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ABSTRACT

The plant derived feedstuffs of poultry contains non-starch polysaccharide (NSP) include celluloses, xylans, mannans, glucans and pectins etc., which are not well digested by the broiler because of their chemical structure and endogenous enzymes insufficiency. Now a days many exogenous enzymes are being used widely in poultry industry for better performance. The supplementation of suitable enzyme in feed enhances the nutrient utilization and performance by counteracting the adverse effect of anti-nutritional factors present in the feed components. The main cause of better performance is due to reduction in digest viscosity, enhanced digestion and absorption of nutrients and available energy. Moreover, the response of exogenous enzymes or NSP enzymes can be variable depending upon the type of enzymes or cocktail enzyme, feed ingredients and their interactive effects. The present comprehensive review paper includes effect of cocktail enzymes supplementation and their possible mechanism of action in broiler.

KEYWORD

Non Starch Polysaccharides (NSPs), Broiler; Enzyme, Cocktail, Phytase, Supplementation, Performance

INTRODUCTION

The feed ingredients of plants origin contain a number of components that cannot be digested by monogastrics species because of the lack of or insufficiency of endogenous enzyme secretions. As a result of unavailable to these components, animal, it leads to depressed animal performance. About 85-90 percent of poultry feed consists of plant materials like grains and their by –products containing large amount of dietary fibre. The principal components of dietary fibre are the structural carbohydrate, which is cellulose, hemicellulose, beta- glucan, arabinoxylans and pectins. Exogenous enzymes have been used to enhance the feeding value of barley, rye and wheat based diet because these feedstuffs are high in insoluble non-starch polysaccharides (NSP) that induce viscosity. Birds are capable of utilizing only 70-80 percent of dietary nutrients and remaining portion is excreted undigested or unabsorbed. Moreover, the use of unconventional feedstuff for poultry production is however limited due to their fibrousness and lack of Cellulase enzyme that can digest the fibre (Adebiyi *et al.*, 2010).Addition of exogenous enzyme in the diets aids the birds to increase the digestion of undigested portion of feedstuffs.

Previously, the research conducted on feed enzymes in poultry nutrition focused on non-starch polysaccharide(NSP) degrading enzymes, especially Xylanase and β-glucanase, in diets containing wheat, rye and barley (Choct, 2006).These, exogenous enzymes capable of degrading non-starch polysaccharides (NSP) in broiler diets based on 'viscous' grains including wheat and barley (Bedford and Schulze,1998). Non-starch polysaccharides mostly present in raw materials used for poultry diets are pectins, cellulose, mixed linked β-glucans and arabinoxylans. Depolymerisation of these NSPs requires specific enzymes, these enzymes are specific to the main and side chain structure of the NSP. The first development and use of NSP-degrading enzymes was applied in barley and then in wheat based broiler diets. Researchers found that the use of NSP-degrading enzymes in barley and wheat based broiler diets improved litter quality and performance. The use of NSP-degrading enzymes in wheat and barley based diets for broilers are therefore well established and accepted (Bedford, 2009).But it is thought that feedstuffs like maize and soybean that induce less viscosity may not benefit from exogenous enzymes. However, Hong *et al.* (2003) and Gracia *et al.* (2003) reported that use of enzyme cocktail (xylanase, amylase and protease) improved digestibility of a maize- soybean meal based diet. Recently, it has been observed that the beneficial effect of exogenous enzyme only possible when enzyme and substrate (dietary ingredients) are compatible to each other. It might be due to some interactions between dietary source and exogenous enzyme (Cowieson *et al.*, 2016).

