A systematic-review on the role of exogenous enzymes on the productive performance at weaning, growing and finishing in pigs

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ABSTRACT

Supplementing exogenous enzymes in pig diets is an alternative solution to increase dietary energy and fiber digestibility to improve pig production performance at a low production cost and to reduce environmental impact with lower N and P excretions. The production stage, diet composition, enzyme source, amount and number of enzymes added, are factors to consider before using them. A database composed by 227 individual diets, resulting from 43 studies with 48 experimental records were divided in different production stages, with 19 records for weaning, 17 records for growing and 12 records for finishing. A descriptive statistical analysis of the chemical composition of the diets and enzyme doses was carried out. The data with normal distribution were analyzed calculating the mean, the minimum and maximum length, the standard deviation and the coefficient of variation. It was found that combined enzymes are the most widely reported enzyme combination in the supplementation of pigs at all stages of production. Phytases and Mannanases are commonly used at weaning and growing stages. Xylanases and Proteases have been reported to be used in all production stages. However, the highest yielding enzymes at weaning, growing and finishing stages were Phytases and Mannanases. Dietary supplementation of exogenous enzymes improves production characteristics at all stages of production. However, an improvement in growth performance and nutrient digestibility is not always observed. Future studies should focus on the interaction between production stages, composition of the diet, origin of the enzyme and the amount and number of enzymes added.

1. Introduction

Food ingredients included in pig diets, especially plant-based cereals, contain large amounts of non-starch polysaccharides (NSPs) (Adeola & Cowieson, 2011; Recharla et al., 2019). These NSPs are an important part of the plant ingredients (10–75%), and most of them are composed by arabinoxylans, cellulose and β-glucans (Choct, 2015). However, NSPs are poorly metabolized by pigs as they lack specific endogenous enzymes for their degradation (Jha & Berrocoso, 2015).

Supplementing exogenous enzymes as additives for pig diets hydrolyze NSPs, break the cell wall that encapsulates them, degrade anti-nutritional factors (protease inhibitors, antigenic proteins, non-protein amino acids) and perform the cleavage of glycolytic bonds that are not hydrolyzed by endogenous enzymatic activity (Kim et al., 2008; Lima, Da Silva, Araujo, Lima & Oliveira, 2007; Masey O’Neill, Smith & Bedford, 2014; Recharla et al., 2019), improving the digestibility of nutrients and thus can be used by the animal.

Most studies on animal diets seek strategies to improve feed efficiency, which are of particular interest to increase productive efficacy and reduce environmental impacts (Aarnink & Verstegen, 2007; Clark & Tilman, 2017). In this sense, exogenous enzymes improve feed efficiency and reduce feeding costs in the animal production industry (Adeola & Cowieson, 2011; Upadhaya, Park, Lee & Kim, 2016a), as pig feed accounts for 55–75% of total production costs (Nguyen, 2017).

Some exogenous enzymes included in diets for pigs are Phytases, Carbohydrases, Proteases and Lipases (Table 1) (Ravindran, 2015). In pigs, Phytase (myo-inositol hexakisphosphate phosphohydrolase) is a phosphohydrolytic enzyme that initiates the phosphate gradual removal

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from phytate (inositol hexakiphosphate), which is the main source of phosphorus found in cereal grains and oil seeds (Dersjant-Li, Awati, Schulze & Partridge, 2014).

Of the world market for feed enzymes for monogastrics, it has been estimated that Phytases and Carbohydrases represent 90% and proteases and lipases 10% (Adeola & Cowieson, 2011). Therefore, the objective of the present systematic-review is to summarize the current knowledge on the use of exogenous enzymes in pig diets, to improve productive performance at weaning, growing and finishing stages with regard to their mode of action and effects. Also, this review aims at reporting the most efficient enzymes in pig productive performance and find the most supplemented exogenous enzymes in pig’s diets at all productive stages (weaning, growing and finishing). The present systematic review evaluated productive variables that are improved with dietary supplementation of exogenous enzymes at each stage of production in pigs.

2. Materials and methods

2.1. Search strategy and selection criteria

Our search for information focused on studies reporting the use of exogenous enzymes in pig diets. A database was created from studies specifying the use of exogenous enzymes in pig diets and the articles used, covered the years 2000 – 2020. The publications were obtained from databases such as World Wide Science, ScienceDirect, Scopus, Springer Link, Wiley Online Library, Dialnet, SciELO, Science Research, PubMEd, Redalyc, Google Academic and ERIC. Obtaining information to find relevant publications was based on a chain of specific topics such as the various exogenous enzymes used in pigs.

The search string with the particular topic was supported by Boolean operators (“and”, “or”), which served to specify the required information. All search terms within a string were checked for a “title, abstract and

Table 1
Exogenous feed enzymes and target substrates.

| Enzyme     | Target substrate                  |
|------------|-----------------------------------|
| Phytases   | Phytic acid                       |
| β-Glucanases | β-Glucan                      |
| Xylanases  | β-Glucan                        |
| α-Galactosidases | Oligosaccharides        |
| Amylase    | Starch                           |
| Mannanases | Cell wall matrix (fiber components) |
| Cellulases |                                    |
| Hemicellulases |                            |
| Pectinases |                                    |
| Proteases  | Proteins                         |
| Lipases    | Lipids                           |

Adapted from Ravindran, 2013.

