Use of Exogenous Enzyme in Animal Feed

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Abstract: The constant population increase and the continued need for feed of animal have originated rise in demand for livestock products and continuous research on the use of additives for growth. In last years, the addition of exogenous enzymes has become a good solution for improving animal performance, and it is most widely studied and reported disciplines in animal science showing rapid growth worldwide in the industry. Nowadays, consumer and producers increase knowledge concern to the use of secure growth promoters and antibiotics in livestock production attend these reasons; many researchers have been investigated the use of exogenous enzymes in ruminant and non-ruminant animal production. The aim of this paper is to review about exogenous enzymes add and their use on different animal livestock species in most recent years.

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The intensive and rapid grow on world population are caused on increased demands of animal origin. Meat and meat end-products consumption (including beef, pork, goat, mutton and poultry) has increased gradually, by almost 60% between 1990 and 2009; from 175,665 thousand to 278,863 thousand tons, driven in part by a growing world population (Henchion *et al*., 2014).

The need of methods for animal rapid growth has been derived in investigation for an increase the livestock production with the human food safety and animal welfare requirements. On this topic, animal nutritional researchers are in constant investment about improvements breeding strategies, based on improvement genomics (Rothschild and Plastow, 2014), or applied growth promoters, as well as the possible alternative strategies for their use in livestock feeding; nevertheless, the use of chemical substances like β-agonist whose increase lean meat to fat ratio and improve feed conversion efficiency it is actually illegal since the 90´s decade (Cayetano *et al.*, 2013; Johnson *et al*., 2014). For these reasons, many researchers were investigated the use of other additives for improving efficiency on animal livestock (Cedillo *et al*., 2014; Gado *et al*., 2014; Salem *et al*., 2014; Zhao and Kim, 2015), natural antioxidants (Rossi *et al*., 2014) and exogenous enzymes (Salem *et al*., 2013, 2015; Valdes *et al*., 2015).

The popular attention on the use of exogenous enzyme it is due to their capacity to increase the efficiency of digestion by help break down anti-nutritional factors (e.g. fiber, phytate and non-starch polysaccharide (NSP)) (Adeola and Cowieson, 2011; Alsersy *et al*., 2015), or do improve the digestibility of many ingredients, which remains low because of a lack of the enzymes needed for breaking down the complex cell wall structure that encapsulates nutrients presents in plant based feed.

The use of dietary exogenous enzymes on livestock production could be a promising strategy for improve animal digestibility and feed efficiency; however, many articles have been published every year in enzyme and animal nutrition disciplines, with counterpoised results, reveled new challenges, and opportunities for this topic research. The purpose of this review article summarizes current research regarding exogenous enzymes and their use on different animal livestock species for a show a general understanding on this topic.

Types of Enzymes Utilized in Animal Feed

Since the late 1980, feed enzymes have played a major part in helping to improve the efficiency of livestock products. The use of exogenous enzymes, specifically non-starch polysaccharidase and phytase, is almost ubiquitous in poultry and common place in pigs feeds because the inclusion of feed of plant origins, which compounds with low digestibility, considered anti nutritional factors such as NSP (Schedle *et al*., 2012) and phytic acid (Abudabos, 2012). However, recently many researchers have directed their work on other species like ruminants, actually exists many types of enzymes utilized in animal feed; most popular are shown in Table 1. Farm animals include diverse species; each of them possesses different physiological systems and forms to digest their food, the use of specific enzymes is explained below.

Table 1. Distinct Type of Enzymes and Action Mode

Type	Enzymes	action mode
	Xylanases (EC 3.2.1.8) β-glucanase (EC 3.2.1.4)	Added especially diets containing cereals rich in non-starch polisacharides NSP such as wheat, barley, and rye, reduced viscosity in the diet and digest (Bedford and Partridge, 2010).
Vegetal cell degrading enzymes	α- amylases (EC 3.2.1.1)	Rapid digestion of excessive amounts of starch present in different diets (Dilorenzo *et al*., 2011).
	β-mannanase (EC 3.2.1.78)	Degrade β-mannan, which is an antinutritional factor existing in many legumes, including soybean and canola meals (Mohammad and Torki, 2010).
	endoglucanase (EC 3.2.1.4) α-1-4-exoglucanase (EC 3.2.1.91) cellobiohydrolase (EC 3.2.1.91) α-glucosidase (EC 3.2.1.20) cellobiase (EC 3.2.1.21)	Increase potentially the hydrolysis of degradable fractions of neutral detergent fiber and acid detergent fiber (Gallardo *et al*., 2010).
Protein degrading	Protease (EC 3.4.21.62)	Increase protein digestibility, increased availability of amino acids present in animal diets (Yan *et al*., 2012).
Phosphatases	Phytase (3.1.3.8)	Increasing release of organic phosphorous present in plant cells, through hydrolysis of myo-inositol (phytic acid) (Abudabos, 2012).

Application of Exogenous Enzymes in Animal Farm.

