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Authors: A. Torres-Pitarch, E.G. Manzanilla, G.E. Gardiner, J.V. O'Doherty, P.G. Lawlor

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Systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: effect of enzyme type and cereal source

A. Torres-Pitarch[1,2], E.G. Manzanilla[1,3] , G.E. Gardiner[4], J.V. O'Doherty[2], P.G. Lawlor[1]

[1]*Teagasc, Pig Development Department, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co Cork, Ireland.*

[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

[3]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.*

[4]*Department of Science, Waterford Institute of Technology, Waterford, Ireland.*

Corresponding author: Peadar Lawlor, Teagasc, Pig Development Department, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co Cork, P61 C996, Ireland. Phone: +353 (0)25 42217 (Peadar.lawlor@teagasc.ie)

Highlights:

- Dietary supplementation with enzymes increased nutrient digestibility in pigs
- Mannanase supplementation improved feed efficiency in pigs
- Multi-enzyme supplementation improved feed efficiency in pigs
- The cereal source used in the diet influences the response to feed enzymes

ABSTRACT:

Dietary supplementation of pig diets with exogenous enzymes has been suggested as a strategy to increase nutrient digestibility and improve feed efficiency in grow-finisher pigs. However, inconsistent results are found in the literature. Ingredient composition of the diets is one of the most important sources of variation that may affect enzyme efficacy and consistency of results. A systematic review and a meta-analysis was therefore conducted to determine which exogenous enzymes with which diet type most consistently improve pig growth, nutrient digestibility and feed efficiency. Enzyme type and dietary cereal source were the main explanatory variables included in the models. The mean difference effects of enzyme supplementation on average daily gain (ADG), average daily feed intake (ADFI), gain to feed (G:F), apparent ileal digestibility (AiD) and apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), and gross energy (GE) were calculated for each study and these were used as the effect size estimates in the meta-analysis. A dataset with 139 comparisons from 67 peer-reviewed publications was used in the meta-analysis. In response to enzyme supplementation, G:F was improved in 38 of the 120 comparisons reporting pig growth data, remained un-changed in 78 and deteriorated in 4. Overall, DM and GE AiD and ATTD were improved by xylanase, xylanase and β-glucanase, mannanase and protease dietary supplementation ($P<0.05$). Crude protein AiD was only improved by protease dietary supplementation ($P<0.001$). Dietary supplementation with xylanase alone improved ADG of maize- ($P<0.05$) and co-product- ($P<0.05$) based diets but had no effect on the G:F of grow-finisher pigs. Dietary supplementation with xylanase + β-glucanase had no effect on ADG, ADFI and G:F. Protease supplementation tended to improve the ADG of co-product- ($P=0.08$) based diets but had no effect on the G:F of grow-finisher pigs. Dietary supplementation with multi-enzyme complexes improved the ADG ($P<0.05$) and G:F ($P<0.01$) of maize-, wheat-, barley- and co-product-based diets. In conclusion, dietary supplementation with all enzyme types improved nutrient digestibility depending on ingredient content, while mannanase and multi-enzyme complex supplementation most consistently improved growth and feed efficiency.

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; AiD, apparent ileal digestibility; ATTD, apparent total tract digestibility; CP, crude protein; DDGS, distillers dried grains with solubles; DM, dry matter; GE, gross energy; G:F, gain to feed ratio; RSM, rapeseed meal,

Keywords: carbohydrases; mannanase; protease; swine; xylanase; β-glucanase.

1. Introduction

Nutrient digestibility and feed efficiency in pigs can be increased by supplementation with exogenous feed enzymes (Bedford and Schulze, 1998; Kiarie et al., 2013). With feed representing ~72% of the total cost of producing pigs (Teagasc, 2016) and pigs being unable to utilize all dietary components, strategies to improve feed efficiency are of particular interest as a means of increasing environmental as well as economic sustainability (Aarnink and Verstegen, 2007; Clark and Tilman, 2017). Feed enzymes are substrate-specific. They target specific chemical bonds present in the undigestible components of feed ingredients, normally plant materials, converting them into substrates that can be digested by the pig (Adeola and Cowieson, 2011). Phytase is the most widely used feed enzyme. It degrades phytic P naturally present in plant materials, increasing P digestibility and reducing the necessity to use expensive inorganic P in diets (Campbell and Bedford, 1992; Dersjant-Li et al., 2015; Humer et al., 2015). After phytase, carbohydrases and proteases are the two enzyme groups most commonly used in monogastric diets (Adeola and Cowieson, 2011; Cowieson and Roos, 2016). In-feed supplementation of carbohydrases (i.e. xylanase, β-glucanase, β-mannanase, α-galactosidase, cellulase, amylase) can increase the digestibility of substrates present in the non-starch polysaccharide (NSP) fraction of the diet such as arabinoxylans, glucans, mannans or galactans among others (Bedford and Schulze, 1998; Masey O'Neill et al., 2014). Plant-based diets are rich in NSPs that are poorly digested by the pig's endogenous enzymes but the amount and type of NSPs vary by plant species (McDonald et al., 1999; Högberg and Lindberg, 2006). Protease may improve the digestibility of amino acids and it has been tested alone as well as part of enzyme complexes (Cowieson and Roos, 2016). However, the *in-vivo* response to dietary enzyme supplementation is inconsistent in grow-finisher pigs. Nutrient digestibility and growth was increased and feed efficiency improved in some studies (Barrera et al., 2004; Woyengo et al., 2008; Emiola et al., 2009; Ndou et al., 2015; Upadhaya et al., 2016a), whereas no beneficial effect of enzyme supplementation was found in others (Cervantes et al., 2001; Willamil et al., 2012). A systematic review and meta-analysis where the overall responses to carbohydrase and protease enzyme supplementation are summarised and factors influencing the direction and magnitude of responses are investigated can be particularly instructive. Phytase supplementation to pig diets is widely used and the economic and environmental benefits associated to their use have already been well proven. Therefore, phytase will not be further investigated in this study.

