Animal Feed Science and Technology 264 (2020) 114481

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet

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ARTICLE INFO

Keywords:
Broilers
Functionality
Phosphorus availability
Phytase

ABSTRACT

The addition of phytase enzyme to broiler feed as a means of improving phosphorus (P) availability and reducing P excretion is widespread practice. New phytases with enhanced functionality continue to be developed. We tested the effects of a next generation biosynthetic bacterial 6-phytase on growth performance, tibia ash and P digestibility in broilers. Treatments included a nutritionally adequate positive control (PC) diet, a negative control (NC) diet formulated with reductions in Ca and available P (avP) of 2.0 g/kg and 1.9 g/kg (starter phase) and 2.0 g/kg and 1.8 g/kg (finisher phase), respectively, and three further diets comprising the NC supplemented with three levels of phytase (250, 500 or 1000 FTU/kg). Diets were fed in mash form to day-old Cobb 500 broilers housed in pens (9 pens for NC, 10 pens for all other treatments; 24 birds/pen), in two phases (starter, days 1–21; finisher, days 22–42). Tibias were collected from 4 birds on day 21 and from 6 birds on day 42 for determination of defatted tibia ash. Ileal digesta was collected on day 21 for determination of apparent ileal digestibility (AID) of nutrients. Compared to PC, the NC exhibited reduced tibia ash at day 21 and 42, resulting in reduced average daily gain (ADG) and average daily feed intake (ADFI) during starter, finisher, and overall phases, and increased feed conversion ratio (FCR) during finisher phase and overall ($P < 0.05$). Phytase at any dose-level during both phases improved tibia ash vs. NC ($P < 0.05$) and maintained feed intake and growth parameters equivalent to the PC. At a dose-level of 500 FTU/kg or above, phytase supplementation increased AID of P vs. NC ($P < 0.05$) and at 1000 FTU/kg, phytase improved the AID of P compared with PC ($P < 0.05$). For all measures, response values were numerically highest with 1000 FTU/kg and increased linearly or quadratically with increasing phytase dose ($P < 0.05$). Phytase at 1000 FTU/kg produced birds with an average day 42 body weight of 2.74 kg and overall FCR of 1.626, comparable to PC. On a grams per kilogram diet basis, phytase at 1000 FTU/kg improved ileal digestible P by 1.76 g/kg above NC (at day 21).

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; AID, apparent ileal digestibility; avP, available P; BW, body weight; Ca, calcium; CF, crude fat; CP, crude protein; DCP, dicalcium phosphate; DM, dry matter; FCR, feed conversion ratio; FTU, phytase units; IP6, myo-inositol hexaphosphate; K, potassium; MCP, monocalcium phosphate; Mg, magnesium; Na, sodium; NC, negative control; P, phosphorus; PC, positive control; SBM, soybean meal

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https://doi.org/10.1016/j.anifeedsci.2020.114481
Received 19 June 2019; Received in revised form 11 March 2020; Accepted 14 March 2020

This is equivalent to 2.07 g P from monocalcium phosphate (MCP-P), based on digestible P improvement. The results suggest the novel biosynthetic phytase has high functionality in the tested dietary setting.

1. Introduction

The addition of phytase to the feed of monogastric farmed animals is widespread practice. Phytase has been used commercially

Table 1
Ingredient and nutrient composition (g/kg, as fed basis) of the negative control (NC) and positive control (PC) diets in the starter (days 1 to 21) and finisher (days 22 to 42) phases.