Poultry

Nevertheless enzymes have been used extensively in poultry diets for performance in different species like turkeys, duck, and laying hens, broilers are received much more attention on research about enzyme addition because they cannot digest adequately NSP and subsequently the ingestion of high levels of soluble NSP leads to increased digest viscosity and reduced nutrient digestibility and absorption (Hajati, 2010), the works are focusing on diverse proposes like develop performance and nutrient digestibility when added to poultry diets containing cereals, improving indicators like body weight gain (27.9 gr/day -control- vs 32.2 gr/day-enzyme add) in broiler supplemented whit Endofeed® (Xylanase and β-glycanase) (García *et al*., 2008), dietary apparent metabolizable energy from 3588 to 3752 Kcal kg^{-1} with β-mannanase supplementation (Kong *et al*., 2011) and decreases on digests viscosity, increased nutrient retention, and consequently improved the performance of broilers fed supplemented with xylanase (Esmaeilipour *et al*., 2011).

Related to adding of phytase results from recent studies have shown increased digestibility on total phosphorus from 35.81 to 15.87% (Nourmohammadi *et al*., 2012), decreased tibia breaking strength around 10.2 kg less (28.8-18.6 kg) than the breaking strength at 28 days for birds fed with the standard diet (Shaw *et al*., 2010) and act synergistically with other enzymes for the availability of protein as a result of phytase addition to poultry diets. However, Francesch and Geraert (2009) obtained a decrease from 3.4% an average in the feed gain ratio, although reduction on feed intake in broilers supplemented with xylanase and phytase. The authors conclude that impairment could be attributed to the dietary energy and crude protein dilution applied or to the negative effect of the phytate on apparent metabolizable energy, ileal protein and amino acid digestibility, and endogenous amino acid flow.

It is important to mention than the enzyme application on broiler feed implicates considered other factors than enzyme type and diets. Figueiredo *et al*. (2012) realized an experiment whit 1-day-old chicks feed wheat-based diet supplemented with a commercial xylanase for 28 days. Significant differences in body weight emerged at 21–28 days of age, suggesting that the response to xylanase supplementation occurs primarily during the latter stages of broiler growth, showing that the age of broiler is an important factor for good results. Other factors less considered, is the size of particle of feed. Jia and Slominski (2010) compared flax seeds diets (whole, grinding and pellet) in broilers supplemented with cellulase, mannanase, pectinase, xylanase and glucanase.

Pelleting showed more pronounced and beneficial effects on growth performance, particularly when whole seeds were used, indicating a potential for using whole flaxseed in the pelleted diets. Enzyme addition resulted in an increase on the total tract fat digestibility by 3 to 6%, which was reflected in an improved feed conversion ratio by 1 to 3%, regardless of the processing method used; the authors conclude that physical rupture of the seed and disruption of cell wall structure via diet pelleting, and the addition of NSP-degrading enzyme offers practical solutions to improve the nutritive value of flaxseed in poultry feed.

Other works about exogenous enzyme addition on poultry performance (Table 2), mentioned the improvements in different indicators, with the use of exogenous enzymes. Only a few papers are reference to egg. Wen *et al.* (2011) proved phytase, xylanase, cellulase, α-amylase and acidic protease on egg production, nutrient retention, digestive enzyme activities and pancreatic enzyme. Poultry fed diets supplemented with enzymes tended to have higher lipase and protease activity in duodenal and jejunal digest, but did not affect productive performance of laying hens in the late phase of egg production. Similar results were founded by Kashani *et al.* (2014), who not observe any effect on egg quality parameters with addition of phytase on laying hens.

Pigs

Regarding of pig exogenous enzymes have been applied on diets for different proposes; like diminish the manure odor emissions measured in European odor units (OuE) in the barley diet from 5,543.2 - 4,426.1 OuE/m^3 (72 h) and oats from 2,366.2 - 1,993.0 OuE/m^3 (72 h) (O´Shea *et al.*, 2010); improvement in bone development. Varley *et al.* (2011) founded a linear increase in bone ash ($P<0.01$), bone calcium ($P<0.01$) and bone phosphorous ($P<0.05$) concentrations at 33 kg body weight in pigs feed with phytase, and improvement on nutritive value of vegetable feedstuffs contained a considerable amount of NSP (De Lange *et al.*, 2010). Wang *et al.* (2008) observed improvement in daily gain of growing pigs fed the rough rice-based diet supplemented with enzymes showing increased by 10.1% ($P<0.05$) and feed: gain improved by 9.4% ($P<0.05$) compared to the control group.

However, unlike the poultry industry in pig, it is livestock has been more cautious in accepting feed enzymes because the pig response is less consistent compared with the poultry. In pigs, passage through the digestive tract is much slower than poultry; time that food stays on a small intestine allow nutrient better absorption (Bedford and Partridge, 2010) which diminish the observable effects for enzyme added.

Previous research has found that carbohydrates in wheat, particularly starch and the non-digestible carbohydrate components such as NSP, influence energy digestibility (Kim *et al.*, 2008). Supplementation of pig diets with NSP-degrading enzymes improved the utilization of these compounds, but not all works are consistent (Table 3).

Table 2. Different Researches About Enzyme Addition on Poultry Diets.