Feed for grow-finisher pigs is mainly manufactured as a mix of plant material (i.e. soybean meal and cereals), a fat source (i.e. soya oil and tallow), synthetic amino acids and a vitamin and mineral premix. Traditionally, wheat and barley are the most widely used cereals for pig diets in Europe; however, depending on availability and volatility of price, the range of plant materials used as feed ingredients is much wider. Maize, drought-adapted cereals (i.e. sorghum and rye) and by- and co-products from the biofuel industry (i.e. distiller dried grains with soluble [DDGS] and rapeseed meal [RSM]) and the milling industry (i.e. wheat bran, pollard) are available for use in pig diets. Due to this potential for substitution of ingredients to produce least-cost diets, the presence and concentration of potential substrates for exogenous enzymes can vary widely from diet to diet. The objective of this systematic review and meta-analysis was to determine which exogenous enzymes are most consistent in improving feed efficiency in grow-finisher pigs and with which cereal source. It was hypothesized that the type of enzyme supplemented, and the cereal source used in the diet during supplementation would influence the nutrient digestibility, growth and feed efficiency response to in-feed enzyme supplementation.

2. Material and Methods

A systematic literature review was conducted using peer-reviewed publications compiled from the on-line database Web of Science™. Several searches were performed in November 2017 to find the publications relevant to the following enzymes: xylanase, β-glucanase, α-amylase, mannanase, α-galactosidase, cellulase and protease. The keywords used to perform each search were: "name of the enzyme" and "growth" and "pig". The on-line database contained publications from 1987 on, and the search constrained results from patents and publications not written in English. Once all publications were collected, only those fulfilling the following selection criteria were retained: a) *in-vivo* swine studies including a control treatment group with the same dietary composition as the treatment diet that did not receive an exogenous enzyme, b) published in English, c) report growth performance results [average daily gain (ADG, g/day), average daily feed intake (ADFI, g/day), feed to gain or feed conversion efficiency or gain to feed ratio (G:F)] d) report sample variance (SD or SEM), sample size (n), age, sex of pigs and duration of the study. All feed efficiency metrics recorded were converted to G:F so that feed efficiency could be compared between experiments. Each study was assigned a publication number according to the peer-reviewed publication from which the information was extracted. For each study two categorical variables were created in the dataset to describe the enzyme type supplemented to the diet and the main cereal source used in the diet formulation. If a diet contained >35% of a specific cereal (maize, wheat, barley, rye or sorghum), it was assigned to that cereal type category. Where diets did not contain >35% of a specific cereal they were assigned to the category "co-products". Where a number of enzyme inclusion rates were used in individual studies, only the data relating to the highest of these were included in the meta-analysis so as to avoid an overweighting of that particular enzyme/study in the meta-analysis. The dose-response effect is summarised and discussed independently from the meta-analysis. The retained publications were used in the meta-analysis to summarize the effect size of enzyme supplementation on ADG, ADFI, G:F, apparent total tract digestibility (ATTD) and apparent ileal digestibility (AiD) of dry matter (DM), crude protein (CP) and gross energy (GE). The metafor package in R (R Core Team, 2015) was used to conduct the meta-analysis (Viechtbauer, 2010) and to construct forest plots. Figures summarizing the forest plots were constructed with the ggplot package in R and are presented in the manuscript. The complete set of forest plots is given on-line in a PDF file as supplementary material. The independent variables (y) included in the linear mixed models of the meta-analysis were: ADG, ADFI, G:F, DM ATTD, GE ATTD, CP ATTD, DM AiD, CP AiD and GE AiD. Mean difference (MD) was the effect size, calculated by subtracting the mean of the control group (CON) from the respective enzyme supplemented group (ENZ) following a similar methodology to Bougouin et al. (2014) and according to the formula:

$$MD_y = y_{ENZ} - y_{CON}$$

The pooled SEM of each study was considered for standardization and weighting of the different comparisons. The linear mixed model used included the interaction between two categorical explanatory variables as described in the following formula:

$$MD_{Estimate} = \mu + x_i z_j + u + e$$

The first explanatory variable was enzyme type (x) and comprised i categories: 1) xylanase, 2) xylanase+β-glucanase, 3) mannanase, 4) protease and 5) multi-enzymes complex. The second explanatory variable (z) included in the model was the main cereal source used in the diet and comprised i categories: 1) maize, 2) wheat, 3) barley, 4) sorghum, 5) rye and 6) co-product sources. Publication number was included as a random effect in all models (u) and the error term (e) was also included in the model. Forest plots were constructed to show the MD effect size estimate and its confidence intervals. Studies in the forest plot are presented in sub-groups according to the individual enzyme or enzyme complex supplemented and the main cereal source used in the test diets. Funnel plots were constructed to assess publication bias according to Viachtbauer (2010) and symmetric plots were observed for all models.

3. Results

3.1 Systematic review

A total of 560 publications were retrieved from the search and, after deletion of duplicates and articles not fulfilling the meta-analysis selection criteria, 139 comparisons from 67 peer-reviewed publications were included in the dataset to study the effect of dietary supplementation with exogenous enzymes on pig growth, feed efficiency and nutrient digestibility. The number of studies excluded as a result of not fulfilling the selection criteria and the reason for exclusion are as follows: 124 studies did not test enzyme supplementation in feed, 113 studies were performed in weaned pigs, 79 studies were performed in other animal species (mainly poultry), 66 studies were not *in-vivo* trials, 55 studies were duplicates in the dataset, 44 studies were not written in English and 12 studies did not provide enough details or statistical data. The amount of comparisons found in the peer-reviewed publications for each of the variables of interest and for each enzyme type is shown in Table 1. A higher number of comparisons were found for growth and ATTD compared to AiD and the enzymes with the highest number of comparisons reported were multi-enzyme complexes and xylanase (Table 1). Supplementation of protease to grow-finisher diets was reported least (14 comparisons from

7 peer-reviewed publications). The inclusion percentage of ingredients in the experimental diets is presented in Table 2. Thirty-four comparisons examined dietary supplementation of enzyme complexes containing various combinations of enzymes. The enzymes included in each complex are listed in Table 3.

