Arbeitsgemeinschaft
für Wirkstoffe
in der Tierernährung e.V. (AWT)

Enzymes

in Animal Nutrition

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Trade Association AWT

AWT is the German Animal Feed Additives association with international activities representing the professional, scientific and technical interests of leading manufacturers and processors of feed additives.

Scope and objective:

- *Safeguarding of member interests and acting as their representative when dealing with authorities, governmental departments, legislative bodies, professional organisations and other institutions nationally.*

- *Representation of German interests in additives internationally.*

- *Co-operation with the harmonisation of conditions for the approval of additives.*

- *Briefing and advice of members in all professional matters, particularly on current legislation.*

- *Informing the public on the use, safety and quality of additives in animal nutrition.*

Contents

1. Introduction

1.1 What are enzymes?

Enzymes are:

- proteins
- catalysts
- naturally occurring
- highly specific.

Enzymes are proteins with a highly complex three-dimensional molecular structure. They only act under very specific reaction conditions (temperature, pH, and humidity) and only with their specific substrates. Enzymes are highly effective biological catalysts which can be found in all biological systems. They accelerate chemical reactions sometimes by as much as 10^6-fold which would otherwise, under the usual conditions prevailing in the organism, proceed only very slowly or not at all. Moreover, it is enzymes which make possible a controlled succession of chemical reactions in biological systems. They are not spent during the reaction, but catalyse and return to their original state once the reaction is complete. For this reason the amount of enzyme required is very small compared to substrate.

Enzymes are produced by all living organisms such as micro-organisms, plants and animals and thus are present in all cells as well as in extracellular spaces. Enzymes added to feedstuff are broken down in the digestive tract in the same way as other proteins. Hence there are not any residues to be found in the faeces or liquid manure, nor is it necessary to observe any withdrawal period before animals fed on enzyme supplemented diets can be slaughtered. Because enzymes are highly specific for the reaction they catalyse, it may be beneficial to add a mixture of different enzymes to the feed in order to be able to break down several unfavourable substances at the same time. When using an enzyme mix it has to be ensured that these enzymes will all act under the same reaction conditions. If this prerequisite is met, as for example in multi-enzyme systems, their application is often superior to that of using individual enzymes.

1.2 Historical development

Enzyme activity was used by mankind throughout history for thousands of years in numerous processes of producing and preserving food without having sufficient knowledge of the nature and characteristics of the enzymes they used. Conventional processes used for producing alcoholic drinks (wine) and fermenting dough were already illustrated in old Egyptian wall paintings.

For these processes, primarily living microbes were added to raw materials obtained from plants and animals.

These micro-organisms such as yeasts and lactic acid bacteria (Lactobacillaceae) produced in turn the enzymes required for the desired con- version. Further examples of enzyme applications are the preservation of white cabbage by adding lactic acid bacteria and also the production of cheese in order to preserve milk and milk constituents.

Enzymes play a major role in all vital processes. Their actual existence was only established in the 19th century. In 1857 Pasteur could demonstrate that fermentation is closely associated with the activity of live yeast. The term "enzymes" was coined in 1878 by Kühne for "soluble ferments" which are not bound to the living cell. This term derives from the Greek "en zyme" and has the same meaning as "in leavening". In 1897 Buchner provided the final and decisive evidence for the effect of enzymes when he demonstrated that the cell-free squeezed juice obtained from yeast cells can cause alcoholic fermentation. In 1893 Ostwald in turn recognised the catalytic effect of enzymes. Then, in 1909 Röhm discovered the activity of proteases originating from the animal pancreas when processing hide for the preparation of leather.

The systematic development of industrial enzymes started with the isolation of a mixture of enzymes from a mould (aspergillus oryzae) cleaving carbohydrates (carbohydrases) and proteins (proteases) by the Japanese scientist Takamine in 1894. Only a year later Boidin was able to develop a process for obtaining alcohol from grain which also employed a mould. The enzymes of this mould caused the saccharification of the starch contained in the grain. The sugars thus obtained are subsequently fermented to alcohol by the yeasts.

Despite intensive research in the 19th century it was not possible to establish the chemical structure of enzymes. Not until 1926 James Summers was able to finally demonstrate with urease that enzymes are proteins.

The period after World War II then saw the advent of an intensive development in the production of substances by fermentation technology which in its beginnings mainly served to obtain antibiotics as well as fungal and bacterial amylases. Today the majority of commercially important enzymes are produced are micro-organisms (fungi, yeasts and bacteria). In addition to these, enzyme preparations from animal tissues (e. g. lipases and proteases from the pancreas) and plants (e. g. papain, a protease contained in the papaya fruit) also play an important role in the industrial application of enzymes to this day.

At present the commercially most important groups of enzymes produced by microbes are the proteases and carbohydrases. The main enzyme markets are the detergent industry followed by starch production and dairy processing. Until the middle of the eighties the use of enzymes in animal feeds was only of secondary importance. They were mainly employed in regions such as Canada, Scandinavia and the former GDR where a limited availability of highly digestible raw materials, such as maize, made their use a necessity. The technical effects observed so far by the application of enzymes, which originally were developed for other application purposes, seemed to make their use in animal nutrition very often not attractive enough. This situation only changed when the marketing of enzyme preparations specially developed for animal feeds attracted an increased interest in these products.

Feed enzymes are the result of many years of expensive research and development processes. Due to their gradually increasing importance they were included in the directive 70/524/EEC for additives in animal nutrition as a new group of substances in 1993 and are thus in the European Union legally regulated for use in animal feeds. They are subjected to a rigorous approval procedure by the European Commission, general directorate for agriculture. Only after thorough examination by this regulatory body and scientific panels of the 15 member states is approval by all EU states granted by mutual recognition procedure. In addition to the efficacy and quality of an additive the safety of humans and animals as well as environmental protection is at the heart of these examinations.

2.1 Nomenclature of enzymes

In the early stages of the research into enzymes they were almost exclusively given trivial names. Examples for this are names such as "intermediate ferment" or "pH 5 enzyme". Whilst many of these names are almost forgotten today (such as ptyalin, a starch degrading enzyme in the saliva) other names such as pepsin or trypsin have survived and are still used to this day. From around the turn of the century onwards enzymes were characterised by the suffix ´ase´. This convention has since been adopted worldwide so that all enzymes are named in this way. In 1961 an international commission known as the Enzyme Commission or for short E.C. has drawn up certain rules and regulations for the systematic classification of enzymes. In this classification enzymes are divided into six main classes according to the type of reaction they catalyse.

Main enzyme classes:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases (synthetases)

Enzymes used as additives in animal nutrition are exclusively hydrolases.

The hydrolases with the E.C. No. 3, which break down C-O, C-N, C-C and some other chemical bonds are differentiated by the type of the molecular group to be broken down.
The following hydrolases can play an important role in the use as feed additives:

E.C. 3.1	Phosphatases (e.g. phytase)
E.C. 3.2	Glycosidases (e.g. carbohydrases)
E.C. 3.4	Proteases

Each molecular group has an inherent series of specific types of bonding. Thus the glycosidases are further subdivided into:

E.C. 3.2.1	O-glycoside hydrolases degrading
E.C. 3.2.2	N-glycoside hydrolases degrading
E.C. 3.2.3	S-glycoside hydrolases degrading

Only O-glycoside degrading hydrolases are relevant for use in animal nutrition.

The last digit of the four in the E.C. code stands for the molecule to be converted. In the following there are listed a few enzymes from the glycosidase group by way of example:

E.C. 3.2.1.1 α-amylase
E.C. 3.2.1.3 glucoamylase
E.C. 3.2.1.4 cellulase (1,4-β-D-glucanase)
E.C. 3.2.1.6 β-glucanase (1,3-1,4-β- D-glucanase)
E.C. 3.2.1.8 xylanase (1,3-1,4-β-D- xylanase)

The table below *(table 1)* illustrates by way of example the enzyme classification system according to the E.C. code:

In addition to the common name, the Enzyme Commission also assigns a systematic name for each enzyme in order to prevent ambiguities as far as possible. Thus the systematic name for xylanase for example is 1,4-β-D-xylanxylanohydrolase.

Due to this grading, each enzyme is allocated a four digit classification number (E.C. No.). This system is not specific for feed enzymes but comprises all enzymes known. Instead of the E.C. No. an IUB No., which was published by the "International Union of Biochemistry", is sometimes used. However, E.C. and IUB numbers use the same four digit code and hence are identical.

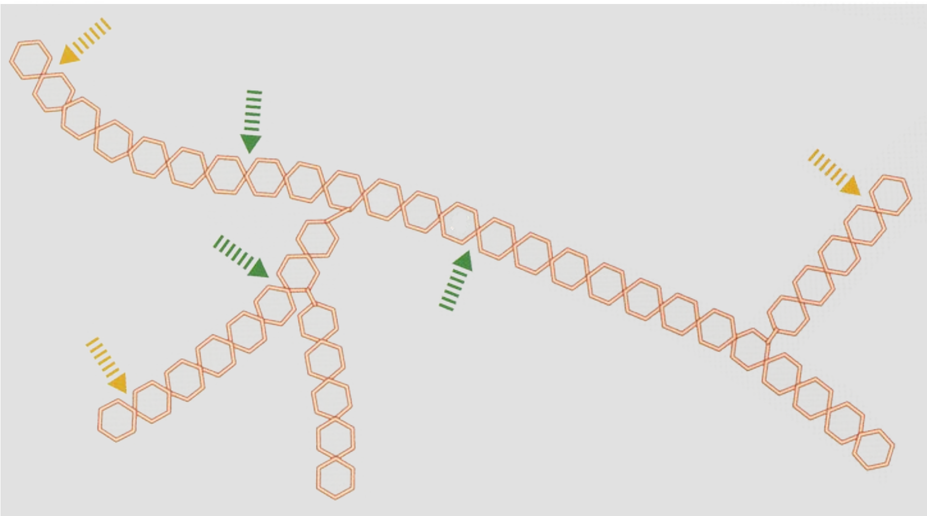
Table 1
Enzyme classification according to the E.C. code

Main Group (1st digit of code)	1st subgroup (2nd digit of code)	2nd subgroup (3rd digit of code)	Specific enzyme (4th digit of code)
The type of catalysed reaction, e.g. hydrolysis (E.C. 3)	The type of substance group to be degraded, e.g. hydrolases, which break down carbohydrates (E.C. 3.2.)	The type of bond to be broken down within a substance group, e.g. carbohydrate degrading hydrolases, which break down the O-glycosidic bonds (E.C. 3.2.1.)	Identification of the enzyme-specific substrate, e.g. xylanase for breaking down the specific carbohydrate xylan (E.C. 3.2.1.8)

2.2 Endo- and exo-enzymes

For enzymes used in animal feeds the degrading site of the substrate plays a major role in the effect to be achieved. There is a fundamental difference between exo- (external) and endo- (internal) enzymes. Exo-enzymes only break down the terminal structural building blocks of the molecular strand whilst endo-enzymes degrade bonds within the molecular strand *(see figure 1)*. Endo-enzymes are thus able to effectively break down large and long-chain molecules into smaller fragments. This is mainly significant for the effect on the digesta viscosity through enzymes which break down non-starch polysaccharides.

