

Role of Enzymes in Animal Nutrition: A Review

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Abstract

Supplementation of the specific digestive enzymes in feed improves its nutritional value by increasing its digestion efficiency and enzymes help in the breakdown of anti-nutritional factors which are present in feed ingredients. The key benefits of enzymes improve feed efficiency and reduce the cost of production of meat and eggs. Enzymes improve the consistency of feed that helps in maintenance of gut health and digestion process results in overcome the growth of disease causing bacteria. Phytases act on phytate and release phosphorus from phytate, while beta Glucanase acts on non-starch polysaccharides and break down the fiber. Proteases act on protein and improve its digestibility. Cellulases act on cellulose polysaccharides and break down the fiber and Alpha Amylases act on starch and improve its digestibility. All enzymes are used extensively in textile industry, leather industry, waste managements, food industry, syrup making, pulp industry, detergents, paper industry, and in animal feeds to increase its digestibility. These digestive enzymes increased growth performance and quality of meat.

Keywords: Cellulase, Phytase, Amylase, Protease, Animal nutrition.

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INTRODUCTION

Digestive Enzymes are required to digest feed in all animals and these enzymes are either produced by the animal body itself or by the beneficial microbes present in their gastro intestinal tract. Animals are not able to digest about 15-25% of the feed they eat, because the feed contain some indigestible components and or the animal bodies lack the specific enzyme which is required for the digestion of those specific feed nutrients (Konietzny and Greiner, 2002). Phytase denotes the phosphomonoesterases that are capable of initiating the stepwise dephosphorylating of phytate (*myoinositol*); the most ample inositol phosphate in landscape. They have been known in plants, microorganisms and animal tissues (Konietzny and Greiner, 2002).

Feed cost is the largest cost (60-70%) in livestock and poultry production systems. To minimize this cost, many producers supplement feed with certain enzyme additives, which enable them to produce more meat per animal or to produce the same amount of meat in cheaper and faster way (Vohra and Satyanarayana, 2002).

Digestive Enzymes are being used in animal feeds since 1980s because of their economic, environmental and health related benefits. The most commonly used enzyme

in animal feed is phytase, which is used worldwide and accounts for 50% of all the enzymes used in feed industry. Other enzymes, mainly polysaccharides degrading enzymes (non-starch) accounts the rest (Selle and Ravindran, 2007).

Anti-nutritional factors are problematic for normal feed digestion, results in low meat and egg production causes low feed efficiency and digestive upsets. Feed enzymes work to make the nutrient (starch, protein, amino acids and minerals, etc.) available from the feed ingredients. Feed enzymes also help to reduce the negative impact of animal production over environment by reducing the animal waste production. These Enzymes are proteins that are ultimately digested or excreted by the animal, leaving no residues in meat or eggs (Greiner and Konietzny, 2006).

Phosphorus is vital mineral for animals to aid several functions. In past, insufficient utilization of phytate P by poultry required addition of inorganic Phosphate (P) in diet to meet their nutritional requirements. Due to less endogenous phytase activity, two thirds of (P) in poultry feeds of plant origin is secreted without digestion. The phytase decrease (P) excretion up to 50% which will not only decrease environmental pollution but also save inorganic (P). Most of the commercially phytases are

derivative of fungi, bacteria and recombinant technology (Greiner and Konietzny, 2006).

Submerged and solid state fermentation used for production of exogenous phytase. One of the major issues to the industry is to improve the utilization of (P) by the birds and reduce its content in their excreta. Supplementation of phytase to feeds is the best solution to overcome these problems. Extensive research led to the use of phytase enzymes in poultry diets and helped to proportionately reduce supplementation of inorganic (P) to the feed. The efficiency of additional phytase in increasing the bioavailability of (P) and other phytate bound minerals, digestibility of energy and amino acids in poultry (Selle and Ravindran, 2008).

