avitech scientific bulletin

JANUARY 2002

ENZYMES

Enzymes are naturally occurring proteins that act as biological catalysts. The complex biochemical reactions that form the metabolism of living organisms are regulated by thousands of enzymes, each promoting a specific reaction that takes place countless times every day. The primary function of the enzymes in the gut of the bird is to breakdown the larger molecules into smaller ones that the bird can utilize.

Feed Enzymes have been available to the poultry Industry for the last four decades, but have found only limited practical use until recently. The main limitation was an unfavorable relationship between the cost of the enzymes and their benefits. Due to advances in biotechnology, production costs of enzyme have reduced. Feed enzyme increase the digestibility of nutrients, leading to improved animal nutrition, and thus can contribute to feeding an ever growing human population. As a result feed enzymes are now playing an essential role in management decisions aimed at optimizing cost effective production of poultry and eggs.

The digestive system of chicken comprises of natural enzymes to digest complex molecules in the feed like proteins, Carbohydrates, Lipids etc. Poultry feed involves many plant and animal origin ingredients. Chickens can easily digest animal residues, however plant material has certain inherent residues, which are not digested by chicken due to lack of endogenous enzymes.

Undigestable plant residues are as follows:

- Non starch Polysaccharides (NSP's)
- Galactosides
- Phytates
- Other Antinutritional factors (ANFs) like lectins, tannins, trypsin inhibitors etc.

All these residues have an adverse effect on the chicken's digestive system.

Non-starch Polysaccharides (NSP)

NSP is composed of hemicelluloses, pectin and gum fractions, the main components being arabinose, xylose, mannose, galactose and glucoronic acids, which are more soluble than cellulose and interact with other nutrients in the gut and produce a viscous medium. NSP of grain is usually associated with the hull and outer layers and is inversely proportional to energy levels. Barley and wheat have cell walls containing building blocks (beta-glucans and arabinoxylans) that absorb water and the intestinal digesta becomes viscous and syrup like. Soluble NSP's lead to following conditions:

- Increase gut viscosity thereby reducing effective contact of substrates and digestive enzymes.
- Modify gut physiology to reduce internal secretion of water, proteins, electrolytes and lipids.
- Bind bile salts, lipids and cholesterol thereby changing digestive and absorptive dynamics of the gut.
- Increase retention time of digesta in the intestine thus decreasing oxygen tension to favour growth of anaerobic toxicogenic microflora causing deconjugation of the bile.

Table 1 : NSP and Arabinoxylan content of some commonly used cereals

Cereals	Arabinoxylans content	Total NSP	Arabinoxylans %
Wheat	8.1	11.4	71
Wheat bran	21.9	35.3	62
Barley	7.9	16.7	47
Rye	8.9	13.2	67
Triticale	10.8	16.3	66
Maize	5.2	8.1	64
Jowar / Sorghum	2.1	4.8	44
Rice	0.2	0.8	25
Rice bran	8.5	21.8	39

Table 2: Content of Phytate phosphorus in feed ingredients

Feed ingredient	Total phosphorus	Phytate phosphorus	Phytate phosphorus %
Maize	2.8 g/Kg	1.9 g/Kg	68
Soybean meal	6.4 g/Kg	4.5 g/kg	70
Wheat middlings	10.6 g/Kg	9.0 g/kg	85

Table 3 : Available exogenous enzymes

Enzymes	Substrates
Amylases	Starch
Pectinases	Pectins
ß-glucanases	ß-glucans
Arabinoxylanases	Arabinoxylans
Cellulases	Cellulose, hemicellulose
Proteases	
Acid proteases	Proteins
Alkaline proteases	Proteins
Others	
Phytases	Phytic acid esters
Esterases	Fats, Esters
Lipases	Fats, Esters.

All these interactions lead to poor assimilation (poor feed conversion, sticky droppings, chronic deficiencies and other health related problems in poultry).

Galactosides

These are short chain carbohydrates usually found in legumes like soybean. Galactose being the main subunit is cross-linked to raffinose, stachyose and verbascose.

