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Effect of increasing *Buttiauxella* phytase dose on nutrient digestibility and performance in weaned piglets fed corn or wheat based diets

Y. Dersjant-Li[a,*], A.L. Wealleans[a], L.P. Barnard[a], S. Lane[b]

[a] *Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK*
[b] *Drayton Animal Health, Alcester Road, Stratford-upon-Avon, Warwickshire, CV37 9RQ, UK*

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ABSTRACT

Two experiments were carried out to determine the effect of increasing phytase levels on apparent total tract digestibility (ATTD) of nutrients, P and Ca retention and growth performance in weaned piglets fed corn or wheat based diets. Five treatments were tested including a positive control (PC) meeting piglet nutrient requirements; a negative control (NC) formulated with a reduction in digestible P (−1.4 g/kg and −1.5 g/kg in Exp. 1 and 2 respectively) and Ca (−1.5 g/kg in both studies); and NC supplemented with a *Buttiauxella* phytase at 500, 1000 or 2000 phytase units (FTU)/kg feed. One FTU is defined as the amount of enzyme required to release 1 μmol of iP (inorganic phosphorus) per minute from sodium phytate at pH 5.5 at 37 °C. A complete randomized design was used in both studies, with individual piglets housed in metabolic crates as the experimental unit. There were 2 runs in Exp. 1 and 4 runs in Exp. 2. Each treatment consisted of 8 replicates (1 piglet/replicate) in Exp. 1 and 12 replicates in Exp. 2. Diets based on wheat and soybean meal in Exp. 1 and corn and soybean meal in Exp. 2 were fed in pelleted form; feed and water were supplied *ad libitum* to the piglets (mean initial BW of 11 ± 1.5 kg) during the 14 day period. Urine and feces production were collected from each crate during d 10 to14. The ATTD of P, Ca, DM, N and energy, and retention of P and Ca were measured using TiO_2 as an indirect marker. In both Exp, increasing phytase dose from 0 (NC) to 2000 FTU/kg linearly increased ($P < 0.05$) ADG and G:F. Phytase at 2000 FTU/kg improved ADG and G:F compared to NC. A linear response was seen for ATTD of P, Ca and GE ($P < 0.05$) in Exp. 1, and for ATTD P and Ca in Exp. 2, with increasing phytase dose. Also there was a tendency for increasing phytase dose to result in a linear increase ($P \le 0.10$) in ATTD of DM in Exp.1, and ATTD of DM and N in Exp. 2. Increasing phytase dose linearly reduced P and Ca excretion and increased the retention of these nutrients in both experiments. The results showed that increasing *Buttiauxella* phytase dose up to 2000 FTU/kg may provide environmental and production benefits in weaned piglets fed either wheat or corn based diets.

1. Introduction

Phosphorus in plant based ingredients is mainly present in the form of phytate (up to 80% of total P), which has limited availability to monogastric animals. Phytate (the salt form of phytic acid, inositol-6-phosphate, IP6) can bind to protein and minerals

Abbreviations: ATTD, apparent total tract digestibility; Ca, calcium; FTU, phytase units; G:F, gain/feed; N, nitrogen; P, phosphorus

* Corresponding author at: Nisco Animal Nutrition DuPont Industrial Biosciences Danisco UK Ltd P.O., UK.
E-mail address: Yueming.Dersjant-Li@Dupont.com (Y. Dersjant-Li).

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as well as interact with endogenous enzymes, thus reducing nutrient utilization (Selle et al., 2012). Phytase has traditionally been used in pig feed at 500 phytase units (FTU)/kg based on historic calculations of economic value. However, the price of inorganic phosphorus (iP) has increased drastically in the last 10 years and more efficient and economical phytases are available in the market. Furthermore, it is recognized that phytase can reduce the anti-nutritional effect of phytate, improve nutrient digestibility such as amino acids, therefore resulting in extra-phosphoric effect (Adedokun et al., 2015; Cowieson et al., 2008; Selle et al., 2012). In grower pigs, increasing phytase dose from 250 to 1000 FTU/kg resulted in a linear increase in ADG (Dersjant-Li et al., 2017), phytase dosed at 1000 FTU/kg improved ADG by 5.3% vs a positive control. All these factors have led to the use of higher doses of phytase in swine diets.

The primary function of phytase is to degrade phytate and to improve P digestibility. However, using the traditional dose of 500 FTU/kg, *in vivo* phytate hydrolysis is incomplete. A review of the literature by Dersjant-Li et al. (2015) showed that approximately 45–60% of phytate was degraded, by the end of the small intestine, in diets containing phytase at 500 FTU/kg, and between 55 and 88% in diets containing 1000 FTU/kg. The phytate degradation rate was related to phytase source, dose, dietary Ca and available P levels, phytate level and grain source.

A high dose (> 1000 FTU/kg) of a bio-efficacious phytase will degrade phytate more thoroughly in the stomach and in the upper part of the small intestine, more effectively eliminating the anti-nutritional effect of phytate and resulting in increased nutrient digestibility and growth performance in piglets. The response of piglets to increasing phytase dose may be related to dietary grain source and phytate levels. The objective of this paper was to evaluate the effects of a *Buttiauxella* phytase at a dose range of 0–2000 FTU/kg on nutrient digestibility, retention and performance in weaned piglets fed wheat- or corn-based diets using data from two studies.

2. Materials and methods

Two digestibility studies were carried out to test the effects of increasing phytase dose on nutrient digestibility and performance in weaned piglets. Both studies were carried out at Drayton, Stratford upon Avon, UK and approved by Animal Welfare committee.

