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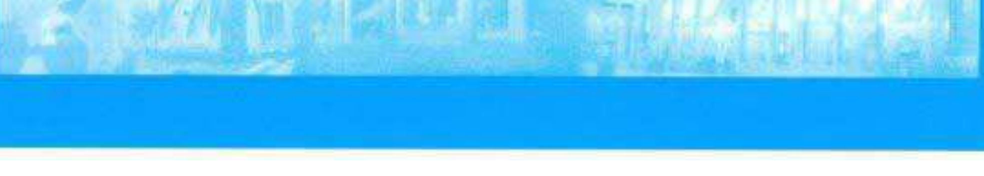

Review Article

The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry

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Summary

The value of dietary exogenous enzymes in promoting growth and efficiency of feed utilization is well recognized in poultry industry. In a case of high-cost yellow corn, several feed producers are choosing to replace yellow corn with other ingredients that have lower nutritional value like triticale, wheat, barley or sorghum. These crops are rich in non-starch polysaccharides (NSPs), and there is an inverse relationship between the content of NSPs in the feed and its nutritional value. Dietary supplementation of enzymes can enhance the nutritional value of crops containing high contents of soluble NSPs. Numerous studies have shown that supplementation of exogenous enzymes in wheat, barley, sorghum or triticale-based rations can improve performance of poultry to a level compared to that obtained by corn-soya-based rations. Naturally, the gastrointestinal tract of poultry produces enzymes to aid the digestion of nutrients. However, the birds do not have enough enzymes to digest fiber completely and need some commercial exogenous enzymes in the diets to improve the digestion. Enzyme is a biological catalyst composed of proteins, amino acids with minerals and vitamins. The advantages of using commercial enzymes in poultry feeds include improved productive performance and feed utilization, minimized environmental pollution due to reduced nutrient of manure. The present review covers the information on enzyme uses and its applications in poultry production. Furthermore, this article demonstrates that the exogenous enzymes are very important factors in the study of metabolic and physiological mechanisms. Such data will improve our understanding regarding the role of commercial enzymes in poultry feeds.

Key words: Digestive enzymes, Enzymes, Nutrition, Performance, Poultry

Introduction

Poultry industry in developing countries, is facing some challenges due to high costs of conventional feed ingredients like yellow corn and soybean meal which are mainly used in poultry rations (Abd El-Hack *et al*., 2015; Alagawany and Attia, 2015). Thus, there is an urgent need for nutritious and affordable feeds. Nowadays, the feed represents about 75% of the total costs of animal production. Increasing prices of poultry feed ingredients caused us to look closely at other crops and agricultural by-products which are less costly than conventional feedstuffs. Therefore, the wastes of fruits and vegetables after harvesting and handling could be used as alternative sources of energy, protein, amino acids, vitamins and other nutrients in feeding poultry. Recently, in developing countries, the benificial uses of untraditional feed ingredients in poultry have received great attention (Alagawany and Attia, 2015; Alagawany *et al*., 2017).

The inclusion of feed ingredients containing anti-nutitional factors may adversely affect poultry performance. Supplementation of commercial enzymes can enhance the nutritional value of crops containing high contents of soluble non-starch polysaccharides (ofNSPs). The NSPs digestibility is very low in poultry and a large amount is voided via the excreta. The NSPs are able to bind large quantities of water and as a result, the fluid viscosity increases. Increasing viscosity may cause some problems in the small digestion of carbohydrate, protein and fat. Furthermore, high viscosity of intestinal content increases the sticky dropping amounts. These problems can be resolve by addition of enzymes to poultry diets. An increased use of exogenous enzymes is expected not only from the nutritional and economic aspects but also from the health and environmental point of view. Since enzymes improve nutrient digestibility and utilization, thereby mitigating the excreta output and lowering nutrient excretion, particularly excess nitrogen, phosphorus, zinc and copper (Moghaddam *et al*., 2012; Abd El-Hack *et al*., 2017a, b; Berwanger *et al*., 2017; Rehman *et al*., 2017).

