

Review



## **Exogenous Enzymes as Zootechnical Additives in Monogastric Animal Feed: A Review**

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**Abstract:** Feed enzymes have been extensively used in livestock diets to enhance nutrient digestion and promote their growth performance. Indeed, recent research has indicated that feed enzymes, notably phytase, protease, and xylanase, function as catalysts, facilitating the breakdown of phytic acid, proteins, and  $\beta$ -1,4-xylan bonds and offering prospective advantages linked to the intestinal wellbeing and microbiota of young pigs and chickens. Various feed enzymes are currently being added to the diets of swine and broiler chickens. The potential enzymes used in the feed industry include cellulase,  $\beta$ -mannanase,  $\beta$ -glucanases, xylanases, phytases, proteases, lipases, and galactosidases. Though significant research has been conducted on phytase, protease, and xylanase, consistent findings, particularly in terms of improving nutrient digestibility and promoting growth performance of monogastric animals, are still limited. Also, the outcome of recent studies raises the question whether phytase and xylanase could play functional roles beyond increasing nutrient digestibility and intestinal health, such as positively modulating the intestinal microbiota and reducing environmental problems. Therefore, in this review we aimed to address the functional roles of exogenous enzyme activities in monogastric animal diets. Also, we sought to explore the advantages of these enzymes in enhancing the nutritional value of both alternative and conventional feedstuffs.

Keywords: exogenous enzyme; performance; nutrition; swine; poultry

### 1. Introduction

Sorghum has emerged as the primary cereal used in animal diets worldwide. However, the presence of various anti-nutritional elements, such as phytic acid and tannins, limits the use of sorghum in feed. Indeed, proteins, carbohydrates, and mineral elements can form complexes with phytic acid and tannins that help the animals to easily digest and absorb the nutrients [1,2]. The removal of anti-nutritional substances may enhance the nutritional value of sorghum and maximize its use in feeds. Since the 20th century, several enzymes have been commercially used in monogastric diets due to their favorable effects on the economy, environment, and human health. All animals need digestive enzymes to break down their food, and these enzymes can either be created by the animal itself or by the helpful bacteria that live in their gastro-intestinal tract. Animals are unable to digest 15–25% of the feed they consume, because the feed contains some undigestible components or the animals' bodies lack the specific enzyme to digest those particular feed ingredients [3]. In addition, anti-nutritional elements in feed are hard to digest and sometimes lead to intestinal distress. Feed enzymes act to release nutrients from feed constituents, such as carbohydrates, proteins, amino acids (AA), and minerals. The foremost enzyme used in monogastric diets is phytase as it accelerates the hydrolysis of phytate and releases phosphorus, which results in minimizing the need for adding pricey inorganic phosphorus sources. The second most common group is carbohydrates, which are primarily utilized in viscous diets with a high inclusion of wheat, barley, and rye compared to diets based on corn



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). and sorghum, with the aim of enhancing nutrient absorption and animal performance [4]. Other enzyme types and applications, such as the utilization of high phytase inclusion rates to lessen the anti-nutritional effects of phytate rather than concentrating just on the release of phosphorus, have recently been developed [5]. According to the Global Animal Feed Enzymes Market Report (LP Information, Inc., Rockville, MD, USA), the use of feed enzymes in animal diet is predicted to increase at a compound annual growth rate (CAGR) of 5.0% from 2022 to 2028 [6]. These statistics suggest enzymes are starting to play a bigger role in the animal feed industry.

In order to increase livestock production, it is necessary to feed the animals with nutritious and highly digestible feed [7]. Such animal feed should be based on cereals, forages, and silage, among other things, depending on the region, but the need to reduce the costs of feeding and animal production has prompted the search for new components. The utilization of agroindustry and agroforestry wastes is currently popular; however, they have drawbacks since the nutritional components are unbalanced or unavailable, necessitating the addition of grains, cereals, legumes, or additives in order to meet the health status [8]. Exogenous enzymes have been demonstrated to have beneficial effects on agro-industrial and agroforestry wastes, and they have been used as animal feed to improve the bioavailability and digestibility of minerals, as well as assisting in the removal of some anti-nutritional elements [9,10]. By specifying the described mechanisms of action and the positive effects of supplemental phytase, xylanase and protease, it was hypothesized that these enzymes may have functional implications related to the intestinal health of livestock. Thus, this review concentrates on the functional roles of exogenous enzyme activities in swine and poultry diets. Additionally, it seeks to explore the advantages of these enzymes in enhancing the nutritional value of both alternative and conventional feedstuffs.