Figure 1
Schematic diagram of the sites attacked by exo-enzymes and endo-enzymes on a branched molecular strand



2.3 Characteristics of enzymes

In addition to the specific degradation site in the molecule, the effectiveness of an enzyme is further largely determined by the prevalent conditions at the site of the reaction. The reaction conditions include the pH value, temperature, water content and presence of activators or inhibitors as well as the substrate concentration. Depending on their origin, i.e. of the producing strain, enzymes vary quite considerably in their activity depending on the reaction conditions.

Whilst the detergent industry is more interested in enzymes that display high activity at 60 °C for example, animal nutrition requires enzymes that are highly effective around 40 °C. Nevertheless stability at high temperature is attempted for enzymes to be used as feed additives so that no inactivation is encountered at pelleting temperatures of 70-80 °C. The effect of the pH value on the enzyme activity is shown in *figure 2*.

Activity profile of various fungal xylanases versus pH value. Measurement of reduced sugars by means of the CVS method over 30 minutes at 50° C using oat spelt xylan as substrate

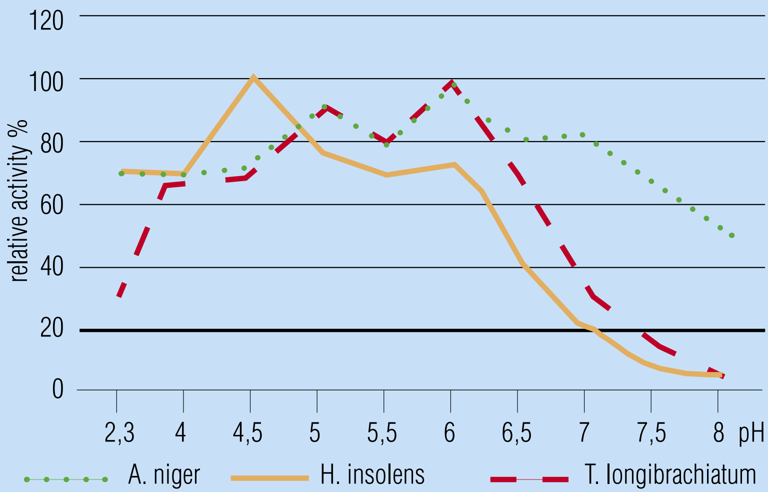


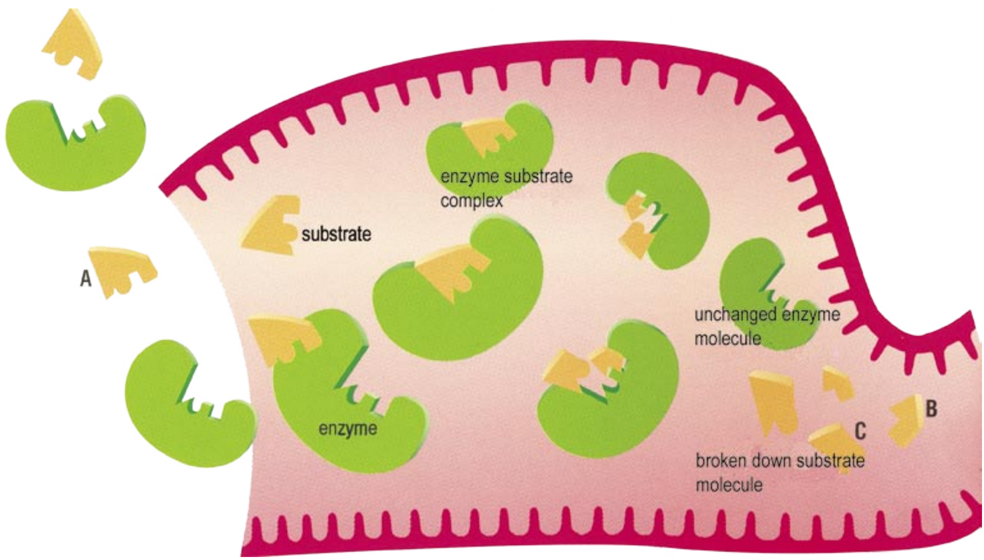
Figure 2
Effect of pH values on the activity of xylanases

Enzymes exhibit a high specificity, which means that each enzyme only breaks down highly specific substrates. This is what is known as the so-called "lock and key" principle. The following diagram *(figure 3)* shows the mode of action of enzymes:

Substrate A forms an enzyme substrate complex with the enzyme from which the reaction products B and C are released much faster than would be possible without enzymes. An example of enzymatic breakdown is that of trypsin breaking down a polypeptide (substrate A) into several smaller peptides (products B and C). At the end of the reaction the unchanged enzyme is available to break down yet another polypeptide.

In summary, the reaction conditions of feed enzymes must be adapted to those conditions which are prevalent in the digestive tract of animals. They must either be able to act under the acidic pH conditions prevalent in the stomach or must be able to resist both the low pH and the proteolytic action of the pepsin in the stomach in order to be able to act in the following sections of the digestive tract. This prerequisite must be considered when selecting enzymes for use in animal nutrition.

Figure 3
Mode of action of enzymes



3.1 Production process

Currently, enzymes are mainly produced with the help of micro-organisms, particularly from fungi and bacteria. This is generally speaking more economical than isolating enzymes from plant or animal source materials. In addition, micro-organisms are able to synthesise a very broad spectrum of hydrolytic enzymes which the animal organism is largely not able to produce itself. As many micro-organisms are adapted to cope with extreme living conditions (temperature, pH, osmolarity) microbial enzymes are in this respect often more stable than enzymes originating from plants and animal. Moreover, microbial enzyme preparations can also be better standardised, which is a further advantage. Only such fungal and bacterial strains are employed which are able to multiply quickly and show a high biosynthesis performance under industrial production conditions. The aim is not only to achieve a high enzyme concentration but also a high yield.

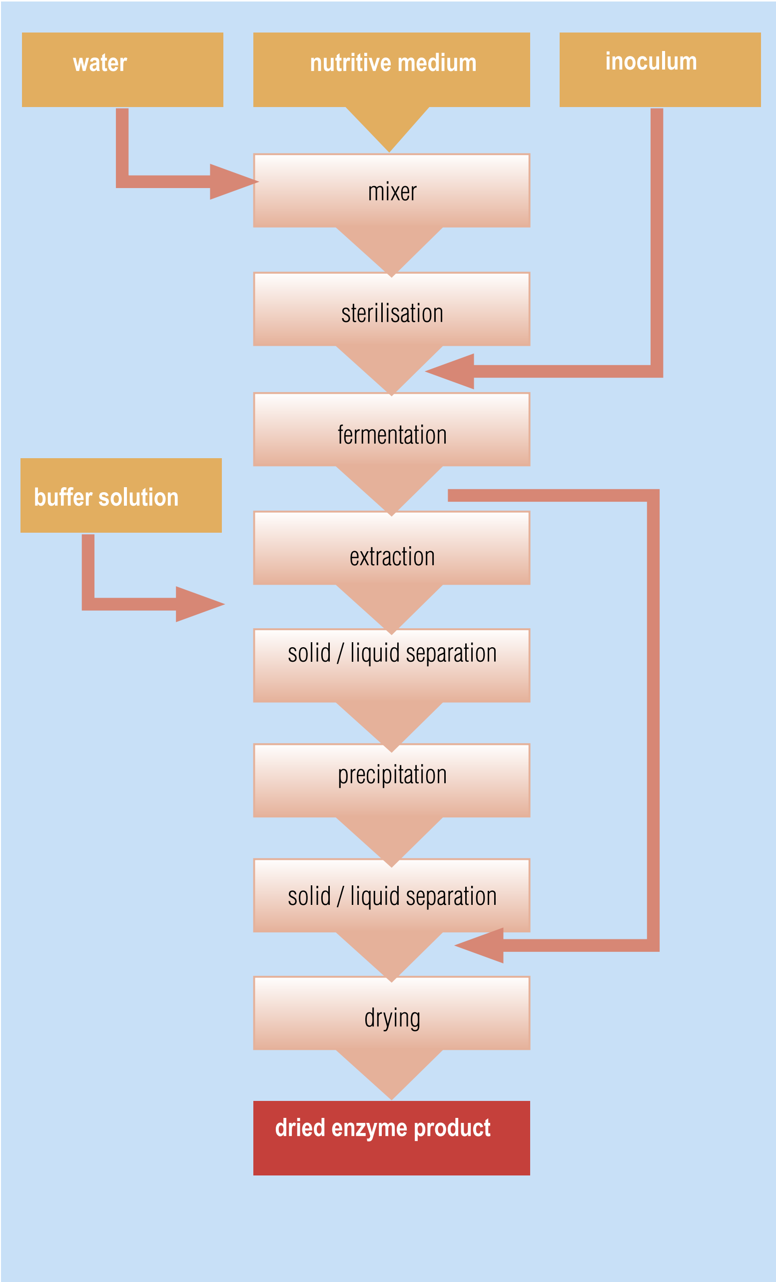
Strains suitable for this purpose are either selected from the wild population and developed or genetic information for certain enzymes is transferred by genetic engineering to suitable production strains. Strains which are particularly sought after for this purpose are those which can synthesise the desired enzyme or enzymes in the largest possible quantities using specific nutritive media. Employing genetic engineering enables production whilst protecting precious resources. In order to find a specially suited strain, hundreds of different ones must be tested by particular screening procedures. The selection of the right micro-organism is of the utmost importance so that in the end the synthesis of the desired enzyme activities is achieved in the production process. In addition the composition of the nutritive medium also has a decisive role to play. Thus the presence, for example, of starch in certain strains induces the production of amylases whilst the presence of casein or albumin induces the formation of proteases.

In the production of enzymes on an industrial scale differentiation has to be between the

- "surface" procedures and
- "submerged" procedures.

