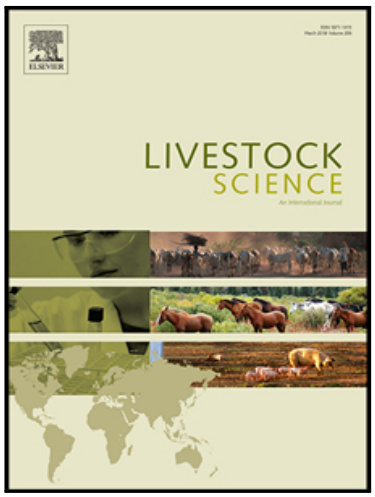

Effect of phytase, carbohydrase, and protease addition to a wheat DDGS and rapeseed based diet on in-vitro ileal digestibility, growth, and bone mineral density of grower-finisher pigs.

A. Torres-Pitarch , U.M. McCormack , V.E. Beattie , E. Magowan , G.E. Gardiner , A.M. Pérez-Vendrell , D. Torrallardona , J.V. O'Doherty , P.G. Lawlor

PII: S1871-1413(18)30201-4
DOI: 10.1016/j.livsci.2018.07.003
Reference: LIVSCI 3494

To appear in: *Livestock Science*

Received date: 9 February 2018
Revised date: 12 June 2018
Accepted date: 5 July 2018

Please cite this article as: A. Torres-Pitarch , U.M. McCormack , V.E. Beattie , E. Magowan , G.E. Gardiner , A.M. Pérez-Vendrell , D. Torrallardona , J.V. O'Doherty , P.G. Lawlor , Effect of phytase, carbohydrase, and protease addition to a wheat DDGS and rapeseed based diet on in-vitro ileal digestibility, growth, and bone mineral density of grower-finisher pigs., *Livestock Science* (2018), doi: 10.1016/j.livsci.2018.07.003

Highlights

- In-vitro digestibility of diets was useful to identify potential responses in-vivo
- Phytase sparing effect was effective to reduce inorganic P and Ca in the diets
- Protease improved feed efficiency when supplemented alone or as part of a complex
- Efficacy of phytase was not reduced when supplemented in combination with protease

Effect of phytase, carbohydrase, and protease addition to a wheat DDGS and rapeseed based diet on in-vitro ileal digestibility, growth, and bone mineral density of grower-finisher pigs.

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A B S T R A C T

The use of rapeseed meal (RSM) and wheat distillers dried grains with solubles (wDDGS) in pig diets is increasing and dietary supplementation with exogenous enzymes has been suggested as means of improving feed efficiency in pigs. The objective of this experiment was to examine the effect of phytase (Phy), a xylanase and β-glucanase complex (XB), protease (Pro) and their various combinations when included in a wDDGS- and RSM-based diet fed to grower-finisher pigs. As the P- and Ca- sparing effect of Phy is well proven, the objective was to examine the additional effects of Phy beyond its P- and Ca-sparing effects. A total of 144 pigs with an initial live weight of 40.1 ± 2.0 kg were assigned to 8 treatments with 9 pens (4 female and 5 male pens) per treatment and 2 females or 2 males per pen. The basal diet was formulated to contain 96 and 200 g/kg of RSM and wDDGS, respectively. The basal diet was supplemented with Phy (0 or 100 mg/kg), XB (0 or 100 mg/kg), and Pro (0 or 200 mg/kg) in a 2 x 2 x 2 factorial arrangement of treatments. Experimental diets were fed for 76 d. Average daily gain (ADG) and average daily feed intake (ADFI) were recorded, and gain to feed (G:F) was calculated. Carcass quality variables were measured at slaughter and the left forelimb feet of pigs from pigs fed the non-supplemented, the Phy supplemented and the Phy + Pro supplemented diets were removed to determine bone mineral density in the third metacarpal. The inclusion of Phy, XB and Pro in the diets increased in-vitro ileal digestibility of dry matter and organic matter ($P < 0.05$). A tendency towards a 3-way interaction among Phy, XB and Pro was observed for ADG ($P = 0.06$) and G:F ($P = 0.06$). The 2-way interactions and main effects did not reveal any improvement for any variable measured in-vivo in response to dietary enzyme supplementation. Bone mineral density was not different for pigs fed the non-supplemented, the Phy supplemented and the Phy + Pro supplemented diets. In conclusion, the in-vitro ileal digestibility

improvements observed were not always reflected in improvements in pig growth and/or feed efficiency. The efficacy of Phy was not reduced when supplemented in combination with Pro, as ADG, G:F, carcass quality and bone mineralization were unchanged.