NON-STARCH POLYSACCHARIDES (NSP)

Non Starch Polysaccharides (NSPs) are polymeric carbohydrates which differ in composition and structure from starch and possess chemical cross linking among them therefore, are not well digested by poultry (Annison, 1993). The fibre component of the grain consists primarily of nonstarch polysaccharides (NSP) which in cereals form part of the cell wall structure. In legumes, NSP also play a role as an energy storage material. A part of these NSPs is water-soluble which is notorious for forming a gel like viscous consistency in the intestinal tract (Ward,1995) thus by reducing gut performance. Predominantly water soluble and viscous arabinoxylans, which belong to pentosans group, are assumed to be the

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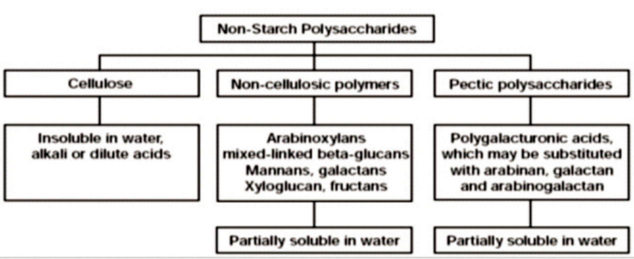
factor responsible. These pentosans also greatly increase the water intake by the birds, which lead to unmanageable litter problems caused by wet and sticky droppings. This deteriorates the hygienic conditions and carcass quality (Dunn, 1996). On the other hand, ß-glucans adversely affect all nutrients, especially protein and starch utilization and are known to give rise highly viscous conditions in the small intestine of the chick.

NSP Classification (chemical basis)



ENZYMES USED IN POULTRY FEEDS

Most of the enzymes currently used in the feed industry are from *Aspergillus,* however, hemicellulases and cellulases are also derived from *Trichoderma* and amylase from *Bacillus* species. Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulase (β-glucanases), xylanases and associated enzymes, phytases, proteases, lipases and galactosidases (Table1). For instance the use of combination of xylanase and β-glucanase has been proved to be beneficial in terms of growth increment in the poultry. Pettersson and Aman (2007) tested the addition of an enzyme cocktail, consisting of xylanase and β-glucanase to anunpelleted poultry diet containing rye and wheat. The enzyme cocktail was added at various inclusion levels.

The addition of the enzyme cocktail in trial resulted in a significant increase in body-weight and feed intake. Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in feed but also reduces environmental pollution (Mandal *et al.,* 2004). Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, β-galactosidases to neutralize certain anti-nutritive factors in non cereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals (Srivastav *et al,* 1997).

Table 1: Main enzymes used in poultry feeds

Enzymes	Substrate
ß-glucanases	Barley Oats
Xylanases	Wheat, Rye, Triticale Rice bran
ß-galactosidases	Grain legumes Lupins
Phytase	Plant feedstuffs
Proteases	Proteins
Lipase	Lipids
Amylases	Starch

The principle rationale for the use of enzyme technology is to improve the nutritive value of feedstuffs. All animals use enzymes in the digestion of food, those produced either by the animal itself or by the microbes present in the digestive tract. However, the digestive process is not 100% efficient. For example, swine are unable to digest 15-25% of the food they eat. Therefore, the supplementation of the animal feed with suitable enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestion process. In many animal production systems, feed is the biggest single cost and profitability depends on the relative cost and nutritive value of the feeds available. Often, the limiting factor when formulating rations is the animal's ability to digest different constituent parts of the feed raw material differently, particularly the fibre. Despite recent advances, the potential nutritional value of feedstuffs is not achieved at the animal level. This inefficiency in the utilization of nutrients can result in an extra cost to the farmer, the food company and the environment. Thus, the feeds need to be supplemented with the exogenous enzymes and these are the four main reasons for using enzymes in animal feed: (i) To breakdown anti-nutritional factors that are present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous digestive enzymes, can interfere with the normal digestion, causing poor performance and digestive upsets,(ii) To increase the availability of the starches, proteins, and minerals that are either enclosed within the fibre-rich cell wall and, therefore, not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (e. g. phosphorus as phytic acid), (iii) To break down specific chemical bonds in raw materials that are not usually broken down by animal's own enzymes, thus releasing more nutrients, (iv) To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate (Sheppy, 2003).

