all phases maintained growth performance equivalent to a nutritionally adequate diet both when applied to a diet reduced in Ca (−0.2 to −0.3% points) (treatment IPF1) as well as when applied to a diet with the full nutrient matrix for PhyG applied (0.2–0.3% points reduction in Ca, 74 kcal/kg reduction in ME, 0.2 to 0.4% points reduction in dig AA, 0.04% reduction in Na) (treatment IPF2). The greater positive effect of PhyG at a lower dose level compared with the phytases in the above-mentioned studies may be due to the combination of a higher inherent efficiency in breaking down phytate (with capacity to replace 2.07 g/kg MCP-P based on digestible P improvement in broilers, Dersjant-Li et al. (2020b)), coupled with a high availability of substrate (IP6) within the test diets (>0.3% phytate-P).

Given the higher P and other nutrient requirements during starter and grower phases, it was expected that a high dose level of PhyG might be needed during these phases in the present study to maximize utilization of the substrate, further reduce the antinutritional effect of phytate and increase the digestibility of P and other nutrients to support normal growth and bone development. This was confirmed in both trials via treatment IPF3

Table 9. Effect of total replacement of added inorganic P by PhyG supplementation with or without xylanase on tibia ash, tibia breaking strength and carcass characteristics of broilers; trial 2 (males only).

	PC	IPF1	IPF2	IPF3	IPF4	SEM	*P*-value[1]
Day 21							
Tibia breaking strength, kgF	17.05	16.42	16.33	18.03	17.95	0.43	0.016
Tibia ash content, % DM	52.36	51.44	52.74	52.40	52.11	0.61	0.664
Day 42							
Tibia breaking strength, kgF	53.34	52.38	52.15	54.41	52.69	1.40	0.796
Tibia ash content, % DM	52.52	51.37	51.34	51.94	51.23	0.55	0.440
Live weight, g	3,044.9^{b}	3,189.7^{a}	3,142.4^{a}	3,118.0^{a,b}	3,150.1^{a}	25.24	<0.001
Carcass weight, g	2,318.6^{b}	2,423.3^{a}	2,387.9^{a,b}	2,377.1^{a,b}	2,407.6^{a}	19.96	<0.001
Breast weight, g	580.9^{b}	630.0^{a}	609.21^{a,b}	611.0^{a}	635.0^{a}	7.62	<0.001
Tender weight, g	114.7^{b}	122.2^{a}	119.4^{a,b}	117.4^{a,b}	121.4^{a}	1.39	<0.001
Leg weight, g	762.3	781.6	791.03	778.5	785.6	7.63	0.093
Fat pad weight, g	21.03	20.72	18.07	16.58	20.14	1.499	0.172
Carcass yield, %	76.1	76.0	76.0	76.2	76.4	0.29	0.850
Breast yield, %	25.0^{c}	26.0^{a,b}	25.5^{b,c}	25.7^{a,b,c}	26.4^{a}	0.21	0.002
Tender yield, %	4.95	5.05	5.01	4.94	5.04	0.05	0.382
Leg yield%	32.9^{a,b}	32.3^{b}	33.1^{a}	32.7^{a,b}	32.6^{a,b}	0.17	0.011
Fat yield, %	0.90	0.86	0.76	0.70	0.84	0.06	0.156

[a-c]Means in the same row with no common superscripts are significantly different at a probability level of $P < 0.05$.
Abbreviation: IPF, inorganic-P-free.
[1]Displayed P values < 0.05 without superscripts next to associated means indicates a statistically significant ANOVA result but nonsignificance from means separation using Tukey's honest significant difference test, at $P < 0.05$.

which contained 3,000 FTU/kg during starter phase and 2,000 FTU during grower phase (and 1,000 FTU/kg during finisher phases). Although PhyG at 1,000 FTU maintained performance compared with PC, indicating that P requirements were met, treatment IPF3 further improved ADG in starter phase (trial 1) and ADG and FCR in grower phase (trial 2) above the level of the PC. This suggests that, in these phases, the beneficial effects of the phase-specific higher phytase dose may have extended beyond simple P-release, that is, a so-called "extra-phosphoric" effect of the phytase. This refers to the capacity of some phytases to improve the digestibility and utilization of nutrients other than P, including Ca, AA, energy, Na, and starch, via a variety of possible mechanisms including the degradation of phytate in the upper GIT before it can form indigestible complexes with these nutrients (Cowieson et al., 2004; Selle et al., 2012; Truong et al., 2015). Other studies of PhyG effects on the digestibility of nutrients other than P have reported and confirmed the extra-phosphoric effects of PhyG (Dersjant-Li et al., 2020c).