Keywords: Feed, Enzyme, Xylanase, β-glucanase, Phosphorus, Calcium

Introduction

Feed represents from 64 to 72% of pig production costs (Rocadembosch et al., 2016; Teagasc, 2016), therefore, nutritional strategies to improve feed efficiency are particularly important. Supplementation of pig diets with exogenous enzymes has been suggested as a tool to improve feed efficiency. These can break down specific chemical bounds in plant ingredients that are not degraded by the pig's endogenous enzymes, thereby increasing nutrient digestibility, inhibiting antinutritional factors and modulating gut health (Adeola and Cowieson, 2011; Bedford and Schulze, 1998). Phytase (Phy) is the most commonly used feed enzyme in pig diets due to its proven efficacy in increasing P digestibility (Campbell and Bedford, 1992; Humer et al., 2015). During diet formulation, a nutrient sparing value for P and Ca is normally used for Phy in the formulation matrix such that the inclusion of Phy increases digestible P and Ca by 1.5 and 1.0 g/kg, respectively. Carbohydrases are the second most commonly used exogenous enzyme in monogastric diets and the complex of xylanase and β-glucanase (XB) accounts for more than 80% of the global carbohydrase market (Adeola and Cowieson, 2011). Exogenous protease (Pro) has also become available in the last 5 to 10 yr. (Cowieson and Roos, 2014) and can be used in pig diets. Exogenous Pro supplementation to pig diets may increase protein and amino acid digestibility, disrupts the feed structure, liberating fat and starch, and inactivates antinutritional factors present in the diet (Cowieson and Roos, 2016; O'Shea et al., 2014; Upadhaya et al., 2016).

Exogenous enzymes such as Phy, XB and Pro are normally supplemented alone or as part of a multi-enzyme cocktail where it is not possible to determine how the

different enzymes interact. The efficacy of Phy can be influenced by its resistance to endogenous Pro degradation in in-vitro experiments (Dersjant-Li et al., 2015). As the efficacy of Phy to spare Ca and P is well proven, the present study examines the effect of Phy beyond its Ca- and P-sparing effect. Due to increasing availability, biofuel co-products such as rapeseed meal (RSM) and wheat distillers dried grains with solubles (wDDGS) are now widely used as feed ingredients in pig diets (Keady and O'Doherty, 2000; Torres-Pitarch et al., 2014). The objective here was to determine the effect of Phy, XB, Pro and their interactions when included in a wDDGS and RSM based diet on in-vitro ileal digestibility, growth performance and bone mineral density of grower-finisher pigs. The hypothesis was that XB, Pro, Phy and their combinations will improve in-vitro ileal digestibility of feed as well as pig growth and feed efficiency. Due to the ability of some Pro to degrade and reduce Phy activity, we further hypothesised that Pro would reduce the response to Phy and therefore reduce the bone mineral density of pigs fed a diet limiting in P and Ca content.

Materials and methods

The care and use of the animals in this study was approved by the Teagasc Animal Ethics Committee. The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

2.1. Animals and experimental design

A total of 144 pigs [Maxgrow x (Landrace x Large White); Hermitage Genetics, Sion Road, Kilkenny, Ireland] with an initial live weight (LW) of 40.1 ± 2.0 kg

were assigned to 8 treatments with 9 pens (4 female and 5 male pens) per treatment and 2 females or 2 males per pen in a randomized complete block design. The 9 blocks were randomly distributed in 5 rooms, 4 of them containing 16 pens (2 blocks in each room) and 1 of them containing 8 pens (1 block). The duration of the experiment was 76 d. The basal diet included 96 and 200 g/kg of RSM wDDGS, respectively, and were formulated to contain 9.4 MJ net energy and 7.9 apparent ileal digestible (AID) Lys/kg. The basal diet was supplemented with Phy (0 or 100 mg/kg; Phyzyme 5000 XP TPT, DuPont-Danisco Animal Nutrition, Marlborough, UK), XB (0 or 100 mg/kg; Rovabio Spiky, ADISSEO, Commetry, France), and Pro (0 and 200 mg/k; Ronozyme ProAct, DSM, Grenzach-Wyhlen, Germany) in a 2 x 2 x 2 factorial arrangement of treatments. Phytase and Pro products contained 5,000 FTU (phytase units) and 75,000 protease units/g, respectively, whereas XB product contained 22,000 xylanase units and 15,200 β-glucanase units/g.