	Starter (days 1–21)		Finisher (days 22–42)	
	PC	NC	PC	NC
Ingredient (g/kg)				
Maize	526	549	627	646
Soybean meal (480 g/kg CP)	338	333.5	242	240.5
Canola meal	50.0	50.0	50.0	50.0
Soy oil	38.9	31.0	43.3	36.1
Monocalcium phosphate	14.9	5.55	10.8	2.15
Limestone	15.3	14.0	15.4	13.8
Sodium bicarbonate	–	–	2.00	2.00
Salt	4.70	4.75	2.78	2.80
DL-methionine	2.83	2.80	2.03	2.00
Lysine HCl	2.13	2.20	1.78	1.80
L-Threonine	0.80	0.80	0.60	0.60
Titanium dioxide	4	4	–	–
Poultry minerals[1]	0.35	0.35	0.35	0.35
Poultry vitamins[2]	2.00	2.00	2.00	2.00
Calculated nutrients (g/kg)				
Dry matter	883	881	883	882
Crude protein (CP)	218	217	181	182
Ash	60.9	52.3	51.8	43.4
ADF	41.3	41.7	39.1	39.6
NDF	75.2	77.1	77.9	79.6
Total calcium	9.99	8.00	9.01	6.99
Total phosphorus	7.15	5.22	5.95	4.18
Available phosphorus	4.5	2.56	3.50	1.70
Metabolizable energy (ME) (kcal/kg)	3025	3025	3175	3175
Available methionine	5.87	5.85	4.67	4.67
Available total sulphur amino acid	9.00	8.99	7.40	7.41
Available lysine	12.00	11.99	9.49	9.51
Available tryptophan	2.09	2.08	1.62	1.62
Available threonine	7.91	7.89	6.45	6.47
Available arginine	13.03	12.97	10.45	10.46
Available valine	9.00	9.00	7.53	7.57
Analyzed nutrients (g/kg)[3]				
Dry matter	886	883	889	886
Crude protein (CP)	221	228	186	184
Crude fat	59.8	54.3	62.2	62.0
Ash	58.8	44.4	51.4	43.6
Phytate	8.32	8.38	8.70	9.09
Phytate-P	2.35	2.4	2.45	2.56
Phosphorus	7.1	5.3	7.0	4.8
Potassium	10.1	10.1	9.6	9.0
Magnesium	1.9	1.9	1.8	1.7
Calcium	10.3	8.6	10.4	8.0

[1] Supplied per kilogram of diet: Vitamin A, 11,023 IU; Vitamin D, 3858 IU; Vitamin E, 46 IU; Vitamin B_{12} 0.0165 mg; riboflavin, 5.845 mg; Niacin, 45.93 mg; d-pantothenic acid, 20.21 mg; Choline, 477.67 mg; Menadione, 1.47 mg; Folic acid, 1.75 mg; Pyroxidine, 7.17 mg; Thiamine, 2.94 m; Biotin, 0.55 mg. The carrier was ground rice hulls.

[2] Supplied per kilogram of diet: Manganese, 149.6 mg; Zinc, 125.1; Iron, 16.5 mg; Copper, 1.7 mg copper; Iodine, 1.05 mg; Selenium, 0.25 mg; Calcium, minimum 6.27 mg - maximum 8.69 mg.

[3] The values are the average values for NC and NC + phytase treatments as one batch of NC basal diet was made. The analyzed phytase activity FTU/kg was 43, 24, 282, 480, 882 in starter phase and < 50, < 50, 253, 594, 1110 in finisher phase for PC, NC, NC + 250FTU/kg, NC + 500FTU/kg and NC + 1000 FTU/kg respectively. Phytase activity in the diets was analyzed by DuPont Feed Technical Service, Brabrand, Denmark.

since the early 1990s (Lei et al., 2013), primarily to reduce unwanted excretion of phosphorus (P) into the environment through its capacity to dephosphorylate phytate (myo-inositol hexaphosphate; IP6), a major constituent of plant-based animal feed ingredients. Since then, new generations of phytase have been developed and commercialized.

