

NON STARCH POLYSACCHARIDE (NSP) HYDROLYSING ENZYMES AS FEED ADDITIVES: MODE OF ACTION IN THE GASTROINTESTINAL TRACT

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1. Introduction

The most abundant NSP-s in animal feed based on cereals are cellulose, 1-3,1-4-β-glucans and pentosans of the arabinoxylan type. β-Glucans and pentosans, in particular are considered as antinutritive factors because of their localisation in the cell wall of endosperm and, more importantly, due to the solubility of a fraction of these NSP-s. Solubilised NSP-s are known to produce high viscosities and it is assumed that elevated digesta viscosities in the upper digestive tract cause impaired nutrient availability.

Because of the ability of endo-β-glucanases and of endo-β-xylanases to partially hydrolyse β-glucans and cereal pentosans, respectively, a class of feed additives containing these enzymes as the main or sole activities was introduced. By June 1999, 36 enzyme products containing NSP hydrolysing activities had been approved in the EU.

Nowadays there is no doubt about the usefulness of microbial NSP hydrolysing enzymes in cereal-based rations. The beneficial effects can be measured in terms of parameters such as daily gain or feed conversion ratio. However, the scale of the effects depends on various factors, i.e. animal species, age of animals, type of cereals, content and solubility of NSP. This is the reason for some discrepancies in reports on the effects and the magnitude of these effects. Most pronounced and best documented are the beneficial effects in young poultry. Considering only experiments done under comparable conditions, Jeroch (1998) reported, for broiler chicken, improvements in weight gain between 0 and 9 % and in feed conversion ratio between 0 and 5 %. Even more inconsistent are the effects reported using these enzymes in piglet feeds.

It is generally believed that beneficial effects are based on the increase in nutrient digestibility or metabolisable energy content of the ration. However, in many cases the effects are higher than would be expected from improvements in nutrient digestibility. Therefore, additional modes of action of the NSP-hydrolysing enzymes are considered to be involved in the overall effect.

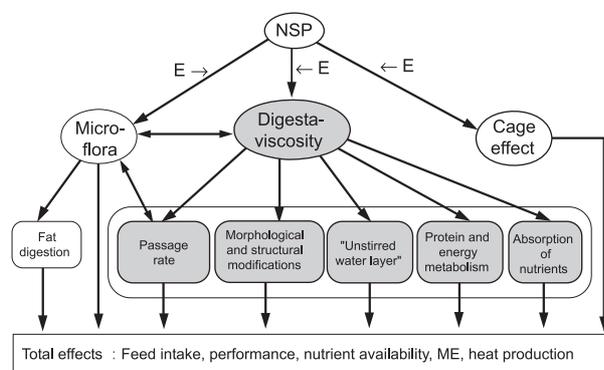
An overview on the subject was given recently at the Workshop on Feed Additives in Jablonna near Warsaw (Simon, 1998). This contribution is based on that meeting and includes, in addition, some new experimental results from our group.

2. Complex model for the possible mode of action of NSP hydrolysing enzymes

In this model the action of the enzymes is mediated by influences on digesta viscosity, on the so-called cage effect and also on the intestinal microflora. All influences are based on the partial hydrolysis of both soluble and insoluble pentosans and/or β-glucans (fig. 1). As mentioned before, soluble NSP-s are responsible for an increase in digesta viscosity, therefore, their partial degra-

ation leads to a decrease in digesta viscosity. Furthermore, another process takes place simultaneously - the partial hydrolysis of insoluble NSP-s, which means solubilisation of NSP-s mainly located in the cell walls. This kind of transformation can be shown by in vitro incubation, but it also occurs in the digestive tract of broilers (Pettersson and Åman, 1989) as well as in piglets (Haberer et al., 1998). Since insoluble NSP-s, as constituents of the cell wall, act to 'fence in' nutrients in the cell lumen, this effect of NSP is frequently called "cage effect".

