平成31年1月10日

農林水産省消費・安全局
畜水産安全管理課　御中

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飼料の公定規格における追加事項の要望について

1．　背景

平成３０年９月１８日の農業資材審議会飼料分科会における審議の結果、MorphΔE8 BP17 4c株を利用して生産されたフィターゼ（製品名：Axtra PHY（アクストラファイ））は、組換えDNA技術応用飼料添加物として、安全性に問題ないとすることは適当であると認める答申が農林水産大臣宛に出されております（30資審第11号）。

また、飼料の公定規格には、「（５）環境負荷低減型配合飼料」に非フィチン態りんの成分量の最小量（％）の規定、及び「第２章アミノ酸および非フィチン態りんの成分量並びに可消化養分総量等の値の計算方法、２　配合飼料の非フィチン態りんの成分量」には、現在指定されている４種類のフィターゼ（フィターゼ（その１）、フィターゼ（その２（１））、フィターゼ（その２（２））及びフィターゼ（その２（３）））に関し、配合飼料において各フィターゼによる分解の結果生じる非フィチン態りん（％）の算出方法の規定があります（飼料の公定規格、農林省告示７５６号）。

2．　要望内容

MorphΔE8 BP17 4c株を利用して生産されたフィターゼを、上記第２章２の規定に追加して頂き、その規定をもって養豚飼料が配合設計されることにより、排泄物中のりん含量を低減する「環境負荷低減型配合飼料」に貢献したいと考えています。具体的には、以下の算出方法を第２章２の規定に追加して頂きたく、要望いたします。

フィターゼの種類	算出方法
同（１４０） MorphΔE8 BP17 4c株を利用して生産されたフィターゼ	飼料1ｋｇあたり500フィチン酸分解力単位を添加した場合、0.14%

３．　参考資料

社内データ

① Axtra®PHY　The complete phytase solution.

② Technical report Axtra PHY Meta.S.80

文献

① A.L. Wealleans, R.M.Bold, Y.Dersjant-Li, and A.Awati.
J. Anim. Sci. 2015. 93: 5283-5290.,
The addition of a *Buttiauxella sp.* Phytase to lactating sow diets deficient in phosphorus and calcium reduces weight loss and improves nutrient digestibility.（査読あり）

② Y. Dersjant-Li, A.L. Wealleans, L.P. Barnard, S. Lane.
Animal Feed Science and Technology 2017. 234: 101-109
Effect of increasing *Buttiauxalla* phytase dose on nutrient digestability and performance in weand piglets fed corn or wheat based diets.（査読あり）

③ Y. Dersjant-Li, R. M. Bold. And W. Li. Danisco Animal Nutrition, Dupont Industrial Biosciences, Marlborough, Unites Kingdom.
J. Anim, Sci Vol.94, E-Suppl. 5 page 443.
The effect of increasing in piglets: Meta-analysis from 5 trial studies.

３．　参考資料

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③ Y. Dersjant-Li, R. M. Bold. And W. Li. Danisco Animal Nutrition, Dupont Industrial Biosciences, Marlborough, Unites Kingdom.
J.　Anim, Sci Vol.94, E-Suppl. 5 page 443.
The effect of increasing in piglets: Meta-analysis from 5 trial studies.

各資料の要約

社内データ

① Axtra®PHY　The complete phytase solution.

基本的な考え方は Phyzyme XP と同じで、実際に豚を利用した [redacted] の消化試験により AxtraPHY がフィチン酸を分解し、P の消化率をどの程度増加させ、非フィチン態 P としてどの程度貢献するか、添加量に幅をもたせ、重回帰分析によってメタ解析から算出した。消化試験は以下のような内容である。

[redacted]

- 最終的にフィターゼによって貢献される有効 P は、増加した P の消化率にフィターゼ無添加の飼料中の全 P を乗じて、さらに 1.28 を乗ずることによって求めた。

フィターゼを 500 単位添加した結果生じる非フィチン態りん（＝有効 P）の計算方法

有効 P (%)＝飼料中の全 P%×フィターゼの添加により改善した P 消化率/100×1.28

＊1.28 は、豚の栄養学から考慮した一般的な P の消化率の逆数

実測計算式　　0.1371＝ 0.45 ×23.81/100 ×1.28

0.14 ≒

② Technical Report

AxtraPHY.Meta.S.80

- 体重 9kg 程度の離乳子豚 380 匹を使用し、40kg まで飼育する間、陽性対照区と陰性対照区（P、Ca を低減）を設定し、陰性対照区に AxtraPHY を 250-2000 単位まで段階的に添加した。
- 飼料設計は、とうもろこし、小麦、大豆粕、大麦を使用した。
- P と Ca の消化率と、排泄物中の P を求めた。
- AxtraPHY の段階的な添加により、P 消化率は陰性対照区と比較し、39.7%～60.9%ま

で改善した。

- 排泄物中Pは、陰性対照区と比較し、段階的に34.0%～51.6%まで減少した。

文献

① The addition of a *Buttiauxalla sp.* Phytase to lactating sow diets deficient in phosphorus and calcium reduce weight loss and improves nutrient digestibility. = Technical Report, PHY.Meta.SO.42 と同等

- 3つの国（オランダ、カナダ、アメリカ）で行われた合計6種類の試験で、母豚271頭を使用し、結果をメタ解析した。
- 試験区は、陽性対照区、陰性対照区、陰性対照区にAxtraPHYを250～2000単位まで段階的に添加した区を設けた。陰性対照区は、平均0.16%の有効Pを低減した。
- 飼料設計は、とうもろこし、メイズ、大豆粕、小麦、大麦を使用した。
- 測定項目は、体重、消化エネルギー、消化性P、消化性Ca
- 消化性Pは、250単位、500単位、1000単位、2000単位のそれぞれの添加によって、陰性対照区と比較して、38.5%、61.5%、69.2%、69.2%改善された。

② Effect of increasing *Buttiauxella* phytase dose on nutrient digestibility and performance in weaned piglets fed corn or wheat based diets.