The improvements in P availability and digestibility that can be achieved with exogenous phytase have been extensively studied and are well accepted for poultry and pigs (Selle and Ravindran, 2007; Selle et al., 2009). It is also widely recognized that phytase can enhance the digestion and utilization of energy and other nutrients, including calcium (Ca), other minerals, amino acids/proteins and starch (Selle et al., 2009; Amerah et al., 2014; Liu et al., 2014; Truong et al., 2015; Dersjant-Li and Kwakernaak, 2019), improving the growth performance of livestock animals. Extensive digestibility trials to quantify the improvements in nutrient digestibility with phytase have enabled the formulation of commercial feed with reduced inclusion rates of inorganic phosphate, Ca and other nutrients in diets containing supplemental phytase. Any reduction in dietary nutrients without loss of performance represents a potential cost saving to producers. Given the continued pressure to reduce feed costs and maximize feed utilization, there remains considerable interest in developing more efficacious phytases and in research to optimize their application in poultry diets.

Current commercial phytases are able to replace 0.3–1.7 g/kg inorganic P from monocalcium phosphate MCP or dicalcium phosphate DCP at a dose range of 500 to 1000 FTU/kg feed in broilers, based on tibia ash and performance parameters Dersjant-Li et al., 2015). New phytases with improved efficacy are being developed using genetic modification technology of the selected enzyme.

The utility of a phytase is typically assessed *in vivo* by the degree to which it can improve P availability by way of measuring bone ash and/or P digestibility, in a diet deficient in nutrients (such as P and Ca) and supplemented with the specific phytase.

This study assessed the utility of a next generation biosynthetic bacterial 6-phytase when added to a basal diet reduced in Ca and P, on broiler tibia ash and apparent ileal digestibility (AID) of P, when compared with a nutritionally adequate, unsupplemented diet. In addition, observations were made on feed intake, growth performance, and feed conversion ratio.

2. Materials and methods

2.1. Experimental and control diets

Positive control (PC) diets based on corn and soybean meal (SBM) were formulated to meet the recommended requirements for nutrients (adequate in P and Ca) of the birds during starter (days 1–21) and finisher (days 22–42) phases (NRC, 1994). Negative control (NC) diets were formulated with reductions in Ca and available phosphorus (avP) of 2.0 g/kg and 1.9 g/kg in starter phase and 2.0 g/kg and 1.8 g/kg in finisher phase diets, respectively (Table 1. All starter diets contained titanium dioxide added at 4 g/kg as an indigestible marker. Negative control diets were tested as stand-alone diets or supplemented with 250, 500 or 1000 FTU/kg of a next generation biosynthetic bacterial 6-phytase containing 5000 FTU/g on a wheat carrier. The phytase is a thermotolerant phytase produced by fermentation with a fungal *Trichoderma reesei*) production strain expressing a biosynthetic bacterial phytase gene. It is a variant of a consensus high melting temperature phytase sequence derived from various bacterial phytase genes including *Buttiauxella sp*. (DuPont Nutrition and Biosciences, The Netherlands). Phytase activity is commonly expressed as phytase units (FTU) and defined as the amount of phytase that liberates 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, 2000, Method 2000.12). Diets and water were provided to birds *ad libitum* in mash form.

2.2. Birds, housing and experimental design

All experimental protocols were approved by the Animal Care and Use Committee of Texas A&M University, USA, where the research was conducted.

Cobb 500 broiler chicks of mixed sex were obtained on day of hatch from a commercial hatchery where they had been vaccinated against Infectious Bronchitis and Newcastle Disease, via drinking water. Vaccination against Infectious Bursal Disease was administered on days 11–14 also via drinking water. Birds were allocated to floor-pens on the basis of initial body weight (BW) so that each pen contained birds of approximately equal BW. A total of 1176 birds were assigned to 49 pens with 24 birds per pen (9 pens for NC and 10 pens for all other treatments) with each pen containing 50 % males and 50 % females, in a completely randomized design. Pens were located in an environmentally controlled broiler house with a lighting regime of L:D 18:6 and an initial temperature of 35 °C, reduced to 24 °C on d 28.