3.2 Effect of feed enzymes on growth, feed intake and feed efficiency

From a total of 120 comparisons, 38 found a positive effect, 78 no improvement and 4 a negative effect on G:F when feed enzymes were supplemented to grow-finisher diets (Fig. 1 and 2). Table 4 summarises the MD estimate effects for ADG, ADFI and G:F in response to enzyme supplementation. Overall, ADG was improved by mannanase, protease and multi-enzyme complex supplementation and G:F was improved by mannanase and multi-enzyme complex supplementation to grow-finisher pig diets. The efficacy of each enzyme differs depending on the main cereal component in the diet; xylanase supplementation to maize- and co-product-based diets improved ADG and supplementation of multi-enzyme complexes to maize-, wheat-, barley- and co-product-based diets improved ADG and G:F.

3.3 Effect of feed enzymes on ATTD digestibility

Table 5 summarises MD estimate effects for ATTD of DM, CP and GE in response to enzyme supplementation. Overall, ATTD of DM was improved in response to xylanase, xylanase + β-glucanase, mannanase, and protease supplementation; ATTD of CP and GE were improved when xylanase, xylanase + β-glucanase, mannanase and protease were supplemented. The efficacy of each enzyme differed depending on the cereal source used in the diet formulation. For instance, DM, CP and GE ATTD was improved by multi-enzyme complex supplementation to maize- and wheat-based diets, but not when supplemented to barley-, rye-, sorghum- and co-product-based diets.

3.4 Effect of feed enzymes on AiD digestibility

Table 6 summarises the MD estimate effects for AiD of DM, CP and GE in response to enzyme supplementation. Overall, AiD of DM was improved by mannanase, protease and multi-enzyme complex supplementation; AiD of CP was only improved when protease was supplemented to pig diets and AiD of GE was improved by xylanase, xylanase + β-glucanase, and protease dietary supplementation. The response to enzyme supplementation differed depending on enzyme type and the cereal source used in the diet formulation. For instance, xylanase improved AiD of DM when supplemented to wheat-based diets but not when supplemented to maize- or rye-based diets.

4. Discussion

The number of studies investigating the individual supplementation of NSP-degrading enzymes (xylanase, xylanase + β-glucanase and mannanase) and multi-enzyme complex preparations was greater than the number of studies investigating protease supplementation. Regarding the variables studied, growth and ATTD data were reported in most of the studies but only a small number of studies reported AiD data. Therefore, the estimates calculated for protease, especially for AiD data must be treated with caution as they are based on a relatively low number of observations.

4.1 Xylanase and xylanase + β-glucanase complex

Xylanase alone or in combination with β-glucanase is the enzyme that has been most studied in the literature to date. The results of the meta-analysis indicate that xylanase improves AiD and ATTD of GE when supplemented to wheat- and maize-based diets. Xylanase supplementation also improved the ATTD of DM and CP, and AiD of DM when supplemented to wheat-based diets. Xylanase degrades the arabinoxylans present in the outer fraction of the cereal grain (Bedford and Schulze, 1998; Huntley and Patience, 2018). The concentration of arabinoxylans in wheat (7.3%) is higher than in maize grains (3.8 - 4.7%; Knudsen, 2014). Therefore, the nutrient digestibility response was, as expected, more pronounced in wheat- (+1.1% DM ATTD, +1.4% CP ATTD, +1.1% GE ATTD, +2.3% DM AiD, +3.6% GE AiD) than in maize-based diets (+1.0% DM ATTD, +1.0% GE ATTD, +3.0% GE AiD). However, unexpectedly, xylanase supplementation improved ADG when supplemented to maize-based diets but not when supplemented to wheat-based diets. Although rye grains are also rich in arabinoxylans (9.5%; Knudsen, 2014), few experiments with rye-based diets reported the AiD of DM and CP, and as a consequence no improvements in nutrient digestibility were found in the meta-analysis. With an arabinoxylan concentration of 8.4% (Knudsen, 2014) barley is a potential substrate for xylanase; however, studies with individual xylanase supplementation to barley-based diets were not found in the literature. Since barley is also rich in β-glucans (5%; Knudsen, 2014), research with barley-based diets has been more focused on combined xylanase + β-glucanase supplementation. However, supplementation with xylanase + β-glucanase had limited success in improving nutrient digestibility in barley-based diets. When supplemented to co-product-based diets, xylanase + β-glucanase increased AiD of GE (+15.1%) and ATTD of DM (+4.3%), CP (+4.8%) and GE (+4.4%). In this instance co-product-based diets were mainly based on wheat-DDGS, corn-DDGS and/or RSM. The arabinoxylan content of RSM is 6% (Knudsen, 2014) and the arabinoxylan content in DDGS, while more concentrated in the DDGS, depends on the particular cereal used for biofuel production co-product (Jaworski et al., 2015).

Despite the multiple improvements found in terms of nutrient digestibility when xylanase or the xylanase + β-glucanase complex were supplemented to pig diets, this was not reflected in significant improvements in G:F. Therefore, from the results of this meta-analysis, it appears that the arabinose, xylose and/or glucans released by xylanase and xylanase + β-glucanase are inefficiently used by the pig. It is well proven in the literature that xylose, arabinose and glucans disappear in the small intestine of monogastric animals (Schutte et al., 1991; Yule and Fuller, 1992; Knudsen and Jorgensen, 2007). However, as summarized by the review of Huntley and Patience (2018) the metabolization of xylose through oxidative pathways is very inefficient in pigs. When pure xylose and/or arabinose is supplemented to pig diets, a high proportion of that absorbed is excreted in the urine (Wise et al., 1954; Yule and Fuller, 1992). The health promoting benefits of β-glucans in monogastrics are well known (Ewaschuk et al., 2012; Laerke et al., 2014), however, their contribution to energy balance upstream of the large intestine has not been well investigated. Products released by xylanase and xylanase + β-glucanase can also contribute to the energy balance of pigs through the absorption of short chain fatty acids produced during microbial fermentation in the large intestine. There is evidence that xylanase and xylanase + β-glucanase supplementation can influence the microbial composition within the gastrointestinal tract of pigs (O'Connell et al., 2005; Reilly et al., 2010; Lan et al., 2017); however, the microbial species that can most efficiently use xylose, arabinose and glucans are unknown. Therefore, the basal intestinal microbial composition of the pigs in each experiment is likely another source of the variability in feed efficiency observed. Other factors that might that may explain the inconsistency in effect on G:F in response to these enzymes are: a) variability in arabinoxylan composition of the cereal sources and b) variability in management and high health conditions between experiments. Arabinoxylan composition of individual cereals is high and depends on cereal quality, harvest time and conditions, level and type of impurities etc. In a recent study, Clarke et al. (2018) observed a positive response to xylanase + β-glucanase when it was supplemented to a diet based on low quality barley (higher crude fibre content but a lower content of β-glucans) but no response was found when supplemented to a diet based on high quality barley. Therefore, studies performed with similar ingredient composition could potentially have a very different concentrations of substrate to be degraded by the enzymes thereby explaining the inconsistent results found. Likewise, *in-vivo* experiments in research facilities are often performed under good management and high health conditions, allowing pigs to grow to their maximum potential which leaves little scope for improvement due to enzyme supplementation.