Phytase as an Animal Feed Additive

Hydrolytic enzymes have arisen as feed additions in order to increase the digestion and absorption of poorly available nutrients from the animal feeds. The first commercial phytase products were introduced on to the market in 1991 (Debnath *et al.*, 2005). The possible uses of phytase in food treating or the production of pharmaceuticals were reported and phytases have been largely used as animal feed additives in diets mostly for swine and poultry and for fish (Debnath *et al.*, 2005). The small intestine of monogastrics has only a very low ability to hydrolyse phytate due to the lack of significant endogenous phytase activity and little microbial population in the upper part of the digestive tract. This fact also explains why phytate phosphorus is poorly available to monogastric animals (Iqbal *et al.*, 1994; Walz and Pallauf, 2002). Phosphorus is absorbed as orthophosphate and use of phytate phosphorus by monogastrics will largely depend on their ability to hydrolyse phytate. The efficiency of additional microbial phytase in improving the utilization of phosphate from phytate is very good to enhance animal nutrition (Simons *et al.*, 1990; Adeola *et al.*, 2006; Augspurger *et al.*, 2006; Garcia *et al.*, 2005). Excretion of phosphate can be decrease by as much as 50%, which is from an environmental viewpoint. The dietary supplementation with a microbial phytase has proved to be the most effective tool for the animal industry to reduce phosphate excretion from animal waste, enabling compliance with clearly useful environmental rules. In adding, phytase supplementation develops amino acid availability. Phytate protein collaboration may bring alterations in protein structure that can decline enzymatic action, protein solubility and digestibility (Garcia *et al.*, 2005).

A negative effect of phytate on the nutritive value of protein was not clearly established in monogastric animals (Sebastian *et al.*, 1998). While some have suggested that phytate does not affect protein digestibility (Peter and Baker, 2001), others have found improved amino acid availability with decreasing levels of phytate (Cowieson *et al.*, 2006). This difference may be at least partly due to the use of different protein sources. In addition, supplemental

phytase was reported to improve use of minerals by animals (Lei and Stahl, 2001). It was hypothesized that phytase addition results in a better energy use in monogastric animals. Enzyme preparations with phytases from *Aspergillus niger*, *Peniophoralycii*, *Schizosaccharomyces pombe* and *Escherichia coli* are available commercially (Debnath *et al.*, 2005b). In general, their large-scale production is based on the use of recombinant strains of filamentous fungi and yeasts. Today, all phytases used for animal feed application belong to the class of histidine acid phytases; β -propeller phytases have been supported for several uses. Impeccable phytases for animal feed should fulfill a sequence of quality criteria: they should be effective in discharging phytate phosphate in the digestive tract, constant to repel inactivation by temperature from feed treating and storage as well as low-cost to yield (Debnath *et al.*, 2005b). Adeola and Cowieson directed (2006) that the effect of microbial phytase on phosphorus release depends on nutritive phytate concentration, the source of phytate, species, age of animals, mineral concentrations in the diet, phytase sources and phytase dosing (Debnath *et al.*, 2005b).

Mode of action of Phytase

Phytase catalyzes the stepwise elimination of phosphate from phytic acid or its salt phytate. The exclusion of the phosphate group starts with a fully phosphorylated phytic acid (IP₆), followed by penta- (IP₅), tetra- (IP₄), tri- (IP₃), di- and mono-esters of inositol in downward order of inclination. This means that the phytases first hydrolyze all of the available fully phosphorylated phytic acid to penta-esters of inositol before they hydrolyze the latter to tetra-esters of inositol and so on (Simon and Igbasan, 2002). In an ideal situation, a complete hydrolysis will result in a myo-inositol and phosphate (plus amino acids, minerals and other nutrients which are linked to phytic acid). However, in the *in vivo* situation, hydrolysis will be incomplete and therefore normally result in a mixture of inositol-phosphate esters (e.g. IP₅, IP₄, IP₃). The phytate binding to Ca is also related to phytate ester composition, with a disproportionate decrease in the capacity of phytate to bind Ca, e.g. IP₃ has approximately 11% of the binding attraction of IP₆. Thus the quick elimination of IP₆ and IP₅ in the stomach will considerably decrease the required of Ca in the small intestine (Simon and Igbasan, 2002).

Phytate hydrolysis in the digestive tract

The capacity of a phytase to hydrolyse phytate in the digestive tract is firm by its enzymatic belongings. With regard to phytate dephosphorylation in the GIT of animals, it is significant to reflect the low pH in the fore stomach (crop) of poultry (pH 4.0–5.0) and in the proventriculus and gizzard of poultry and stomach of pigs and fish (pH 2.0–5.0) (Simon and Igbasan 2002). The small intestine of animals presents a neutral pH environment (pH 6.5–7.5). Two key types of phytases have been known, i) acid

phytases show greatest phytate dephosphorylation around pH 5.0 and ii) alkaline phytases with a pH ideal of around pH 8.0. Animal feeding have convinced that the main functional site of additional phytase in pigs and fish is the stomach (Yan *et al.*, 2002; Yi and Kornegay, 1996). The site of phytase action in the gastrointestinal tract of poultry has received little attention. A phytase that should be active in the small intestine requires a sufficiently high stability under the pH conditions in the stomach and intestine as well as a high resistance to proteolytic activities, mainly of pepsin in the stomach and the pancreatic proteases in the small intestine. To assurance an efficient phytate dephosphorylation in the crop and stomach, stability in an acid environment and conflict to pepsin are properties that are highly desirable for extra acid phytases (Jongbloed *et al.*, 1992).