Enzymes, which may not be produced in large levels by the birds, are suggested to be supplemented to the diets. The anti-nutritional activity of cell wall NSPs had impaired impacts on growth rate and feed efficiency (Kalantar *et al*., 2015). Anyway, current benefits in feed biotechnology could enhance the utilization of high fiber containing feed ingredients (Attia *et al*., 1998). Diets supplemented with olive pulp and enzymes enhanced growth rate of broiler chickens (Attia *et al*., 2003). This article aims to supply some information and results about commercial enzymes as feed additives and their role in poultry nutrition and in improving the productivity, nutrient digestibility, carcass traits and meat quality as well as digestive enzymes.

Enzymes activity

Biologically, Ferket (1993) stated that enzyme is a functional protein that stimulates or accelerates the rate of specific chemical reactions. Enzymes activity is reliant on the substrate in a random way or at a very particular site on the substrate. Some lyric enzymes hydrolyze chemical bonds within several different types of molecules that have common chemical features. For example, in proteins, the hydrolysis of peptide bonds are catalyzed by peptidases, which will react with proteins from several different sources or only one type of molecule and are highly specific. Therefore, enzymes are classified by the substrates upon which they react, and by their specificity. Enzymes have several specificities, e.g. absolute, broad and optical specificity (Rastogi *et al*., 2007). There are currently around 2500 classes of recognized enzymes. Probably, the response to a multi-enzyme complex depends on various factors, including the nutrients level in the diet, diet type, enzymes dose, genetic strain and birds age (Cowieson *et al*., 2006). Using enzyme technology is the principle rationale to improve the nutritive value of feedstuffs (Bedford and Partridge, 2001). Also, the economic considerations determine the importance of feed enzymes domestically and internationally. The high content of fiber limits usage of sunflower meal (SFM) in poultry diets. The solution for this problem may be using exogenous enzymes in accordance with the suggestion by Tavernari *et al*. (2008) that these hydrolyze the NSPs, which could be used by avian and increase energy utilization.

Sources of enzymes

For 10,000 years, and before any awareness of enzymes, the preparation of foods was dependent on enzymes. For 100 years, in the western world, the industrial utilization of microbial enzymes started with the patenting for using fungus (*Aspergillus oryzae*) in the alpha-amylase production (Wallis, 1996). Enzymes are created in each living organism from the simplest unicellular forms of life to the highest developed plants and animals. Most of the enzymes presently used in the beverage and food industry are from *Aspergillus*, but cellulases and hemicellulases are derived from *Trichoderma*. Newly, genes encoding has been used in cloning for various enzymes, including phytases, xylanases and β-glucanases and expressed in various commercial systems (plants and microorganisms). Probably, large amounts production of an inexpensive enzyme by permanently selecting suitable microbes, increasing them in systems of modern fermentation and by efficient regulation of the enzyme extraction and purification (Wallis, 1996).

The enzymes were produced by microorganisms e.g. Bacteria (*Bacillus lentus*, *B. subtilis*, *B. stearothermophils* and *B. amyloliquifaciens*), Yeasts (*Sacharomyces cerevisiae*) and Fungi (*Asperigillus niger*, *A. oryzae* and *Triochoderma longibrachiatum*) (Wallis, 1996).

Enzymes in poultry nutrition

The use of commercial enzymes in poultry nutrition is of great importance. A proportionate increase in feed ingredients price has been the primary impediment in almost all developing countries. As a result, the non-conventional and cheaper feed ingredients should be used which have NSPs at a higher percentage along with the starch. Non-starch polysaccharides are polymeric carbohydrates, which vary from starch in structure and composition (Morgan and Bedford, 1995) and therefore, are not completely digested by birds (Adams and Pough, 1993). A portion of NSPs is water-soluble which is very bad for forming a gel like viscous texture in the intestinal tract (Ward, 1995), thus gut performance is decreased. In most cases, β-glucans had a negative effect on nutrients, especially starch and protein utilization, the conditions within the small intestine of chicks are highly viscous (Hasselman and Aman, 1986). Poultry does not produce enzymes that hydrolyze NSPs of cell wall in grains and they remain without decomposition and cause reduction in feed efficiency (Choct *et al*., 1995). Supplementation of preparations of favorable exogenous enzyme in the diets is considered modifications to overcome the adverse effects of NSPs. Enzymes break down the NSPs, reduce intestinal viscosity, and subsequently get better nutrients digestibility by improving gut performance (Amerah, 2015). Enzymes cause the disruption of the plant cell wall integrity and consequent release of nutrients encapsulated by the cell wall (Ravindran, 2013).