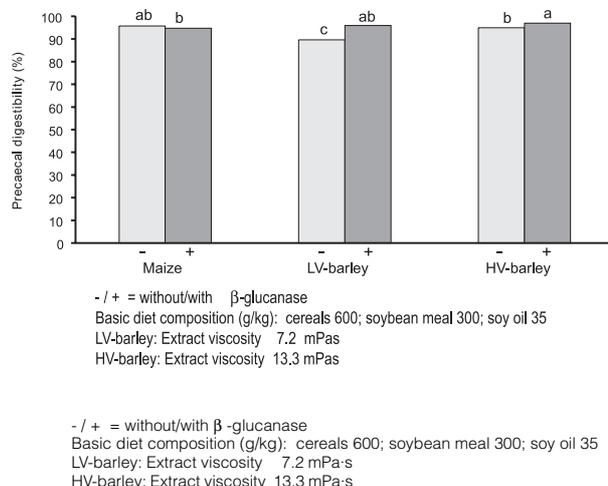
Figure 1: Assumed complex model of action of non starch polysaccharides (NSP) and NSP-hydrolysing enzymes (E→)



2.1. Softening the cage effect by NSP hydrolysing enzymes

It can be imagined that the partial hydrolysis of insoluble NSP-s may soften the cage effect and make the nutrients contained in plant cells more accessible for digestive enzymes. However, it seems difficult to quantify these effects as a proportion of the total effect of enzyme addition. Since starch is the main constituent of endosperm cells in cereals, the improvement of precaecal starch digestibility can indicate in some way the contribution of the cage effect. Almirall et al. (1995) reported significant increases of precaecal starch digestibility by addition of β-glucanases to barley-based rations in broilers (fig. 2). However, due to the already high starch digestibility with the unsupplemented diets, the magnitude of improvement was low.

Figure 2: Precaecal digestibility of starch in broilers (3 weeks old); diet based on maize and barley, respectively; effects of β -glucanase supplementation (according to Almirall et al., 1995)

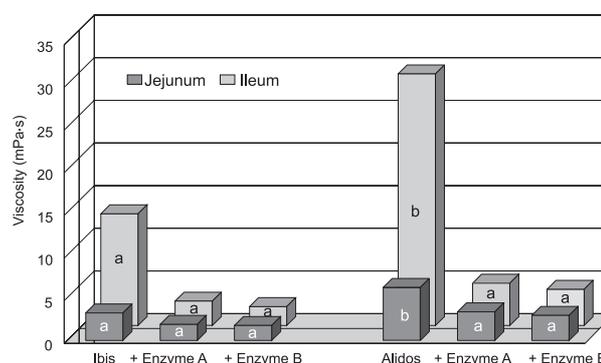


A similar situation was observed in piglets (Inbarr et al., 1993). If we take precaecal starch digestibility as an indicator for all nutrients of the plant cell, one may estimate that the enzyme effect, via softening of the cage effect, only contributes to a limited extent to the total, because improvements of precaecal starch digestibility are in the range of one to two percent. Furthermore, it should be kept in mind that a cage effect is only acting on the nutrients of the cereal itself and not on nutrients of the other constituents of the ration.

2.2. Effects associated with the decrease of digesta viscosity

In practically all experiments with chicken in which digesta viscosity was measured, the decreasing effect of NSP hydrolysing enzymes became evident. This viscosity reduction was not only pronounced when cereals with a high content in soluble pentosans like rye were included in the ration (Dänicke et al., 1999a), but also when wheat-based diets were supplemented with xylanases (fig. 3). Furthermore, this figure shows that digesta viscosity is very much influenced by the variety of wheat and particularly by the content of soluble arabino xylans. Positive correlations between contents of soluble pentosans in wheat, extract viscosity and digesta viscosity at the jejunal and ileal level have been demonstrated (Dusel et al., 1998). For pigs it is generally believed that digesta viscosity is not a significant problem. Nevertheless, in experiments at our institute with piglets receiving diets based on both triticale or wheat, a significant decrease of jejunal and ileal digesta was observed due to the addition of a xylanase-containing product (data not published).