2.3. Sampling and measurements

Representative sub-samples of all diets were analyzed for dry matter (DM), crude protein (CP), crude fat (CF), ash, P, potassium (K), magnesium (Mg), Ca, sodium (Na), phytate and phytase.

Body weight and feed intake (FI) were measured on day 1, 21, and 42 on a pen basis, and used to calculate average daily weight gain (ADG), average daily feed intake (ADFI) and mortality corrected feed conversion ratio (FCR). Mortality was checked and recorded daily.

On day 21 and 42, 4 birds (2 males, 2 females, sex determined at the sampling point) and 6 birds (3 males, 3 females), respectively, were randomly selected per pen, killed by CO_2 gas and their left tibias collected and pooled (by pen) for the determination of defatted tibia ash. Ileal digesta (distal half of the ileal sample) was collected from euthanized birds on day 21, pooled per pen and frozen on a Labconco FreeZone 12+ dehydration machine (Labconco, Kansas City, Missouri). Dried feed and digesta samples were analyzed for P and Ca content in order to calculate nutrient digestibility using titanium dioxide as the inert marker.

2.4. Chemical analysis

Samples were analyzed in duplicate for all analyses. Nutrients in feed and ileal digesta were analyzed according to the following methods: crude protein, NEN-EN-ISO 16634 NEN-ISO, 2008); crude fat, NEN-ISO 6492 (NEN-ISO, 1999); crude fiber, NEN-ISO 6865 (NEN-ISO, 2000). Phosphorus, Ca, Mg, K and Na in feed and P and Ca in digesta were analyzed by microwave digestion and Inductively Coupled Plasma-Optical Emission Spectrometry (OES) in accordance with method AOAC, 2011.14 (AOAC, 2011). Titanium concentration in feed and ileal digesta was analyzed using a modified protocol outlined by Short et al. (1996). Half a gram of each dried sample was weighed and placed in an ashing oven at 450 °C. Following ashing, each sample was titrated with 10 mL of 7.4 M sulfuric acid and boiled at 200 °C for 3 h until dissolved. Samples were then titrated with 10 mL of 30 % hydrogen peroxide. A total sample volume of 100 mL was achieved by addition of distilled water. Samples were analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV–vis (Model 10S UV–vis) Spectrophotometer at 410 nm. Phytate phosphorus (PP [inositol hexaphosphate (IP6)]) concentrations and phytase activities in the diets were determined by DuPont Laboratories (Brabrand, Denmark), using the methods described by Yu et al. (2012).

Tibia ash was measured using the method described below: fibula, muscle and connective tissue were removed and the bones dried at 100 °C for at least 12 h before defatting in diethyl ether for 7 − 8 h and air-drying. Defatted tibias were dried again at 100 °C for at least 12 h and then ashed in ceramic crucibles at 600 °C for 24 h.

2.5. Calculations

Feed conversion ratio was calculated based on total body weight gain (BWG, including the mortality weight) and total feed intake from days 1 to 21, days 22 to 42, and days 1 to 42. Both ADG and AFDI were calculated by correction of mortality, e.g. ADFI was calculated by total feed intake in each phase and divided by the total number of days of feeding. Mortality-corrected ADG was calculated from mortality corrected ADFI divided by mortality corrected FCR.

The apparent ileal digestibility (AID) of P and Ca were calculated based on the following formula, using titanium dioxide as the inert marker:

$$AID = 1 - [(Ti_d/Ti_i) \times (N_i/ N_d)]$$

Where Ti_d is the titanium concentration in the diet, Ti_i is the titanium concentration in the ileal digesta, N_i is the nutrient (P or Ca) concentration in the ileal digesta and N_d is the nutrient concentration in the diet. All analyzed values were expressed as grams per kilogram dry matter.

