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Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to weaned piglets fed a corn-soybean meal-based diet without added inorganic phosphate

Yueming Dersjant-Li [a, *], Boris Villca [b], Vincent Sewalt [c], Arno de Kreij [d], Leon Marchal [a], Deepak E. Velayudhan [a], Robin A. Sorg [a], Trine Christensen [e], Rie Mejldal [e], Igor Nikolaev [a], Sina Pricelius [a], Hye-Sook Kim [c], Svend Haaning [e], Jens F. Sørensen [e], Rosil Lizardo [b]

[a] DuPont Nutrition and Biosciences, Archimedesweg, 30, 2333, CN Leiden, the Netherlands
[b] Institut de Recerca I Tecnologia Agroalimentàries, Centre Mas de Bover, Ctra. Reus-El Morell km. 3.8, E-43120, Constantí, Spain
[c] DuPont Nutrition & Biosciences, 925 Page Mill Road, Palo Alto, CA 94304, USA
[d] DuPont Nutrition & Biosciences, 21 Biopolis Road, Nucleos, South Tower, 138567, Singapore
[e] DuPont Nutrition and Biosciences, Edwin Rahrs Vej 38, DK-8220, Brabrand, Denmark

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ABSTRACT

The utility of a next generation biosynthetic bacterial 6-phytase (PhyG) in restoring bone ash, bone phosphorus (P) content and performance in piglets depleted in P was evaluated. A total of 9 treatments were tested as follows. Treatment 1, a negative control (NC) diet; treatments 2, 3, 4, NC supplemented with 250, 500 or 1,000 FTU/kg of PhyG; treatments 5, 6, NC supplemented with 500 or 1,000 FTU/kg of a commercial *Buttiauxella* sp phytase (PhyB); treatments 7, 8, 9, NC supplemented with monocalcium phosphate (MCP) to provide 0.7, 1.4 and 1.8 g/kg digestible P, equating to a digestible P content of 1.8, 2.5 and 2.9 g/kg. The latter constituting the positive control (PC) diet with adequate P and calcium (Ca). The NC was formulated without inorganic P (1.1 g digestible P/kg) and reduced in Ca (5.0 g/kg). Additional limestone was added to treatments 7 to 9 to maintain Ca-to-P ratio between 1.2 and 1.3. A total of 162 crossed Pietrain × (Large White × Landrace) 21-d-old piglets (50% males and 50% females) were fed adaptation diets until 42 d old and then assigned to pens with 2 pigs/pen and 9 pens/treatment in a completely randomized block design. Piglets were fed mash diets based on corn and soybean meal *ad libitum* for 28 d. At the end of the study, one piglet perpen was euthanized and the right feet collected for determination of bone strength, bone ash and mineral content. Compared with the PC, the NC group had reduced average daily gain (ADG) and increased feed conversion ratio (FCR) during all growth phases and overall, and at d 28 (70 d old) NC pigs had bones with reduced ash, Ca and P content ($P < 0.05$). The PhyG at 250 FTU/kg improved bone ash vs. NC. Increasing PhyG dose linearly or quadratically improved bone ash, ADG and FCR ($P < 0.05$). At $\geq$ 500 FTU/kg, both PhyG and PhyB maintained ADG and FCR equivalent to PC. Linear regression analysis was done to compare the measured response parameters to increasing digestible P from MCP. Based on this analysis it was shown that PhyG and PhyB at 1,000 FTU/kg could replace 1.83 and 1.66 g/kg digestible P from MCP in the diet, respectively, on average across metacarpi bone ash, ADG or FCR. These findings suggest that the biosynthetic phytase is highly effective in the tested dietary setting.

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* Corresponding author.
E-mail address: Yueming.dersjant-li@dupont.com (Y. Dersjant-Li).

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1. Introduction

Exogenous phytase enzymes were first introduced into commercial pig and poultry diets in the 1990s (Lei et al., 2013), primarily as a means of improving phosphorus (P) availability to the animal whilst reducing P excretion into the environment (Selle and

Ravindran, 2008). This is achieved through the enzyme's capacity to release P from the phytate molecules (myo-inositol hexaphosphate; IP6) present in plant-derived feed ingredients. Over the past 20 years, feed ingredient costs have continued to increase, phytase costs have reduced as the enzyme has been commercialized and new, more efficacious phytases have been developed. This has led to the widespread practice of phytase supplementation to commercial pig diets.

Quantification of the improvements in nutrient utilization and consequently growth performance that may be delivered by a specific phytase in a given dietary setting has been achieved via extensive *in vivo* digestibility trials. This has enabled the formulation of phytase-supplemented diets with reduced inclusion of P from expensive inorganic P sources (such as monocalcium phosphate [MCP]), and reduced calcium (Ca) content. The application of such nutrient down specification (also called matrix values) in feed formulations containing phytase is attractive to feed manufacturers because it can confer a reduction in overall feed costs. New phytases with increased efficacy (phytate degrading capacity) as well as other desirable characteristics such as broad spectrum of effectiveness (across multiple dietary settings) are continuously being sought.