- イギリスの研究機関とダニスコの共同試験として行われた離乳子豚合計 100 匹を使用した2つの代謝試験の結果を纏めた。
- 試験区は、陽性対照区、陰性対照区、陰性対照区にAxtraPHYを500、1000、2000単位/kg飼料添加した区を設けた。陰性対照区は消化性P0.14%（試験1）、0.15%（試験2）を低減した。
- 飼料設計は、試験1はとうもろこし、大豆粕主体で、試験2は小麦、大豆粕主体。
- 測定項目は、各栄養素の見かけの消化率および成長成績。
- Pの見かけの消化率は、AxtraPHYの段階的な添加によって改善され、成長成績は陽性対照区と同等以上となった。
- 試験1では、500単位のAxtraPHYの添加により、最大0.17%の全Pが貢献できることが確認された。0.17%の全Pは、0.16%の有効Pとなる。
- 試験2では、2000単位のAxtraPHYの添加により、最大0.24%の全Pが貢献できることが確認された。0.24%の全Pは、0.22%の有効Pとなる。

③ The effect of increasing *Buttiauxella* phytase dose on performance in piglets: Meta-analysis from 5 trial studies.

- 5 つの試験データを統合して、子豚における AxtraPHY による効果を評価した。
- 平均体重 10kg の計 364 匹の子豚を使用し、合計 234 のデータポイントをメタ解析した。
- 試験区は、陽性対照区（充足した栄養成分）、陰性対照区（Ca 0.15%減、P 0.19%減）、陰性対照区＋500、1000、2000 単位/kg のフィターゼを添加した区を設けた。
- 試験飼料の原料構成は、とうもろこし、大豆粕、小麦/大麦を使った飼料で、各穀類とフィターゼ単位に相互作用がないため、データ全体をプールし統計処理をした。（Turkey's HSD）
- フィターゼ 500 単位の添加によって、飼料中 0.19%の有効 P と 0.15%の Ca と置き換え可能であることをデータが示した。
- 陰性対照区にフィターゼの添加単位を 1000～2000 に増加させることによって、さらなる P の増加による成長改善も期待できることが示唆された。
- 陽性対照区よりも、陰性対照区にフィターゼを添加したほうが、コストが節約できた。
- 結論として、AxtraPHY は、2000 単位まで子豚の生産に利益をもたらすことが示された。

社内データ 2

Axtra® PHY improves phosphorus digestibility and reduces total tract phosphorus excretion in weaned piglets (meta-analysis)

Benefits in weaned piglet diets

Phytase breaks down the anti-nutrient phytate to release phosphorus and other key nutrients such as calcium, energy and amino acids. As a result, the addition of Axtra® PHY at 2000 FTU/kg in the diet:

- **improves total tract phosphorus digestibility by 60.9% compared to the negative control**
- **improves total tract phosphorus digestibility by 24.5% compared to the positive control**
- **reduces total tract phosphorus excretion by 51.6% compared to the negative control**
- **reduces total tract phosphorus excretion by 53.5% compared to the positive control**

Design

Number: 380 | Type: weaned piglets | Treatments: 6
Phytate P level %: 0.22 | Trial length: ~9-40 kg

Treatment

- Positive control
- Negative control (NC)
- NC + Axtra® PHY at 250 FTU/kg
- NC + Axtra® PHY at 500 FTU/kg
- NC + Axtra® PHY at 1000 FTU/kg
- NC + Axtra® PHY at 2000 FTU/kg

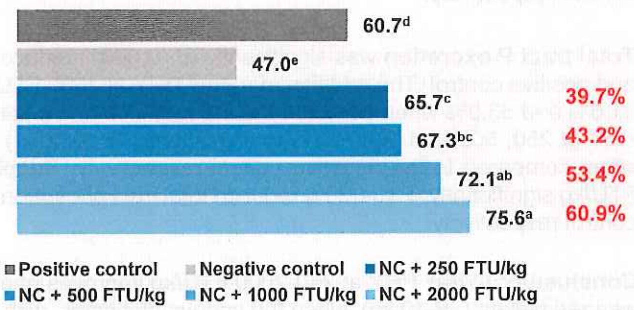
See appendix for detailed breakdown

Trial site

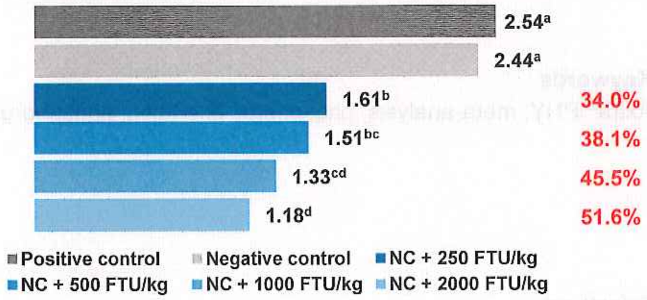
Combination of 7 trials

Results

TOTAL TRACT PHOSPHOSUS DIGESTIBILITY
(%, 9-40 kg)



TOTAL TRACT PHOSPHOSUS EXCRETION
(g/kg, 9-40 kg)



[abc] Values without a common superscript are significantly different ($P<0.05$)

What is Axtra® PHY?
Phytase breaks down the anti-nutrient phytate to release phosphorus and other digestible nutrients such as calcium, energy, amino acids and trace minerals. As a consequence of the additional release of these nutrients parameters such as bone ash, bone strength, bodyweight gain, egg mass, egg production and feed utilisation can be improved [1,2,3,4,5,6,7].

Summary

Seven trials were conducted to evaluate the effect of Axtra® PHY at 250, 500, 1000 and 2000 FTU/kg in various diets on the apparent total tract phosphorus excretion of weaned piglets (~9-40 kg). Results from the seven trials were combined and analysed to calculate total tract phosphorus excretion and phosphorus digestibility (see appendix: detailed trial design).