The beneficial role of exogenous enzymes

Growth enhancer

An earlier study showed that supplementation of exogenous enzyme mixture (xylanase 12000 units/g, β-glucanase 5000 units/kg and pectinase 10 units/g) in broiler diet during the first seven weeks of age did not exhibit significant effects on body weight (BW) gain (Francesch *et al*., 1995). Also, Soliman (1997) showed no significant effects of multi-enzyme supplementation as Optizyme-P5 on BW and BW gain of broiler breeder hens at 28, 40 and 50 weeks of age. Hashish *et al*. (1995) noticed that enzyme addition to diets which contained corn-barley-soybean caused no significant effect on BW and BW gain of broiler chicks.

Elangovan *et al*. (2004) assessed the effect of dietary enzyme supplementation with different levels of energy on the performance of quail during the growing period and showed that growth rate was not improved by adding enzyme. Also, Wu *et al*. (2005) evaluated the effect of dietary β-mannanase on BW during the second-cycle of commercial Leghorns and showed no significant difference when adding the β-mannanase. Yoruk *et al.* (2006) studied the effect of multi-enzyme supplementation at levels 0, 1, or 2 g/kg on hen performance from 30 to 46 weeks of age. They concluded that BW was not negatively influenced by multi-enzyme supplementation. Mikhail *et al.* (2013) demonstrated that the dietary enzyme supplementation with distiller's dried grains (DDGS) of quail diet did not affect BW and BW gain.

Rabie and Abo El-Maaty (2015) clarified that final live BW and BW gain of growing Japanese quails fed diet supplemented with Bio-Feed® Pro enzyme were significantly depressed. The multi-enzyme (3 different enzyme combinations: xylanase, glucanase, protease, and amylase) supplements to broiler diets showed no improvement in BW (Shalash *et al.*, 2009). It has been reported (Slominski, 2011) that the use of commercial non-specific enzyme preparations containing protease, amylase, and xylanase to target the two main nutrients of a corn-soybean diet and its NSPs components has been unsuccessful. Kocher *et al.* (2003) reported that exogenous complex enzyme (protease, xylanase, and amylase) product addition to broiler chickens' diets had little effect on growth performance. Scheideler *et al.* (2005) showed no significant effects of commercial enzyme supplementation on BW gain. Sharifi *et al.* (2012) showed that growth performance was not improved by adding enzyme. Enzyme supplementations, in some cases, did not increase BW (Ahmed *et al.*, 2017).

In contrast to the above studies, supplementation with multi-enzyme tended to improve the nutritive value of corn-soybean diet in broiler chicks (Shirmohammad and Mehri, 2011), the good incorporation between enzyme and Japanese quail diet plays an important role in improving growth (Chimote *et al.*, 2009). Sherif (2009) declared that the diet supplemented with Avizyme, Sicozyme, Natuzyme or phytase had a significant positive effect on final BW of chicks compared to unsupplemented diet. Chimote *et al.* (2009) and Goli and and Shahryar (2015) studied the effect of adding enzyme on growth performance of quail and found that supplementary multi-enzyme improved BW gain significantly. Raza *et al.* (2009) reported that the use of enzyme in broiler diets containing 6% crude fibre significantly increased BW compared to without enzymes. Enzyme supplemented wheat and barley diets with multi-enzymes give rise to significant (P<0.05) increase in BW gain compared to without enzymes (Kalantar *et al.*, 2015; Zeng *et al.*, 2015). Enhancement of locally created enzymes in broiler chicks diet significantly (P<0.05) boosted the BW and BW gain (Zamani *et al.*, 2017).