2.1. Animals

Two studies were carried out using weaned piglets individually housed in metabolic crates in an environmentally controlled facility. Lighting was set at 16:8 h light: dark and temperature was set to gradually reduce from 27 to 23 °C over the course of the experiment. In Exp. 1 a total of 40 weaned entire male Large White x Landrace piglets with initial age between 28 and 32 days were used. The study was completed in 2 runs, with 4 replicates/treatment in each run, 8 replications per treatment. In Exp. 2, a total of 60 entire male Landrace x Duroc piglets aged between 21 and 28 days were used; 15 piglets/run in 4 runs, with 1 piglet/crate and 3 piglets/treatment in each run, 12 replications per treatment. In both trials, after a 7d acclimatization period (on a commercial diet) piglets were randomly allocated to metabolic crates with an average initial BW of 11 ± 1.5 kg.

2.2. Experimental design

The treatments included a positive control (PC) diet formulated to meet the nutrient requirements of the piglets; a negative control (NC) with a reduction in digestible P and Ca; and NC diet supplemented with a 6-phytase from *Buttiauxella* sp. (Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK) at 500, 1000 or 2000 FTU/kg respectively. One FTU is defined as the quantity of enzyme that releases 1 μmol of inorganic P per minute from 5.0 mM sodium phytate at pH 5.5 at 37 °C (AOAC, 2000). Negative control diets were formulated with 1.5 g/kg lower Ca in both trials and with 1.4 g/kg and 1.5 g/kg lower digestible P in Exp. 1 and 2 respectively compared to PC. However, the analyzed total P content was 1.7 g/kg and 2.4 g/kg lower in NC compared to PC, for Exp. 1 and 2 respectively (Table 1).

2.3. Experimental diets

In Exp. 1 a wheat and soybean meal based diet was used and in Exp. 2, a corn and soybean meal based diet was used. The composition of PC and NC diets is summarized in Table 1. One batch of NC diet was produced and split into 4 batches before phytase was added to the treatment batches. Diets were fed in pelleted form (pelleting temperature < 80 °C); feed and water were provided *ad libitum* throughout the 14d period. Animals were weighed at the start and the end of each run. The amount of feed offered to each crate was measured daily and any feed wasted or removed was weighed to provide a total feed intake during the study. Feed samples were analyzed for phytase activity and phytic acid content by Danisco Innovation Laboratories (Brabrand, Denmark), using the method described by Yu et al. (2012, 2014).

2.4. Sampling and measurements

Average daily feed intake, ADG and G:F were calculated for each piglet. Total collection of feces and urine was conducted during days 10–14; urine and fecal productions were recorded twice daily for each of the crates for each run. Fresh feces were collected from each crate at least twice daily and refrigerated at approximately 4 °C. At the end of the collection period the total 4 day collection for each animal was weighed. After thorough mixing and homogenization a sample from each animal was weighed and dried at 55 °C to

Table 1
Ingredients, calculated and analyzed nutrients composition (as fed).

	Exp. 1		Exp. 2	
	PC[1]	NC[2]	PC	NC
Ingredients, g/kg				
Wheat	583.1	595.8		
Corn			556.7	571
Soybean meal	207.9	205.4	267	266
Dried whey	100	100	100	100
Skimmed milk	50	50		
Soya protein concentrate			25	25
Fish meal 60	10	10		
Soybean oil	23.7	20	17	12
Dicalcium phosphate	10.6	0	14.7	2.9
Limestone	2.7	6.7	6.3	9.8
Salt	1	1	1.2	1.2
Lysine HCl	3.8	3.8	1.35	1.35
DL-Methionine	0.8	0.8	1.1	1.1
L-Threonine	1.6	1.6	0.6	0.6
V/TM Premix[3]	5	5	5	5
Titanium dioxide	4	4	4	4
Calculated nutrients, g/kg				
Protein	206	206.3	205	205
DE, MJ/kg	14.58	14.58	14.5	14.5
Ca	6.4	4.9	8	6.5
Total P	6.5	4.6	6.8	4.7
Digestible P	3.6	2.2	3.5	2
Dig Lys	12.4	12.4	10.9	10.9
Dig Met	3.6	3.6	4	4
Dig Met + Cys	6.5	6.5	6.5	6.5
Dig Thr	8	8	7.1	7.1
Dig Trp	2.1	2.1	2	2
Analyzed phytate P, g/kg	2.3	2.3	2	2

[1] PC: positive control.
[2] NC: negative control.
[3] V/TM Premix: vitamin and trace mineral premix, supplied the following per kg of finished feed: vitamin A, 5.0 mg; vitamin D3, 2 mg; vitamin E, 200 mg; vitamin B1, 4.2 mg; vitamin B2, 5.6 mg; vitamin B6, 5.0 mg; vitamin B12, 50.0 mg; pantothenic acid, 19.9 mg; niacin, 40 mg; Cu, 160 mg; Zn, 100 mg; Fe, 200 mg; Mn, 62 mg; I, 2.2 mg; Se, 0.3 mg.

determine individual sample DM. After drying, samples for each individual animal were sent to Eurofins Ltd (Wolverhampton, UK) and analyzed for total P, Ca, N, ash, TiO_2 and GE, using the methods described below.