2.2. Diets

The 8 experimental diets were formulated to be 6% below the estimated energy and 4% below the AID amino acid requirements of pigs used in this study (De Blas et al., 2013), the ingredient content, energy and nutrient composition of each is given in Table 1. Phytase, when included at 100 mg/kg of feed, provided 500 FTU/kg of finished feed and according to the manufacturers advice this dose should allow a sparing effect of 0.12% and 0.09% for digestible P and total Ca, respectively. When included in the experimental diet, the digestible P and total Ca concentrations were reduced by 0.08 and 0.09%, respectively compared to the control diet. Diets that were supplemented with Phy had reduced total P (no

inorganic P source was added to the diet) and Ca (sparing effect of 0.09% Ca) to allow for the sparing effect of Phy. Diets were steam conditioned at 60°C and pelleted to a diameter of 3 mm.

2.3. Housing and feeding

Pairs of pigs were penned in fully slatted pens (1.81 m x 1.18 m) with steel rail partitions. Air temperature was maintained at 20 to 22°C. The feeders were stainless steel dry feed hoppers, 30 cm in width (O'Donovan Engineering, Coachford, Co. Cork, Ireland). Ad-libitum access to feed (dry pellets) and water (one drinking bowl per pen; DRIK-O-MAT, Egebjerg International A/S, Egebjerg, Denmark) was provided. Pigs were observed closely twice daily. Any pig showing signs of ill-health was treated as appropriate. All veterinary treatments were recorded including identity of pig, symptom, medication used, and dosage.

2.4. Measurements and sampling

Individual pig weight and feed disappearance per pen were recorded on 0, 25, 42, 56, 63, and 76 d of the experiment. Average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were calculated. The 63 d was considered the last day of the experimental period for the growth performance study. At 63 d of the experimental period, one pig per pen was randomly selected and transported to a commercial abattoir (DAWN Pork and Bacon, Waterford, Ireland), stunned with CO_2, and killed by exsanguination. From 63 to 76 d the remaining pigs continued to receive the experimental diets and at 76 d of the experiment, were slaughtered under the same conditions. At slaughter, carcass hot weight was recorded, and back-fat thickness and muscle depth measured at 6 cm

from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean content was estimated according to the following formula (Department of Agriculture Food and Rural Development, 2001): Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm). In order to determine if Pro has the ability to degrade and reduce Phy effects on bone mineral density, on 63 d of the experimental period, the left forelimb foot from pigs fed the non-supplemented, Phy, and Pro supplemented diets were removed at the carpal joint after slaughter and frozen at - 20˚C in polythene bags for bone density analysis.

2.4. Laboratory analysis

2.4.1. Feed Analysis

Prior to analysis, samples were ground through a 1 mm screen in a Cyclotec^{TM} mill (FOSS Electric, Hilleroed, Denmark). Dry matter was determined by oven drying for 4 h at 103 ºC. Ash was determined by incineration in a muffle furnace (Gallenkamp, London, UK) at 550 ºC overnight. The crude protein (CP) was determined as N x 6.25 with a LECO FP - 2000 analyser (Leco Instruments Ltd., Stockport, Cheshire, UK). Fat was determined according to the method described by Usher et al. (1973) by extraction with perchlorethylene in a Foss Let 15300 (A/S N. Foss Electric, Hillerod, Denmark). Crude fiber (CF) was measured by a Fibertec semi-automatic system (Tecator, Hoganas, Sweden). Diets were analyzed by ADISSEO France for xylanase activity using a colorimetry assay. One visco-unit of endo-1,4-β-xylanase activity was defined as the amount of enzyme reducing the viscosity of the solution, to give a change in relative fluidity of 1

dimensionless unit per minute per mL (or per g) under the conditions of the assay (pH 5.5 and 30°C). Diets were analyzed by DuPont-Danisco Animal Nutrition for Phy activity using the laboratory procedure based on a colorimetry assay and described by Yu et al. (2014). One phytase unit (FTU) is defined as the amount of enzyme that liberates 1 μmol inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37ºC. Diets were analyzed by DSM for Pro activity using a colorimetry assay. One Pro unit was defined as the amount of enzyme that released 1 μmol of p-nitroaniline from a 1 millimolar substrate (Suc-Sala-Ala-ProPhe-pNA) per minute at pH 9.0 and 37°C.