Total tract phosphorus digestibility was significantly (P<0.0001) improved with the addition of Axtra® PHY at all doses compared to the negative and positive control. The addition of Axtra® PHY at 2000 FTU/kg significantly (P<0.0001) improved phosphorus digestibility by 60.9% and 24.5% when compared to the negative and positive control respectively. Supplementing weaned piglet diets with Axtra® PHY at 250, 500 and 1000 FTU/kg significantly (P<0.0001) improved phosphorus digestibility by 39.7%, 43.2%, and 53.4% when compared to the negative control respectively. Supplementing weaned piglet diets with Axtra® PHY at 250, 500 and 1000 FTU/kg significantly (P<0.0001) improved phosphorus digestibility by 8.2%, 10.9%, and 18.8% when compared to the positive control respectively.

Total tract P excretion was significantly (P<0.0001) reduced with the addition of Axtra® PHY at all doses compared to the negative and positive control. The addition of Axtra® PHY at 2000 FTU/kg significantly (P<0.0001) reduced total tract phosphorus excretion by 51.6% and 53.5% when compared to the negative and positive control respectively. Supplementing weaned piglet diets with Axtra® PHY at 250, 500 and 1000 FTU/kg significantly (P<0.0001) reduced total tract phosphorus excretion by 34.0%, 38.1%, and 45.5% when compared to the negative control respectively. Supplementing weaned piglet diets with Axtra® PHY at 250, 500 and 1000 FTU/kg significantly (P<0.0001) reduced total tract phosphorus excretion by 36.6%, 40.6%, and 47.6% when compared to the positive control respectively.

Conclusion: Axtra® PHY at 250-2000 FTU/kg improves phosphorus digestibility and reduces total tract phosphorus excretion in weaned piglets (~9-40 kg), when fed various diet types, with the greatest reduction of phosphorus excretion at 2000 FTU/kg.

Comments

Axtra® PHY is a bacterial (*Buttiauxella sp.*) phytase expressed in *Trichoderma reesei.*

Keywords

Axtra® PHY, meta-analysis, phosphorus excretion, phosphorus digestibility, phytase, phytate, swine

References

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Detailed trial design

Seven individual trials were incorporated into a meta-analysis (see table below for summary of each trial). All seven trials measured total tract phosphorus excretion. In total, 380 weaned piglets (~9-40 kg) of different commercial breeds were randomly allocated to 6 dietary treatments (see table below). The negative control diet was fed either unsupplemented or supplemented with Axtra® PHY at 250, 500, 1000 or 2000 FTU/kg. The positive control was formulated to meet nutritional requirements compared to the NRC (2012) recommendation containing Ca in a range of 0.66-0.97% and digestible phosphorus in a range of 0.20-0.39%. The negative control diet was formulated to be reduced in digestible phosphorus (digestible phosphorus content in a range of 0.16-0.35%) and calcium (Ca content in a range of 0.49-0.65%). Diets were fed either as mash or pellets *ad libitum*. At the end of the trial faecal and urinary samples were collected and analysed for phosphorus. Total tract phosphorus excretion was then calculated. Phosphorus and calcium digestibility, retainable phosphorus and retainable calcium were also calculated.

Trial design:

Trial no.	Total pigs	Replicates/ treatment	Pigs/pen	Breed	Experimental phase (Overall)
1	48	8	1	Large White x Landrace	11- 18 kg
2	70	10	1	Great Yorkshire x Landrace	12-15 kg
3	54	9	1	PIG	10-15 kg
4	72	12	1	(Yorkshire x Landrace) x Duroc	20-40 kg
5	20	8	1	Talent x (Great Yorkshire x Landrace)	9-22 kg
6	20	8	1	Talent x (Great Yorkshire x Landrace)	9-22 kg
7	96	12	1	Landroc (Landrace cross Duroc)	11-20 kg

Diet used in trials:

Trial no.	Diet form	Diet	Axtra® PHY (FTU/kg)	Phytate phosphorus level
1	Pellet	Wheat/ SBM	250, 500, 1000, 2000	0.22
2	Pellet	Barley/ corn/ SBM	250, 500, 750, 1000, 2000	0.22
3	Mash	Corn/ SBM	250, 500, 1000, 2000	0.23
4	Mash	Corn/ SBM	250, 500, 1000, 2000	0.22
5	Mash	Wheat/ corn/ SBM	250, 500, 1000	0.22
6	Mash	Wheat/ corn/ SBM	250, 500, 1000	0.22
7	Mash	Corn/ SBM	500, 1000, 2000	0.23

Results: Summary of 7 trials in weaned piglets (9-40 kg BW)

	Positive control	Negative control (NC)	NC + 250 FTU/kg	NC + 500 FTU/kg	NC + 1000 FTU/kg	NC + 2000 FTU/kg
Total tract Phosphorus excretion (g/kg feed)	2.54^{a}	2.44^{a}	1.61^{b}	1.51^{bc}	1.33^{cd}	1.18^{d}
Phosphorus digestibility (%)	60.7^{d}	47.0^{e}	65.7^{c}	67.3^{bc}	72.1^{ab}	75.6^{a}
Retainable Phosphorus (% intake)	58.9^{d}	45.7^{e}	64.6^{c}	66.7^{bc}	70.7^{ab}	74.6^{a}
Calcium digestibility (%)	65.4^{b}	60.7^{c}	76.5^{a}	74.7^{a}	79.2^{a}	78.2^{a}
Retainable calcium (% intake)	60.3^{b}	48.0^{c}	64.7^{ab}	66.0^{ab}	69.8^{a}	73.2^{a}

^{abc} Values without a common superscript are significantly different ($P<0.05$)