From d 10–14 urine was collected twice daily and the weight of urine recorded. Urine was placed into an air tight container containing 25 ml of 25% v/v sulphuric acid. To prevent volatilization of the N fraction, an additional 25 ml of sulphuric acid was added to each urine container every morning during the collection period. On d 14 the total 4 d collection for each animal was weighed and a representative sample (100 g) taken and sent chilled on ice packs to Eurofins Ltd (Wolverhampton, UK). The samples were analyzed for total N (using the Dumas method), total P, Ca (using inductively coupled plasma optical emission spectrometry) and GE (using bomb calorimetry). TiO_2 analysis was according to the method described by Vogel (1961). Feed samples were analyzed for DM, GE, CP, Ca and P by Sciantec Analytical Services Ltd (Cawood, UK). Dry matter was measured by weighing a fixed quantity of sample and dried overnight (> 4 h) in a drying oven at a temperature of 70–80 °C to receive a constant weight. GE, CP, Ca and P content were measured using the same methods as mentioned above

The formula to calculate total tract digestibility coefficients was:

$$\text{Apparent nutrient digestibility, \%} = 100 - [(N_f/N_d) \times (T_{id}/T_{if}) \times 100],$$

Where N_f is equal to nutrient concentration in feces (mg/kg DM), N_d is equal to nutrient concentration in diets (mg/kg DM), T_{id} is equal to TiO_2 concentration in diets (mg/kg DM) and T_{if} is equal to TiO_2 concentration in feces (mg/kg DM).

P and Ca retention (expressed as g/d) were calculated based on total fecal and urine collection data. P and Ca intake were calculated using daily feed intake multiplied by analyzed dietary P and Ca levels. P and Ca retention were calculated by the intake of these nutrients minus fecal plus urinary excretion, expressed as g/d.

2.5. Statistical analysis

The data were analyzed for each experiment, with individual pig as the experimental unit and run considered as random effect. Treatment means were compared by Tukey's HSD (Honest Significant Difference) test using JMP 11 (SAS Institute Inc., Cary, NC,

Table 2
Analyzed phytase activity (phytase units, FTU/kg), nutrient content in Exp. 1 and Exp 2[1].

	PC	NC	500 FTU/kg	1000 FTU/kg	2000 FTU/kg
Exp. 1 (wheat)					
DM, g/kg	922	921	921	921	919
Crude protein, g/kg	211	210	212	210	211
Gross energy (MJ/kg)	17.49	17.28	17.27	17.44	17.40
P, g/kg	5.8	4.1	4.3	3.7	4.1
Ca, g/kg	5.7	4.5	4.6	4.1	4.6
Phytase activity, FTU/kg	< 50	< 50	538	938	1901
Exp. 2 (corn)[2]					
DM, g/kg	890	889	888	889	889
Crude protein, g/kg	197	213	209	209	214
Gross energy (MJ/kg)	16.33	16.45	16.45	16.44	16.48
P, g/kg	6.8	4.4	4.2	4.5	4.4
Ca, g/kg	8.8	6.6	5.9	6.8	6.2
Phytase activity, FTU/kg	< 50	< 50	522	1096	1994

[1] *Buttiauxella* phytase added on the top of NC.
[2] The values are average values of diets from different runs, for the analyzed P and Ca content.

USA). Linear and quadratic responses were analyzed with increasing phytase dose from 0 (NC) to 2000 FTU/kg. Significance was determined at $P < 0.05$; $P < 0.10$ was taken to indicate a trend.

3. Results

Phytase activity in all diets was within the target dose range, as shown in Table 2.

3.1. Performance

Performance results are presented in Table 3. In Exp.1, pigs fed P and Ca deficient NC diets showed 12% lower ADG than PC, however, the difference was not statistically significant. Phytase at 2000 FTU/kg increased ($P < 0.05$) ADG (24%) and ADFI (7.7%), showing greater G:F (15%, $P = 0.06$) compared to the NC. When compared to PC, phytase at 2000 FTU/kg resulted in 9.5% greater ADG and 5% greater G:F but the differences were not statistically significant. In Exp. 2, pigs in NC group had lower ADG and G:F than PC ($P < 0.05$). Phytase at 2000 FTU/kg increased ADG and ADFI compared to the NC ($P < 0.05$) and all phytase treatments had the

Table 3
Performance of weaned piglets fed wheat or corn based diets in response to increasing phytase dose during the 14 d test period.

Diets	Treatments	BW d0 kg	BW d14 Kg	ADG, g	ADFI,g	G:F, g:g
Exp1	PC[1]	10.9	17.4	466.5ab	578.3ab	0.804AB
	NC[2]	11.1	16.9	410.7b	562.7b	0.733B
	500 FTU/kg[3]	11.3	17.9	473.2ab	576.6ab	0.825AB
	1000 FTU/kg[3]	11.4	18.0	474.1ab	574.9ab	0.831AB
	2000 FTU/kg[3]	11.2	18.3	510.7a	605.9a	0.845A
	SEM	0.475	0.865	55.4	81.7	0.035
	P	0.94	0.54	0.019	0.039	0.06
	P linear[4]		0.14	0.019	0.34	0.037
	P Quadratic[4]		0.43	0.44	0.87	0.14
Exp2	PC	11.4	19.9	607.6a	785.2	0.776a
	NC	11.1	17.9	480.1b	691.9	0.695b
	500 FTU/kg	11.1	18.8	547.4ab	760.6	0.721ab
	1000 FTU/kg	11.1	19.0	567.6ab	745.1	0.760ab
	2000 FTU/kg	11.1	19.6	604.5a	778.5	0.778a
	SEM	0.633	0.971	31.6	47.2	0.021
	P	0.98	0.19	0.008	0.16	0.0076
	P linear		0.08	0.002	0.09	0.0035
	P Quadratic		0.61	0.28	0.49	0.41

a–b Values within column with different superscript are significantly different ($P < 0.05$).
A–B Values within column with different superscript tend to be different ($P \leq 0.10$).
[1] PC: positive control.
[2] NC: negative control.
[3] A *Buttiauxella* phytase added on the top of NC.
[4] Linear or quadratic effect was tested with increasing phytase dose from 0 (NC) to 2000 FTU/kg.