Enzyme	**Diet**	**type of poultry**	**Improvement over control**	**Reference**
Lipase and phospholipase	Sunflower seeds	Broiler	Increase on BWG	Brenes *et al*. (2008)
Multicarbohydrase	Wheat – soy bean meal	Broilers	Growth improved , health promoter	Jia *et al*. (2009)
β-mannanase	Guar meal	Laying hens	Increased FCR	Mohammad and Torki (2010)
β-mannanase	Corn-soybean meal	Broilers	Improve CTTAD	Kong *et al*. (2011)
xilanases	Corn- wheat	Laying hens	Improve the digestion of xylans and arabinoxylans	Cufadar *et al*. (2010)
multi-enzyme	Multi ingredient diet	Broiler	Improved relative growth, energy efficiency and protein efficiency	Hajati (2010)
β-mannanase	Corn-soybean meal	Broiler	Increase CTTAD	Li *et al*. (2010)
Natuzyme®	Multi ingredient diet	Broiler	Improved the feed conversion ratio	Khalaji *et al*. (2011)
Protease	Corn–soybean	Broiler	Improve content in protein digestibility	Angel *et al*. (2011)
Microbial phytase	Corn-soybean meal	Broiler	Increased ileal digestibility of nutrients, increasing on mineral contents in tibia ash	Nourmohammadi *et al*. (2012)
β-mannanase	Whole	Broiler	Improved BWG and feed conversion ratio	Zangiabadi and Torki. (2010)
Thermotolerant xylanase	Wheat and soybean meal	Broiler	Improved performance	Masey *et al*. (2012)
xylanase, β-glucanase, and phytase	Corn-soybean	Broilers	Reduction of phytate	Francesch and Geraert (2009)
Protease	Corn, soybean	Poultry	Increase protein digestibility	Yan *et al*. (2012)
Natuzyme®	Sorghum –Wheat	Broilers	Improve IDE 1.5%- 2.9%	Sultan *et al*. (2012)
Phytase	Yellow corn and soybean	Laying hens	Increasing release of organic phosphorous	Abudabos (2012)

Ileal digestible energy (IDE), coefficient of total tract apparent digestibility (CTTAD), body weight gain (BWG), feed conversion ratio (FCR).

Woyengo *et al*. (2008) were conducted an experiment with the addition of xylanase and phytase on wheat based diets, but there were no dietary effects on growth performance. Phytase increased the apparent total tract digestibility of phosphorus and calcium by 51 and 11% at 20 kg of body weight (BW) or by 54 and 10% at 60 kg of BW, respectively, but increasing the level of phytase and the apparent ileal digestibility (AID) of phosphorus and calcium by 21 and 12%. The addition of xylanase increased AID of lysine, leucine, phenylalanine, threonine, glycine, and serine. Phytase improves digestibility of phosphorus and xylanase enhances amino acid digestibility, but no interaction between the two enzymes was noted. The authors, concluded that phytase supplementation to wheat based diets for growing pigs improved phosphorus, and calcium digestibility, but they cannot influence on grow performance.

Adeola and Cowieson in 2011, reviewed investigation work about NSP-degrading enzyme addition on growth swine, and report variable responses on body weight gain (BWG). The differences could be explained for type and quantity of cereal grains used the extent of deficiency of limiting nutrient, and the extent to which the enzyme increased digestible nutrient content.

Enzyme supplementation on pigs' diets depends of the survivor of the enzyme in digestive tract, age of swine or the synergism between different enzyme types (Emiola *et al*., 2009a), for example; the gastrointestinal tract of a piglet is a complex environment. Opposite adult pigs, the piglet digestive system is immature and is more susceptible to suffer affectations and quickly alters in digestive and immune. The weaning of piglets usually takes place between 3 and 4 week of life, when the majority of nutrients are ingested with milk. Poor digestion of solid feed by piglets weaned at 21-28 of age is especially obvious in the first 2-3 weeks after weaning inappropriate choice of feed ingredients at this time can be produced diarrhea (Bedford and Partridge, 2010).

In this case, works realized with addition of exogenous enzymes founded good responses in the treatments applied. Tapingkae and Yachail (2008) diminished the incidence of diarrhea in weanling piglets from the 3-4 week post weaning, with a diet based on local ingredients and multi-enzyme treatments. In the same way, Zhou *et al*. (2011) proved the inclusion of enzymolitic soy bean (ESBM) on piglet diets; the inclusion of ESBM at 5 to 15% increased the digestibility of crude protein (CP) by 5 to 16%, and ESBM at 15% increased the digestible energy (DE), calcium and phosphorus compared with the control diet. The results suggest that ESBM can be a better protein source in improving growth performance, nutrient digestibility and immune function of weaned piglets. Owusu-Asiedu *et al*. (2010) measured the effect of xylanase in piglet´s wheat-barley based-diets. On average, piglets grew 525 g/day, consumed 845 g/day, and exhibited mean feed conversion ratio (FCR) of 1.59. Weight gain and feed intake did not differ among treatments; however, FCR for the overall period was better for pigs fed diets supplemented with 200 g/MT enzyme compared with those fed the control diet.

Table 3. Different Works of Enzymes Addition on Pig Diets.

Enzyme	Diet	Age of swine	Observation	Reference
Carbohydrases phytase	Corn, wheat-middling, soybean, canola	Weanling-finisher	Improved ADG in pigs whit 10-kg of body weight but not in pig with 23-kg , improving phosphorus digestibility in pigs of all ages	Olukosi *et al*. (2007)
Non-starch polysaccharides (NSP)enzymes	Rough rice	Growing Pigs	Improved performance of pig reduced viscosity and increased digestive activity in the small intestine.	Wang *et al*. (2008)
β-glucanase	Barley-oat	Finishing Pigs	Increased manure ammonia on oat diets, not influence on the oat-based diets.	O´Shea *et al*. (2010)
Phytase	Wheat-barley-soya	Weanling	Improved Ca and P digestibility feed conversion ratio and bone mineralization at 33 kg BWG.	Varley *et al*. (2011)
Carbohydrase	Palm kernel meal	Growing Pigs	Improve the growth performance and energy and nutrient digestibility's	Ao *et al*. (2011)
Easyme Mixer®, Hemicell-W®, Porzyme 93010®	Dried distillers grains	Nursery	Not improve growth performance	Jones *et al*. (2011)
Axtra® XB,	Mixed grains	Fattening pigs	Not improve growth performance	Kiarie *et al*. (2012)
Multicarbohydrase	Distillers dried grains	Finishing Pigs	Improved growth performance and apparent total tract digestibility	Emiola *et al*. (2009b)

