Enzymes & Potassium diformate in animal nutrition

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Enzymes in animal nutrition

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The last few years the world markets have seen an over-supplementation with cereals as well as protein rich feedstuffs due to good harvests in recent years. However, size middle of last year supply of raw materials for the feed industry decreased significantly and price are on the way up. There is no doubt that cereals will play a key role in human nutrition in the future and there will be a deficit within the next 20-30 years. On a long term scale, supply of the growing world population may not be a problem of distribution but may be increasingly a problem of production. Today's population is about 6 bn. and will have risen to 8 bn. in 2020. However, not only the number of people but also the individual consumption of food will increase. The growing prosperity will dramatically change the eating habits, thus, also increase the consumption of cereals in refined form (meat). This increasing demand can be covered by changes in eating habits or higher production of grains. But the latter alternative will not be able to solve the tightness in the world food situation. A rapid need for considerable additional efforts have to be made to improve the production of grains as well as the efficacy of feed in animal production.

One possibility to increase the efficacy of animal production is the use of feed enzymes in feed production. Up to now, only a fraction of compound feed is supplemented with enzymes. This situation will rapidly change as soon as the development of new feed enzymes or new application forms for these products make progress. The current generation of enzymes has proven to be highly beneficial but their usefulness will increase when new forms of enzymes become available. Modern biotechnology represents a possibility to produce specific enzymes for certain fields of application. Also many other problems need to be solved before the full market potential of enzymes is penetrated.

The objective of this paper is to characterize substances present in common feed ingredients which have a negative impact on animal performance and to show ways to overcome these negative effects by supplementing the feed with exogenous enzymes.

Anti-nutritional factors in animal feed

Today animal feed mainly consists of two to three ingredients which make up more than 75 % of the complete feed. The main energy sources are corn, wheat and sometimes barley; as protein sources mainly soybean meal, canola, animal by products or sunflower meal are used. Each of these ingredients contains variable quantities of anti-nutritional factors. Their concentration in the final feed is minimal if the raw material is included at low inclusion rates but considerable with the nowadays usual 2-3 component diets where one ingredient easily can reach an inclusion rate of 60%. Modern feed formulation is mainly driven by providing feed with a maximum nutrient density to reach high animal performance and at the same time achieving minimum costs. Least cost formulation of feed is not taking dietary concentration of anti-nutritional factors into account due to the economical attractive nature of grains and protein meals as well as due to the natural variation in the content of anti-nutritional factors in the raw materials.

The properties of anti-nutritional factors depend on the anti-nutrient concerned and the concentration in the final feed formulation. Per definition anti-nutritional factors are those generated in natural feedstuffs by normal metabolism of the species from which the material originates and by different mechanisms (decomposition or inactivation of some nutrients, diminution of the digestive or metabolic utilization of feed), which exert effects contrary to optimum nutrition. Anti-nutritional factors are not toxic for animals but their presence in the feed results in reduced growth, poor feed conversion, hormonal changes and occasional organ damages. For most of the known anti-nutritional factors the physical-chemical properties and the mode of action are known. Based on this knowledge it is clear that any activity to reduce the quantity of anti-nutritional factors will effect animal performance. Besides technical treatment during processing of raw materials, enzymes play a mayor role in eliminating the negative effects of anti-nutritional factors. Exogenous enzymes supplemented to the feed may simply support the endogenous enzyme system (amylase, lipase) or supply enzymes which are not present on the animal's digestive system (phytase, xylanase).

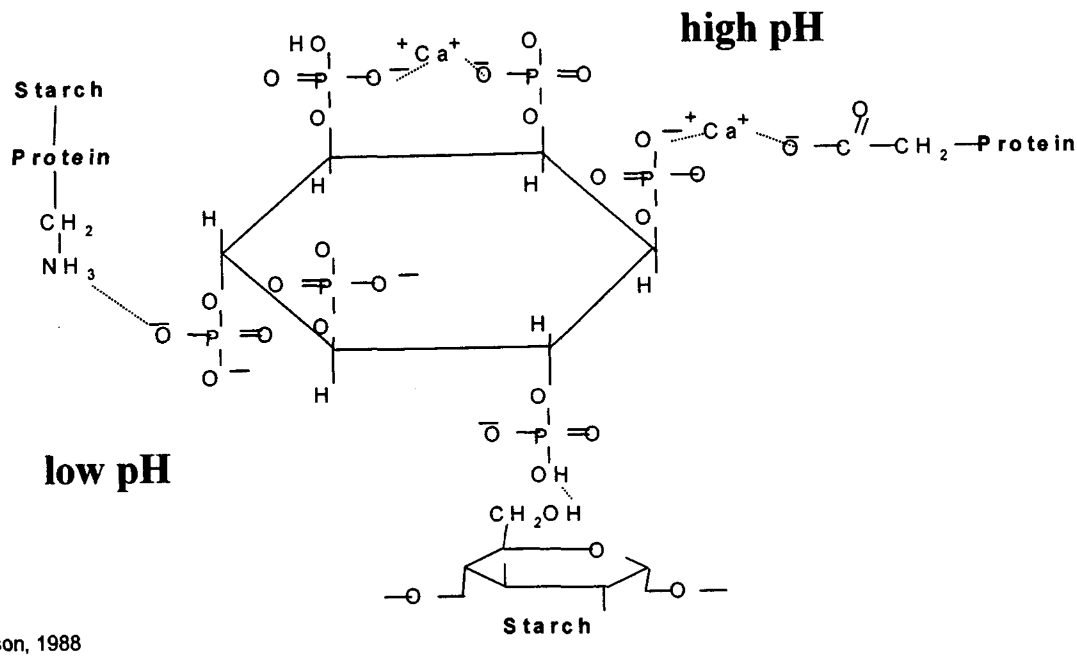
Based on the importance of the different anti-nutritional factors their effects and possible counteraction of exogenous enzymes will be described.