2.6. Statistical analysis

Data are reported by pen as the experimental unit. Data were analyzed by analysis of variance (ANOVA) using the Fit Model

Table 2

Effect of the next generation biosynthetic bacterial 6-phytase on growth performance and defatted tibia ash content in broilers, by phase.[1]

	PC	NC	NC + Phytase (FTU/kg)			SEM	*P* - value	*P linear*[2]	*P quadratic*[2]
			250	500	1000				
Starter (days 1–21)									
BW day 21 (kg/bird)	0.90[a]	0.71[b]	0.86[a]	0.88[a]	0.89[a]	0.010	< 0.001	< 0.001	< 0.001
ADFI (g/day)	56.6[a]	46.3[b]	54.1[a]	54.3[a]	55.4[a]	0.764	< 0.001	< 0.001	< 0.001
ADG (g/day)	40.6[ab]	31.7[c]	39.0[b]	39.5[ab]	40.8[a]	0.431	< 0.001	< 0.001	< 0.001
FCR (g/g)	1.396[ab]	1.460[a]	1.390[b]	1.377[b]	1.358[b]	0.016	< 0.010	< 0.001	0.014
Tibia ash day 21 (g/kg DM)	503.9[ab]	443.5[c]	493.1[b]	499.1[ab]	508.5[a]	3.08	< 0.001	< 0.001	< 0.001
Finisher (days 22–42)									
BW day 42 (kg/bird)	2.70[a]	1.85[b]	2.64[a]	2.70[a]	2.74[a]	0.031	< 0.001	< 0.001	< 0.001
ADFI (g/day)	157.7[a]	121.5[b]	154.4[a]	156.1[a]	156.0[a]	1.89	< 0.001	< 0.001	< 0.001
ADG (g/day)	86.9[a]	58.2[b]	84.7[a]	87.2[a]	87.8[a]	1.135	< 0.001	< 0.001	< 0.001
FCR (g/g)	1.815[b]	2.093[a]	1.823[b]	1.792[b]	1.779[b]	0.029	< 0.001	< 0.001	< 0.001
Tibia ash day 42 (g/kg DM)	463.9[a]	422.7[b]	464.5[a]	464.7[a]	469.9[a]	7.03	< 0.001	0.002	0.010
Overall (day 1–42)									
ADFI (g/day)	118.3[a]	92.9[b]	115.1[a]	116.4[a]	116.7[a]	1.186	< 0.001	< 0.001	< 0.001
ADG (g/day)	71.3[ab]	50.6[c]	69.1[b]	70.9[ab]	71.8[a]	0.648	< 0.001	< 0.001	< 0.001
FCR (g/g)	1.661[b]	1.835[a]	1.666[b]	1.643[b]	1.626[b]	0.019	< 0.001	< 0.001	< 0.001

a,b,c, Least square means within a row with no superscript letters in common differ ($P < 0.05$, Tukey test).

[1] All performance data are corrected for mortality; PC, positive control; NC, negative control; BW, body weight; ADFI, average daily feed intake per bird; ADG, average daily gain per bird; FCR, feed conversion ratio.

[2] *P* values for linear and quadratic contrast were analyzed with increasing phytase dose from 0 NC to 1000 FTU/kg.

platform of JMP 14.0 (JMP, 2019) to investigate the effect of treatments in a randomized design. Means separation was achieved using Tukey's Honest Significant Difference test. Linear and quadratic responses to increasing phytase dose were analyzed using orthogonal polynomials. Differences were considered statistically significant at $P < 0.05$; $P < 0.10$ was considered a tendency.

3. Results

3.1. Diet analysis

Analyzed phytase activities in the final diets confirmed the target dose-levels (Table 1). Analyzed values of CP in the basal (control) diets were within 10 % of calculated values. Achieved reductions in P content in the NC diets adhered well to targeted reductions; based on analyzed values, total P content was reduced by 1.8 g/kg in starter and 2.2 g/kg in finisher diets.