4.3 Mannanase

The results of the meta-analysis indicate that mannanase supplementation to maize-based pig diets can increase nutrient digestibility (+3.1% DM AiD, +0.8% DM ATTD, +1.0% CP ATTD and +1.0% GE ATTD) and increase ADG (+19.4 g/day) and feed efficiency (+0.7% G:F).. Despite the positive effects found here with mannanase, its supplementation alone is not commonly practiced and as such this deserves more attention in the future. Mannanase degrades the galactomannans in mono-oligosaccharides. Galactomannans are present as a reservoir polysaccharide in the cell walls of legumes and palm seed (Gidley and Reid, 2006; Buckeridge, 2010). The principal source of galactomannans in pig diets is the soya bean meal with a galactomannan content of up to 2% (Hsiao et al., 2006). Ten peer-reviewed publications included in this meta-analysis investigated the effect of mannanase supplementation to grow-finisher pigs. The content of soybean meal in the respective experimental diets varied between 20 and 42%. Two studies also included palm kernel as a dietary ingredient (Kim et al., 2013a; Mok et al., 2015). Very consistent responses to mannanase supplementation were found in this meta-analysis. All diets to which mannanase was supplemented were formulated with maize as the main cereal.. The use of mannanase in diets based on other cereals has not yet been investigated and research with diets based on other type of cereals is needed.

4.4 Protease

The results of the current meta-analysis indicate that protease supplementation to grow-finisher pig diets can increase nutrient digestibility and growth; however, the number of peer-reviewed publications in the meta-analysis was low and consequently results should be treated with caution. For example, the MD estimates for protease supplementation to co-product- and barley-based diets relies on one publication for each, however, this is the best estimate that can currently be determined with the available data. When diets were formulated with maize, the AiD and ATTD of DM, GE and CP were improved due to protease supplementation; however, no improvements in G:F were found. On the other hand, when protease was supplemented to diets formulated with co-products, ATTD was unchanged but ADG (+68.1 g/day) tended to increase. The variability in results due to protease supplementation could also be due to differences in amino acid digestibility. Two meta-analyses using collated data from poultry and pigs previously reported improvements in the apparent ileal digestibility of amino acids in response to supplementation with different sources of protease (Cowieson and Roos, 2014; Lee et al., 2018). Both of these studies used a merged dataset for poultry and pigs at all growing stages and consequently the specific effect of protease in grow-finisher pigs cannot be extrapolated. Lee et al. (2018) also summarized the MD estimate effect for growth in response to protease supplementation to pig diets using a dataset (mixture of published and unpublished internal data) including all growth stages of pigs. They found no improvements in ADG, ADFI or G:F; however, the specific effect of protease in grow-finisher pigs cannot be determined from this study either. In comparison to our dataset, Lee et al. (2018) included data for protease supplementation to grow-finisher pig diets from only 5 peer-reviewed publications (O'Doherty and Forde, 1999; O'Shea et al., 2014; Pan et al., 2016; Upadhaya et al., 2016b; Pan et al., 2017) compared with the 11 peer-reviewed publications included in our meta-analysis. The latter highlights the importance of performing a structured systematic review prior to dataset compilation when conducting a meta-analysis.

4.5 Enzyme complexes

Dietary supplementation with multi-enzyme complexes had the most consistent effect in terms of improving nutrient digestibility and feed efficiency in grow-finisher pigs. The results from this meta-analysis indicate that G:F was improved when a multi-enzyme complex was supplemented to maize- (+1.9%), wheat- (+2.1%), barley- (+2.1%) and co-product- (+2.5%) based diets. The multi-enzyme complexes used in the experiments had combinations of 2, 3 or 4 different enzymes and the enzyme composition of the complexes varied between experiments, comprising phytase, cellulase, xylanase, β-glucanase, protease, mannanse, α-galactosidase, and α-amylase. It can be speculated from the results of this meta-analysis that synergies exist between enzymes and beneficial additive effects can be observed when enzymes are supplemented together. In a previous meta-analysis of enzyme supplementation to weaner pig diets, multi-enzyme supplementation also consistently increased G:F of piglets (Torres-Pitarch et al., 2017). However, as more than one enzyme is included in the product, the contribution of each individual component and their additivity cannot be separated in most of the experimental designs used. Additive improvements to ADG (Lyberg et al., 2008) and G:F (Kim et al., 2008) have been found when phytase and xylanase were supplemented together; however, other studies found no additive effect on G:F (Olukosi et al., 2007; Woyengo et al., 2008). Mok et al. (2013) found no additive effects when mannanase and phytase were supplemented to grow-finisher pig diets. O'Shea et al. (2014) found an additive response from xylanase and protease for AiD of GE in grow-finisher pigs, but none was observed for G:F. More studies in which enzymes are supplemented both individually and in combination are needed to determine the additive effect of enzyme supplementation in grow-finisher diets.