The phosphoric effect of phytase is typically assessed by estimation of its inorganic P replacement value. This can be done either directly via *in vivo* trials in which the digestible P improvement with supplemental phytase is evaluated relative to a P-deficient, negative control (NC) diet using meta-analysis of data from large number of *in vivo* studies, and/or indirectly by estimate of the available P equivalence of the phytase relative to that of a reference source of inorganic P, such as MCP, using mainly bone ash, but also average daily gain (ADG) or feed conversion ratio (FCR) as response parameters (Dersjant-Li et al., 2019). A review of the literature showed phytases are estimated to be able to replace 0.49 to 1.6 g/kg inorganic P at a dose range of 500 to 1,000 FTU/kg feed in pigs, based on bone ash and performance outcome measures (Dersjant-Li et al., 2015), whilst nutritionally complete diets for weaned pigs typically contain >1.8 g/kg of digestible P from inorganic phosphate sources, when diets are formulated without meat and bone meal. Given this, a phytase that is more efficacious in releasing P from phytate could enable a further reduction in the need to add expensive inorganic P to pig diets, or lead to complete replacement of inorganic P, while maintaining bone mineralization and performance.

The improvements in P utilisation that can be achieved with supplemental phytase in pig diets have already been extensively described (Selle and Ravindran, 2008; Humer et al., 2015). Biologically relevant improvements in the digestibility of other nutrients by phytase have also been described, e.g., for Ca (Traylor et al., 2001; Almeida et al., 2013), protein and amino acids (Adedokun et al., 2015; Zouaoui et al., 2018; Dersjant-Li and Dusel, 2019). However, not all phytases can effectively improve digestibility of amino acids and energy (Adeola and Sands, 2003). Phytase is included in animal feed based on the analyzed activity at pH 5.5, however, a phytase needs to be highly active at upper gastrointestinal tract (GIT), to break down the phytate quickly and completely, to reduce the negative effect of phytate on amino acids digestibility. A recent publication (Dersjant-Li and Kwakernaak, 2019) demonstrated that a *Buttiauxella* sp. phytase is more effective in improving the ileal digestibility of amino acids and energy than an *Escherichia coli* phytase. Phytase breaks down phytate (the salt of phytic acid, consisting of an inositol ring linked to 6 phosphate groups, IP6) in a step wise manner to IP5 (inositol ring with 5 phosphate groups), IP4, and IP3 and IP2. It is well known that IP6 and IP5 are the most important anti-nutritional esters of the phytic acid, which have strong binding capacity to amino acids and

minerals, however, for maximal alleviation of pepsin inhibition, IP6 needs to be broken down to IP1–2 (Yu et al., 2012). Thus, the efficacy of both phosphoric and extra-phosphoric effect of phytase can be further improved by developing a new phytase that can breakdown phytate quickly and more completely in the upper GIT.

Recently, several bacterial phytase sequences were developed by DuPont utilizing bioinformatics and other synthetic biology tools. The aim of this study was to assess the utility of dietary supplementation with one of these next generation biosynthetic bacterial 6-phytases in weaned piglets fed a commercial corn-soybean meal-based diet without added inorganic phosphate, compared to addition of inorganic P from MCP, on bone ash and mineralization and on maintaining ADG and FCR. An existing commercial phytase was included in the study for comparative purposes. The second objective was to determine the digestible P-equivalence value of the biosynthetic phytase in the tested setting.

2. Materials and methods

The experimental procedures were in compliance with European Directive 2010/63/EU and the Spanish guidelines for the care and use of animals in research (B.O.E. number 252, Real Decreto 2010/2005).

2.1. Experimental and control diets

A total of 9 dietary treatments were tested. A positive control (PC) diet based on corn, soybean meal (SBM), rice and rice bran was formulated to meet the nutritional requirements of piglets weighing 10 to 25 kg (NRC, 2012), containing 2.9 g/kg digestible P and 7.0 g/kg Ca (Table 1). A negative control (NC; treatment 1) diet was formulated without inorganic phosphate (1.1 g/kg digestible P) and reduced in Ca (5.0 g/kg). The NC was tested as a stand-alone diet and also when supplemented with 250, 500 or 1,000 FTU/kg of a next generation biosynthetic bacterial 6-phytase (PhyG; treatments 2 to 4), 500 or 1,000 FTU/kg diet of a commercial phytase (PhyB; treatments 5 to 6), or with added MCP at 3 levels (+0.7, +1.4 and + 1.8 g/kg digestible P from MCP vs. NC; treatments 7 to 9), equating to a digestible P content of 1.8, 2.5 and 2.9 g/kg (the treatment 9 constituting the PC diet). Additional limestone was added to the MCP-supplemented diets in order to maintain Ca to P ratio within the range 1.2 to 1.3 (Table 1). Diets were provided to piglets *ad libitum* in mash form, and water was freely available.

The PhyB was a commercial bacterial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (PhyB, Axtra PHY, DuPont Nutrition and Biosciences). The PhyG was produced by fermentation with a fungal (*T. reesei*) production strain expressing a biosynthetic variant of a consensus bacterial phytase gene assembled via ancestral reconstruction with sequence bias for *Buttiauxella* sp. (DuPont Nutrition and Biosciences). Both phytases are characterized as highly active at low pH compared to other commercial phytases. However, the PhyG has high activity in a wider pH range. It has a relative activity of e.g. 82% at pH 1.5, 158% at pH 2.5, 221% at pH 3.5 and 241% at pH 4.5 compared to the activity at pH 5.5 (100%).

2.2. Pigs, housing and experimental design

A total of 162 crossed Pietrain × (Large White × Landrace) 21-d-old piglets of mixed sexes (50% males, 50% females) were obtained at weaning (initial body weight [BW] = 6 ± 1 kg) and fed a common pre-starter adaptation diet until 42 d old (~10 to 11 kg BW). Piglets were then blocked based on BW and gender and allocated to pens, with 2 pigs/pen and 9 pens/treatment, in a completely randomized block design. Test diets were administered to pigs from 42 d old until 70 d old. Pens were grouped together in an environmentally

Table 1
Ingredient and nutrient composition (g/kg, as fed basis) of NC and NC with increased level of digestible P from MCP inclusion diets fed to weaned piglets (42 to 70 d of age).