Fig. 1. PRISMA study flow diagram of the systematic review from initial search and screening to final selection of publications to be included in the study.
The pig is a monogastric animal that does not produce endogenous enzymes, action mode, effects, productive performance, treatment (control vs enzyme or combined-enzymes), pig production stage (weaning, growing and finishing), dosage of enzyme in the diet (g/kg), average daily gain weight (ADG kg/day), gain: feed ratio (G: F kg/kg), average daily feed intake (ADFI, kg/day) and digestibility of dry matter (DDM). Each of the obtained values was homogenized in the database to be able to be calculated: dosage of enzyme in the diet (g/kg), ADG (kg/day), G: F ratio (kg/kg), ADFI (kg/day) and DDM (g/kg).

A total of 43 studies and with different enzymes doses were included in the database: Agyeikum et al., 2015, Ao et al., 2010, Castro et al., 2011, Cho & Kim, 2013, Cho et al., 2017, Choe et al., 2017, Dersjant-Li, Plumstead, Awati & Remus, 2018, He et al., 2020, Jang, Kim, Jang, & Kim, 2020, Jo et al., 2012, Kiarie, Nyachoti, Slominski & Blank, 2007, Kim et al., 2004, Kim et al., 2008, Kim et al., 2013, Kim et al., 2017, Lan, Li & Kim, 2017, Lee et al., 2011, Lei, Cheong, Park & Kim, 2017, Li, Gabler, Loving, Gould & Patience, 2018, Lu et al., 2016, Lv et al., 2013, Martínez, Figueroa, Cordero, Sánchez & Martínez, 2017, Nguyen, Upadhaya, Lei, Yin & Kim, 2019, O’Shea et al., 2014, Olukosi, Sands & Adeola, 2007a, Omobogeniun, Nyachoti & Slominski, 2003, Omobogeniun, Nyachoti & Slominski, 2004, Owusu-Asiedu et al., 2012, Park et al., 2020, Recharla et al., 2019, Tsai, Dose, Bedford & Azain, 2019, Upadhaya et al., 2016a, 2016b, Voyengo, Dupe, Akinremi & Nyachoti, 2016, Yañez, Ladero, Owusu-Asiedu, Cervantes & Zijlstra, 2013, Yi et al., 2013, Yoon et al., 2010, Zeng et al., 2011, Zeng et al., 2014, Zeng et al., 2015, Zhang, Yang, Wang, Yang & Zhou, 2014, Zijlstra, Li, Owusu-Asiedu, Simmins, & Patience, 2004, Zuo et al., 2015. Experiments were treated individually even when published within one article. Experiments were treated individually even when published within one article.

2.2. Data extraction and analysis

Our database consisted of 227 individual diets, resulting in 43 studies with 48 experimental records that were divided by production stages, with 19 records for weaning, 17 records for growing and 12 records for finishing (Figure 1). A statistical descriptive analysis of the chemical composition diets and enzyme doses was performed: enzymes, number of animals, dosage of enzyme in the diet, initial body weight, average daily feed intake, average daily gain weight, gain: feed ratio, digestibility of dry matter, to determine the effect of enzymes strains alone or in combined-enzymes on those variables. The datasets were analyzed for bifurcation by computing basic indices such as number of studies. The analysis was repeated for the length of each segment with statistical analyses such as mean, minimum, maximum length, standard deviation and coefficient of variation. The analysis was carried out using the SAS statistical software (SAS, 2004). The analysis to obtain the means was with Fisher’s F test and the comparison of means was with Tukey’s test.

3. Results and discussion

The pig is a monogastric animal that does not produce endogenous enzymes capable of digesting dietary NSPs and this lead to increases in digesta viscosity, alterations in epithelial morphology of the intestine and reduced nutrient digestibility (Lindberg, 2014; Passos, Park, Ferket, von Heimendahl & Kim, 2015). Therefore, the purpose of exogenous enzymes is to improve pig productive performance by dietary means. Although the purpose of dietary supplementation of exogenous enzymes is to improve growth performance and nutrient digestibility, pigs receiving enzymes do not always show constant improvements (Barrera, Cervantes, Sauer, Araiza & Torrentera, 2004; Leek, Callan, Reilly, Beattie & O’Doherty, 2007; Olukosi et al., 2007a).

In this review, we found that in pig diets, the most supplemented enzymes at all productive stages (weaning, growing and finishing) are Phytases, Carbohydrases (Mannanases, Xylanases), Proteases and Combined-enzymes. In the next sections we will discuss and describe the function of each enzyme.

3.1. Phytases

Adeola and Cowieson (2011), reported that the best-selling enzymes are Phytases with 60% of the sale market, Carbohydrases with 30% and Proteases and Lipases with 10%. After the introduction of phytate-degrading enzymes in 1991, the use of microbial Phytases had a great boost, so their inclusion in pigs surpassed NSPs enzymes, which was predictable since phytate is present in diets with Phytases and they represent a viable alternative source of P and reduce its excretion (Selle & Ravindran, 2008). For this reason, its sale in the market had surpassed the use of other enzymes.

Adeola and Cowieson (2011), mentioned that Phytase inclusion level greater than 2500 FTY/kg of feed characterizes a high Phytase inclusion dose. Efficacy depends on various factors, such as pig growth stage, type of diet and source of Phytase (Jongbloed, Van Diepen, Kemme & Broz, 2004). Increasing the level of enzyme inclusion does not necessarily represent a linear improvement in nutrient utilization (Da Silva et al., 2019).