In addition to improving diet utilization, enzyme addition can reduce the variability in nutritive value between feedstuffs and improving the accuracy of feed formulations. Experimental trials have shown that ensuring feed consistency in this way can increase the uniformity of groups of animals, thus aiding management and improving profitability. The general health status of animals can also be indirectly influenced, resulting in fewer of non-specific digestive upsets that are frequently provoked by the fibre components in the feed (Elangovan *et.al.,* 2003; Sheppy, 2003).The inclusion of feed enzymes in poultry diets to enhance nutrient utilization and performance by counteracting the negative influence of targeted substrates has become common place within the last two decades. The role of exogenous enzymes capable of degrading nonstarch polysaccharides (NSP) in broiler diets based on 'viscous' grains, including wheat and barley has been elucidated by Bedford and Schulze (1988).Thus, enzyme supplementation in the feed plays an important role in increasing the availability of nutrients and retarding the adverse effect of anti –nutritional factors present in the feed components.

MODE OF ACTION OF NSP ENZYMES

The NSP enzymes work through a composite of activities, the contribution of each activity varying with ingredients and individual birds. Thus the response of NSP enzymes can be variable depending on what enzyme or cocktail of enzymes used, the quality of feed ingredients or substrate and if the enzymes are thermo-tolerant. The two main key functions are plant (cereal) cell wall destruction, and stimulation of beneficial bacteria with changes in the fiber composition. The target for such a feed enzymes product for corn-soya diets is not based on lowering intestinal viscosity derived from soluble cell wall polysaccharides since corn and soya contain low levels of soluble material. Instead of soluble fiber, it has been suggested that in corn based diets the target is the insoluble fiber component to help break open the cell wall material. Research has shown that in addition to the impact of soluble fiber, the insoluble fraction can have a direct and indirect effect on digestive function. That causes lower digestive efficiency and increase the level of nutrients used for gut maintenance, thus pulling nutrients away from growth. Whether the target is the soluble or insoluble component, the benefit brought about is due to enhanced digestibility and absorption, not only of the nutrients present in grain (like starch) but also from other ingredients, particularly added fats by improving the diffusion of nutrients within the intestinal lumen. The main key functions of NSP enzymes described as follow.

1. Cell walls destruction:

Encapsulation of nutrients - The cell wall material in the starchyendosperm of corn and sorghum is constructed mainly of small amounts of celluloseen crusted with hemicellulose, the bulk of which is arabinoxylans with minor B-glucan components and lesser contents of mannans (Wyatt *et. al*., 2007). Since poultry do not possess the necessary enzymatic capacity to degrade plant cell walls, a lot of the content (especially starch and protein) within this material can effectively bypass digestion or not be broken down until the lower gut by bacteria.

Effective degradation of this material requires the addition of sufficient amounts of the appropriate NSP enzyme activity such that "holes" are created in the cell wall. This allows water hydration and large enough amounts of pancreatic proteases and amylases enabling better digestion of the starch and protein more rapidly .When enzymes acted on cell wall resultant breaking down the cell wall material exposing more starch and protein for enzymatic digestion, reducing endogenous secretion (i.e. lowers mucin production) and altering the lower gut microbial populations. These effects of exposing more nutrients in the upper intestine, reducing the physical damage and lowering endogenous secretions by the gutvilla does lower maintenance requirement spent on digestion and improve nutrient retention. However, in a corn-soya based diet one will need to use NSP enzymes (i.e. xylanase, glucanase) these are more effective at targeting and breaking down the insoluble fiber fraction. A direct benefit of feeding these enzyme products is through reducing the variability in birds and improvements in bird uniformity across the different feed batches.