Khan *et al.* (2006) reported that enzyme supplementation with corn based diets had significant improvements in broiler growth. Supplementation with enzyme tended to improve broiler performance which fed diets including SFM (Mushtaq *et al.*, 2009). Alagawany *et al.* (2017) noticed that enzyme addition to diets which contained SFM up to 50% substitution for soybean meal caused significant (P<0.01) improvement in BW and BW gain. Also, BW gain was increased by 3.17% (1-21 days of age), 2.65% (1-42 days of age) in broiler fed diets supplemented with exogenous enzymes and improved by 8.6% (P<0.05) and 4.1% (P<0.05) with supplementation of Axtra XAP or Avizyme, respectively (Tang *et al.*, 2014). Ahmad *et al.* (2013) declared that the live weight of chicken was improved with xylanase supplementation. Diets supplemented with xylanase had significantly higher total weight gain of chicks compared to unsupplemented diet (Nikam *et al.*, 2016; Gade *et al.*, 2017). Kocher *et al.* (2015) showed that supplementation of enzyme mixture (xylanase and protease) to broiler diet during the first three weeks of age improved the performance. The addition of phytase in broiler feeds can mitigate the effects of anti-nutritional factors and ameliorate bird performance (Shirley and Edwards, 2003), and significantly increase weight gain of broiler when added with a phosphorus insufficient diet (Cowieson *et al.*, 2006; Karimi *et al.*, 2013).

Improved feed utilization

Enzyme addition at the level of 0 or 0.4 g/kg had a positive effect on the nutritive value of feed (Vukic and Wenk, 1995). Badawy (1996) stated that laying hens fed Arolen (0.5%) consumed significantly higher feed. Furthermore, Gorden and Roland (1997) used five levels of non-phytate P (NPP, 0.1 to 0.5%) and two levels of phytase (0 and 300 U/kg feed) in laying hen diets at 21 weeks of age. Feed intake of hens fed on 0.1% NPP without phytase decreased by 5.8% over 17 weeks and by 13.0% over the last 4 weeks. They added that feed intake was maintained at the level of other treatments without phyatse when the 0.1% NPP diet was supplemented with phytase (82.1% and 82.4 g/head). Gorden and Roland (1998) used 0.1 and 0.3% NPP without or with supplemental phytase (300 U/kg diet) and reported that the improvement in feed consumption was greatest when the chicken fed diet contained 0.1% NPP diet and was supplemented with phytase, during 3 to 6 weeks of age.

With regard to using Avizyme, in studies using the different types of Avizyme in layer diets, Ghazalah *et al.* (2011) showed that adding Avizyme in laying hen diets had no significant effect on feed intake. Regarding the use of Kemzyme, Hataba *et al.* (1994) obtained improvement in feed efficiency (13%) by feeding birds diet supplemented with 0.05% Kemzyme. Hashish *et al.* (1995) showed that a significant decrease in daily feed consumption was noticed for chicks fed diets supplemented with Kemzyme (0.5 or 1.0 g/kg) and using Roxazyme. Brenes *et al.* (1993) stated that addition of B-glucanase/pentosanase enzyme complex (*Trichoderma* viride, Roxozyme, RG and cellulase, TV) in poultry diets by 0.2 and 0.4 g/kg feed improved feed efficiency. Francesch *et al.* (1994) fed hens a diet based on 42% barley + 10% soybean meal + 18% peas + 14% SFM with 16% NSPs. An enzyme complex (Roxazyme-G) was added to the diets at increasing level (0, 80 and 160 ppm), and found that enzyme improved feed efficiency significantly. Addition of enzyme preparation (0.1% Roxazyme-G) to the birds' diet had significant effect on feed efficiency. Um and Paik (1999) added Roxazyme (microbial enzyme, multi-carbohydrases) preparations to four dietary treatments:

T1: 10% wheat
T2: 25% wheat
T3: 25% wheat + 0.01% Roxazyme
T4: All wheat + 0.01% Roxazyme

They found that feed intake of the second treatment was lower (P<0.01) than that in other treatments. Brufau *et al.* (2006) used *Trichoderma* viride enzyme at levels of 0, 100 and 200 mg/kg in a diet based on 68.93% barley and found that feed conversion ratio was not affected by enzyme supplements.

