Age influence on effectiveness of a novel 3-phytase in barley-wheat based diets for pigs from 12 to 108 kg under commercial conditions

Cambra-López, M.[a], A. Cerisuelo [b], P. Ferrer [b], L. Ródenas[a], R. Aligue[c], V. Moset [d], J.J Pascual.[a]

[a] Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

[b] Centro de Investigación de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, 12400 Segorbe, Castellón, Spain

[c] Departamento de Biomedicina, Universitat de Barcelona, 08036 Barcelona, Spain

[d] Herbiteh Agro SLU, Plaza Señores de la Cuba y Clemente, 16192 Molinos del Papel, Cuenca, Spain

Corresponding author: Cambra-López M.

e-mail: macamlo@upvnet.upv.es

phone: +34 963 879 388

fax: +34 963 877 439

Abstract

The main objective of this study was to evaluate the influence of age on the effectiveness of a new microbial 3-phytase, produced by *Komagataella pastoris,* under commercial conditions in barley-wheat based diets. Two experiments were conducted; firstly, to determine phytase efficacy on dry matter, organic matter, energy, protein and mineral (phosphorus, P and calcium, Ca) digestibility (n=48; Experiment 1), and secondly, to evaluate the effect of phytase on growth performance and bone mineralization (n=312; Experiment 2) in weaned, growing and finishing pigs. For each experiment, three barley-wheat based diets were formulated following the recommendations for each animal age, of which two versions were manufactured, including 0 and 1000 phytase units (FTU)/kg of feed of the new 3-phytase to be tested. The new phytase has the potential to increase the digestibility of Ca and P (on av. +0.05 and +0.06, respectively; P<0.01), especially P digestibility in growing pigs (+0.10; P<0.001), consequently decreasing P and Ca excretion. Digestible energy (DE) of the diet increased with the addition of phytase in weaned pigs (+0.69 MJ/kg of dry matter (DM); P<0.001). Dietary inclusion of new 3-phytase enhanced average daily gain from 46 to 94 days of age (+0.07 kg/d; P<0.05) and decreased feed conversion ratio from 46 to 154 days of age (on av. −0.13; P<0.05), although no significant effect was observed from 154 to 185 days of age. Addition of the new 3-phytase also promoted bone mineralization, increasing the weight of the bones (+3.99 and +3.64 g of tibia at 95 days and metacarpus at 100 days of age, respectively; P<0.05) and the ash, Ca and P content in these bones (e.g. +0.46 and +0.33 g of P in tibia at 95 days and metacarpus at 100 days of age, respectively; P<0.001). In conclusion, pig age affected the efficacy of a new 3-phytase on P and Ca digestibility both in weaned and growing diets and DE content of the weaned diets, which also resulted in improvements in growth, feed conversion and bone development until 154 days of age. These effects seem to be reduced during the finishing period, although the advantages of the new 3-phytase on bone mineralization were maintained until 185 days of age.

Keywords

Enzyme; age; swine; minerals; digestibility; phytase

List of Abbreviations

ADF, acid detergent fibre
ADFI, average daily feed intake
ADG, average daily gain
BW, body weight
Ca, calcium
CP, crude protein
CTTAD, coefficients of total tract apparent nutrient digestibility
DE, digestible energy
DM, dry matter
DP, digestible protein
EE, ether extract
FCR, feed conversion ratio
FTU, phytase units
GE, gross energy
GLM, general lineal model
IP6, *myo*-inositol hexaphosphate ester
NDF, neutral detergent fibre
OM, organic matter
P, phosphorous
SCD, standard commercial diet

Introduction

Phytase represents about 60% of the global feed enzyme market (Adeola and Cowieson, 2011). Although phytase is the most commonly used exogenous enzyme in the feed for monogastric animals, there is a competitive market for enzymes in animal nutrition. Consequently, there is an ongoing development of phytases with varying efficiencies and optimal pH, thermal tolerance and resistance to endogenous proteolytic enzymes (Konietzny and Greiner, 2002) in order to decrease production costs and improve enzyme stability.

The efficacy of phytase supplementation in animal diets is mainly assessed by comparing total tract nutrient digestibility, bone mineralization and growth performance in animals fed low phosphorus (P)-diets with and without phytase addition (Dersjant-Li et al., 2015). The effect of phytase supplementation in pig diets on calcium (Ca) and P digestibility has been widely described (Selle and Ravindran, 2008; Almeida and Stein, 2012). Phytase can liberate phytate-P by hydrolysis of the phytate molecule (*myo*-inositol hexaphosphate ester, IP6) and more P will thus be available in the stomach and small intestine of these non-ruminants. The hydrolysis of IP6 also liberates the inorganic dietary Ca bonded (Gonzalez-Vega et al., 2015).

The main consequence of the higher P and Ca digestibility due to the use of phytase in pig diets is the reduction of inorganic P and Ca sources in their formulation, resulting in economic and environmental benefits (the latter mainly due to reductions in P excretion) (Selle and Ravindran, 2008). In addition, better growth performance and improvements in bone mineralization characteristics have been reported in diets supplemented with phytase (Braña et al., 2006, Varley et al., 2011a).

The capacity of phytase to enhance P availability and hence growth performance of pigs with lower than required levels of P is well documented in the literature (Varley et al., 2011a; Almeida et al., 2013; She et al., 2015). However, very few works have explored the effect of phytase in commercial conditions, where theoretically there are no P deficiencies, as the total P concentration of the diet is adjusted considering only the P amount released by the enzyme. In addition, a wide range of variation in the effect of added phytase on P availability at the same dose and type of diet can be found among studies, probably due to differences in basal diet and animals among studies, contributing to unreliable diet formulations. For these reasons, nutritionists nowadays use higher phytase inclusion rates and P levels in practice, which may indicate that commercial diets contain a surplus of P as a safeguard (Partridge and Bedford 2001).

Concerning diet-related factors, feed ingredients, Ca and P content and phytase type and inclusion level are among the main ones influencing phytase efficiency. Feed ingredients can contribute to differences in the rate of hydrolysis of IP6 and total P retention, due to differences in levels and location of IP6 and endogenous phytase among cereals and oilseeds (Selle and Ravindran, 2008; Desjant-Li et al., 2014). The presence of this plant phytase activity could limit responses of exogenous phytase (Rodehutscord et al. 1996), but it would help to enhance total P retention (Blaabjerg et al., 2012). However, very little information about this can be found in the literature. This may be because most of the research using supplemental microbial phytase has been carried out using maize/soya bean meal-based diets, which have much lower phytase activity than wheat and barley-based diets (Eeckhout and De Paepe 1994; Desjant-Li et al., 2014), the latter two being the most frequently used for pig feed in Europe and Canada (Blaabjerg et al., 2012).

Concerning animal-related factors, nowadays it is widely acknowledged that age is a factor affecting the effect of supplemental microbial phytase on P and Ca digestibility among swine categories, due to differences in gut maturity, gastric emptying, stomach capacity and pH of gastric contents (Kemme et al., 1997; Selle and Ravindran, 2008, Dersjant-Li et al., 2015). However, phytase supplementation formulations in pig diets are generally not tailored to the animal category, probably due to the lack of consistent results.

Given all this, it would be important to determine the true effectiveness of the new phytases at different ages, when available P levels used are close to the recommendations (reducing only the P that is expected to be released by the phytase), and with diets based on barley and wheat (mainly based on European swine feed), as well as their impact on the digestive efficiency of nutrients, growth development and bone mineralization in pigs. Therefore, the main objective of this study was to evaluate the age influence on the effectiveness of a new microbial 3-phytase when supplemented in barley-wheat diets under commercial P supplementation conditions. To this end, we conducted two experiments to determine phytase efficacy in dry matter (DM), organic matter, energy, protein and mineral (P and Ca) digestibility, bone mineralization and growing performance in weaned, growing and finishing pigs.

Material and methods

All experimental procedures used in this study were approved by Universitat Politècnica de Valencia's Animal Experimentation Ethics Committee and authorized by the Valencian Ministry of Agriculture, Environment, Climate Change and Development, Spain, with code 2015/VSC/PEA00172.

Enzyme

A new 3-phytase (myo-inositol-hexakisphosphate 3-phosphohydrolase) from *Serratia odorifera*, *AppAs-r-op*, synthesized at the Department of Biomedical Sciences (Universitat de Barcelona, Spain) was used in this study (ePhyt 1000® Global Feed; Spain – EU zootechnical additive authorization number 4a25). A liquid formulation of the 3-phytase (FLF1000) was obtained by fermentation of a genetically modified strain of *Komagataella pastoris* (strain CECT 13094) (Salaet et al. 2019, personal communication). The minimum phytase activity of the liquid formulation of FLF1000 was 1000 FTU/mL.