3.2. Feed intake and growth performance

The effect of dietary treatment on ADFI, BW, ADG and FCR is presented in Table 2. Treatment affected all response measures during all growth phases (starter, finisher, overall; $P < 0.01$ in all cases). No significant differences were observed for mortality (data not shown).

Compared to PC, birds fed the NC diet exhibited reduced BW at day 21 and day 42, increased FCR during finisher phase and overall, and reduced ADG and ADFI during all phases ($P < 0.05$).

Supplementation with the phytase, at any dose-level, allowed the birds to overcome the P deficiency in NC diets with improved ADFI, BW and ADG, and FCR during all phases ($P < 0.05$) such that they were equivalent to the PC during all phases, regardless of phytase dose. A dose-level of 1000 FTU/kg of the phytase produced birds with a mean BW at day 42 of 2.74 kg and a mean overall FCR of 1.626 (vs. 1.661 in PC). Increasing phytase dose from 0 (NC) to 1000 FTU/kg resulted in linear and quadratic response in ADFI, ADG and FCR in all phases ($P < 0.05$).

3.3. Tibia ash

The effect of dietary treatment on tibia ash was highly significant ($P < 0.001$) and is also presented in Table 2. Compared to PC, birds fed the NC diet exhibited reduced tibia ash at day 21 and at day 42 (-6.0 and -4.1 percentage points, respectively; $P < 0.05$). Compared to NC, phytase supplementation improved tibia ash sampled at both day 21 and day 42, at all three dose-levels ($P < 0.05$). At day 21, the greatest tibia ash was seen with phytase at 1000 FTU/kg, which was greater $P < 0.05$) than the NC or phytase at 250 FTU/kg, whereas PC and phytase at 500 FTU/kg showed intermediate tibia ash values. At day 42, tibia ash in all phytase treatments was equivalent to PC.

3.4. Nutrient digestibility

The AID of P was not significantly reduced in birds fed the NC vs. PC diets (Table 3). At a dose-level of 500 FTU/kg or above, phytase supplementation increased AID of P vs. NC ($P < 0.05$) and at 1000 FTU/kg, phytase improved the AID of P compared with PC ($P < 0.05$). Expressed on a g/kg basis, ileal digestible P in the diets was improved by phytase when dosed at 500 FTU/kg or higher (+ 1.39 g/kg vs. NC at 500 FTU/kg and + 1.76 g/kg vs. NC at 1000 FTU/kg; $P < 0.05$). At these dose-levels, digestible P expressed as g/kg in the diet was equivalent to that of the PC diet. The AID of Ca was unaffected by dietary treatment but tended to increase linearly ($P = 0.052$) with increasing phytase dose from 0 to 1000 FTU/kg. Increasing phytase dose from 0 (NC) to 1000 FTU/kg increased AID P in a linear ($P < 0.001$) and quadratic manner ($P = 0.048$).

4. Discussion

This study has shown that the biosynthetic bacterial 6-phytase added to the P and Ca deficient NC diets at dose levels of 250, 500

Table 3
Effect of the next generation biosynthetic bacterial 6-phytase on apparent ileal digestibility (AID) coefficient of P and Ca and digestible P in the diets as g/kg, in broilers at 21 days of age.

	PC	NC	NC + Phytase (FTU/kg)[1]			SEM	P - value	P-linear[1]	P quadratic[1]
			250	500	1000				
AID P	0.501[bc]	0.390[c]	0.577[abc]	0.652[ab]	0.722[a]	0.0577	< 0.001	< 0.001	0.048
AID Ca	0.402	0.392	0.470	0.511	0.553	0.0493	0.240	0.052	0.520
Digestible P (g/kg diet)	3.55[a]	2.06[b]	3.06[ab]	3.46[a]	3.82[a]	0.3	< 0.001	< 0.001	0.048

a,b,c, Least square means within a row with no superscript letters in common differ ($P < 0.05$, Tukey test).
PC, positive control; NC, negative control.
[1] P values for linear and quadratic contrast were analyzed with increasing phytase dose from 0 NC to 1000 FTU/kg.

or 1000 FTU/kg improved performance and tibia ash above the NC, to a level equivalent to a nutritionally adequate PC diet. The improved ileal P digestibility was the clear driver of performance and tibia ash improvements; the phytase dosed at 500 and 1000 FTU improved digestible P as g/kg diet vs. NC, to a level comparable to the PC.

