Feed Enzymes: Source and Applications

17

Poonam Singh and Sanjay Kumar Yadav

Abstract

Enzymes are found in all living organisms, including microorganisms, plants, animals, and humans; and control all vital reactions. Enzymes can be produced by a diverse group of organisms through various production processes such as submerged and solid-state fermentation through microorganisms. Among all organisms, microorganisms are the more prominent and suitable host to produce stable and industrially important enzymes. Enzymes are used in different industries including the animal feed industry. Some common enzymes that participate in the preparation of animal feed are phytase, protease, alpha-amylase, xylanases, beta-glucanases, xyloglucanases, galactomannanases, pectinases, arabinofuranosidases, and ferulic acid esterases. Hydrolytic enzymes remove antinutritional factors from feed ingredients and enhance feed digestibility in animal gut.

Keywords

Microorganism · Submerged · Phytase · Hydrolyzed · Enzyme · Feed · Xylanase · Animal · Fermentation

17.1 Introduction

Feed ingredients and improver enzymes have become a crucial part of animal feeds, which help to increase the production of milk and meat worldwide. Increased human populations demand increases in milk and meat production, leading to growth in the

P. Singh (✉) · S. K. Yadav
Department of Molecular and Cellular Engineering, SHIATS, Allahabad, India

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feed industry. Feed enzymes improve feed quality and enhance feed conversion by animals. The worldwide feed enzyme market was $899.19 million in 2014 and will increase up to $1371.03 million in 2020. Feed is an expensive item in livestock and poultry production and accounts for 60–70% of the total cost of production. Enzyme-supplemented feed is cheaper and more cost effective, which enhances the rate of meat production per animal (Walsh et al. 1993). Enzymes catalyze chemical reactions, which combine with substrate and are converted into product, before the assimilation of nutrients in animal bodies needed to convert complex forms of nutrients into the simplest form. Some common enzymes used in animal feed formulation are phytase, protease, alpha-amylase, xylanases, beta-glucanases, xyloglucanases, galactomannanases, pectinases, arabinofuranosidases, and ferulic acid esterases (Walsh et al. 1993). Feed enzymes need to have some specific features like long shelf life and stability at high pH and temperature; during the feed pelletization process temperature increases, and feed enzymes need to be stable at the high temperature, otherwise they will become denatured. Commercial feed enzymes are obtained from various bacterial and fungal species through submerged or solid-state fermentation processes, but a small quantity of feed enzymes can be obtained from animals and plants. The first enzymatic product protozymes used in poultry diets were reported in 1920 (Ewing 1963). Grain cell walls are made up of nonstarch polysaccharides (NSPs), and poultry do not produce NSP-hydrolyzing enzymes, like xylanase, β-glucanase, cellulose, and pectinase. Feed enzymes help break down NSPs like cellulose, pectins, and glucans. Feed enzymes overall improve ingredient digestion in poultry and cattle.

17.2 Sources of Animal Feed Enzymes

The application of enzymes in food processing was first reported around 10,000 years ago; commercially viable microbial enzyme use started about 100 years ago, when alpha-amylase enzyme was produced from *Aspergillus oryzae.* All living organisms produce enzymes for their essential metabolic processes, either catabolism or anabolism. Plants, animals, and microorganisms are the main sources of enzymes, but microorganisms are concerned with commercially viable enzyme production at the industrial level. Some commonly known bacterial species such as *Bacillus licheniformis, Bacillus amyloliquifaciens,* and *Acedothermus* have been used for enzyme production using a submerged fermentation method. Fungal species like *Triochoderma viride, Trichoderma reesei, Asperigillus fumigatus*, and *Asperigillus niger* are commonly used for enzyme production through a solid-state fermentation method. The enzymes production increases much fold at commercial level with the help of genetic engineering (enzymes encoded gene are clone in microorganism), advance fermentation techniques and suitable enzymes purification method (Wallis 1996).