An essential characteristic of the surface procedure (solids or surface fermentation) is the cultivation on solid or pasty nutritive media with surface venting. After completion of the fermentation process the solids are homogenised, adjusted to a moisture content of 10-12% and then ground. The powder thus obtained can then be used as an enzyme preparation for industrial purposes.
It has to be noted that viable forms of production organisms must not be contained in the final product. This is ensured by putting relevant precaution-

Figure 4
Schematic diagram of enzyme production by means of the surface process



ary measures into place. However, some applications require further processing of the fermentation product. An extract is produced for this purpose from which the enzyme can be obtained in various ways (e.g. by ultrafiltration, precipitation etc.) *(figure 4)*.

In contrast, the submerged process is much more frequently employed than the laborious and cost intensive surface process. In this process the enzyme producing micro-organisms are not cultivated on the surface but within a liquid nutritive medium. This method offers a number of advantages, among others a better control of the nutritive medium composition, the pH value, the temperature and the venting. Furthermore there is also a reduced risk of foreign infection. This method can be used with almost all relevant micro-organisms. The procedure of a submerged fermentation can be seen schematically in *figure 5*.

The fermentation products obtained by this method are purified, standardised and subjected to quality control. They are marketed both in solid and liquid form.

3.2 Classification of enzyme producing micro-organisms

Various fungi, bacteria and to some extent also yeasts are employed as enzyme producing micro-organisms. Enzyme production is essential for these micro-organisms since they sustain their own viability by producing enzymes to break down substrates for further metabolisation. In addition specifically selected strains or organisms modified by genetic engineering can result in a far greater yield of enzyme.

Enzymes used in feedstuff production are derives from micro-organisms which commonly occur in nature or there exists long-term experience on their use in the food and feedstuff industry. Comprehensive tests have demonstrated their safety in production and application.

According to the agreement reached at Budapest, each production strain must be deposited in an approved strain bank. The name and location of this strain bank, the storage reference number as well as all properties necessary for the identification of the relevant production strain must be submitted to the regulatory authority prior to an approval being granted for the enzyme to be used as a feed additive.

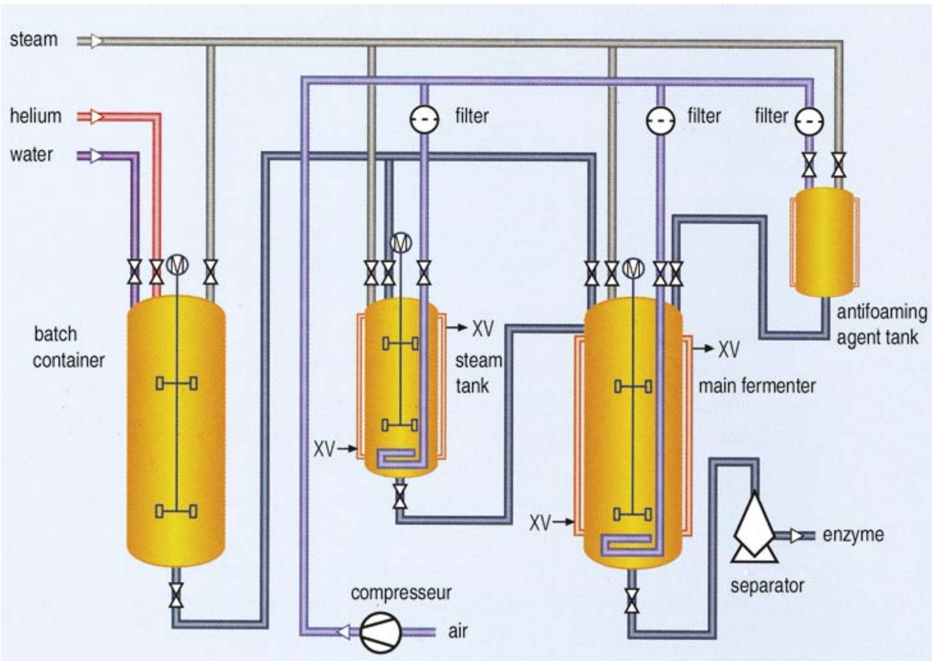
Fungi represent the largest group among enzyme producing micro- organisms. The most important genera are: *Aspergillus ssp. (e.g. A. niger), Penicillium ssp., Humicola ssp. (e.g. H. insolens)* as well as *Trichoderma ssp. (e.g. T. longibrachiatum)*. These fungi produce enzymes for the degradation of various substrates. However they all have one thing in common, that is the production of enzymes for the breakdown of plant cell wall components in the form of high polymer carbohydrates.

In the bacteria group, *Bacillus ssp.* is of particular importance for animal nutrition. α-Amylases and proteases are mainly produced by *Bacillus ssp.* of which *Bacillus licheniformis* and *Bacillus subtilis* are frequently used; but also β-glucanases and xylanases can be produced by Bacillus species.

3.3 Areas and importance of use

The demand of the world market for industrially produced enzymes has increased more than 10-fold during the past 25 years and today is worth more than DM 1 billion. The market for feed enzymes still has a compara-

Figure 5
The principle of enzyme production with the submerged procedure



tively small share in this but is among the fastest growing sectors today.

Enzymes are used in the following sectors:

- Diagnostics and medical therapy
- Cosmetics Food industry (dairy processing, production of alcohol, production of fruit juice, bakery products)
- Leather industry
- Paper industry
- Starch industry
- Textile industry
- Animal nutrition
- Detergent industry

Enzymes contribute in many ways to the economic viability of production processes within these fields of application. Thus enzymes are able to improve the yield and utilisation of raw materials, reduce manufacturing costs, assist the preservation of products as well as technical properties of process aids or to positively alter end products.

3.4 Use in animal nutrition

Enzymes specially developed and produced for application in animal nutrition are within the broad range of industrial enzymes, still a very young product group even though the first trials, particularly in poultry and piglets, go back as far as 50 years.

At the beginning, enzymes did not catch on because the preparations used were originally developed for different purposes. They therefore showed an insufficient and varying efficacy when used in animal feeds as they frequently did not meet the physiological and digestive requirements.

Moreover, the above mentioned fermentation technologies in the earlier days resulted in much smaller enzyme yields than is the case today making the enzymes too expensive and hence of no interest for use in animal nutrition.

Continuous development of the production processes using genetic engineering together with an application oriented product development has resulted in the use of enzymes as a regular component of modern feeding systems today. Phytase as well as the enzymes for breaking down non-starch polysaccharides (NSP degrading enzymes) represent the two most significant product categories of currently used enzymes in animal nutrition.

4.1 Classification of substrates

Substrates to be broken down by feed enzymes can be mainly divided into three main groups:

a) Substrates for which monogastric animals synthesise suitable enzymes in their own digestive tract (starch, proteins and lipids).

Starch for example is made up of amylose and amylopectin which are glucose molecules linked by α-glycosidic bonds *(figure 6)* and combined in a 1,4- or 1,4- and 1,6-configuration respectively. All enzymes (α-amylase, glucoamylase, maltase, isomaltase, maltotriase, β-glucosidase) which are necessary for the complete degradation of starch to glucose and subsequently its absorption, are formed by the monogastric animal but may not be available in sufficient amounts under certain conditions such as in young animals, particularly under stress. The same also applies to proteases and lipases.

b) Substrates for which enzymes are not produced by the animal's organism and which have a very low digestibility (e.g. cellulose).

Cellulose comprises linear chains of several thousand glucose molecules. These are linked by β-glycosidic bonds *(figure 7)* and therefore nearly indigestible by monogastric animals and only partly broken down by micro-organisms in the digestive tract (low metabolisable energy).

c) Substrates for which enzymes are not produced by the animal's organisms and which in addition have antinutritive effects (e.g. 1,3-1,4-β-glucans, pentosans and phytate).

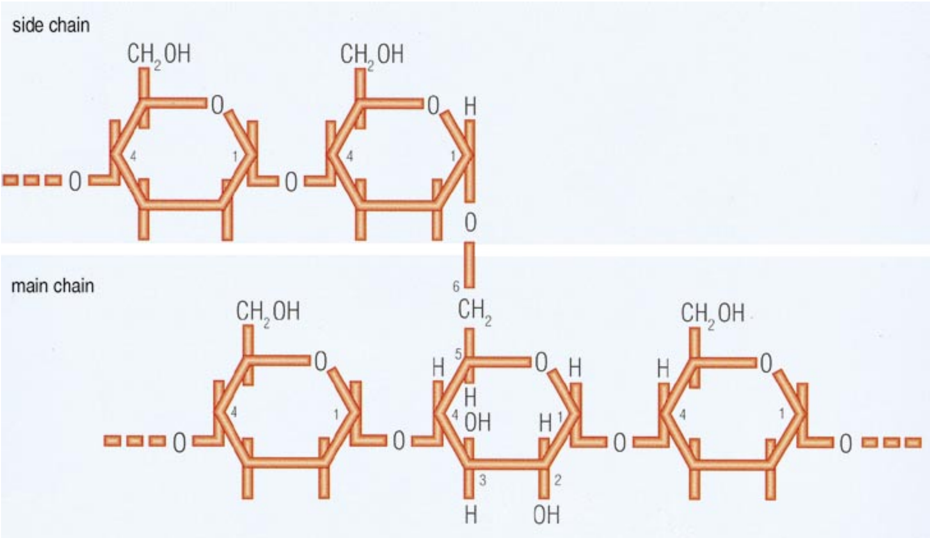


Figure 6
Section from an amylopectin formula. The glucose molecules are linked together in a 1,4- or 1,6-bonding.