2.4.2. In-vitro ileal digestibility analysis

The in-vitro ileal digestibility of dry matter (DM), organic matter (OM), and CP of all diets was determined following a 2-step in-vitro incubation procedure adapted from Boisen and Fernandez (1995) and Akinsola (2013). The 8 diets were ground through a 0.5 mm screen in a centrifugal mill (ZM200, Retsch, Haan, Germany). Milled samples were incubated inside ANKOM F-57 nylon bags using a DAISY II incubator at 39˚C (Ankom, USA). Each sample was incubated in duplicate and each incubation consisted of 22 nylon bags per incubation. The first step, simulating the digestion in the stomach was an enzymatic hydrolysis with a pepsin solution at pH 2.0 and 39°C for 5h. This step was followed by hydrolysis with a multi-enzyme pancreatin (mixture of Pro, amylase, and lipase, from porcine pancreas; Sigma-Aldrich ref. P1750, Germany) at pH 6.8 and 39°C for 17h. After the incubation, the nylon bags with the residues were dried for 4 h at 103°C and the DM, CP and OM of the residues were analyzed to determine digestibility.

2.4.3. Mineral density analysis

Area bone mineral density (aBMD; g/cm^2) was measured using dual energy X-ray absorptiometry (DXA) with a Hologic QDR 4500 (Hologic, Bedford MA 01730, USA). The bones were scanned in a dorso-palmar position and the bone mineral density of the third metacarpal was analyzed using the spinal application as described by Ryan et al. (2011).

2.5. Statistical analysis

All data were analyzed using the MIXED procedure of SAS® software version 9.4 (SAS Institute, Inc., Cary, NC, US). Phytase, XB, Pro, and their 2 and 3-way interactions were included in the model as fixed effects. For final LW, ADG, ADFI, and G:F, initial LW was included as a covariate in the model and day was regarded as a repeated variable with pen as the experimental unit. For kill out percentage, muscle depth, fat depth, and lean meat percentage; carcass weight was included as a covariate in the model. A compound symmetry covariance structure was fitted to all data. Model suitability was investigated by checking normality of scaled residuals using the Shapiro-Wilk test within the UNIVARIATE procedure of SAS. Significance was reported for $P \leq 0.05$ and tendencies towards significance were reported for $P \leq 0.10$.

Results

The analyzed composition of diets and ingredients is presented in Tables 1 and 2, respectively. The experimental diets had a slightly lower content of CF (4.61 vs. 3.71 %) and a slightly greater content of ash (4.1 vs. 3.2 %) and oil (3.61 vs. 3.32 %) than the calculated values. According to product specifications at the dose

used, diets supplemented with Phy should provide 500 FTU/kg and the Phy activity analysis showed an average 531 FTU/kg feed in diets containing Phy (Table 1). Diets supplemented with XB should provide 2200 xylanase units/kg feed and the xylanase activity analysis showed an average 1,374 xylanase units/kg in diets containing XB (Table 1). Diets supplemented with Pro should provide 15,000 Pro units/kg feed and the Pro activity analysis showed on average 11,677 Pro units/kg in diets containing Pro (Table 1).

3.1. In-vitro ileal digestibility

The in-vitro ileal digestibility results are presented in Table 3. A 3-way interaction among Phy, XB, and Pro was observed (Table 3, $P = 0.08$). Diets with XB supplementation alone increased DM and OM in-vitro ileal digestibility and supplemented with a combination of enzymes tended to have greater DM and OM in-vitro ileal digestibility than the non-supplemented diets or diets supplemented only with Phy or Pro. Two-way interactions were not observed. The results of the in-vitro determination indicates that the main factors Phy, XB, and Pro increased in-vitro ileal nutrient digestibility (Table 3). The in-vitro ileal DM and OM digestibility was increased by Phy, XB, and Pro supplementation (Table 3). The in-vitro ileal CP digestibility was improved by XB and tended to be improved by Phy and Pro.