Table 4
Effect of increasing phytase dose on% apparent total tract digestibility (ATTD) of nutrients in piglets fed wheat or corn based diets.

Diet	Treatments	Nitrogen	Phosphorus	Calcium	Dry matter	Gross energy
Exp 1	PC[1]	92.2xy	73.6bc	77.3b	91.9xy	92.0
	NC[2]	91.4y	65.5c	72.6b	91.3y	91.2
	500 FTU/kg[3]	93.8x	84.9a	88.6a	93.5x	92.7
	1000 FTU/kg[3]	93.2xy	81.1ab	85.3a	92.9xy	93.0
	2000 FTU/kg[3]	93.5xy	86.4a	88.8a	93.4x	93.3
	SEM	1.77	4.06	3.08	1.12	1.10
	P	0.039	< 0.0001	< 0.0001	0.029	0.32
	P linear[4]	0.18	< 0.0001	< 0.0001	0.07	0.041
	P Quadratic[4]	0.20	0.0025	0.005	0.134	0.227
Exp 2	PC	88.8	75.8ab	81.3a	91.0	91.0
	NC	87.5	57.3c	71.0b	89.5	89.4
	500 FTU/kg	89.4	75.0b	82.0a	91.5	91.2
	1000 FTU/kg	89.2	79.3ab	84.9a	91.4	91.0
	2000 FTU/kg	91.0	86.5a	89.3a	92.3	91.8
	SEM	1.73	3.58	2.71	1.36	1.43
	P	0.37	< 0.0001	< 0.0001	0.22	0.41
	P linear	0.10	< 0.0001	< 0.0001	0.10	0.18
	P Quadratic	0.82	0.03	0.016	0.48	0.58

a–c Values within column with different superscript are significantly different ($P < 0.05$).
x-y Based on general model, values within column with different superscript are significantly different ($P < 0.05$). However, Tukey HSD tested showed difference only at $P < 0.10$.
[1] PC: positive control.
[2] NC: negative control.
[3] A *Buttiauxella* phytase added on the top of NC.
[4] Linear or quadratic effect was tested with increasing phytase dose from 0 (NC) to 2000 FTU/kg.

same performance level as PC ($P > 0.05$). In both experiments, increasing phytase dose from 0 (NC) to 2000 FTU/kg linearly increased ADG and G:F ($P < 0.05$). A tendency of a linear response ($P = 0.09$) was observed for ADFI in Exp. 2.

3.2. Nutrient digestibility

Apparent total tract digestibility (ATTD) results are shown in Table 4. In Exp. 1, phytase at all three doses increased ($P < 0.05$) ATTD P and Ca compared to NC. Phytase at 500 and 2000 FTU/kg increased ($P < 0.05$) ATTD P vs PC, increased ATTD of N and DM vs NC ($P < 0.05$, based on general model). Increasing phytase dose from 0 (NC) to 2000 FTU/kg resulted in a linear increased in P, Ca and GE ($P < 0.05$), while a tendency for linear increase was found for ATTD DM ($P = 0.07$). In Exp. 2, all phytase treatments increased ($P < 0.05$) ATTD P and Ca vs NC. Linear increase was found for ATTD P and Ca with increasing phytase dose from 0 (NC) to 2000 FTU/kg, while a tendency for linear response for ATTD of DM and N ($P = 0.10$) was observed.

The ATTD of P was greatest at 2000 FTU/kg with improvements of 32% over the NC in Exp. 1, and 51% over the NC in Exp. 2.

3.3. Phosphorous and calcium retention

The P balance data are presented in Table 5. In both Exp., NC showed lower ($P < 0.05$) P retention vs PC, increasing phytase dose from 0 (NC) to 2000 FTU/kg linearly reduced ($P < 0.01$) fecal and total P excretion and increased P retention. In Exp 1., all three phytase doses reduced fecal and total tract P excretion vs PC and NC, while phytase at 500 and 2000 FTU/kg improved P retention vs NC and comparable to PC. Urinary P excretion was lower with phytase treatments at 500 and 1000 FTU/kg vs PC, while phytase at 2000 FTU/kg showed intermediate response. However, a linear increase in urinary P excretion was seen in Exp. 1 with increasing phytase dose from 0 (NC) to 2000 FTU/kg. In Exp. 2, NC and phytase treatments had lower P intake vs PC. All phytase treatments reduced fecal and total P excretion vs both PC and NC. Phytase at 500 and 2000 FTU/kg had lower urinary P excretion than PC. The retention of P was greater with phytase at 1000 and 2000 FTU/kg vs NC, but lower than PC.

Phytase at 2000 FTU/kg reduced ($P < 0.05$) total P excretion by 56 and 62% compared to PC, in Exp. 1 and 2 respectively. Compared to NC, phytase at 2000 FTU/kg increased ($P < 0.05$) P retention by 43 and 78% in Exp. 1 and 2 respectively.

Similar response was seen with Ca retention (Table 6). In both Exp., a linear response was seen for fecal, urinary, total Ca excretion and Ca retention with increasing phytase dose from 0 (NC) to 2000 FTU/kg. In Exp. 1, NC treatment had greater urinary Ca excretion than all other treatments. All three phytase treatments reduced ($P < 0.05$) fecal Ca, total Ca excretion compared to PC and NC, reduced urinary Ca excretion and improved Ca retention vs NC. Phytase at 500 and 2000 FTU/kg had the same Ca retention as PC. In Exp. 2, NC and phytase treatments had lower ($P < 0.05$) Ca intake than PC. NC had greater urinary Ca excretion than PC and 2000 FTU phytase treatment, and greater total Ca excretion than all other treatments. All phytase treatments reduced fecal Ca excretion vs PC and NC, reduced ($P < 0.05$) total Ca excretion vs NC. Phytase at high dose of 1000 and 2000 FTU/kg improved ($P < 0.05$) Ca retention vs NC, and at dose of 2000 FTU/kg reduced urinary Ca excretion vs NC.