#### 2. Exogenous Enzymes as Feed Additives

Energy and protein are the major nutrients in animal diet. The main energy and protein sources in swine diet are corn and soybean meal. In addition, barley, wheat, sorghum and oats are considered as energy ingredients that have higher fiber content than corn, while alternative protein sources are oilseeds such as rapeseed, canola, flaxseed, etc. Generally, corn and soybean meal (SBM) diets have relatively higher non-starch polysaccharides (NSPs) and anti-nutritional factors. These anti-nutritional factors pose challenges for the regular digestion of feed and lead to reduced meat and egg production, lower feed efficiency, and digestive disturbances. Feed enzymes function to enhance the availability of nutrients, such as starch, protein, amino acids, and minerals, from feed ingredients [3]. Traditional poultry diets mainly comprise grains such as corn, wheat, and SMB that contain 10% to 22.7% of NSPs; sometimes these NSPs lead to physiological impacts in chickens. Previously, Elangovan et al. [11] reported that quails fed dietary enzyme supplementation at various energy levels improved their growth rate over the growing period, while Shalash et al. [12] reported that the inclusion of multi-enzyme supplements containing xylanase, glucanase, protease, and amylase had no impact on growth performance. Similarly, Kocher et al. [13] reported that broiler-fed diets supplemented with exogenous complex enzymes (protease, xylanase, and amylase) had minimal impact on their growth performance. Exogenous enzymes are frequently added to the diets to help with nutrient digestion and the utilization of energy from animal feed in an effort to reduce negative effects [14,15].

In the context of animal nutrition, commercial enzymes can be divided into three main categories based on their intended function (Table 1). Phytase targets phytate molecules [16] released from phosphorus, while beta-glucanase and cellulases target NSPs and cellulose polysaccharides, respectively, effectively breaking down fiber into smaller components. On the other hand, proteases act on proteins, leading to enhanced digestibility. Lastly, alpha-amylase enzymes act as starch and improve digestion in animals [17].

Enzymes	Substrates	Effect	Example	References
Phytase	Phytates	Phytase degrades phytate bonds, liberating trapped nutrients, which leads to enhanced livestock efficiency. Additionally, it increases phosphorus absorption, reducing the risk of soil and water contamination through excreta. Moreover, phytase supplementation increases amino acid availability.	Histidine acid phytase (pH 5.0) mainly applied to feed for poultry or pigs.	Ojha et al. [10]
Proteases	Proteins	Certain proteases have been found to enhance the apparent ileal nitrogen digestibility and apparent nitrogen retention in both broiler chicks and broiler cockerels. When added exogenously, proteases can further enhance the digestibility of proteins in feed ingredients by solubilizing and hydrolyzing dietary proteins. As a result, levels of antinutritional factors decrease. These proteases can originate from animal, vegetable, or microbial sources.	Proteases isolated from microorganisms such as <i>Aspergillus niger</i> and <i>Bacillus</i> spp. Chymosin, pepsin A Bromelain, papain, ficine, aminopeptidase, bacillolysin 1, dipeptidyl peptidase III, chymotrypsin, subtilisin, trypsin.	Ghazi et al. [18]; Marsman et al. [19].
Carbohydrases	Carbohydrates (fiber and/or starch)	Exogenous enzymes, such as carbohydrases and proteases, improve the digestibility of plant biomass, leading to an increase in energy availability. This beneficial effect extends to both poultry and pig diets.	Xylanases and β-glucanases (degrade cell walls, used in poultry), β-mannanases Pectinases α-galactosidases α-amylase (improves digestibility of starch, body weight gain has been observed in poultry)	Nortey et al. [20]; Yin et al. [21].