3.2. Growth performance, carcass quality, and bone mineral density

Live weight, ADG, ADFI, and G:F results are presented in Table 3. There was a tendency towards an interaction among Phy, XB, and Pro for ADG (Table 3, $P = 0.06$) and G:F ($P = 0.06$). Pigs fed the Phy, XB, and Phy + XB + Pro supplemented diets tended to have a

greater ADG than pigs fed non-supplemented diets, whereas the ADG of pigs fed diets with Pro or combinations of the two enzymes (Phy + XB, Phy + Pro, XB + Pro) were not different to that of pigs fed non-supplemented diets (data not shown). Pigs fed the Pro and Phy + XB + Pro tended to have better G:F than that of pigs fed non-supplemented diets whereas the G:F of pigs fed diets with Phy, XB or combinations of two enzymes were not different to that of pigs fed non-supplemented diets (data not shown). There was no difference among main factors or any 2-way interactions. Carcass weight, kill out percentage, muscle depth, fat depth, and lean meat percentage results are presented in Table 3. There was no difference among main factors, or any 2-way and 3-way interactions for any carcass quality measure (Table 3). Bone mineral density measured in the third metacarpal of the left front foot was not different (P = 0.89) for pigs fed the non-supplemented diet (0.52 ± 0.01 g/cm^2), the diet supplemented with Phy (0.52 ± 0.01 g/cm^2), and the diet supplemented with Phy and Pro (0.53 ± 0.01 g/cm^2).

Discussion

4.1. Enzyme supplementation to RSM and wDDGS-based diets

The success of improving feed efficiency in pigs by dietary feed enzyme supplementation is highly dependent on achieving a good match between the substrate in the diet and the enzyme used, and having a sufficient concentration of that substrate available in the diet (Adeola and Cowieson, 2011; Bedford and Schulze, 1998). The availability of co-products from biofuel production such as RSM and wDDGS has increased substantially in recent years, but in comparison with traditional ingredients (e.g. soya bean meal, wheat, corn and barley), these ingredients are richer in fiber and non-starch polysaccharides (Knudsen, 2014; Sauvant et al., 2004). They also have lower fiber and CP digestibility (Keady and

O'Doherty, 2000; Nanclares et al., 2017; Smit et al., 2014; Torres-Pitarch et al., 2014) than traditional ingredients. Therefore, carbohydrase and Pro supplementation to RSM- and wDDGS-based diets has the potential to improve the feeding value of diets based on these ingredients.

4.2. The use of in-vitro *ileal digestibility techniques to predict enzyme efficacy*

Despite some limitations due to factors not accounted for in the in-vitro models (microbiota degradation, digesta flow rate, diet density, etc.), in-vitro digestion techniques are an inexpensive and fast procedure to screen enzymes or enzyme combinations for their ability to degrade a specific substrate and to increase nutrient digestibility. The Boisen and Fernández (1995) technique and its version adapted to the Daisy II incubator (Akinsola, 2013) used in this study has previously shown good correlation (R^2=0.95) between in-vitro and in-vivo DM digestibility when different ingredients and pig diets were tested (Akinsola and Fushai, 2014; Pujol and Torrallardona, 2007). In the current experiment, in-vitro ileal digestibility of DM, OM, and CP was improved by Phy, XB, and Pro supplementation. A 3-way interaction among Phy, XB, and Pro was found for DM and OM digestibility, thereby indicating an additive effect. Some substrates (e.g. proteins) present in the plant cell wall that are not normally accessible by specific enzymes (e.g. Pro), might be liberated by the action of other enzymes (e.g. carbohydrases) and thereby made available for subsequent degradation by other enzymes. In agreement with our in-vitro results, Kong et al. (2015) observed increased in-vitro DM ileal digestibility when ingredients such as wheat, barley, and wDDGS were supplemented with an enzyme complex consisting of xylanase, Pro, and Phy. Kim et al. (2008) and Lyberg et al. (2008) observed additive effects

in pig growth when Phy and Xyl were supplemented together. O'Shea et al. (2014) observed additive effects on nutrient digestibility when Pro and Xyl were supplemented together in grower-finisher pigs. In a recent meta-analysis, Torres-Pitarch et al. (2018) reported that the most consistent results in nutrient digestibility, growth, and feed efficiency are achieved when multi-enzyme complexes are supplemented.