Experiment 1: Digestibility trial

Diets

Table 1 shows the three control diets that were formulated and manufactured for the digestibility trial, following the main recommendations for swine feed (FEDNA, 2013), but including total P levels 13, 24 and 23% below recommendations for weaned, growing and finishing pigs, respectively (i.e. 5.2 g P/kg DM in weaned piglets, 4.2 g /kg DM in growing pigs and 4.1 g /kg DM in finishing pigs). Thus, controls were diets with low levels of P and 0 phytase units (FTU)/kg of exogenous phytase dosage (Diets 0). Additionally, versions of each of the three control diets with 1000 FTU/kg of exogenous phytase dosage were also formulated and manufactured (Diets 1000). Analysis of phytase content of the diets using ISO 30024: 2009 confirmed the treatments, showing 36, 0 and 130 FTU/kg in Diets 0 for piglets, growing pigs and finishing pigs, and 894, 1038 and 1182 FTU/kg in Diets 1000, respectively. All diets were provided *ad libitum* in mash form.

Animals

Forty male castrated weaned piglets 35 days of age (Pietrain x ACMC-Meidam), 24 male castrated growing pigs 94 days of age (PIC line 65, PIC Camborough 22) and 24 male castrated finishing pigs 168 days of age (PIC line 65, PIC Camborough 22) were used in the digestibility trial. An outline on the management of animals and diets throughout the digestibility trial is shown in Figure 1. Digestibility trials were conducted in the Research and Technology Animal Centre (CITA-IVIA) located in Segorbe, Spain

Piglets were divided into two subsequent batches of 20 animals each. On arrival, pigs from each batch were identified with an ear tag and distributed by weight (similar weight among treatments) into 4 pens (5 animals/pen) and housed in an isowean-type room provided with an environment control system. Over the experimental period, ventilation rates and room temperature were adapted to the age of the animals. The lighting schedule provided consisted of 12 hours of light and 12 hours of darkness throughout the study. The first 11 days of the trial, piglets were fed a pre-starter standard commercial diet (SCD) based on cooked cereals (barley and wheat), barley, wheat and maize (3350 kcal metabolizable energy, 190 g crude protein (CP), 6.5 g P and 6.9 g Ca per kg). At 46 days of age, the experimental weaned pig diets with two different phytase concentrations (0 and 1000 FTU/kg) were randomly provided to the animals for 18 days (2 pens per diet in each batch). At 53 days of age (after 7 days of diet adaptation in pens), all animals were housed in pairs in 10 metabolism cages (2 × 1.2 m^2; 5 cages per treatment and batch; 10 replicates per treatment) in an environmentally controlled room for a period of 11 days. Average ± standard deviation of body weight (BW) of pigs in the two batches at starting the faeces collection for the digestibility trial (at 60 days of age) was 17.99 ± 1.86 kg.

Growing pigs were divided into two subsequent batches of 12 animals each. On arrival, pigs from each batch were identified with an ear tag and distributed by weight (similar weight among treatments) into 2 pens (6 animals/pen) and housed in an isowean-type room provided with an environment control system. The experimental growing pig diet with two different phytase concentrations (0 and 1000 FTU/kg) was randomly provided to the animals for 18 days. After 9 days, 6 animals per treatment and batch (12 replicates per treatment) were individually housed in 12 metabolism pens (same metabolism pens as for piglets) for a period of 9 days. Average ± standard deviation of BW of pigs in the two batches at allocation in metabolic cages (at 103 days of age) was 38.66 ± 3.57 kg BW.

The same procedure was used for the twenty-four finishing pigs 168 days of age, but using the corresponding experimental finishing pig diet. Average ± standard deviation of BW of pigs in the two batches at allocation in metabolic cages (at 177 days of age) was 115.10 ± 7.86 kg BW.

Digestibility procedure

The metabolic cages allowed the measurement of individual feed intake and faeces collection. Cages were equipped with a conventional nursery feeder (with five spaces, or piglets) and a conventional fattening feeder (for growing and finishing pigs). Each cage had a single nipple drinker. During the first 7 days, animals were adapted to metabolism cages followed by a collection period for the last 4 days, where feed intake and total faecal output were daily weighed per cage to determine apparent digestibility coefficients. At the end of the collection period, faeces kept at 4ºC were pooled by cage and homogenized, and representative samples of faeces and diets were then stored at -20ºC until analysis. Additionally, representative samples of diets were taken directly from the feeders at the end of the digestibility period in order to measure *in situ* DM to calculate the DM intake during this period. Coefficients of total tract apparent nutrient digestibility (CTTAD) of DM, organic matter (OM), CP, gross energy (GE), Ca and P of diets were determined for each animal as:

$$CTTAD\ of\ Nutrient = 1 - \frac{Nutrient\ faecal\ excretion}{Nutrient\ intake}$$

Experiment 2: Performance and mineralization trial

Diets

From 35 to 46 days of age, all the piglets were fed an SCD (described at the digestibility trial). At 46 days of age, the experimental diets with two different phytase concentrations (0 and 1000 FTU/kg) were randomly provided to the corresponding group of animals until the end of the experiment (185 days of age). Figure 1 shows the versions of these experimental diets (0 and 1000 FTU/kg) for weaned piglets, growing pigs and finishing pigs that were supplied from 46 to 94, from 94 to 154 and from 154 to 185 days of age, respectively.

Table 2 shows the three control diets that were formulated and manufactured for the performance and mineralization trial, following the main recommendations for swine feed (FEDNA, 2013), but including total P levels 13, 28 and 10% below recommendations for weaned, growing and finishing pigs, respectively (i.e. 5.2 g P/kg DM in weaned, 3.8 g /kg DM growing pigs and 4.4 g /kg DM in finishing pigs).

Analysis of phytase content of the diets confirmed the treatments, showing 36, 406 and 444 FTU/kg in Diets 0 for piglets, growing pigs and finishing pigs, and 894, 1322 and 1430 FTU/kg in Diets 1000, respectively. Growing and finishing pig diets presented higher phytase analyse values due to the higher endogenous phytase levels of diets based on barley and triticale. Diets were prepared in mash form for weaned piglets and provided *ad libitum* in solid form (35 to 95 days of age). Then, from 95 to 185 days of age, feed was prepared as mash and offered in liquid form three times a day. Total amount of feed was adjusted daily per pen to avoid residual feed.

Animals

Three hundred and twelve castrated male weaned piglets at 35 days of age (Landrace × Duroc) were used in the performance and mineralization trial. Average ± standard deviation of BW of pigs when starting the experiment 2 was 8.30 ± 0.77 kg BW. Animals were housed in 24 pens (12 pens/treatment) with 13 animals per pen in an isowean-type room in a commercial fattening pig facility located in Teruel (Spain). Ventilation rates and room temperature were adapted to the age of the animals. The lighting schedule provided consisted of 12 hours of light and 12 hours of darkness from 35 to 94 days of age.

At 95 days, animals were transferred to a growing-finishing building until pigs were 185 days of age. The house was naturally ventilated to provide ambient temperature adapted to the age of the animals. Animals received natural light. Pens of fully slatted floor and manure pit were 4.0 × 2.5 m in floor area. Pigs were fed in a 4 m feeder.

Animal health was adequate during the experimental period. Animals received all necessary vaccinations at the start of the experiment. Animals' health status recorded daily by veterinary health checks can be considered good. Only two animals were treated for lameness (one in the weaning period and one in the growing period) and another for a tail wound in the growing period. These animals were treated with medicinal substances under veterinary supervision and were removed from the experiment. Necropsies were performed on all dead animals.

Performance and mineralization traits

Individual body weight (BW) and feed intake by pen were recorded weekly. BW and feed intake were used to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). To evaluate the effect of phytase on bone mineralization degree, one animal per pen (12 pigs per treatment) was randomly selected and slaughtered by stunning and exsanguination at 95 days. At 185 days of age, all animals were slaughtered by stunning and exsanguination, selecting one animal per pen randomly to evaluate bone mineralization. The left tibia in piglets (95 days of age) and the III and IV metacarpus bone of the left forelimb in finishing pigs (185 days of age) were removed and, after removing all the soft tissues, frozen at -20ºC until analyses.

Analytical methods

Feed samples were dried at 105ºC for 24 h and faecal samples at 80ºC for 48 h, after which they were ground and stabilized. Feeds and individual faeces were analysed for DM, ash, CP, GE, Ca and P. Feeds were also analysed for ether extract (EE), neutral detergent fibre not assayed with a heat stable amylase and expressed exclusive of residual ash (NDF), acid detergent fibre expressed exclusive of residual ash (ADF) and phytate-P. All feed samples were analysed in quadruplicate. Faeces samples were assayed in simple.