2. Bacterial population stimulation

Exogenous NS Pase(s) breakdown plant cell wall carbohydrates and reduce chain length producing smaller polymers and oligomers. At some point the fragments become small enough (ie. short chain oligosaccharides) and numerous enough to act as a substrate (pre-biotic) for bacterial fermentation. Xylanases, mannanases and cellulases produce xylo-, manno- or gluco- oligosaccharides respectively. The benefit of such end products depends upon the type and quantity of the oligosaccharides produced, with different enzymes producing different oligosaccharides. These short chain oligosaccharides travel to the lower gut and become substrates for bacterial fermentation in the ileum and cecum, which can be beneficial with VFA production and altering the bacterial population. Several papers have shown that use of enzymes significantly alters VFA production and the population profiles of bacteria in both the ileum and cecum (Apajalahti and Bedford, 1999; Choct *et. al*., 1999).

BENEFITS OF ENZYMES SUPPLEMENTATION

Benefits of using feed enzymes to poultry diets include; reduction in digesta viscosity, enhanced digestion and absorption of nutrients especially fat and protein, improved Apparent metabolizable Energy (AME) value of the diet, increased feed intake, weight gain, and feed–gain ratio, reduced beak impaction and vent plugging, decreased size of gastrointestinal tract, altered population of microorganisms in gastrointestinal tract, reduced water intake, reduced water content of excreta, reduced production of ammonia from excreta, reduced output of excreta, including reduced N and P (Gracia *et al*., 2003; Saleh *et al*., 2003; Odetallah *et al*., 2005 and Wang *et al*., 2005).

Reduction in digesta viscosity

Enzymes added to poultry diets; especially diets containing cereals rich in NSP such as wheat, barley, and rye, reduced viscosity in the diet and digesta. Muramatsu *et al*.(1992) found that enzyme supplementation of wheat based diets significantly reduced foregut digesta viscosity of birds. The reduction in foregut digesta viscosity was achieved primarily by reducing the molecular weight through hydrolysis of xylan backbone by endo-xylanase into smaller compounds and thus reduction in viscous effects of the feed because foregut digesta viscosity is directly proportional to the molecular weight of wheat arabinoxylans (Bedford and Classen, 1993). As a result of endo-xylanase and ß-glucanase supplementation, the long backbones of the arabinoxylans and ß-glucans are cleaved into shorter fragments, thereby reducing their viscosity. Further, Bhatt *et al*. (1991); Dunn (1996) and Rani *et al*.(2003) reported that the high viscosity in the gut contents caused by the pentosans led to increased water intake of the birds, which resulted in the wet and sticky droppings.

Increase in available energy

One of the main reasons for supplementing wheat- and barley-based poultry diets with enzymes is to increase the available energy content of the diet. Increased availability of carbohydrates for energy utilization is associated with increased energy digestibility (Partridge and Wyatt, 1995).

Enzyme supplementation improves this range by enhancing carbohydrate digestibility, reducing gut viscosity, and improving fat utilization (Almirall et. al., 1995). The improvements in (Apparent Metabolizable Energy) AME resulting from enzyme supplementation are variable because of the variability in the NSP content of wheat. The increase in AME with the use of enzymes is difficult to predict, as nutrient ratios, such as energy–protein, and other factors also play an important part in poultry-feed formulations. The AME value of wheat has been correlated with its content of water-soluble NSPs (Annison and Choct, 1991), which in turn affects gut viscosity (Bedford et al., 1991). Adding adequate activity levels of α-amylase, β-glucanase, and xylanase to broiler starter and grower corn-soybean diets with a 3% reduction in dietary ME allowed full restoration of growth performance of broilers comparable to those fed the adequate energy (Yu and Chung, 2004). However, (Mandal et al., 2005) found increased value of AME of sunflower meal, while rapeseed meal did not increase. The addition of enzyme complex to toasted guar meal significantly improved AME as compared to without enzymes treatment (Dinani et al., 2010).