Combination of 7 trials:
PB0804, PB0812, PB0819, PB0916_F, PB0921A, PB0921B, PB1006

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文献 1

The addition of a *Buttiauxella* sp. phytase to lactating sow diets deficient in phosphorus and calcium reduces weight loss and improves nutrient digestibility

A. L. Wealleans,[1] R. M. Bold, Y. Dersjant-Li, and A. Awati

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ABSTRACT: Improving the efficiency of P use by pigs is especially important for lactating sows, whose metabolic requirements for P and Ca are high. The effect of a *Buttiauxella* sp. phytase on lactating sow performance and nutrient digestibility was investigated using the combined data set for 6 studies. Treatments included a nutritionally adequate positive control diet (PC), a negative control diet (NC; with an average reduction of 0.16% available phosphorous and 0.15% Ca vs. PC), and NC supplemented with a *Buttiauxella* sp. phytase at 250, 500, 1,000 or 2,000 phytase unit (FTU)/kg, respectively. Phosphorus and Ca deficiency in the NC resulted in significantly higher BW loss compared with the PC. All phytase treatments maintained BW loss at the same level as the PC. Increasing doses of phytase significantly ($P < 0.05$) reduced sow BW loss and increased energy intake, with improvements most apparent in sows older than parity 5. The positive effects on BW and energy intake were not observed in first-parity sows. This may be a consequence of fewer first parity sows in the data set. The apparent total tract digestibility of DM, OM, and CP were not affected by phytase supplementation. Digestible P and Ca were significantly improved (linear, $P < 0.0001$; quadratic, $P < 0.0001$) by increasing the dose of phytase supplementation. Significantly lower apparent total tract digestibility of energy, Ca, and P was found in the NC treatment vs. the PC treatment, whereas no significant differences were found between phytase treatment and the PC treatment. In conclusion, phytase supplementation at a level of 250 FTU/kg can replace 0.16% available phosphorous and 0.15% Ca; however, increasing the phytase dose can further reduce BW loss in sows fed P- and Ca- deficient diets.

Key words: digestibility, lactation, performance, phytase, sows

 J. Anim. Sci. 2015.93:5283–5290
doi:10.2527/jas2015-9317

INTRODUCTION

Weight loss in lactating animals is a direct consequence of the metabolic demands of milk production. This is especially high in sows, whose milk is nutritionally dense compared with other domesticated animals (Perrin, 1958; Jenness, 1982). Modern sows are required to nurse increasingly larger litters to be financially viable—the average litter size in France increased by 25% between 1986 and 2006 (Prunier et al., 2010)—creating a demand for nutrients that cannot be matched by the sow's relatively limited feed intake (Aherne and Williams, 1992; Clowes et al., 1998; Lawlor and Lynch, 2007). This differential between energy demand and intake leads to a state of catalysis and the mobilization of body reserves (McNamara, 1997). Phosphorus availability is complicated by the reduced bioavailability of organic P: about 60 to 80% of P in cereal grains is present in the form of phytate phosphorus (Maga, 1982). In this form, the P is largely inaccessible to pigs, which inherently do not possess enough of the enzymes required to hydrolyze the phytate into its component parts (Golovan et al., 2001). Accordingly, the utilization of P by pigs is poor: only between 20 and 40% of dietary P is metabolized without the aid of exogenous enzymes (Jongbloed et al., 2004). In approximately 70% of pig diets, this

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Received May 15, 2015.
Accepted August 25, 2015.

problem is solved by the addition of phytase: phytase in P-deficient diets will improve performance as well as reduce environmentally harmful levels of P in manure (Poulsen, 2000; Sands et al., 2001). However, as both Kemme et al. (1997b) and Jongbloed et al. (2004) noted, there is a paucity of literature on phytase efficacy in lactating sows, despite wide adoption of phytase in sow diets. Early work suggested that the optimum phytase dosage in sows was lower than for piglets and growing/finishing pigs, as more ideal conditions in the sow stomach allow for greater phytase efficacy (Kemme et al., 1997a), and subsequent studies have shown increases in P and Ca digestibility in sows fed P- and Ca-deficient diets, although there have been few demonstrated effects on sow or piglet performance (Lantzsch and Drochner, 1995; Jongbloed et al., 2004, 2013). The aim of this study was to evaluate the ability of a *Buttiauxella* sp. phytase to reverse losses in performance and digestibility caused by P and Ca deficiency in lactating sow diets.

MATERIALS AND METHODS

Experiments were conducted in accordance with all relevant institutional and national animal care guidelines at each participating experimental station.

Animal Trials

Six separate trials, as detailed in Table 1, were run to assess to efficacy of a *Buttiauxella* sp. phytase in P- and Ca-deficient diets for lactating sows. The following design criteria were used:

- The phytase must have been fed to lactating sows of known parity;
- The phytase must have been fed for entire study period, and the inclusion rates must have been available;
- Weight loss and feed intake must have been recorded;
- Nutrient digestibility for P and Ca must have been recorded;
- There must have been a negative and positive control treatment; and
- There must have been 2 or more replicates per treatment.

Individual sow data from the 6 separate trials were then collated in a database, providing 255 treatment means across 5 phytase inclusion levels (0, 250, 500, 1,000 or 2,000 FTU/kg. One FTU is defined as the quantity of enzyme that releases 1 μmol of inorganic P/min from 5.0 mM sodium phytate at pH 5.5 at 37°C. The phytase used in each study was a microbial 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* (Danisco Animal Nutrition, Marlborough, UK). Within the study, information on the parity and diet was also recorded for each replicate used.

In each study, individual sows were the sampling unit and the experimental unit for digestibility and feed intake. Within each study, sows were randomly allocated to the dietary treatments and parity of the sows was balanced as much as possible.