The overall improvement (above PC) in FCRc observed in treatment IPF3 was consistent and substantial across both trials: −12 points in trial 1; −11 points in trial 2. However, above-PC improvements in treatment IPF3 were seen in a greater number of individual response measures and individual growth phases in trial 2 than in trial 1. This could have been due to differences in the sex composition of the trials. The higher nutrient requirements of males compared with females might have resulted in a greater nutritional and performance benefit being derived from the higher PhyG dosing regimen of IPF3 which would have been more evident in trial 2 (all males) than in trial 1 (mixed males and females).

The addition of xylanase in combination with the PhyG phytase, via treatment IPF4, is also of interest for maximizing PhyG efficacy in commercial dietary formulations. Xylanase is commonly added to broiler diets to improve digestion of the NSP fraction of the diet, particularly but not exclusively in wheat-based diets or those containing industrial by-products such as corn dried distillers grains with solubles that contain high levels of NSP. Xylanase can improve nutrient digestibility and retention, leading to improvements in apparent metabolizable energy (AMEn) and growth performance (Liu et al., 2011; Kiarie et al., 2014). Xylanase is often added in combination with phytase due to the complementary modes of action of the 2 enzymes (Cowieson and Bedford, 2009; Welleans et al., 2017). In the present study, overall FCR and FCRc in treatment IPF4 were equivalent to treatment IPF3. Given that treatment IPF4 contained a lower dosing regimen of PhyG than IPF3, this implies that the addition of the xylanase complemented the PhyG in supporting performance with a lower dose level of PhyG than PhyG given alone in treatment IPF3.

Comparison of growth performance outcomes by treatment against performance objectives set by the breeder provides a further means of evaluating the effectiveness of PhyG in the tested setting. The day 35 and day 42 BW and FCR values, expressed as a percentage of the relevant performance objectives for Ross 308 broilers set by the breeder (Aviagen, 2014b) are given in Tables 6 and 7. These performance objectives were met or exceeded in all PhyG treatments in both trials, except for FCR in treatment IPF2, which was slightly below the performance objective at day 35 and day 42 (range 98.0–99.6% of breeder's objective across the 2 time points and trials). In both trials, the performance objectives were exceeded to the greatest extent in treatment IPF3, followed by treatment IPF4, providing further evidence for the effectiveness of these treatments in supporting growth performance. Interestingly, on a cost-benefit basis, the treatment in which a full nutrient matrix was applied in combination with PhyG at 1,000 FTU/kg in all phases (treatment IPF2) delivered the greatest feed cost saving per kg BWG vs. PC in trial 2 (0.032 USD/kg BWG vs. PC, Table 7; calculation of the costs of the experimental diets based on market ingredient prices in 2019). In trial 1, maximum feed ingredient cost savings were delivered by the treatment containing PhyG (tiered-dosing regimen by phase) in combination with xylanase (treatment IPF4) (0.033 USD/kg BWG vs. PC, Table 6). These cost savings are largely brought about by the ability to use cheaper, phytate-rich ingredients such as rapeseed meal, rice bran, and wheat bran in the PhyG supplemented diets, which is exactly in line with the goal of many feed producers to increase sustainability by using more local raw materials and industrial by-products. The capacity of a phytase to maximize P-release from these more complex, higher phytate, diets, without the need to add Pi, could help to further reduce feed costs at the same time as improving the sustainability of broiler feed formulations. Based on the inorganic phosphorus inclusion level in the current commercial practical diets, it is estimated that with the total replacement of Pi, the industry should be able to reduce the usage of monocalcium-phosphate in broiler feeds by at least 1 million tonnes/year. This represents a massive step toward more environmentally sustainable broiler production and a significant decrease in phosphorus excretion. In this first study, to maintain a relative consistent feed composition, we have used relatively high phytate level through all phases. As the digestible phosphorus requirement is lower in grower and finisher phases, the phytate P level could be further reduced in the grower and finisher phases to further reduce the phosphorus excretion. Further studies are in progress to test this hypothesis.