4.3. In-vivo results and correspondence with in-vitro ileal digestibility

Despite the improvements observed in-vitro, main effects or 2-way interactions were not observed in any variable measured in-vivo. This suggests that factors not accounted for by the in-vitro ileal digestion simulation (e.g. impact on gut microbes, nutrient absorption) affect the response to enzyme supplementation in-vivo. In agreement with our in-vivo results, when a combination of xylanase and Pro was supplemented to RSM- and wDDGS- based diets, no improvements in growth or feed efficiency were observed by O'Shea et al. (2014). Similarly, Xie et al. (2012) and Thacker (2001) observed no growth effects of carbohydrase and Pro supplementation to RSM based diets. Furthermore, when a combination of Phy and xylanase was supplemented to diets containing RSM, no improvements in growth were reported by Shim et al. (2003).

However, a tendency towards and interaction was observed for ADG and G:F. When supplemented alone, XB tended to increase the in-vitro ileal digestibility and increased ADG when supplemented in-vivo. In agreement with our results, xylanase alone or XB were previously shown to improve growth when supplemented to diets based on RSM (Fang et al., 2007a; Fang et al., 2007b) or wDDGS (Emiola et al., 2014). Phytase supplementation alone had no effect on the

in-vitro ileal digestibility of DM, OM, and CP, but tended to increase the daily gain of pigs in-vivo, possibly indicating that improvements in P and Ca digestibility rather than improvements in the digestibility of other nutrients were primarily responsible. The sparing effect of Phy was effective in the current in-vivo experiment. Diets with reduced P and Ca that were supplemented with Phy did not result in impaired growth, feed efficiency or bone mineral density. This agrees with the literature where Phy has been consistently shown to increase P digestibility and consequently replace inorganic P sources in pig diets (Selle and Ravindran, 2008; Torres-Pitarch et al., 2017; Varley et al., 2010). This gives us confidence in our experimental design and that any additional benefit to Phy inclusion observed here was additional to that of the P- and Ca-sparing effect expected with Phy. Prot supplementation alone failed to improve in-vitro ileal digestibility or increase growth in the in-vivo experiment.

The combination of Phy, XB, and Pro tended to increase the in-vitro ileal DM and OM digestibility and tended to improve G:F in pigs. This supports the results reported by Torres-Pitarch et al. (2017; 2018) in their systematic review and meta-analysis including 205 studies. These authors reported that cocktails of different enzymes give the most consistent results in improving feed efficiency in weaning and grower-finisher pigs. Omogbenigun et al. (2004) reported improved feed efficiency in piglets when diets were supplemented with a cocktail containing Phy, Pro, and XB enzymes. In addition, the additive effects of carbohydrases, Pro, and Phy have also been reported when supplemented in combination to poultry diets (Cowieson and Adeola, 2005).

Conclusion

Main effects or 2-way interactions were not observed when Phy, XB, and Pro were supplemented to pig diets in a factorial design experiment. A tendency towards a 3-way interaction shows that dietary supplementation with Phy, XB or a combination of Phy + XB + Pro tended to improve ADG and diet supplementation with Pro or a combination of Phy + XB + Pro tended to improve feed efficiency in pigs. There was no interaction observed between Phy and Pro for growth, feed efficiency and/or carcass quality, and Pro addition to a Phy supplemented diet did not reduce bone mineralization, indicating that the efficacy of Phy is not reduced when supplemented in combination with Pro.

Declaration of interests:

None.

Acknowledgments:

We thank T. Ryan, S.G Buzoianu and the technical/farm staff at the Pig Development Department at Teagasc, Moorepark, for assistance with pig management. Research leading to these results received funding from the European Union's Seventh Framework Programme (ECO-FCE project no. 311794) for research, technological development and demonstration. A. Torres-Pitarch and U.M. McCormak were funded by the Teagasc Walsh Fellowship programme. The exogenous enzymes (Rovabio Spiky, Ronozyme ProAct, Phyzyme 5000 XP TPT) were provided on request by ADISSEO (Commetry, France), DSM (Grenzach-Wyhlen, Germany) and DuPont-Danisco Animal Nutrition (Marlborough, United Kingdom), respectively. In particular, we wish to acknowledge the assistance of Pierre Cozannet (ADISSEO), Tom Hughes (DSM), and Angela

Riemensperger and Maria Walsh (DuPont-Danisco Animal Nutrition) for their help with the analysis of enzyme activities.