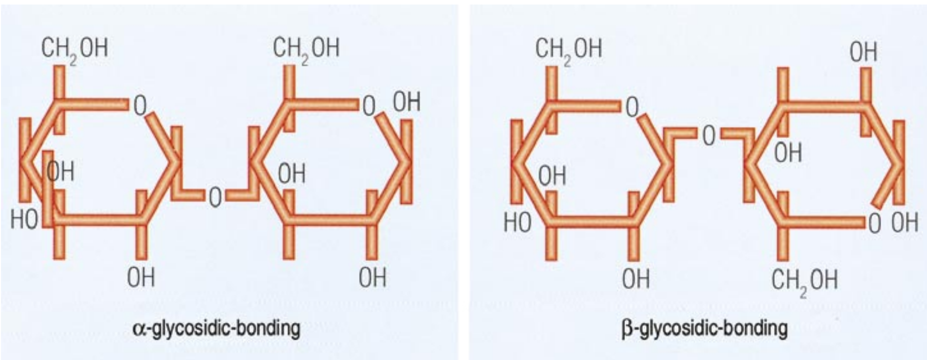
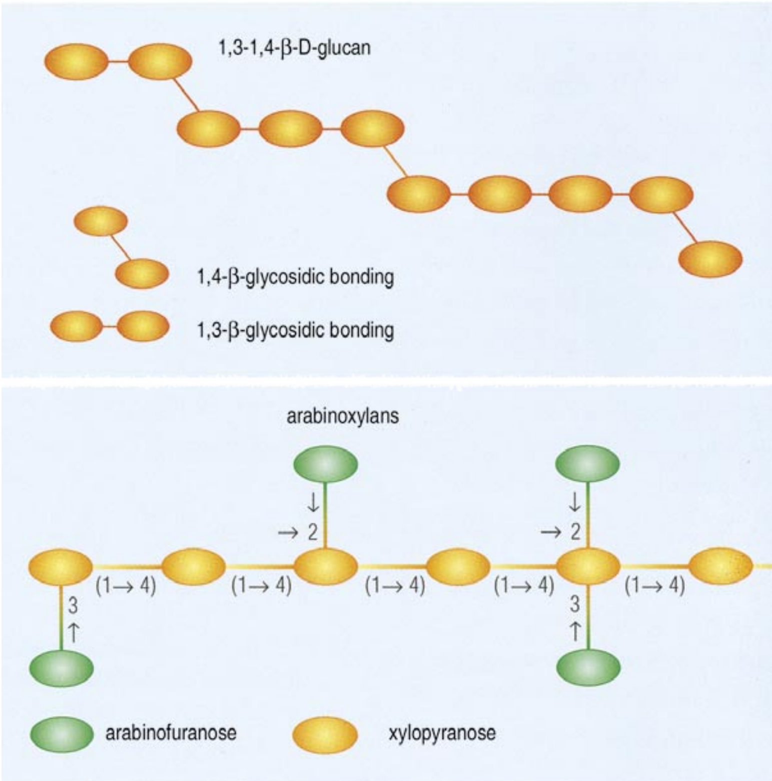


Figure 7
α- and β-glycosidic bonding

Figure 8
Schematic diagram of the structure of 1,3-1,4-β-glucans and arabinoxylans



Just like cellulose, 1,3-1,4-β-glucans are also glucose molecules linked by β-glycosidic bonds *(figure 8)*. In addition to the 1,4-bonds which are characteristic for cellulose they also have 1,3-bonds which are responsible for the strong branching of these β-glucans and the likelihood of water inclusion (swelling) and thus an antinutritive effect (increased viscosity).

Pentosans which are mainly present as arabinoxylans in rye and wheat also show a strong viscosity increase and hence antinutritive effect. Arabinoxylans consist of a main chain of xylopyranose and side chains of arabinofuranose. The structure of 1,3-1,4-β- glucans and arabinoxylans is shown in *figure 8*.

In addition to these substances which belong to the non-starch polysaccharides (NSP) there are higher concentrations of specific indigestible oligosaccharides (α-galactosides, e.g. raffinose, stachyose, verbascose) present, mainly in vegetable protein carriers (legumes, rape seed). These are essentially short-chain compounds (3 to 5 molecules) made up of glucose and galactose molecules.

Another important and poorly digestible substance contained in feedstuffs is phytic acid and its salts (ester of the hexaphosphoric acid of inositol) *(figure 9)*.

The salts of the phytic acid are called phytates. A maximum of six phosphate groups of the inositol ring can bind various cations such as calcium, magnesium, iron and zinc in fixed complexes and thus interfere with their availability.

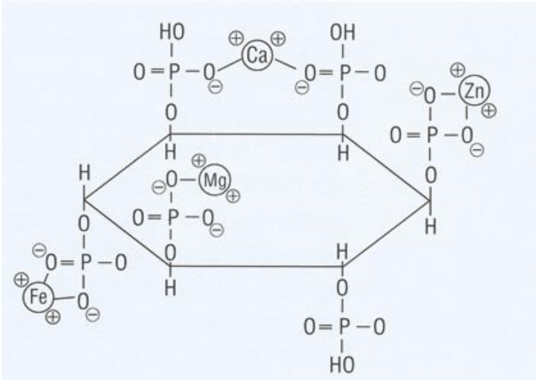


Figure 9
Phytate structure

4.2 Concentrations in feedstuffs

The concentration of NSP-fraction in feedstuff varies quite considerably. In addition to the plant species and type it is mainly dependent on climatic conditions, the cultivation site and the harvest period. The NSP concentrations of selected feedstuff are shown in *table 2*.

Table 2
Concentrations of phosphorus, phytate phosphorus, crude fibre and various NSP-fractions in selected feed ingredients (in g/kg dry matter)

Ingredient	Crude fibre	β-Glucans	Pentosans	Total NSP	Phosphorus	Phytat phosphorus
Wheat	20-34	2-15	55-95	75-106	3,8	2.3-2.9
Rye	22-32	5-30	75-91	107-128	3,9	2.5
Triticale	30	2-20	54-69	74-103	4,5	*
Barley	42-93	15-107	57-70	135-172	4	2.2-2.9
Oats	80-123	30-66	55-69	120-296	3,9	2.1
Maize	19-30	1-2	40-43	55-117	3,1	2.1
Wheat bran	106-136	*	150-250	220-337	12	7.2-9.2
Soybean extr. meal	34-99	*	30-45	180-227	7,4	4.4
rape seed meal	109-159	*	*	187	11,4	6.8-8.3
Feed peas	56-72	*	*	156	4,8	1.9-2.4

** No data available*

Pentosans are predominantly present in native crops. Rye, oats and barley contain in addition significant proportions of β-glucans. In this case it has to be taken into account that under the conditions of the European climate the extreme values listed in table 2 will not be reached. Hence the level of β-glucan in barley in Germany is mainly in the range of 30-45 g/kg dry matter. However, the total level of β- glucans or pentosans in the crop alone still does not allow for any conclusions to be drawn regarding a possible antinutritive effect. The soluble part of these compounds is also important, since only soluble β-glucans or pentosans result in the development of viscosity. Hence an antinutritive effect of barley is primarily due to the 1,3-1,4-β-glucans because their soluble portion is considerably higher than that of pentosans. Furthermore the soluble part of pentosans in wheat can be dependent on the type and the batch of wheat present.

In plant based feedstuff approximately 50-80 % of the phosphorus present is bound to phytate (compound with calcium, magnesium, zinc and other bivalent cations) *(table 2)*. Phosphate from the phytic acid can only be broken down by the enzyme phytase (which is virtually not produced by monogastric animals) and thus be made digestible for the animal.

Plant fibre components can be chemically defined as the total of NSP and lignin. In addition to the pentoses (arabinose and xylose) and hexoses (glucose, mannose and galactose) they contain desoxyhexoses (rhamnose, fucose) as well as hexauronic acids (galacturonic acid).

The possibilities of characterising the poorly digestible viscous feed materials have very much improved in the course of the last few years. Thus it is possible today to analyse for example phytate and phytate phosphorus, β-glucans, pentosans, pectins and cellulose with various methods. In this way a better assessment of feeds with respect to their utilisation in the animal is possible, than e.g. by analysing crude fibre. However, when assessing the crude fibre level of the feeds, the fraction of the soluble NSP, which is of particular importance, is not detected. As can be seen in ***table 2*** the NSP level is significantly higher than the corresponding crude fibre level.

It has however to be assumed that these NSP fractions detected by analysis are present in plant cell walls in various complex bonds within themselves and with other nutrients (proteins, minerals) which makes a characterisation with respect to the effect on the feed value more difficult.

4.3 Effects of NSP compounds and phytate in animals

According to the present knowledge the feed materials can be assigned the following negative effects:

Influence on energy density ("dilution effect")

They are poorly digestible and hence dilute the metabolisable energy and nutritive content in the feed (this applies to all fractions, oligosaccharides, lignin and phytate phosphorus).

Entrapping of nutrients ("cage effect")

Some materials which are the main components of the plant cell wall exhibit a so-called "cage effect", there by entrapping other nutrients such as starch, fat and protein which would be highly digestible nutrients otherwise (this applies mainly to the insoluble parts of the NSP which are present in various cell wall structures).

Increasing the viscosity of digesta

Certain NSP fractions (mainly the soluble components of the β-glucans and pentosans as well as the pectins), certain glycoproteins (i.e. compounds of carbohydrates and proteins) act in the digestive tract by increasing the viscosity. This means they store large amounts of water (swelling) and the digesta will become more or less viscous and sticky. This increase in viscosity hinders the intestinal absorption of nutrients and can result in a negative effect on the consistency of faeces and even symptoms of diarrhoea. The increased digesta viscosity can in turn lead to a slowing down of the feed passage rate and possibly to a decreased feed intake. In addition, the thorough mixing of the digesta with endogenous enzymes and bile is also adversely affected. The following scheme in *figure 10* will illustrate once again the possible consequences of the viscosity increasing effect on NSP.

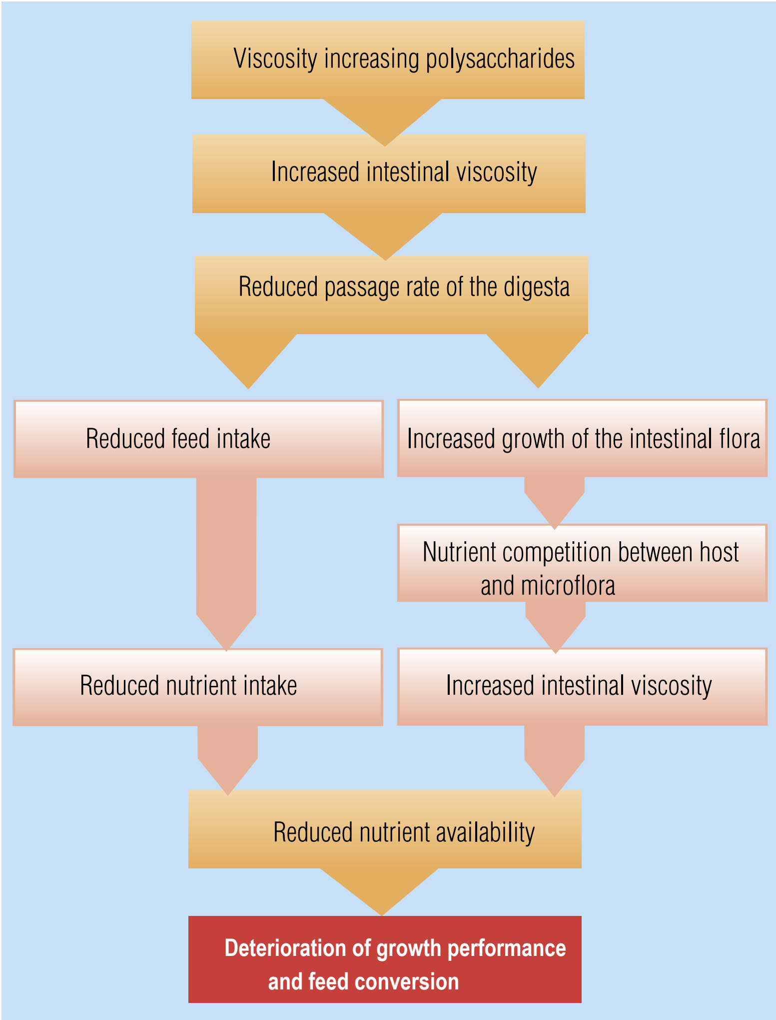
Complex formation

Due to complex formation (e.g. phytate with Ca, Mg, Zn as well as proteins) other nutrients and minerals are more difficult to digest.