Item	NC	NC + digestible P from MCP		
		0.7 g/kg	1.4 g/kg	1.8 g/kg (PC)
Ingredients				
Corn	400	400	400	400
Soybean meal (48% CP)	293.35	292.85	292.65	292.65
Rice	150	150	150	150
Rice bran	50.0	50.0	50.0	50.0
Sugar beet pulp	30.0	30.0	30.0	30.0
Animal fat	36.7	36.7	36.7	36.7
MCP	–	3.30	6.70	8.80
Calcium carbonate	6.70	7.40	8.20	8.60
Salt	4.10	4.10	4.10	4.10
L-lysine HCl	4.00	4.00	4.00	4.00
DL-methionine	1.70	1.70	1.70	1.70
L-threonine	1.50	1.50	1.50	1.50
L-tryptophan	0.50	0.50	0.50	0.50
Noxyfeed[1]	0.20	0.20	0.20	0.20
Titanium dioxide	5.00	5.00	5.00	5.00
Filler (diatomaceous earth)	10.0	6.50	2.50	–
Vitamin-mineral premix[2]	6.00	6.00	6.00	6.00
Test product with carrier[3]	0.25	0.25	0.25	0.25
Calculated nutrients				
Metabolizable energy, Mcal/kg	3.35	3.35	3.35	3.35
Net energy, Mcal/kg	2.52	2.52	2.52	2.52
Crude protein	194	194	194	194
Ether extract	63.3	63.3	63.2	63.2
Total Ca	5.00	5.75	6.53	7.00
Total P	4.00	4.76	5.53	6.00
Digestible P	1.06	1.76	2.46	2.90
Non-phytate P	1.28	2.00	2.80	3.30
Total lysine	13.4	13.4	13.4	13.4
SID lysine	12.3	12.3	12.3	12.3
SID threonine	7.70	7.70	7.70	7.70
SID methionine	4.43	4.43	4.43	4.43
SID tryptophan	2.42	2.42	2.42	2.42

NC = negative control; P = phosphorus; MCP = monocalcium phosphate; PC = positive control; SID = standardized ileal digestible.

[1] Noxyfeed is an antioxidant, containing butylated hydroxytoluene, propyl gallate and citric acid.

[2] Vitamin-mineral premix supplied per kilogram of diet: iron from $FeSO_4 \cdot H_2O$, 120 mg; iodine from $Ca(IO_3)_2$, 0.75 mg; cobalt from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$, 0.6 mg; copper from $CuSO_4 \cdot 5H_2O$, 6 mg; manganese from MnO, 60 mg; zinc from ZnO, 100 mg; selenium (E8) from Na_2SeO_3, 0.37 mg; vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E (alfa tocopherol), 25 mg; vitamin B_1, 1.5 mg; vitamin B_2, 3.5 mg; vitamin B_6, 2.4 mg; vitamin B_{12}, 20 μg; vitamin K_3, 1.5 mg; calcium pantothenate, 14 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; biotin, 50 μg.

[3] The test product is mixed with wheat carrier to get the targeted dose, the control treatment received only carrier without test product.

controlled animal room in which the temperature was maintained at 30 °C initially and thereafter reduced by 1 °C per week.

2.3. Sampling and measurements

Representative sub-samples of all diets were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), ash, minerals, phytate and phytase activity.

Pigs were weighed individually before the start of the experiment, and again at d 14 and 28 of trial to calculate ADG. Feed disappearance was assessed on d 14 and 28 and used to calculate average daily feed intake (ADFI). Feed conversion rate was calculated from ADFI and ADG.

On d 28 of the trial, one piglet per pen was euthanized by intravenous overdose of sodium pentobarbital and the right feet from the fore- and hindleg was excised to determine metacarpi/metatarsi bone ash and mineral content (Ca and P). Feet were stored at −20 °C until analysis.

2.4. Chemical analysis

All samples were analyzed in duplicate. Dry matter, ash, CP and EE in feed were analyzed according to the AOAC (2000a) methods (925.09, 942.05, 968.06 and 920.39, respectively). Nitrogen content was determined by the Dumas procedure, by means of Nitrogen FP-528 analyzer (LECO corp., St Joseph, Mo, USA). Organic matter was calculated as the difference between DM and ash. Analysis of exogenous phytase activity in feeds was performed according to Engelen et al. (1994). One phytase unit (FTU) was defined as the amount of phytase that liberated 1 mmol of inorganic phosphate per minute from 0.0051 mol/L of sodium phytate at a standard pH of 5.5 and temperature of 37 °C (AOAC, 2000b).

Bone ash was determined on both metacarpi III/IV and metatarsi III/IV from the right fore- and hindfoot, respectively. After extraction, bones were first used to characterize their integrity in a 3-point mechanical test using an Instron testing system (Norwood, MA, US) model 2519-106 equipped with a 2 kN load cell. Biomechanical parameters including extrinsic stiffness, ultimate force, displacement and work to failure were used to characterize integrity of bones (Turner, 2006). Then, bones were used to determine their DM content in an oven at 103 °C for 4 h. The ash content was determined in an oven-dryer for 3 h at 200 °C previous to their introduction into a muffle furnace at 550 °C for 72 h. Ashes from metacarpi bones were then ground using a pestle and a mortar, and sent to SCT lab (University of Lérida, Spain) for mineral determination after sulfuric acid digestion. Mineral composition from feed (Ca, P, Mg, Fe, Zn and Cu) and bone (Ca, P) was analyzed on ash samples by inductively-coupled plasma mass spectrometry (ICP-MS; Agilent Technologies model 7700X) at SCT lab (Pacquette et al., 2018).