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Table 1

Ingredient composition of experimental diets (on DM basis)[a].

Item	Phy (mg/kg):	0	0	0	0	100	100	100	100
	XB (mg/kg):	0	0	100	100	0	0	100	100
	Pro (mg/kg):	0	200	0	200	0	200	0	200
Ingredient composition, g/kg									
Wheat		337.8	337.6	337.7	337.5	298.8	298.6	298.7	298.5
Barley		338.0	338.0	338.0	338.0	383.5	383.5	383.5	383.5
Wheat DDGS		200.0	200.0	200.0	200.0	199.4	199.4	199.4	199.4
Rapeseed		96.3	96.3	96.3	96.3	95.2	95.2	95.2	95.2
Soya oil		1.5	1.5	1.5	1.5	1.2	1.2	1.2	1.2
Limestone		10.3	10.3	10.3	10.3	10.0	10.0	10.0	10.0
L-lysine		4.9	4.9	4.9	4.9	4.8	4.8	4.8	4.8
L-threonine		1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.6
L-tryptophan		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt		1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.0
Premix[b]		4.30	4.30	4.30	4.30	4.30	4.30	4.30	4.30
Mono-dicalcium phosphate		0.42	0.42	0.42	0.42	-	-	-	-
Phytase[c]		-	-	-	-	0.1	0.1	0.1	0.1
Xylanase + β-Glucanase[d]		-	-	0.1	0.1	-	-	0.1	0.1
Protease[e]		-	0.2	-	0.2	-	0.2	-	0.2
Energy and nutrient composition									
Dry matter, g/kg		879.1	880.3	882.0	879.5	879.3	879.7	880.6	878.9
Crude protein, g/kg		174.4	173.7	173.6	174.3	173.7	174.2	174.4	173.9
AID Lys, g/kg[f]		7.90	7.90	7.90	7.90	7.90	7.90	7.90	7.90

Oil, g/kg	37.8	34.5	34.3	37.6	34.5	38.9	36.2	35.3
Net energy, MJ/Kg[f]	9.43	9.43	9.43	9.43	9.43	9.43	9.43	9.43
Ca, g/kg[e]	6.60	6.60	6.60	6.60	5.70	5.70	5.70	5.70
Av P, g/kg[f]	3.00	3.00	3.00	3.00	2.20	2.20	2.20	2.20
Ca:P, g/kg[e]	11.7	11.7	11.7	11.7	12.0	12.0	12.0	12.0
Crude fiber, g/kg	36.0	34.0	37.0	37.0	39.0	38.0	37.0	39.0
Acid detergent fiber, g/kg	55.4	53.0	55.7	55.7	59.9	52.0	47.5	53.9
Acid detergent lignin, g/kg	15.0	18.2	16.2	17.7	14.6	15.1	17.7	18.4
Ash, g/kg	41.8	42.4	42.8	42.7	39.9	39.6	39.5	40.5
Analyzed enzyme activity[g]								
Phytase	138	210	194	206	510	509	530	576
Xylanase	12	15	1,541	1,353	45	24	1,266	1,334
Protease	< DL	10,816	< DL	12,587	< DL	11,836	< DL	11,467

[a] Phy = Phytase, XB = xylanase and β-glucanase, Pro = Protease, AID = apparent ileal digestible, DDGS = distillers dried grains with solubles

[b] Premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate 0.7 mg; cholecalciferol, 12.5 µg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B_{12}, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10mg; vitamin B_1, 2 mg; vitamin B_6, 3 mg; and celite, 300 mg.

[c] Phytase (Phyzyme 5000 XP TPT, Danisco Animal Nutrition, Marlborough, United Kingdom) providing 5,000 phytase units (FTU)/g of product.

[d] Xylanase and β-glucanase (Rovabio Spiky, Adisseo France, Commetry, France) providing 22,000 xylanase units/g of product and 15,200 β-glucanase units/g of product.