DM (930.15), ash (923.03), EE (920.39), CP (990.03) were analysed according to the methods of AOAC (2003). The NDF and ADF were analysed as described Van Soest et al. (1991). GE was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, Loughborough, UK). Mineral (Ca and P) content were analysed by inductively coupled plasma atomic emission spectrometry (ICP-OES) (model Varian 720-ES, Varian Inc., California, USA). In brief, a dried and ground subsample of 3 to 5 g of each feed and faecal sample was ashed at 550ºC for 3.5 h in a muffle furnace. Samples were cooled and 4 mL concentrated HCl (37%), 1 mL HNO_3 and 1 mL of Ytrium solution (100 mg/L) was added to 0.1 g-ashed sample. Samples were then filtered through a nylon 0.45 μm filter and the filtered solution was analysed in the ICP-OES. Phytate-P was analysed by spectrophotometry according to the method described by Haug and Lantzch (1983).

For the determination of ash, Ca and P in tibia and metacarpus, bones were dried at 110º C for 12 h, defatted with an ether solution for 48 h and dried at 110ºC for 12 h, as described by Català-Gregori (2006). Bones were then weighed and ashed at 550ºC for 12 h in a muffle furnace. The ash content was expressed as a percentage of dry fat-free bone weight. Mineral (Ca and P) content in tibia bones was then analysed using ICP-OES, adding 0.05 g-ashed sample to the acid solution instead of 0.1 g as in feed and faeces.

Statistical analysis

CTTAD and mineralization data were analysed according to the general lineal model (GLM) procedure of SAS (SAS, 2002), in a completely randomized design with a model accounting for the fixed effect of the phytase dose (0 and 1000 FTU), the age (60, 100 and 175 days of age for the CTTAD and 95 and 185 days of age for the performance traits), their interaction and if so the batch (1 and 2) as a block factor. For digestibility parameters experimental unit was the metabolic cage housing two piglets and one growing-finishing pig (N= 20 and 24, respectively).

Data on performance were analysed using a repeated measures model. A mixed SAS model was used, according to a repeated measures design that takes into account the variation between animals and covariation within them. Covariance structures were objectively compared using the most severe criteria (Schwarz Bayesian criterion; Littell et al., 1998). The model included the phytase dose (0 and 1000), the age (6 periods), and their interactions as fixed effects. Random terms in the model included a permanent effect of each animal (*p*) and the error term (*e*), both assumed to have an average of zero, and variance σ_p^2 and σ_e^2. Results were presented as least square means with their standard errors. Statistical significance level was set at 5% (0.05).

Results

Digestibility trial

Table 3 shows the effect of phytase supplementation and age of the animals on CTTAD and nutritive value of the feeds. Supplementation of the new 3-phytase (1000 FTU/kg) significantly increased CTTAD of Ca by around 9% (+0.052 ± 0.015; P=0.004) and P by around 12% (+0.061 ± 0.015; P=0.001), as well as the DE content by around 2% (+0.29 ± 0.09; P<0.004), of the diets compared to Diets 0. There was also a tendency (P < 0.10) for pigs fed Diets 1000 to have a higher CTTAD of GE than pigs fed Diet 0.

Weaned piglets at 60 days of age showed significantly higher CTTAD of DM, OM, CP, GE and Ca, as well as digestible protein (DP) content of their diets than growing pigs 100 days of age and finishing pigs 175 days of age (P<0.05). In addition, growing pigs showed significantly lower CTTAD of DM, OM, CP, GE, as well as lower DE in their diets than finishing pigs ($P<0.05$). However, higher DP levels were found in growing pigs diets compared to finishing pigs ($P<0.05$).

Interactions between phytase dose and age were observed on the CTTAD of P and the DE content of the diet (Figure 2). The observed increase on the CTTAD of P with the dietary addition of phytase was mainly due to its improvement at 100 days, where digestible P increased by around 24% with the addition of the enzyme ($+0.102 \pm 0.025$; $P<0.001$), and 60 days of age, where digestible P increased by 9.4% with addition of the enzyme ($+0.056 \pm 0.024$; $P<0.001$). No significant differences on P digestibility were observed at 175 days of age ($+0.024 \pm 0.027$), where digestible P only increased by 5% with addition of the enzyme. The improvement in DE content of diets observed when the new 3-phytase was supplemented was due to the increase noted at 60 days of age ($+0.690 \pm 0.152$ MJ DE/kg; $P<0.001$), while no significant differences were found at 100 and 175 days of age ($+0.226 \pm 0.144$ and -0.017 MJ DE/kg, respectively).

Supplementation of the new 3-phytase (1000 FTU/kg) decreased the total Ca excreted per animal and day at 60, 100 and 175 days by 7.8, 9.4 and 3.2%, respectively (data not shown). With the addition of phytase to the diets total P excreted per animal and day decreased by 3.6, 9.7 and increased 2.8% in in pigs aged 60, 100 and 175 days, respectively.

Performance and mineralization trial

Average mortality was 1.9% during the 150-day experimental period. A total of 6 animals died (3 animals in treatment 0 and 3 animals in treatment 1000). Necropsies did not reveal any signs that could be related to the experimental treatments. Additionally, 2 animals were eliminated from the trial, one due to leg problems (treatment 1000, 146 days of age) and the other due to an open wound in the tail (treatment 0, 158 days of age).

Figure 3 summarizes the main results of the effect of supplementation with the new 3-phytase on pig performance traits. No significant differences between treatments were observed in ADFI of animals overall the experiment (on av. 1.841 ± 0.107 kg/d). Although ADG of animals fed Diet 1000 was not significantly higher than those fed Diet 0 during the overall period (+0.051 ± 0.032 kg/d; P=0.1421), it was higher from 46 to 94 days of age (+0.065 ± 0.031 kg/d; P=0.0458) and from 94 to 154 days of age (+0.056 ± 0.028 kg/d; P=0.0611). In a same way, FCR was not significantly different in the overall period (on av. 2.527 ± 0.054), but animals fed Diet 1000 improved the FCR compared with Diet 0, from 46 to 94 (–0.109 ± 0.046; P=0.0355) and from 94 to 154 days of age (–0.149 ± 0.043; P=0.0017).

Table 4 shows the mineralization of pig bones for the two diets. At 95 days of age and compared to animals fed Diets 0, piglets fed Diets 1000 had significantly higher tibia weight (+3.99 ± 1.03 g; P<0.01), as well as higher ash, Ca and P in tibia, both total (+2.56 ± 0.05, +0.97 ± 0.22 and +0.46 ± 0.10 g, respectively; P<0.001) and in proportion of dry tibia (+0.015 ± 0.006, +0.006 ± 0.003 and +0.033 ± 0.001 g, respectively; P<0.05). At 185 days of age, finishing pigs fed Diet 1000 had significantly higher metacarpus weight (+3.64 ± 0.92 g; P<0.05), as well as higher ash, Ca and P in metacarpus (+1.87 ± 0.41, +0.62 ± 0.15 and +0.33 ± 0.08 g, respectively; P<0.001) compared to pigs fed Diet 0.

Discussion

Digestibility trial

This study showed that Ca, P, and energy availability could be improved by the addition of this new 3-phytase. However, interactions between phytase and age were found in P digestibility and DE, meaning that age should be considered when supplementing diets with phytase. In this work, age also affected CTTAD of all nutrients analysed (DM, organic matter, CP, GE, DE, DP, Ca and P).

The effect of age could be being confounded with the effect of the diet. Indeed, diets offered at each age were different, to meet the requirements of each age and because the main purpose of this study was to evaluate the age influence on the effectiveness of this new 3-phytase under commercial supplementation conditions. Although all diets were barley-wheat-based diets and included oilseed meals, their choice and inclusion level depended on the suitability of each ingredient, considering their inclusion limitations and pig requirements at each age.

As expected, taking into account that dietary composition is the main factor responsible for its digestibility, the diet for piglets (richer in corn, wheat and soybean concentrate) had greater CTTAD of DM, OM, GE and CP compared to the growing and finishing diets (richer in barley and sunflower meal with a lower nutritive value; FEDNA, 2010).

On the other hand, the higher non-phytic P content of the diet for weaned piglets (the only one including an inorganic source of P, monocalcium phosphate) compared to growing and finishing diets could be the main factor responsible for the higher CTTAD of P in piglets. The weaned piglets' diet (including both calcium carbonate and monocalcium phosphate) also showed a clear greater CTTAD of Ca than those of growing and finishing pigs (including only calcium carbonate). González-Vega et al. (2015) previously observed, in growing pigs, that the CTTAD of Ca from a diet whose Ca source was calcium carbonate was significantly lower than that obtained when using monocalcium phosphate (0.70 and 0.79, respectively, $P < 0.05$). In any case, independently of the Ca and P sources, the highest Ca and P requirements in pigs occur in the first 12 weeks of their life, mainly due to the requirements for bone and muscle structures (Varley et al., 2011b), which could promote a higher absorption of these minerals. In this regard, Kiela and Ghishan (2016) stated that rapid phosphate absorption clearly occurs during early life to meet the demands of growth, and Ilchev et al. (2010) observed a higher CTTAD for Ca in a starter feed compared to growing and finishing feeds (0.61, 0.43 and 0.36, respectively).