Body weight gain (BWG); Average Daily Gain (ADG)

The variability in the responses founded in diverse woks implicated the needs for more research into the mode of action, survivor, degree of breakdown and mixed types of exogenous enzymes in a digestive tract, for pig growth improvement.

Ruminants

Cellulose and hemicellulose are quantitatively the most important structural carbohydrates present in ruminant diets (Khattab *et al*., 2013; Kholif *et al*., 2014). Rumen microorganisms produce enzymes that catalyze their hydrolysis, but the complex network formed by structural carbohydrates and lignin can reduce their digestibility and restricts efficient utilization. Fiber degradation in the rumen is not optimal, since the fiber fraction recovered from feces is fermentable (Gallardo *et al*., 2010).

The use of exogenous fibrolytic enzymes holds promise as a means of increasing forage utilization and improving different parameters in ruminal species. Several studies have reported increases in enhancing in different responses (Khattab *et al*., 2011). Togtokhbayar *et al*. (2015) tested xylanase and cellulose in vitro ruminal degradation, all enzymes tested still increased the degradability of substrate acid detergent fiber and the production of acetate, propionate and total volatile fatty acids (VFA). Gado *et al*. (2009) proved the commercial enzyme complex ZADO® in dairy cows, founded increases on milk and milk protein production 12.8–15.7 and 0.45–0.57 kg/day respectively. Pinos-Rodríguez *et al*. (2008) improving ruminal disappearance rates of dry matter (DM) in lambs. Carreón *et al*. (2010) increased potentially disappearance fractions and total disappearance of DM and starch on steers.

Although studies showed improved animal performance with enzyme treatment, others not show effects, like Phakachoed *et al*. (2012) reported that dry matter intake, ruminal pH, in sacco ruminal disappearance of feedstuff (rice straw), and hemicellulose degradability were unaffected by xylanase treatment. Their inconsistent response is due to a lack of standardization of methodology used to assay enzyme activity of ruminant feed enzymes. The resulting enzymatic activity on ruminal environment is affected by conditions like the substrate used, temperature, and pH.

These inconsistencies might be attributable to different factors like mode of application or the portion of the diet to which the enzyme was added. The methods of the enzyme mixture application are possible responsible for inconstancies responses (Khattab *et al*., 2011). Quantities of doses are an important factor for a different responses (Phakachoed *et al*., 2012) reported that dose response was an enzyme dependent for most variables, for example, increase a similar result were found by Avellaneda *et al*. (2009) who not observe effects on dry matter intake, nitrogen balance, ruminal degradation, total tract digestion, ruminal fermentation, as well as ruminal protozoal count with exogenous fibrolytic enzyme treatment. Seed fiber disappears in proportion to the increase in enzyme concentration.

Nevertheless, the inconsistencies of the results obtained with exogenous enzyme addition ruminal species, recent investment is applied for prove other non-conventional feedstuffs. Awawdeh and Obeidat (2011) improve nutrient digestibility and increases on feed intake and growth on lamb performance feed with olive by product derived of olive oil production (residue obtained after the pressure extraction of oil from the entire olive fruit and contains skin, crushed pulp, stone wall and kernel). Salem *et al*. (2013) applied enzyme on crossbred steers diet in order to evaluated the effects of exogenous enzyme (ENZ) addition on nutrient intake, digestion, ruminal fermentation and feed conversion in beef steers. The magnitude of improvement in digestibility varied among nutrients, with the highest improvement occurring in digestibility of NDF and ADF (21.8% and 26.7%, respectively). Addition of ENZ also increased ($P<0.05$) concentrations of rumen ammonia N and total short chain fatty acids (SCFA) before and 3 h post-feeding

Enzyme addition did not affect ($P=0.1$) DM intake, whereas it increased ($P<0.05$) total tract apparent digestibility of all nutrients. The magnitude of improvement in digestibility varied among nutrients, with the highest improvement occurring in digestibility of NDF and ADF (21.8% and 26.7%, respectively). Addition of ENZ also increased ($P<0.05$) concentrations of rumen ammonia N and total short chain fatty acids (SCFA) before and 3 h post-feeding. Allantoin concentration total purine derivates were increased ($P=0.04$) with enzyme addition while uric acid was not affected ($P=0.05$). Live-weight gain was also higher ($P<0.01$) in steers supplemented with ENZ. In conclusion, adding the exogenous enzyme product increased live-weight gain by 16% due to increased nutrient digestibility.

Another research about the employ of exogenous enzyme for improving nutrient digestibility is applied in the region with forages with low levels of nutrients like tree and shrub. Salem *et al*. (2012) proved the effect of sun-drying an enzyme adds in leaves of desert shrubs showed that there are beneficial impacts of sun-drying and/or dietary exogenous enzyme addition for sheep fed leaves. The authors attributed the best response to the enzyme preparation may have stimulated and/or increased total viable rumen bacterial numbers because rumen microbial nitrogen synthesis was increased; which may be due, at least in part, to increased fiber digestion and an improved capacity of rumen bacteria to digest feed and degrade secondary metabolites present in the diet (Kholif and Aziz, 2014).