Table 5
Effect of increasing phytase dose on P balance in piglets fed wheat or corn based diets.

		P intake, g/d	P feces, g/d	P urine, g/d	Total P Excreted, g/d	P retention, g/d
Exp1	PC[1]	3.49a	1.00a	0.23a	1.23a	2.27a
	NC[2]	2.43bc	0.92a	0.04c	0.96b	1.47b
	500 FTU/kg[3]	2.57b	0.42b	0.05c	0.47c	2.10a
	1000 FTU/kg[3]	2.24c	0.46b	0.10bc	0.56c	1.68b
	2000 FTU/kg[3]	2.64b	0.39b	0.15ab	0.54c	2.10a
	SEM	0.092	0.142	0.021	0.146	0.101
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	P linear[4]	0.20	0.0004	< 0.0001	0.0093	0.002
	P quadratic[4]	0.057	0.0011	0.88	0.0019	0.39
Exp2	PC	7.08a	1.70a	0.23a	1.93a	5.15a
	NC	3.93b	1.64a	0.10b	1.74a	2.19c
	500 FTU/kg	4.20b	0.99b	0.08b	1.08b	3.12bc
	1000 FTU/kg	4.27b	0.88b	0.14ab	1.02b	3.25b
	2000 FTU/kg	4.63b	0.63b	0.11b	0.73b	3.90b
	SEM	0.315	0.186	0.027	0.186	0.311
	P	< 0.0001	< 0.0001	0.003	< 0.0001	< 0.0001
	P linear	0.04	< 0.0001	0.53	< 0.0001	< 0.0001
	P quadratic	0.92	0.03	0.3	0.04	0.26

a–c Values within column with different superscript are significantly different ($P < 0.05$).
[1] PC: positive control.
[2] NC: negative control.
[3] A *Buttiauxella* phytase added on the top of NC.
[4] Linear or quadratic effect was tested with increasing phytase dose from 0 (NC) to 2000 FTU/kg.

Table 6
Effect of increasing phytase dose on Ca balance in piglets fed wheat or corn based diets.

		Ca intake, g/d	Ca feces, g/d	Ca urine, g/d	Total Ca Excreted, g/d	Ca retention, g/d
Exp1	PC[1]	3.43a	0.84a	0.06b	0.90a	2.53a
	NC[2]	2.66c	0.80a	0.37a	1.17a	1.49c
	500 FTU/kg[3]	2.75bc	0.34b	0.11b	0.45b	2.30ab
	1000 FTU/kg[3]	2.49c	0.41b	0.11b	0.52b	1.97b
	2000 FTU/kg[3]	2.96b	0.36b	0.06b	0.43b	2.53a
	SEM	0.099	0.113	0.025	0.101	0.076
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	P linear[4]	0.038	0.0031	< 0.0001	0.0001	< 0.0001
	P quadratic[4]	0.016	0.0025	< 0.0001	0.0001	0.18
Exp2	PC	9.16a	1.72a	0.39c	2.11b	7.05a
	NC	5.85b	1.65a	1.27a	2.92a	2.93c
	500 FTU/kg	6.03b	1.01b	0.99ab	2.00b	4.03bc
	1000 FTU/kg	6.52b	0.94b	1.10ab	2.04b	4.48b
	2000 FTU/kg	6.54b	0.71b	0.72bc	1.43b	5.10b
	SEM	0.582	0.218	0.131	0.228	0.571
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	P linear	0.21	0.0003	0.006	< 0.0001	0.0007
	P quadratic	0.62	0.065	0.87	0.18	0.26

a–c Values within column with different superscript are significantly different ($P < 0.05$).
[1] PC: positive control.
[2] NC: negative control.
[3] A *Buttiauxella* phytase added on the top of NC.
[4] Linear or quadratic effect was tested with increasing phytase dose from 0 (NC) to 2000 FTU/kg.

4. Discussion

The two studies reported in this paper had similar designs but differed in main grain source and dietary analyzed P levels. The results showed that the phytase added to P, Ca deficient diets was effective in improving nutrient digestibility, P and Ca retention and performance of piglets.

4.1. Effect of phytase on performance

Compared to PC, the P and Ca deficient NC diet reduced ADG numerically in Exp. 1 (−56 g) and significantly in Exp. 2 (−127 g).