Phytate:

It is well known that considerable amounts of some nutrients in feeds are not utilized and absorbed by monogastric animals. Among other things the availability of nutrients can be influenced by natural complexing agents. This is particularly the case in cereals, oils seeds and legumes containing phytate. Also known as phytic acid it is an organic complex being the storage form of phosphorus in plants. Phytate is a reactive anion which can form salts with nutritionally important minerals like Ca, Mg, Zn or Fe. Today there is increasing evidence that the complexing properties of phytate go much beyond than just limiting P and mineral availability.

In a similar way as for minerals, phytate is able to complex protein, amino acids or endogenous enzymes. The interaction between phytate and proteins seems to be an ionic binding which depends on the pH conditions. At low pH , phytate forms electrostatic linkages with the basic arginine, lysine and histidine residues resulting in an insoluble complex. With an increasing pH getting close to the isoelectric point, the charge of the protein is neutral and it will not longer be bound to the phytate. Under basic circumstances phytate complexes with protein in the presence of divalent cations. These cations (Ca, Mg, Zn) act as a bridge between the negatively charged protein carboxyl group and the phytate.

Figure 1: Phytate-protein interaction at different pH condition



Thompson, 1988

In vitro studies have shown that phytate-protein complexes are insoluble and have a lower accessability to proteolytic enzymes than the same protein alone. The relevance of this complex had no impact on the formulation of poultry diets up to now. The presence of phytate-protein complexes might have a negative influence on the digestibility and absorption of protein and amino acids. The significance of such an effect depends on the property and configuration of the complex which can vary with the protein source. Different studies showed that protein from soybeans, corn, wheat, rape seed, sunflower meal or rice bran complexes with phytate (Ravindran et al., 1999a).

Phytate is also known to inhibit several endogenous digestive enzymes like pepsin, amylase or trypsin. These effects are likely due to the unspecific nature of phytate-protein complexes or an inhibition due to chelation of Ca ions necessary for the activity of the endogenous enzymes. Recent results show evidence on a formation of phytate-protein complexes in the gut and an interaction between free amino acids and phytate. An in vitro trial reported by Jongbloed et al. (1997) suggested that phytate protein complexes are formed post feeding in the gut at a pH of 2-3. Rutherfurd et al. (1997) found that phytate forms complexes with supplemented free amino acids.

The content of phytate phosphorus in raw materials frequently used in swine and poultry nutrition is quite variable. The phytate phosphorus content of a range of ingredients is shown in Table 1.

Table 1: Phytate P content of different feed ingredients:

Ingredient	Phytate P (g/100 g DM)	Phytate P (as % of tP)
Cereals		
Corn (Zea mays)	0.24	72
Barley (Hordeum volgare)	0.27	64
Wheat (Triticum aestivum)	0.27	69
Oats (Avena sativa)	0.29	67
Sorghum (Sorghum vulgare)	0.24	66
Foxtain millet (Setaria ital ica)	0.19	70
Finger millet (Eleusine coracana)	0.14	58
Rice (Oryza sativa), unpolished	0.27	77
Rice, polished	0.09	51
Cereal by products		
Rice bran	10.31	80
Wheat bran	0.92	71
Rice polishings	20.42	89
Roots and tubers		
Cassava (Manihor esculenta) root meal	0.04	28
Sweet potato (Ipomea batatas) tumer meal	0.05	24
Taro (Colocasia esculenta) corn meal	0.09	24

Ravindran, Bryden and Kornegay, 1995 (modified)

The effect of different phytate levels on broiler performance was evaluated by Ravindran et al. (1999b). With increasing phytate levels from 1.04 to 1.57 % weight gain of broilers fed from day 7 to day 25 on a phosphorus sufficient diet (0.45% aP) decreased from 691 g/bird to 593 g/bird (see Figure 2). In the same experiment (Ravindran et al., 1999c) the influence of phytate content on ileal nitrogen digestibility was measured. With the increasing level of phytate the digestibility of nitrogen was reduced from 81.5 % to 79.3 %.