Galactosides interfere with the gut physiology leading to flatulence and poor assimilation of nutrients.

Phytates

All feed grains, their by-products and oil seed meals used, contain phosphorous in organic form as phytate phosphorus and non-phytate phosphorus. The non-phytate phosphorus is easily digestible and hence bioavailable for the chicken and monogastric animals. However phytate phosphorus is largely unavailable to the chicken due to inadequate phytase enzyme activity present in the intestines, required to hydrolyse the phytate bond. Due to this most of the organic phosphorus is passed undigested in the faeces.

Phytates chelate minerals, calcium, starch, amino acids making them biologically unavailable.

Since phytate phosphorus is not absorbed by chicken additional organic or inorganic phosphorus source has to be supplemented to the feed.

Undigestible phosphorus excreted in the faeces results in environmental pollution.

Other anti nutritional factors include chemically varied type of residues, which usually occur in low concentrations. They affect digestion by way of interfering with the action of endogenous enzymes.

The enzymes available for addition to animals diets (exogenous) are;

Amylase

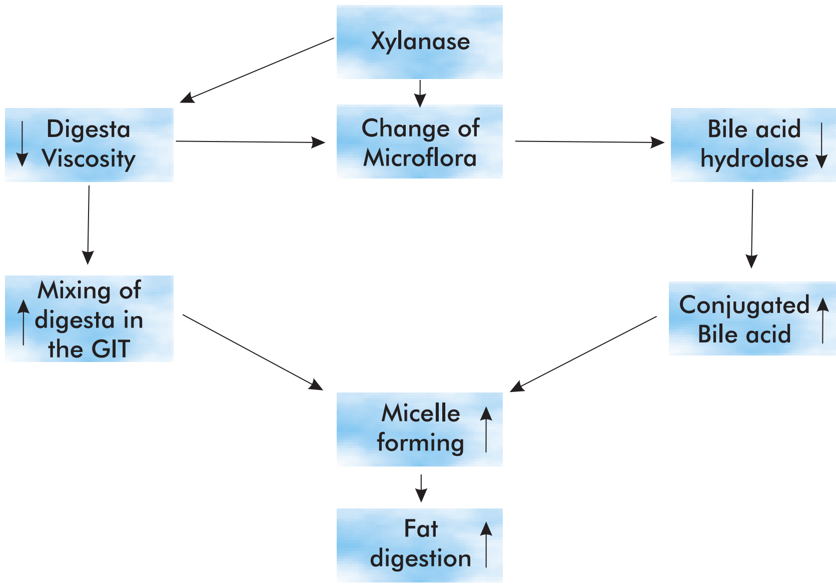
It is the enzyme required to digest starch. An amylase is an enzyme that hydrolyses α- (1-4)-glucosidic bonds in starch and glycogen. There are three different kinds of amylases:
α-amylase, ß-amylase and glucoamylase. During the digestion of starch, α-amylase and glucoamylase are produced by the animal and secreted into small intestines. However all the starch fraction is not completely digested as starch in plants is stored in granules. The shape and size of these granules differ from source to source partly explaining the differences in digestibility that are found between different starch sources.

Xylanase

Xylanases hydrolyses arabinoxylans, which are the major component of NSP. Arabinoxylans increases viscosity in the intestine due to their enormous water binding capacity. Due to this viscosity, large quantities of well-digested nutrients by the animals also get entrapped in it thereby remaining unavailable for digestion in the small intestine.

Some studies showed that commercial xylanase preparations differ widely in the degradation of soluble and insoluble arabinoxylans.

Fig : 1 Relation of Xylanase and Fat digestion.