β-Glucanase

The soluble non starch polysaccharides (NSP), Pentosans and mixed linked beta-glucans cannot be hydrolyzed by endogenous enzymes and result in poor feed conservation ratio, reduced body weight gain and wet litter conditions. The primary mechanism of the anti-nutritional effects of the soluble NSP activity is related to their viscous properties, which consequently affect the viscosity of the aqueous fraction in the small intestine contents. The exaggerated intestinal viscosity affects the digestion and absorption of nutrients in chicks by reducing glucose and sodium transport into the epithelial cells and reducing the release rate of pancreatic enzymes and bile acids. The enzyme widely used in poultry industry is xylanase and beta glucanases. These enzymes cleave the non-starch polysaccharides in cereal grains such as barley or hull-less barley (Whitcomb and Lowe, 2007). Several mechanisms have been proposed to explain the beneficial effects of these exogenous enzymes in improving energy and nutrient utilization of hull less barley based diets (Whitcomb and Lowe, 2007). Beta glucans are a polysaccharide made of glucose molecules linked together into long chains that monogastric animals cannot readily digest. In more familiar terms they are cellulose plant fiber, cereal bran fiber, and parts of certain types of fungi, yeast, and bacteria. As a kind of indigestible fiber, they may become viscous in the intestinal tract and slow peristalsis (intestinal contractions). These glucans are also present in feed ingredients like barley, wheat and rye which commonly used mostly in commercial diets, causing increase in the viscosity of ingesta thus results in decrease in the availability of nutrients required for the animal growth (Whitcomb and Lowe, 2007).

Importance of Beta-glucanase

Beta Glucanase digests fiber and helps in better digestion of heavy cereal grains such as wheat, barley and rye in diet. Because Beta glucanase hydrolyzes the glucans present in these ingredients thus reducing viscosity

of digesta, and helps to revitalize natural peristalsis, enhances the digestive process, increasing the overall nutritional value of diet. Beta Glucanase is a very important enzyme because monogastric animals cannot produce it on its own, Beta Glucanase helps in the breakdown of plant walls (cellulose), and increases the overall efficiency of binding excess cholesterol and toxins in the intestines for their removal. Beta glucanase are also particularly useful in industrial and agriculture processing applications due to its high degree of stability. This enzyme is resistant to denaturation by higher than moderate temperature and pH extremes make it particularly durable. It hydrolyze beta D glucans components, by passing branch points which often serve as blocking points to other exo-acting enzymes. Beta Glucanase is used for commercial food processing in coffee; it performs hydrolysis of cellulose during drying of beans, Beta Glucanase also used in textile industry as a fading agent. Beta-Glucanase has also been found to be particularly useful in reducing the coating or biofilm of *Candida* (fungus) that can grow in the digestive tract (Whitcomb and Lowe, 2007).

Endogenous proteases

Proteases are the enzymes which degrade proteins; these are categorized by their ability to break down the bonds at certain sites of amino acids. The activities from endogenous proteases are site specific because their activity in the incorrect location can lead to destruction of the animal's own tissues. Pancreas is the chief source of proteases in the body. Major portion of proteases are produced as inactive pro-enzymes. These proteases include trypsinogen, chymotrypsinogen, pro-carboxypeptidases and proelastase (Kraut, 1977).

Exogenous proteases

Proteases are actually protein-degrading enzymes which are utilized in poultry and livestock nutrition to degrade storage proteins present in plant materials and protein containing anti-nutrients in them. Seeds, predominantly belonging to legume family such as soy, contain high amount of storage proteins. These are proteins which are mainly generated during production of seeds and deposited in the seed to give a nitrogen source for the emerging embryo. Storage proteins can attach with starch (Yu *et al.*, 2007). Proteases can cleave storage proteins hence help in releasing bound energy-rich starch which can then be assimilated by the animal. Major protein based anti-nutrient factor is trypsin inhibitor. This is present in untreated plant based protein sources, such as soybeans. They impair digestion process as they inhibit trypsin, secreted by the pancreas and play role in the breakdown of protein in small intestine. Processed soybean meal contains minimal levels of trypsin inhibitor, thus enhances protein digestibility (Kraut, 1977).