Sow BW was recorded at farrowing and weaning. The BW of piglets were recorded at birth and weaning. The measurements of piglets within a litter were combined to provide information on litter birth weight, litter weaning weight, and litter weight gain until weaning. At farrowing, total number of pigs born, live born, and stillborn were recorded and cross-fostering of pigs was done to equalize litter size as much as possible; limitations on the extent of cross-fostering occurred when farrowing dates were disparate.

The experimental diets were provided ad libitum throughout lactation. Sows were fed treatment diets from Day 108 of gestation. To calculate ADFI, feed added and wastage (as detected) was recorded daily throughout the experimental period.

All sows and piglets were monitored daily for abnormalities and clinical signs of sickness as well as the availability of feed and water. Dead piglets, when they occurred, were recorded and piglet mortality was calculated by subtracting the number of piglets at the end of the experiment from the number of piglets at the beginning of the experiment. Mortality was expressed both as an absolute number and as a percentage.

All experimental diets were analyzed for marker, DM, Ca, and P. Available P was calculated using the slope ratio method (Soares, 1995). In each study, apparent total tract digestibilities of DM, OM, GE, N, fat, ash, NDF, Ca, and P were determined by the indirect method, using SiO_2 or TiO_2 as the marker and the concentration of Ti or Si in the calculations. The formula to calculate nutrient digestibility coefficients was

$$\text{DC nutrient} = [1 - (\text{nutrient/indicator})_{\text{feces}} / (\text{nutrient/indicator})_{\text{diet}}] \times 100\%,$$

in which DC is the digestibility coefficient of the nutrient (DC nutrient), $(\text{nutrient/indicator})_{\text{feces}}$ is the ratio of marker compound to nutrient in the feces, and $(\text{nutrient/indicator})_{\text{diet}}$ is the ratio of marker compound to nutrient in the feed.

Table 1. Trials used in this analysis

Trial	Country	Pigs per replicate	Number of replicates	Length of lactation	Dietary main grains[1]	Treatments used[2]	Analyzed phytase levels, FTU[3]/kg	Analyzed total P	Calculated AvP[4]	Analyzed total Ca
1	Netherlands	1	12	21	Maize/sunflower meal/RSM/SBM/ sugar beet pulp	PC	244	0.71	0.34	0.79
						NC	141	0.51	0.18	0.64
						250 FTU/kg	368	0.51	0.18	0.64
						2,000 FTU/kg	2,402	0.51	0.18	0.63
2	Netherlands	1	8	17	Maize/sunflower meal/RSM/SBM	PC	113	0.50	0.18	0.64
						NC	98	0.50	0.18	0.64
						250 FTU/kg	391	0.63	0.32	0.68
						500 FTU/kg	684	0.47	0.16	0.56
						1,000 FTU/kg	1,399	0.45	0.16	0.54
						2,000 FTU/kg	1,735	0.46	0.16	0.55
3	Canada	1	8	15	Wheat/barley/SBM	PC	252	0.45	0.16	0.55
						NC	256	0.45	0.16	0.53
						250 FTU/kg	447	0.45	0.16	0.52
						500 FTU/kg	603	0.60	0.35	0.81
						2,000 FTU/kg	1,746	0.44	0.19	0.66
4	United States	1	8	18	Maize/maize gluten meal/RSM/SBM	PC	<50	0.44	0.19	0.68
						NC	<50	0.45	0.19	0.66
						250 FTU/kg	347	0.44	0.19	0.67
						1,000 FTU/kg	1,632	0.64	0.35	1.03
						2,000 FTU/kg	2,747	0.45	0.16	0.78
5	Canada	1	8	15	Wheat/barley/SBM	PC	262	0.45	0.16	1.20
						NC	284	0.45	0.16	0.95
						250 FTU/kg	417	0.45	0.16	0.81
						500 FTU/kg	681	0.62	0.35	1.08
						2,000 FTU/kg	1,537	0.44	0.19	0.68
6	United States	1	13	15	Maize/SBM/DDGS	PC	57	0.45	0.19	0.72
						NC	56	0.45	0.19	0.69
						250 FTU/kg	355	0.44	0.19	0.68

[1]RSM = rapeseed meal; SBM = soybean meal; DDGS = dried distillers grains with solubles.

[2]PC = positive control diet; NC = negative control diet.

[3]FTU = phytase units.

[4]AvP = available phosphorous.

Statistics

Outlier removal was conducted using jackknife distances (Tukey, 1958; Miller, 1974): data rows where the jackknife distances for the multidimensional mean of improvements in body weight change, feed intake, and daily energy intake were more than 2.5 SD were excluded from the data set, resulting in the removal of 5 treatment rows: 2 removed rows came from the positive control diet (**PC**) treatment, 2 from the negative control diet (**NC**) treatment, and 1 from the 250 FTU/kg treatment.

The effect of length of lactation and main grain, as these variables differed between studies, on parameters of interest were analyzed using the REML method; trial was considered the random effect, as this accounts for the underlying heterogeneity between studies (Lean et al., 2009). Means separation was achieved using Tukey's honest significant difference test in the Fit Model platform of JMP 11 (SAS Inst. Inc., Cary, NC) and significance was determined at $P < 0.05$. Dietary main grain had no effect on performance parameters, DM digestibility, ash digestibility, digestible P, Ca, or energy. However, CP digestibility was significantly increased in wheat-based diets as compared with maize-based diets (88.91 vs. 85.83%; $P < 0.05$). Length of lactation was not significantly correlated with any digestibility or performance parameters. Therefore, the data from the 6 individual trials was combined into a single data set for further analysis.