[e] Protease (Ronozyme ProAct, DSM Nutritional Products, Grenzach-Wyhlen, Germany) providing 75,000 protease units/g of product.

[f] Calculated values

[g] Xylanase activity in units/kg feed and measured as visco-units of endo-1,4-β-xylanase (amount of enzyme reducing the viscosity of the solution, to give a change in relative fluidity of 1 dimensionless unit per minute per g at pH 5.5 and 30°C). Phytase activity in FTU/kg feed

measured as FTU (amount of enzyme that liberates 1 μmol inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37ºC). Protease activity in units/kg feed and measured as protease units (amount of enzyme that releases 1 μmol of p-nitroaniline from a 1 millimolar substrate (Suc-Sala-Ala-ProPhe-pNA) per minute at pH 9.0 and 37°C). DL = Detection limit.

Table 2

Analyzed nutrient composition of ingredients used in the experimental diets (g/kg, as-fed basis).

Item	Wheat	Barley	Rapeseed meal	Wheat DDGS[a]
Dry matter	865	861	884	886
Crude protein	102	92	334	311
Fat	15	15	29	39
Crude fiber	30	45	106	62
Ash	16	20	73	49

[a] DDGS = distillers dried grains with solubles

Table 3

Effect of phytase (Phy), xylanase + β-glucanase complex (XB) and protease (Pro) dietary supplementation alone or in combination on in-vitro ileal digestibility of feed, pig growth and carcass quality of grower-finisher pigs[a]

	Phy (mg/kg)		XB (mg/kg)		Pro (mg/kg)		SEM	P-values						
Item	0	100	0	100	0	200		Phy	XB	Pro	Phy*XB	Phy*Pro	XB*Pro	Phy*XB*Pro
In-vitro digestibility														
Dry matter, %	73.1	75.4	71.6	77.0	73.2	75.4	0.6	0.03	0.01	0.04	0.74	0.68	0.62	0.01
Organic matter, %	73.6	76.0	72.0	77.6	73.7	75.9	0.6	0.02	0.01	0.04	0.84	0.69	0.65	0.01
Crude protein, %	85.4	87.1	84.3	88.3	85.4	87.2	0.5	0.07	0.02	0.06	0.80	0.81	0.38	0.04
Pig growth														
Initial LW, kg	40.1	40.0	39.9	40.0	40.1	40.0	0.9	0.65	0.88	0.74	0.61	0.55	0.77	0.73
Final LW, kg	10	10	10	10	10	10	1.1	0.	0.	0.	0.50	0.40	0.66	0.10

	7.0	7.0	7.0	7.3	7.0	7.0		9.8	5.2	4.1				
	1,04	1,05	1,04	1,05	1,04	1,04		0.7	0.8	0.9				
ADG, g/day	6	2	7	0	8	9	11	1	6	6	0.59	0.68	0.84	0.06
	2,86	2,84	2,85	2,86	2,85	2,85		0.4	0.7	0.8				
ADFI, g/day	7	5	3	0	9	4	19	0	9	5	0.22	0.14	0.42	0.36
	0.37	0.37	0.37	0.37	0.37	0.37	0.1	0.4	0.9	0.8				
G:F, g/g								5		4	0.35	0.61	0.94	0.06
Carcass quality														
								0.2	0.9	0.8				
Carcass weight, kg	88.6	87.5	88.0	88.1	88.0	88.1	0.7	9	7	8	0.51	0.70	0.86	0.60
	77.0	76.6	76.8	76.8	76.9	76.4		0.2	0.7	0.6				
Kill out, %							0.3				0.30	0.51	0.38	0.49
	54.6	55.9	55.3	55.2	55.8	54.7		0.1	0.9	0.9				
Muscle depth, mm							0.6	2	6	9	0.45	0.80	0.37	0.97
	13.0	13.0	13.0	13.0	12.9	13.0		0.8	0.6	0.6				
Fat depth, mm							0.2	9	8	3	0.32	0.70	0.41	0.64
	57.4	57.5	57.3	57.6	57.6	57.4		0.9	0.5	0.6				
Lean meat, %							0.3				0.63	0.82	0.91	0.90

1 0 3

[a] SEM = Standard error mean, ADG = Average daily gain, ADFI = Average daily feed intake, G:F = Gain to feed ratio.