Phytases are supplemented in the same way at all productive stages, as well as Mannanases, with a higher use at weaning and growing pig stages (Tables 5 – 7), acting on the hydrolysis of phytate (myo-inositol 1,2,3,4,5,6-hexakis [dihydrogen phosphate]) to release the phosphate from this complex, improving the digestibility of phosphorus (P), calcium, amino acids, energy and reduced inorganic P excretion into the environment (De Faria et al., 2015; Dersjant-Li et al., 2014; EFSA, 2012). The most used Phytases in animal feed are histidine acid phosphatases (HAPs), followed by other classes of Phytase such as Phytase of helix β (BPPhy or alkaline Phytase), purple acid Phytase and protein tyrosine phosphatase (Lei, Weaver, Mullaney, Ullah & Azain, 2012). Improved availability of phosphorus and other minerals in pig’s diets with the use of Phytase, reduces soil contamination (Sefer et al., 2012). Phytase in pig diets is generally added at 2.5 g/kg, but less than 50% of the Phytate in the diet is hydrolyzed (Dersjant-Li, Schuh, Weallean, Awati & Dusel, 2017; Selle, Cowieson & Ravindran, 2009). In the present review, the inclusion of Phytases (g/kg diet as DM) vary among productive stages (Table 5, 6 and 7), with 2.50 ± 0.88 g/kg diet at weaning, growing, and finishing stages, respectively (Table 8). Similar effects (P > 0.05) between studies were found to ADFI (kg/d), ADG (kg/d), G: F ratio while DDM showed 840.6 ± 25.5 g/kg at weaning, 862.5 ± 7.4 at growing and 802.0 ± 1.41 at finishing stages. The average daily gain with phytases supplementation at weaning stage was 11.9% higher and at growing stage was 7.3% higher compared to the control group. While at finishing stage, this effect becomes negative (~15.4%) possibly due to an improved efficiency of P utilization in younger pigs. At weaning stage, Zeng et al. (2014), reported on average an increase in ADG of 10.76%, an ADFI of 6.89% and a G: F of 3.50%, with phytase supplementation at 0.5–20 g/kg, this effect was higher than the present results. Váñez et al. (2013), reported similar results to the present study on average an increase in ADG of 7.29%, a G: F of 7.46% and a decrease in ADFI of ~1.36%, with phytase supplementation at 0.1 g/kg. In the growing stage, Zeng et al. (2011), reported on average an increase in ADG of 5.88%, a ADFI of 3.65% and a DDM of 0.13% with a Phytase supplementation at 0.25–2 g/kg, which is lower than the
present study. On the contrary, at finishing stage, Olukosi et al. (2007a), reported on average an increase in ADG of 11.96%, a ADFI of 0.86%, a G: F of 7.69% and a decrease in DDM of 0.95%, with Phytase supplementation at 0.5–1 g/kg. This variation in production responses may be due to the amount of calcium/phosphorus in the diet and its interaction with other factors, as well as the concentration of phytases in the diet as a function of the pig’s production stage, so the amount of P vs. enzyme supplemented in the diet should be reviewed. The amount of P vs. enzyme supplemented in the diet should be checked in order to observe optimal performance.

3.2. Carbohydrases

Carbohydrases are enzymes that catalyze the breakdown of complex carbohydrates into oligosaccharides, disaccharides, monosaccharides and are used as a method to help overcome the limitations of pigs to effectively utilize non-starch polysaccharides (NSPs) such as arabinoxylans and β-glucans (Campbell & Bedford, 1992). These enzymes hydrolyze plant cell wall components such as xylan, mannan and beta-glucan and assist in the release of nutritional constituents such as proteins, starch, lipids and other minerals that are trapped within the cell wall matrix (Li, Sauer, Huang & Gabert, 1996; Meng & Slominski, 2005; Nortey, Patience, Simmins, Trottier & Zijlstra, 2007). After hydrolysis of the NSPs and digestibility of the trapped nutrients, the resulting products are readily accessible to the gut microflora, which can have multiple beneficial effects on the gastrointestinal functionality of the animals (Yin, Zhang, Huang & Yin, 2010). Fiber-degrading enzymes should be applied to fibrous diets to improve efficient production of swine, especially considering low fiber digestibility of fiber-rich ingredients (Zhao, Zhang, Liu, Wang & Zhang, 2020). Carbohydrases work best in young pigs, due to their intestinal incapacity (Patience & DeRouche, 2010), and to the negative effects caused by high fiber levels, thus improving growth performance (Tsai et al., 2017). This supplementation favors nutrient digestion at the most proximal portion of the digestive tract (Mathlouthi, Lalies, Lepercq, Juste & Larbier, 2002), (Figure 2). Limitations imposed by intestinal incapacity make Carbohydrases supplementation an essential dietary intervention in young pigs, but the use in sows is still scarce (Adeola & Cowieson, 2011).

3.2.1. Mannanases

Mannanases use is due to the fact that the tract of pigs lacks the enzymes that target the links β-1,4-manosyl and α-1,6-galactosyl, so nutrient utilization and growth performance are limited and supplementation with β-mannanase or enzyme complex with β-mannanase has the potential to improve them, in addition to eliminating the negative effect of mannan (Ao et al., 2011; Jo et al., 2012; Kim et al., 2013, 2017; Pettey, Carter, Senne & Shriver, 2002; Veum & Odle, 2001).

The most widely used Carbohydrases found in the present review are Mannanases (Table 5, 6 and 7), which have become biotechnologically important since they target the hydrolysis of complex polysaccharides of plant tissues into simple molecules such as oligosaccharides, manose (Dhawan & Kaur, 2007), and Xylanases that enhance energy use by the pig (Nortey et al., 2007).