4.6 Dose effect on response to enzyme supplementation

Exogenous enzymes are usually supplemented to pig diets at the manufacturer's recommended inclusion level. The recommended dose of a commercial enzyme product is based on the purity of the product (enzyme activity) and cost-benefit estimations. It was not possible to include the concentration of enzyme activity as a variable in the meta-analysis for several reasons: activity of enzyme products is measured under different conditions, often expressed in different units and sometimes the activities recovered in the feed are not even reported in publications. In the current systematic review and meta-analysis, 8 publications used more than one dose when testing the effect of exogenous enzyme supplementation. In general, positive linear growth and nutrient digestibility responses were found with increasing dietary enzyme inclusion rates in grow-finisher pigs. Positive linear increases in ADG and G:F were found when increasing doses of xylanase were supplemented to wheat-based diets (Barrera et al., 2004; Yang et al., 2017) and RSM-based diets (Fang et al., 2007a; Fang et al., 2007b). Barrera et al. (2004) also found a positive linear response for AiD of CP when increasing doses of xylanase were supplemented to a wheat-based diet. Woyengo et al. (2008) found no effect of xylanase supplementation at any of the two doses they supplemented to wheat-based diets. Increasing the dietary inclusion of mannanase resulted in a positive linear response for ADG, G:F, ATTD of GE and ATTD of CP when supplemented to maize-based diets (Yoon et al., 2010; Kim et al., 2017). No effect was found when increasing doses of enzyme complexes were supplemented to maize- (Ao et al., 2010) or RSM-based diets (Fang et al., 2007a). Contrary to this, Fang et al. (2007b) found an increased ADG response when a higher dose of a multi-enzyme complex was supplemented to a RSM-based diet.

5. Conclusions

Dietary supplementation with mannanase, and multi-enzyme complexes increased growth and feed efficiency in grow-finisher pigs. Despite the improvements found in nutrient digestibility in response to xylanase or xylanase + β-glucanase supplementation, they did not improve feed efficiency in grow-finisher pigs. The response to enzyme supplementation is influenced by the main cereal source used in the diet formulation. Dietary supplementation with mannanase increased feed efficiency with maize-based diets and dietary supplementation with multi-enzyme complexes improved feed efficiency when maize-, wheat-, barley- and co-product-based diets were fed to grow-finisher pigs.

6. Aknowledgments

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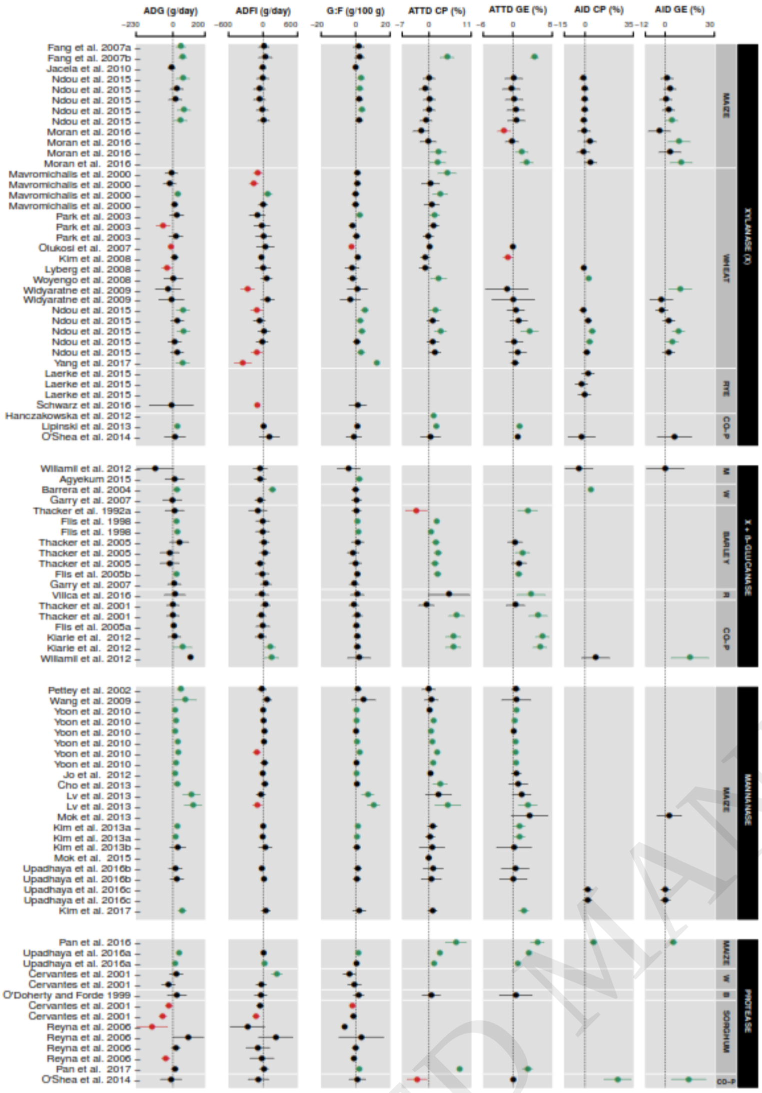
Figure 1. Forest plots showing mean difference effect of xylanase (X), xylanase and β-glucanase, mannanase and protease supplementation on average daily gain (ADG), average daily feed intake (ADFI), gain to feed (G:F), apparent total tract digestibility (ATTD) of gross energy (GE) and crude protein (CP), and apparent ileal digestibility of GE and CP. M = maize, W = wheat , B = Barley, CO-P = co-products. Green dot (•) indicates significantly increased, red dot (•) indicates significantly reduced, black dot (•) indicates not significant, straight horizontal lines indicate the confidence interval.