Figure 3: Digesta viscosity in broilers receiving diets including wheat with low (Ibis) or high (Alidos) content in soluble pentosans (Dusel et al., 1998)

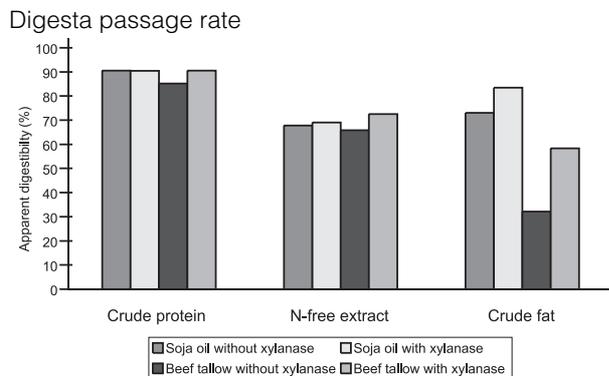


2.2.1. Well documented effects

Nutrient digestibility

It was shown that precaecal digestibility of starch, crude protein and fat were significantly depressed when wheat pentosan fractions were added to commercial broiler diets (Choct and Anison, 1992) and that partial degradation of the NSP constituents, by addition of appropriate enzyme preparations, increases apparent digestibility of these nutrients measured at the ileal level in broilers (e.g. Almirall et al., 1995; Martinez et al., 1992; Dänicke et al., 1999a) or pigs (e.g. Bach Knudsen et al., 1993; Inbarr et al., 1993; Jørgensen et al., 1996) and for the total digestive tract (e.g. Friesen et al., 1992). Another important observation was that the site of absorption was shifted towards upper segments of the small intestine (Inbarr et al., 1993). Improvements of nutrient digestibility due to enzyme addition were combined with increased contents of metabolisable energy of the diets (e.g. Dänicke et al., 1999b). Furthermore, it was shown that these modifications were associated with reductions in digesta viscosity of the upper digestive tract. Of the crude nutrients, fat digestibility is most depressed by elevated digesta viscosity in broilers and the enzyme effects are therefore most pronounced for crude fat. In a model experiment in which rye-based diets were formulated with 10 % soya oil and 10 % beef tallow and which were fed unsupplemented and xylanase supplemented, respectively, these interactions were demonstrated (fig. 4). These results indicate that the fat type (probably because of different melting points) is an important factor determining the effect of digesta viscosity and of NSP hydrolysing enzymes on fat digestibility. Similar findings were reported by Smulikowska and Mieczkowska (1996) and Langhout et al. (1997).

Figure 4: Interactions between dietary fat type and xylanase supplementation in rye based broiler diets - effects on nutrient digestibility (according to Dänicke et al., 1997b)



Another consequence of increased digesta viscosity is a delayed digesta passage rate. Increasing inclusion of soluble and highly viscous cellulose (carboxy-methyl-cellulose = CMC) of 0, 10 and 30 g/kg into a semisynthetic diet prolonged the digesta passage rate in terms of T₅₀ systematically, being 4.6 h, 5.4 h and 7.2 h respectively (Van der Klis and Van Voorst, 1993). This is particularly important for broiler and turkey feeding, since the first consequence of reduced digesta passage rate is a decrease in feed intake (Bedford and Classen, 1992; Brenes et al., 1993).

Stimulated digesta passage rates due to enzyme addition of rye-based or barley-based diets for chicken were indeed shown in several experiments (Almirall and Esteve-Garcia, 1994; Dänicke et al., 1997a; Jeroch et al., 1990; Salih and Campbell, 1991), which may be the explanation for reduced feed intake when diets with high NSP contents are fed.