#### Table 1. Enzymes used in animal feed processing.

### 3. Application of Phytase Additive in Monogastric Animal Nutrition

Enzymes were developed as feed additives in order to improve the digestion and absorption of nutrients. The first phytase products entered the market in 1991 [22] and were particularly used in swine, poultry, and fish diets [23]. The small intestine of monogastric animals has a limited capacity to break down phytate due to the absence of potent internal phytase enzymes and a relatively small number of microbes in the upper digestive tract. This phenomenon can also explain the low bioavailability of phosphorus from phytate [24,25]. The utilization of phosphorus in monogastric animals primarily relies on the absorption of orthophosphate, and their ability to utilize phytate phosphorus depends largely on their capacity to hydrolyze phytate. Earlier studies [26–29] have shown that the inclusion of microbial phytase in animal diets enhanced the utilization of phosphate from phytate, thereby improving overall animal nutrition. The addition of microbial phytase to animal diets has been demonstrated as the most effective strategy for the animal industry to reduce phosphate excretion in animal waste. It also enhances the availability of amino acids. The interaction between phytate and proteins can lead to changes in protein structure, which can negatively impact enzymatic activity, protein solubility, and digestibility [26]. However, the detrimental impact of phytate on monogastric animals has not been definitively established. Diverse perspectives exist regarding the impact of phytate on protein digestibility [30]. Some researchers [31] have suggested that phytate does not affect protein digestibility, while other researchers [32] have pointed out that amino acid's availability improves when phytate levels decrease. It has been proposed that the inclusion of phytase

enzymes could improve energy utilization in animals. For instance, the phytase enzymes derived from *Aspergillus niger*, *Peniophoralycii*, *Schizosaccharomyces pombe*, and *Escherichia coli* improved energy utilization in animals [22]. Currently, the phytase utilized in animal feeds is classified as histidine acid phytase. Phytase supplements should be cost-effective and exhibit resistance to temperature-induced inactivation during feed processing and storage, so that they can serve as suitable feed additive for livestock.

In monogastric animals, phytate is destroyed during the digestion process. To investigate the effects of intrinsic feed phytases and endogenous gut phytases on phytate hydrolysis, a diet containing barley, wheat, and soybean was tested. The presence of InsP1,2,3,4 and InsP5 were considered as the predominant plant-based intrinsic feed phytases (6-phytases), and they were responsible for phytate degradation in the stomach. Previously, Kemme et al. [33] found minimal phytate degradation in the stomach and proximal duodenum with a maize and soybean meal-based diet supplemented with low Ins-phytase activity (35 FTU/kg DM). The authors also observed significantly increased hydrolysis of intermediate inositol phosphates (InsP4 and InsP5) in pigs fed high levels of phytase supplementation. Similarly, Rapp et al. [34] found that diets with high intrinsic plant phytase exhibited greater phytate hydrolysis in the stomach compared to very low intrinsic phytase activity (43 FTU/kg DM). Some anti-nutritional elements derived from plant origins, including phytate, can lower animal performance [35]. Because of its prevalence in diets, phytate restricts the utilization of minerals like phosphorus, which is crucial for animal development. Additionally, it restricts the use of energy and the digestibility of amino acids not only because it binds to nutrients or prevents the access of digestive enzymes to the bolus, but also because of endogenous losses brought by its aggression towards the intestinal mucosa [2].

