EFFECT OF XYLANASES ON THE INTESTINAL FLORA

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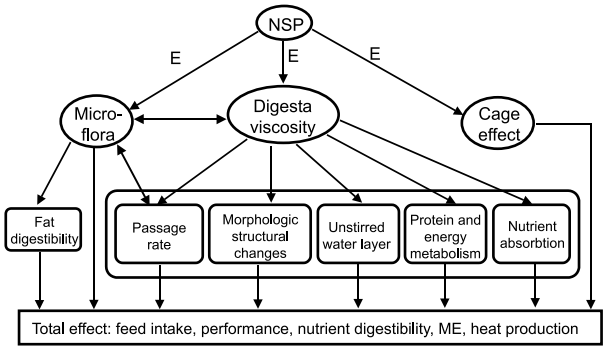
1. Introduction

Incorporation of xylanases into livestock rations, especially diets for broilers, turkeys and to a lesser extent piglets, is commonly practised today where wheat and triticale (and possibly rye) are used as cereal components. In the European Union some 40 products containing xylanase activity either alone or in combination with other enzyme activities have been granted provisional approval.

The overall effect of these feed additives is an improvement of performance parameters such as liveweight gain and feed conversion ratio (FRIESEN et al., 1992; BEDFORD, 1995), a reduction in the incidence of sticky droppings (CHOCT and ANNISON, 1992) and an increase in the level of metabolisable energy of the cereal or the ration as a whole (DÄNICKE et al., 1999; DUSEL et al., 1997; WARD, 1996). The mechanisms of action responsible for these effects were poorly understood initially and some have only recently been investigated. Figure 1 gives an overview of the mechanisms which are most probably involved in the overall effect.

Figure 1: Assumed mode of action of non starch polysaccharides (NSP) and NSP-hydrolysing enzymes (E®)



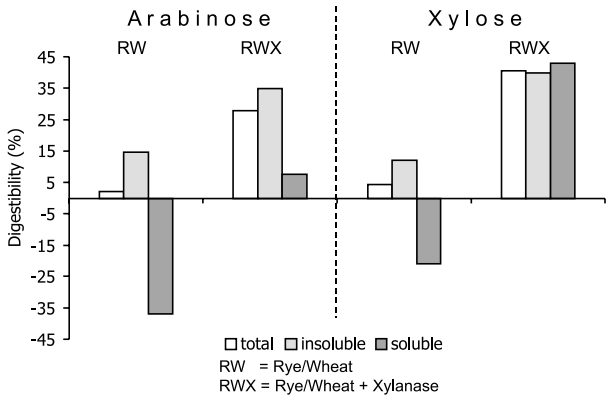
Key effects of xylanases and other non starch polysaccharide (NSP)-hydrolysing enzymes are a reduction in the viscosity of the digesta and probably also the softening of the so called "cage effect". These reactions are due to the partial hydrolysis of both soluble and insoluble NSP. While the softening of the cage effect may result in a direct improvement in the nutrient digestibility of cereal, the viscosity reduction exerts effects on numerous morphological, structural and functional parameters of the digestive tract. Responses associated with a reduction of chyme viscosity are: Accelerated passage of digesta (ALMIRALL and ESTEVE-GARCIA, 1994), lower relative gut mass and length (SIMON, 1998), altered villous morphology in the small intestine and reduced proliferation rate of enterocytes (GEE et al., 1996), reduced protein synthesis rate of the tissues of the digestive tract (DÄNICKE et al., 2000) and improved precaecal nutrient digestibility, especially of fats (DÄNICKE et al., 1997; ALMIRAL et al., 1995).

Relatively little is known about the effect of xylanase supplementation on the gut flora and the implications of this for the overall response and the host animal. Results by our research team on these aspects are presented below.

2. Microbial breakdown of pentosans in the small intestine - Effect of xylanase supplementation

These mechanisms were studied in piglets (24 kg LW) fed a ration with wheat and rye as cereal components (without and with supplemental xylanase-ZY68) and fitted with a duodenal re-entrant cannula and a postvalve T-shaped cannula in the caecum (BARTELT et al., 2001). As the pentosans in these cereal varieties occur almost exclusively as arabinoxylans, the precaecal digestibility was determined with reference to the digestibility of arabinose and xylose from the total fraction and the soluble and insoluble arabinoxylans (Fig. 2).