Alsersy *et al*. (2015) reported the effects of feeding *Atriplex halimus* (AH) silage treated with two developed enzyme cocktails to sheep on feed intake, nutrient digestibility and ruminal fermentation. Ensiling AH with enzymes reduced its contents of neutral detergent fiber and acid detergent fiber. The dry matter intake of AH of AH silage treated with ZAD1® and barley (D2), AH silage treated with ZAD2® and barley (D3) and AH silage treated with a combination of ZAD1® and ZAD2® (1:1) and barley (D4) decreased ($P < 0.001$) as compared to AH silage and barley (D1). However, enzyme-treated diets had greater total digestible nutrients intake ($P < 0.001$) as compared to D1. The nutrients digestibility for D2, D3 and D4 were higher than those for D1 ($P < 0.001$), and were higher for D3 as compared to both D2 and D4. Sheep fed on D3 had highest ($P < 0.001$) ruminal total volatile fatty acids concentration.

However, not only fibrolytic enzymes are applied in ruminal feed, phytase and their role like an additive are less studied. In no-ruminant animals like a pig or poultry, phytate is not fully utilized, and this resulting excretion of phosphorus contributes to phosphorus pollution. In contrast, the rumen is a source of highly active phytase, and thus ruminants can use phytate as a source of phosphorus (Bedford and Partridge, 2010). Buendia *et al*. (2010) proved the inclusion of phytase in sorghum diets on lamb digestion and pollutants excretions, and conclude that the enzymes not modified in vitro kinetic characteristics of diets and did not diminish the negative impact on the environment.

Inclusion of exogenous enzymes in ruminants needed more investigation of the mode and level of application on ruminant diets. However, there is evidence that fibrolytic enzymes only enhance for stomach digestion and fermentation of fiber.

Other Species: Aquaculture.

Nevertheless, the aquaculture it is not considered in livestock products fish farming is the fastest-growing sector of world food production (Soler-Villa *et al*., 2009). In recent years, aquaculture has been relatively slow adopting feed enzyme technology. Normally, research in carnivorous fish nutrition focuses on the replacement of the fish meal with plant-based protein ingredients in order to support the globally expanding aquaculture (Dalsgaard *et al*., 2011).

The looking for replacing a fish meal by cheaper and more sustainable protein sources is essential for reducing the cost of fish feeds. In order to develop sustainable, economical, and viable aquaculture production; alternate sources of high-quality proteins must be identified to replace unavailable high-cost fish meal. Various plant protein sources such as soybean, corn gluten, cotton seed and canola, are major candidates for the substitution of the fish meal in fish diets due to availability and low cost (Soler-Villa *et al*., 2009). However, carnivore fish such as trout and salmon have a high protein requirement and do not have the capacity to utilize energy from complex fibrous carbohydrates, for these reason exogenous enzymes are frequently used to increase the nutritive value of feed ingredients of plant origin and improve growth performance.

Denstadli *et al*. (2011) tested the pretreatment with carbohydrates in three different diets (sunflower, soybean meal and whole) in rainbow trout *Oncurhynchus mykiss* diets concluding than the enzyme add has significant effects on reductions in total NSP in vitro, but the released substrates did not contribute to an improvement in fish performance under the current experimental conditions. Contrary to these authors, Imanpoor and Bagheri (2012) proved soy bean meal diet with the addition of phosphorous and microbial phytase in Persian sturgeon *Acipenser persicus* founded that the soy bean meal could be partly an alternative protein source if phosphorous supplied for fish by incorporation with microbial phytase or phosphorous.

Future research on exogenous enzymes in fish feed could focus on effects of enzymes on amino-acid availability, because in fish feed, it is normally used high protein content stuffs like a fish meal (FM) for the favorable amino acid profile (Shina *et al*., 2011). Inclusions of proteases which can promote the release of amino-acids or diminish the protease inhibitors presents in fish diets could be a good response in fish growth.

Conclusion

The inclusions of exogenous enzyme in animal feed are a vast area of research, and promise turn is a good solution for the use of safe additives in animal nutrition. Mostly of investments were demonstrated good responses in the increases of nutrient digestibility, health and improved animal performance. However, before the inclusion of enzymes in any diet, it is necessary considered many factors than can affect the response in animal growth, at this point it is clear the need to continue to clarify the mode of action in the different pathways in animal digests, for the purpose to development new products or methods specifically to improve feed digestion through the exogenous enzyme addition.