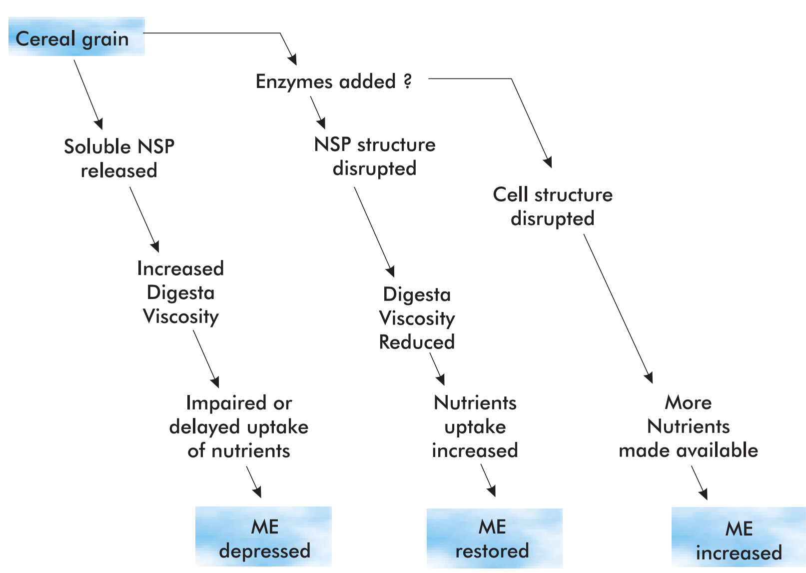


Courtin et al (2000) screened 10 different xylanase preparations in their selectivity for soluble and insoluble arabinoxylans, they found that xylanase produced by *Bacillus subtilis* had a higher activity on insoluble arabinoxylans, compared to the others. By this action insoluble arabinoxylans were solubilised and finally degraded. As high quantities of insoluble arabinoxylans are present in raw materials, these type of Xylanase are more efficient in application in feeds.

A distinct relationship between the ileal viscosity and fat digestion in broilers is found with respect to improvement in fat digestibility by dietary endoxylanase addition, which was related to decrease in chyme viscosity. (Klis et al 1995).

Conjugated bile acids emulsify fat molecules to facilitate absorption in the intestine. This emulsifying effect of the conjugated bile acids is bactericidal in action. Some bacteria like Clostridium species produce bile acid hydrolases, which weakens the emulsifying action therby interfering with micelle formation, and hence fat absorption. Xylanase supplementation reduces the production of bile acid hydrolases by the bacteria thereby reducing the microbe population in the gut. This might result in more conjugated bile acids becoming available and enhancing fat digestibility.

Fig 2 : Summary of the possible effects of the addition of NSP ase- enzyme preparations to cereal based diets for poultry.



In various experiments (Hubenar et al. 1998-1999) xylanase administration in the feeds has shown reduction in bacterial bile acid hydrolase activity and a slight increase in endogenous lipase activity.

Beta glucanase

It acts on ß-glucans present in cereals like barley and oats. High ß-glucans results in viscosity of intestinal contents. Endo ß-glucanase hydrolyses ß-glucans in the rations and thereby decreases the intestinal viscosity.

Cellulase

It acts on fibre which is undigestible to the birds. Cellulose is a small proportion of grain cell walls and is thought to be of little nutritional consequence. Glucan chains of cellulose are held together in an organized manner by inter and intramolecular hydrogen bonding which renders this carbohydrate insoluble and resistant to enzymatic hydrolysis.

Pectinase

It acts on the pectin fraction of carbohydrates.

Lipase

Lipases results in breakdown of oils and fats into free fatty acids , partial glycerides and glycerol.

Proteases

Acts on proteins to liberate peptides and amino acids. It neutralizes negative effects of proteinaceous anti nutritional

Table 4 : Enzymes sources

Fungal species	*Bacterial Species*
Trichoderma longibrachiatum	*Bacillus subtilis*
Trichoderma Viride	*Bacilus amyloliquefaciens*
Aspergillus Spp.	*Arthrobacter ureafaciens*
Rhizopus Spp.	
Saccharomyces cerevisiae	
Candida cylindracea	

(Source: Enzyme Technical association)

factors. *Frequent cases where adverse effects of protease supplementation have been observed* (Naveed etal, 1999). This may be due to number of factors like

- Increased exogenous protease may give negative feed back to animal where proteases in the gut exceed a particular threshold value and animal secretes less proteases (endogenous)
- Supplemental protease may alter the texture and size of antinutritional proteins such as Lectins and enzyme inhibitors so that they are able to diffuse more effectively within the GIT and produce adverse effects.