Figure 2: Ileal digestibility of arabinoxylans in piglets - Effect of xylanase supplementation



It should be noted that hydrolysis of arabinoxylans in the small intestine can only take place through the action of enzymes of microorganisms as endogenous enzymes for this purpose do not exist in the animal's body. Without supplemental xylanase the digestibility of both arabinose and xylose from the total fraction was below 5 %. But if we consider insoluble and soluble pentosans separately, a different picture emerges. While the precaecal digestibility of the insoluble fraction is 10 to 15 %, the digestibility of the soluble fraction is highly negative. This finding shows that the microbial population of the small intestine primarily causes a solubilisation of pentosans by converting insoluble arabinoxylans into a soluble form. When xylanase is added to the diet the digestibility of both the total fraction and the insoluble pentosans to the terminal section of the small intestine is increased to 30 or 40 %. The digestibility of the soluble fraction is also in the positive range. This suggests that xylanase supplementation considerably increases the breakdown of both insoluble and soluble arabinoxylans in the small intestine. But these results do not tell us whether the activity of the supplemented xylanase alone is responsible for this effect or whether the development of a specific flora capable of utilising NSP also plays a role.

3. Effect of xylanases on the microbial population in the small intestine and its metabolic activity

3.1 Use of classic microbiological methods

The effect of dietary factors on the microbial colonisation of the digestive tract can be assessed by growing specimens from the digestive tract on selective culture media. By setting up dilution series the number of colony forming units (CFU) of specific groups of bacteria can be determined, not just those belonging to one genus like enterobacteria, Gram-positive cocci or lactobacilli. It is also possible to differentiate between luminal bacteria (digesta sample) and tissue-associated bacteria (mucosal sample after washing). One of the first studies of this type in broilers (VAHJEN et al., 1998) fed wheat with high extract viscosity as the sole cereal component with and without supplemental xylanase (ZY68) demonstrated that xylanase supplementation evidently causes shifts in the spectrum of microbial species in the digestive tract (Fig. 3 to 5).

Figure 3: Development of luminal enterobacteria in the small intestine of broilers

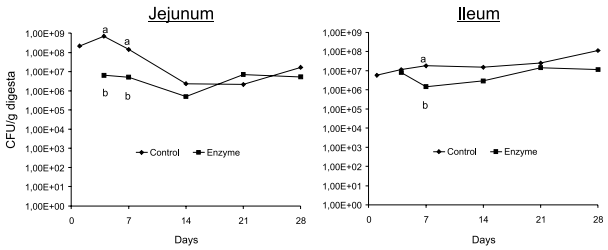


Figure 4: Development of tissue-associated Gram-positive cocci in the small intestine of broilers

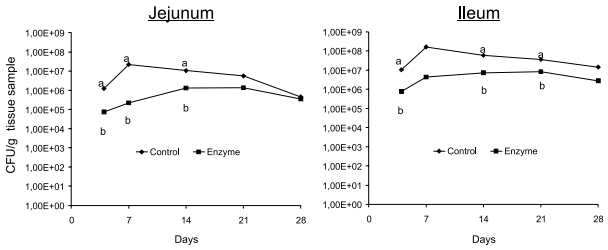
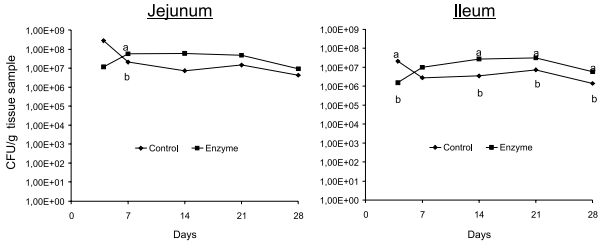


Figure 5: Development of tissue-associated lactobacilli in the small intestine of broilers