For the effect of phytase, data were analyzed using the REML method; the model considered treatment as a main effect, parity was considered a covariate, and trial code was included as a random effect. Treatment means separation was achieved using Tukey's honest significant difference test in the Fit Model platform of

Table 2. Effect of supplementary phytase on lactating sow and piglet performance

Performance parameter	PC[1]	NC[1]	NC+250[1]	NC+500[1]	NC+1000[1]	NC+2000[1]	SEM	ANOVA	Linear	Quadratic
Weight after farrowing, kg	248.85	244.31	249.34	254.18	243.64	248.89	4.45	0.72	0.66	0.78
Weight after weaning, kg	233.42	219.97	230.95	235.75	222.21	230.22	6.20	0.23	0.12	0.37
BW change, kg	−16.05[a]	−24.66[b]	−19.68[ab]	−17.06[ab]	−16.94[ab]	−15.89[a]	6.55	<0.05	<0.05	0.18
BW change, %	−6.41[a]	−10.11[b]	−7.67[ab]	−6.49[ab]	−6.41[ab]	−6.36[a]	2.68	<0.05	<0.01	<0.1
Daily BW change, kg/d	−0.89[a]	−1.36[b]	−1.06[ab]	−0.93[ab]	−0.92[ab]	−0.86[ab]	0.18	<0.05	<0.05	0.26
Feed intake, kg/d	5.86	5.43	5.66	5.53	5.64	5.63	6.23	0.57	0.22	0.37
Daily energy intake, MJ	79.57	74.13	76.02	77.42	77.95	77.44	3.50	0.64	0.21	0.35
Piglets born	13.84	14.07	13.45	13.78	13.86	13.57	1.19	0.97	0.89	0.86
Born alive	12.74	12.78	12.10	13.15	13.23	12.60	1.17	0.83	0.57	0.75
Born dead	1.13	1.31	1.36	0.77	0.55	1.01	0.19	0.28	0.12	0.26
Average birth weight, kg	1.50	1.48	1.49	–[2]	1.43	1.48	0.13	0.97	0.60	0.74
Litter birth weight, kg	18.78	20.03	18.29	–	19.28	18.56	1.08	0.48	0.42	0.47
Preweaning deaths/litter	0.95	0.76	0.67	0.44	0.64	0.40	0.37	0.42	0.45	0.92
Preweaning deaths/litter, %	7.47	5.66	5.23	3.38	5.08	3.30	2.52	0.34	0.56	0.96
Piglets weaned/litter	10.72	11.16	10.80	10.73	10.64	11.16	0.41	0.30	0.14	0.42
Average weaning weight, kg	7.04	6.96	6.93	–	6.64	6.82	0.60	0.81	0.47	0.70
Litter weaning weight, kg	75.74	78.33	72.51	–	71.86	77.32	2.10	0.18	0.71	0.39

[a–c]Values within rows with the same superscript are not significantly different ($P < 0.05$).

[1]PC = positive control diet; NC = negative control diet; NC+250 = negative control + 250 FTU/kg; NC+500 = ; negative control + 500 FTU/kg; NC+1000 = negative control + 1,000 FTU/kg; NC+2000 = negative control + 2,000 FTU/kg.

[2]No data available for cells containing "–".

JMP 11 (SAS Inst. Inc.) and significance was determined at $P < 0.05$.

Data were split into "parity groups": primiparous sows ($n = 38$), sows on parity 2 through 4 ($n = 151$), and sows on parity 5 or above ($n = 67$). Performance parameters that were significantly affected in the overall data set were then investigated in each of these parity groups through REML analysis with trial as a random effect; Tukey's honest significant difference was used to separate treatment means. Significance was determined at $P < 0.05$.

RESULTS

The main effects of the level of *Buttiauxella* sp. phytase on sow and piglet growth performance are outlined in Table 2. Sow weight loss was negatively affected by the reduction of P and Ca in the diet, with NC sows losing 24.66 kg compared with 16.05 kg for PC sows ($P < 0.05$). Equivalence to the positive control was achieved at the lowest dose, 250 FTU, and a significant difference from the negative control was achieved at 2,000 FTU/kg ($P < 0.05$). There were no significant effects on piglet performance until weaning.

The data on sow BW change and feed intake were then split by parity group (first parity, parity 2–4, and parities 5+), as shown in Table 3. There were no significant differences between treatments in primiparous sows.

In midparity sows (parities 2, 3, and 4), there were significant linear relationships between phytase dose and all investigated parameters: BW change in kilograms and percent ($P < 0.001$), daily feed intake ($P < 0.05$), and daily energy intake ($P < 0.05$). Percent BW change also had significant quadratic effects ($P < 0.05$). The effect of phytase in maintaining body condition was greatest in older sows (parities 5+), reducing BW loss versus the negative control by 13.20, 13.89, 15.77, and 15.36 kg at 250, 500, 1,000, and 2,000 FTU/kg, respectively, with significant linear effects ($P < 0.05$).

The effect of supplementary phytase on nutrient digestibility is presented in Table 4. No differences were found between treatments for OM digestibility. Dry matter and CP digestibility were significantly affected by treatment: in both cases, the positive control reported the lowest digestibility coefficients (84.39 g/100 g DM and 86.15 g/100 g CP) and the 500 FTU/kg treatment reported the highest (86.81 g/100 g DM and 87.52 g/100 g CP). Phytase inclusion had no linear or quadratic effects on DM or CP digestibility.