In the present work, CTTAD values increased (3 to 5 percentage points) for all the evaluated nutrients between 100 and 175 days of age, some of them significantly (DM, OM, CP and GE). Most authors maintain that the CTTAD of the main nutrients in the pig diet increases between 90 and 180 days of age (Ilchev et al., 2010, Atakora et al., 2011). Concerning Ca and P digestibility, however, no significant differences were observed in the present work between animals aged 100 and 175 days. Digestibility of P has been shown to increase with BW of pigs, as indicated in a meta-analysis by Létourneau-Montminy et al. (2012). However, Harper et al. (1997) and Kim et al. (2017) reported that the digestibility of P and Ca was lower in the finisher phase than in the grower phase.

Concerning the interaction between age and phytase inclusion in P digestibility found in this work, the higher increment in P digestibility due to phytase supplementation was observed in growing pigs, followed by weaned piglets. However, no significant differences in P digestibility and faecal P excretion were found in finishing pigs fed low-P diet, with and without phytase supplementation. According to Selle et al. (2003), a higher effect of phytase can be expected in animals with an immature gut (i.e. piglets) compared to adult animals, where phytase would decrease its effect as the gut maturity is reached, as the anti-nutritive effects of the diets would then be overcome. In addition, it seems that P requirements for finishing pigs could be being slightly overestimated, which could contribute to their lowest effect of phytase. In this regard, Mavromichalis et al. (1999) and Peter et al. (2001) removed two-thirds and 100% of the supplemental P, respectively, in finishing pigs (from 80 and 87 kg BW) without finding any effect on weight gain, feed intake, feed efficiency and carcass characteristics. The higher effect of phytase on growing pigs compared to piglets could be explained by the higher P-phytate content of their diets (Kemme et al. 1997) or the lower digestibility of the diet, although differences in gastric emptying, stomach capacity and pH of gastric contents between piglets and growing pigs could also affect the efficacy of phytase Kemme et al. (1997). According to Harper et al. (1997), the greatest potential for commercial application of phytase is in growing-finishing pigs, as the volume of both feeds consumed, and manure excreted is substantially higher than during other phases of production. However, there would be a justification to use phytase in piglets because there is evidence that the effects of increasing Ca and P absorption in piglets could benefit the whole fattening period (Heaney et al., 2000; Varley et al., 2011b).

In the present work, the addition of phytase increased energy digestibility, especially DE in the diets at 60 days. Phytase has previously been reported to increase DE of the diets (Brady et al., 2002; Adedokun et al., 2015, Arredondo et al., 2019). However, a clear explanation to elucidate the energy effect of phytase has not yet been developed. It seems that phytase collectively increases utilization of energy derived from protein, lipid and starch (Selle and Ravindran, 2008). In this regard, protein digestibility increased numerically in piglets' diets due to phytase addition (from 0.851 to 0.875, results not shown). In growing and finishing pigs, however, similar CP digestibility were found between Diets 0 and Diets 1000, which could explain the lower effect on DE of their diets when supplementing with phytase. Increments in DE and CP with phytase addition in piglets could have been influenced by the synergistic effect of some feed additives with phytase in their diet (xylanase, amylase, glucanase, Table 1). A complementary action of carbohydrase and phytase has been shown in pigs by Oryschak et al. (2002) and Nortey et al. (2007). A possible mechanism for synergism between phytase and carbohydrase is explained because carbohydrase degrades cell walls, permitting endogenous enzymes (amylase and proteases) to access starch and proteins inside the aleurone and endosperm, and supplemental phytase to access phytate that binds proteins and starch. However, it seems that the synergistic effect of carbohydrase and phytase requires high fibre contents in diet (Kim et al., 2005 and Moehn et al., 2007) which is not the case in weaner´s diet.

Another reason for the effect of phytase on energy digestibility was given by Kies et al. (2005). These authors found reductions in energy expenditure of the digestive tract for the mineral absorption due to phytase supplementation in young pigs fed with maize-soyabean meal diets, but decreased metabolizable energy due to the higher heat production of the animals supplemented with phytase. However, in Moehn et al. (2007), phytase addition only increased metabolizable energy (and not DE) in growing-finishing pigs fed wheat-based diets ad libitum. More research is needed to clarify the energy effect of microbial phytase, as it might depend on different factors such as the inherent digestibility of the nutrients and their partition of energy before phytase intervention, the phytase inclusion level, the total P of the diets, the nature of the IP6 and, age of the animals (Partridge and Bedford 2001; Adeola and Cowieson, 2011, Arredondo et al., 2019) and the presence of other feed additives.

Performance and mineralization trial

In the present work, ADFI and FCR increased with pigs' age; ADG also increased during the first two months of age, but from this moment on it remained stable. A statistically significant increase of ADFI with age was also found by Ilchev et al. (2010). Dietary inclusion of the new 3-phytase did not affect feed intake, with animals showing similar ADFI values in Diets 0 and Diets 1000 throughout the trial. However, higher ADG and lower FCR were observed in the pigs supplemented with phytase.

In the present work, the effect of phytase could have been influenced by feed additives other than phytase commonly found in commercial diets, and thus included in our experimental diets. As mention above, weaner diets included carbohydrase enzymes (xylanase, amylase, glucanase, Table 1) which could have promoted the effect of phytase (Oryschak et al., 2002 and Nortey et al., 2007). Growing and finishing diets in experiment 2 were included formic acid and essential oils (Table 2). Improvements of mineral absorption by the addition of organic acids has been previously reported (Suiryanrayna et al., 2015).

Controversial results can be found in the literature regarding the effect of phytase on moderate reductions of the P content. In this regard, no effect on growth performance was found in pigs when reductions on dietary P of the diet were from 4.7 to 4.4, 5.8 to 4.7, 6.3 to 5.2 and 5.4 to 4.7 g/kg (Brady et al., 2002; Lyberg et al., 2008; Emiola et al., 2009 and Atakora et al., 2011, respectively).

However, Beers and Jongbloed (1992) and Campbell et al. (1995) found phytase enhancing performance in pigs offered P-adequate diets. These authors justified those results by an extra phosphoric effect of phytase (potential increase in CP and energy digestibility). Similarly, control diets of this study were formulated close to the animals' requirements for piglets and growing pigs (with 3.6 and 2.3 g/kg of digestible P, respectively) and significant improvements in growth performance were observed with the dietary inclusion of the phytase, probably due to the effect of the phytase on energy, P and Ca availability observed in the digestibility trial.

In this work, the effects of the 3-phytase on bone mineralization were even more evident than in growth performance. According to Koch and Mahan (1985), bone criteria are more sensitive and reliable indicators of P bioavailability than growth performance, as the storage of Ca and P in bone continues even after the dietary needs for other functions have been met (Vipperman et al., 1974; Mahan, 1982).

Results from this work showed that piglets 95 days of age fed Diets 1000 had significantly higher tibia weight, and ash, as well as higher Ca and P contents in their tibia in DM and total Ca and P content compared to piglets fed Diets 0. At 185 days, the effect of phytase was only evident in total bone weight, total ash and total P and Ca, but not in the mineral concentration. Li et al. (2015) stated that bone ash weight is a more sensitive parameter than bone ash percentage to evaluate the effect of phytase or non-phytate content in broiler diets. According to these authors, total ash weight reflects the absolute amount of mineral contained in the bone and is affected by both bone size and bone mineralization.

However, controversial results can be found in pigs in this regard. In Varley et al. (2011a; 2011b), phytase enhanced bone mineralization by increasing P and Ca concentrations in two metacarpal bones in weaned pigs (from 8 to 33 kg BW). However, She et al. (2015) found improvements on the content of P and Ca in the third and fourth metacarpals in weaned pigs (11 kg BW), but very little improvement in P and Ca concentration was detected in these bones. In our study, a more pronounced effect of phytase was found in piglets compared to finishing pigs, as both mineral concentration and mineral weight was improved in piglets with Diets 1000, but only mineral weight was affected in finishing pigs using phytase. Due to the early allometry of the bones, the introduction of a phytase like the one used in this work can improve both the amount of bone and its mineral concentration. However, the effect on the bone composition seems to diminish with age. In any case, the effectiveness of the phytase was maintained until the end, as the effect is observed in the total amount of bone, ash Ca and P. This higher effect of phytase on weaned piglets was also corroborated in the digestibility trial.

Increments in total P and Ca amount in bone are beneficial not only because they are important storage mineral sources (She et al., 2015), which might help to prevent damage when future P and Ca deficiencies arise, but also because P and Ca contents are correlated to bone strength (Brady et al., 2002). This latter fact is especially relevant in fast growing pigs, where the prevention of skeletal irregularities is critical (Jørgensen, 1995).