### 4. Application of Xylanases Supplementation in Swine

The digestive system of early-weaned pigs is not completely matured [36], making it less capable of effectively processing the transition from a milk-based diet to solid foods. Previously, Himmelberg et al. [37] stated that digestive enzymes are responsible for breaking down carbohydrates, proteins, and fats. Weaned pigs may experience decreased nutrient digestibility, which can negatively affect the integrity of the intestinal epithelial layer. However, the undigested starch and protein in the digestive tract can serve as substrates that promote the growth of pathogens [38]. These factors frequently lead to inadequate utilization of nutrients, compromised health, and impaired growth performance in young weanlings. To improve the nutrient digestibility of weanling pigs, exogenous enzymes, like xylanases [39], were incorporated in their diets. Xylanase is an enzyme that breaks down xylans, which are significant carbohydrate constituents present in the cell walls of various dietary ingredients commonly used in pig feed [40]. In an earlier study, Woyengo et al. [41] proposed that xylanases exert their effects through several potential mechanisms, including cell wall degradation, reduction in viscosity, and the provision of fermentable xylo-oligosaccharides. These actions collectively modify nutrient availability in the small intestine and result in increased growth performance and nutrient digestibility [42,43]. Feeding high levels of co-products has a greater risk compared with traditional diets. This risk can be minimized by using modern feed formulation, feed enzymes, and feed processing to attain predictable swine growth performance, carcass characteristics, and pork quality. For instance, increased energy digestibility in swine was observed with the inclusion of xylanase in a diet containing wheat co-products from flour milling having high NSP and arabinoxylan contents [20]. Also, the supplementation of xylanase in growing pigs' diets containing wheat co-products improved apparent total tract digestibility (ATTD) and ileal digestibility of dry matter (DM), crude protein (CP) and energy [21]. The addition of enzyme cocktails. such as xylanase and beta-glucanase, to energy-deficient diets containing distilled dried grain solubles (DDGS) as a grain coproduct has also been reported in improving the total tract digestibility of DM, GE and nitrogen [44]. Phytase supplementation increased the ATTD and standardized total tract

digestibility (STTD) of P and diminished P excretion in the feces of pigs fed diets containing rice co-products such as rice bran, rice feed mill and broken rice [45]. The inclusion of pectinolytic enzymes in rapeseed meals has been shown to have significant improvement in carbohydrate digestion [46]. Also, xylanase addition to rapeseed meal improved nutrient digestibility and growth performance in pigs [47]. However, supplemental xylanase does not always seem to improve the energy digestibility of DDGS, even though phytase does increase phosphorus digestibility [48]. Several studies failed to show the positive effects of carbohydrate enzyme supplementation in the diets containing DDGS of nursery pigs [49] and grower-finishing pigs [50,51]. Thus, more research is necessary to prove the exact mechanism of action.

#### 5. Application of Xylanase Supplementation in Poultry

Xylanases are categorized as carbohydrase and belong to the glycosyl hydrolase enzyme family. This classification implies that xylanases facilitate the hydrolysis of glycosidic bonds present in complex sugar compounds [52]. As such, xylanase plays a crucial role in catalyzing the hydrolysis of 1,4- $\beta$ -D-xylopyranosyl linkages present in xylan, which helps in reducing the anti-nutritional effects associated with this type of NSP [53]. Consequently, it aids in decreasing the digesta viscosity and releasing nutrients that would remain trapped. This increased accessibility of endogenous enzymes to their substrates results in improved nutrient digestibility and enhanced growth performance, providing significant benefits. Recently, more studies have been conducted evaluating the supplementation of xylanase for poultry, and they have been showing promising results, especially related to enhancements in nutrient digestibility and intestinal health [54,55]. The positive outcomes in growth performance and nutrient digestibility, along with the modulation of mucosa-associated microbiota in the jejunum and changes in jejunal oxidative stress and morphology parameters, can be attributed to the decrease in digesta viscosity and bulkiness. This reduction leads to the release of trapped nutrients and bioavailability of fermentable NSP-derived compounds. Previously, McCormick et al. [56] found a close link between fermentable non-starch NSP compounds and increased fragmentation in the xylan structure. These compounds are believed to include phenolic substances such as ferulic acid. Ferulic acid possesses antioxidant properties [57] and antimicrobial functions [58]. Research on the supplementation of xylanase in broiler chickens has consistently demonstrated positive effects, such as reduced digesta viscosity and improved nutrient digestibility [59]. However, it should be noted that broiler chickens might exhibit varying responses to the utilization of xylanase concerning intestinal health parameters.