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References

1. Abudabos AM. Phytate phosphorus utilization and intestinal in laying hens. Italian Journal of Animal Science 2012;11:41–46.
2. Adeola O, Cowieson AJ. Opportunities and challenges in using exogenous enzymes to improve non ruminant animal production. Journal of animal science 2011; 89(10):3189-218.
3. Alsersy H, Salem AZM, Borhami BE, Olivares JG, Ado HM, Mariezcurrena MD, Yacuot, MHK, Holif AE, El-Adawy M, Hernandez SR. Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. Animal Science Journal 2015;86:51–58.
4. Angel C, Saylor W, Vieira S, Wards N. Effects of a mono component protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poultry Science 2011;90:2281–2286.
5. Ao X, Zhou T, Meng Q, Lee J, Jang H, Cho J, Kim I. Effects of a carbohydrase cocktail supplementation on the growth performance, nutrient digestibility, blood profiles and meat quality in finishing pigs fed palm kernel meal. Livestock Science 2011; 137(1):238-243.
6. Awawdeh M, Obeidat B. Effect of supplemental exogenous enzymes on performance of finishing Awassi lambs fed olive cake-containing diets. Livestock Science 2011; 138:20-24.
7. Bedford M, Partridge G. Enzymes in farm animal nutrition 2nd edition, © CAB International, 2010. ISBN-13: 978 1 84593 674 7.
8. Brenes A, Centeno C, Viveros A, Arija I. Effect of Enzyme Addition on the Nutritive Value of High Oleic Acid Sunflower Seeds in Chicken Diets. Poultry Science 2008;87:2300–2310.
9. Buendia G, Mendoza G, Pinos-Rodriguez J, Gonzalez-Muñoz S, Aranda E. Influence of supplemental phytase on growth performance, digestion and phosphorus balance of lambs fed sorghum-based diets. Italian Journal of Animal Science 2010;9:187-189.
10. Carreón L, Pinos-Rodríguez JM, Bárcena R, González SS, Mendoza G. Influence of fibrolytic enzymes on ruminal disappearance and fermentation in steers fed diets with short and long particle length of forage. Italian Journal of Animal Science 2010;9(1):e17.
11. Cayetano JA, Salem AZM, Mariezcurrena BMA, Rojo R, Cerrillo-Soto MA, Gado H, Camacho LM. Effect of Adding Salix babylonica Extracts and Exogenous Enzymes to Basal Diets on the Meat Quality of Growing Suffolk Lambs. Anim Nutr Feed Technol 2013; 13, 373-380.
12. Cedillo J, Vázquez-Armijo JF, González-Reyna Salem AZM, Kholif AE, Hernández-Meléndez J, Martínez-González JC, Jiménez RM, Rivero N, López D. Effects of different doses of *Salix babylonica* extract on growth performance and diet in vitro gas production in Pelibuey growing lambs. Ital J Anim Sci. 2014;13:609-613.
13. Cufadar Y, Yildiz Ö, Olgun O. Effects of xylanase enzyme supplementation to corn/wheat-based diets on performance and egg quality in laying hens. Canadian Journal of Animal Science 2010;90(2):207-212.
14. Dalsgaard J, Verlhac V, Hjermitslevc N, Ekmannc K, Fischer M, Klausen M, Pedersen P. Effects of exogenous enzymes on apparent nutrient digestibility in rainbow trout (Oncorhynchus mykiss) fed diets with high inclusion of plant-based protein. Animal Feed Science and Technology 2011;171:181-191.
15. De Lange, CFM, Pluske J, Gong J, Nyachoti CM. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 2010;134(1-3):124–134.
16. Denstadli V, Hillestad M, Verlhac V, Klausen M, Øverland M. Enzyme pretreatment of fibrous ingredients for carnivorous fish: Effects on nutrient utilization and technical feed quality in rainbow trout (< i> Oncurhynchus mykiss</i>). Aquaculture 2011;319(3):391-397.
17. Dilorenzo N, Smith D, Quinn M, May M, Ponce C, Steinberg W, Engstrom M, Galyean M. Effects of grain processing and supplementation with exogenous amylase on nutrient digestibility in feed lot diets. Livestock Science 2011;137:178-184.
18. Emiola A, Akinremi O, Slominski B, Nyachoti M. Nutrient utilization and manure P excretion in growing pigs fed corn-barley-soybean based diets supplemented with microbial phytase. Animal Science Journal 2009(a);80:19–26.
19. Emiola A, Opapeju F, Slominski B, Nyachoti M. Growth performance and nutrient digestibility in pigs fed wheat distillers dried grains with solubles-based diets supplemented with a multicarbohydrase enzyme. Journal of Animal Science 2009(b); 87:2315-2322.