Improvement in nutrient digestibilities

Enzymes supplementation increased nutrient digestibilities and decreased nutrient waste in the excreta. In monogastric excretion of large amounts of organic matter, especially those containing high levels of nitrogen and phosphorus, presents serious environmental threats. The large amount of excreta output in the poultry industry is a serious concern for the environment as well as it results in slower growth performances. Dry-matter digestibility (DMD) in animals ranges from 50 to 80%; the remainder of the dry matter (DM) is lost via the excreta. In the poultry industry, the amount lost via excreta is about 9 000–22 000 t of high-N manure per million birds annually. The effect of enzyme supplementation on DMD in poultry depends on the type of diet and the type of animal. An increase in DMD ranges from 0.9 to 17% in poultry has been reported by enzyme supplementation (Annison and Choct, 1991). The enzyme supplementation improved DMD by 17%, apparent metablizable energy (AME) by 24%, and feed conversion ratio (FCR) by 31% (Annison and Choct, 1993). Further Tiwari et al., (2010) reported that when a cocktail of xylanase, amylase and peotease supplemented to nutritionally marginal diet of chicks, improved ileal N digestibility. ME level of diet affect digestibility of nutrients and most profound effect on digestibility in diet with reduced ME level (Horvatovic et al., 2012).

Effect on growth performance

The role of exogenous enzyme capable of degrading NSP in broiler diets based on 'viscous' grains, including wheat and barley has been studied by Bedford and Schulze (1998). Supplementation of the maize/soy-based diets with both glucanase and xylanase displayed improvement in feed conversion ratio (FCR) and ideal nutrient digestibility (Cowieson et al., 2010). When both enzymes were added simultaneously, a synergistic effect resulted in greater benefits than either enzyme independently but less than the sum of the individual effects was observed. The best performance was achieved with the combination of xylanase (16 000 BXU/kg) and glucanase (30 000 BU/kg) (Cowieson et al., 2010).The enzyme (Yemzim B) containing 300 IU of xylanase/g, 20 IU of β-glucanase/g, 20 IU of hemicellulase/g and 260 IU of amylase/g with or without essential oil was incorporated in wheat—soybean meal based, in mash form diet for broilers. Birds fed on diets containing enzyme, essential oil (250 mg/kg) and enzymes plus essential oil (250 mg/kg) had significantly higher weight gain than those given control diet from day 0 to 7 (Basmaciog et al., 2010). Supplementation with enzyme significantly decreased viscosity and increased dry matter of digesta, but did not alter pH of digesta. Enzymes and essential oil had different modes of actions. The supplementation of enzyme with essential oil in diets is more likely to be effective in view of performance, nutrient digestibility, enzyme activities and immune system (Basmaciog et al., 2010).

In another study, Maize-soyabean based diet supplemented with mixture of xylanase, amylase, and protease (XAP) at 650, 1650 and 4000 I.U., respectively, or *Escherichia coli*-derived phytase (1000 phytase units/kg) individually or in combination was fed to the chicks for 21 days. It was found that a cocktail of XAP alone did not improve performance, but phytase supplementation improved weight gain (Tiwari et al., 2010). The enzymes were additive in their effects on growth performance of the chicks. The enzymes had no effect on ileal digestible energy. Ileal N digestibility was higher in diets with XAP or phytase individually compared with negative control diet. Both phytase and XAP individually and in combination improved ileal P digestibility compared with negative control (Tiwari et al., 2010). This study shows that a combination of XAP and phytase improved performance, but the enhancement in performance appears to be due mainly to phytase. Both XAP and phytase were effective in improving P digestibility and retention of chicks receiving nutritionally marginal maize—soybean meal (Tiwari et al., (2010); Rambabu et al., (2011) reported that the supplementation of fibre degrading enzymes (cellulase-420 IU, xylanase 402 IU and pectinase-53 I.U. / kg) and proteolytic enzyme (protease-5000 IU /Kg) to high fibre diets resulted significant improvement in body weight gain of broiler (0-6 week).