The inclusion of Mannanases used in the pigs diet at any productive stage (Table 6 and Table 7), showed a DDM (g/kg) of 827.4 ± 24.5 and 836.5 ± 34.62 at growing and finishing stages, respectively. However, when these data are expressed in percentage, it is observed that compared to control groups, there is an increase in the average daily gain of 3.8% and 1.3%, at weaning and at growing stages, respectively, and an improved G:F ratio (2.7%) at growing stage which can be explained by improved efficiency of energy utilization.

At growing stage, some authors have reported productive variables in pig performance with the use of Mannanases, Lv et al. (2013), reported on average an increase in ADG of 16.96%, a G:F of 22.19%, a DDM of 2.78% and a decrease in ADFI of –4.98%, with Mannanase supplementation, which is higher than the present study at 0.2–0.6 g/kg (Table 9). A lower response was reported by Kim et al. (2017), on average an increase in ADG of 7.16%, a ADFI of 2.61% and a DDM of 2.33%, with Mannanase supplementation, which is higher than the present study at 0.2–0.6 g/kg (Table 4).

3.2.2. Xylanases

Xylanase is another carbohydrase used in pig diets, the inclusion covers all stages of production (Table 5, 6 and 7), and is within the 80% of the best-selling Carbohydrases worldwide for use in monogastric diets (Adeola & Cowieson, 2011). Endo-1,4-β-Xylanase is produced by a genetically modified strain of Bacillus subtilis TD160 (229) (European Union Reference Laboratory for Feed Additives, 2014), Xylanase has the ability to hydrolyze the xylan content of 1,4-β-D-xylans and can improve energy use by the pig (Nortey et al., 2007). The magnitude of effect of exogenous Xylanases depends on the nutritional value of the diet to which they are added (Cowieson & Bedford, 2009).

The inclusion of Xylanases used in the pigs diet at any productive
stage (Table 5 and 7), showed an average dry matter digestibility (g/kg) of 829.5 ± 7.14 at weaning and 759.1 ± 6.93 at finishing stages. Table 10 shows the effects of supplementing Xylanases exogenous enzymes in pig diets. Overall, no significant differences (P > 0.5) were observed. However, when these data are expressed in percentage, it was observed that the best response is at weaning stage, with an increase in the average daily gain of 2.5% compared to the control group. With regard to performance, at weaning stage, Lan et al., (2017), reported on average an increase in ADG of 3.88%, a ADFI of 0.34%, a G: F of 3.50% and a DDM of 2.25%, with Xylanase supplementation at 0.05–0.1 g/kg. At finishing stage, Cho et al. (2017), reported on average an increase in ADG of 1.81%, a ADFI of 0.18%, a G: F of 1.58% and a decrease in DDM of −0.62%, with Xylanase supplementation at 0.1 g/kg, which correspond to the present results.

3.3. Proteases

The productive results when proteases used in the pig’s diet at any productive stage (Table 6 and 7), showed an average in DMD (g/kg) 882.7 ± 12.20, 754.2 ± 3.61, 722.9 ± 3.47, in weaning, growing and finishing stages respectively. The effects of supplementing Proteases in pig diets are shown in Table 11. Dietary inclusion of Proteases did not affect (P > 0.05) ADFI, ADG, G: F and DDM at all productive stages. On another hand, the fact that no improvement in CP and AA digestibility is observed in Protease-treated soybean meal (SBM) compared with untreated SBM, is because pigs fed a diet containing pretreated SBM with Protease enzyme had no change in G: F ratio compared with pigs fed with untreated SBM (Rooke, Slessor, Fraser & Thomson, 1998). However, when these data are expressed in percentage, it is observed that there is a reduction of 2.7% in the ADG at weaning stage, possibly due to a better utilization of protein, leading to a reduction in feed consumption, without affecting their productive parameters, when including proteases compared to the control group, showing an increase in the ADG of 45% and a better G:F ratio (56%). This effect decreased at growing stage, showing an increase in the ADG of 2.5% and a better G:F ratio (4.5%) possibly due to a better efficiency of protein utilization at younger stages. In terms of performance, at weaning stage, Zuo et al. (2015), reported on average an increase in ADG of 6.31%, in ADFI of 5.62% and a decrease in DDM of −0.26%, with a Protease supplementation at 0.1–0.3 g/kg, which is lower to the present results. At finishing stage, Lei et al. (2017), reported on average a decrease in ADG of −0.11%, a ADFI of −1.90%, a DDM of −0.47 and an increase in G: F of 1.79%, with Protease supplementation at 0.5 g/kg, better utilization of protein (essential amino acids) leads to a reduction in feed intake, without affecting their productive parameters, with a better G:F ratio.

The mode of action of protease in its productive stages of the pig will improve the digestibility of the nutrients (Table 5, 6 and 7 ), as well as the intestinal fermentation capacity and the longer transit time (Choe et al., 2017; Lei et al., 2017; Nguyen et al., 2019; Tacitacan, Cho & Kim, 2016; Zuo et al., 2015). Pigs have the ability to produce digestive proteases such as pepsin, trypsin, chymotrypsin and carboxypeptidases that digest proteins included in the diet. A fraction of these proteins included in the feed that is intake it, are excreted in the feces, which means that an exogenous protease can improve the use of the proteins (Lemme, Ravindran & Bryden, 2004; Parsons, Castanon & Han, 1997).