2.5. Statistical analysis

Data were based on pen as the experimental unit, except for bone ash and bone strength, which were based on pig as the experimental unit. Data were analyzed by analysis of variance (ANOVA) using the Fit Model platform of (JMP 14.0) to investigate the effect of treatments in a randomized block design. Means separation was achieved using Tukey's Honest Significant Difference test. In addition, a 2-way ANOVA analysis was carried out with factors 'phytase' (PhyG vs. PhyB) and dose (500 and 1,000) to compare 2 phytases at 2 dose levels of 500 and 1,000 FTU/kg. Linear and quadratic response with increasing PhyG dose were analyzed using orthogonal polynomials. In addition, linear regression was performed with increasing level of digestible P from MCP (e.g. NC, NC + 0.7, NC + 1.4 and NC + 1.8 g/kg digestible P from MCP) for metacarpi bone ash, ADG and FCR. The digestible P equivalence was calculated by applying the response parameters (y, e.g. bone ash) values at a given phytase dose and calculate the corresponding MCP-P replacement (x) values. Differences were considered significant at $P < 0.05$; $P < 0.10$ was considered a tendency.

3. Results

3.1. Diet analysis

Analyzed values of dietary nutrients are presented in Table 2. Phytase activities in the NC diets were ≤ 50 FTU/kg indicating the absence of phytase cross-contamination. Activities in the phytase supplemented diets were within 10% of target values, except for treatment NC + PhyG at 250 FTU/kg and NC + PhyG at 500 FTU/kg in which activities were respectively −20% and +27% vs. target dose. The analyzed P content of the NC diets containing added P

Table 2
Analyzed nutritional values of the experimental diets (g/kg, as fed basis).

Item	NC	NC + PhyG			NC + PhyB		NC + digestible P from MCP		
		250 FTU/kg	500 FTU/kg	1,000 FTU/kg	500 FTU/kg	1,000 FTU/kg	0.7 g/kg	1.4 g/kg	1.8 g/kg (PC)
Dry matter	893	899	896	896	897	897	898	897	893
Metabolizable energy, Mcal/kg [1]	3.18	3.19	3.17	3.19	3.20	3.22	3.19	3.18	3.18
Net energy, Mcal/kg [1]	2.33	2.34	2.32	2.34	2.35	2.36	2.34	2.34	2.34
Organic matter	834	839	834	831	835	835	834	834	833
Crude protein	200	200	202	199	199	201	200	200	199
Ether extract	61.3	61.6	69.0	65.8	65.5	63.8	66.9	66.5	68.1
Ash	59.7	61.2	62.5	65.4	61.8	61.6	63.4	63.4	59.5
Ca	5.96	5.94	6.32	6.41	6.29	6.15	7.26	7.65	8.73
P	4.29	4.50	4.67	4.89	4.63	4.48	5.48	5.65	6.33
Analyzed Ca-to-P ratio	1.39	1.32	1.35	1.31	1.36	1.37	1.32	1.35	1.38
Magnesium	2.14	2.20	2.25	2.42	2.28	2.26	2.46	2.26	2.37
Iron	0.21	0.22	0.23	0.24	0.23	0.37	0.24	0.19	0.19
Copper, mg/kg	10	9	9	15	10	10	13	14	16
Zinc, mg/kg	83	90	93	96	102	102	115	109	95
Phytate-P	2.6	–	–	–	–	–	–	–	–
Analyzed phytase, FTU/kg[2]	<50	201	635	1,058	552	1,083	<50	<50	<50

NC = negative control; PhyG = a next generation biosynthetic bacterial 6-phytase (DuPont Nutrition and Biosciences); PhyB = a commercial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (DuPont Nutrition and Biosciences); MCP = monocalcium phosphate; PC = positive control.
[1] Metabolizable and net energy were calculated as 0.79 and 0.58 of gross and digestible energy, respectively, according to AFZ-INRA tables (Sauvant et al., 2004).
[2] Phytase activity in the diets was analyzed by DuPont Laboratories, Brabrand, Denmark.

from MCP were close to the expected values based on the intended levels of MCP addition.

3.2. Bone ash minerals and bone strength

At d 70 (d 28 of the experiment), metatarsi and metacarpi bone ash, metacarpi Ca and P content were reduced in piglets fed the NC diet vs. PC ($P < 0.05$; Table 3). Supplementation with both phytases and at all dose levels improved bone ash (%) compared to NC ($P < 0.05$). At 500 and 1,000 FTU/kg, metatarsi and metacarpi bone ash, and metacarpi bone P content were equivalent to PC. Increasing the dose of PhyG from 0 (NC) to 1,000 FTU/kg resulted in linear and quadratic increases in metatarsi and metacarpi bone ash at d 28 ($P < 0.05$, Fig. 1). Metacarpi bone Ca content was unaffected by phytase supplementation. A linear response was observed for metatarsi and metacarpi bone ash and metacarpi P content with increasing MCP-P levels in the diets ($P < 0.05$).