17.3 Production Process of Animal Feed Enzymes

The feed enzyme production process consists of fermentation of a suitable microbial culture in growth media, recovery of enzymes through filtration or centrifugation, and purification of the enzymes before formulation with a suitable carrier (Fish and Lilly 1984). The fermentation process is very specific and its main aim is to obtain high-yield, high-quality products in minimum time. The fermentation process is carried by two methods, solid-state fermentation and submerged fermentation (Rana and Bhat 2005). Commercially viable feed enzymes are produced by a submerged fermentation process because the process of solid-state fermentation and their downstream processing is slightly difficult compared to the submerged fermentation process (Aunstrup 1979). Submerged fermentation of enzyme production consists of inoculant preparation from production culture and transferal of inoculants into a fermenter, where physical parameters like temperature, pH, agitation, and dissolved oxygen are controlled for maximum enzyme production. Submerged fermentation is categorized into three different fermentation processes: batch fermentation, fed batch fermentation, and continuous fermentation. With batch fermentation, all growth nutrients are added at the start of fermentation, and fed batch fermentation is very similar to batch fermentation, the only difference being that inoculant culture is fed with extra production nutrients in the fermentation process, while in continuous fermentation a constant state is obtained by supplying a new fresh production medium with fermented broth harvested from a fermenter. After a certain duration of the fermentation process, the fermented broth contains a mixture of cell biomass, remaining nutrients, enzymes, and other metabolites, and now pure enzymes need to be separated to form fermented broth by downstream processing. The initial steps of the downstream processing are the separation of supernatant containing enzyme solution from the biomass, through either centrifugation or filtration, after separation, the enzyme solution should be concentrated by means of an ultrafiltration, diafiltration, or evaporation method. The purification of enzymes is necessary to increase enzyme activity; a commercially viable purification method is column chromatography (Linder et al. 2004). Depending upon the application of the enzymes it can be formulated in the form of powder, granules, or liquid. Enzymes should be stable and be easily released at the site of action, and there should be no dust formation, which can cause allergic reactions.

17.4 Mode of Action of Animal Feed Enzymes

Exogenous feed enzymes that are capable of breaking down complex carbohydrates, proteins, and fats into simplest form become more available to animals. Enzymes facilitate the availability of nutrients and remove structural barriers to microbial digestion of feed in rumen. Enzymes enhance the digestion of feed in rumen and in

postruminal digestive tract, they provide favorable conditions for the growth and multiplication of ruminal microorganisms. Fibers are mostly digested by enzymes in the area of the lower digestive tract; enzymes improve nutrient absorption and reduce the viscosity of digestive ingesta. The main role of enzyme supplementation is to increase the efficiency of feed digestion in ruminants and decrease waste production.

17.5 Application of Enzymes in Animal Feed Industry

The main parts of cell walls are made up of lignocelluloses and hemicelluloses. The cellulose made up of insoluble fibers of 1,4-glucan chains and hemicelluloses consist of noncellulosic polysaccharides. The lignin of plant cell walls is made up of a composite polyphenolic structure. Carbohydrases improve the digestibility of carbohydrates in feed and energy release during metabolism, so animals use released energy for daily vital activity and growth. Carbohydrases are categorized into two groups, NSP-degrading carbohydrases and starch-polysaccharide-degrading carbohydrases. Cellulase and xylanase are two major NSP-degrading carbohydrases that can digest complex carbohydrates found in dietary fibers. Alpha-amylase is a main starch-polysaccharide-degrading carbohydrase that breaks down complex starch into fructose, maltose, glucose, and other simple sugars. Phytase and protease play a major role in the digestion of animal and chicken feed, phytase releases phosphorus from grains and other complex sources of phosphate. Phytase enhances the absorption of phosphorus from feed and improves animal performance. Poultry produce insufficient amounts of digestive enzymes but require specific amounts of enzymes for the proper digestion of all complex nutrient components.