Improvements in P availability coincided with improvements in growth performance parameters, which were evident in both starter and finisher phases and were produced with diets that contained phytate at normal commercial levels. This indicates that the phytase was highly effective in the tested dietary setting.

Whilst responses in performance at all phytase dose-levels were statistically equivalent to PC, there was a clear dose-response effect observed in both starter and finisher phases, as evidenced by a linear and quadratic increase in all performance response measures including tibia ash and in AID of P with increasing phytase dose within the tested dose-range. Several recent studies have noted similar dose-response effects of phytase on performance and/or nutrient digestibility in broilers within the range 0–2,000 FTU/kg (Kiarie et al., 2015; Dersjant-Li et al., 2018; Dersjant-Li and Kwakernaak, 2019). Dersjant-Li and Kwakernaak (2019) observed a curve-linear response in ADG with increasing phytase dose from 0 to 1000 FTU dosed at 250, 500, 750 and 1000 FTU/kg for *Buttiauxella* phytase in broilers fed corn SBM based diets between 5 and 20 days of age. In the current study, we observed that at a dose level of 1000 FTU/kg, while ADG was equivalent to PC, FCR was reduced by 2.72 %, 1.98 % and 2.11 %, in starter, finisher and overall phases, respectively compared with PC. This may indicate an extra-phosphoric effect of the phytase.

The tibia ash results are consistent with the growth performance results. Tibia ash in all three phytase treatments 250, 500 and 1000 FTU/kg was statistically equivalent to PC at both time-points day 21 and day 42, indicating that the phytase was effective in compensating for the reduced inorganic P content of the phytase-supplemented diets. Nevertheless, the response was numerically highest with 1000 FTU/kg tibia ash 508.9 g/kg DM, vs. 443.5 NC and 503.9 PC. Based on analyzed values of total P in the final diets, the phytase dosed in the range of 250 to 1000 FTU/kg was effective in replacing 1.8 g/kg total P in the starter diets and 2.2 g/kg total P in the finisher diets, using tibia ash, ADG and FCR as response parameters.

There was a clear dose-response effect of the phytase on AID of P. At a dose-level of 500 and 1000 FTU/kg, AID of P was improved by 26.2 and 33.2 percentage points respectively vs. NC. This compares well with the existing literature relating to other phytases in corn SBM based broiler diets. Truong et al. (2015) found an AID of P increase of 14 percentage points by *Buttiauxella* phytase supplemented at 500 FTU/kg in older birds fed corn SBM based diets from 28 to 40 days of age. Amerah et al. (2014) reported improvements in ileal P digestibility of 12.3–19.9 percentage points (dependent on the dietary Ca:avP ratio) by *Buttiauxella* phytase supplemented at 1000 FTU/kg in broilers fed corn SBM based diets from 5 to 21 days of age. Bello et al. (2019) observed that in broilers fed corn SBM based diets, *Buttiauxella* phytase supplemented at 1000 FTU/kg increased ileal P digestibility of 27.3 percentage points measured at 22 days of age. In broilers at 28 days of age fed corn SBM based test diets for 32 h (without adaptation), Kim et al. (2018) observed an increased in AID of P of 29–43 percentage points (dependent on the dietary Ca level and limestone particle size) by *Buttiauxella* phytase supplemented at 1000 FTU/kg. Ravindran et al. (2006) observed improvements in ileal P digestibility coefficients of 13–21% dependent on dietary phytate content with 1000 FTU/kg of an *E. coli* phytase, when compared to an unsupplemented NC diet. In the current study, on a grams per kilogram diet basis, the phytase at 500 and 1000 FTU/kg improved the amount of digestible P in the diet by 1.39 and 1.76 g/kg, respectively, above the NC on day 21. For feed formulation purposes, the P digestibility of MCP is commonly taken as 85 % (CVB, 2018. On this basis, it is estimated that the phytase at 500 and 1000 FTU/kg could replace 1.64 and 2.07 g MCP-P per kilogram of diet, respectively, when using digestible P improvement as the response parameter. This suggests that the phytase is highly effective in the breakdown of phytate and at increasing P availability. AID of Ca was not affected by dietary treatments, however increasing phytase dose tended to linearly increase AID of Ca, indicating the phytase can also benefit Ca availability.