Conclusions

Supplementing barley-wheat based diets with the evaluated microbial 3-phytase at 1000 phytase units, under commercial dosage conditions, has a clear potential to significantly increase the digestibility of calcium and phosphorous in weaned and growing pigs, but its effectiveness seems to be less relevant in finishing pig diets. The breakage of the phytate group by the phytase action also allowed us to increase the digestible energy content in piglet feed, helping to improve its nutritive value. As a consequence of the better mineral and nutritive value, and despite using basal phosphorous levels only slightly lower than the current recommendations, dietary inclusion of new 3-phytase enhanced average daily gain from 46 to 94 days of age and decreased feed conversion ratio from 46 to 154 days of age. Finally, inclusion of the new 3-phytase from 46 days of age already allowed better tibia development and greater mineralization of the tibia in the piglets at 95 days of age. The advantages in bone development were maintained until 185 days of age. Therefore, we can conclude that the use of this type of enzymes, in commercial dosage conditions, can be recommended from the beginning of the life, as they improve the availability of nutrients, bone mineralization and pig performance. However, their effectiveness seems to be reduced during the finishing period, where animals consume more, so their inclusion in finishing diets with moderate phosphate levels could be avoided to reduce costs.

Acknowledgements

We thank the technical staff at the experimental farms of the Research and Technology Animal Centre (CITA-IVIA), the Institute of Animal Science and Technology (Universitat Politècnica de Valencia) and Javier Gómez (Crianzas Campovivo) for expert technical assistance and experimental support.

References

Adedokun, S.A., Owusu-Asiedu, A., Ragland, D., Plumstead, P., Adeola, O., 2015. The efficacy of a new 6-phytase obtained from *Buttiauxella spp*. expressed in *Trichoderma reesi* on digestibility of amino acids, energy, and nutrients in pigs fed a diet based on corn, soybean meal, wheat middlings and corn distillers dried grains with solubles. J. Anim. Sci. 93, 168-175. https://doi.org/10.2527/jas.2014-7912

Adeola, O., Cowieson, A.J., 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89, 3189-218. https://doi.org/10.2527/jas.2010-3715

Almeida, F.N., Stein, H.H., 2012. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn coproducts fed to pigs. J. Anim. Sci. 90(4), 1262-1269. https://doi.org/10.2527/jas.2011-4144

Almeida, F.N., Sulabo, R.C., Stein, H.H., 2013. Effects of a novel bacterial phytase expressed in Aspergillus Oryzae on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. J. Anim. Sci. Biotechnol. 4, 8. https://doi.org/10.1186/2049-1891-4-8

AOAC, 2003. Official methods of analysis of the association of official's analytical chemists. Official Method 945.18. Cereals Adjuncts, 17th edn. Association of official analytical chemists, Arlington, Virginia, USA.

Atakora, J.K., Moehn, S., Sands, J.S., Ball, R.O., 2011. Effects of dietary crude protein and phytase–xylanase supplementation of wheat grain based diets on energy metabolism and enteric methane in growing finishing pigs. Anim. Feed Sci. Technol. 166, 422-429. https://doi.org/10.1016/j.anifeedsci.2011.04.030

Arredondo, M.A., Casas, G.A., Stein, H.H., 2019. Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs.

Anim. Feed Sci. Technol. 248, 27-36. https://doi.org/10.1016/j.anifeedsci.2019.01.001

Blaabjerg, K., Nørgaard, J.V., Poulsen, H.D., 2012. Effect of microbial phytase on phosphorus digestibility in non-heat-treated and heat-treated wheat–barley pig diets. J. Anim.Sci. 90(4), 206-208. https://doi.org/10.2527/jas.53920

Beers, S., Jongbloed, A.W., 1992. Effect of supplementary *Aspergillus niger* phytase in diets for piglets on their performance and apparent digestibility of phosphorus. Anim. Prod. 55, 425-430. https://doi.org/10.1017/S0003356100021127

Brady, S.M., Callan, J.J., Cowan, D., McGrane, M., O'Doherty, J.V., 2002. Effect of phytase inclusion and calcium/phosphorus ratio on the performance and nutrient retention of grower–finisher pigs fed barley/wheat/soya bean meal-based diets. J. Sci. Food Agric. 82(15), 1780-1790. https://doi.org/10.1002/jsfa.1262

Braña, D.V., Ellis M., Castaneda E.O., Sands J.S., Baker D.H., 2006. Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs. J. Anim. Sci. 84 (7), 1839-1849. https://doi.org/10.2527/jas.2005-565

Campbell, R.G., Harrison, D.T., Butler, K.J., Selle, P.H., 1995. Effect of dietary available phosphorus and phytase (Natuphos) on the performance of pigs from 19–40 days post-weaning. Manipulating Pig Production V. Australasian Pig Science Association, Werribee, Vic. Australia, p. 193.

Dersjant-Li, Y., Awati, A., Schulze, H., Partridge, G., 2015. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95, 878-896. https://doi.org/10.1002/jsfa.6998

Eeckhout, W., De Paepe, M., 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Technol. 47(1-2), 19-29 https://doi.org/10.1016/0377-8401(94)90156-2

Emiola, A., Akinremi, O., Slominski, B., Nyachoti, C.M., 2009. Nutrient utilization and manure P excretion in growing pigs fed corn-barley-soybean based diets supplemented with microbial phytase. Anim. Sci. J. 80(1), 19-26. https://doi.org/10.1111/j.1740-0929.2008.00590.x

FEDNA, 2010. Tablas de la Federación Española de Nutrición Animal (FEDNA) de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos (3rd ed). Ed. De Blas, J.C., González-Mateos, G., García-Rebollar. Escuela Técnica Superior de Ingenieros Agrónomos de Madrid, Spain. 502 pp.

FEDNA, 2013. Fundación Española Desarrollo Nutrición Animal. Necesidades nutricionales para ganado porcino 2ª ed .Fund. Esp. Desarro. FEDNA, Madrid, Spain.

González-Vega, J.C., Walk, C.L., Stein, H.H., 2015. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93(5), 2255-2264. https://doi.org/10.2527/jas.2014-8215

Harper, A.F., Kornegay, E.T., Schell, T.C., 1997. Phytase supplementation of low-phosphorus growing-finishing pig diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion. J. Anim. Sci. 75(12), 3174-3186. https://doi.org/10.2527/1997.75123174x

Haug, W., Lantzsch, H.J., 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34, 1423-1426. https://doi.org/10.1002/jsfa.2740341217

Heaney, R.P., Abrams, S., Dawson-Hughes, B., Looker, A., Looker, A., Marcus, R., Matkovic, V., Weaver, C., 2000. Peak bone mass. Osteoporosis Int. 11(12), 985-1009. https://doi.org/10.1007/s001980070020

Ilchev, A., Ganchev, G., Chobanova, S., Kanakov, D., Petkov, P., Nikiforov, I., 2010. Age-related changes in mineral retention and excretion in starter and finisher pigs diets with and without exogenous phytase. Agric. Sci. Technol. 2(4), 183-190.

ISO 30024:2009. ISO 2009. International Standard ISO 30024. First edition 2009-07-15. Animal feeding stuffs — Determination of phytase activity.

Jørgensen, B., 1995. Effect of different energy and protein levels on leg weakness and osteochondrosis in pigs. Livest. Prod. Sci. 41(2), 171-181. https://doi.org/10.1016/0301-6226(94)00048-C

Kemme, P.A., Jongbloed, A.W., Mroz, Z., Beynen, A.C., 1997. The efficacy of Aspergillus niger phytase in rendering phytate phosphorus available for absorption in pigs is influenced by pig physiological status. J. Anim. Sci. 75(8), 2129-2138. https://doi.org/10.2527/1997.7582129x

Kiela, P.R., Ghishan, F.K., 2016. Physiology of intestinal absorption and secretion. Best Pract. Res. Clin. Gastroenterol. 30(2), 145-159. https://doi.org/10.1016/j.bpg.2016.02.007

Kies, A.K., Gerrits, W.J., Schrama, J.W., Heetkamp, M.J., van der Linden, K.L., Zandstra, T., Verstegen, M.W., 2005. Mineral absorption and excretion as affected by microbial phytase, and their effect on energy metabolism in young piglets. J. Nutr. 135(5), 1131-1138. https://doi.org/10.1093/jn/135.5.1131

Kim, J.C., Simmins, P.H., Mullan, B.P., Pluske, J.R., 2005. The effect of wheat phosphorus content and supplemental enzymes on digestibility and growth performance of weaner pigs. Anim. Feed Sci. Tech. 118(1-2), 139-152. https://doi.org/10.1016/j.anifeedsci.2004.08.016

Kim, J.W., Ndou, S.P., Mejicanos, G.A., Nyachoti, C.M., 2017. Standardized total tract digestibility of phosphorus in flaxseed meal fed to growing and finishing pigs without or with phytase supplementation. J. Anim. Sci. 95(2), 799-805.