On average across the two experiments, the NC diets reduced ADFI, ADG and G: F by 17.1, 8.0 and 9.6% respectively compared to the PC. This is in agreement with literature, reductions in growth performance in pigs fed a similar nutrient deficient diet have been reported (Kies et al., 2006; Zeng et al., 2015). These reductions in growth performance due to nutrient deficiency were reversed with the addition of phytase. Across two experiments, exogenous *Buttiauxella* phytase at 500, 1000 and 2000 FTU/kg increased ADG by 14.6, 16.9 and 25.2% and increased G:F by 8.2, 11.3 and 13.5% compared to the NC diet. At the highest dose of phytase fed in this study, 2000 FTU/kg, numerical improvements above the nutritionally adequate PC were seen in ADG (3.6%) and G:F (2.7%) across two studies. The performance response to increased phytase dose observed in the current study is in agreement with literature. Zeng et al. (2015) found, in piglets (initial BW of 9.5 kg) fed a corn and soybean meal based diet for four weeks, the reduction of 1.8 g/kg non-phytate P and 1.6 g/kg Ca in NC diet significantly reduced ADG and increased feed:gain ratio compared to the PC that was formulated with adequate available P and Ca. Supplementation of 500, 1000 and 20000 FTU/kg *Buttiauxella* phytase linearly improved ADG and reduced feed:gain ratio, which was closely related to increased phytate degradation rate with increasing phytase dose. Furthermore, in a study in nursery pigs (Jones et al., 2010), a linear response on ADG and feed:gain ratio was observed with increasing doses (from 200 to 1000 FTU/kg) of an *E. coli* phytase. Pigs fed the low available P, corn and soybean meal based NC diet had reduced performance during the 21 day trial period. Similarly, Brana et al. (2006) observed a linear response in ADG and feed:gain ratio with increasing an *E. coli* phytase dosing from 250 to 1000 FTU/kg in nursery, grower and finisher phases. In grower/finisher pigs fed European type wheat, corn, barley and SBM based diet, supplementation of *Buttiauxella* phytase at 250, 500 and 1000 FTU/kg to a P and Ca deficient negative control diet improved ADG by 3.5, 7.2 and 8.1% respectively compared to the negative control and by 0.8, 4.5 and 5.3% respectively compared to the positive control (Dersjant-Li et al., 2017).

The addition of phytase at 2000 FTU/kg increased ADG in both corn based (125 g/d, Exp. 2) and wheat based (100 g/d, Exp. 1) diets compared to the NC. In the wheat based diet (Exp. 1) ADG and G:F were numerically greater than the PC at all levels of phytase supplementation, whereas in the corn based diet in Exp. 2, only pigs fed 2000 FTU/kg had a similar ADG and G:F compared to the PC diet. This might be explained by the more severe reduction in dietary P was used in the corn based NC diet in Exp. 2. The NC diets were formulated with 1.4–1.5 g/kg lower digestible P compared to PC diets. However, the analyzed total P level was 1.7 g/kg lower in the wheat based NC diet in Exp 1 but 2.4 g/kg lower in the corn based NC diet in Exp 2, compared to the respective PC.

The results from Exp 1 indicate that phytase at 500 FTU/kg could replace 1.7 g/kg total P in wheat based diets and maintain the performance at the same level as the PC. In the corn based diet in Exp 2, phytase at 2000 FTU/kg could replace 2.4 g/kg total P and maintain performance at a similar level to the PC. Logically, a high phytase dose will be needed to recover the performance when the diet is highly deficient in phosphorus, as in this case. Similar results were observed by Kies et al. (2006) where increased levels of an *Aspergillus niger* derived phytase up to 15000 FTU/kg resulted in a linear increase in ADG and G:F in weaned piglets (7.8 kg BW) fed a corn, barley and soybean meal based diet. The phytase was added to a highly P deficient control diet with a digestible P level of 1.25 g/kg which may explain the continued performance response at a phytase supplementation level as high as 15000 FTU/kg.

Comparing the results from two experiments with different grain sources, the wheat based diet had a lower ADFI, which resulted in lower ADG and final BW. This could be partially related to a high NSP (non-starch polysaccharides) level in the wheat based diet, as no NSP degrading enzymes were included in the diets. The high fiber (arabinoxylans) content in wheat based diet may result in increased water holding capacity, increased digesta retention time and reduced feed intake (Partridge, 2000). The addition of xylanase enzymes could break down arabinoxylans and reduce viscosity, increase nutrient digestibility and improve voluntary feed intake, ADG and G:F in pigs (Partridge, 2000; Lindberg et al., 2007; Ndou et al., 2015). In addition, the corn based PC diet had greater analyzed P level which may contribute to greater feed intake. Moreover, the wheat based diets contained skimmed milk powder and fish meal, whereas the corn based diet contained soy protein concentrate, the difference in ingredients composition may also contribute to the different feed intake level between the two diet types observed in these two studies.

4.2. *Effect of phytase on nutrient digestibility, P and Ca retention*

In both experiments, increasing phytase dose linearly improved the ATTD of P. The ATTD of P increased from 65.5% in the NC to 86.4% with phytase at 2000 FTU/kg in the wheat based diets in Exp 1 and from 57.3% in the NC to 86.5% with phytase at 2000 FTU/kg in the corn based diets in Exp 2. It is worth noting that high P digestibility was observed in the NC of both diet types, which may be due the inclusion of 10% whey in the diet that provided a highly digestible P source. Kies et al. (2006) reported an ATTD of P of 33.5% in a corn, barley and soybean meal based diet in weaned piglets, and the addition of phytase linearly improved ATTD of P, with the greatest ATTD of P (84%) attained with *Aspergillus niger* phytase at 15000 FTU/kg. The effect of increasing phytase dose on ATTD of P has also been observed in growing pigs in many other studies (Rutherfurd et al., 2014; Lizardo et al., 2015).

The total P excretion was significantly lower in phytase treatments compared to PC and NC, in a linear reduction manner with increasing phytase dose in both experiments. This indicates that increased phytase supplementation to P deficient diets can have environmental benefit compared to PC. Across two studies, a phytase dose of 2000 FTU/kg reduced P excretion by 61.5% compared to the PC. Similarly, Bento et al. (2012) reported that increasing *Buttiauxella* phytase up to 2000 FTU/kg increased the ATTD of P from 28.4 to 70.9%, in piglets fed a corn, barley, soybean meal and canola meal based diet.