3.4. Combined enzymes

The pig industry will continue to seek cost-effective alternative food ingredients, such as cereal co-products from the biofuel and milling industries (Kiarie & Nyachoti, 2009). In the present review, from 50 different diets, the most used combined enzymes in pig diets were Phytases (34 diets), Mannanases (25 diets), Xylanases (13 diets) and Proteases (12 diets).

Carbohydrases mixture can produce a greater benefit than each of the individually acting enzymes (Juanpere, Perez-Vendrell, Angulo &

Table 2

| Enzyme | Action Mode | Main Effects |
|--------|-------------|--------------|
| Mannanase | Improves the viscosity of the ileal digesta. | Increases AID of DM and NSPs. Lactobacillus and lactate count. |
| Phytase | Improves AID of DM, GE, CP, starch, NSPs, phytate, Ca, P, Na, K, Mg, and Zn as well as the retention of Mg and Zn. | Decreases fecal P excretion. |
| Protease | Improves AID of DM, GE, CP, starch, NSPs, phytate, Ca, P, Na, K, Mg, and Zn as well as the retention of Mg and Zn. | Increases ADG, ADFI and G: F ratio. Treponema and Barmesielia bacteria in the intestine. |

Table continued on next page
Table 2 (continued)

| Enzyme | Action Mode | Main Effects | Reference |
|--------|-------------|--------------|-----------|
| Nutrient digestion and modification of microbial communities in the posterior intestine. | Improved the digestibility of N. | -Decreases ADFI. | Kiarie et al., 2007; Nortey et al., 2010; Upadhaya et al., 2016a |
| Glucanase | Improves the viscosity of the ileal digesta. AID of DM, GE, CP, starch, NSPs, pectinase, and BUN | -Increased glucose. | Kiarie et al., 2007; Kwagugho et al., 2017; Owusu-Asiedu et al., 2018; Recharla et al., 2019 |
| Amylase | Improves AID of DM, GE, CP, starch, NSPs and pectinase, ATTD of DM, GE, CP, starch, NSPs, pectinase, and utilization of P. | -Decreases ADFI and G. F ratio. | Recharla et al., 2018; Li et al., 2018 |
| Xylanase | Improves AID of DM, starch, NSPs, pectinase, and BUN | -Decreases ADFI. | Nortey et al., 2016; Owusu-Asiedu et al., 2018; Upadhaya et al., 2016a |

Table 3

Mode of action and main effects of enzymes used in pig diets at growing stage.

| Enzyme | Action Mode | Main Effects | Reference |
|--------|-------------|--------------|-----------|
| Mannanase | Improves AID of AA, ATTD of DM, NDF, ADF, GE, CP, Ca, mannose, galactose, phosphorus, blood glucose concentration and BUN. Digestibility of N. | -Decreases ADFI. | Ao et al., 2010; Jo et al., 2012; Kim et al., 2013; Lv et al., 2013; Yoon et al., 2016; Kim et al., 2017; Upadhaya et al., 2016a |
| Phytase | Improves AID of DM, lysine, threonine, serine, isoleucine, isolucine and asparagine and valine. ATTD of P, Ca, DM, GE, leucine, lysine, phenylalanine, alanine and cysteine. | -Decreases ADFI and FCR and G. F ratio. | Nortey et al., 2007; Kim et al., 2008; Zeng et al., 2011; Woyengo et al., 2016 |
| Protease | Improves ATTD of DM, GE, CP and BUN. Nutrient digestibility. | -Decreases ADFI and G. F. Blood creatinine levels. | Jo et al., 2012; Nguyen et al., 2019 |
| Xylanase | Improves AID of DM, AA, isoleucine, P and nutrient transport. Digestibility of N. | -Decreases ADFI and FCR and glucose. | Nortey et al., 2007; Owusu-Asiedu et al., 2018; Upadhaya et al., 2016a |
| Mannosidase | Improves AID of AA. Digestibility of N. | -Decreases ADFI. | Ao et al., 2010 |
| Galactosidase | Improves AID of the DM and AA. Digestibility of N. | -Decreases ADFI and BUN and glucose. | Ao et al., 2010 |
| Galactomannanase | Improves AID of the DM and AA. Digestibility of N. | -Decreases ADFI and G. F. | Ao et al., 2010 |

AID: Apparent ileal digestibility; DM: Dry matter; GE: Gross energy; CP: Crude protein; ADF: Apparent total digestibility; DM: Dry matter; NSPs: Non-starch polysaccharides; GE: Gross energy; CP: Crude protein; AID: Apparent ileal digestibility; DM: Dry matter; NSPs: Non-starch polysaccharides; GE: Gross energy; CP: Crude protein; ATTD: Apparent total tract digestibility; P: Phosphorus; ADG: Average daily weight gain; G:F ratio: Gain Feed ratio; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; FCR: Feed conversion ratio; ADFI: Average daily feed intake; Ca: Calcium; AA: Amino acids; Na: Sodium; K: Potassium; Mg: Magnesium; Zn: Zinc; N: Nitrogen; BUN: Blood urea nitrogen.
Table 5