ADG (g/day)
ADFI (g/day)
G:F (g/100 g)
ATTD CP (%)
ATTD GE (%)
AID CP (%)
AID GE (%)
−230
0
200
−600
0
600
−20
0
20
−7
0
11
−6
0
8
−15
0
35
−12
0
30
Fang et al. 2007a
Fang et al. 2007b
Jacela et al. 2010
Ndou et al. 2015
Ndou et al. 2015
Ndou et al. 2015
Ndou et al. 2015
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Moran et al. 2016
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Mavromichalis et al. 2000
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Park et al. 2003
Park et al. 2003
Park et al. 2003
Olukosi et al. 2007
Kim et al. 2008
Lyberg et al. 2008
Woyengo et al. 2008
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Ndou et al. 2015
Yang et al. 2017
Laerke et al. 2015
Laerke et al. 2015
Laerke et al. 2015
Schwarz et al. 2016
Hanczakowska et al. 2012
Lipinski et al. 2013
O'Shea et al. 2014
MAIZE
WHEAT
RYE
CO-P
XYLANASE (X)
Willamil et al. 2012
Agyekum 2015
Barrera et al. 2004
Garry et al. 2007
Thacker et al. 1992a
Fils et al. 1998
Fils et al. 1998
Thacker et al. 2005
Thacker et al. 2005
Thacker et al. 2005
Fils et al. 2005b
Garry et al. 2007
Villca et al. 2016
Thacker et al. 2001
Thacker et al. 2001
Fils et al. 2005a
Kiarie et al. 2012
Kiarie et al. 2012
Willamil et al. 2012
M
W
BARLEY
R
CO-P
X + β-GLUCANASE
Pettey et al. 2002
Wang et al. 2009
Yoon et al. 2010
Yoon et al. 2010
Yoon et al. 2010
Yoon et al. 2010
Yoon et al. 2010
Yoon et al. 2010
Jo et al. 2012
Cho et al. 2013
Lv et al. 2013
Lv et al. 2013
Mok et al. 2013
Kim et al. 2013a
Kim et al. 2013a
Kim et al. 2013b
Mok et al. 2015
Upadhaya et al. 2016b
Upadhaya et al. 2016b
Upadhaya et al. 2016c
Upadhaya et al. 2016c
Kim et al. 2017
MAIZE
MANNANASE
Pan et al. 2016
Upadhaya et al. 2016a
Upadhaya et al. 2016a
Cervantes et al. 2001
Cervantes et al. 2001
O'Doherty and Forde 1999
Cervantes et al. 2001
Cervantes et al. 2001
Reyna et al. 2006
Reyna et al. 2006
Reyna et al. 2006
Reyna et al. 2006
Pan et al. 2017
O'Shea et al. 2014
MAIZE
W
B
SORGHUM
CO-P
PROTEASE

Figure 2. Forest plots showing mean difference effect and confidence interval of multi-enzyme complex supplementation on average daily gain (ADG), average daily feed intake (ADFI), gain to feed (G:F), apparent total tract digestibility (ATTD) of gross energy (GE) and crude protein (CP), and apparent ileal digestibility of gross energy and GE. M = maize, W = wheat, B = barley, S = sorghum, CO-P = co-products. Green dot (•) indicates significantly increased, red dot (•) indicates significantly reduced, black dot (•) indicates not significant, straight horizontal lines indicate the confidence interval.

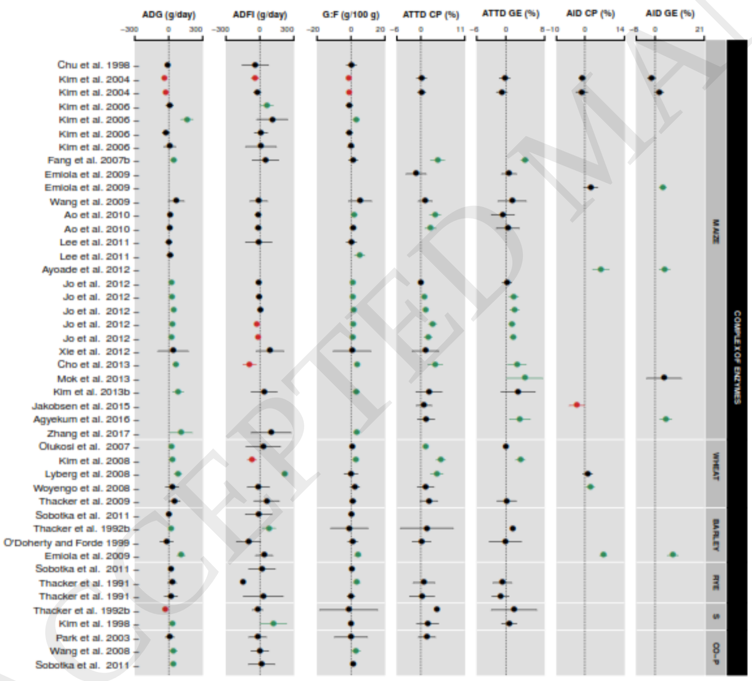

Table 1. Number of comparisons reporting each variable of interest in the dataset used to perform the meta-analysis.

	Growth performance[1]			**ATTD**[2]			**AiD**[3]		
	ADG	ADFI	G:F	DM	CP	GE	DM	CP	GE
Total number of comparisons	120	120	120	81	96	82	29	36	32
Comparisons by enzyme type[4]									
Xyl	30	30	30	22	29	22	14	20	17
XB	19	19	19	9	12	10	2	3	2
Mann	18	18	18	20	19	19	3	2	3
Prot	12	12	12	3	5	5	1	2	2
Cplex	40	40	40	26	30	26	9	9	8
Comparisons by cereal source									
Maize	52	52	52	46	49	48	17	18	20
Wheat	29	29	29	17	21	13	7	10	7
Barley	13	13	13	5	10	8	1	1	1
Rye	6	6	6	4	4	4	3	3	0
Sorghum	9	9	9	3	3	2	0	0	0
Co-products	11	11	11	6	8	7	1	3	3

[1] ADG = average daily gain, ADFI = average daily feed intake, G:F = gain to feed ratio.

[2] ATTD = apparent total tract digestibility, DM=Dry matter, CP=Crude protein, GE=Gross energy.

[3] AiD = Apparent ileal digestibility

[4] Xyl = xylanase, XB = xylanase+β-glucanase, Mann = mannanase, Prot = protease, Cplex = complex of enzymes

Table 2. Cut-off value for cereal source categorisation, minimum (Min), maximum (Max) and mean inclusion percentage (%) of ingredients included in the experimental diets.

	Cut-off	Min	Max	Mean
Maize	35	35.0	75.2	56.1
Wheat	35	41.2	96.8	73.2
Barley	35	35.6	84.2	59.1
Rye	35	50	96.8	79.3
Sorghum	35	73.4	94.4	83.8
Co-products[1,2]				
wDDGS		0.0	30.0	6.9
mDDGS		0.0	8.0	1.3
Wheat bran		0.0	22.0	2.6
RSM		0.0	21.0	7.1
Peas		0.0	35.0	3.9

[1] Studies with a diet below the cut-off value in all of the above cereal source categories were included in the co-products category.