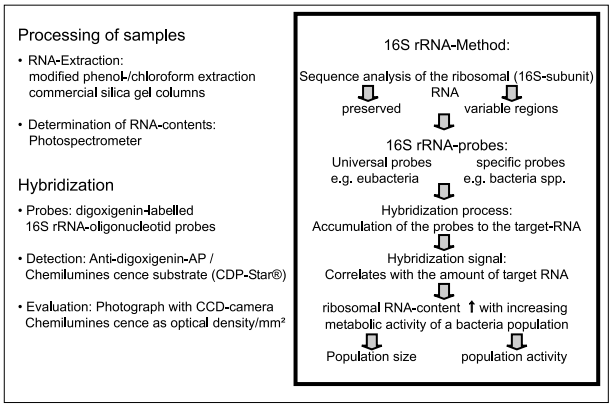
The main observations of this trial were reduced numbers of luminal and tissue-associated enterobacteria and Gram-positive cocci during the first 2 to 3 weeks of life and increased numbers of tissue-associated lactobacilli from 2 weeks of age as a result of the xylanase supplementation. The findings concerning enterobacteria and Gram-positive cocci were confirmed in subsequent, similar studies, but not those for lactobacilli (HÜBENER et al., 1998).

Results of this kind confirm the assumption that xylanases have an effect on bacterial colonies in the small intestine but they do not allow any inferences to be drawn about their significance for the host animal. One reason for this is that the selective culture media detect groups of microorganisms whose representatives could either be completely harmless or else could carry pathogenicity factors, which may be the case for both enterobacteria and Gram-positive cocci. To differentiate these further is very demanding of time and resources and hardly practicable for serial tests. But there is another reason why the methodology described is unsatisfactory. The number of microbial species colonising the digestive tract is believed to be in the region of 300 to 500, but only 10 to 50 % of these can be cultivated outside the gut. This means that we learn considerably less than “half the truth” through using these methods.

3.2 Use of hybridization by 16S rRNA probe for analysis of the intestinal flora at species level

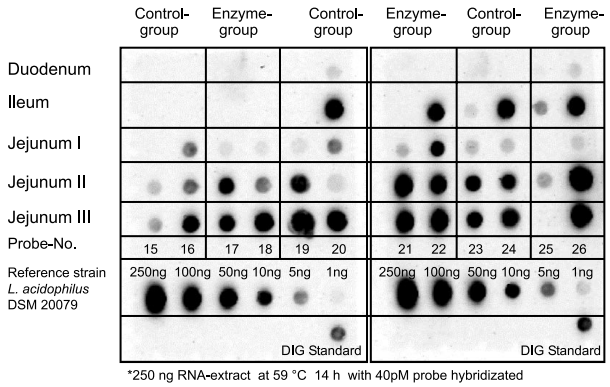
By using molecular biological methods it is possible to perform a semiquantitative (quantitative) determination of population size and activity of microorganisms at species level in the digestive tract even if the microorganisms are impossible or hard to grow in vitro. This is done by developing labelled oligonucleotide probes which consist of 18 to 30 bases whose sequence is specific for one particular genus (group probe) or a particular species (species-specific probe). Probes for ribosomal RNA of the 16S subunit of ribosomes (16S rRNA) have proved particularly suitable for this purpose. As well as highly preserved areas of the base sequence these probes also possess highly variable areas, so that in most cases both group probes and species-specific probes can be found which do not hybridise with nucleic acids of other microorganisms. The methodological procedure is described schematically in Figures 6 and 7. As ribosomes constitute the “protein factories” of the bacterial cell, the results of 16S rRNA hybridization correlate with the metabolic activity of a specific bacterial species or group of bacteria.