Figure 2: Weight gain of broilers fed a wheat-sorghum-soybean meal based diet containing varying amounts of phytate

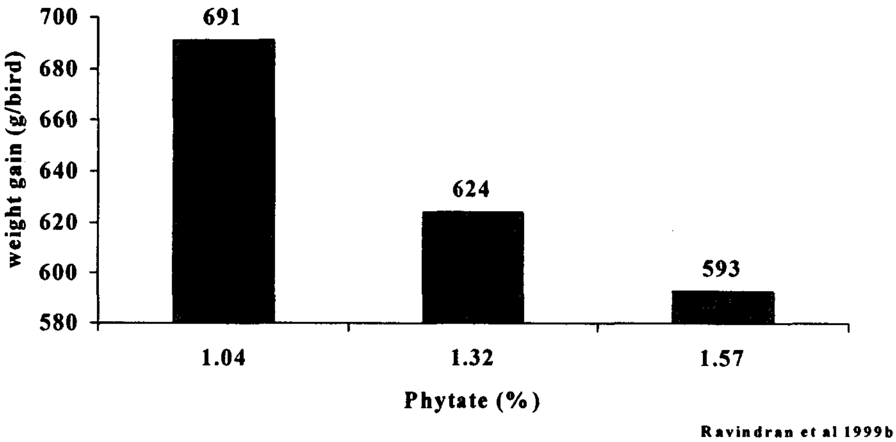
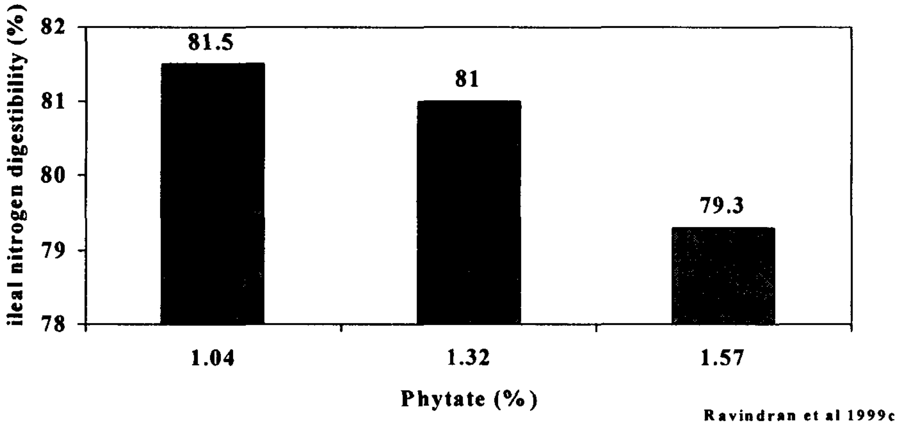


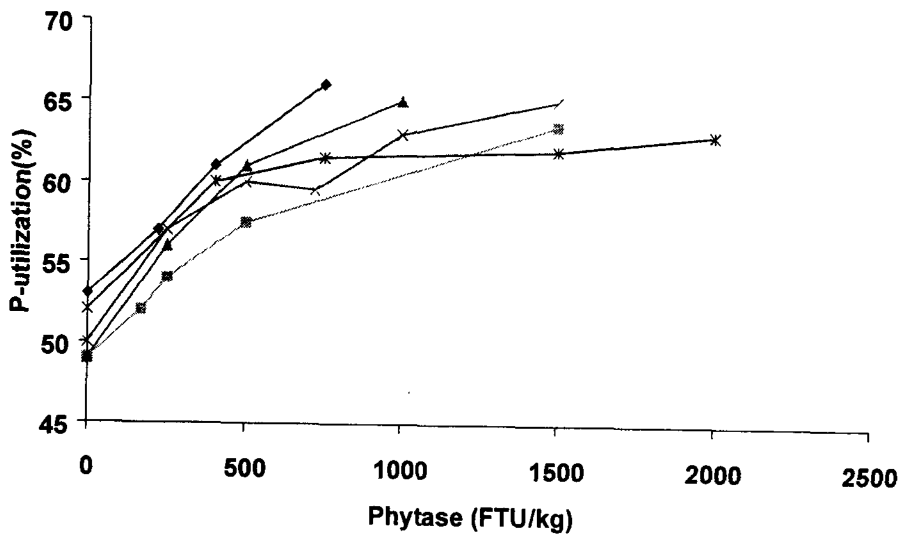
Figure 3: Nitrogen digestibility of broilers fed a wheat-sorghum-soybean meal based diet containing varying amounts of phytat



A solution for the negative effects of phytate is provided by nature itself. The enzyme phytase is produced by many species of bacteria, yeast and fungi and capable to eliminate the anti-nutritional properties of phytate. On a commercial scale this enzyme is produced by a limited number of organisms, Aspergillus being the most important one. In animal nutrition phytase is being used for many years to improve phosphorus availability.

Many trials have been conducted during the last 10 years to demonstrate the effects of microbial phytase on phosphorus utilization by swine an poultry. Figure 4 shows the result of 5 trials evaluating the dose response of phytase on phosphorus utilization in broilers. The results demonstrate that up to a dosage of 500 phytase units the response of phosphorus utilization is almost linear. Higher phytase dosage rates result in a lower improvement per unit of phytase.