Digestible P and Ca were significantly improved (linear, $P < 0.0001$; quadratic, $P < 0.0001$) by phytase supplementation. The reductions in dietary P and Ca led to significant reductions in digestible P and Ca (0.07 g/100 g digestible P and 0.08 g/100 g digestible Ca) compared with the positive control. For digestible P, this reduction was reversed with the addition of 250 FTU/kg, with all levels of phytase supplementation

Table 3. Effect of supplementary phytase on lactating sow performance by parity

Performance parameter	PC[1]	NC[1]	NC+250[1]	NC+500[1]	NC+1000[1]	NC+2000[1]	SEM	ANOVA	Linear	Quadratic
Primiparous										
BW change, kg	−21.24	−20.83	−19.18	–[2]	−5.73	−14.12	6.12	0.46	0.43	0.14
BW change, %	−9.87	−9.60	−9.62	–	−2.92	−6.41	3.01	0.42	0.43	0.12
Feed intake, kg/d	4.84	5.22	5.14	–	7.10	5.66	0.56	0.18	0.16	0.29
Daily energy intake, MJ	66.24	72.56	71.47	–	98.83	78.61	13.4	0.16	0.16	0.29
Parities 2–4										
BW change, kg	-16.71^{ab}	-25.85^{b}	-21.38^{ab}	-15.76^{ab}	-12.17^{a}	-13.35^{a}	6.84	<0.01	<0.001	<0.1
BW change, %	-6.66^{ab}	-10.66^{b}	-8.64^{ab}	-5.81^{ab}	-4.57^{a}	-5.48^{a}	2.78	<0.01	<0.001	<0.05
Feed intake, kg/d	6.02^{a}	5.20^{b}	5.53^{ab}	5.39^{ab}	5.87^{ab}	5.63^{ab}	0.17	<0.05	<0.05	<0.1
Daily energy intake, MJ	82.27^{a}	71.21^{b}	75.40^{ab}	75.89^{ab}	80.81^{ab}	77.06^{ab}	3.04	<0.05	<0.05	<0.1
Parities 5+										
BW change, kg	-6.73^{a}	-32.00^{b}	-18.30^{ab}	-18.11^{ab}	-16.23^{ab}	-16.64^{a}	8.29	<0.05	<0.05	0.11
BW change, %	-2.07^{a}	-11.78^{b}	-6.77^{ab}	-6.53^{ab}	-5.36^{ab}	-5.74^{a}	3.01	<0.05	<0.05	0.13
Feed intake, kg/d	6.11	5.68	5.98	5.67	4.36	5.45	0.34	0.39	0.16	0.14
Daily energy intake, MJ	83.86^{a}	58.00^{b}	74.92^{ab}	79.04^{ab}	83.52^{ab}	76.07^{ab}	4.52	<0.05	<0.01	0.15

a–cValues within rows with the same superscript are not significantly different ($P < 0.05$).

[1]PC = positive control diet; NC = negative control diet; NC+250 = negative control + 250 FTU/kg; NC+500 = ; negative control + 500 FTU/kg; NC+1000 = negative control + 1,000 FTU/kg; NC+2000 = negative control + 2,000 FTU/kg.

[2]No data available for cells containing "–".

providing significantly more digestible P than the NC. Digestible Ca was improved with the addition of all levels of phytase to within statistical insignificance of the positive control, with significant difference from the NC achieved at 500 FTU/kg. Digestible energy was also significantly improved by the addition of phytase (linear, $P < 0.0001$; quadratic, $P < 0.0001$), with each treatment releasing in excess of 0.5 MJ to the sow.

DISCUSSION

Body weight loss affects the sow's current lactation and also her future reproductive and piglet performance (Vesseur et al., 1994). Sows with excessive weight losses during lactation have extended remating intervals (Sterning et al., 1990; Zak et al., 1997, 1998), are less likely to return to estrus within 10 d of weaning, and have reduced ovulation rates (Zak et al., 1997) and reduced embryonic survivals (Close and Mullan, 1996).

Overall, the addition of *Buttiauxella* sp. phytase to sow diets significantly reduced both absolute ($P < 0.05$) and percent ($P < 0.01$) change in BW compared with the negative control. Equivalence to the positive control was achieved at the lowest dose, 250 FTU/kg, with further reductions with increasing dose. Nasir et al. (2014) reported that supplementation with an *Aspergillus oryzae* 6-phytase improved nutrient digestibility but not performance in lactating sows. This finding is echoed elsewhere in the literature, with Jongbloed et al. (2013) also finding no effect of phytase supplementation on sow BW change throughout lactation. This may suggest that the older generation phytases studied in previous papers were not able to release sufficient nutrients to impact weight loss, only recovering those removed from the NC. Previous studies with *Buttiauxella* sp. phytase in growing–finishing pigs have demonstrated dose-dependent improvements in energy, AA, nitrogen, and mineral digestibil-

Table 4. Changes in nutrient digestibility following phytase supplementation

Digestibility parameter	PC[1]	NC[1]	NC+250[1]	NC+500[1]	NC+1000[1]	NC+2000[1]	SEM	ANOVA	Linear	Quadratic
Ash, g/100g	45.57^{ab}	43.72^{b}	46.66^{ab}	47.74^{ab}	47.45^{ab}	48.71^{a}	11.36	<0.05	<0.001	<0.05
CP, g/100g	86.56^{b}	87.30^{a}	87.40^{a}	87.50^{a}	87.11^{ab}	86.65^{ab}	0.83	<0.01	0.06	0.08
DM, g/100g	84.39^{c}	86.38^{ab}	86.28^{ab}	86.29^{a}	86.09^{ab}	85.90^{b}	1.19	<0.0001	0.10	0.12
OM, g/100g	88.55	89.24	89.17	88.88	88.70	88.81	1.03	<0.1	0.59	0.66
DE, MJ	15.31^{a}	14.76^{b}	15.26^{a}	15.46^{a}	15.29^{a}	15.37^{a}	0.41	<0.001	<0.0001	<0.0001
Digestible Ca, g/100g	0.24^{ab}	0.16^{c}	0.20^{bc}	0.25^{ab}	0.27^{ab}	0.25^{a}	0.03	<0.0001	<0.0001	<0.0001
Digestible P, g/100g	0.20^{ab}	0.13^{c}	0.18^{b}	0.21^{ab}	0.22^{a}	0.22^{a}	0.01	<0.0001	<0.0001	<0.0001

a–cValues within rows with the same superscript are not significantly different ($P < 0.05$).