Figure 6: Methodology



In the example below the use of several species-specific 16S rRNA probes for the genus *Lactobacillus* is demonstrated. The sample material was small intestinal digesta of piglets which had been fed a ration with wheat/rye as

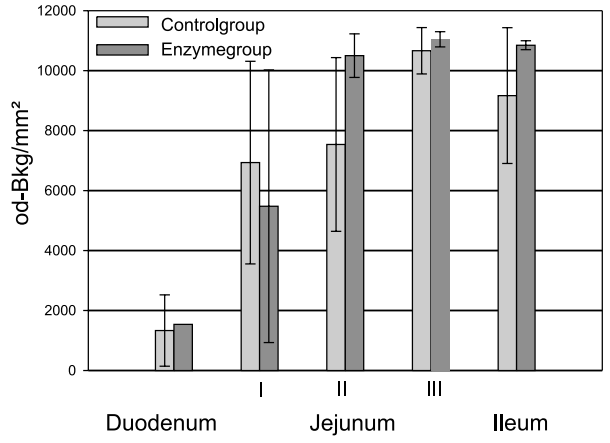
Figure 7: Hybridization of RNA extracts from the small intestine with 16S rRNA oligonucleotide probes using *L. acidophilus as an illustrative example**



*250 ng RNA-extract at 59 °C 14 h with 40pM probe hybridizated

cereal component for three weeks after weaning - two groups of 6 animals each, one group with and one without supplemental xylanase (ZY68). The results obtained with the group probe for the species *Lactobacillus* are shown in Figure 8. It reveals increased metabolic activity of the bacteria of this genus in the middle and terminal jejunum and the ileum under the influence of the xylanases.

Figure 8: Metabolic activity of the Lactobacillus population in the small intestine of piglets



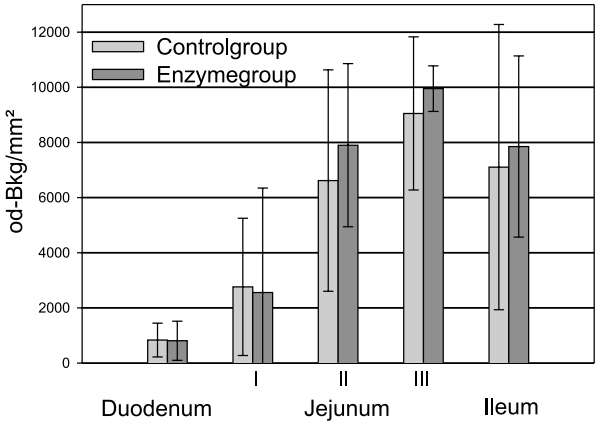
Average controlgroup: Duodenum n=3 Jejunum n=6 Ileum n=3
Average enzymegroup: Duodenum n=1 Jejunum n=5 Ileum n=2

The use of species-specific probes for *L. acidophilus, L. amylophilus* and *L. reuteri* (Fig. 9 to 11) demonstrates that the metabolic activity of *L. reuteri* in particular was stimulated by the xylanase supplement. This Lactobacillus species is known as a bacteriocin producer and has also been tested for its probiotic activity. But the wide variations in the results also reveal that microbial communities in the small intestine of individual animals differ widely in their response to such nutritive factors.

3.3 Adaptation of the intestinal flora to NSP utilization - or how xylanases trigger a "domino effect"

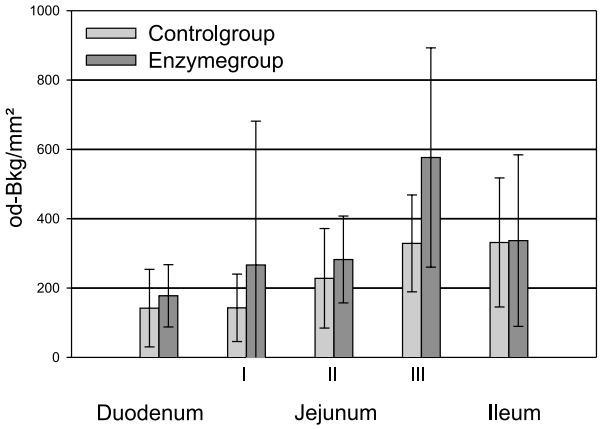
Metabolic activities of microorganisms are of major interest in evaluating the physiological significance of nutritionally induced changes of the intestinal flora. As outlined above,

Figure 9: Metabolic activity of *L. acidophilus* in the small intestine of piglets