The influence of dietary treatments on metacarpi bone biomechanical parameters is presented in Table 3. Ultimate force (N) to break the metacarpi bone was lower in NC ($P < 0.05$) compared to all other treatments. Both phytases at 1,000 FTU/kg restored bone strength to the same ultimate force as for PC. Both phytases at 500 FTU and 1,000 FTU improved stiffness (mPa) vs. NC and at 1,000 FTU/kg restored stiffness (mPa) and work to failure (J) to the same as for the PC diet that contained an additional of 1.8 g

digestible P from MCP per kg diet. On comparison of 2 dose levels across 2 phytases, phytase at 1,000 FTU/kg showed greater bone ash, ultimate force (N), stiffness (mPa) and work to failure (J) compared to 500 FTU/kg ($P < 0.05$). No interaction was found between phytase source and dose levels.

3.3. Growth performance

The effect of dietary treatment on growth performance is presented in Table 4. Except for ADFI during d 0 to 14 (tendency, $P = 0.08$), all growth performance response measures were impaired (ADG and ADFI reduced; FCR increased) in piglets fed the NC diet compared to the PC diet ($P < 0.05$).

During the first phase of the experiment (d 0 to 14), both PhyG and PhyB at 1,000 FTU/kg produced a greater ADG and a reduced FCR ($P < 0.05$) vs. NC, which were equivalent to the PC diet that contained 1.8 g/kg added digestible P from MCP.

During the second phase of the experiment (d 15 to 28), PhyG at 250 FTU/kg or higher improved ADG vs. NC, and at 500 FTU/kg or higher improved FCR vs. NC ($P < 0.05$). PhyB also improved ADG and FCR vs. NC at both dose levels ($P < 0.05$). At 500 FTU/kg or higher, both phytases produced ADG and FCR values equivalent to PC that contained 1.8 g/kg added digestible P from MCP.

During the overall phase (d 0 to 28), both phytases at all dose levels improved ADG vs. NC, and both phytases improved FCR vs.

Table 3
Effect of increasing dose of 2 phytases and inorganic P content on metatarsi and metacarpi bone ash and mineralization and metacarpi bone strength in piglets at 70 d old.

Item	NC	NC + PhyG			NC + PhyB		NC + digestible P from MCP			SEM	*P*-value
		250 FTU/kg	500 FTU/kg	1,000 FTU/kg	500 FTU/kg	1,000 FTU/kg	0.7 g/kg	1.4 g/kg	1.8 g/kg (PC)		
Bone ash and minerals, % DM basis											
Metatarsi ash	22.1[d]	26.1[c]	27.5[bc]	30.1[ab]	27.1[bc]	29.3[ab]	25.8[c]	29.4[ab]	30.6[a]	0.68	<0.001
Metacarpi ash	25.1[d]	28.5[bc]	29.9[abc]	32.0[a]	30.0[abc]	32.0[a]	27.8[cd]	31.0[ab]	32.7[a]	0.63	<0.001
Metacarpi Ca	7.7[b]	9.0[ab]	8.9[ab]	9.3[ab]	8.5[ab]	9.5[ab]	8.6[ab]	9.7[a]	10.0[a]	0.44	0.008
Metacarpi P	4.9[c]	5.6[bc]	6.1[ab]	6.5[a]	6.1[ab]	6.6[a]	5.4[bc]	6.1[ab]	6.8[a]	0.21	<0.001
Bone strength											
Ultimate force, N	188[d]	258[c]	293[bc]	365[a]	290[bc]	373[a]	251[c]	328[ab]	371[a]	12.8	<0.001
Stiffness, mPa	112[d]	158[cd]	194[abc]	224[a]	182[abc]	225[a]	159[bc]	202[ab]	224[a]	9.5	<0.001
Work to failure, J	0.60[d]	0.79[bcd]	0.78[cd]	1.11[a]	0.95[abc]	1.16[a]	0.78[cd]	1.03[ab]	1.11[a]	0.05	<0.001
Displacement, mm	4.5	4.3	3.7	4.3	4.5	4.4	4.3	4.3	4.3	0.18	0.156

NC = negative control; PhyG = a next generation biosynthetic bacterial 6-phytase (DuPont Nutrition and Biosciences); PhyB = a commercial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (DuPont Nutrition and Biosciences); MCP = monocalcium phosphate; PC = positive control.
[a,b,c,d] Least square means within a row with different superscript letters differ ($P < 0.05$, Tukey test).
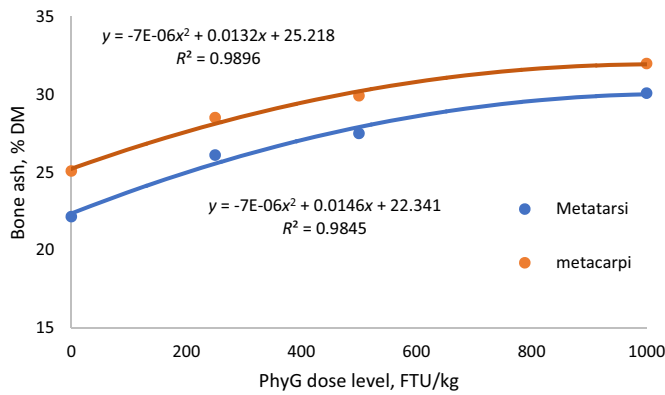
Fig. 1. Effect of increasing PhyG dose from 0 (NC) to 1,000 FTU/kg on metatarsi and metacarpi bone ash at the end of the study. Metatarsi bone ash: P linear < 0.0001, P quadratic = 0.049; metacarpi bone ash: P linear < 0.0001, P quadratic = 0.028. PhyG = a next generation biosynthetic bacterial 6-phytase.