17.5.1 Carbohydrases

Carbohydrases are divided into two groups, NSP-degrading enzymes and starch-polysaccharide-degrading enzymes. The NSP-degrading enzymes hydrolyze NSPs like cellulose, xylan, beta-glucans, xyloglucans, galactomannan, and pectin present in plant cell walls. The main NSP carbohydrases are xylanases, beta-glucanases, xyloglucanases, galactomannanases, pectinases, arabinofuranosidases, and ferulic acid esterases. Probably the most important and widely used enzymes in this class are xylanases, as arabinoxylans constitute a major part of the NSPs in cereals used as feed ingredients (Duy et al. 2013). Enzymes reduce the antinutritional factors of NSPs s in plant material by the degradation of fibers to reduce gut viscosity and improve nutrient absorption (Ravn et al. 2015). The most common starch-polysaccharide-degrading carbohydrases are alpha-amylases, which promote fast-growing broilers to improve starch digestion (Svihus and Hetland 2001). Fast-growing modern broiler breeds digest starch less efficiently than slow-growing breeds. During the grower and finisher phases, the excretion of pancreatic amylase is limited; therefore, more alpha-amylase is needed in diet.

17.5.1.1 Xylanases

Xylanases are a major feed enzyme that breaks down xylan into xylose, which is a unit of pentose sugar; xylans are the core bonding agent among lignin and cellulose. Xylans are readily available in nature, more than 10,000 xylose units are polymerized by 1,4-ß-linkages to form xylan strands (Collins et al. 2005). Different side chains are attached to a linear backbone. Based on their composition xylans are grouped into four major families: (1) arabinoxylans (AX), (2) glucoronoxylans (GX), (3) Glucuroarabinoxylans (GAX), and (4) galactoglucuronoarabinoxylans (GGAX). Xylanases help in the bioconversion of hemicelluloses, which are an important part of lignocellulosic material. Xylanases are produced by microbial species of bacteria, filamentous fungi, yeast, and actinomycetes; filamentous fungi are the predominant producer of xylanases (Beg et al. 2001). Xylanases are used as feed improvers in combination with other NSP-degrading enzymes like glucanases, pectinases, and cellulases and starch-polysaccharide-degrading enzymes like alpha-amylases and galactosidases. Xylanases are also used along with proteases, phytase, and lipases, which hydrolyze arabinoxylans (Twomey et al. 2003). A small quantity of endogenous enzyme is produced in young fowl and swine, but they are not sufficient for the digestion of feed ingredients and need to be supplemented by extra exogenous enzymes to improve the performance of livestock. A lack of proper enzymatic balance and undigested ingredients of feed like phosphorus, nitrogen, copper, and zinc pass through fowl and swine excreta, which causes environmental problems. Xylanases along with other enzymes play an important role in the reduction of environmental contamination (Polizeli et al. 2005). In poultry, xylanases affect feed transit time and help in nitrogen and fiber absorption (Babalola et al. 2006).

17.5.1.2 Beta-Glucanases

Beta-glucanases have the capability to break down (1− > 3) (1− > 4)-β-glucosidic bonds. Beta-1,3–1,4-D-Glucans are mostly found in endosperm (McCleary 1988). The nonstarch unbranched diverse linkage (1− > 3) (1− > 4)-β-D-glucans are the polysaccharide part of the endosperm and aleurone layer of plant cell walls belonging to the Graminiae family. The enzyme β-1,3–1,4-glucanase breaks down the beta-glucosidic bond of β-D-glucans (Autio and Salmenkallio-Marttila 2001). β-Glucan is a water-soluble polysaccharide that can be used to form viscous solutions (Wood 2010). *Bacillus* spp. and *Streptomyces* spp. are the best-known bacterial species that produce β-glucanases, which are capable of degrading lichenan (a polysaccharide consisting of (1− > 3)-β- and (1− > 4)-β-glycosidic bonds), but however it has no activity against barley glucan. Some common fungal species such as *Orpinomyces* sp. (Chen et al. 1997), *Cochibolus carbonum* (Gorlach et al. 1998), *Talaromyces emersonii and Phaffia rhodozyma* are the good sources of β-glucanase, which are producing ß-glucanase through a solid-state fermentation process at the commercial level. The first industrial success came from the supplementation of β-glucanase to barley-based feed diets and contained β-glucans, which caused more viscosity in chicken gut. The beneficial effect of enzyme-supplemented feed is to increase animal weight from an equal quantity of barley, resulting in increases in the feed conversion ratio.