20. Esmaeilipour O, Shivazad M, Moravej H, Aminzadeh S, Rezaian M, Van Krimpen MM. Effects of xylanase and citric acid on the performance, nutrient retention, and characteristics of gastrointestinal tract of broilers fed low-phosphorus wheat-based diets. Poultry science 2011; 90(9):1975–82.
21. Figueiredo A, Correia B, Ribeiro T, Ponte P, Falcão L, Freire J, Prates J, Ferreira L, Fontes C, Lordelo M. The effects of restricting enzyme supplementation in wheat-based diets to broilers. Animal Feed Science and Technology 2012;172:194-200.
22. Francesch M, Geraert P. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poultry Science 2009;88:1915–1924.
23. Gado HM, Almustafa SS, Salem AZM, Khalil FA, Abdalla EB. Influence of Vitamins and Exogenous Enzymes Combination on alleviating Heat Stress in lactating Ewes under Egyptian Summer Conditions. Anim Nutr Feed Technol 2014; 14: 195-203.
24. Gado H, Salem AZM, Robinson P, Hassan M. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Animal Feed Science and Technology 2009;154:36-46.
25. Gallardo I, Bárcena R, Pinos-Rodríguez J, Cobos M, Carreon L, Ortega M. Influence of exogenous fibrolytic enzymes on in vitro and in sacco degradation of forages for ruminants. Italian Journal of Animal Science 2010;9:34-38.
26. García M, Lázaro R, Latorre M, Garcia M, Mateos G. Influence of Enzyme Supplementation and Heat Processing of Barley on Digestive Traits and Productive Performance of Broilers. Poultry Science 2008;87:940–948.
27. Hajati H. Effects of Enzyme Supplementation on Performance, Carcass characteristics, Carcass Composition and Some Blood Parameters of Broiler Chicken. American Journal of Animal and Veterinary Sciences 2010;5(3):221-227.
28. Henchion M, McCarthy M, Resconi VC, Declan T. Meat consumption: Trends and quality matters. Meat Science 2014;98(3):561–568.
29. Imanpoor M, Bagheri T. Effects of replacing fish meal by soybean meal along with supplementing phosphorus and magnesium in diet on growth performance of Persian sturgeon, *Acipenser persicus*. Fish Physiology Biochemistry 2012;38(2):521-528.
30. Jia W, Slominski A, Bruce H, Blank G, Crow, Jones O. Effects of diet type and enzyme addition on growth performance and gut health of broiler chickens during subclinical Clostridium Perfringens challenge. Poultry Science 2009;88:132–140.
31. Jia W, Slominski B. Means to improve the nutritive value of flaxseed for broiler chickens: the effect of particle size, enzyme addition, and feed pelleting. Poultry science 2010;89(2):261–269.
32. Johnson BJ, Smith SB, Chung KY. Historical Overview of the Effect of β-Adrenergic Agonists on Beef Cattle Production. Asian-Australasian Journal of Animal Sciences 2014; 27(5):757-766.
33. Jones C, Bergstrom J, Tokach M, De Rouchey J, Goodband R, Nelssen J, Dritz S. Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with solubles for nursery pigs. Journal of Animal Science 2011;88(6):2084-2091.
34. Kashani S, Mohebbifar A, Habibian M, Torki M. Effects of Phytase Supplementation of Low Protein Diets on Performance, Egg Quality Traits and Blood Biochemical Parameters of Laying Hens. Annual Research & Review in Biology 2014;4(4):684-698.
35. Khalaji S, Zaghari M, Shivazad M. Estimation of standardized ileal threonine equivalency values of a multi-enzyme and its effects on broiler chick's performance. Italian Journal of Animal Science 2011;10.
36. Khattab HM, Gado HM, Kholif AE, Mansour AM, Kholif AM. The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. Inter J Dairy Sci. 2011;6:267-277.
37. Kholif AE, Khattab HM, El-Shewy AA, Salem AZM, Kholif AM, El-Sayed MM, Gado HM, Mariezcurrena MD. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with *Pleurotus ostreatus*. Asian Austral J Anim Sci. 2014;27:357-364.
38. Kholif AM, Aziz HA. Influence of feeding cellulytic enzymes on performance, digestibility and ruminal fermentation in goats. Anim Nutr Feed Technol 2014;14:121-136.
39. Kiarie E, Owusu-Asiedu A, Peron A, Shimins P, Nyachoti C. Efficacy of xylanase and β-glucanase blending mixed grain sand grain co-products-based diets for fattening pigs. Livestock Science 2012;148:129-133.
40. Kim J, Sands J, Mullan B, Pluske J. Performance and total-tract digestibility responses to