Efficacy of exogenous Proteases

The efficacy of mixtures of enzymes together with protease has been widely reported. Yu *et al.*, (2007) studied effect of including protease in broiler study, where both high and a low protein corn–soy diets were used. The protease enhanced soy protein digestion *in vitro* in a classic system that imitated the actual digestive tract results fish meal and maize were similarly influenced (Yu *et al.*, 2007). Many modes of action have been recommended to explain the positive effects of proteases in poultry diets. Proteases may enhance endogenous peptidase formation hence reduces the necessity for amino acids and energy or augment the degradation of dietary protein. In addition to it, proteases may cleave proteinacious anti-nutrient like trypsin inhibitors enhancing the effectiveness with which bird uses amino acids and reduces protein turnover rate (Huo *et al.*, 1993; Marsman *et al.*, 1997).

To confirm previous results which recommended that exogenous protease are a useful entity in animal nutrition, it is suggested that further research work should be done to clarify mechanism of action, ultimate dose, ideal substrate, as well as to discover the relations between supplemental and endogenous enzyme systems (Marsman *et al.*, 1997).

Mechanism of Action of Exogenous Amylase and Protease

The composition of the diet can affect the functioning of digestive system (Starck, 1999). The true value of exogenous proteases may reduce maintenance energy demands (also amino acid requirements) rather improving ideally digestible energy only. Proteases are rarely fed separately rather found commonly as portion of an enzyme cocktail, mainly involving glucanases, xylanases, proteases and phytases. The efficacies of such enzymes are intricately linked to the assimilation of the diet to which these enzymes are included (Cowieson, 2010).

It can be concluded that exogenous proteases are valuable in poultry nutrition. The effects with other enzymes like glucanases, xylanases, phytases, growth-enhancing antibiotics, etc. remains uncertain. Planned intervention at secretory level may be of larger extent than modest for improvements in ileal energy recovery. Further studies are necessary to comprehend how the animal reacts to what it ingests (Cowieson, 2010).

Cellulases

Enzyme that hydrolyzes β -1, 4 linkage present in a cellulose chain is known as Cellulase. That is produced by plants, animals, bacteria, protozoans and fungi. Catalytic activity classification of cellulases is based on its crystal structures and arrangement of amino acid. These are divided into many families according to the classification (Arnold, 2001). Carbohydrate binding molecules CBM are present in cellulases which are located at N or C terminal of catalytic module of the enzyme. Enzymes may contain

other modules of known or unknown function other than CBM. Three types of cellulases are required for complete hydrolysis of cellulose which are; endoglucanases, exoglucanases and beta-glucosidase BG (Bayer *et al.*, 1995).

There is an increased trend of using cellulases in many industries. Mostly, cellulases are used in food industry, pulp industry, paper industry, textile industry and also used in detergents as an additive. These are mainly used in animal feeds to improve feed digestibility Cellulases have a larger share in world enzyme industry at present. But their prices are relatively high than other enzymes so that it's used can be further increased. Its catalytic activity should be further increased to make it economically better and to make animal feed more digestible. In dairy, digestibility can be somewhat bearable but in poultry, it should increase starch digestibility to overcome energy deficiency. Cellulases performance should be further increased for the better cellulose hydrolysis understanding. In that way we can also understand the relationship of molecular structure, and function of cellulose (Bayer *et al.*, 1995).

Cellulase Enzyme Systems for Cellulose Hydrolysis

To utilize cellulase, two strategies are adopted by cellulolytic microorganisms. First one is discrete non-complexed cellulase and second is complexed cellulase. Mostly cellulose is degraded by secretions of cellulolytic micro-organisms. A set of cellulase has a CBM and a catalytic module. CBM and catalytic modules are attached with each other by a linker peptide which is flexible in nature. CBM is present at C terminal or N terminal of module and its position doesn't affect its function in most cases. Large complexes of multi-enzymes having more than one million molecular mass are produced by anaerobic micro-organisms mostly that are known as cellulosomes. These cellulosomes are bounded to the surface of micro-organisms mostly. These cellulosomes can also be produced by some bacteria. Bacteria can also produce free cellulases. CBM is not present in the cellulosome enzymes mostly. CBM is present in a protein called scaffoldin (Arnold, 2001; Bayer *et al.*, 1995).