Bone Quality and Carcass Characteristics

Bone ash and breaking strength are important indicators of the capacity of a phytase to release phosphorus for use by the bird and, as already mentioned, P requirements during starter phase when bone deposition and mineralization is occurring are high. These measures can also provide an indication of whether Ca requirements have been met since Ca is equally critical in bone development and mineralization (Rath et al., 2000; Li et al., 2020). Phytase efficacy in monogastric

animals can be affected by the Ca to total P ratio especially in low P diets (Qian et al., 1996, 1997) due largely to the capacity of Ca to bind to phytate in the GIT, forming insoluble complexes that are resistant to digestion by phytase (Selle et al., 2009). Measures of bone strength and quality can provide an indirect confirmation of whether Ca and P were in balance in the test diets. Previous broiler trials involving reduced dietary nPP or Pi levels have sometimes reported reduced tibia ash (weight or percentage) or breaking strength even where no apparent impairment to growth performance has been observed (Yan et al., 2000; Dhandu and Angel, 2003). The study by Scholey et al. that examined the effect of total removal of Pi (during grower and finisher phases only) with phytase supplementation, reported that both tibia ash and strength were impaired compared with a control diet, except in the group that received the highest phytase dose of 1,000 FTU/kg, in which tibia strength (N) was equivalent to PC but ash (%) remained below the level of the control (Scholey et al., 2018). In a previous study of the PhyG phytase in broiler diets reduced in Ca and available P (by 2.0 g/kg and 1.9 g/kg (starter phase) and 2.0 g/kg and 1.8 g/kg (finisher phase)), it was reported that tibia ash at day 21 was maintained equivalent to PC with PhyG at 500 FTU or 1,000 FTU (but not 250 FTU), while at day 42 all of these dose levels were effective at maintaining equivalence to PC (Dersjant-Li et al., 2020b). The present study has extended these findings by demonstrating that the phytase with or without xylanase, at a dose level of 1,000 FTU/kg or higher was able to release enough P from the test diets that contained no Pi, to meet requirements for normal bone mineralization and strength, suggesting that P was not limiting during any growth phase and that Ca and P were in balance in the test diets. A note of caution should be applied when interpreting tibia breaking strength values at day 21 in trial 1 as they were uniformly unexpectedly high (range 31.25–33.66 kgF) with no obvious explanation, which could be related to the Intron equipment calibration settings issue. Nevertheless, equivalence of all PhyG treatments to PC was maintained. There was additional evidence from trial 1, but not trial 2, that day 42 tibia breaking strength in treatments IPF3 and IPF4 exceeded that of the PC, and the size of the effect was notable (+14.05% and +19.19%, respectively). To the author's knowledge, these data are the first to show that normal, or superior, bone quality and strength can be supported by a phytase in diets containing no added Pi in broilers.

The carcass characteristics results were obtained from a relatively small number of birds in each treatment group (6 birds per pen, 60 birds per treatment) but are broadly consistent with the growth performance and bone quality results; birds who received PhyG supplementation (regardless of dose, with or without xylanase) exhibited carcass characteristics at 42 d of age that were equivalent to birds fed the nutritionally adequate control diet on both a weight and percentage yield (percentage of carcass weight) basis. This was consistent across both trials. Treatment IPF3 again appeared to be the most effective; weights of several carcass parts were increased in IPF3 compared with PC, especially breast meat weight (+13.79% in trial 1 and + 5.17% in trial 2). It is presumed that the difference in effect size between the 2 trials was due to the absence of females in trial 2. Breast meat yields were also increased in this treatment group in both trials, suggesting that this effect was not due to differences in BW. The increased breast meat weight and yield could have been related to increased protein deposition. It is already known that phytase supplementation can increase the digestibility of amino acids (Cowieson and Bedford, 2009). Previous studies have reported a positive dose-response relationship between microbial phytase supplementation and breast meat and yield, that is also related to the amino acid composition of the diet, especially the content of the essential amino acid lysine (Walk and Rao, 2020). It is possible that the comparatively high dose of PhyG during starter phase (when amino acid requirements of the birds are high) in treatment IPF3 further reduced antinutritional effect of phytate and further improved AA digestibility, this could have enabled increased protein deposition in the breast meat in treatment IPF3 vs. PC. Overall, PhyG was able to compensate the total removal of Pi in all phases and maintained and even improved growth performance, bone quality, and carcass yield through day 42, indicating high phosphoric and extra-phosphoric bioefficacy.

In conclusion, results from the 2 trials presented here have demonstrated, for the first time, that a novel consensus phytase variant was effective in maintaining normal growth performance in broilers during the entire growth cycle, in wholly vegetable diets containing no added inorganic phosphorus and >0.3% phytate-P. Bone strength and quality, and carcass characteristics, were also maintained or better compared with positive control while the inorganic phosphorus free treatments were also more economical.

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L. Marchal, Y. Dersjant-Li, and A. Bello are employees of DuPont Nutrition and Biosciences.

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