Figure 4: Effect of different phytase inclusion rates on phosphorus utilization in broiler diets



As already described above, phytate is also able to complex Ca or trace elements. Schoener et al. (1993) reported a dose dependent increase in Ca utilization by supplemented phytase in broilers. The addition of 500 phytase units improved Ca utilization from 21 % to 25% (Figure 5). The influence of phytase on trace element availability (Zn) was evaluated by Yi et al. (1996a) in broilers fed a low Zn diet The addition of graduated levels of phytase linearly improved Zn retention from 38% to 45% when phytase was added at 600 units/kg feed (Figure 6).

Figure 5: Effect of phytase on Ca utilization of broilers

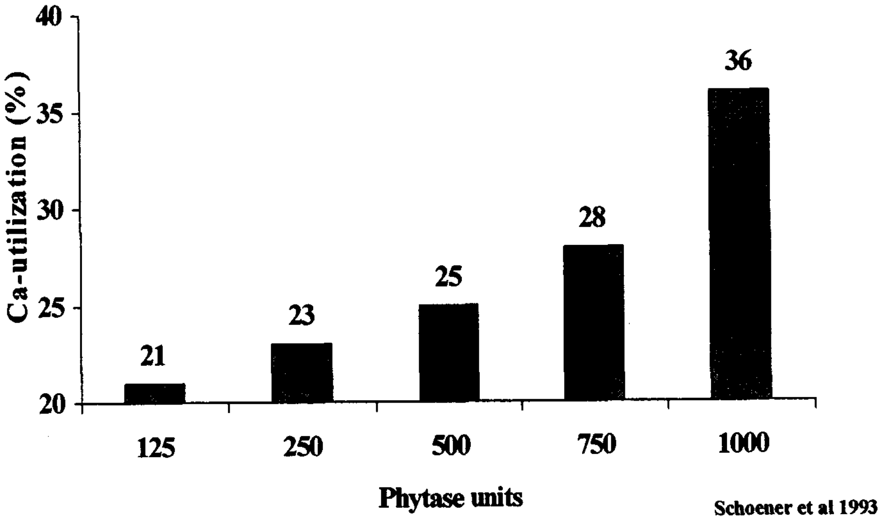
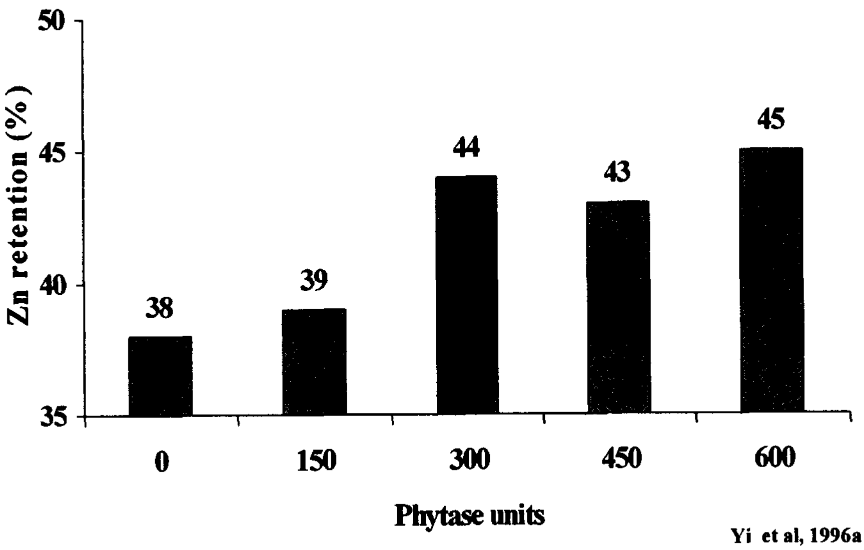


Figure 6. Effect of supplemental phytase on zinc retention of broilers



Based on the in vitro knowledge on the interactions between phytate and proteins a phytase supplementation should result in a release of phytate bound protein and amino acids for utilization in the animal. Several published data support this hypothesis. Already in the early 1990's van der Klis and Versteegh (1991) reported that the supplementation of a layer diet with 300 phytase units/kg significantly improved ileal nitrogen absorption from 79.3% to 80.9%.

Also in pigs, broilers, ducks and turkeys a positive effect of phytase addition on nitrogen digestibility could be demonstrated (Farrell et al. 1993, Martin and Farrell, 1994,Yi et al. 1996b).

The protein/amino acid effect of microbial phytase is of considerable practical interest and needs to be quantified to enable its inclusion in modern feed formulation.

A study was conducted by Ravindran and Bryden in 1999 to investigate the effects of graduated levels of microbial phytase on ileal amino acid digestibility. A wheat-sorghum based diet was formulated to contain 80% of the NRC (1994) recommended level of lysine. This diet was supplemented with 125, 250, 375, 500, 750 and 1000 phytase units/kg. The supplementation with phytase significantly improved weight gain reaching a plateau at 500 units/kg. With respect to feed conversion ratio phytase addition had no effect up to 250 units but then linearly improved feed conversion with further phytase

Table 2: Ileal digestibility of nitrogen and amino acids as influenced by varying phytase levels

Phytase units	**0**	**125**	**250**	**375**	**500**	**750**	**1000**
Nitrogen	78.1	78.7	78.9	79.8	81.2	81.0	82.2
Lysine	79.4	81.2	81.6	82.5	83.0	83.4	84.1
Methionine	91.0	90.5	91.2	91.6	91.7	91.3	91.8
Arginine	82.1	82.4	82.8	84.9	85.6	85.2	86.2

Ravindran and Bryden, 1999

supplementation. The determined amino acid digestibility is shown in Table 2.