# 6. Functional Role and Mode of Action of Protease Supplementation in Swine and Poultry

Gut health and growth performance of animals are significantly influenced by the balance of dietary proteins and amino acids [60]. Diets low in protein have the potential to make a profit by lowering the cost of feed, nitrogen excretion, and environmental impacts [61]. The nutrients in poultry feed that are relevant to the environment include crude protein and phosphorus [62]. Even after receiving all of their nutritional needs, broilers fed low-protein diets (more than 3%) showed slower developmental rates and poorer carcass composition [63,64]. According to Zulkifli et al. [65], broilers fed low-protein diets had lower growth performance under heat stress. Supplementing feed with enzymes can aggravate nutrient consumption of broilers [66,67]. Earlier studies showed that the utilization of dietary proteins and amino acids may enhance animal performance [68,69]. In order to maintain growth and improve the sustainability of poultry production, lowprotein diets with protease are suggested [70]. Protease supplementation enhanced amino acid digestibility, feed conversion, and intestinal integrity in broiler chicks [71]. Another investigation verified that adding proteases may change the substrates that are available for bacterial growth in the gut [72]. Supplemental protease's effects are still debatable. Protease supplementation in broilers and turkeys has been reported to improve [73], decrease [74], or have no effect [75] on AA digestibility. The impact of protease on amino acid digestibility varies depending on the product [76] and dosage [77].

Soybean meal is one of the major ingredients for pigs as a protein source, but it contains several anti-nutritional factors, such as lectins, oligosaccharides, haemagglutinin, goitrogenic factors, trypsin inhibitors, and antigenic proteins, that cause reduction in protein utilization of pigs by inhibiting the secretion of pancreatic digestive enzymes and increasing the loss of endogenous secretions of pigs [78-80]. Protein sources, such as soybean meal, used in pig diets are more expensive than other ingredients, and the pigs' inability to consume them drives up the cost of feed. Pigs' inability to metabolize protein contributes to environmental damage from their nitrogen emissions. The swine industry has therefore been exploring solutions to these problems. A method to increase nutrient availability and lower feed costs in the swine business may be to add dietary protease to pig diets. Dietary protease is commonly used to decrease the amount of protein that is not digested and help pigs better utilize their nutrients. Proteases have been routinely included in swine diets for many years as part of enzyme cocktails containing xylanases, cellulases, amlyases and glucanases [81–84]. The proteases promote the animal's usage of additional energy by efficiently separating the protein–starch links during digestion [27,85]. Dietary supplementation protease fed to broilers had a higher protein utilization than those fed a diet lacking a dietary protease, according to research by Adeola and Cowieson [27]. Zulkifli et al. [65] demonstrated that numerous proteases with various pH optimums and substrate specificities can enhance soybean meal digestion. This would increase the amount of protein consumed and utilized, enhance poultry physiology, and have a less environmental impact.

#### 7. The Application of Enzyme Cocktails in Swine and Poultry

Enzymes are commonly used in animal diets as supplements, and their physical effects are widely known. However, the precise mechanisms underlying their actions are still the subject of ongoing research. The application of enzyme cocktails has been utilized to establish and anticipate the ideal dosage of xylanase and  $\beta$ -glucanase in diets primarily composed of wheat and barley. Several attempts have been made to establish the optimal dosage of phytase in diets predominantly based on maize [86]. While it is widely established that adding supplemental carbohydrases, proteases, and phytases to poultry diets is effective, there remains a significant lack of clarity regarding the precise mechanisms through which exogenous enzymes exert their actions. The response to combinations of enzymes can be influenced by various factors, encompassing enzyme specificity towards the target substrate, dosage levels, interactions between different enzymes, quality and composition of ingredients, as well as the age of the animals. Several mechanisms have been suggested to elucidate the beneficial effects of glucanase in enhancing the energy and nutrient utilization of diets primarily based on wheat [87]. The effect of single- or multiple-enzyme supplementation on the growth and health performance in swine and poultry is summarized in Table 2.