Comparing phytase P-equivalence/replacement values across studies/phytases is not straightforward. Differences in assessment criteria, age of animals, adaptation period, dietary phytate content and Ca:P ratio, as well as phytase dose and source, may all influence the magnitude of responses observed (Dersjant-Li et al., 2015). Nevertheless, a MCP-P replacement of 2.07 g/kg based on digestible P improvement with this new phytase indicates a high efficacy compared with other commercial phytases. The first generation fungal-derived phytases of the 1990s were capable of replacing approximately 1 g P from MCP (Yi and Kornegay, 1996; Selle and Ravindran, 2007), whilst second generation *E. coli* phytases developed in the 2000s with improved efficacy were effective in replacing between approximately 1.2–1.5 g P from MCP (Rodriguez et al., 1999a, b; Tran et al., 2011; Dersjant-Li et al., 2015). Studies in a range of dietary settings incorporating different cereal, phytate and Ca contents would provide further information, but the results from this study suggest that the biosynthetic bacterial 6-phytase experimental phytase has high efficacy.

5. Conclusions

This study demonstrated that the next generation biosynthetic bacterial 6-phytase was effective at maintaining growth performance, tibia ash and ileal phosphorus digestibility equivalent to a nutritionally adequate diet, when added to diets formulated with a 1.8–1.9 g/kg reduction in inorganic phosphorus from monocalcium phosphate and administered at dose levels between 250 and 1000 FTU/kg. The phosphorus replacement value from monocalcium phosphate was estimated to be 1.64 and 2.07 g/kg of diet respectively at 500 and 1000 FTU/kg (equal to 1.39 and 1.76 g/kg digestible P from monocalcium phosphate), based on the observed increase in ileal digestible phosphorus.

CRediT authorship contribution statement

Y. Dersjant-Li: Conceptualization, Formal analysis, Writing - original draft. **G. Archer:** Data curation, Project administration,

Investigation, Writing - review & editing. **A.M. Stiewert:** Data curation, Project administration, Investigation, Writing - review & editing. **A.A. Brown:** Data curation, Project administration, Investigation, Writing - review & editing. **E.B. Sobotik:** Data curation, Project administration, Investigation, Writing - review & editing. **A. Jasek:** Data curation, Project administration, Investigation, Writing - review & editing. **L. Marchal:** Methodology, Writing - review & editing. **A. Bello:** Methodology, Writing - review & editing. **R.A. Sorg:** Methodology, Writing - review & editing. **T. Christensen:** Methodology, Writing - review & editing. **H.-S. Kim:** Methodology, Writing - review & editing. **R. Mejldal:** Methodology, Writing - review & editing. **I. Nikolaev:** Methodology, Writing - review & editing. **S. Pricelius:** Methodology, Writing - review & editing. **S. Haaning:** Methodology, Writing - review & editing. **J.F. Sørensen:** Methodology, Writing - review & editing. **A. de Kreij:** Methodology, Writing - review & editing. **V. Sewalt:** Methodology, Writing - review & editing.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgement

The authors would like to thank Dr Joelle Buck (Reading, UK) for her assistance with the writing of this manuscript.

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