Reduction of nutrient absorption

Multiple factors take part in the reduction of the nutrient absorption caused by NSP (e.g. viscosity, altered composition of the intestinal flora, increased absorption of bile, influence on the intestinal mucosa). This could particularly be observed with respect to the digestion of fat. Moreover, a higher then normal excretion of digestive enzymes into the small intestine might be caused by the impaired nutrient absorption resulting in the increased endogenous loss of protein.
According to the purpose of the application, feed enzymes can be fundamentally divided into:

Figure 10
Effects of increased intestinal viscosity



a) Enzymes which are to quantitatively supplement endogenous digestive enzymes of monogastric animals (e.g. proteases, amylases, lipases).

b) Enzymes which are not produced by monogastric animals (e.g. β-glucanases, pentosanases, β-galactosidases, phytases).

5.1 Supplementing endogenous enzymes

The aim of using these enzymes is to balance the gradually occurring sub-optimal synthesis of endogenous enzymes by farm animals. In this context amylases and proteases are primarily addressed. The use of these enzymes is mainly discussed in young animals which eat larger amounts of plant based feeds.

5.2 Supply of enzymes, which are not produced by the animal

Of the enzymes employed in animal nutrition and which are different from those produced in the digestive tract the following effects are known:

Breakdown of components which cannot be digested into absorbable nutrients by endogenous enzymes
This could be demonstrated with phytate phosphorus where phosphates are released by using phytase. Various oligosaccharides can be also broken down to glucose and galactose by α-galactosidases and then absorbed. In the case of complex NSP a number of specific enzymes are required to achieve their complete breakdown. The relatively short retention time of the digesta and the enzymes contained in the digestive tract are normally not sufficient for completely breaking down NSP. In the case of the pentosans (main group of the NSP in cereal) a complete breakdown is not necessary since the breakdown products xylose and arabinose are absorbed but contribute only marginally to the energy supply of the animal due to the inadequate metabolic utilisation. The partial hydrolysis of NSP compounds in the upper parts of the digestive tract however can result in an increased microbial digestion and the production of short-chain fatty acids in the large intestine.

Lowering of the gastrointestinal viscosity in the digestive tract
For lowering the viscosity in the digesta the breakdown of soluble NSP into smaller units is necessary which then in turn loose their property of binding water and swelling. For this purpose the so-called endo-enzymes, i.e. enzymes which attack the internal bonds of long-chain molecules are suitable. Within a short period of time these enzymes (endo-β-glucanases, endo-xylanases) are able to break down these soluble NSP to the extent that the viscosity increasing property of these fractions is largely reduced. Exo-enzymes which attack the molecule at the endings require a much longer reaction time to achieve the same effect.

Due to the reduced viscosity, a better mixing of the digesta is possible thereby increasing the efficacy of the endogenous enzymes. Thus, the digestibility of nutrients as well as the utilisation of the energy contained are improved. In addition, the reduced viscosity results in an increased passage rate of the digesta as well as in drier and less sticky faeces and litter.

Reduced nutrient entrapment

This can be achieved by the breakdown of cell wall structures on order to release the nutrients contained therein, such as starch, protein and fats, and to make them accessible to the digestive enzymes. Again, endo-enzymes are most suited for this purpose as they are able to break open complex structures from within and hence to make the cell wall porous within a short time. This leads to an acceleration of the enzyme-substrate contact. These endo-enzymes have to primarily break down the insoluble NSP which are firmly anchored in the cell wall. A complete breakdown is not necessary for this purpose. The positive influence of this enzyme effect is mainly based on an improvement of the digestibility of the entrapped nutrients.

Releasing other nutrients

NSP, proteins, phytic acid and various minerals are present as complex compounds in the cell walls of plants. Nutrients bound to NSP or phytate are released by NSP degrading enzymes or phytases so that the digestibility of the protein and of various minerals (Ca, Mg, Zn) can be improved as a concomitant effect.

6.1 NSP degrading enzymes

The effects of NSP degrading feed enzymes described under chapter 5 primarily result in a better conversion of the nutrients contained in the feedstuff. From this two different strategies of application can be derived:

"On top" application

Using the same energy and nutrient values for feed ingredients despite the use of enzymes means that there are in fact more nutrients available to the animal compared to a diet without enzymes. This improved diet results in an improved growth performance. This is called an "on top" application.

"Energy uplift" application

If however the improved metabolisable energy resulting from the added enzymes is taken into account by re-evaluating a certain feedstuff (e.g. wheat with the application of a feed enzyme with xylanase activity) then we talk of an "energy uplift". These two application strategies will be briefly illustrated with the help of a trial carried out in broilers *(see table 3)* with four treatment groups of 200 animals each. Group 1 received a wheat based diet without added enzymes whilst group 2 was offered the same diet however with the enzymes added. Group 3 also received a wheat based diet without added enzymes, but the wheat had a 6 % energy uplift. Group 4 finally received the same feed as group 3 but with enzymes added. As can be seen in ***table 3*** group 2 is clearly superior to the control (group 1) with respect to the fattening performance.

Table 3
Effect of a feed enzyme on the fattening performance and viscosity in the small intestine of broilers (0-42 days)

	Group 1	Group 2	Group 3	Group 4
«Energy uplift»	No	No	Yes	Yes
Enzyme application «on top»	No	Yes	No	Yes
Liveweight gain (g)	2187[b]	2321[a]	2130[b]	2403[a]
Feed : gain	1.99[b]	1.88[a]	2.01[b]	1.97[b]
Foregut viscosity (mPa · s)	13.4[c]	4.2[a]	10.5[bc]	4.6[ab]

[a.b.c] Mean values not sharing a superscript in one row differ statistically significantly from each other ($P < 0.05$)

As group 1 and group 2 were fed on the same diet with the exception of the added enzymes, the increase in performance is directly due to the enzyme application. In group 3 the wheat was uplifted with 6% more energy even though the diet did not contain any enzymes. Due to overrating the energy of the wheat, the diet really has a lower energy content which is shown in the poorer growth performance of group 3 compared to group 1. But if a suitable feed enzyme for wheat is added (as shown in group 4) then the same feed conversion is achieved as in group 1.

Whilst for poultry the viscosity lowering effect of NSP-degrading enzymes is generally recognised as an important mode of action this seems not to be so clear-cut for the pig. In this case the effect is considered an interaction of various modes of action (reduction of viscosity, nutrient release through opening up of cell walls).

6.1.1 Effects on the animal and its performance

Besides the effects already mentioned in chapter 5, enzymes have a positive effect on animal performance. In addition to the enhanced availability of nutrients, the enzymes due to a faster digesta passage rate counteract the multiplication of bacteria, which could migrate from the hindgut upwards.

The reduction of the digesta viscosity also results in a decreased stickiness as well as a higher dry matter content of the faeces which gives rise to a drier litter and hence cleaner animals. This effect is particularly important for the rearing of broilers and turkeys due to the usual floor husbandry on litter since in this way the incidence of leg weaknesses and breast blisters can be reduced. This effect also leads to a lower proportion of dirty eggs in laying hens.

When compared with the daily protein turnover, the production of endogenous enzymes in the tissues of the digestive tract is about 25%. These enzymes are able to digest up to 30 times the daily intake of nutrients. There is now evidence that the reduction of viscosity also results in decreased secretion of endogenous digestive enzymes. This assumption prompted a trial with piglets which were fed on a barley based diet with or without enzyme supplements *(table 4)*. Although the mechanism of regulation is not yet completely understood it appears that due to the reduction in viscosity the contact of the enzyme with the substrate is increased. Hence less endogenous enzymes would be required for breaking down the same amount of substrate. Consequently the energy which is not needed for endogenous enzyme production is now available for the deposition of body mass, resulting in an energy and protein saving effect.

Further studies are necessary for substantiating this hypothesis.

6.1.2 Possible applications in compound feed

Although the NSP levels not only vary greatly between the types of cereal but also within a type of cereal depending where it is grown as well as the type and harvest conditions, a more constant feed quality can be produced by mixing enzymes into the feed.

Table 4
Effect of supplemental β-glucanase on the diet for piglets (9-15 kg), high in barley, on their performance, the intestinal viscosity as well as the concentration of endogenous digestive enzymes

	Control Group	Enzyme Groupe	Significance
Daily gain (g)	212	219	p=0.07
Feed : gain	1.64	1.59	p=0.06
Digesta viscosity in the small inestine (mPa · s)	3.3	2.2	p<0.05
Endogenous enzyme concentration (mU/g digesta dry matter)			
Trypsin	10.0	5.8	p<0.05
Chymotrypsin	0.15	0.11	p<0.10
Lipase	95.8	51.4	p<0.10
Amylase	866	451	p<0.05
Faeces dry matter (%)	13.6	14.3	p<0.05

Due to the improved digestibility of the nutrients the feed value can be increased by the application of enzymes whilst having the same dietary composition. *Figure 11* shows the variation of the energy level of five different wheat sources as well as the effect of enzyme addition on the energy level. With reference to the diagram it can be seen that there are considerable differences in the level of metabolisable energy between wheats of different origins. The addition of enzymes always results in an increase in the energy which is the larger the lower the baseline energy level was.

Thus differences occurring under conditions in practice are reduced, which leads to a more homogenous feed quality.

Moreover, the maximal limits for the inclusion of certain raw materials to compound feeds can be raised because the antinutritive properties of various components contained in feeds can be reduced. Thus diets with a high proportion of barley can for instance be employed in broiler diets. In summary it can be said that more cost effective compound feed can be produced by using feed enzymes.