https://doi.org/10.2527/jas.2016.1045

Koch, M.E., Mahan, D.C., 1985. Biological characteristics for assessing low phosphorus intake in growing swine. J. Animal Sci. 60(3), 699-708. https://doi.org/10.2527/jas1985.603699x

Konietzny, U., Greiner, R., 2002. Molecular and catalytic properties of phytate-degrading enzymes (phytases). Int. J. Food Sci. Technol. 37, 791-812. https://doi.org/10.1046/j.1365-2621.2002.00617.x

Létourneau-Montminy, M.P., Jondreville, C., Sauvant, D., Narcy, A., 2012. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. Animal 6, 1590-1600. https://doi.org/10.1017/S1751731112000560

Li, W., Angel, R., Kim, S.W., Jiménez-Moreno, E., Proszkowiec-Weglarz, M. Plumstead, P.W., 2015. Impact of response criteria (tibia ash weight vs. percent) on phytase relative non phytate phosphorus equivalance. Poultry Sci. 94(9), 2228-2234. https://doi.org/10.3382/ps/pev156

Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76, 1216-1231. https://doi.org/10.2527/1998.7641216x

Lyberg, K., Andersson, H.K., Sands, J.S., Lindberg, J.E., 2008. Influence of phytase and xylanase supplementation of a wheat-based diet on digestibility and performance in growing pigs. Acta Agric. Scand A Anim. Sci. 58(3), 146-151. https://doi.org/10.1016/j.livsci.2007.01.114

Mahan, D.C., 1982. Dietary calcium and phosphorus levels for weaned swine. J. Anim. Sci. 54(3), 559-564. https://doi.org/10.2527/jas1982.543559x

Mavromichalis, I., Hancock, J.D., Kim, I.H., Senne, B.W., Kropf, D.H., Kennedy, G.A., Hines, R.H., Behnke, K.C., 1999. Effects of omitting vitamin and trace mineral premixes and (or) reducing inorganic phosphorus additions on growth erformance, carcass characteristics, and muscle quality in finishing pigs. J. Anim. Sci. 77, 2700-2708. https://doi.org/10.2527/1999.77102700x

Moehn, S., Atakora, J.K.A., Sands, J., Ball, R.O., 2007. Effect of phytase-xylanase supplementation to wheat-based diets on energy metabolism in growing–finishing pigs fed ad libitum. Livest. Sci. 109(1-3), 271-274. https://doi.org/10.1016/j.livsci.2007.01.118

Nortey, T.N., Patience, J.F., Simmins, P.H., Trottier, N.L., Zijlstra, R.T., 2007. Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower pigs fed wheat-based diets containing wheat millrun. J. Anim. Sci. 85(6), 1432-1443. https://doi.org/10.2527/jas.2006-613

Oryschak, M.A., Simmins, P.H., Zijlstra, R.T., 2002. Effect of dietary particle size and carbohydrase and/or phytase supplementation on nitrogen and phosphorus excretion of grower pigs. Can. J. Anim. Sci. 82(4), 533-540. https://doi.org/10.4141/A02-016

Partridge, G.G., Bedford, M.R., 2001. Enzymes in farm animal nutrition. Partridge, G.G., Bedford, M.R. eds. CAB International 2001, Wallingford, UK, 406 pp.

Peter, C.M., Parr, T.M., Parr, E.N., Webel, D.M., Baker, D.H., 2001. The effects of phytase on growth performance, carcass characteristics, and bone mineralization of late-finishing pigs fed maize-soyabean meal diets containing no supplemental phosphorus, zinc, copper and manganese. Anim. Feed Sci. Technol. 94, 199-205. https://doi.org/10.1016/S0377-8401(01)00300-5

Rodehutscord, M., Faust, M., Lorenz, H., 1996. Digestibility of phosphorus contained in soybean meal, barley, and different varieties of wheat, without and with supplemental phytase fed to pigs and additivity of digestibility in a wheatsoybeanmeal diet. J. Anim Physiol. Anim. Nutr. 75(1), 40-48. https://doi.org/10.1111/j.1439-0396.1996.tb00466.x

Salaet, I., Marques, R., Yance, T., Macias, J., Giménez-Zaragoza, D., Pujol, M.J., Bachs, O., Aligue, R., 2019. A novel long-term phytase from *Serratia odorifera*: Cloning, expression, characterization and production. Personal communication.

SAS, 2002. Statistical Analysis Systems Version 9.1. SAS Institute Inc., Cary, North Carolina, USA

Selle, P.H., Cadogan, D.J., Bryden, W.L., 2003. Effects of phytase supplementation of phosphorus-adequate, lysine-deficient, wheat-based diets on growth performance of weaner pigs. Aust. J. Agric. Res. 54, 323-330. https://doi.org/10.1071/AR02121

Selle, P.H., Ravindran, V., 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113, 99-122. https://doi:10.1016/j.livsci.2007.05.014

She, Y., Su, Y., Liu, L., Huang, C., Li, J., Li, P., Li, D., Piao, X., 2015. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. Anim. Feed Sci. Technol. 208, 132-144. https://doi:10.1016/j.anifeedsci.2015.07.011

Suiryanrayna, M.V.A.N., Raman, J.V., 2015. A review of the effects of dietary organic acids fed to swine. J. Anim. Sci. Biotechnol. 6 (45), 1-11. https://doi:10.1186/s40104-015-0042-z

Van Soest, P.V., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10), 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Varley, P.F., Callan, J.J., O'Doherty, J.V., 2011a. Effect of dietary phosphorus and calcium level and phytase addition on performance, bone parameters, apparent nutrient digestibility, mineral and nitrogen utilization of weaner pigs and the subsequent effect on finisher pig bone parameters. Anim. Feed Sci. Technol. 165, 201-209. https://doi:10.1016/j.anifeedsci.2011.02.017

Varley, P.F., Flynn, B., Callan, J.J., O'Doherty, J.V., 2011b. Effect of phytase level in a low phosphorus diet on performance and bone development in weaner pigs and the subsequent effect on finisher pig bone development. Livest. Sci. 138(1-3), 152-158. https://doi.org/10.1016/j.livsci.2010.12.014

Vipperman Jr, P.E., Peo Jr, E.R., Cunningham, P.J., 1974. Effect of dietary calcium and phosphorus level upon calcium, phosphorus and nitrogen balance in swine. J. Anim. Sci. 38(4), 758-765. https://doi.org/10.2527/jas1974.384758x

Table 1. Digestibility trial: Ingredients and chemical composition of the control diets (no exogeneous phytase inclusion) used at each period.

	Piglets	Growing pigs	Finishing pigs
Ingredients (g/kg)			
Barley grain	300	650	698
Wheat grain	330	44.1	9.8
Corn grain	102	-	-
Soybean meal 440 g/kg CP	152	-	-
Soybean meal 470 g/kg CP	-	120	90.3
Soy concentrate 630 g/kg CP	44.0	-	-
Sunflower meal 280 g/kg CP	-	192	17.9
Rice bran	-	40.0	50.0
Bakery byproduct	-	-	70.0
Sugarcane	-	70.0	10.0
Soybean oil	33.0	-	-
Soy lecithine	-	8.1	-
Tallow	-	24.5	24.0
L-lysine	5.5	6.4	5.5
DL-methionine	1.5	1.2	0.8
L-threonine	-	1.7	1.4
L-tryptophan	1.7	0.8	0.5
Choline hydrochloride	-	0.1	0.2
Calcium carbonate	14.4	11.0	11.0
Monocalcium phosphate	6.4	-	-
Hydrogen sulphate	-	0.6	-
Sodium bicarbonate	6.0	0.7	1.9
Sodium chloride	-	2.5	2.9
Vit mineral premix[1,2]	4.5	4.0	4.0
Chemical composition (g/kg DM)			
Dry matter (DM), as fed	903	904	907
Ash	55	59	52
Crude protein (CP)	196	175	160
Ether extract	41	53	54
Neutral detergent fibre	153	205	206
Acid detergent fibre	34	60	51
Gross energy (MJ/kg DM)	18.46	18.97	19.45
Calcium	8.6	7.8	7.7
Total-phosphorus	5.2	4.2	4.1
Phytate-phosphorus	2.0	2.1	2.4

[1] Provided per kg of piglet diet: $CaCO_3$, 45 mg; Mn, 35mg (MnO); Zn, 72.3 mg (ZnO); Cu, 29.8 mg ($CuSO_4(H_2O)_5$); Copper chelate of amino hydrate, 18 mg; I, 153 mg (KI); Se, 18.3 mg (Na_2SeO_3); Fe, 9.64 g ($FeCO_3$); Vit A, 2000000 IU; Vit D3, 400000 IU; Vit E, 8000 IU; Vit K, 0.20 g; Vit B1, 0.90 mg; Vit B2, 4.5 mg; Vit B6, 2.7 mg; Vit B12, 36 µg; Niacinamide, 27 mg; D-calcium pantothenate, 13.5 mg; Pantothenic acid, 12.42 mg; Folic acid, 0.45 mg; Biotin, 36 µg; Betaine, 180 µg; Sepiolite, 727.70 mg; Mixture of flavouring compounds, 211.5 mg; E320 Butylhydroxyanisole, 346.5 µg; Butylhidroxytoluene, 38.12 µg; Ethoxyquin, 5.54 µg; Endo-1,3(4)-beta-glucanase (150 U/g), 135 U; Endo-1,4-beta-xylanase (4000 U/g), 3600 U; Alpha-amylase 1 (1000 U/g), 900 U; Polygalacturonase (25 U/g), 22.5 U.