Enzyme	Level	Animals	Effects	Reference
Phytase	1000 FYT/kg	Laying hens	Enhanced overall shell quality and a beneficial influence on reproductive hormones to sustain and support continuous egg production.	Eltahan et al. [88]
Phytase	250 ftu/kg and 500 ftu/kg	Broiler chickens	Reduction in nutritional levels improves bird performance	Lelis et al. [89]
Phytase	50 g/ton diet	Weaned pig	Improved ATTD of energy and protein; improved standard ileal digestibilty (SID) of histine	Pluske et al. [38]

**Table 2.** Effect of single or cocktail exogenous enzyme supplementation on health performance in swine and poultry.

Enzyme	Level	Animals	Effects	Reference
Phytase	500 units/kg diet	Growing pigs	Increased the ATTD and STTD of Ca and ATTD of P	Gonzalez-Vega et al. [90]
Phytase	1500 units/kg diet	Growing pigs	Increased digestibility of P in Canola meal, 00-rapeseed meal and 00-rapeseed expellers	Maison et al. [91].
Phytase	500 units/kg 250 units/kg diet	Growing pigs	Fecal output, as well as the output of Ca in feces, was reduced. Increased ATTD of Ca and P and increased STTD of Ca. Endogenous loss of Ca decreased. Daily P output reduced	Gonzalez-Vega et al. [92]
Phytase	1500 FTU/kg diet	Growing-finishing pigs	Increased body weight and the ATTD of P but no effects on meat quality	Dang and Kim et al. [93]
Xylanase	4000 unit/kg diet	Growing pigs	No effect on nutrient digestibility	Yanez et al. [48]
Xylanase	4000 unit/kg diet	Growing- finishing pigs	Improved apparent ileal digestibilty (AID) of energy and threonine in wheat but no improvement in growth performance	Widyaratne et al. [50]
Xylanase	45,000 XU/kg	Weaning Pigs	Enhanced growth performance and gut morphology, reduced digesta viscosity, and reduced intestinal oxidative stress	Duarte et al. [94]
Protease	125 g/ton	Finishing pigs	Improved growth performance and ATTD of nutrients and reduced stress-related hormones	Upadhaya et al. [95]
Protease	1 to 3 g/kg feed	Finishing pigs	Linear reduction in feed conversion during overall experimental period; linear increase in nutrient digestibility; linear reduction in serum total protein concentration	Liu et al. [96]
Protease	150–300 mg/kg	Weaned Piglets	Promoted nutrient absorption, improved small intestine morphology and enhanced digestive enzyme activity	Zhu et al. [97]
Phytase, Xylanase	250 and 500; 200 and 4000 units/kg diet respectively	Growing pigs	No effect of phytase on AID of amino acids, and xylanase improved AID of some AA	Woyengo et al. [41]
Cellulase + xylanase + beta-glucanase + protease	10,000, 6000, 5000 and 12,000 units/g respectively	Finishing Pigs	No effect on growth performance. Improved IgG and reduced malondialdehyde levels in serum in extruded RSM	Xie et al. [98]
Xylanase + glucanase + cellulase	2200, 1100 and 1200 unit/kg diet respectively	Finishing pigs	Increased AID of DM, organic matter, energy, threonine, proline and serine	Emiola et al. [99]
Multienzyme (beta-glucanase and beta-xylanase)	1 g/kg feed	Growing-finishing pigs	No effect on growth performance and carcass characteristics for both barley types	Prandini et al. [100]

Table 2. Cont.

STTD, standardized total tract digestibility; ATTD, apparent total tract digestibility; AA, Amino Acid, RSM, Rape seed meal; DM, Dry matter.

A single enzyme or an enzyme cocktail can be supplemented to an animal diet depending on particular needs [101,102]. For instance, the conventional utilization of xylanase, glucanase, phytase, and more recent multi-carbohydrase preparations, have been suggested for employing regular digestive tract enzymes as well [77,103–105]. The combination of different enzymes catalyzes a greater proportion of the entrapped nutrients from a diet, which eventually contributes to improved feed efficiency (Figure 1).



Figure 1. Benefits of exogenous enzymes on poultry health and digestibility.