[1]PC = positive control diet; NC = negative control diet; NC+250 = negative control + 250 FTU/kg; NC+500 = ; negative control + 500 FTU/kg; NC+1000 = negative control + 1,000 FTU/kg; NC+2000 = negative control + 2,000 FTU/kg.

ity over and above the expected improvements in P and Ca (Adedokun et al., 2015; Zeng et al., 2015), and this, combined with the differential pH optimum of *Buttiauxella* sp. phytase, which allows for nutrient release higher in the digestive tract (Menezes-Blackburn et al., 2015), will contribute to the reduction in weight loss demonstrated in this study.

Body weight losses can be especially detrimental to first parity sows, which are especially sensitive to body reserve depletion. In general, gilts are not physiologically mature at the time of first mating (Everts, 1994) and so do not have enough body reserves at first farrowing, and their feed intake capacity is not sufficient to fulfill energy needs during lactation (Mejia-Guadarrama et al., 2002). The results of this analysis did not show significant effects of phytase inclusion on weight loss in first parity sows, although a trend was observed for both absolute and relative BW change ($P < 0.1$). The nonsignificance of the data may be due to the low number of first parity animals ($n = 39$) compared with older animals ($n = 249$). In midparity sows (parities 2, 3, and 4), there were significant linear relationships between phytase dose and all investigated parameters: BW change in kilograms and percent ($P < 0.001$), daily feed intake ($P < 0.05$), and daily energy intake ($P < 0.05$). Percent BW change also had significant quadratic effects ($P < 0.05$).

The effect of phytase in maintaining body condition in P-deficient diets was greatest in older sows (parity 5+), reducing BW loss by 13.20, 13.89, 15.77, and 15.36 kg at 250, 500, 1,000, and 2,000 FTU/kg, respectively, with significant linear effects ($P < 0.05$). This is likely linked to the high BW loss of older sows on the NC (32.00 kg, 11.78%), suggesting that older sows are less capable of adapting to Ca- and P-deficient diets than younger sows.

Although higher parity sows can recycle and conceive with higher lactation weight losses compared with first-parity animals (Thaker and Bilkei, 2005), reduced weight losses in higher parity sows will reduce culling and replacement rate. Maintaining these indicators of reproductive performance will improve sow longevity, as culling rates increase with decreasing reproductive performance (Stalder et al., 2004; Sasaki and Koketsu, 2008), especially for young sows, where reproductive failure is the main reason for removal from the herd (Lucia et al., 2000). Increasing longevity will also reduce overall costs to the producer. Replacing sows at the end of their productive life incurs a financial cost: the difference in value between cull sows and replacement gilts is known as “livestock depreciation” and varies from herd to herd and between years.

Strategies to reduce lactating weight loss often involve increasing the fat and energy intake by sows (Eissen et al., 2003; Smits et al.., 2013). No significant differences in DM, OM, or CP digestibility were seen in this study, in line with the results of Jongbloed et al. (2004) and Kemme et al. (1997a,b).

No significant differences were seen in daily energy intake in the overall data set, but significant differences were seen in both mid-parity (parities 2–4) and older (parities 5+) sows. In both cases, energy intake was reduced in the NC treatment compared with the PC ($P < 0.05$), and the addition of phytase restored equivalence to the positive control ($P > 0.05$). However, a significant increase in energy digestibility was seen with all levels of supplementary phytase (Table 4; $P < 0.0001$). This, which supports the ability of the *Buttiauxella* sp. phytase to reduce BW loss, both in absolute and percentage terms, will likely improve sow reproductive performance, although no significant effects on piglet performance were seen in this study.

These results show that the *Buttiauxella* sp. phytase is effective at improving the digestibility of both P and Ca in lactating sows. Previous sow studies (Lantzsch and Drochner, 1995; Kemme et al., 1997a,b) using an *Aspergillus niger* phytase reported increased total tract digestibility of P, with no significant effects on Ca. Jongbloed et al. (2004) did find a significant effect of a *Peniophora lycii* phytase supplementation on Ca digestibility, although the authors attributed this to supplemental limestone in the NC. Unlike Jongbloed et al. (2004), the diets in the experiments investigated in this analysis did not try to balance the Ca:P ratios of the PC and NC; therefore, the improvement cannot be the result of supplemental limestone. Phytate is known to chelate with Ca and other trace minerals, reducing their bioavailability (Wise, 1983), and phytase-driven improvements in Ca digestibility are widely reported in younger pigs (Traylor et al., 2001; Braña et al., 2006; Rutherford et al., 2014).

Each study in this analysis used cross-fostering procedures, reducing the variation in litter size and piglet weight for each sow. However, piglet performance is often dependent on the level of sow health and nutrition before farrowing and in early lactation (Revell et al., 1998). As the *Buttiauxella* sp. phytase reduces weight loss and improves digestive capability, continuous supplementation of sow diets throughout the reproductive cycle may, therefore, be able to improve long-term indicators of sow and piglet performance, including pigs per sow/year, litters per sow/year, and kilograms of pig meat raised per sow/year.

In conclusion, this study shows that the addition of phytase to sow diets at 250 FTU/kg is able to replace 0.16% available P and 0.15% Ca in lactating sows diets, enabling producers to safely lower the level of Ca and P in sow diets. However, higher doses up to 2,000

FTU/kg will have further beneficial effects on BW loss, especially in older sows. The effects on younger sows and on subsequent reproductive parameters are not yet conclusively described and merit further attention.

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Animal Feed Science and Technology 234 (2017) 101–109

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Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

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

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ARTICLE INFO

Keywords:
Buttiauxella phytase
Digestibility and retention
Performance
Piglets

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 ($P \leq 0.10$) 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

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http://dx.doi.org/10.1016/j.anifeedsci.2017.09.008
Received 7 May 2017; Received in revised form 12 September 2017; Accepted 14 September 2017
0377-8401/

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.

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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.

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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
doi: 10.2527/jam2016-0920

0921 Effects of dietary β-mannanase supplementation with soybean meal in the performances in weanling pigs.

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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 ($P =$