Figure 11
Effect of NSP degrading enzymes on the level of metabolisable energy (ME) of five different wheat sources

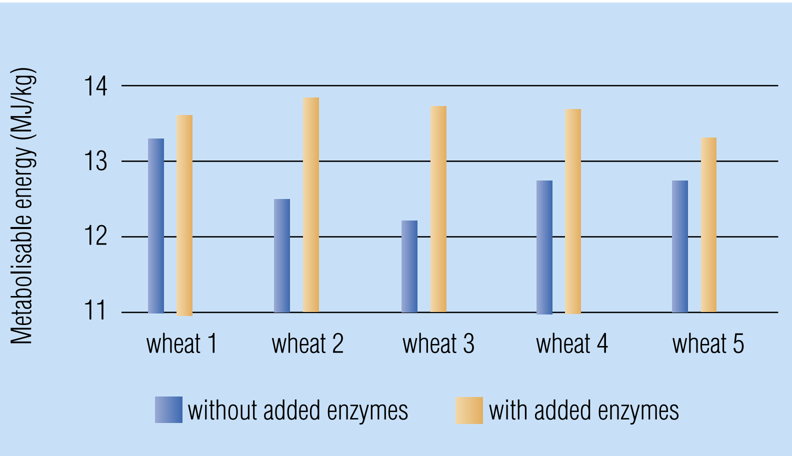


Table 5 contains data of the possible cereal proportion in complete diets for various poultry types using feed enzymes.

A further area of application is the use of feed enzymes in diets with new harvest cereals. The main problem with freshly harvested cereal is the high proportion of the NSP fraction, compared with cereal stored over a longer period. The NSP are only reduced to a small degree within the first four to six weeks of storage due to the enzymes contained in the cereal grains. Therefore recently harvested cereal is conventionally blended with longer stored cereal in gradually increasing amounts in order to utilise the cost advantages of a new harvest. The addition of feed enzymes enables recently harvested cereal to be directly used in higher proportions without incurring any loss of performance.

6.1.3 Economical evaluation of the effects

The economic success in rearing pigs and poultry essentially results from a high growth performance and beneficial feed utilisation as well as meeting the carcass characteristics demanded by the market. Thus the use of feed enzymes can make a significant contribution to the increase of productiveness. The degree of the economic effect depends of course quite considerably on the actual raw material and compound feed prices, as well as the sales per unit weight. The main economic advantages of adding enzymes are shown below for the example of rearing broilers.

Table 5
Recommendations for cereal proportions in complete diet mixes for poultry with or without feed enzymes

	Cereal type (% of the diet)									
	Barley		Oats		Rye		Triticale		Wheat	
Added Enzymes	-	+	-	+	-	+	-	+	-	+
Chicken Feed	10	40	20	ad lib.	5	20	20	ad lib.	30	ad lib.
Pullet Feed	30	ad lib.	30	ad lib.	15	30	30	ad lib.	40	ad lib.
Laying Hen Feed	40	ad lib.	20	40	20	40	30	ad lib.	40	ad lib.
Broiler Feed	10	40	20	ad lib.	5	20	20	40	20	ad lib.

w.r. = without restrictions

a) Effect on feed production cost

Feeds high in energy with an energy level of at least 13.4 MJ AME/kg are used in the rearing of broilers. Wheat and maize are the two main energy sources in the broiler feed. The energy and nutrient levels are derived from tables where the energy level for wheat is stated as being 12.6 MJ/kg and that for maize being 13.4 MJ AME/kg.

With the aid of Linear Programming and establishing the usual dietary requirements for broiler feeds a feed without and one with added enzymes were formulated. In the first instance values of feeding tables were used for all raw material components. In this case the diet contained 17.5% wheat and 36.5% maize. In the second case the wheat was assigned a 6% energy uplift due to the added enzymes. When considering this higher energy level the dietary calculations gave a wheat proportion of 54.2% whilst no maize was introduced into the diet. After subtraction of the cost of enzymes a cost advantage of the feed supplemented with enzymes could be shown.

b) Effect on the production cost

However if energy uplifting is not used, supplementing with enzymes via the modes of action described above results in a higher growth performance. With a finishing weight of 1.7 kg and an improvement of the feed conversion from 1.85 to 1.76 (-5%), 153 kg less feed per 1000 broilers is required, corresponding to a definite cost advantage. Thus in a complete productivity calculation the faster turnaround time per farm resulting from the shorter production cycle must also be taken into account since due to the added enzymes not only the feed conversion but also the growth performance (daily gains) are improved.

These associations between animal performance and productivity are similarly valid in the rearing of piglets and finisher pigs. In addition, individual factors affecting the use of enzymes can have a considerable significance for the various areas of improving economics. In this context must be mentioned among other things the positive effect of an improved litter quality on the carcass quality of broilers or the reduced proportion of dirty eggs of laying hens. However, in the rearing of piglets the uniformity of the animal group also plays a role when calculating the cost.

6.2 Phytases

The phosphorus from plant-based feeds, such as cereal and soybeans can only be utilised in small amounts by pigs and poultry compared with other phosphorus sources *(see figure 12)*. This is primarily due to the fact that approximately two thirds of the total phosphorus contained is present as bound phytate-phosphorus *(table 2)*.

This phosphorus compound represents the main storage form for phosphorus in plant seeds and can only be broken down by phytases which are not present in the digestive tract of pigs and poultry. A certain phytase activity can only be detected in some plant-based feedstuff such as wheat and rye which primarily causes the differences in the phosphorus digestibility of various plant-based feed components. The effect of native phytases can however only be taken into account in the determination of the digestible or available phosphorus in the total diet if the feed is not pelleted or subjected to other hydrothermal processing procedures as these processes result in the inactivation of native phytase. A prognosis of the effect is difficult to make since the native phytase activity is also prone to great variation.

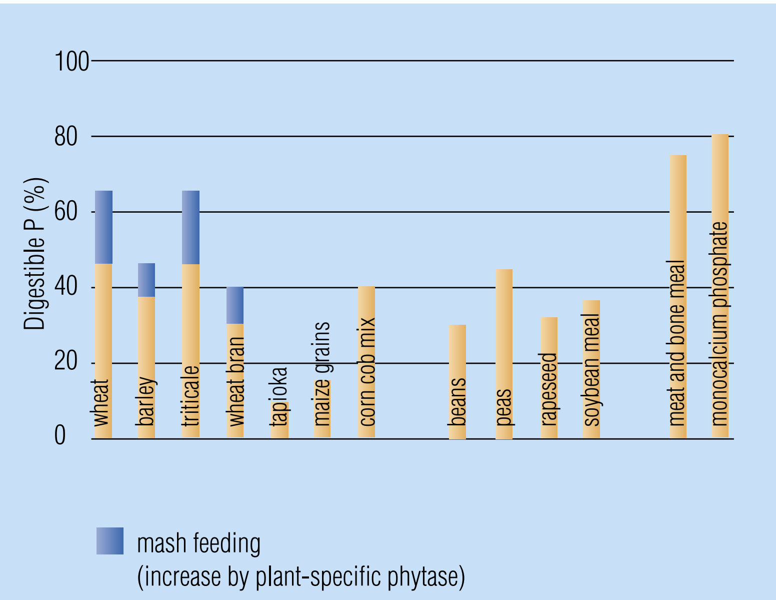


Figure 12
Phosphorus digestibility of selected feedstuff in pigs

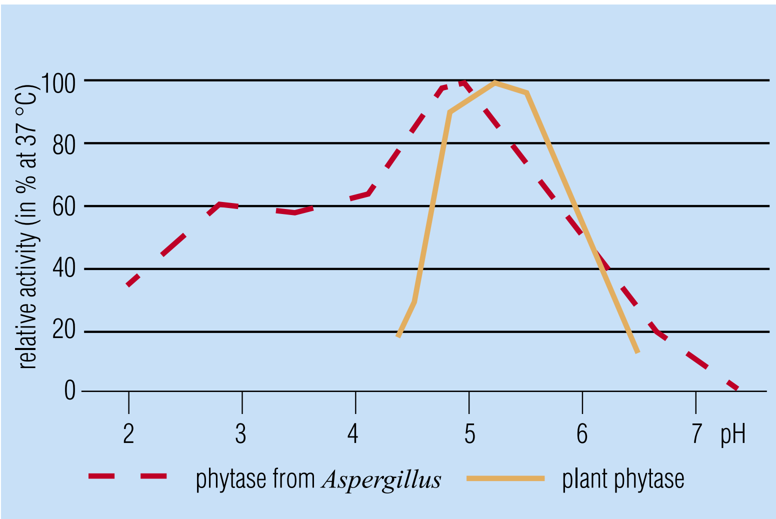
In addition it can be shown that plant-based phytase at lower pH values has a lower activity than microbial phytase *(see figure 13)*. In phosphorus digestibility studies in the pig it could be shown that phytase from wheat shows only about 55% of the efficacy of microbial phytase.

In addition to the binding of phosphorus phytate displays further antinutritive effects in pigs and poultry which are due to the molecular structure. Thus the six phosphate groups of the phytic acid have twelve negative charges when completely dissociated which are able to bind various elements in fixed complexes in a weakly acidic to neutral environment and hence their availability can be reduced. There are also reports of interactions between phytic acid and proteins. In the acidic environment of the stomach a negative effect of phytic acid on the solubility of proteins and the action of the protein degrading enzyme pepsin can be expected with certain proteins.

6.2.1 Effects of phytase

The effect of microbial phytase in the pig and in poultry has often been demonstrated. As can be seen from the investigations of various authors summarised in *figure 14* the digestibility or availability of the phosphorus in entirely plant-based diets in pigs and broilers can be increased by maximally 25 and 15% respectively by adding

Figure 13
pH profile of microbial phytase (from aspergillus) and plant phytase (from wheat)



microbial phytase. In both animal species it could be shown that the addition of 500 units of active microbial phytase to the diet can effectively replace 1.15 g phosphorus from dicalcium phosphate or 1 g phosphorus from monocalcium phosphate.

6.2.2 Possible applications in compound feed

Depending on the animal species, type of usage and the amount of phosphorus to be substituted, up to 600 units phytase activity *(definition see chapter 8.2)* is added per kg complete diet.

Higher calcium levels in the feed have a negative effect on phosphorus deficient diets but not on diets usually used in practice. Nevertheless 0.9 % calcium in the broiler diet should not be exceeded. For the optimisation of feed for piglets, finisher pigs and sows, it is recommended to adjust the calcium/digestible phosphorus ratios according to *table 6*. Trials carried out to date showed no age related effects on phytase efficacy.