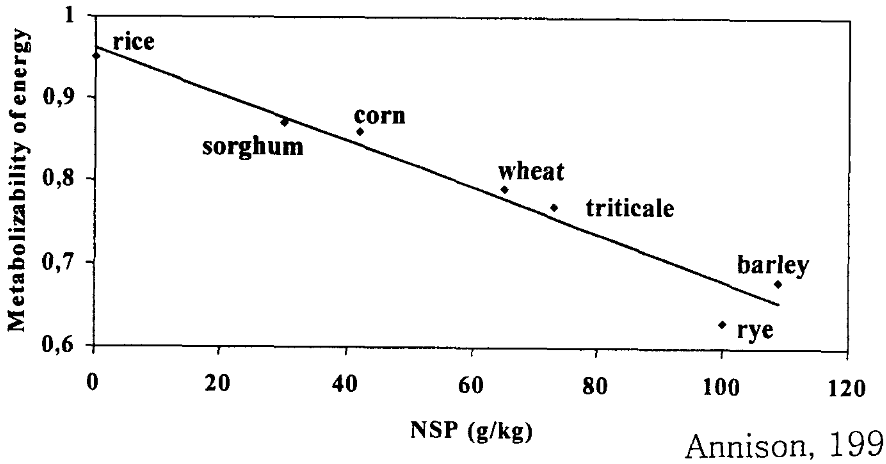
In the same experiment the effects of microbial phytase on apparent metabolizable energy (AME) was determined. The AME content of the diet was linearly increased with increasing levels of supplemental phytase. The addition of 500 phytase units/kg improved the AME content by 2.3% from 13.06 to 13.35 MJ/kg diet. The highest response could be shown with an addition of 750 units/kg. These results confirm the results reported earlier (Farrell et al. 1993, Ravindran et al. 1999c, Ledoux, 1999). However, the mode of action is still not completely known, but clearly improved protein and amino acid digestibility is partly responsible for these responses.

The relevance of negative effects of phytate on phosphorus, minerals, protein and energy utilization in monogastric animals is extensively described in the literature and the ability of the enzyme phytase to release these phytate bound nutrients received increasing acceptance in the scientific world as well as under practical feed formulation conditions. The enzyme phytase is proven to be a tool to reduce the supplementation of feed with inorganic phosphorus, protein and energy. At the same time excretion with the manure is decreased and therefore the enzyme contributes to a protection of the environment.

Non-Starch-Polysaccharides:

Non-Starch-Polysaccharides (NSP's) exist in huge varieties in nature and are components of the cell wall. The content of NSP's has been shown to be negatively correlated with metabolizability of energy of cereals (Figure 7).

Figure 7: Relation between metabolizability of energy and NSP content in cere



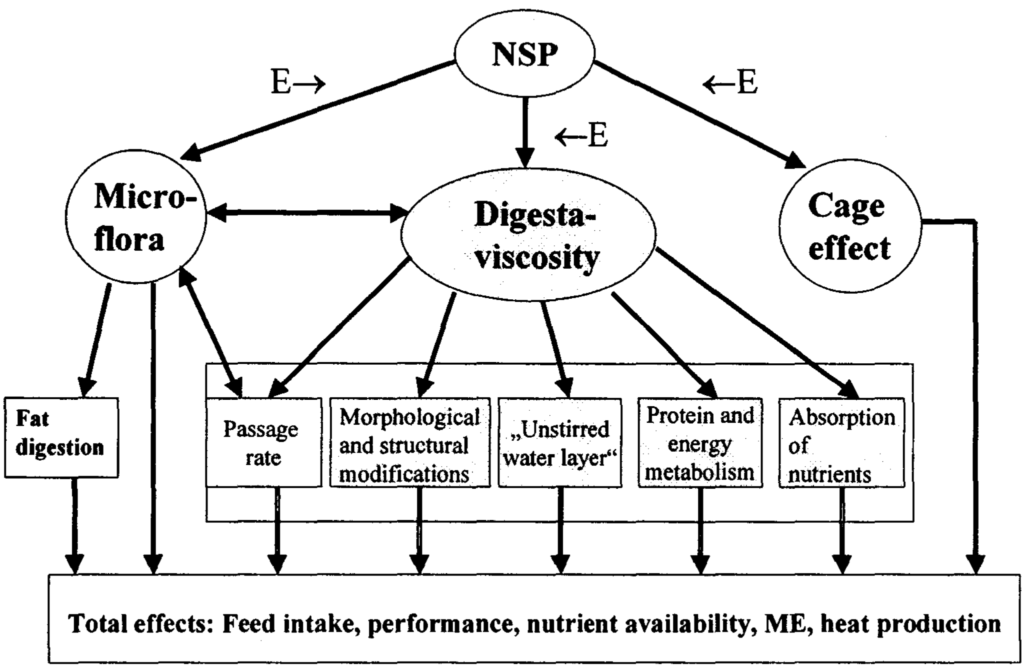
Annison, 1993

Reasons for the anti-nutritional properties of NSP's are their ability to bind large amounts of water and as a result increase the viscosity of the intestinal content when feed containing NSP's is consumed. The increased viscosity may cause problems in the small intestine because nutrients like fat, starch or protein become less accessible and available for endogenous enzymes. The result is a depressed digestibility of these nutrients. Additionally, high viscosity of the digesta increases the amounts of sticky droppings.