Catalytic Mechanisms of Cellulases

Acid–base catalysis is used by glycosides to cleave glucosidic bonds in most cases. Two catalytic residue of the cellulase takes part in hydrolysis which is a general acid and a nucleophile or base. During hydrolysis, anomeric configuration is either inverted or retention takes place. That hydrolyses depends upon catalytic residues spatial positions. Configuration of anomeric carbon that has a target glucosidic bond is retained after hydrolysis in retaining cellulases. In that reaction hydrolysis takes place in two steps that are glycosylation and deglycosylation. On the other end, configuration of anomeric C is inverted after hydrolysis in inverted cellulases. In inverted cellulases,

single displacement occurs during hydrolysis (Doi *et al.*, 1998).

Endoglucanase

New ends are generated by endoglucanases or CMCase by cutting β -1, 4-bonds of cellulose randomly. Different endoglucanases are produced by fungi, plants, animals, archaea and bacteria produce different types of endoglucanases. These types have different catalytic modules in different families. A catalytic module with or without a CBM, is present in fungal endoglucanases generally. In contrast, multiple catalytic modules, CBMs, and other modules with unknown function are present in bacterial endoglucanases (Den Haan *et al.*, 2007).

Exoglucanase

These enzymes cut the cellulose at reducing or non-reducing end and it works in a row on cellulose. After the reaction of these enzymes, cellobiose or glucose is removed from polysaccharide chain as a product. Microcrystalline cellulose can be worked effectively by these enzymes (Den Haan *et al.*, 2007).

Cellulase Activity Assays

Many assays are used to check cellulase activity. These assays are divided into two major groups which are initial rate assays and end point assays. Initial rate assay is similar to regular enzyme assay which is done on soluble substrates. End point assay is somewhat unique because it is used for cellulases only. Enzymes should be diluted in series to perform that assay (Doi *et al.*, 1998). Three things are most important in engineering of cellulose; (1) cellulase directed evaluation, (2) cellulase rational design and (3) the reconstitution of cellulase mixtures or designer cellulosome (cocktails) active on insoluble cellulosic substrates (Doi *et al.*, 1998).

Industrial Applications of Cellulases

These enzymes are used extensively in detergents, pulp industry, paper industry, textile industry and in animal feeds to increase its digestibility (Figure 1). Cellulases have a larger share in world enzyme market now a day. Its demand in market is growing briskly. In near future, this enzyme will be the commonest enzyme in the world market as compared to the all the other enzymes (Den Haan *et al.*, 2007).

Cellulase Production

These enzymes are produced by submerged or solid state fermentations. Submerged fermentation is used mostly by all the companies to decrease production cost. In that way, more than 100 grams of crude cellulase can be produced per liter of broth. Almost all the enzyme companies produce commercial cellulases based on *Trichoderma* and *Aspergillus* and their derivative strains except Dyadic's *Chrysosporium lucknowense*. In near past,

Genencor and Novozymes claimed a 20 to 30 fold reduction in cellulase production costs to 20–30 cents per gallon of cellulosic ethanol. That achievement is implemented mainly by (1) sugar costs lessen from lactose to glucose and sophorose' small amount, (2) for higher specific activity, enzyme cocktail is used and (3) enhanced thermostability. That achievement seems over-claimed. It is foreseen that present cellulase prices may range from 1.00 to 1.50 U.S. dollars per gallon of cellulosic ethanol (Doi *et al.*, 1998).

Amylase

Amylase is a group of enzymes that catalyze the breakdown of starch and sugar. Amylase breaks polysaccharides (carbohydrates) into smaller disaccharides, finally converting them into monosaccharides such as glucose, fructose, galactose etc. People who can't digest fats mostly eat sugar and carbohydrates to fulfill the lack of fat in their diet. If the diet is abundant in carbohydrates, then they develop an enzyme deficiency (Fierobe *et al.*, 2002). Amylase does not digest only carbohydrates but also dead pus cells. For example, when someone is deficient in amylase then there is chance of abscesses (Heinzelman *et al.*, 2009).

Amylases are enzymes that degrade starches. They are extensively distributed in microbial, plant and animal kingdoms. They degrade starch to produce products of individual amylolytic enzymes. The term amylase was used to choose enzymes capable of breaking the glucosidic linkages present in glycogen, starches (amyloses and amylopectins) and their catalyzed products. They act by breaking bonds between adjacent glucose units, producing products of the particular enzyme involved (Freer, 1993).