Numerous studies examined the influence of nutrient restriction on leg abnormalities in broilers and found a decrease in leg problems when fed phytase supplements. It can be proposed that phytase additives heightened bird activity during a crucial phase in leg bone development [106,107]. Previously, Pintar et al. [108] observed that the addition of phytase in broiler diets led to enhanced levels of Fe and Mg in the tibia bone but had no significant impact on the concentrations of Ca, P, and Zn. Emiola et al. [99] demonstrated that the inclusion of an enzyme cocktail containing xylanase, beta-glucanase and cellulase in a diet containing 30% wet-distilled dried-grain soluble DDGS and 65% barley improved the ATTD of DM, N and energy in grower pigs. However, the inclusion of a 0.01% dietary mixture of protease and probiotics to a corn–soybean meal-based diet did not have any beneficial effects on growth performance and carcass characteristics in growing-finishing pigs [109]. The addition of enzymes has been found to confer better efficiency in diets containing relatively higher fiber such as barley, oats, triticale, peas, rye or wheat, whereas a corn–soybean meal-based diet is not suggested due to lower NSP contents.

An enzyme cocktail ( $\beta$ -glucanase, xylanase and protease) improved the total tract digestibility of crude protein and ileal energy in pigs fed hull-less barley-based diets [84]. In addition, Yin et al. [82] reported that enzyme cocktail (arabinoxylanase and protease) dietary supplementation improved the nutritional value of diets containing wheat bran or rice bran in growing pigs. Moreover, weaned pigs' growth performance and nutrient utilization were increased by dietary supplementation with enzyme cocktails that included proteases [83]. Though abovementioned studies showed beneficial effects, it is still unclear how much protease contributed to these enhancements. The exogenous enzymes and the mode of action on non-ruminant animals are presented in Figure 2. NSPs increase the viscosity of diets due to their capacity to bond to great amounts of water and to form a viscous gel, reducing the rate of substrate and digestive enzyme diffusion which provides a better litter quality.





Figure 2. Exogenous enzymes: a mode of action in non-ruminant animals.

The optimal dose of xylanase and  $\beta$ -glucanase enzymes in wheat- and barley-based diets has been determined or predicted using research findings to date, and efforts have been undertaken to set the equivalent dose for phytase in corn-based diets [86]. Although the benefits of adding more carbohydrases, phytases, and proteases to chicken diets are well known, it is still unclear how these exogenous enzymes work. The ranging of an enzyme to the target substrate, dosage, interactions between enzymes, ingredient quality, component composition, and animal age are just a few of the variables that might affect how an animal reacts to a combination of enzymes. The beneficial effects of glucanase in enhancing the energy and nutritional consumption of wheat-based diets have been explained by a number of mechanisms [87]. Carbohydrase in poultry diets may increase availability of endogenous enzymes to cell content hydrolysis of cell wall arabinoxylan [27] and increase viscosity, which encourages a healthier microbiota.

#### 8. Conclusions

The data from the literature summarized in this review article, including the utilization of exogenous enzymes as a feed additive in animal nutrition, has grown significantly in recent years. Over the past few years, extensive research has been carried out to investigate the impact of incorporating exogenous enzymes into swine and poultry diets. In general, the addition of phytase, xylanase and proteases to pig and broiler chicken diets has been shown to reinforce their nutritional significance by improving growth performance and nutrient digestibility. Moreover, these enzymes have demonstrated a functional benefit by reducing the oxidative stress response and potentially influencing the composition of the mucosal microbiota in the small intestine. In addition, inclusion of either individual enzymes or a combination of enzymes in animal diets that contain high-fiber ingredients, such as cereal and its by-products, can lead to enhanced growth performance and improved nutrient digestibility in the animals. In addition to the aforementioned benefits, enzyme supplementation, particularly with phytase and protease, has been found to play a significant role in reducing environmental pollution. By aiding in the breakdown of phytate compounds, these enzymes contribute to decreased excretion of phosphorus, nitrogen, and other minerals in animal waste. As a result, these nutrients become more readily available and accessible for the animals, minimizing their release as pollutants into the environment. Moreover, the ongoing trend in the field involves actively seeking novel sources of enzymes for utilization in animal feed, aiming to advance future research. This includes exploring

the modification of existing enzyme-producing strains to withstand the harsh conditions of the gastrointestinal tract, as well as exploring innovative techniques to encapsulate and safeguard enzymes. These aspects hold significant importance and warrant thorough investigation and application within the agroindustry to enhance animal feed production.

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