exogenous xylanase and phytase in diets for growing pigs. Animal Feed Science and Technology 2008;142:163-172.
41. Kong C, Lee JH, Adeola O. Supplementation of β-mannanase to starter and grower diets for broilers. Canadian Journal of Animal Science 2011;91(3):389–397.
42. Li Y, Chen X, Chen Y, Li Z, Cao Y. Effects of β-mannanase expressed by *Pichia pastoris* in corn–soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. Animal Feed Science and Technology 2010;159:59-67.
43. Masey H, Neill O, Bedford M, Graham H, Kumar A. Comparing a thermo tolerant xylanase with a multi-enzyme blend in wheat-based broiler diets. Australian Poultry Science Symposium 2012.
44. Mohammad E, Torki M. Effects of Dietary Inclusion of Guar Meal supplemented by β-Mannanase on Performance of Laying Hens, Egg Quality Characteristics and Diacritical Counts of White Blood Cells. American Journal of Animal and Veterinary Sciences 2010;5(4):237-243.
45. Nourmohammadi R, Hosseini S, Farhangfar H, Bashtani M. Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed corn-soybean meal diets. Italian Journal of Animal Science 2012;11(1):36-40.
46. O´Shea C, Haham D, Lynch M, Callan J, O´Doherty J. Effect of β-glucan source and exogenous enzyme supplementation on intestinal fermentation and manure odour and ammonia emissions from finisher boars. Livestock Science 2010;134:194-197.
47. Olukosi O, Sands J, Adeola O. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing pigs. Animal Science 2007;85(7):1702-1711.
48. Owusu-Asiedu A, Simmins P, Brafau J, Lizardo R, Perón A. Effect of xylanase and β-glucanase on growth performance and nutrient digestibility in piglets fed wheat–barley-based diets. Livestock Science 2010;134:76-78.
49. Phakachoed N, Lounglawan P, Suksombat W. Effects of xylanase supplementation on ruminal digestibility in fistulated non-lactating dairy cows fed rice straw, Livestock Science 2012;149(1-2):104-108.
50. Pinos-Rodríguez JM, Moreno R, González SS, Robinson PH, Mendoza G, Álvarez G. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Animal feed science and technology 2008,142(3):210-219.
51. Rossi R, Ratti S, Pastorelli G, Crotti A, Corino C. The effect of dietary vitamin E and verbascoside on meat quality and oxidative stability of Longissimus Dorsi muscle in medium-heavy pigs. Food Research International 2014;65:88-94
52. Rothschild MF, Plastow GS. Applications of genomics to improve livestock in the developing world. Livestock Science 2014;166:76–83.
53. Salem AZM, Buendia-Rodríguez G, Elghandour MMM, Mariezcurrena MMA, Peña JF, Pliego AB, Chagoyan JCV, Cerrillo AM, Rodríguez MA. Effects of cellulase and xylanase enzymes mixed with increasing doses of Salix babylonica extract on in vitro rumen gas production kinetics of a mixture of corn silage with concentrate. Journal of Integrative Agriculture 2015;14(1):131–139.
54. Salem AZM, Gado HM, Colombatto D, Eghandour MMY. Effect of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. Livest. Sci. 2013;154:69–73.
55. Salem AZM, Hassan A, Khalil M, Gado H, Alsersy H, Simbaya J. Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed Atriplex halimus foliages. Anim Feed Sci Technol 2012;171:128-135.
56. Salem AZM, Kholif AE, Olivares M, Elghandour MMY, Mellado M, Arece J. Influence of *S. babylonica* extract on feed intake, growth performance and diet in vitro gas production profile in young lambs. Trop Anim Health Prod 2014,46: 213–219
57. Schedle K, Pecina J, Punz C, Mair C, Windisch W. Inclusion of NSP-hydrolyzing enzymes in diets for grower-finisher pigs containing two levels of distillers dried grains with solubles. Animal Review 2012;18:129-134.
58. Shaw AL, Blake JP, Gordon RW. Evaluation of commercial phytase enzymes on performance and tibia-breaking strength of male broiler chicks. The Journal of Applied Poultry Research 2010;19(4):415–421.
59. Shina A, Kumar V, Makkar H, Boeck G, Becker K. Non-starch polysaccharides and their role in fish nutrition: A review. Food Chemistry 2011;127:1409–1426.
60. Soler-Villa A, Coughlan C, Guiry M, Kreaan S. The red alga Porphyra dioica as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effects on growth, feed efficiency, and

carcass composition. Journal of Applied Phycol 2009;21:617-624.

61. Sultan A, Alhassany A, Mahat H, Oramary S, Li X, Zhang D, Bryden W. Nutrient digestibility of sorghum and wheat by broilers: effect of an enzyme cocktail. Australian Poultry Science Symposium 2012.
62. Tapingkae W, Yachai M. Influence of crude xylanase from *Aspergillus niger* FAS128 on the in vitro digestibility and production performance of piglets. Animal Feed Science and Technology 2008;140(1-2):125-138.
63. Togtokhbayar N, Cerrillo SMA, Jigjidpurev S, Shinekhuu J, Urantulkhuur D, Nergui D, Elghandour MMY, Odongo NE, Kholif AE. Effect of exogenous xylanase on rumen in vitro gas production and degradability of wheat straw. Anim Sci J 2015; doi: 10.1111/asj.12364.
64. Valdes KI, Salem AZM, Lopez S, Alonso MU, Rivero N, Elghandour MMY, Domínguez IA, Ronquillo MG, Kholif AE. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J Agr Sci. 2015; doi. 10.1017/S0021859614000975.
65. Varley P, Callan J, O´Doherty J. Effect of dietary phosphorus and calcium level and phytase addition on performance, bone parameters, apparent nutrient digestibility, mineral and nitrogen utilization of weaner pigs and the subsequent effect on finisher pig bone parameters. Animal Feed Science and Technology 2011;165:201-209.
66. Wang M, Xu Z, Sun J, Kim B. Effects of Enzyme Supplementation on Growth, Intestinal Content Viscosity, and Digestive Enzyme Activities in Growing Pigs Fed Rough Rice-based Diet. Asian-Aust. Journal of Animal Science 2008;21(2):270-276.
67. Wen C, Wang LC, Zhou YM, Jiang ZY, Wang T. Effect of enzyme preparation on egg production, nutrient retention, digestive enzyme activities and pancreatic enzyme messenger RNA expression of late-phase laying hens. Animal Feed Science and Technology 2012;172:180-186.
68. Woyengo T, Sands J, Guenter W, Nyachoti C. Nutrient digestibility and performance responses of growing pigs fed phytase- and xylanase-supplemented wheat-based diets. Journal of Animal Science 2008;86(4):848-857.
69. Yan F, Garribay L, Arce J, Lopez-Coello C, Camacho D, Disbennet P, Vazquez-Anon, Manandi M, Odetaallah N, Carter S. Effects of protease supplementation on broiler performance in vitro protein digestibility. Australian Poultry Science Symposium 2012.
70. Zangiabadi H, Torki M. The effect of a β-mannanase-based enzyme on growth performance and humoral immune response of broiler chickens fed diets containing graded levels of whole dates. Tropical Animal Health Production 2010;42(6):1209-1217.
71. Zhao PY, Kim IH. Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. Animal Feed Science and Technology 2015;200:86–92.
72. Zhou S, Sun W, Ma L, Yu J, Ma C, Ru Y. Effect of Feeding Enzymolytic Soybean Meal on Performance, Digestion and Immunity of Weaned Pigs. Asian-Aust. Journal Animal Science 2011;24(1):103-109.