| Enzyme | Mode of Action | Main Effects | Reference |
|--------|----------------|--------------|-----------|
| Mannanase | Improves ATTD of GE, N, DM and CP. | -Increased ADG; G: F; ADFI and blood glucose concentration. -Decreased fat thickness of rib number 10. | Yoon et al., 2010; Cho & Kim, 2013; Kim et al., 2013 |
| Phytase | Improves digestibility of P. | -Increases ADG and Ca. -Decreased P excretion. | Olukosi et al., 2007 |
| Protease | Improves AID of GE. ATTD of CP. | -Decreases ADG and GE. -Decreases ADFI. Fecal ammonia emission. | Olukosi et al., 2014; Choe et al., 2017; Let et al., 2017 |
| Xylanase | Improves AID of GE. ATTD of CP. | -Increases ADG and GE. -Decreases P excretion. Manure odor emissions. Fat thickness of rib number 10. | Olukosi et al., 2017; Choe et al., 2017 |
| Amylase | Improves the digestibility of P. | -Decreases ADFI. | Olukosi et al., 2007 |
| Galactosidase | Improves ATTD of DM and N. | -Decreases ADFI. | Kim et al., 2013 |

AID: Apparent ileal digestibility; DM: Dry matter; GE: Gross energy; CP: Crude protein; ATTD: Apparent total tract digestibility; P: Phosphorus; ADFI: Average daily feed intake; G:F: Feed gain; ADG: Average daily gain; ADFI: Average daily feed intake; Ca: Calcium; N: Nitrogen.

The inclusion of combined-enzymes, used in the pig diet at any productive stage (Tables 5 – 7), showed an average in dry matter digestibility (g/kg) of 716.6 ± 139.9, 785.3 ± 59.8 and 811.5 ± 29.58 at weaning, growing and finishing productive stages, respectively, which target different antinutritional compounds in food to obtain the maximum benefit from the enzyme (Adeola & Cowieson, 2011). The effects of supplementing combined enzymes (Table 12) had no effect (P > 0.05) on ADG, ADFI, G: F, and DDM at all productive stages in the present study. However, it was observed that there is a reduction of 1.4% in the ADFI at weaning stage, when including combined-enzymes, resulting in an increase in the ADG of 4.9% and a better G: F ratio (1.6%). This effect was more visible at finishing stage, showing a better G: F ratio (8.0%) possibly due to a higher ADFI (4.6%) compared to the control group. The enzyme combination has led to better nutrient utilization, showing in all studies a reduction in ADFI, and a slight increase in DDM.

4. Conclusion

Nowadays, the use of combined enzymes in pig diets has been widely
### Table 6
Effects of supplementing exogenous enzymes in pig diets at growing productive stage on animal performance.

| Variable                        | Phytase | Xylanase | Mannanase | Protease | Combination of exogenous enzymes | Control |
|---------------------------------|---------|----------|-----------|----------|----------------------------------|---------|
| **Number of animals**           |         |          |           |          |                                  |         |
| Mean ± SD                       | 5       | 4        | 7         | 5        | 11                               | 23      |
| Min – Max                       | 120.8 ± | 39.5 ±   | 29.43 ±   | 24.16 ±  | 41.60 ± 6.69                     | 717.27 ± 9.69 |
| CV (%)                          | 144.7   | 1.74 ±  | 16.28 ±    | 16.09 ±  | 56.08                            | 148.26  |
| **Dosage of Enzyme in the diet, (g/kg)** |         |          |           |          |                                  |         |
| Mean ± SD                       | 1.34 ±  | 1.74 ±   | 2.90      | 1.14 ±  | 0.33                              | 0.84 ± 0.48 |
| Min – Max                       | 0.54 ±  | 0.21 ±   | 0.08      | 0.53 ±  | 1.61                              | 0.405 ± 1.37 |
| CV (%)                          | 65.93   | 167.0 ±  | 28.66     | 83.07    |                                  | 0.525 ± 6.795 |
| **Digestibility of Dry Matter, (%)** |         |          |           |          |                                  |         |
| Mean ± SD                       | 1.01 ±  | 0.16 ±   | 0.28      | 0.043 ± | 0.013                             | 0.05 ± 0.16 |
| Min – Max                       | 0.055 ± | 0.019 ±  | 0.58      | 0.018 ± | 0.005                             | 0.024 ± 0.065 |
| CV (%)                          | 59.89   | 171.89   | 31.42     | 43.51    |                                  | 0.053 ± 0.647 |

### Table 7
Effects of supplementing exogenous enzymes in pig diets at finishing productive stage on animal performance.

| Variable                        | Phytase | Xylanase | Mannanase | Protease | Combination of exogenous enzymes | Control |
|---------------------------------|---------|----------|-----------|----------|----------------------------------|---------|
| **Number of animals**           |         |          |           |          |                                  |         |
| Mean ± SD                       | 5       | 4        | 7         | 5        | 11                               | 23      |
| Min – Max                       | 120.8 ± | 39.5 ±   | 29.43 ±   | 24.16 ±  | 41.60 ± 6.69                     | 717.27 ± 9.69 |
| CV (%)                          | 144.7   | 1.74 ±  | 16.28 ±    | 16.09 ±  | 56.08                            | 148.26  |
| **Dosage of Enzyme in the diet, (g/kg)** |         |          |           |          |                                  |         |
| Mean ± SD                       | 1.34 ±  | 1.74 ±   | 2.90      | 1.14 ±  | 0.33                              | 0.84 ± 0.48 |
| Min – Max                       | 0.54 ±  | 0.21 ±   | 0.08      | 0.53 ±  | 1.61                              | 0.405 ± 1.37 |
| CV (%)                          | 65.93   | 167.0 ±  | 28.66     | 83.07    |                                  | 0.525 ± 6.795 |
| **Digestibility of Dry Matter, (%)** |         |          |           |          |                                  |         |
| Mean ± SD                       | 1.01 ±  | 0.16 ±   | 0.28      | 0.043 ± | 0.013                             | 0.05 ± 0.16 |
| Min – Max                       | 0.055 ± | 0.019 ±  | 0.58      | 0.018 ± | 0.005                             | 0.024 ± 0.065 |
| CV (%)                          | 59.89   | 171.89   | 31.42     | 43.51    |                                  | 0.053 ± 0.647 |