NSP's are not an uniform substance in feed ingredients but the level, type and composition can differ largely between plant seeds. In cereals, NSP's mainly consist of pentosans, glucans and cellulose whereas, in legumes pectins and galactosides are the predominant NSP's. Pentosans consist of a backbone xylose residue and are branched with arabinose. Pentosans occur mainly in wheat, rye or triticale and exhibit their anti-nutritional effects by their water solubility. Pentosans available in sorghum or corn are mostly water insoluble and therefore, less anti-nutritional. Barley or oats contain high amounts of glucans, which are chains of glucose residues. In legumes like soybeans, beans or peas higher concentrations of pectins are measured. Fed to chicken, pectines, a backbone of galacturonic acid and rhamnose have anti-nutritional properties. Galactosides like verbascose, stachyose or raffinose can cause digestive and diarrhea problems in poultry nutrition.

Microbial Non-Starch Polysaccharide hydrolizing enzymes are successfully used since many years in the feed industry to reduce the anti-nutritional properties of NSP's in cereal based poultry and swine diets. The positive effect of these enzymes can be measured in terms of improved performance parameters like weight gain or feed conversion ratio. However the magnitude of these effects depends very much on the species, age, type of feed, cereal inclusion rate or concentration and solubility of the NSP's. Up to now the mode of action of NSP degrading enzymes has not been clarified completely. Figure 8 shows different factors influencing the action of NSP's and of NSP degrading enzymes.

Figure 8: Mode of action of NSP's and of NSP degrading enzyme



Simon, 1998

Soluble NSP's produce viscosity in aqueous solutions and the degradation of this fraction by exogenous NSP enzymes decreases digesta viscosity. In parallel the enzymes are also able to hydrolize insoluble NSP's which are primarily located in the cell walls, and transfer them into a soluble form. NSP's have been shown to lock in nutrients in the cell lumen which in the literature is often described as cage effect. The beneficial effect of NSP enzymes is mainly explained by the viscosity reducing action on the one hand and by the release of nutrients by softening the cage effect.

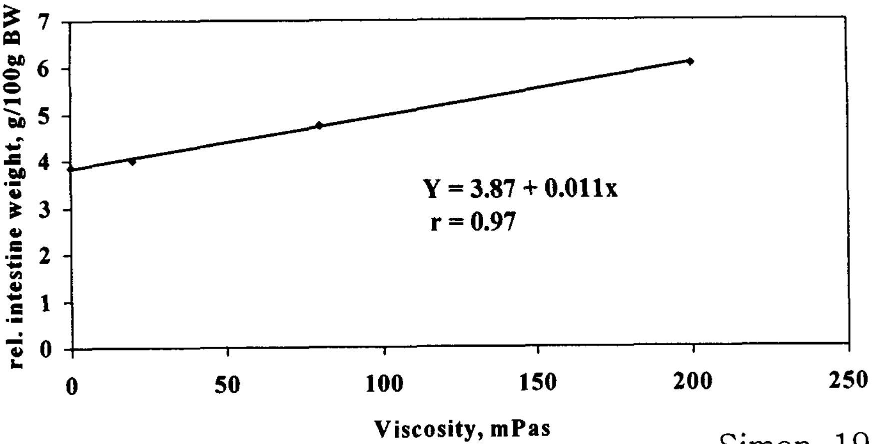
Simon (1998) explained an increase of nutrient digestibility due to a reduction of intestinal viscosity by:

- Improved convection of the intestinal content by contractions
- Improved contact of absorbable nutrients with the surface of enterocytes
- Facilitated diffusion of substrates, digestive enzymes and products of digestion

An indication for the contribution of the cage effect might be the effect of exogenous enzymes on ileal starch digestibility, since starch is the main nutrient in the endosperm of cereals. Almirall et al. (1995) reported an improved starch digestibility by glucanase supplementation to a barley based diet.

As shown in Figure 8 several other parameters are influenced by digesta viscosity. In the literature there is indication that digesta viscosity might have an influence on morphological and structural properties of the digestive tract. A trial with growing broilers showed that an increase in digesta viscosity by replacing corn by rye resulted in an increase in weight, length and protein content of the digestive tract. Figure 9 shows that there was a highly positive correlation ($r = 0.97$) between the relative weight of the intestine and the jejunal viscosity (Simon, 1998).