Increasing phytase dose also linearly improved the ATTD of Ca. In both Exp., phytase treatments increased ATTD Ca compared to the NC. In the GIT, one phytate molecule may bind up to 5 Ca atoms, thus reduce the availability of both P and Ca (Selle et al., 2009). *Buttiauxella* phytase is highly active at low pH (Menezes-Blackburn et al., 2015) and can breakdown phytate early in the GIT (e.g. stomach), this may reduce the number of Ca-phytate complexes in the small intestine and improve ATTD Ca. Some literature studies reported that microbial phytase imcreased ATTD of Ca in pigs (Almeida et al., 2013; González-Vega et al., 2015). However, Bournazel et al. (2017) showed that supplementation of 500 FTU/kg of a fungal phytase did not affect ATTD Ca but increased retainable Ca. In that study, phytase treatments had higher dietary Ca level compared to PC. The different observations may be related to Ca level and source, phytase type and level, as well as other factors such as phytate level and Ca: P ratio, which may all have an impact on ATTD of Ca.

An interesting observation in this study was the different response on P and Ca balance between two experiments. In Exp. 1, P excretion and retention reached a plateau with phytase at 500 FTU/kg, whereas in Exp. 2, the lowest P excretion and highest P retention was found with phytase at 2000 FTU/kg. This might be related to the P deficiency levels in the diets. In Exp. 1, NC had 1.7 g/kg lower analyzed P levels vs PC, while in Exp. 2, NC had 2.4 g/kg lower analyzed P levels. Thus in Exp.1, it seems that phytase at 500 FTU already met the P requirement (when compared to PC), this could also explain the linear increase in urinary excretion with increasing phytase dose in Exp. 1. The low P digestibility and retention observed with phytase at 1000 FTU/kg in Exp. 1 is unexpected, which might be related to the low analyzed P level in this diet (3.7 vs 4.1 g/kg in NC, see Table 2). This might be due to possible error in feed mixing. In Exp. 2, due to the severe P reduction in NC diet, a stronger linear response was seen with increasing phytase dose from 0 (NC) – 2000 FTU/kg, on reduction of P excretion and increase in P retention, while no dose response was seen with urinary excretion. These data indicated that when using higher phytase dose it is beneficial to replace more inorganic phosphate in the diets, to reduce both P excretion and feed cost. In a study in turkeys, Wealleans et al. (2016) demonstrated that the optimal phytase dose in terms of inorganic P replacement was around 996 FTU/kg, but in this study the P digestibility and retention data indicate that increasing phytase dose to 2000 FTU/kg can further degrade phytate and increase P availability, allowing further reduction of inorganic P in the diet, compared to the traditional dose of 500 FTU/kg.

In Exp.2, P retention in the highest phytase dose treatment (e.g. 2000 FTU/kg) did not reach the level of PC, which could be partially due to a lack of accessible substrate (phytate) to release 2.4 g/kg retainable P by phytase, as the analyzed phytate level was only 2 g/kg in NC diet in Exp. 2. In addition, this may also be explained by that the PC diet in Exp. 2 contained high level of P and Ca which resulted in greater retention of these nutreints compared to the PC treatmemt in Exp. 1. However, phytase at 2000 FTU/kg maintained the same ADG and G:F levels compared to PC. This might imply that either PC exceeded P requirement, or phytase at 2000 FTU/kg resulted in some extra-phosphoric effect. In Exp. 1, a numerically higher ADG and G:F was found with phytase at 2000 FTU/kg vs PC, while P retention reached plateau at 500 FTU/kg, this also could imply an extra phosphoric effect at 2000 FTU/kg.

Ca balance is closely related to P balance data. In Exp. 1, a lower Ca intake was seen in NC and all phytase treatments, however, phytase at 2000 FTU/kg maintained the same Ca retention as PC. An interesting result was the high urinary Ca excretion rate in NC diet, when the Ca intake and retention was low in this treatment. This might be because animals need to maintain Ca and P balance (Angel et al., 2002; González-Vega and Stein, 2014), the P deficiency in the NC diet meant that excess Ca could not be retained and was excreted. The similar response was seen in Exp 2., e.g. NC diet had greater urinary and total Ca excretion vs PC and phytase treatments due to P deficiency. This implies that the NC diet had an imbalanced Ca:P ratio, due to the non-proportional P and Ca reduction levels in the NC diets (more P reduction than Ca reduction, based on the requirement). The greater fecal P and Ca excretion in the NC diets indicated phytate-Ca formation, in the absence of phytase, which resulted in low availability of both minerals. Increasing phytase dose degraded phytate more completely, thus reduced Ca-phytate formation, restoring Ca and P balance and reducing Ca excretion.

In the current study, increased phytase levels tended ($P = 0.10$) to linearly increased GE digestibility in Exp. 1 and tend to increase N and DM digestibility in Exp.2. These findings are in agreement with previous studies. Lizardo et al. (2015) showed that with the same phytase, increasing the dose up to 2000 FTU/kg improved DM and N digestibility in growing pigs. Adedokun et al. (2015) reported that the addition of *Buttiauxella* phytase at 500 FTU/kg and above improved the ATTD of minerals including Na, Mg and K compared to an un-supplemented control diet in growing pigs. These data indicated that the increased phytate degradation rate at high doses could further reduce the anti-nutritional effect of phytate and improve the digestibility of other nutrients, resulting in extra-phosphoric effect (Zeng et al., 2016).

6. Conclusion

The results from this study showed that increasing *Buttiauxella* phytase doses up to 2000 FTU/kg could increase nutrient digestibility and retention and improve production performance in weaned piglets fed wheat or corn based diets. The addition of *Buttiauxella* phytase at 1000 or 2000 FTU/kg to wheat or corn based diets for weaned piglets could provide both environmental and production benefits, compared to traditional dose of 500 FTU/kg.

Author declaration template

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.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from Yueming.Dersjant-Li@dupont.com.