[2] wDDGS = wheat distillers dried grains with solubles, mDDGS = maize distillers dried grains with solubles, RSM = rapeseed meal.

Table 3 Enzymes present in the multi-enzyme complexes used for each study included in the meta-analysis[1]

Publication	Xyl	β-glu	Phy	Cel	Prot	Man	α-amy	α-gal
Zhang et al., 2017	*	*		*				
O'Shea et al., 2014	*				*			
Cho et al., 2013	*					*		
Kim et al., 2013a						*		*
Jo et al., 2012					*	*		
Jo et al., 2012					*	*	*	
Jo et al., 2012						*	*	
Xie et al., 2012	*	*			*			
Ao et al., 2010						*		*
Lee et al., 2011			*			*		
Lee et al., 2011			*			*		
Ao et al., 2010	*	*				*		*
Wang et al., 2009	*	*				*		*
Emiola et al., 2009	*	*		*				
Kim et al., 2008	*		*					
Lyberg et al., 2008	*		*					
Thacker et al., 2009	*	*		*	*			
Wang et al., 2008	*	*		*				
Woyengo et al., 2008	*		*					
Fang et al., 2007b	*	*				*		
Kim et al., 2006						*		*
Olukosi et al., 2007	*		*					
Kim et al., 2006						*		*
Kim et al., 2004	*	*			*		*	
Kim et al., 2004	*	*					*	
Park et al., 2003				*			*	
Chu et al., 1998	*	*			*			
Kim et al., 1998				*			*	
Thacker et al., 1992b		*			*			
Thacker et al., 1992b		*			*			
Thacker et al., 1991		*			*			
Thacker et al., 1991		*			*			
Ayoade et al. 2012	*	*		*	*	*		*
O'Doherty and Forde 1999					*			*
Agyekum et al. 2016	*	*			*		*	
Mok et al. 2013			*		*			
Jakobsen et al. 2015	*	*			*			
Sobotka et al. 2011	*	*		*	*	*		
Emiola et al. 2009	*	*		*				

[1]Xyl = xylanase, β-glu = β-glucanase, Phy = phytase, Cel = cellulase, Prot = protease, α -amy = α-amylase, α-gal = α-galactosidase

Table 4 Summary of mean difference (MD) estimate effects of enzyme dietary supplementation on average daily gain (ADG, g/day), average daily feed intake (ADFI, g/day) and gain to feed (G:F, g/100g) of grow-finisher pigs.

	ADG			**ADFI**			**G:F**		
	MD	SE	p-value	MD	SE	p-value	MD	SE	p-value
Xylanase									
Xyl*Maize	22.1	10.90	<0.05	-6.5	25.00	0.79	0.4	0.65	0.57
Xyl*Wheat	2.8	9.02	0.75	-32.1	18.08	0.08	0.1	0.51	0.84
Xyl*Rye	-10.0	76.51	0.90	-100.0	46.93	<0.05	1.2	2.92	0.67
Xyl*Co-products	57.6	24.73	<0.05	38.4	42.34	0.36	0.8	1.30	0.55
Overall	18.1	20.6	0.38	-25.1	18.09	0.17	0.6	0.84	0.46
Xylanase + Glucanase									
XB*Maize	-33.6	35.76	0.35	-66.2	48.45	0.17	0.9	1.56	0.58
XB*Wheat	13.0	24.43	0.60	53.4	39.19	0.17	0.6	1.27	0.65
XB*Barley	16.1	15.34	0.29	11.6	28.05	0.68	0.5	0.75	0.50
XB*Rye	12.0	45.20	0.79	-20.0	72.69	0.78	1.0	2.40	0.68
XB*Co-products	19.4	18.53	0.30	39.2	30.36	0.20	0.7	0.93	0.49
Overall	5.4	13.71	0.67	3.6	21.42	0.87	0.7	0.68	0.30
Mannanse									
Mann*Maize	19.4	7.96	<0.05	2.2	13.29	0.87	1.0	0.40	0.01
Overall	19.4	7.96	<0.05	2.2	13.29	0.87	1.0	0.40	0.01
Protease									
Prot*Maize	25.0	31.18	0.42	15.0	43.04	0.73	1.0	1.42	0.48
Prot*Wheat	22.6	24.27	0.35	137.5	46.42	<0.01	-1.0	1.66	0.55
Prot*Barley	57.2	37.45	0.13	4.0	65.38	0.95	2.3	1.88	0.21
Prot*Sorghum	-15.5	18.67	0.41	-44.3	32.83	0.18	-0.1	0.90	0.91
Prot*Co-products	68.1	39.12	0.08	-47.2	97.11	0.63	2.4	2.66	0.38
Overall	31.5	14.9	<0.05	13.0	28.65	0.65	0.9	0.84	0.28
Complex of enzymes									
Cplex*Maize	31.2	7.69	<.0001	-13.9	12.85	0.28	1.9	0.39	<0.001
Cplex*Wheat	36.7	9.50	0.00	-29.2	22.86	0.20	2.1	0.55	<0.01
Cplex*Barley	43.5	12.17	0.00	17.9	29.41	0.54	2.1	0.77	<0.01
Cplex*Rye	-5.4	14.40	0.71	-61.2	35.30	0.08	1.7	1.58	0.27
Cplex*Sorghum	21.2	24.346	0.38	34.1	45.69	0.46	-0.1	1.40	0.90
Cplex*Co-products	47.4	13.4611	0.00	-55.4	40.31	0.17	2.5	0.90	<0.01
Overall	29.1	7.67	<0.001	-17.9	14.82	0.23	1.7	0.45	<0.001

[1] Xyl = xylanase, XB = xylanase+β-glucanase, Mann = mannanase, Prot = protease, Cplex=multi-enzyme complex

Table 5 Summary of mean difference (MD) estimate effects of enzyme dietary supplementation on apparent total tract digestibility (ATTD, %) of dry matter (DM), crude protein (CP) and gross energy (GE) of grow-finisher pigs.