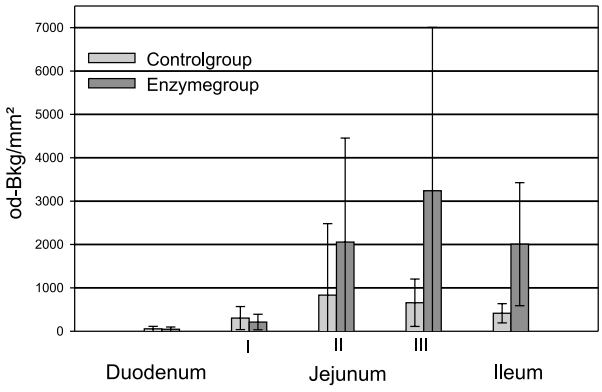
Average controlgroup: Duodenum n=2 Ileum n=3
Average enzymegroup Duodenum n=2 Jejunum III n=5 Ileum n=3

Figure 10: Metabolic activity of *L. amylophilus* in the small intestine of piglets



Average controlgroup: Duodenum n=2 Ileum n=3
Average enzymegroup: Duodenum n=3 Jejunum III n=5 Ileum n=3

Figure 11: Metabolic activity of *L. reuteri* in the small intestine of piglets

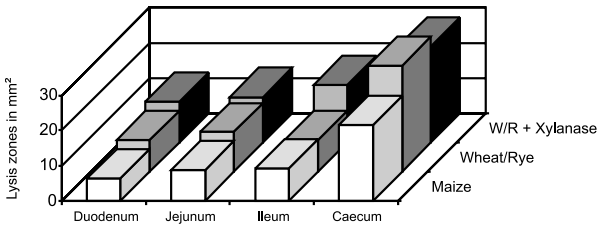


Average controlgroup: Duodenum n=3 Ileum n=3
Average enzymegroup: Duodenum n=2 Ileum n=3

one of these metabolic activities is the degradation of NSP. As xylanases supplied with the diet induce a partial hydrolysis of pentosans (see Fig. 2) it was logical to assume that microorganisms which are specifically capable of utilising arabinoxylans and are themselves xylanase producers and potentially also producers of other NSP-hydrolysing enzymes will be stimulated. This hypothesis was investigated in several experiments. The test animals in each case were broiler chicks which were fed from day-old one of the rations with maize or wheat/rye as cereal component, the latter ration with or without supplemental xylanase.

One possibility is to measure the ß-glucanase activity, which is of microbial origin in the digestive tract, in the digesta of the different gut sections after feeding the aforementioned rations (Fig. 12).

Figure 12: Bacterial enzyme activity of ß-glucanases

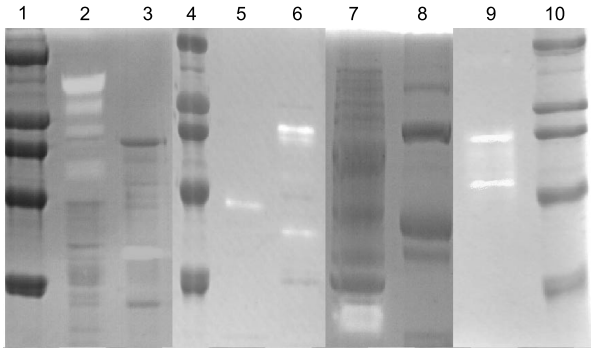


- Site of highest activity is the Caecum
- Wheat-Rye-Feed enhances the activity
- Xylanase increases NSP-digestion (ß-glucan) in the small intestine

Compared with the maize-based ration, the wheat/rye diet caused an increase in the ß-glucanase activity in the caecum. Supplementation of the same diet with a xylanase (free of secondary activities of other NSP-hydrolysing enzymes) leads to an increase in the ß-glucanase activity in the small intestine as well, in particular the ileum, which suggests the establishment of a NSP-utilising microbial population in the small intestine in response to xylanase. In a DFG project (German Research Council) by our research team ß-glucanase producers have been isolated in the digestive tract of chicks. Several species of the genera *Clostridium, Bacteroides, Streptococcus* and *Enterococcus* have been identified as ß-glucanase producers (Fig. 13, the pale lysis zones indicate ß-glucanase activity in these zymograms). In quantitative terms, *Enterococcus faecium* seems to be the major glucanase producer in the anterior digestive tract.