Galactosidase

Hydrolyses galactosides found in legumes like soybeans. It neutralizes anti-nutritional factors like trypsin inhibitors from soy meal.

Phytase

It hydrolyses phytic acid to myoinositol and phosphoric acid, thereby releasing phosphorus. Phytase frees phosphorus contained in cereals and oilseeds, and by breaking down the phytate structure also achieves the release of other minerals, such as calcium, amino acids, which are bound to phytate.

How enzymes are of help to chicken

Nutrient digestibility is a hydrolytic process initiated by endogenous enzymes in the animal's gastro intestinal tract. However monogastric animal do not produce enzymes capable of hydrolyzing all feed ingredients. No animals can synthesize the enzymes needed for breaking down cellulose and NSPs. Instead animals rely on microorganisms in their digestive tract to accomplish this job. In chickens the microbial population is located in the cecae. The major site of nutrient digestion and absorption is small intestine and cecae is located at the terminal end of small intestine. Approximately 80-90 % of all dietary nutrients are absorbed prior to the intestinal contents reaching the last one-third of small intestine. Considering normal rate of passage, the digesta reaches this section within 1.5 hours following feed consumption. Many of nutrients released by microbes in the ceacae are unavailable to the bird, since they are beyond the major site of absorption, and they are simply excreted with the faeces. It would be advantageous, therefore to introduce specific enzymes into the diet that breakdown and release cell wall constituents early during the digestive process. If the cell wall constituents can be broken down prior to reaching the terminal end of small intestine, the nutrients released are available to the bird. For example, non-starch polysaccharides, beta glucans, cellulose and pentosans, cannot be normally broken in the gastro intestinal tract of mono-gastric animals. Addition of enzyme such as beta glucanase may release extra nutrients from the feed. Other exogenous enzymes such as xylanase reduce the viscosity of

Table 5 : Comparisons between two enzymes sources

Parameters	Fungal Enzyme	Bacterial Enzyme
Activity in gut pH range	3-5	Above 6
Heat stability during pelletisation	Unstable	Improved stability
Sensitivity to Xylanase inhibitors	Highly sensitive	Resistant
Selectivity	Acts on soluble arabinoxylans	Acts on both insoluble and soluble arabinoxylans
Duration of activity	Low (acts only in crop and gizzard)	High (Acts in intestine)

gut contents. The presence of polysaccharides in the diet produces viscosity and is thought to increase the size and stability of the unstirred layer at the mucosal surface of the digestive tract. This reduces the contact between the feed and the digestive enzymes and slows the uptake in the fore gut of released sugars, amino acids and lipids resulting in impaired digestibility of the major nutrients (Bedford,1995 ;Van der Klis et al., 1995).The increase in the viscosity of the digesta promotes bacterial proliferation to the detriment of both overall digestive efficiency and bird health. (Choct et al.1996)

Mode of action of Enzymes

The mode of action of enzymes can be described by the "Lock and Key" principle. Imagine a substrate (the molecule on which an enzyme acts) as a kind of lock, and an enzyme as the only key, which will open it. Put the two together and a rapid reaction takes place, which breaks apart, the substrate into two or more smaller parts. The enzyme key is then removed intact to play its role in another reaction. The Enzyme, itself, is not consumed in the reaction.

To be fully effective within the bird, feed enzymes must function quickly in the gastrointestinal region and/or very efficiently within the duodenum and jejunum of the small intestine. Ishiki and co workers (1989) reported that more than 85 % of all nutrients were absorbed prior to the terminal jejunum of the chicken and feed reaches this part of the gut in approximately 100 minutes.

What are the Enzyme sources?

Feed enzymes mainly are derived from fungus and bacterial species. Bacterial enzymes appear to have some distinct advantages over fungal origin enzymes as described in table 5.