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文献3

0919 Terroir: Science based or marketing gimmick.

L. Goddik*, *Oregon State University, Corvallis.*

The concept of terroir is rapidly gaining importance in the U.S. marketplace in alignment with the growth of the local food sector. Terroir for products such as coffee and wine are well recognized, but there is little science to support terroir for dairy products. Nevertheless, it's common to see promotions for regional cheeses such as Wisconsin cheese or New York Cheddar. This presentation will evaluate factors that influence farm and regional milk sources and their impact on cheese characteristics. Our current research has focused on the simple question: If all other factors are kept constant, will milk from different farms and regions produce cheeses that are different? Initial results have demonstrated that milk from farms, selected due to similar herd management principles, produce Cheddar cheeses that are different based on sensory and flavor chemistry profiles. Non-starter lactic acid bacteria (NSLAB) profiles are unique to individual farms. The link to the individual farms (terroir effect) is more pronounced in 5 mo aged Cheddar than in 9 mo aged Cheddar. Milk from coastal regions appears to be particularly suited for cheese production, likely due to complex NSLAB profiles and flavor development.

Key Words: milk source, cheese, flavor
doi: 10.2527/jam2016-0919

NONRUMINANT NUTRITION: ENZYMES

0920 The effect of increasing *Buttiauxella* phytase dose on performance in piglets: Meta-analysis from 5 trial studies.

Y. Dersjant-Li, R. M. Bold, and W. Li*, *Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, United Kingdom.*

The effect of *Buttiauxella* phytase on the performance of piglets was evaluated combining the datasets of five trials. A total of 234 data points (364 piglets, average initial BW 10 kg) were used in the analysis. Treatments included a nutritionally adequate positive control diet (PC), a negative control diet (NC, with an average reduction of 0.15% calcium and 0.19% phosphorus compared to the PC), and NC supplemented with *Buttiauxella* sp. phytase at 500, 1000, or 2000 phytase units (FTU)/kg feed. One FTU was defined as the amount of enzyme required to release 1 μmol of iP per minute from sodium phytate at pH 5.5 at 37°C. Piglets received the test diets (based on corn/SBM, wheat/SBM or wheat/barley and SBM) for 14 d. No grain source × phytase dose interaction was found, thus data from the 5 trials were pooled for statistical analysis (JMP 11.0, SAS). Treatment means were separated using Tukey's HSD test, trial was used as a random factor. Linear or nonlinear response was tested with increasing phytase dose from 0 (NC) to 2000 FTU/kg. Phytase dose at 1000 and 2000 FTU/kg improved ADG by 12.3 and 19.3% respectively vs. NC ($P < 0.05$), and by 3 and 9.4% vs. PC ($P > 0.05$). No significant differences were seen in feed intake. FCR was improved with phytase at 1000 and 2000 FTU/kg by 8.8 and 10.2% vs. NC ($P < 0.05$), and by 5.5 and 6.3% vs. PC ($P > 0.05$). Increasing phytase dose from 0 (NC) to 2000 FTU/kg increased ($P < 0.05$) ADG linearly and reduced FCR in a nonlinear manner. The data demonstrated that phytase at 500 FTU/kg could replace 0.19% P and 0.15% Ca. Increasing phytase dose to 1000 or 2000 FTU/kg could further improve performance of piglets fed P and Ca deficient diets, most likely due to the extra-phosphoric effects of the phytase. Cost calculation (based on feed cost and 14-d performance data) showed a net value of $0.11, 0.35, and 0.62 per pig ($11.8, 39, and 65/ton of feed) with *Buttiauxella* phytase at 500, 1000, and 2000 FTU/kg respectively compared to PC. In conclusion, increasing *Buttiauxella* phytase dose up to 2000 FTU/kg may provide production benefits in piglets.

Key Words: piglets, meta-analysis, performance
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0921 Effects of dietary β-mannanase supplementation with soybean meal in the performances in weanling pigs.

B. Balasubramanian*, H. M. Yun, Y. M. Kim, J. K. Kim, and I. H. Kim, *Department of Animal Resource & Science, Dankook University, Cheonan, South Korea.*

Soybean meal (SBM) is by far the most popular protein source used for feeding livestock. The objective of the present study was to test the efficacy of supplementation of β-mannanase in diets containing de-hulled or conventional hulled SBM (44% and 48%) as well as to evaluate the interactive effects of SBM and enzyme on growth performance, nutrient digestibility, fecal microflora, and noxious gas emission in weanling pigs. In total, 140 pigs [(Landrace × Yorkshire) ×Duroc] with a initial BW of 5.97 ± 1.01 kg were used in a 6-wk feeding trial, randomly allotted in a 2 × 2 factorial arrangement, with feed consisting of hulled or de-hulled SBM with or without β-mannanase [T1 (SBM 44%), T2 (SBM 44% + 0.05% β-mannanase), T3 (SBM 48%), and T4 (SBM 48% + 0.05% β-mannanase)]. Pigs were allocated randomly to 4 treatment groups consisting of 7 replicate pens per treatment with 5 pigs per pen. Pen was the experimental unit. In this study, pigs fed diets containing 0.05% β-mannanase had greater BW, ADG, G:F, and ADFI than pigs fed diets without β-mannanase, but the differences were not statistically significant; however, interactions of SBM diets showed significant differences for ADFI ($P = 0.0334$) at the second week and showed significant effects on DM ($P = 0.0077$), N ($P = 0.0082$), E ($P = 0.0362$), P ($P = 0.0472$) at the sixth week. Furthermore, when compared with SBM, β-mannanase had effects on DM ($P = 0.0105$), N ($P = 0.0416$), P ($P = 0.0591$), but not E and Ca. There were no significant differences for serum BUN, WBC, Lymphocytes, however observed significance on RBC ($P =$