2.2.2 Expected effects under study

Morphological-histological modifications of the intestinal tract

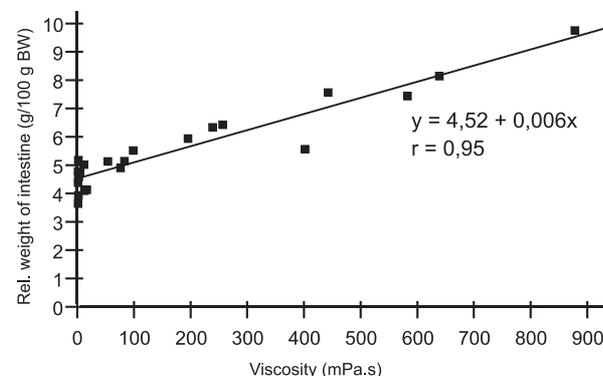
From a theoretical point of view, relatively small changes in relative protein contents of tissues of the intestinal tract (as referred to total protein content of the body) or of the protein turnover rates of these tissues might influence energy expenditure of the animal considerably. The reason is the high contribution (20 to 30 %) of protein synthesis in tissues of the gastrointestinal tract compared to the whole body synthesis, which is combined with a high heat production. It was calculated that an increase of protein synthesis rate in tissues of the gastrointestinal tract by 30 % would increase heat production of the animal by 5 % (Simon, 1998).

Ikegami et al. (1990) described dramatic morphological and structural modifications in rats when digesta viscosity was elevated by model substrates. In particular, increased weights of small intestine, caecum and pancreas, stimulated pancreas secretion and increased activities of various pancreatic enzymes were observed. Other reports indicate that similar changes can be expected in broilers (e.g. Savory, 1992; Van der Klis and Van Voorst, 1993; Viveros et al., 1994; Almirall et al., 1995; Veldman and Vahl, 1994).

In order to study these phenomena, a series of experi-

ments on broiler chickens were initiated at our institute. In these experiments digesta viscosity was modulated over a wide range (jejunal digesta: 4 to 900 mPa.s) by replacing crystalline cellulose with various soluble NSP-s or by gradual replacing of maize by rye in experimental diets. As shown in figure 5, under these conditions the relative weight of the intestine was almost doubled at highest viscosity and correlated with high significance with digesta viscosity. The relative length of the small intestine was influenced to a similar degree. As the protein content of intestinal tissue was not changed, this means that one can expect an increased relative amount of protein in this tissue combined with an increased contribution of these tissues to the total protein turnover of the animals.

Figure 5: Relationship between viscosity in jejunal lumen and the relative weight of intestinal tissues in broilers



In an extended study, more detailed morphological parameters of the jejunum were measured in chickens receiving a maize-soya protein isolate diet (low viscosity) or diets producing high viscosities (CMC substitution or rye inclusion). It was found that at the jejunum, under conditions of elevated viscosity, the villi were shorter and thicker, which points towards a reduction of the absorption surface compared to the control animals (low digesta viscosity). Furthermore, proliferation rates of epithelial cells were measured in jejunum by incorporation of dibromo-desoxyuridine. According to these measurements the ratio of proliferating to total epithelial cells in the crypt region increased significantly from 0.33 at low digesta viscosity to 0.36 at increased digesta viscosity by CMC. This is in line with studies on rats by Mathers (1995) and Southon et al. (1985) who measured stimulated proliferation rate in small intestine as a response to increased NSP contents of the diet. However, no significant changes were measured at increased digesta viscosity by inclusion of rye in our study. Furthermore, Southon et al. (1985) found an increased protein synthesis rate in the mucosa of the small intestine under the same conditions.

Several reports indicate a stimulated mucine formation and an increased number and size of goblet cells when diets with high NSP contents were fed to rats (Schneemann et al., 1982; Satchithanandam et al., 1990) or broilers (Viveros et al., 1994). The intensity of mucine formation may influence the thickness of the so-called unstirred water layer, which is expected to be especially important for the movement of fat micelles during the absorption process.