Factors determining the usage of appropriate type of enzymes in the ration

For determining the appropriate enzyme to be added in the feed, following factors should be considered in the composition of feeds.

- Type of cereals used
- Inclusion rate of cereals.
- Level of Antinutritional components in the cereals. (Differences due to variety, climate, soil etc.)

A second factor is the animal for which the feed is intended. Especially age and species has to be taken consideration.

Generally in chicken diets following combinations of enzymes are used.

- Use of Xylanase in arabinoxylan rich rations.
- Use of ß-glucanase in ß-glucan rich rations.
- Use of α–amylase in rations rich in starch.

However the choice of enzymes is not as simple. For Example; Maize, which contains 69 % starch, is likely to be combined with α–amylase. On the other hand maize also contains 5.1% insoluble arabinoxylans. The arabinoxylans in the cell wall enclose starch and other nutrients. The presence of xylanase in the feed will set free these captured nutrients. Exogenous α–amylase make sure that this extra starch is digested in the small intestines. So a combined use of xylanase and á–amylase will increase the digestibility of corn based rations.

Factors affecting efficacy of enzymes when used as a feed additive

Since enzymes are proteins, the structure of the enzyme is critical to its activity. pH, heat or certain organic solvents can alter enzyme structure . Changes in the structure of the protein can decrease or negate enzymatic activity. The temperature which feeds are exposed during the pelleting process can range from 60° to 90° C. under normal conditions. These temperatures and pressure can therefore lead to loss of feed borne and added enzyme activity (Rexen, 1981) . Recent studies reveal that enzyme activity begins to decrease as pelleting temperatures reach 80°C. The data suggests that cellulase, fungal amylase, and pentosanase can be pelleted at temperatures up to 80°C and bacterial amylase up to 90°C without any considerable loss of activity. (Spring et al.1996).

When enzymes have been applied to diets in the absence of antibiotics, it is clear that the microbial challenge is greater. This is partly due to increased concentration of rapidly fermentable oligomers in the posterior sections of the GIT. In such cases birds may be more susceptible to microbial challenge and thus to disease. (Marounek etal., 1999)

Factors affecting enzymatic activity.

- Presence of respective substrates
- Optimum temperature
- Pelleting and storage stability
- Resistance against gastric acidity and proteolytic attack
- Optimum pH
- Spectrum of enzymatic activity
- Age of the bird.

Advantages of addition of enzymes in the diets

- Improved metabolizable energy
- Increased mineral utilization, especially phosphorus
- Improved feed conversion
- Increased growth rate
- Decreased viscosity of intestinal digesta resulting in more normal rate of passage.
- Fat absorption from saturated fats is increased in chicks due to reduced intestinal viscosity.
- Reduced sticky droppings
- Fewer downgrades and cleaner eggs
- Improved litter conditions
- Breakdown of anti-nutritive factors that have a negative effect on performance
- Improved flock uniformity.

Considerations in incorporating enzymes in commercial diets

- What is the target ingredient?
- What enzyme activity is required?
- What is the stability of the enzyme source during feed processing and within the digestive tracts?
- Will an enzyme cocktail be more effective than a single type of enzyme?
- Do different commercially enzyme preparations have the same efficacy?
- Should the enzymes be added to the dry mix or applied as a liquid spray?
- What are the costs and benefits of using enzymes?

Conclusion

Feed manufacturers should look to use enzymes as a means to alter the nutrient worth of the ingredients under consideration in order to obtain the optimal benefit from the specific feed enzyme. A broiler company can benefit from using feed enzymes by reducing feed cost per kg of meat produced through improved broiler performance on standard diets or by altering the nutrient specifications of the cereal grains. Feed enzymes allow a wider range of ingredients to be used in a diet for desired outcome. This gives the producer a great deal of flexibility in formulating a nutritionally balanced, least cost diet. Reduction in manure output would have environmental benefits extending far beyond the broiler industry, and be particularly relevant to producers in areas of intensive animal production.

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