17.5.1.3 Xyloglucanases

Xyloglucanases are hydrolyzes the xyloglucans and lead to improved saccharification processes of plant matter (Hayashi 1989). Xyloglucans are made up of a cellulosic backbone; nearly 70% of 1, 4-linked β-D-glucose from this backbone are substituted by shorter chains, which mainly have galactose, xylose, and fucose (Kato et al. 1981). Gramineae and Solanaceae families of dicotyledonous plants have additional unsubstituted glucose residues in xyloglucans compared to other dicotyledonous plants (Eda and Kato 1978). Soluble galactoxyloglucans are found in tamarind seed and are made up of β-1, 4-linked D-glucose residues, the xylose residues are joined by α-1, 6 linkage β-1, 2 linkage to D-galactose, due to that specific linkage they have a high degree of substitution properties (Powlowski et al. 2009). Diverse genera and species of microorganisms produce xyloglucanases, bacterial spp. *Bacillus licheniformis* and *Ruminococcus favfaciens*, and fungal spp. such as *Fusarium* and *Cephalosporium* and actinomycetes. The xyloglucan favorable endo-β-1,4-glucanases are produced by *Aspergillus aculeatus* (Pauly 1999).

17.5.1.4 Galactomannanases

Galactomannans are a heteropolysaccharide, usually present in the plant (seed) of the Leguminoseae family (soybean, guar, sunflower, and sesame). Galactoglucomannans are the main hemicellulolytic constituent of softwood. Galactoglucomannans are made up of β-1,4-linked d-mannose residues and may be replaced by d-galactose residues through an α-1,6-linkage. Water-soluble galactoglucomannans have a high amount of galactose compared to water-insoluble galactoglucomannans. Through the acetyl groups nearly 20–30% of glucose or mannose residues are esterified (Mccleary and Matheson 1976). Galactomannanase enzymes are obtained from microorganisms, from fungal species like mold and yeast, and bacterial species such as *Bacillus* sp*p., Sporotrichum cellulophilum*, and *Pseudomonas.* Commercial-level galactomannanases are produced from fungal species of *Trichoderma* and *Aspergillus* from solid-state fermentation.

17.5.1.5 Pectinases

Pectinase is a biological catalyst that hydrolyzes pectin molecules. Pectin is obtained from an area of the middle lamella from plant cell walls; pectinase hydrolyzes pectin to break down plant cell walls and release nutrients. Pectins are made up of complex colloidal galacturonic acid polysaccharides, which are linked with (1 ± 4) linkages, and the lateral chains of pectin backbone molecules are made up of L-rhamnose, arabinose, galactose, and xylose (Miller 1986). Pectinase enzymes are obtained from microorganisms, plants, and animals, commercially 50% of pectinases are obtained from fungus species *(Aspergillus aculeatus and Rhizopus* spp.) by solid-state fermentation and the remaining part are obtained from bacterial, plant, and animal sources (Anisa and Girish 2014). In nature pectic materials are more accessible than other fibers in plant tissue; pathogenic microorganisms typically start the degradation of plant materials with the help of pectinolytic enzymes (Gummadi and Panda 2003). Hydrolytic enzymes added to ruminant feed enhance the breakdown of organic substrates, increasing animal performance through faster nutrient digestion,

resulting in increased digestible energy intake (Hoondal et al. 2000; Arambel et al. 1987). During the feed formulations, the combination and amount of hydrolytic enzymes depend upon the nature of the feed ingredients (Ghorai et al. 2009).