Enzyme mixture supplementation containing amylase 23 U/g, protease 4.6 U/g, lipase 210 U/g and cellulase 75 U/g at levels of 250, 500 and 1000 g/ton improved feed conversion of pullet during 22-34 weeks (Fayek *et al.*, 1995). Badawy (1996) found that addition of Arolen at levels of 0.5 or 0.1% to layer diets improved feed efficiency. However, Soliman (1997) found that supplementing diets of broiler breeder hens with multi-enzyme (Optizyme P5) had no significant effect on feed conversion ratio, feed per settable egg, feed per egg and feed per hatched chick at 28, 40 and 50 weeks of age. Chotinsky (2015) indicated that the use of beta-glucanase and pentosanase in poultry diets containing Rye (ground or whole) supported productive performance comparable to the wheat control diet with respect to feed conversion ratio. Using β-mannanase enzyme in hens' diet had no significant effect on feed intake (Wu *et al.*, 2005). No negative effects on feed consumption of birds if multi-enzyme supplementation was observed by Yoruk *et al.* (2006). Mourão and Pinheiro (2009) indicated that feed conversion ratio (FCR) was not improved by adding xylanase enzyme to rye or wheat based diets in comparison to maize-based diet (the control). Enzyme supplementation had no significant influence on feed consumption or feed conversion (Scheideler *et al.*, 2005), and did not significantly improve feed conversion at days 14 and 35 (Zeng *et al.*, 2015), during the breeding period and significantly increased (P<0.05) FCR values (Ahmed *et al.*, 2017).

Chimote *et al.* (2009) studied the effect of adding enzyme on performance of quail and found that supplementary multi-enzyme improved feed efficiency of quail significantly. Supplementing wheat and barley diets with multi-enzymes increased total feed intake and decreased feed conversion ratio significantly (P<0.05) compared to without enzymes (Kalantar *et al.*, 2015). Feed conversion was significantly improved by xylanase supplementation (Gade *et al.*, 2017) and the addition of locally created enzymes (Zamani *et al.*, 2017). Feed efficiency was significantly improved by xylanase supplementation (Nikam *et al.*, 2016). On the contrary, xylanase supplementation had no significant effects on feed efficiency throughout the overall growth period (Wu *et al.*, 2005; Anuradha and Roy, 2015). Also, different studies have found that feed intake was not affected by the enhancement of exogenous enzymes in quail (Chimote *et al.*, 2009), broiler (Lu *et al.*, 2013; Vieira *et al.*, 2015) diets. Rabie and Abo El-Maaty (2015) observed that total feed intake of quail significantly increased when the diet was supplemented with Bio-Feed® Pro enzyme and with decreasing dietary CP level from 24 to 20%. Feed conversion ratio of quail was not affected by feeding with the lowest dietary CP levels (24, 22 and 20%). The significant increase in feed consumption was reported by Anuradha and Roy (2015), Zeng *et al.* (2015) when different enzyme preparations are supplemented in broiler diets.

Improved nutrient digestibility and intestinal enzyme activities

Seven enzymes activities e.g. pentosanase, protease, cellulase, betaglucanase, phytase, pectinase and amylase are capable of degrading pentosans, protein, cellulose, starch and phytate, subsequently improving the nutrient digestibility and its absorption in the avian intestine (Ramesh and Devegowda, 2004). Higher figures of apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) were recorded for broiler chicks fed enzyme-supplemented diets as compared to control group (Khan *et al.*, 2006). Supplementation of commercial enzymes in poultry diets containing SFM can stimulate digestion of fiber and decrease their harmful effects (Alagawany *et al.*, 2015).