[2]Provide per kg of growing and finishing diet: Vit A, 7200 IU; Vit D3, 1350 IU; Vit E, 13 mg; Vit K-3, 0.9 µg; Vit B1, 0.453 mg; Vit B2, 0.448 mg; Vit B12, 12 µg; Niacinamide, 18 mg; Pantothenic acid, 700 mg; Choline chloride, 180 mg; Fe, 32.82 mg ($FeSO_4 \cdot H_2O$); I, 0,32 mg ($(IO_3)_2$); Cu, 2,0 mg ($CuSO_4(H_2O)_5$); Mn, 28.9 mg (MnO); Zn, 100.4 mg (ZnO); Se, 0.07 mg (Na_2SeO_3); Diatomaceous earth, 600 mg; $CaCO_3$, up to 4 g.

Table 2. Performance and mineralisation trial: Ingredients and chemical composition of the experimental diets used at each period.

	Piglets	Growing pigs	Finishing pigs
Ingredients (g/kg)			
Barley grain	300	550	513
Wheat grain	330	31.1	-
Triticale grain	-	120	158
Corn grain	102	-	-
Rye grain	-	33.6	-
Soybean meal 440 g/kg CP	152	-	-
Soybean meal 470 g/kg CP	-	69.2	88
Soy concentrate 630 g/kg CP	44.0	-	-
Sunflower meal 280 g/kg CP	-	60	70
Wheat bran	-	57	100
Soybean oil	33.0	-	-
Tallow	-	42.3	44.4
L-lysine	5.5	9.4	5.2
DL-methionine	1.5	2.1	0.4
L-threonine	-	2.1	0.8
L-tryptophan	1.7	-	-
Calcium carbonate	14.4	9.9	9.1
Monocalcium phosphate	6.4	-	-
Sodium bicarbonate	6.0	-	-
Sodium chloride	-	4.3	4.1
ProPhorce® PH 101[1]	-	3	3
Pediococcus acidilactici	-	2	-
Vit mineral premix[2,3]	4.5	4.0	4.0
Chemical composition (g/kg DM)			
Dry matter (DM), as fed	903	901	907
Ash	55	49	56
Crude protein (CP)	196	174	182
Ether extract	41	74	81
Neutral detergent fibre	153	173	184
Acid detergent fibre	34	65	70
Gross energy (MJ/kg DM)	18.46	19.40	19.46
Calcium	8.6	7.1	6.6
Total-phosphorus	5.2	3.8	4.4
Phytate-phosphorus	2.0	2.4	2.6

[1] Blend of formic acid and essential oils (thyme, clove, oregano, eugenol and carvacrol) (ProPhorce® PH101, Perstorp, The Netherlands)

[2] Provided per kg of piglet premix: (see Table 1)

[3] Provided per kg of growing and finishing diet: Vit A, 6500 UI; Vit D3, 1500 UI; Vit E, 300 mg; Vit B12, 15 µg; Vit B6 1 mg; Vit B2, 3 mg; Nicotinic acid, 15 mg; Calcium pantothenate, 10 mg; Choline chloride, 50 mg; Anhydrous betaine, 23.04 mg; Fe, 48.21 mg ($FeCO_3$); Mn, 37.9 mg; (MnO_2); Se, 0.18 mg ($NaSeO_3$); Zn, 80,34 mg (ZnO); I, 0.59 mg (KIO_3); Butylhydroxytoluene, 4 mg; Citric acid, 15 mg; Sodium citrate, 0.4 mg; Sepiolite, 400 mg; $CaCO_3$, 2.84 g.

Table 3. Digestibility trial: Least square means of apparent total tract nutrient digestibility coefficients, digestible protein (g/kg dry matter, DM) and digestible energy (MJ/kg DM) in function of phytase inclusion (0 and 1000 FTU/kg) and age in pigs

	Phytase dose (FTU/kg)				Age (days)				
	0	1000	SEM	P-value	60 (18 kg)	103 (39 kg)	177 (115 kg)	SEM	P-value
Dry matter	0.84	0.84	0.003	0.333	0.87[c]	0.81[a]	0.84[b]	0.004	<0.001
Organic matter	0.85	0.85	0.003	0.330	0.88[c]	0.82[a]	0.85[b]	0.004	<0.001
Crude protein	0.83	0.83	0.005	0.951	0.86[c]	0.79[a]	0.84[b]	0.007	<0.001
Gross energy	0.83	0.84	0.003	0.088	0.86[c]	0.80[a]	0.83[b]	0.004	<0.001
Calcium	0.60	0.65	0.012	0.004	0.71[b]	0.56[a]	0.60[a]	0.015	<0.001
Phosphorus*	0.51	0.57	0.012	0.001	0.64[b]	0.48[a]	0.51[a]	0.015	<0.001
Digestible protein (g/kg DM)	146.6	146.9	0.90	0.825	169.1[c]	137.7[b]	133.6[a]	1.10	<0.001
Digestible energy (MJ/kg DM)*	15.7	16.0	0.07	0.004	16.1[b]	15.2a	16.2b	0.09	<0.001

[a,b,c] Means for age in a row not sharing superscript are significantly different at P<0.05.
Experimental unit in all cases is the metabolic cage housing two piglets and one growing-finishing pig.
Treatment 0 = animals fed control diets with low levels of P and 0 phytase units (FTU)/kg of exogenous phytase dosage (Replicates/treatment=34); Treatment 1000 = animals fed diets with low levels of P and 1000 phytase units (FTU)/kg of exogenous phytase dosage (Replicates/treatment=34).
Age 60 = piglets starting faeces collection at 60 days old, 18 kg live weight. Replicates/treatment=10
Age 103 = growing pigs starting faeces collection at 103 days old, 39 kg live weight. Replicates/treatment=12
Age 177 = finishing pigs starting faeces collection at 177 days old, 115 kg live weight. Replicates/treatment=12
* Interaction phytase dose x age at P<0.05

Table 4. Performance and mineralisation trial: Mineralization of tibia or metacarpus in piglets and finishing pigs in function of phytase supplementation (least square means ± standard error).

		Phytase dose (FTU/kg)			
		0	1000	SEM	P-value
Piglets at 95 days:					
Tibia weight,	g	27.9	31.9	0.82	0.004
Ash in tibia,	g	14.4	16.9	0.40	0.001
	g in g DM[1] bone	0.52	0.53	0.005	0.040
Calcium in tibia,	g	5.4	6.3	0.17	0.001
	g in g DM bone	0.19	0.20	0.002	0.049
	g in g ash	0.37	0.37	0.002	0.808
Phosphorus in tibia,	g	2.5	3.0	0.08	0.002
	g in g DM bone	0.090	0.093	0.0010	0.024
	g in g ash	0.17	0.18	0.001	0.488
Finishing pigs at 185 days:					
Metacarpus weight,	g	30.3	33.9	0.74	0.001
Ash in metacarpus,	g	14.9	16.7	0.33	<0.001
	g in g DM bone	0.49	0.49	0.007	0.926
Calcium in metacarpus,	g	5.4	6.0	0.12	0.001
	g in g DM bone	0.18	0.18	0.003	0.762
	g in g ash	0.37	0.36	0.001	0.066
Phosphorus in metacarpus,	g	2.6	2.9	0.06	<0.001
	g in g DM bone	0.09	0.09	0.001	0.834
	g in g ash	0.18	0.18	0.001	0.673

[1] DM, dry matter

Experimental unit is the individual animal in all cases.

Treatment 0 = animals fed control diets with low levels of P and 0 phytase units (FTU)/kg of exogenous phytase dosage (Replicates/treatment=12); Treatment 1000 = animals fed diets with low levels of P and 1000 phytase units (FTU)/kg of exogenous phytase dosage (Replicates/treatment=12).