17.5.1.6 Arabinofuranosidases

D-L-arabinofuranosidases (D-L-AFases) are accessory enzymes that hydrolyze the D-L-arabinofuranosidic linkages (Spagna et al. 1998; Margolles-Clark et al. 1996). D-L-AFase hydrolyzes the arabinosyl side chain from cell wall materials like pectic substrate and hemicellulose, and after the removal of arabinosyl side chains, these materials are more susceptible to attack by other glycanase enzymes (Saha 2000). D-L-AFase enzymes are obtained from several species of bacteria, fungi, and plants (Hashimoto and Nakata 2003; Lee et al. 2003; Rahman et al. 2003). D-L-AFases are produced by diverse groups of species like *Bacillus pumilus* PS213 (Degrassi et al. 2003), *Pseudomonas cellulosa* (Beylot et al. 2001), *Rhodothermus marinus*, and fungal species *Aspergillus niger* mutants, *Penicillium purpurogenum*, and *Aspergillus kawachii* (Koseki et al. 2003). Most animal forage is made up of hemicelluloses (mostly xylans) but provide minimum nutritional energy to livestock. D-L-AFases are capable of raising the hydrolysis of hemicellulose (Dehority and Scott 1967). For maximum digestion of cell wall polysaccharides, animal feed is supplemented by D-LAFases enzymes along with cellulases, pectinases, and xylanases (Coen and Dehority 1970). D-LAFase enzymes help in the removal of arabinose side chains, which inhibit the proper action of glycanases, which is breaking down cell wall polysaccharides (De-Vries et al. 2000).

17.5.1.7 Ferulic Acid Esterases

Ferulic acid esterases are debranching enzymes that degrade plant cell wall polymers along with other hydrolytic enzymes. Ferulic acid is made up of phenylpropenoid compounds. The extensive cross-connected linking of ferulic acids affects the extensibility, plasticity, and digestibility of cell walls (Borneman et al. 1990). The proper hydrolysis of hemicelluloses, lignin, pectic substrates, and other cell wall components needs to break down the cross-linking of ferulic acid (Nethaji and Pattabhi 1988). Usually in nature a commercial level or bulk amount of ferulic acid esterases are produced by microorganisms, and some important producers of ferulic acid esterases are *Aspergillus niger*, *Penicillium* spp., and *Sporotrichum thermophile* (Panagiotou et al. 2006). The maximum production of ferulic acid esterases is obtained from fungal species *Penicillium brasilianum* through solid-state fermentation. The prominent application of ferulic acid esterase in animal feed leads to improvement in the digestibility and nutritive value of feed through the breakage of chains among lignin and other cell wall substrates (Krueger et al. 2008).

17.5.2 Alpha-Amylase

Alpha-amylase enzymes are a hydrolytic enzyme that hydrolyzes the internal α-1,4-glycosidic bond of starch molecules (Gupta et al. 2003). Starch is a polymer

of hexose sugar, and molecules joined to each other by a glycosidic bond. Two different types of glucose chains are found in starch molecule, first amylose and second amylopectin. The linkage pattern of glucose differs in amylose and amylopectin. Amylose contains 6000 glucose units linked to each other by α-1,4 glycosidic bonds and amylopectin has two types of bond, α-1,4 glycosidic and α-1,6 glycosidic bonds. Alpha-amylase is obtained from several microorganisms, plants, and animals. From an industrial point of view microorganisms are the best source of alpha-amylase (Tanyildizi et al. 2005). Commercially, α-amylases are mainly obtained from *Bacillus* species, *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens*, through submerged fermentation (Konsoula and Liakopoulou-Kyriakides 2007). Other well-known sources of alpha-amylase are fungal species; *Aspergillus* spp. and *Penicillium* spp. are the most prominent alpha-amylase producers, through solid-state fermentation (Kathiresan and Manivannan 2006; Couto and Sanroman 2006). Alpha-amylases are secreted endogenously by animals and birds, but extra alpha-amylase supplementations improve digestibility and energy intake (Barletta 2010; Anguita et al. 2006).