NC at or above 500 FTU/kg ($P < 0.05$). For either phytase, at 500 FTU/kg or higher, ADG and FCR were equivalent to PC that contained 1.8 g/kg added digestible P from MCP. In addition, increasing dose of PhyG from 0 to 1,000 FTU/kg resulted in a linear increase in ADG and reduction in FCR during the overall phase ($P < 0.05$, Fig. 2). A linear response was observed for ADG and FCR with increasing MCP-P levels in the diets ($P < 0.05$). On comparison of 2 dose levels across 2 phytases, FCR was lower at 1,000 FTU/kg vs. 500 FTU/kg (1.59 vs. 1.66, $P < 0.05$). A tendency of greater ADG was observed at 1,000 FTU/kg vs. 500 FTU/kg (635 vs. 590 g/pig, $P = 0.08$), no difference was found on feed intake (data not shown). No interaction was found between phytase source and dose levels.

3.4. Inorganic P equivalence

The dietary digestible P equivalence values (g/kg diet) of PhyG and PhyB were calculated based on metacarpi bone ash, ADG and FCR as response parameters, using the observed responses to increasing digestible P from MCP as a reference, as described in section 2.5. Responses to increasing digestible P from MCP were linear and positive for all 3 response measures ($P < 0.001$; Fig. 3). Regardless of the response parameter used, calculated digestible P equivalence values

increased with increasing phytase dose and were highest at 1,000 FTU/kg (Table 5). At this dose-level digestible P equivalence values were higher for PhyG than PhyB (average across response parameters 1.83 g/kg vs. 1.66 g/kg, respectively) and were highest for ADG and lowest for metacarpi bone ash as the response parameter.

4. Discussion

The efficacy of a phytase, as can be measured by its inorganic P replacement capability and extra-phosphoric effect on performance, can vary markedly from one phytase to another, dependent on the unique enzymatic properties of the phytase (Menezes-Blackburn et al., 2015), the dietary phytate levels, host genetics, and the dose-level of supplementation (Dersjant-Li et al., 2015). It is therefore important to determine the efficacy of each specific phytase separately, and to draw comparisons with other phytases at equivalent (FTU) dose-levels of inclusion.

In the present study, at an inclusion level of 500 FTU/kg or 1,000 FTU/kg, the biosynthetic bacterial phytase (PhyG) was able to compensate for a 1.8 g/kg reduction in digestible P (from MCP) and restore ADG and FCR at a level equivalent to that produced by a nutritionally adequate PC diet. This indicates that this member of a clade of next generation biosynthetic bacterial 6-phytases was highly effective in the tested dietary setting. The magnitude of the growth performance response to PhyG (as measured by the improvements in ADG and FCR vs. NC) were numerically higher but statistically equivalent to that produced by PhyB. Previous studies reported that PhyB is effective in improving digestible amino acids and energy and demonstrated an extra-phosphoric effect in broilers and pigs (Dersjant-Li and Kwakernaak, 2019; Dersjant-Li and Dusel, 2019). The results from the current study may suggest an equal or greater dose-equivalent efficacy of PhyG on piglet performance, which could be speculated to also be due to the extra-phosphoric effect of this phytase. This hypothesis needs to be further evaluated.

The positive dose–response relationship observed for PhyG on FCR is consistent with existing studies that have reported linear and/or quadratic positive dose–response effects in pigs. In particular, dose–response effects across the same dose-range (0 to 1,000 FTU/kg) have previously been reported for PhyB on mineral, amino acid and protein digestibility (Adedokun et al., 2015; Dersjant-Li and Dusel, 2019), as well as growth performance and carcass characteristics (Dersjant-Li et al., 2017a; b). These previous studies have mainly focused on

Table 4
Effect of increasing dose of 2 phytases or inorganic P content on performance in weaned piglets from 42 to 70 d old.

Item	NC	NC + PhyG			NC + PhyB		NC + digestible P from MCP			SEM	P-value
		250 FTU/kg	500 FTU/kg	1,000 FTU/kg	500 FTU/kg	1,000 FTU/kg	0.7 g/kg	1.4 g/kg	1.8 g/kg (PC)		
d 0 to 14 on trial											
BW d 0, kg	10.46	10.52	10.46	10.54	10.45	10.47	10.49	10.52	10.43	0.6	0.999
ADG, g	436[c]	480[abc]	490[abc]	562[a]	505[abc]	526[ab]	460[bc]	470[bc]	541[ab]	39.8	<0.001
ADFI, g	721	760	743	811	783	776	745	732	783	59.8	0.08
FCR, g/g	1.65[a]	1.58[abc]	1.53[ab]	1.45[c]	1.56[abc]	1.48[bc]	1.64[ab]	1.58[abc]	1.45[c]	0.04	<0.001
d 15 to 28 on trial											
BW d 14, kg	16.3[c]	17.0[abc]	17.1[abc]	18.1[a]	17.3[abc]	17.6[ab]	16.7[bc]	16.8[bc]	17.8[ab]	1.4	<0.001
ADG, g	491[c]	604[b]	664[ab]	713[a]	663[ab]	702[a]	610[b]	666[ab]	708[a]	37.1	<0.001
ADFI, g	1,011[b]	1,104[ab]	1,160[ab]	1,181[a]	1,129[ab]	1,193[a]	1,152[ab]	1,101[ab]	1,178[a]	74.0	0.015
FCR, g/g	2.07[a]	1.82[ab]	1.76[b]	1.66[b]	1.71[b]	1.71[b]	1.88[ab]	1.65[b]	1.67[b]	0.08	0.004
d 0 to 28 on trial											
BW d 28, kg	23.2[d]	25.4[bc]	26.4[abc]	28.1[a]	26.5[abc]	27.4[ab]	25.2[c]	26.2[abc]	27.7[a]	1.9	<0.001
ADG, g	463[c]	542[b]	577[ab]	637[a]	584[ab]	614[a]	535[b]	568[ab]	624[a]	37.3	<0.001
ADFI, g	866[b]	932[ab]	952[ab]	996[a]	956[ab]	985[a]	948[ab]	916[ab]	980[a]	34.3	0.011
FCR, g/g	1.86[a]	1.72[abc]	1.66[bc]	1.57[c]	1.65[bc]	1.61[bc]	1.78[ab]	1.62[bc]	1.58[c]	0.04	<0.001