Enzyme supplementation caused positive effects in energy and protein digestibility of broiler chickens (Pourreza *et al.*, 2007). Supplementation of xylanase significantly reduced the competition for nutrient utilisation from gut micorbiota and more nutrients were available for the birds (Hosseini and Afshar, 2017). Rutherfurd *et al.* (2007) noticed that ileal nitrogen digestibility was significantly increased with addition of mixture of enzymes (amylase, glucanase, and xylanase) in a corn-soy diet of broiler chickens at 29 days old. Sherif (2009) noticed that the addition of Avizyme, Sicozyme, Natuzyme, or phytase in the broiler diet led to significant improvements in digestibility of EE and CP and nitrogen retention rate compared to the control group. Oukosi *et al.* (2008) observed that total tract retention of all nutrients improved and metabolisable energy (ME) increased by addition of combination of phytase with amylase, protease and xylanase in the negative control diet. Zhou *et al.* (2009) found that supplementing diets with the multi-enzyme (xylanase, amylase, and protease) showed improvement in energy utilization, specially in the diets formulated with the lowest levels of energy. The addition of α-galactosidase enzyme in broilers diet led to improvements in energy digestibility of soybean meal (Kidd *et al.*, 2001). Mehri *et al.* (2010) noticed that exogenous enzymes may support the process of digestion usefully. Enzyme significantly increased retention of excreta calcium, improved ME figures compared to without enzyme. Diet supplemented with enzyme had a positive effect on nutrient absorption of birds fed diets containing SFM (Mushtaq *et al*., 2009). Tang *et al*. (2014) stated that the addition of Avizyme significantly decreased (P<0.05) fresh faeces output. Also, supplementation of enzymes improved digestibility of DM and N retention. In Japanese quail, digestibility of nutrients (DM, OM, CF, EE, CP, and NFE) were influenced positively by addition of exogenous enzyme (Bio-Feed® Pro) to diets (Rabie and Abo El-Maaty, 2015). Pure enzyme supplementation increased the protein metabolisability, NSPs digestibilities, apparent metabolizable energy (AME) and retention of calcium, phosphorus, phytate phosphorus and nitrogen in birds, which helps in better utilization of alternate feed ingredients (Ramesh and Chandrasekaran, 2011). Masádeh (2011) stated that Allzyme supplementation decreased total phosphorous and nitrogen excreted per kg of DM intake when DDGS were increased in the diet.

Elangovan *et al*. (2004) noticed that supplementation of enzyme to Japanese quail diets had no significant effect on DM digestibility or N retention. Bedford (2009) noticed that good and acceptable use of NSP degrading enzymes in wheat and barley based broiler diets. Multi-enzymes supplementation with wheat and barly diets significantly decreased the adverse effects of soluble NSPs on viscosity of intestinal content (Mirzaie *et al*., 2012; Kalantar *et al*., 2015). Zeng *et al*. (2015) showed that phytase and NSP-degrading enzymes were able to improve the availability of P, Ca, ash, CP, EE and energy, thus boosting bird performance. Digestibility of dietary P and any other minerals were improved due to phytase function (Cowieson *et al*., 2004). Calcium and protein retention were significantly positively affected by Avizyme supplementation (Scheideler *et al*., 2005). Enzyme supplementation had no significant effect on values of digestibility coefficient of EE, CF, CP, and NFE (Shalash *et al*., 2010).

Supplementation with cellulase or phytase alone or both of them did not show any significant influence on digestibility of NSP (Hartini and Purwaningsih, 2017). While a previous trail by Meng *et al*. (2005) illustrated that multi-enzyme preparations statistically improved digestibility of NSP. Scheideler *et al*. (2005) noticed that the viscosity of gut content and AME were not affected by Avizyme supplementation, however, P retention was negatively affected. Also, the digestibility of OM, protein, starch and metabolizable energy were not improved by xylanas and β-glucanase supplementation with the diets that contained hull-less barley. Yuan *et al*. (2017) suggested that appropriate supplementation of exogenous enzyme complex (protease, xylanase, and amylase) was useful for the bird, while exorbitance enzyme complex had negative effects on endogenous enzyme secretion and destroyed the small intestine structure, then little effects on growth performance. All nutrients digestibility which was studied by Ghazalah *et al*. (2011) were not significantly influenced by Avizyme supplementation. Sharifi *et al*. (2012) stated that enzyme supplementation to broiler diets showed no improvement in nutrient digestibility. Cowieson *et al*. (2017) concluded that phytase is effective in enhancing the digestibility of amino acid and that these impacts originate from the removal of the anti-nutritional impacts of phytic acid.

Conclusion

Poultry industry is becoming increasingly receptive to the use of exogenous enzymes supplementation. Enzyme supplementation to the poultry rations has a positive effect on feeds digestibility and leads to better productivity and performance. Moreover, supplementation of commercial enzymes can increase the nutritive value of feed ingredients and diets as well as allow greater flexibility in diet formulation. It has also a potential effect on mitigation of the environmental pollution by reducing the excretion of some elements such as nitrogen and phosphorus in poultry manure.

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