Figure 14
Influence of phytase on the digestibility and the utilisation (in % of the intake) of phosphorus in pigs and poultry

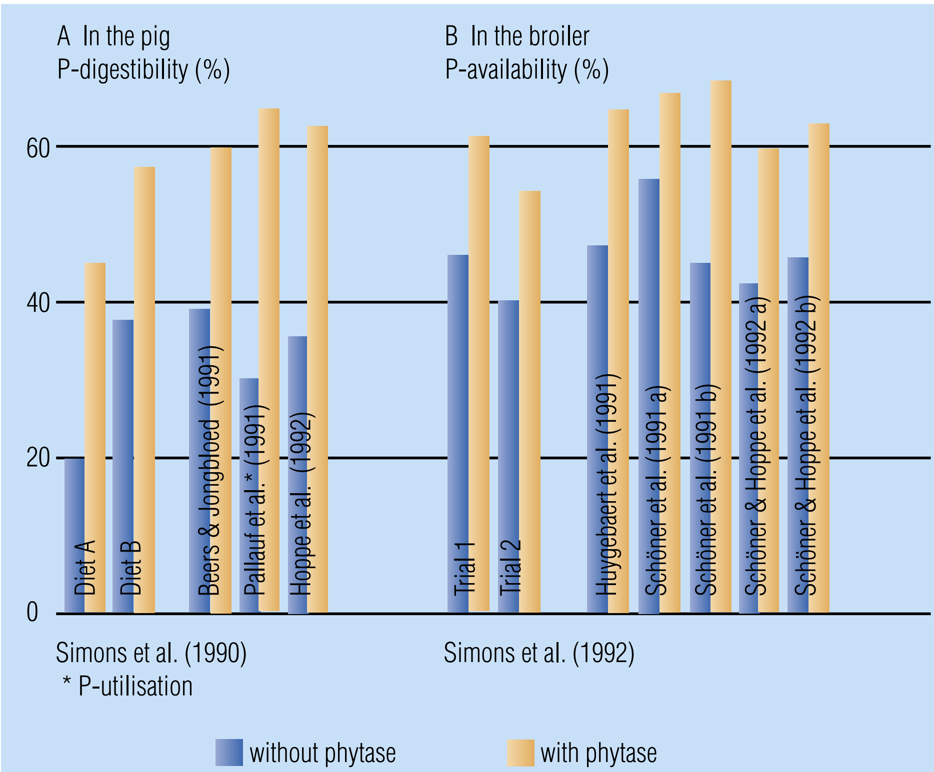
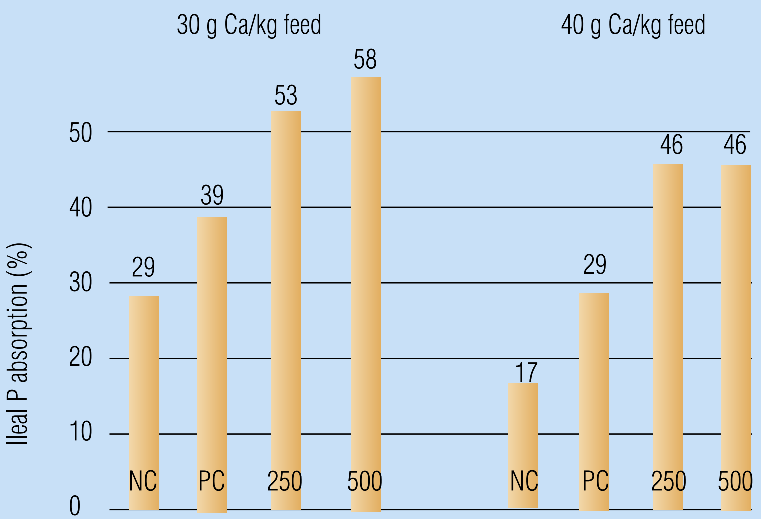


Table 6
Recommendations for the calcium/digestible phosphorus ratio in pig diets

Piglets	2.5 – 3.0 : 1
Finisher pigs	2.7 – 3.5 : 1
Sows	2.7 – 3.5 : 1

The release of P from phytic acid can also be clearly improved by the addition of microbial phytase to the diet and P in mineral form can be significantly reduced in laying hens. Microbial phytase is even more effective in laying hens than in broilers and pigs. Contrary to the general expectation, in this case the effect of phytase on breaking down phytate appears to be largely independent of the Ca level of the diet. Although higher calcium levels in the diet have a generally negative effect on the P digestibility *(see figure 15)*, the improvement in the ileal P digestibility caused by microbial phytase is largely independent of the calcium level of the feed.

Figure 15
Ileal P absorption in laying hens



NC = negative control
PC = positive control = NC + 1 g P from MCP/kg feed
250 = NC + 250 FTU/kg feed   500 = NC + 500 FTU/kg feed
P level of the basic diet = 3.4 g/kg

Depending on the supply level a positive effect of microbial phytase on the absorption rate could be demonstrated – primarily with the help of feeding trials in pigs – also for other minerals (e.g. of calcium and magnesium) as well as various trace elements (e.g. zinc). Therefore it is advised to monitor not only a reduction of the P level but also the supplementation rate for other minerals and trace elements when adding microbial phytase to the feed.

Recent trials in the pig show that microbial phytase can also have a positive effect on the protein and amino acid digestibility. However, these data are still not sufficient in order to derive additional feeding recommendations for the use of phytase.

6.3 Other enzymes

Besides NSP degrading enzymes and phytase, further enzymes are used as feed additives including e.g. proteases, amylases and galactosidases. Investigations have shown that nutrients for the breakdown of which the animal itself can produce enzymes are not always optimally digested. This sub-optimal nutrient utilisation can under certain conditions be due to insufficient endogenous enzyme production. This is particularly the case in young animal as well as during phases of changing diet composition. Here a supplement with amylase and protease can result in an improved nutrient conversion.

7. Enzymes and environment

During the conversion of dietary nutrients such as protein and phosphorus into animal products, relatively high losses occur even under optimal conditions in pigs and poultry.

Numerous feeding measures can contribute to minimise these losses and thus to largely reduce the excretion of nutrients in the manure. The most effective measures include a feed which is better adapted to the requirements and the addition of pure amino acids whilst simultaneously reducing the level of crude protein in the feed as well as the addition of feed enzymes.

Table 7
Reducing P-excretion in finisher pigs by adding phytase

	Control group	Trial group (500 FTU phytase per kg feed)
Feed intake		
- Starter (kg)	74.4	76.4
- Finisher (kg)	142.3	142.6
P-level per kg feed		
- Starter (g)	5.5	4.6
- Finisher (g)	4.4	3.4
P-intake, total (g)	1035	836
P-excretion per animal (g)	625	428
Reduced P-excretion due to the addition of phytase (%)		32

The addition of phytase might be of the greatest value in the reduction of phosphate excretion. Due to the increase of digestibility or availability of phosphorus contained in the feed the amount of supplemented phosphorus required to cover the demand can be reduced. Both factors together gave a significant effect on the level of phosphorus excretion. Under conditions usually encountered in practice, with the addition of microbial phytase a reduction of the P-excretion of 30% on average can be expected *(see table 7)*. The use of NSP degrading enzymes contributes via an improvement of the feed conversion and thus nutrient utilisation, which reduces nutrient excretion (N, P etc.) by an average of 5% and further results in higher fecal dry matter level.

By using enzymes, in particular NSP degrading enzymes, the feed conversion can be improved and hence the nutrient expenditure per kg product (meat, eggs) produced can be clearly reduced. When adding NSP degrading enzymes the water consumption relative to the feed quantity also drops. Hence enzymes make a significant contribution to a better environmental and resources friendly animal production (substainable animal production).

The measurement of the enzyme activity is different to the analysis of other additives for animal nutrition. Whilst during the chromatographic analysis of for example vitamins, individual, chemically precisely defined molecules are determined according to type and amount, this is not adequate in the quantification of enzymes. The effect of enzymes must be demonstrated with respect to their activities.

8.1 Measurement of the NSP enzyme activities

Nature produces quite a variety of enzymes which have the ability to break down NSP. However, if the added feed enzymes are considered in practice then this diversity is reduced to a very few. The measurement of activities in the enzyme preparations is undertaken by incubation with a suitable polymer substrate and the subsequent determination of the breakdown products released therefrom. Suitable substrates are, for example, specific xylans or β-glucans. As is the case with all enzymatic measurement procedures, they are influenced by temperature, pH value, incubation period and enzyme to substrate ratio and these affect the result in a decisive manner. Therefore the measurement conditions must be adhered to precisely.

The determination of enzyme activities in feedstuffs or in premixes is also carried out according to the principle of measuring the molecules broken down in a substrate. However for this purpose the reducing sugars cannot be used as a measurement criterion as they are already naturally present in high concentration in the feed sample. An alternative possibility is the use of dye marked substrates. These are broken down by the enzymes into smaller, soluble units which carry the dye. These are separated from the insoluble substrate by a simple filtration step and can be measured photometrically. In addition there are insoluble chromogenic substrates, for which the release of a colour can be measured without requiring a prior filtration step. As interactions with feed components can occur, it is necessary to either work according the internal standard method (addition of a known amount of the used enzyme preparation) or with the help of a calibration curve with a corresponding blank feed.

A further possibility is to measure the viscosity. The principle of the viscosity measurement method uses the ability of the enzyme to reduce the viscosity of a standard substrate solution (under defined conditions). For the activity measurement the reduction of viscosity per time unit is determined by enzyme addition.

8.2 Determination of phytase activity

The activity of phytase as with all other enzymes – is essentially dependent on temperature, pH value and type of substrate as well as the substrate concentration. When establishing the parameters, nutritional and physiological conditions under which phytase acts in vivo were closely observed. The incubation temperature is adjusted to 37 °C and the pH value to 5.5 by adding buffer. Sodium phytate, which is readily soluble in water, was selected as the substrate and also represents the naturally occurring substrate. From the mode of action, i.e. the release of phosphate, the measurement principle for the activity determination (i.e. analysis of phosphate released) is derived. As the test material usually already contains phosphate, a measurement of the difference is required in any case. It is carried out in such a way that the phosphate level of sample is measured prior to (corresponds to the blank value of the sample) and after (corresponds to the sample value) an incubation period. This offers the advantage that at the same time there is compensation for sample-specific differences. The photometric measurement of phosphate is carried out according to the official EC method with the vanadate-molybdate-complex coloured yellow. A phytase unit (FTU) is defined as the enzyme activity which under optimised conditions (pH 5.5 at 37 °C) can release 1 µmol inorganic phosphate per minute from an excess of Na-phytate.

For the determination of the phytase activity in enzyme preparations, premixes and compound feeds there are available two VDLUFA association devised procedures. The reference method is mainly used for the validation and monitoring of the phytase standard or control samples and the level of phosphate released is deter- mined by an external phosphate calibration curve. The relative method which is used in daily routine analyses compares the enzyme activity with that of a phytase standard preparation of known enzyme activity.

Enzymes can generally be used in feed mixes in dry or liquid form.