However, (Meng et al., 2005) when carbohydrases enzymes are applied to corn–SBMbased diets, less-than-optimum performance responses have been noted. Further he analyzed constituent NSP in corn and SBM and concluded that an extensive blend of carbohydrases must be supplemented if any performance response is to be achieved. This is likely why minimal or inconsistent performance improvements have been reported in corn–SBM-based poultry diets supplemented solely with xylanase and β-glucanase or a combination of xylanase, amylase, and protease. Rather, a complex blend of multiple carbohydrases is required to depolymerize the NSP present in the diet to ultimately lead to predictable improvements in BW gain, FCR, and nutrient digestibilities. Thus, to achieve viable and consistent economic returns in commercial poultry feeding programs, the correct blend of multiple carbohydrases, including cellulases, pectinases, xylanases, glucanases, mannanases, and galactanases must be applied (Slominski, 2011).

Effect on the overall performance of the poultry

A reduction in the moisture content of poultry excreta is often noted when glycanases are included in the diet. Supplementing the NSP-enriched diet with three different commercial glycanase products improved performance, but their effectiveness in reducing the moisture levels of the excreta differed from 10 to 29% (Choct and Annison, 1990). Ascoccidiosis is major problems in poultry farming due to excessive moisture inside farm ,it could be prevented by using enzymes. Birds fed a wheat-based diet with and without glycanase supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme group (Morgan and Bedford,1995) which also had a much better lesion score. An increase in digesta passage rate and a reduction in excreta moisture are often noted when glycanases are added to poultry diets, which may be detrimental to the life cycle of the organism.

Jo Zefiak *et al.,* (2010) reported that the cereal type as well as the exogenous enzyme supplementation influence the microbiota in broiler chicken caeca, and may have the effect of reducing potentially pathogenic entrobacteriaceae populations. Influence of supplementation of xylanase to rye-based diets and β-glucanase to barley-based diets is not only limited to the ileal phase. B-glucanase supplementation could have an important impact on gut health in chickens, in particular with respect to the caecallumen reflux and possible migration of some of the bacterial species to upper parts of the gastro-intestine. Exogenous enzyme supplementation might attenuate the secretory function of responding organs and decrease the size of the digestive organs and the gastrointestinal tract to some extent which had direct economic benefit as result increase in dressing percentage proportionally (Wang *et al.,* 2005). Further, Cowieson *et al.,* (2016) reported that the beneficial effect of exogenous enzyme supplementation might be linked both to enhanced protein and energy digestibility and improved intestinal morphological characteristics, secretory and absorptive dynamic and immune resilience.

FACTORS AFFECTING THE BENEFITS OF ENZYME

The degree of performance obtained by adding enzymes to the diet depends on many factors (Bedford, 1996), including the type and amount of cereal in the diet; the level of anti-nutritive factor in the cereal, which can vary within a given cereal (for example, low- versus high-ß-glucan barley); the spectrum and concentration of enzymes used; the type of animal (poultry tend to be more responsive to enzyme treatment than pigs); and the age of the animal (young animals tend to respond better to enzymes than older animals); type of gut micro flora present and the physiology of the bird. In older birds, because of the enhanced fermentation capacity of the micro flora in their intestines, have a greater capacity to deal with negative viscosity effects (Choct *et al.,* 1995).

CONCLUSION

Nonstarch polysaccharide (NSP) enzymes or in combinations or cocktail enzymes had beneficial effect on diets like wheat and bajra in terms of growth, body weight and health performance. However, inconsistent performance was observed on corn-SBM based diets. The benefit of exogenous enzyme or cocktail enzyme supplementation only possible, when enzyme and substrate (dietary source) are compatible to each other. Hence there is a need for a development of substrate specific NSP cocktail or extensive blend of carbohydrates must be supplemented to maximize body weight gain, feed conversion ratio and nutrient digestibility.

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