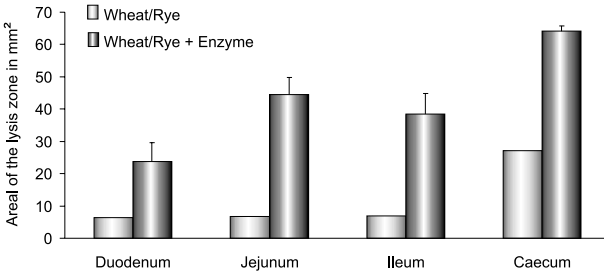
Xylanase supplementation of the wheat/rye ration increased the xylanase activity in all tested gut sections by several times (Fig. 14). This must be attributed primarily to exogenously supplemented xylanase. Here, too, a domino effect can be observed, i.e. stimulated microbial expression of xylanase in the digestive tract through exogenously supplied xylanase. Proving this was complicated, but we eventually succeeded by using the zymogram technique. As well as the active band in the low molecular range representing the enzyme supplied with the diet, the test detected the additional presence of a xylanase activity in the high molecuar range in the digesta of xylanase-supplemented animals. Digesta of chicks fed the wheat/rye ration without xylanase lacked both xylanase activity bands.

Figure 13: SDS / PAGE zymograms of 1,3-1,4-ß-glucanase-degrading bacterial isolates (combination of several zymograms)



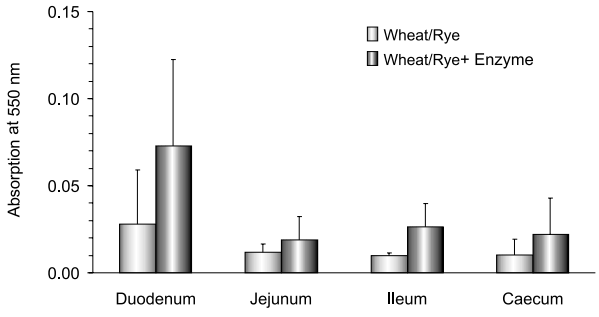
1, 4, 10 = high molecular protein standard, 8 = low molecular protein standard
2 = *Clostridium oroticum*, 3 = *Clostridium viride*, 5 = *Streptococcus bovis*,
6 = *Clostridium perfingens*, 7 = *Bacteroides ovatus*, 9 = *Enterococcus faecium*.

Figure 14: Xylanase activity in the digesta supernatant of 28-day-old broilers



Further evidence that dietary xylanases stimulate the adaptation of a specific bacterial population with the ability to utilise arabinoxylans is supplied by experiments on the growth capacity of specimens from the small intestine and the caecum in minimal media with wheat-derived arabinoxylan as the sole energy source (Fig. 15). This technique involves incubation at 40 °C for 18 hours followed by turbidity measurement, which is an indicator of cell growth. The stimulated cell growth of batches containing digesta of animals fed xylanase with the diet suggests the presence of a large number of microorganisms capable of using arabinoxylans as an energy source.

Figure 15: Growth capacity of luminal xylan utilizers at 6 weeks of age



4. Conclusions

- The presence of xylanases as a feed additive induces modifications of the luminal and the tissue-associated microbial population in the intestinal tract of both broilers and piglets.
- Cultivation of intestinal bacteria from xylanase treated animals on selective media consistently showed a reduction of enterobacteria and Gram-positive cocci and (less easily reproducible) an increase in the number of lactobacilli.
- The response of different species within the genus *Lactobacillus* to dietary xylanase supplementation varies in intensity. The metabolic activity of *L. reuteri* in particular seems to be highly stimulated.
- Dietary xylanase supplementation stimulates the development of a bacterial population in the small intestine that is capable of utilising pentosans and presumably also other NSP for energy generation. A shift occurs in the localisation of these specific bacteria towards anterior sections of the intestinal tract.

5. Literature

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