Figure 9: Correlation between digesta viscosity and the relative weight of the intestine



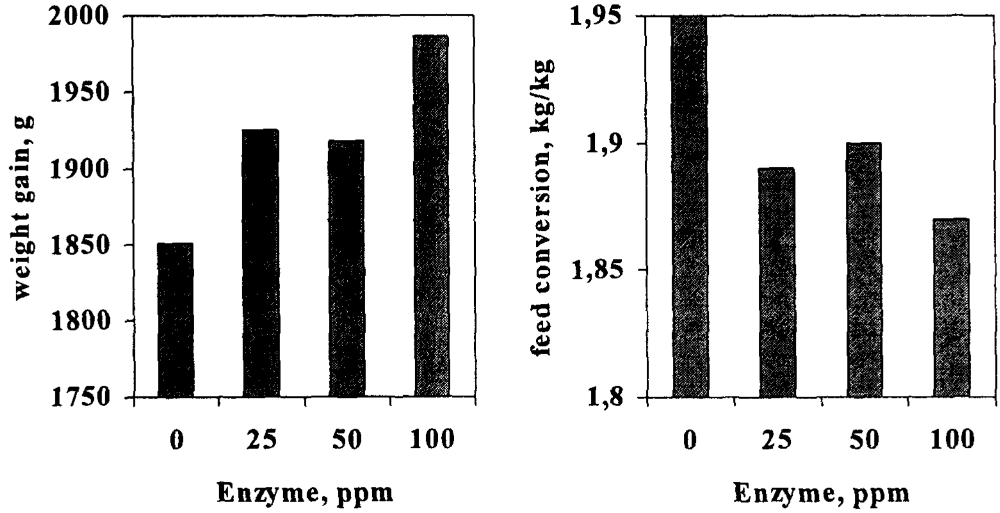
Simon, 1998

Concerning the effects of exogenous NSP enzymes an alternative hypothesis claims modifications of the intestinal microflora which is discussed as another mode of action of NSP hydrolizing enzymes. However, experimental data to support this theory are very limited. Potentially enzyme supplementation can modify the quantity and composition of the microbial population in the digestive tract. Mechanisms might be a accelerated passage rate of the digesta, improved absorption of nutrients and a shift of the site of the absorption, reduced length of the intestine or reduced viscosity which modifies the adhesion conditions for tissue associated bacteria. A recent study (Vahjen et al. 1998) examined the effect of a xylanase supplementation to a wheat based diet on colonisation of lactobacilli, enterobacteria and facultative anaerobic gram positive cocci.

The enzyme addition significantly reduced enterobacteria and total gram positive cocci in luminal and mucosa samples and increased the mucosa associated lactobacilli. At the present these results are difficult to interpret with respect to the effect on nutrient utilization in the animal.

NSP degrading enzymes which are currently in commercial use world-wide are mainly xylanases and glucanases. Pectinases for the use in diets containing legumes and galactosidases to hydrolize galactosides in legumes are of much lower commercial importance. The economic benefits of xylanase and /or glucanase in terms of performance improvement are extensively described in the literature. Figure 10 describes the effect of graduated levels of a xylanase and glucanase preparation on weight gain and feed conversion ration in broilers feed a diet containing 45% wheat (Heindl and Steenfeldt, 1999). The addition of 100 ppm of the enzyme preparation improved weight

Figure 10: Performance of broiler fed a wheat based diet with increasing levels of an enzyme supplementation



Heindl and Steenfeldt, 1999

gain during the period from day 0 to day 42 by 7.3% and feed conversion ration by 4.1 %.

The supplementation of low viscous cereals like corn or sorghum with NSP degrading enzymes is currently not common practice in the feed industry. The mayor reason is the inconsistency of the effects of NSP enzymes in these feedstuffs. Pack and Bedford (1997) reported that an enzyme combination consisting of xylanase, amylase and protease could improve weight gain and feed conversion ratio of broiler fed a corn-soy based diet. In contrast to these results, Sherif et al. (1997) observed no effect of different enzyme preparations containing either only glucanase or glucanase, hemicellulase and pectinase or amylase, glucanase, lipase and protease on performance of broilers fed diets containing either soybean meal or sunflower meal in combination with corn.