SD: Standard deviation; Minimum: Min; Maximum: Max; CV: Coefficient of variation.
studies are necessary to understand the interaction between diet composition, productive stage, origin of the enzyme, quantity, and although the latter are less frequently supplemented in pig diets. More enzymatic activities that can be carried out against antinutritive com-


doing the use of Mannanases and Xylanases, as well as Proteases, reported at all productive stages. Their use is due to the multiple enzymatic activities that can be carried out against antinutritive compounds in the diet, which can benefit animal performance. Phytases are the most supplemented enzymes at all productive stages of pigs, surpassing the use of Mannanases and Xylanases, as well as Proteases, although the latter are less frequently supplemented in pig diets. More studies are necessary to understand the interaction between diet composition, productive stage, origin of the enzyme, quantity, and number of added enzymes, since all those variables interfere with the mode of action and have specific effects at different productive stages. Although most research using exogenous enzyme supplementation in pig diets has shown to produce positive results compared to control diets, there not consistent improvements in growth, performance, and nutrient digestibility and this deserves further attention.

5. Authors contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: “Conceptualization, MGR, E.A., and E.V.-B.-P; methodology, MGR, E.A.A.; software, MGR; LERJ; E.A.A.; validation, MGR, E.V.-B.-P and LERJ; formal analysis, EAA, LERJ; investigation, EAA, LERJ, MGR; resources, MGR, JOA; data curation, MGR, E.A.A., LERJ; writing—original draft preparation, LERJ, MGR, E.V.-B.-P and EAA; writing—review and editing, LERJ, MGR, E.V.-B.-P; visualization, MGR; supervision MGR; project administration, MGR; funding acquisition, MGR. All authors have read and agreed to the published version of the manuscript”, please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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7. Availability of data and materials

At the request to the corresponding author.

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### Table 8

Effects of supplementing Phytases exogenous enzymes in pig diets at different growing stages on animal performance.

| Variable         | Weaning Control | Phytases | P-value | % Increment | Growing Control | Phytases | P-value | % Increment | Finishing Control | Phytases | P-value | % Increment |
|------------------|-----------------|----------|---------|-------------|-----------------|----------|---------|-------------|-------------------|----------|---------|-------------|
| Number of animals| 309             | 287      | 0.03    | 367         | 367             | 359      | 0.03    | 0.75        | 39 ± 0.75         | 0.03    | 0.75    | 0.03        |
| Dosage of Enzyme in the diet, (g/kg) | 2.51 | 2.49 | 2.43 | 2.50 | 3.97 | 3.91 | 0.03 |
| Dosage of Enzyme in the diet, (g/d) | 2.00 | 2.00 | 2.00 | 2.01 | 3.17 | 3.14 | 0.02 |
| Dosage of Enzyme in the diet, (g/kg) | 0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.001 | 0.01 |
| Initial body weight, (kg) | 9.11 | 9.10 | 0.83 | 0.8 | 20.69 | 20.72 | 0.82 | 0.1 | 44.95 | 39.90 | 0.73 | 11.2 |
| Average Daily Feed intake, (kg/d) | 4.99 | 4.98 | | 5.99 | 5.98 | | 9.46 | 9.43 |
| Average Daily Gain, (kg/d) | 0.71 | 0.71 | 0.93 | 0.0 | 1.44 | 1.47 | 0.85 | 2.1 | 2.06 | 1.97 | 0.84 | 4.3 |
| Gain: Feed, (kg/kg) | 0.21 | 0.98 | | 0.21 | 0.21 | | 0.35 | 0.34 |
| Digestibility of Dry Matter (g/kg) | 0.08 | 0.07 | | 0.07 | 0.08 | | 0.12 | 0.12 |
| Table 9

Effects of supplementing Mannanase exogenous enzymes in pig diets at different growing stages on animal performance.

| Variable         | Weaning Control | Mannanase | P-value | % Increment | Growing Control | Mannanase | P-value | % Increment | Finishing Control | Mannanase | P-value | % Increment |
|------------------|-----------------|-----------|---------|-------------|-----------------|----------|---------|-------------|-------------------|----------|---------|-------------|
| Number of animals| 32              | 32        | 0.85    | 243         | 215             | 106      | 0.85    | 0.75        | 70                | 0.85    | 0.75    | 0.85        |
| Dosage of Enzyme in the diet, (mg/kg) | 6.96 | 6.96 | 0.98 | 0.0 | 41.1 | 40.6 | 6.38 | 0.93 | 1.2 | 70.6 | 72.7 | 9.04 |
| Average Daily Feed intake, (kg/d) | 15.6 | 0.05 | | 6.39 | | | | |
| Average Daily Gain, (kg/d) | 0.56 | 0.54 | 0.82 | -3.6 | 2.12 | 2.09 | 0.20 | -1.4 | 2.58 | 2.60 | 0.28 |
| Gain: Feed, (kg/kg) | 0.05 | 0.05 | | 0.20 | | | | |
| Digestibility of Dry Matter (g/kg) | 0.09 | 0.08 | | 0.89 | 3.8 | | | |
| % Increment, compared with the control diet.