These results indicate that morphological, metabolic and functional modifications of the intestinal tissue might also be involved in the overall effect of NSP hydrolysing enzymes.

2.3 Effects mediated by modifications of the intestinal microbial communities

Dietary effects on quantity and composition of microbial communities in the intestinal tract are known (Goodson et al., 1988; Sharma and Schumacher, 1995; Zentek, 1995) and it is frequently claimed that modifications of the microbial populations in the intestine are involved in the effect of various feed additives. From a theoretical point of view it seems very probable that contents and characteristics of NSP-s on the one hand and partial degradation of these substrates by addition of xylanases or β -glucanases on the other hand will modify the intestinal microflora. It is a specific subject of research to study these modifications although it remains difficult to interpret changes in the microbial community, because of complex interactions between bacteria, host and feed. Specific metabolic activities of particular groups or strains of bacteria have to be taken into consideration in this context.

The following mechanisms might be involved in the interaction between NSP hydrolysing enzymes, intestinal microflora and the host:

- Reduced retention time of digesta: This may shift bacterial populations towards genera with shorter generation time.
- Improved precaecal nutrient digestibility and shift of the site for nutrient absorption towards upper segments of the intestine: This would mean a shortage of specific substrates for bacterial growth particularly in the distal segments.
- Reduced length of intestine: Under these conditions the area for tissue-associated bacteria is restricted and the absolute number of bacteria in a segment will be reduced in this way.
- Reduced digesta viscosity and mucine formation: Adhesion conditions for tissue-associated bacteria would be changed.
- Partial degradation of NSP-s and solubilisation of insoluble NSP-s: Resulting oligomers (and monomers) improve the availability of these substrates for specific groups of bacteria and shifts their availability towards upper segments of the intestine.

All these are hypothetical mechanisms of how NSP hydrolysing enzymes might modify the intestinal flora. Another, even more important question, is: What are possible consequences of a modified intestinal flora for the animal? Changes in quantity and composition of the intestinal population can influence the nutrient availability for the host. Furthermore, formation of metabolic products (toxins) or colonisation of enterocytes are potential factors for mediation of the enzyme effects via microbial populations.

Intestinal bacterial populations may affect fat digestibility in a specific way. Some strains are known to produce enzymes capable of deconjugating bile acids (Cole and Fuller, 1984; Feighner and Dashkewicz, 1988). According to Feighner and Dashkewicz (1988), the activity of these enzymes was increased in ileal digesta of broiler chickens 10 to 20 times when rye-based diets were fed compared to maize-based diets. Therefore, it is of particular interest to see whether or not addition of xylanases to pentosan-

rich diets influences the activity of these bacterial enzymes in the intestinal lumen.

However, all these possible consequences will remain a speculation as long as modifications of intestinal microbial communities by NSP hydrolysing enzymes are not clearly shown. In a recent study by our group (Vahjen et al., 1998) the colonisation of lactobacilli, enterobacteria and facultative anaerobic gram positive cocci, both luminal and tissue associated, was monitored in broiler chickens during day 1 to 28 post hatching using conventional cultivation and identification techniques. Lactobacilli are known to dominate the anterior small intestine and their beneficial effect against pathogenic micro-organisms such as enterobacteria is discussed (Salminen and Wright, 1992). Anaerobic gram-positive cocci were facultatively chosen, because they include many lactic acid producing strains and occur at high counts, particularly in the terminal small intestine. Enterobacteria multiply at a high rate and many strains produce adhesion factors (Tarkkanen et al., 1990; Krogfelt, 1991) and some strains are pathogenic (Noldner, 1989; Westerlund and Korhonen, 1993).