	ATTD DM			ATTD CP			ATTD GE		
	MD	SE	p-value	MD	SE	p-value	MD	SE	p-value
Xylanase									
Xyl*Maize	1.0	0.49	<0.05	0.0	0.53	0.94	1.0	0.45	<0.05
Xyl*Wheat	1.1	0.50	<0.05	1.4	0.44	<0.01	1.1	0.46	<0.01
Xyl*Rye	-	-	-	1.3	0.78	0.10	-	-	-
Xyl*Co-products	1.4	1.02	0.16	-	-	-	1.3	1.15	0.25
Overall	1.2	0.45	<0.01	0.9	0.38	<0.05	1.4	0.71	<0.05
Xylanase + Glucanase									
XB*Maize	2.8	1.09	<0.01	-	-	-	-	-	-
XB*Wheat	-	-	-	-	-	-	-	-	-
XB*Barley	-	-	-	1.1	0.67	0.10	1.6	0.75	<0.05
XB*Rye	3.2	1.98	0.11	5.2	3.00	0.08	3.7	1.86	<0.05
XB*Co-products	4.3	1.09	<.0001	4.8	0.99	<.0001	4.4	0.91	<.0001
Overall	3.4	0.84	<0.001	3.7	1.07	<0.001	3.4	0.97	<0.001
Mannanse									
Mann*Maize	0.8	0.36	<0.05	1.0	0.34	<0.01	1.0	0.38	<0.01
Overall	0.8	0.36	<0.05	1.0	0.34	<0.01	1.0	0.38	<0.01
Protease									
Prot*Maize	2.9	1.06	<0.01	3.5	1.01	<0.01	3.3	0.87	<0.01
Prot*Wheat	-	-	-	-	-	-	-	-	-
Prot*Barley	-	-	-	0.7	1.66	0.67	0.7	2.03	0.74
Prot*Sorghum	3.0	1.48	<0.05	8.0	1.20	<.0001	3.0	1.25	<0.05
Prot*Co-products	-	-	-	-2.7	1.62	0.10	0.1	5.32	0.98
Overall	3.0	0.91	<0.01	2.4	0.69	<0.001	3.7	1.09	<0.01
Multi-enzyme complex									
Cplex*Maize	1.1	0.35	<0.01	1.1	0.33	<0.01	1.1	0.36	<0.01
Cplex*Wheat	3.5	0.61	<.0001	3.1	0.47	<.0001	1.4	0.48	<0.01
Cplex*Barley	0.0	3.34	1.00	0.4	1.53	0.79	0.1	1.81	0.94
Cplex*Rye	-0.3	1.30	0.80	0.9	1.52	0.55	-0.4	1.18	0.73
Cplex*Sorghum	0.7	1.11	0.51	1.6	1.22	0.19	0.7	1.39	0.62
Cplex*Co-products	-	-	-	-2.5	1.62	0.12	0.3	5.32	0.95
Overall	1.0	0.79	0.21	0.78	0.50	0.12	1.2	0.99	0.23

[1] Xyl = xylanase, XB = xylanase+β-glucanase, Mann = mannanase, Prot = protease, Cplex=multi-enzyme complex

Table 6 Summary of mean difference (MD) estimate effects of enzyme dietary supplementation on apparent ileal digestibility (AiD, %) of dry matter (DM), crude protein (CP) and gross energy (GE) of grow-finisher pigs.

	AiD DM			**AiD CP**			**AiD GE**		
	MD	SE	p-value	MD	SE	p-value	MD	SE	p-value
Xylanase									
Xyl*Maize	-1.4	1.23	0.25	-0.6	1.39	0.65	3.0	1.33	<0.05
Xyl*Wheat	2.3	1.15	<0.01	1.9	1.33	0.16	3.6	1.38	<0.01
Xyl*Rye	-0.7	1.64	0.68	-0.3	2.80	0.92	-	-	-
Xyl*Co-products	-	-	-	-2.4	5.58	0.67	5.8	5.53	0.29
Overall	0.14	0.88	0.87	-0.4	1.69	0.83	4.1	2.02	<0.05
Xylanase + β-Glucanase									
XB*Maize	-6.3	3.77	0.09	-4.3	5.70	0.45	0.2	6.05	0.97
XB*Wheat	14.1	3.77	<0.01	4.1	2.50	0.10	-	-	-
XB*Barley	-	-	-	-	-	-	-	-	-
XB*Rye	-	-	-	-	-	-	-	-	-
XB*Co-products	-	-	-	7.7	5.70	0.18	15.1	6.05	<0.01
Overall	3.9	5.32	0.46	2.5	3.04	0.41	7.7	4.42	0.08
Mannanse									
Mann*Maize	3.1	1.41	<0.05	1.9	2.70	0.48	0.6	1.71	0.73
Overall									
Protease									
Prot*Maize	5.0	1.68	<0.01	6.0	2.73	<0.05	5.0	1.90	<0.01
Prot*Wheat	-	-	-	-	-	-	-	-	-
Prot*Barley	-	-	-	-	-	-	-	-	-
Prot*Sorghum	-	-	-	-	-	-	-	-	-
Prot*Co-products	-	-	-	23.6	5.58	<.0001	14.4	5.53	<0.01
Overall	5.0	1.68	<0.01	14.8	3.11	<0.001	9.7	2.93	<0.001
Multi-enzyme complex									
Cplex*Maize	2.5	0.73	<0.01	0.9	1.37	0.53	2.8	0.90	<0.01
Cplex*Wheat	1.9	1.26	0.13	2.3	1.44	0.12	-	-	-
Cplex*Barley	0.6	0.87	0.46	5.6	1.69	<0.01	7.2	1.54	<.0001
Cplex*Rye	-	-	-	-	-	-	-	-	-
Cplex*Sorghum	-	-	-	-	-	-	-	-	-
Cplex*Co-products	-	-	-	0.8	5.58	0.89	-3.2	5.53	0.56
Overall	1.6	0.67	<0.05	2.39	2.59	0.14	2.3	1.97	0.25

[1] Xyl = xylanase, XB = xylanase+β-glucanase, Mann = mannanase, Prot = protease, Cplex=multi-enzyme complex