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For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used: “Conceptualization, MGR, E.A., and E.V.-B.-P; methodology, MGR, E.A.A.; software, MGR; LERJ; E.A.A.; validation, MGR, E.V.-B.-P and LERJ; formal analysis, EAA, LERJ; investigation, EAA, LERJ, MGR; resources, MGR, JOA; data curation, MGR, E.A.A., LERJ; writing—original draft preparation, LERJ, MGR, E.V.-B.-P and EAA; writing—review and editing, LERJ, MGR, E.V.-B.-P; visualization, MGR; supervision MGR; project administration, MGR; funding acquisition, MGR. All authors have read and agreed to the published version of the manuscript”, please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

6. Funding

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7. Availability of data and materials

At the request to the corresponding author.
### Table 10
Effects of supplementing Xylanases exogenous enzymes in pig diets at different growing stages on animal performance.

| Variable       | Weaning Control | Xylanases | P-value | % increment | Growing Control | Xylanases | P-value | % increment | Finishing Control | Xylanases | P-value | % increment |
|----------------|----------------|-----------|---------|-------------|----------------|-----------|---------|-------------|-----------------|-----------|---------|-------------|
| Number of animals | 98            | 98        | 0.001   | ±            | 0.08 ±         | 0.05     | ±       | 0.08 ±      | ±              | 0.01 ±    | ±       | 0.001 ± ±   |
| Dosage of Enzyme in the diet, (g/kg) | 0.50 | 0.49 | 0.74 | 0.75 | 0.07 | 0.07 |
| Dosage of Enzyme in the diet, (g/d) | ± | ± | ± | ± | ± | ± |
| Dosage of Enzyme in the diet, (g/kg) | 0.07 | 0.07 |
| Dosage of Enzyme in the diet, (g/LW<sup>0.75</sup>) | ± | ± | ± | ± | ± | ± |
| Initial body weight, (kg) | 2.35 | 2.35 | 1.40 | 1.40 | 0.94 | 0.94 |
| Average Daily Feed Intake, (kg/d) | 0.15 | 0.15 | 0.25 | 0.25 | 0.25 | 0.25 |
| Average Daily Gain, (g/kg) | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| Gain: Feed, (kg/kg) | 0.39 | 0.39 | 0.42 | 0.42 | 0.42 | 0.42 |
| Digestibility of Dry Matter, (kg/g) | 15.6 | 15.6 | 54.1 | 54.1 | 54.1 | 54.1 |

### Table 11
Effects of supplementing Proteases exogenous enzymes in pig diets at different growing stages on animal performance.

| Variable       | Weaning Control | Proteases | P-value | % increment | Growing Control | Proteases | P-value | % increment | Finishing Control | Proteases | P-value | % increment |
|----------------|----------------|-----------|---------|-------------|----------------|-----------|---------|-------------|-----------------|-----------|---------|-------------|
| Number of animals | 23            | 23        | 0.001   | ±            | 0.25 ±         | 0.01     | ±       | 0.01 ±      | ±              | 0.04 ±    | ±       | 0.001 ± ±   |
| Dosage of Enzyme in the diet, (g/kg) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Dosage of Enzyme in the diet, (g/d) | ± | ± | ± | ± | ± | ± |
| Dosage of Enzyme in the diet, (g/kg) | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Dosage of Enzyme in the diet, (g/LW<sup>0.75</sup>) | ± | ± | ± | ± | ± | ± |
| Initial body weight, (kg) | 16.3 | 16.3 | 16.3 | 16.3 | 16.3 | 16.3 |
| Average Daily Feed Intake, (kg/d) | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 |
| Average Daily Gain, (g/kg) | ± | ± | ± | ± | ± | ± |
| Gain: Feed, (kg/kg) | ± | ± | ± | ± | ± | ± |
| Digestibility of Dry Matter, (kg/g) | ± | ± | ± | ± | ± | ± |

% increment compared with the control group.

### Table 12
Effects of supplementing combination of exogenous enzymes in pig diets at different growing stages on animal performance.

| Variable       | Weaning Control | Multi-enzyme | P-value | % increment | Growing Control | Multi-enzyme | P-value | % increment | Finishing Control | Multi-enzyme | P-value | % increment |
|----------------|----------------|-------------|---------|-------------|----------------|-------------|---------|-------------|-----------------|-------------|---------|-------------|
| Number of animals | 283           | 283        | 1.32    | ±            | 0.001 ±         | 0.03     | ±       | 0.001 ±      | ±              | 0.001 ±    | ±       | 0.001 ± ±   |
| Dosage of Enzyme in the diet, (g/kg) | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 |
|Dosage of Enzyme in the diet, (g/d) | ± | ± | ± | ± | ± | ± |
|Dosage of Enzyme in the diet, (g/kg) | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 |
|Dosage of Enzyme in the diet, (g/LW<sup>0.75</sup>) | ± | ± | ± | ± | ± | ± |
| Initial body weight, (kg) | 4.90 | 4.90 | 4.90 | 4.90 | 4.90 | 4.90 |
| Average Daily Feed Intake, (kg/d) | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Average Daily Gain, (g/kg) | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |
| Gain: Feed, (kg/kg) | ± | ± | ± | ± | ± | ± |
| Digestibility of Dry Matter, (kg/g) | ± | ± | ± | ± | ± | ± |
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