17.5.3 Phytases

Phytase enzymes have the ability to hydrolyze phytate or myo-inositol hexakisphosphate. The majority of grains contain phosphate but they are unavailable to animals and birds, because they are tightly bound with minerals or proteins (Ravindran et al. 1995). Phosphate groups of phytic acid have negatively charged oxygen atoms at neutral pH; therefore, various cations and amino acids are strongly chelated with phytic acid (Reddy et al. 1982). On the basis of liberation of phosphate groups from phytate rings, phytase enzymes are divided into two categories, 3-phytase (phosphate group are released at the 3 carbon position of the phytate ring) and 6-phytase (phosphate group are released at the 6 carbon position of the phytate ring). Microorganisms are the source of phytase, and *Aspergillus niger* is the best commercially viable 3-phytase producers. The phytase enzyme is very sensitive to temperature, and during feed formulation the pelleting temperature needs to be less than 80 °C to avoid the denaturation of phytase ((Ravindran et al. 1995). Phosphate present in the cell walls of endosperm occurs in unavailable form like phytate, which lacks endogenous phytase secretion in the digestive systems of poultry, so phosphorus is biologically less available to poultry (Coelho and Kornegay 1999). Phytase-supplemented feed enhances poultry performance (Selle et al. 2000). Ruminant or digastric animals having microorganisms in their gut metabolize phytic acid as a source of phosphorus, but monogastric animals are not able to metabolize phytic acid. The major portion of phosphorus in monogastric animals is excreted in feces, which causes water and environmental pollutions (Zeng et al. 2014). Supplementation of phytase enzymes in poultry feed enhances the hydrolysis of more phosphorus from feed; phosphorus plays a major role in growth,

development, cell division, tissue restoration, and animal performance (Cowieson et al. 2015).

17.5.4 Proteases

Protease enzymes hydrolyze peptide bonds and convert complex proteins into simple amino acids and peptide. Protein present in animal feed generally attaches to carbohydrates. Protease enzymes enhance protein digestibility through the hydrolysis of the storage and structural form of proteins. Proteases overcome the antinutritional factors like residual trypsin inhibitors, lectins, and other vegetable proteins, promoting better nutrient digestibility (Yu et al. 2007). Different groups of microorganisms are in commercially produced feed proteases through both submerged and solid-state fermentation. *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudonomonas fluorescens biovar*, *Vibrio parahaemolyticus, Pseudomonas aeruginosa, Aspergillus niger*, and *Serratia proteamaculans* HY-3 are commonly known protease producers. Ruminants and monogastric animals both secrete digestive protease enzymes for the digestion of feed proteins (Parsons et al. 1997). Some exogenous proteases are used in broiler feed to improve the digestion of protein (Lemme et al. 2001).

17.6 Future Prospects of Animal Feed Enzymes

In livestock and poultry production, feed is the costliest item, accounting for about 60–70% of total expenses. Animal producers demand cheaper and more efficient animal feed. Hydrolytic enzymes that supplement feed seem to have solved these widespread problems and save money, energy, and time of animal producers. Enzymes are commonly used in feed and are important not only for animal or poultry production but also for improving environmental health. The most common feed enzymes are carbohydrase, xylanase, glucanase, phytase, pectinase, protease, and alpha-amylase. The animal feed industry is one of the fastest growing fields in the world, and producers are expected to provide a cheaper, more effective, and more natural enzyme-supplemented feed in the future. Advances are needed in animal feed sectors such as improving the efficiency of enzymes currently used in the market with regard to cost of production, thermal stability, resistance to digestion, and enhanced activity in the target section of the gastrointestinal tract. Proper formulations of animal feed are also needed. Additional researches are required in the area of feed formulation and combinations of enzymes and feed ingredients to the proper proportion (Marquardt and Brufau 1997).

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