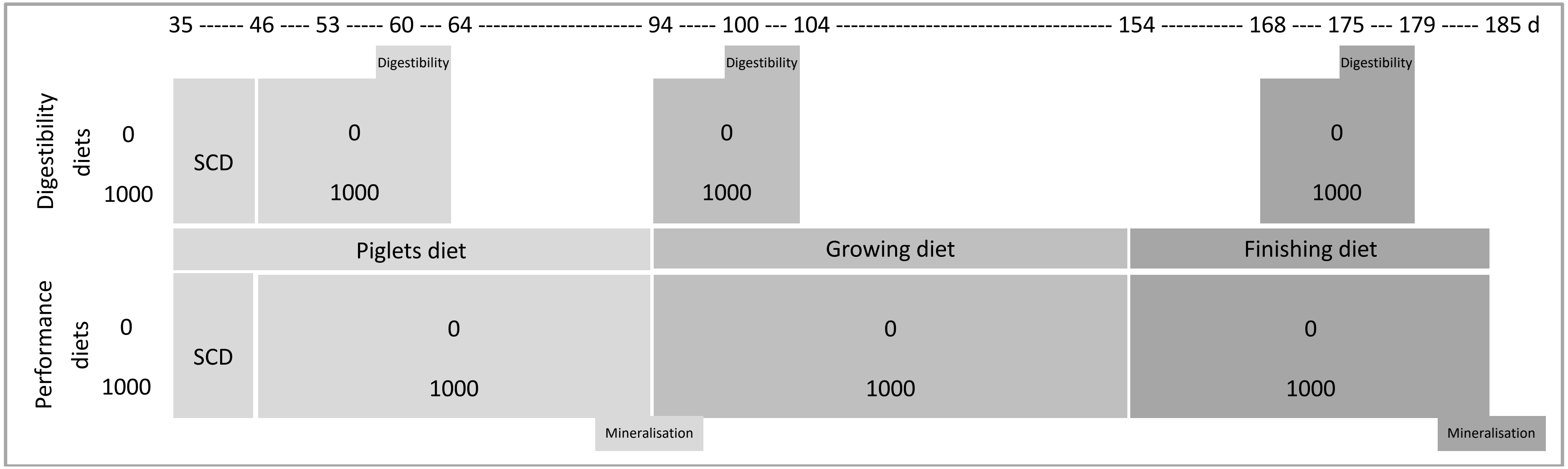


Figure 1. Outline on the management of experimental diets throughout the digestibility and performance trials. It is detailed when the digestibility and mineralization tests were carried out. Experimental diets: 0, negative controls with low level of Ca and P and 0 FTU of exogenous phytase added; 1000, diets with low levels of Ca and P and 1000 FTU of exogenous phytase added. Commercial diets: SCD, standard commercial diet.

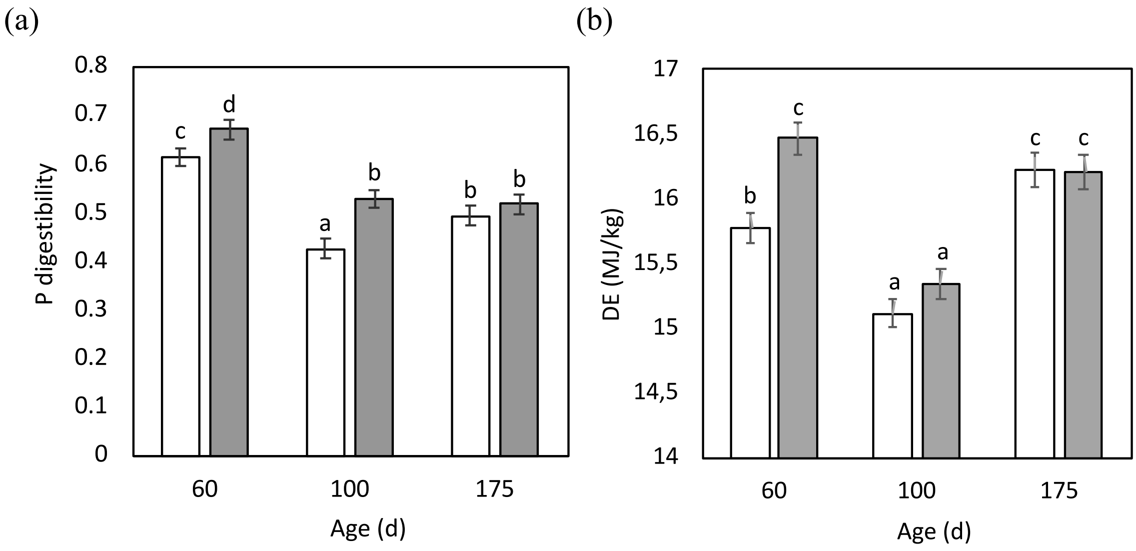
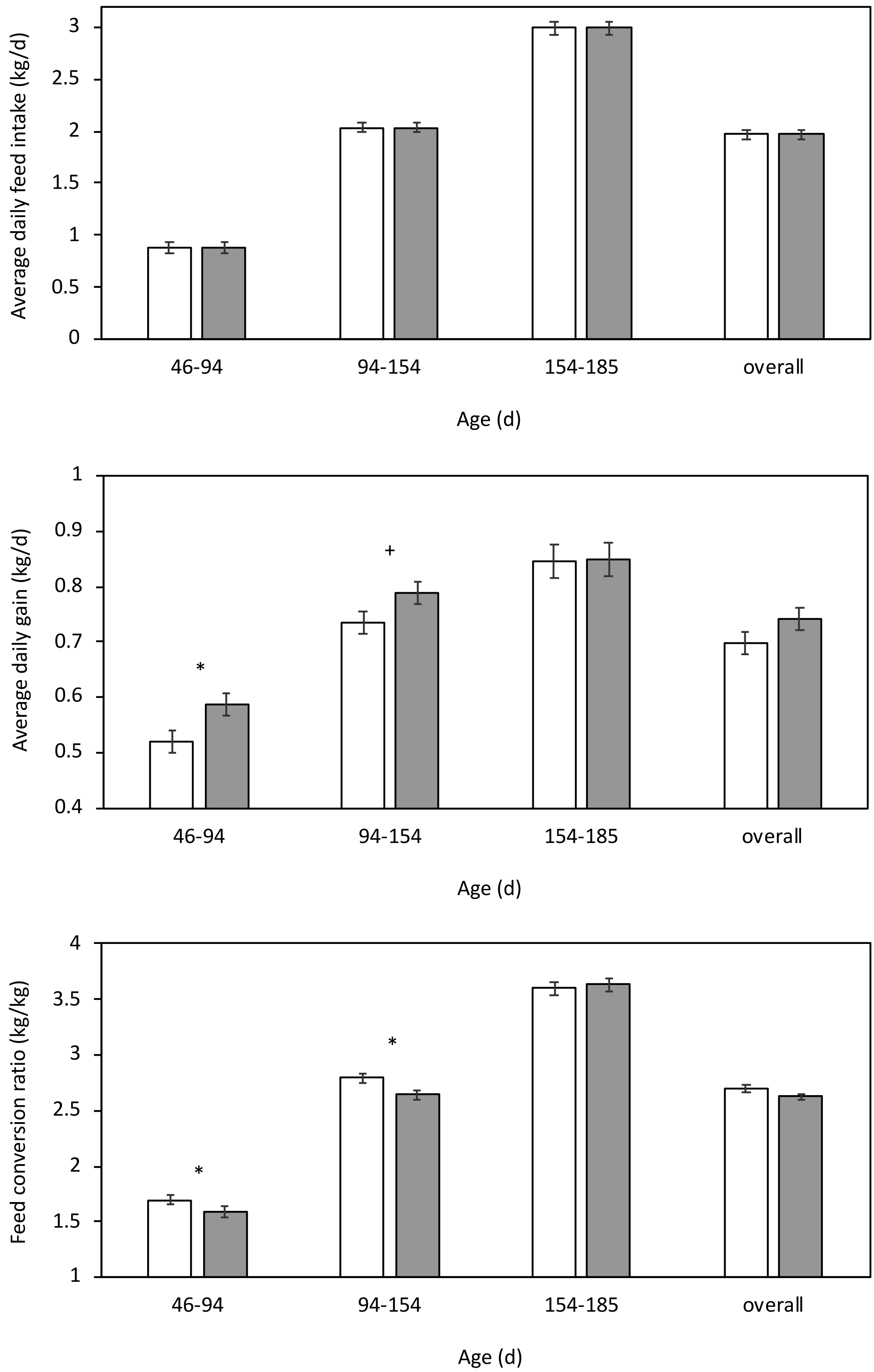


Figure 2. Digestibility trial: Evolution of (a) the apparent faecal digestibility of the P and (b) digestible energy of the feed when phytase was added at 0 and 1000 FTU/kg (white and grew bars, respectively). [a,b,c,d] Means in a figure not sharing letter are significantly different at P<0.05.



(a)

(b)

(c)

Figure 3. Performance and mineralisation trial: Evolution of (a) average daily feed intake, (b) average daily gain and (c) feed conversion ratio when phytase was added at 0 and 1000 phytase units, FTU/kg (white and grew bars, respectively). Bars within an age significantly different at *P<0.05 or $^{+}$P<0.10.