NC = negative control; PhyG = a next generation biosynthetic bacterial 6-phytase (DuPont Nutrition and Biosciences); PhyB = a commercial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (DuPont Nutrition and Biosciences); MCP = monocalcium phosphate; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.
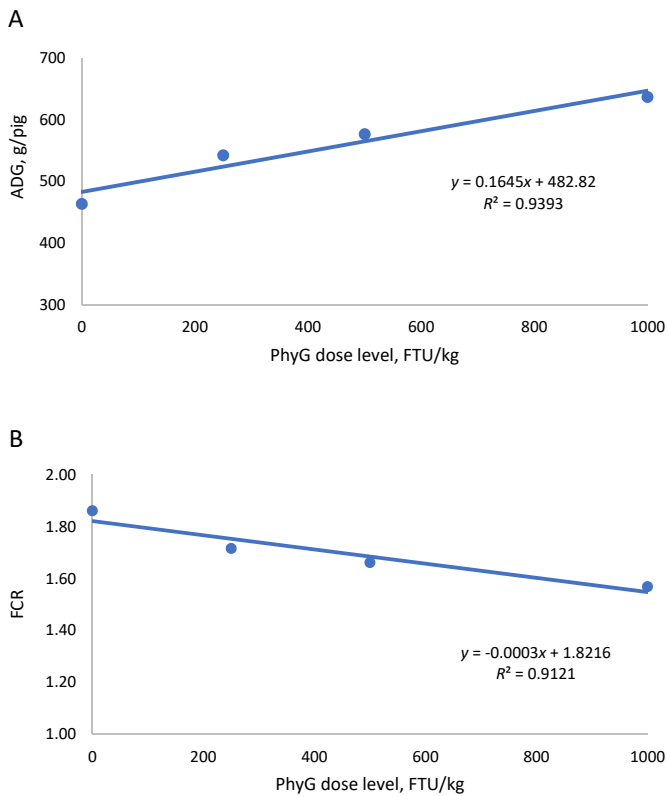[a,b,c,d] Least square means within a row with different superscript letters differ ($P < 0.05$, Tukey test).

Fig. 2. Effect of increasing PhyG dose from 0 (NC) to 1,000 FTU/kg on ADG (A) and FCR (B) for overall period. ADG: *P* linear < 0.0001, *P* quadratic = 0.27; FCR: *P* linear < 0.001, *P* quadratic = 0.23. PhyG = a next generation biosynthetic bacterial 6-phytase; ADG = average daily gain; FCR = feed conversion ratio.

growing-finishing pigs. The present data suggest a positive dose–response effect of the tested phytase on performance (ADG and FCR) in piglets. Further, the results suggest that the inclusion of PhyG or PhyB in piglets is warranted up to at least 1,000 FTU/kg.

The metacarpi bone ash and bone P content results are consistent with the growth performance results. At dose-levels of 500 FTU/kg or higher, both phytases added to P-deficient diets maintained metacarpi bone ash and P content to levels equivalent to the PC with 1.8 g/kg added digestible P from MCP. This suggests that at these dose-levels the biosynthetic phytase may be able to replace 1.8 g/kg of digestible P from MCP without negative impact on bone mineralization. However, data showed that 1,000 FTU/kg of the tested phytases are needed to maintain bone strength to the level of PC with 1.8 g/kg added digestible P from MCP. This indicates that the P requirement is higher for bone strength than bone ash.