In our experiment the diets were based on wheat selected for high extract viscosity (variety "Aron", 4.25 mPa·s), soya bean meal and fish meal. One group received the diet unsupplemented and the other supplemented with a xylanase preparation (production strain *Thermomyces longinosus*; 1200 IU/kg diet).

Examples of the results are presented in figures 6 and 7 and the results can be summarised briefly:

- Enterobacteria and gram positive cocci in luminal and mucosa associated samples were significantly reduced by xylanase addition.
- Mucosa associated lactobacilli counts were higher for animals receiving the supplemented diet, but luminal CFU were not influenced.
- All observed differences occurred mainly during the first three weeks of life.

Except changes for tissue associated lactobacilli, the effects of xylanase were reproduced when the enzyme was added to a wheat/rye-base diet (data not published).

A further series of experiments was designed to demonstrate modifications of the metabolic activity of the intestinal flora due to xylanase addition. Two examples of this kind of result will be presented.

Figure 6: Development of mucosa associated lactobacilli in the small intestine of broilers

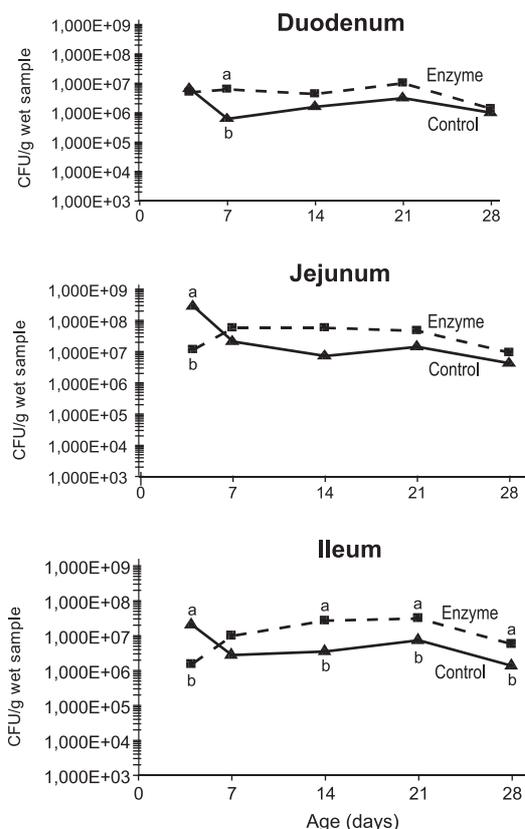
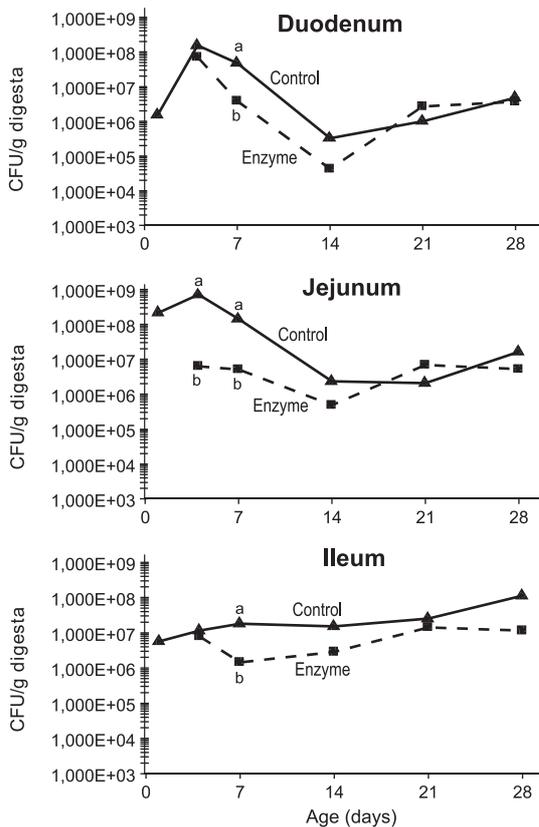
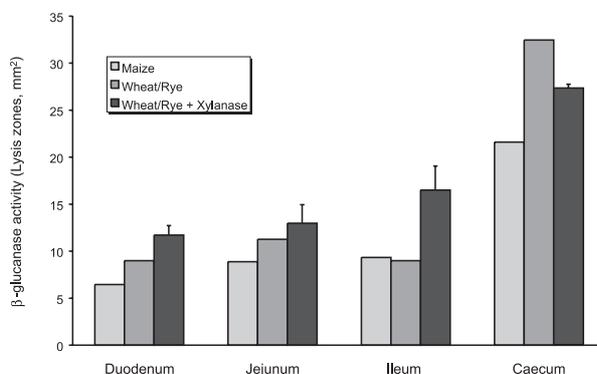