Firstly, enzymes, in line with most other feed additives, are admixed to the feed in powder or granular form. Due to their excellent properties with respect to storage stability and mixability these product forms have proved to perform well in conventional feed productions. They are primarily used in mash feeds but also in pelleted feedstuff. Depending on the dosage, the enzyme product can either be directly added or admixed vie premixes into the compound feed.

In recent years compound feeds have in some countries, been increasingly pelleted or expanded at very high temperatures as a preventive measure for microbial contamination. However, there is the danger that the enzymes, being high molecular proteins and relatively sensitive substances, are easily destroyed. Enzymes for liquid application were developed based on these considerations. These liquid products are only brought into use following hydrothermal treatment of the feed which means that liquid enzymes are sprayed onto the cooled feed following the pelleting or expansion process.

For this process a variety of equipment for continuous or batch application is supplied by several manufacturers. This product type appears to be the only form of application for particularly sensitive enzymes. Since enzymes differ in their tolerance to high temperatures, the specific limitations for particular products stated by manufacturers with respect to each maximal temperature tolerance have to be observed when they are used in pelleted feeds.

Dosages

The dosage of enzyme products is very different depending on their degree of dilution and the concentration of the relevant enzyme activities. It ranges from 50 to 2000 mg/kg complete feed. As the enzyme activity is dependent on the substrate available, statements on minimal dosages are not required neither are maximal values, since, on the one hand, with an overdose no intolerability can be observed and on the other hand a normal proteolytic breakdown of the enzymes takes place in the digestive tract of the animal.

In general it can be said that following the selection of a suitable form, enzyme products can be used with all technologies in the production of compound feed.

User Safety

Allergic reactions can occur on contact with the concentrated enzyme as is the case with many other exogenous proteins. Therefore it is an important part of all approval procedures for enzyme products that the safety of their use is tested. This is carried out according to the usual regulations in force for the registration of feed additives. These tests are undertaken to the highest safety standards.

However, the manufacturers of these products have decided on additional safety, in particular for highly sensitive persons, to label their products with the St. Andrew's Cross and also the hazard symbol "Harmful to health" as well as the sentences "Sensitisation possible on inhalation" and depending on the state "Dust must not be inhaled" or "Aerosol must not be inhaled" as a warning of possible health risks so that appropriate hygiene measures can be followed when handling enzymes.

It is a general rule that with the application of enzyme products the same care has to be applied as is standard with all other feed additives. When admixing enzymes to premixes and compound feedstuff appropriate safety measures have to be put into place.

Amylases. Enzymes which catalyse the breakdown of starch. We differentiate between α-amylase (breakdown of α-glycosidic bonds inside the starch molecule) and β-amylase (splitting off of maltose molecules).

Amylose. Soluble, non-starchy component of the starch grain enveloping → amylopectin. Linear polysaccharide of → glucose molecules, which are linked via α-1,4-bonds.

Amylopectin. The starchy envelope substance of starch grains which swells in water. Branched → polysaccharide of → glucose molecules which are linked via α-1,4- and α-1,6-bonds.

Antinutritive factors. Materials/ substances having a negative effect on the nutritive value of feed raw material, e.g. → NSP in cereal, trypsin inhibitors in soybean extraction meal, and → phytate in plant seeds (abbreviation ANF)

Apoenzyme. Protein component of an enzyme. Forms together with the → coenzyme the → holoenzyme.

Arabinose. → Pentose. Component often found in → pentosans.

Aspergillus. Fungus family of the fungus subclass of ascomycetes (tubular type fungi). Aspergillus (A.) niger is a fungus type with black spores which synthesises for example the enzyme phytase.

Bacillus subtilis. Ubiquitary, aerobic, starch degrading and proteolytic bacterium (so-called hay bacillus) which produces enzymes. The spores are also used as a probiotic for the colonisation of intestinal flora.

Carbohydrase. Generic term for enzymes which catalyse the → hydrolytic breakdown of carbohydrates (also: carbohydratase).

Carbohydrates. Collective name for the widely spread natural substances polyhydroxy aldehydes (aldoses) and polyhydroxy ketones (ketoses). The carbohydrates make up the greatest proportion in feedstuff.

Cellulase. Enzyme which catalyses the → hydrolytic breakdown of cellulose (β-1,4-glycosidic bonds).

Cellulose. Long-chain linear carbohydrate. Consists of → glucose molecules in β-1,4-bonds. It is found in increased amounts in cell walls and effects the stability of a plant.

CMC. Carboxy methyl cellulose. Standardised substrate which is used for measurement of the → cellulase activity.

Coenzyme. Low-molecular non-protein organic compound which in addition to an → apoenzyme is required for the initiation and maintenance of the catalytic efficacy. Together with the → apoenzyme forms the → holoenzyme.

Endosperm. Internal part of a seed. In cereal known as starch endosperm. The enveloping cell walls contain → NSP.

Enzyme. Protein which acts as a biological catalyst to accelerate biochemical reactions.

Enzyme activity. Describes the ability of an enzyme to convert a certain amount of substrate under defined conditions per time unit. It is dependent on external factors such as → the substrate concentration, → pH value or the temperature.

Enzyme cocktail. Enzyme mixture.

Enzyme mixture. Enzyme product with several → enzyme activities, which are synthesised by different micro-organisms.

Enzyme complex. Enzyme product with several → enzyme activities which are synthesised by the same micro-organism.

Enzyme action. Result of a chemical reaction catalysed by an enzyme.

Ferment. Older name for → enzyme (particularly for digestive enzymes). Example: Rennin which is used for clotting the milk in cheese making.

Fermentation. A process in which defined products are obtained by aerobic or anaerobic metabolisms of micro-organisms, by microbial enzymes but also with the help of plant or animal cell cultures.

Galactose. Stereoisomeric monosaccharide (aldohexose) to → glucose (dextrose).

Galactosidases. → Enzymes, which catalyse the breakdown of → galactose contained in → oligosaccharides.

Glucans. Long-chain carbohydrates (polymers of glucose) that are mainly found in cereal (barley, oats, triticale). → ANF for monogastric animals.

Glucanase. → Enzyme which specifically catalyses the hydrolytic breakdown of → glucans (example 1,3-, 1,4-β-glucanase).

Glucose. → Hexose. Most important monosaccharide which as a monomer component of → cellulose, represents the highest proportion of the biomass on earth. It is also found as a component of starch and other carbohydrates.

Hemicellulase. Generic term for → enzymes which catalyse the → hydrolysis of → hemicellulose.
Hemicellulose. Group of long-chain carbohydrate molecules which contribute to the texture of plants. They include for example the → glucans and → xylans.

Hexose. Sugar with six carbon atoms.

Holoenzyme. Enzyme acting as a catalyst comprising → apoenzyme (protein part) and → coenzyme.

Hydrolases. Enzymes which break down substrates by taking up water (hydrolytic).

Hydrolysis. Breakdown of chemical compounds by taking up water.

IU. International unit to describe enzyme activity.

Lipase. → Enzyme which catalyses the → hydrolyses of fats.

ME. Metabolisable energy. Energy which can be converted by the metabolism of animals.

Monosaccharide. Single sugar molecule, also saccharide, component of → oligosaccharides and → polysaccharides. Examples: → pentoses (xylose, arabinose), → hexoses (glucose, galactose).

NSP. Non-starch polysaccharides, also present as fibre components in plants (→ endosperm).

Oligosaccharide. Carbohydrate of two to a maximum of ten → monosaccharide molecules. Example: lactose (disaccharide, raffinose (trisaccharide).

Osmolarity. Measure for an osmotically acting concentration of dissolved substances (e.g. sugars, salts). Determined by freezing point depression for example.

Pentosanase. Enzyme catalysing the hydrolyses of → pentosans.

Pentosans. Long-chain polysaccharides of pentoses. Mainly found in wheat and rye. → ANF for monogastric animals.

Pentose. Sugars with five carbon atoms.

Phosphatase. → Hydrolase which breaks down the ester of organic phosphoric acid thereby liberating phosphate and alcohol.

pH value. Negative common logarithm of water ion concentration of a solution, which states the degree of acidity on a pH scale of 0-14; (acidic: pH 0 - 7, alkaline: pH 7 - 14). Factor which influences the activity of enzymes.

Phytase. → Enzyme which catalyses the hydrolytic cleavage of phosphate from phytic acid to lower inositol phosphate esters and inorganic phosphate. It is naturally found in plants. Microbial phytase is obtained as a final product of fermentation of → aspergillus strains.

Phytate. Salts of → phytic acid: phosphorus compounds which mainly are found in plants and constitute approximately 2/3 of the amount of phosphorus present. Poor digestibility for humans and animals.

Phytic acid. Hexaester of the phosphoric acid of the sugar alcohol inositol (myo-inositol hexa-dihydrogen phosphate). Occurs naturally in plant seeds.

Polysaccharide. Multiple sugar, long-chain carbohydrate of at least 10 → monosaccharide molecules, e.g. pentosans, → cellulose, starch.

Protease. Enzyme which catalyses the → hydrolysis of protein.

Proteolysis. Breakdown of proteins.

Strain. Genetically, morphologically and biologically uniform subtype (subspecies) of an organism (bacterium, yeast, fungus) which in addition to other metabolic products synthesises → enzymes. Stored in official collections (banks) subsequent to its precise description and definition (→ systematics).

Submerged procedure. Fermentation procedure for the production of enzymes in which micro-organisms are grown within a liquid nutritive medium.

Substrate. Substance which is converted in an enzymatically catalysed reaction into one or more products.

Substrate specificity. Dependence of enzyme action on the type of → substrate (lock and key principle).

Surface procedure. Fermentation process for the production of enzymes in which the micro-organisms are grown on the surface of solid or pasty nutritive media with surface ventilation.

Swelling. Uptake of water by molecular binding.

Systematics. Division of biology which is directed to the meaningful, comparative order of organisms on the basis of their combined morphological and biochemical characteristics. The objective is a taxonomic classification of organisms as an expression of a graded genetic relationship.

Taxonomy. → Systematics.

Trichoderma ssp. Fungus family (e.g. T. viride, T. longibrachiatum) which procedure a variety of different enzymes.

Viscosity. Tenacity. Measurable parameter for the description of the flow property of gaseous and liquid materials. Deformation of these materials at a rate (c) following the influence of shear (τ) taking place according to the formula $\tau = f(c)$. The viscosity of the intestinal content is also dependent on the proportion of water soluble → NSP in feeds capable of swelling.

Xylan. Long-chain carbohydrate of → pentoses (polymer of xylose) which is mainly present in wheat and rye.

Xylanase. Endoenzyme which specifically catalyses the → hydrolytic breakdown of xylan (splits β-1,4-bonds between xylose molecules).