Table 3. Anti-nutritional factors in gain legumes

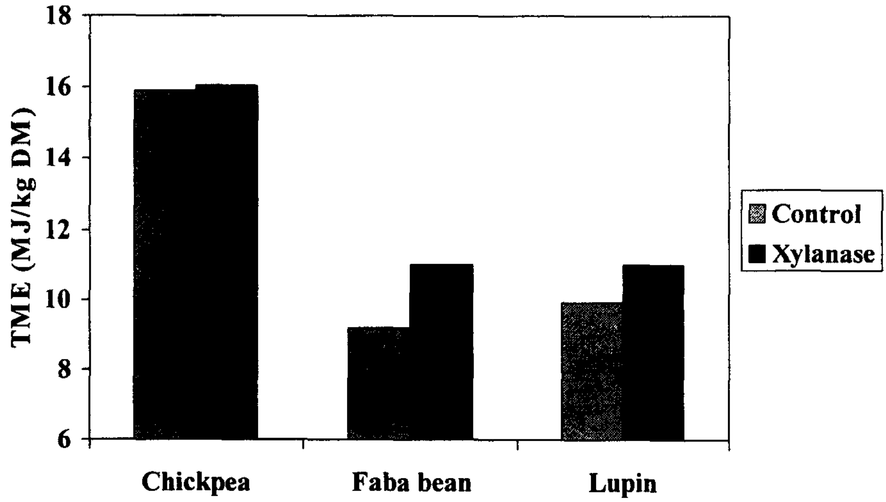
Anti-nutritional factors	Physiological effects	Distribution
Protease inhibitors	Depressed growth, pancreatic hypertrophy/hyperplasia, acinar nodules, interference with protein digestion	Most legumes
Tannins	Interference with protein and starch digestion	Most legumes
Lectines	Depressed growth, death	Most legumes
Amylase inhibitors	Interference with starch digestion	Most legumes
Glycosides		
- Oligosaccharides	Flatulence	Most legumes
- Saponins	Affect intestinal permeability	Most legumes
- Cyanogen	Respiratory failure	Lima bean
- Vicine/Convicine	Haemolytic anamia	Faba bean
Alkaloids	Reduce palatability, depressed growth	Lupins

Wiryawan et al., 1997

Major constrains for the use of grain legumes like beans, peas or lupins in poultry diets are the concentrations of anti-nutritional factors in these raw materials which may depress poultry performance. Table 3 shows the distribution and physiological effects of ingestion of anti-nutritional factors present in grain legumes.

The most commonly found factors are protease inhibitors, tannins, lectins amylase inhibitors, glycosides or alkaloids. A number of techniques like heat treatment, mechanical treatments or enzyme treatment for elimination of these anti-nutritional factors, thus improving the nutritive value of legumes are used by the industry. Studies (Wiryawan et al. 1995) on the effect of xylanase supplementation of beans, peas and lupins showed an improvement of true metabolizable energy (TME) for growing chicken (Figure11).

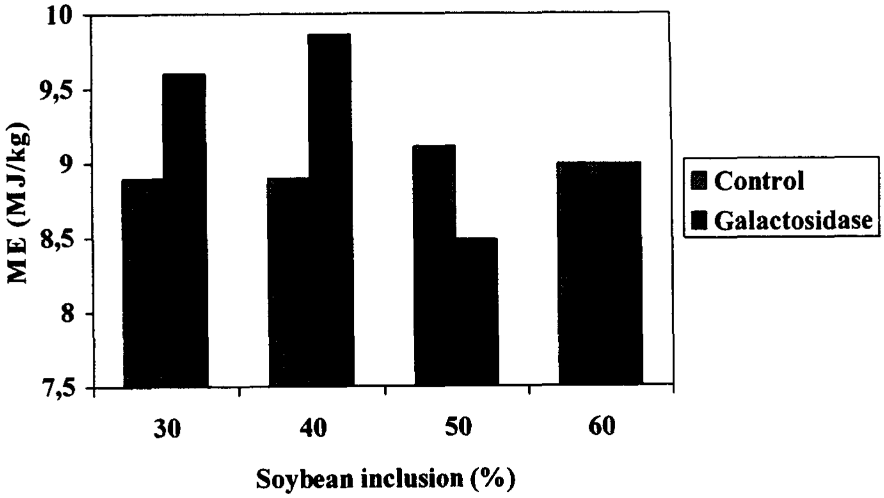
Figure 11: Effect of enzyme supplementation on true metabolizable energy of grain legumes for growing chicken



Wiryawan et al., 1995

Soybean meal is the most frequently used protein source for poultry diets. It is known since many years that soybean meal contains significant amounts of alpha galactosides. These galactosides can impair nutrient digestibility by raising the osmolarity of the intestinal content and by stimulating the intestinal motility reflex, thus decreasing nutrient hydrolisation and increasing intestinal feed transit. Vila and Mascarell (1999) investigated the effect of an alpha galactosidase supplementation on the energy value of soybean meal included at different levels (30, 40, 50 or 60 %) in a broiler diet. The results show (Figure

Figure 12: Effect of alpha galactosidase on the energy content of soybean meal



Vila and Mascarell, 1999

12) that at inclusion rates of 30 and 40% soybean meal alpha galactisidase supplementation increased the AME content by 8 and 11 %, respectively. At higher inclusion rates of soybean meal there was no effect of enzyme supplementation on the energy value.

Conclusions:

Both production and consumption of meat have increased sharply over the past decade. The annual growth rate has amounted to about 6% and has been more rapidly than for other agricultural products.

With respect to the nutritional part of animal production enzymes can contribute to optimize feeding systems. The above discussed enzymes offer a possibility to reduced the effects of anti-nutritional factors present in almost all currently used feedstuffs. Furthermore enzymes help to reduce the impact of modern animal production on the pollution of the environment which will certainly have a more pronounced influence on animal production in the future. The development of new technologies like genetic engineering will help to develop specific enzymes to solve nutritional problems with certain feedstuffs and to make poultry nutrition more profitable.

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