Another way of estimating the digestible P-replacement value of the phytase is to use linear regression analysis to compare the observed response (bone ash content, ADG or FCR) to increasing phytase dose with that from increasing digestible P from MCP, as a reference. This analysis suggested that PhyG at 1,000 FTU/kg could replace 1.64, 2.04 or 1.81 g/kg and PhyB could replace 1.65, 1.77 and 1.58 g/kg digestible P from MCP, when metacarpi bone ash, ADG and FCR were used as the response measure, respectively. The corresponding average digestible P-replacement values are 1.83 g/kg for PhyG and 1.66 g/kg for PhyB dosed at 1,000 FTU/kg, indicating that, under the tested conditions, the digestible P-replacement capacity of the PhyG at a dose-level of 1,000 FTU/kg could be somewhat greater than that of an existing *Buttiauxella* sp., phytase. Wider comparison with studies of other phytases in pigs is not straightforward because differences in dietary composition,
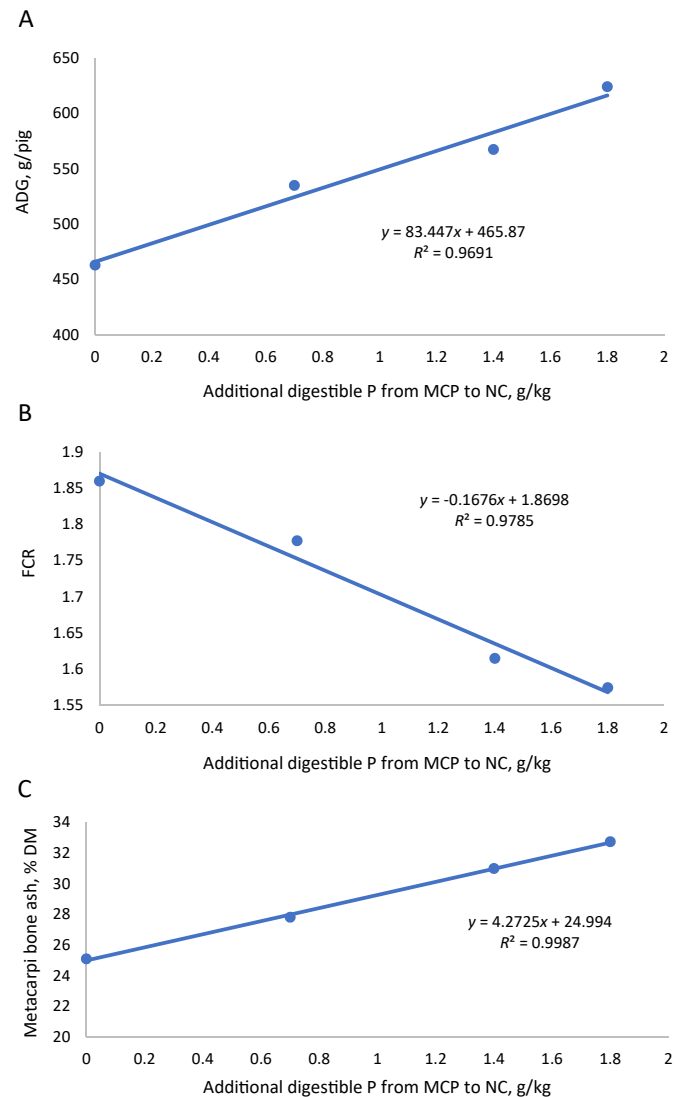

Fig. 3. Linear regression on dose response of ADG (A), FCR (B) and metacarpi bone ash (C) with increasing supplemental digestible P from MCP to the negative control (NC). Linear regression was performed with increasing added digestible P from MCP (e.g. NC, NC+0.7, NC+1.4 and NC+1.8 g/kg digestible P from MCP) against metacarpi bone ash, ADG and FCR, with an equation of *y* = a + b*x*, where *y* is response parameters and *x* is the increasing added digestible P from MCP. *P* < 0.001 for all 3 parameters; R^2 is based on the regression from treatment means. MCP = monocalcium phosphate.

Table 5
Calculated dietary digestible P equivalence values (g/kg diet) of PhyG and PhyB based on bone ash, ADG and FCR as response parameters, using increasing digestible P from MCP as a reference[1]

Item	NC + PhyG			NC + PhyB	
	250 FTU/kg	500 FTU/kg	1,000 FTU/kg	500 FTU/kg	1,000 FTU/kg
Metacarpi bone ash	0.83	1.16	1.64	1.19	1.65
ADG	0.91	1.32	2.04	1.41	1.77
FCR	0.93	1.26	1.81	1.34	1.58
Mean	0.89	1.24	1.83	1.31	1.66

P = phosphorus; PhyG = a next generation biosynthetic bacterial 6-phytase (DuPont Nutrition and Biosciences); PhyB = a commercial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (DuPont Nutrition and Biosciences); ADG = average daily gain; FCR = feed conversion ratio; MCP = monocalcium phosphate;
[1] The digestible P equivalence was calculated by applying *y* values at a given phytase dose (targeted dose) and calculate the corresponding *x* values based on the linear regression model presented in Fig. 3.

animals used and phytase dose-levels across studies are all likely to influence phytase efficacy leading to different P-replacement estimates, as discussed above. However, it is noted that the estimated digestible P-replacement value of PhyG at 1,000 FTU/kg (1.83 g/kg on average from MCP) is higher than the range of values reported across multiple phytase studies in pigs in the recent review by Dersjant-Li et al. (2015).

Further testing of this biosynthetic bacterial 6-phytase is needed in order to confirm its efficacy in terms of P-replacement capacity and capacity to enhance the digestibility of other nutrients such as amino acids, in a range of dietary and animal host settings and throughout the entire production cycle. Nevertheless, the swine industry is currently moving away from the lower dose of 500 FTU/kg feed incorporation level for phytase, towards higher levels that could replace all inorganic P in pig diets, which was achieved with the biosynthetic phytase with an estimated digestible P-replacement capacity of 1.83 g/kg at a dose-level of 1,000 FTU/kg.

5. Conclusion

In conclusion, this study has shown that a next generation biosynthetic bacterial 6-phytase (PhyG) produced in *T. reesei* was effective at maintaining piglet metacarpi bone ash, bone P content and growth performance equivalent to a nutritionally adequate diet (containing 2.9 g/kg digestible P, with 1.8 g/kg dig P from MCP), when added to a corn-soybean meal-based diet without added inorganic P, at a dose-level of 500 or 1,000 FTU/kg. Responses were greatest at a dose-level of 1,000 FTU/kg, at which it was estimated that this phytase could replace an estimated 1.83 g/kg of digestible P from MCP in weaning piglets fed corn-SBM based diets containing rice and rice bran.

Conflict of interest

Yueming Dersjant-Li, Vincent Sewalt, Arno de Kreij, Leon Marchal, Deepak, E. Velayudhan, Robin Anton Sorg, Trine Christensen, Rie Mejldal, Igor Nikolaev, Sina Pricelius, Hye-Sook Kim, Svend Haaning, Jens Frisbæk Sørensen are employee of DuPont Nutrition & Biosciences. We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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