Figure 7: Development of luminal enterobacteria in the small intestine of broilers



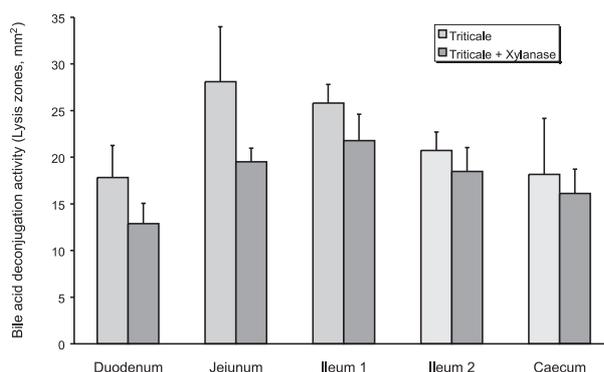
The aim of the first experiment was to study the effect of xylanases on the β -glucanase activity in the intestinal tract of broilers. Three dietary treatments were applied. Treatment I was a diet based on maize producing a low digesta viscosity (5.9 mPa·s at the ileum), treatment II was a wheat/rye-based diet producing a viscosity in ileal digesta of 62.5 mPa·s and treatment III was the same diet as treatment II but supplemented with a xylanase preparation free of β -glucanase activity, which decreased ileal digesta viscosity to 8.4 mPa·s. After three weeks of adaptation, β -glucanase activity was measured along the intestinal tract (fig. 8). The addition of xylanase induced elevated activities of another NSP hydrolysing enzyme, the β -glucanase, in all segments of the small intestine. We interpret this as a shift of a specific flora capable of utilising NSP-s toward upper parts of the intestine due to partial hydrolysis of pentosans by the exogenously supplied xylanase.

Figure 8: Effect of cereal basis of diets and of xylanase addition to β -glucanase activities in the intestine of broiler chicken (according to Hübener et al., 1998)



The aim of the second experiment was to study the effects of xylanase in a broiler diet based on triticale on the activity of other enzymes also expressed only by intestinal bacteria, i.e. bile acid deconjugating enzymes. The animals were adapted post hatching to the unsupplemented and the supplemented diets, respectively, and measurements were made after two weeks. In all segments the bile acid deconjugating activity was decreased when xylanase was added to the diet (fig. 9). One explanation for these findings is that growth of bacteria which produce this enzyme (e.g. clostridia, bifidobacteria or bacteroides spp.) are repressed under the conditions of xylanase addition.

Figure 9: Effect of xylanase addition to a triticale based diet on the bile acid deconjugation activity in the intestinal tract of broilers (according to Hübener et al., 1998)



3. Conclusion

The mode of action of NSP hydrolysing enzymes is very complex. Effects associated with the reduction of digesta viscosity include, in addition to the well documented improvement of nutrient digestibility, morphological and histological modifications of the small intestine as well as the protein turnover of these tissues. Furthermore, the intestinal microbial populations are influenced in terms of count numbers and composition of the microbial communities which may influence the activity of specific microbial enzyme activities in the intestine.

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