Starch hydrolysis may be carried out using either acid or enzyme as catalyst. Enzyme hydrolysis has numerous advantages. 1) It is more specific, therefore less by products are formed, and hence yields are higher. 2) Conditions for enzyme hydrolysis are milder that is why refining stages to remove ash and color is minimized. 3) The enzymatic hydrolysis of starches has been practiced on an industrial scale for many years and is steadily replacing the traditional acid hydrolysis process (Freer, 1993).

In the history of industrial enzymes production, Dr. Jhokichi Takamine began the production of digestive enzyme preparation from wheat bran koji culture of *Aspergillus oryzae* in 1894. Commercial production of dextrose crystals and dextrose powder from starch by using amylase and glucoamylase began in 1959. In starch processing industry, conversion of starch into sugar, syrups and dextrins becomes the major part of that industry (Agrawal *et al.*, 2005). The hydrolyzed states of starches are used as carbon sources in fermentation and other sources of sweetness in many commercially manufactured food products and beverages. Hydrolysis of starch to

products such as glucose and maltose is brought about by controlled degradation (Sonia *et al.*, 2009).

Process of dispersion of insoluble starch granules in aqueous solution (Liquefaction) followed by partial law of hydrolysis using heat resistant amylases. In commercial industrial processes, the starch suspension for the process of liquefaction is in surplus of 35% (weight/volume). Therefore the viscosity is tremendously high following gelatinization. Heat resistant amylases are used as thinning agents, which cause reduction in viscosity and partial hydrolysis of starch. Retro gradation of starch is thus avoided during consequent cooling. Other important applications of amylases are 1) Manufacturing of maltose, 2) Manufacture of high fructose containing syrups, 3) Manufacture of oligosaccharides mixture, 4) Manufacture of maltotetraose syrup, 5) Production of anomalously linked oligosaccharides mixture, 6) Manufacturing of high molecular weight branched dextrans, 7) Removal of starch sizer from textile, 8) Direct fermentation of starch to ethanol. Amylases used as detergents are alkaline amylases. Sometimes amylases are used as digestive tools to increase the digestibility of animal feed ingredients (Amoozegar *et al.*, 2003).

economic and social benefits of enzymes have been well understood, but this segment needs further exploration in future. Therefore, further research is required to determine that either current enzyme reached their full potential in the industry or there are some deficiencies. This is also need of time to discover new enzymes which will lead to maximum nutrient utilization from our available feed resources. Regarding this, we have to take the aid of the present review will helps in the development of different latest techniques and formulation of best rations using a wider range of our present ingredients. Consequently advances in this field will improve the welfare of animals, atmosphere, efficient utilization of nutrients and better farm economics.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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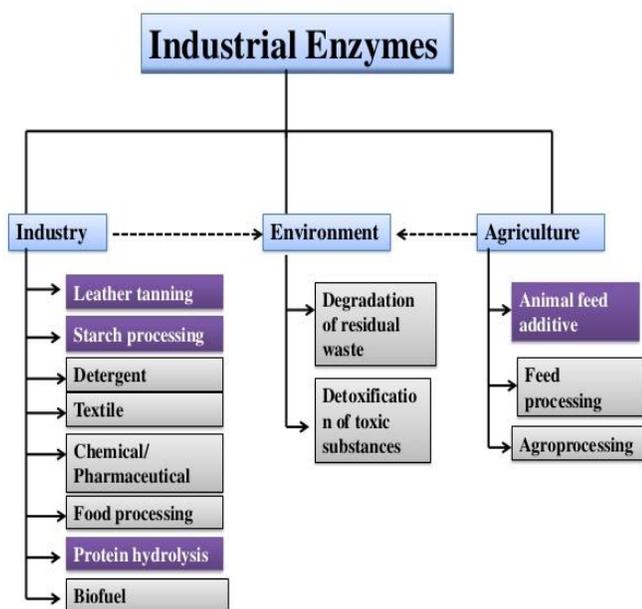


Fig. 1. Enzyme applications

Conclusion

Digestive Enzymes usage in animal feed as a feed additive has rapidly expanded. In the last few years, extensive studies have been conducted to study the effects of feeding exogenous enzymes on the performance of animals especially poultry. By seeing these studies, this review provides proof that enzyme is a beneficial instrument for the use in animal feed. Although, the

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