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Influence of different non starch polysaccaride degrading feed enzymes on the intestinal microbiota in piglets

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ABSTRACT

In order to estimate the growth potential of intestinal bacteria under the influence of different types of enzymes, 1,4 â-arabinoxylan from wheat, 1,3 – 1,4 â-glucan from barley or modified wheat extracts were preincubated for 1 hour with an endo 1,4 â-xylanase- or a multienzyme preparation before incubation with intestinal contents of weaned piglets (56d).

Compared to controls, both enzyme preparations inhibited bacterial growth in stomach contents in incubations with 1,4-â arabinoxylan as substrate, but the multienzyme preparation led to higher bacterial growth in jejunum and colon contents. A growth increase was noted for the multienzyme preparation and 1,3 – 1,4-â glucan as substrate in all intestinal segments, while the monoenzyme showed no difference with this substrate. A trend for growth reduction for both enzyme preparations in stomach- and in part in jejunum contents was also visible in incubations with a large molecular size wheat extract (> 30 kD). Only minor differences were observed for its low molecular counterpart (< 30 kD) and endo 1,4 â-xylanase preincubation, but the presence of the multienzyme preparation showed increased growth.

This in vitro study has shown that intestinal bacteria react differently to the presence of non – starch polysaccharide (NSP) degrading enzymes. Production of multiple NSP fragments by the multienzym preparation from the wheat extracts may enhance bacterial growth in the jejunum, while the hydrolysis of 1,4-â arabinoxylans with the 1,4-â arabinoxylanase seemed to inhibit bacteria in all tested intestinal segments.

Keywords: piglet, bacteria, NSP, feed enzymes, growth

INTRODUCTION

Like any other microbial habitat, the microbiota of the porcine intestinal tract is largely determined by amount and type of available substrates. Dietary carbohydrates are the main energy sources for intestinal bacteria, but due to a very efficient starch digestion, bacterial fermentation is focussed on structural carbohydrates, namely non-starch-polysaccharides (NSP). While insoluble NSP pass the small intestine without physiological consequences, soluble NSP are known as viscosity inducing- and hence antinutritive components in cereal based diets for monogastric animals. NSP – degrading enzymes have been used successfully to circumvent problems with high intestinal viscosity in broiler chicken (Bedford, 2000). However, the influence of soluble NSP on digestion in piglets is less clear. Soluble â-glucans have been shown to be fermented in the porcine small intestine to a rather large extent (Bach-Knudsen et al., 1991, 1993), while arabinoxylans are fermented in the hind gut. However, solubilisation of arabinoxylans already takes place in the small intestine (Bartelt et al., 2002). Thus, the addition of NSP degrading enzymes modifies bacterial populations in the whole intestinal tract.

Several NSP – degrading enzyme preparations are commercially available. Two types can be distinguished: mono enzyme preparations, which contain only a single enzyme activity and multienzyme preparations, which are processed fermentation supernatants of selected fungal production strains. Contrary to the defined enzyme activity of mono enzyme preparations, multiple NSP – degrading enzyme activities can be found in multi enzyme preparations.

This in vitro study was carried out to investigate the modifications of a mono enzyme preparation and a multi enzyme preparation on the intestinal microbiota in weaned piglets.

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MATERIAL AND METHODS

Samples

Pigets (56d old) used for this study were fed a standard diet based on maize and soja bean meal. After slaughter, samples from the stomach, central jejunum and colon ascendens were taken and kept on ice until further incubation.

Enzymes

According to the reducing sugar assay, the main activities in the multienzyme preparation (production strain: Trichoderma longibrachiatum) were as follows: 3000 IU/g arabinoxylanase; 2800 IU/g 1,3 – 1,4 ß-glucanases, 190 IU/g 1,4-ß-Galactomannanase; 140 IU/g 1,4-ß-Arabinogalactanase and 160 IU/g cellulose (Commercial product: Roxazyme G2 G; DSM Nutritional Products, Basel, Switzweland). The monoenzyme preparation (production strain: Aspergillus oryzae, genetically modified with a xylanase gene from Thermomyces lanuginosus) yielded 1100 IU/g (Commercial product: Ronozyme WX CT, DSM Nutritional Products, Basel, Switzweland).

Wheat extract preparation

Ground wheat was shaken in water (1:4) at 37°C for 2 hours. Supernatants were precipitated in ethanol (4:1) and dried to constant weight at 85°C. Extraction of NSP proceeded as described by Englyst and Cummings (1989).

Resulting NSP – extracts were ultrafiltrated in a tangential flow device equipped with 30 kD cutoff polysulfone ultrafiltration membranes (Minitan, Millipore, Schwalbach, Germany). After filtration, both eluate and retentate were collected, dried to constant weight at 85°C and consequently used as substrates in the in vitro growth assays.

In vitro assays on bacterial growth

A minimal medium without a carbon source was supplemented with a single carbohydrate substrate. Carbohydrate substrates (1% (w/v)) consisted of either arabinoxylan from wheat (Megazyme, Ireland), 1,3 – 1,4 ß-glucan from barley (Megazyme, Ireland), the large molecular wheat extract or the low molecular wheat extract. The media were preincubated with a 1mg/ml sterile filtrated enzyme solution (1h, 37 °C). After preincubation, decimal dilutions of digesta samples were added at 1/10 volume in quadruplicates. Microtiter plates were sealed with parafilm and incubated for 24 h at 37 °C. Optical density at 690 nm was recorded and controls (0h, uninoculated controls) were substracted to calculate bacterial growth.

RESULTS AND DISCUSSION

1,4 ß-arabinoxylan

Compared to controls, the presence of the monoenzyme in the arabinoxylan supplemented medium led to growth reduction in all digesta samples (Table 1). The multienzyme also reduced growth in stomach samples, however jejunual and colon samples showed higher bacterial growth than the respective controls.

Growth inhibition of bacteria due to the degradation of 1,4 ß-arabinoxylan by the endo 1,4 ß-xylanase may have several explanations. First of all, osmotic pressure in the closed in vitro incubation system could have been increased due to the enzymatic generation of oligosaccharides. However, it is questionable, if the preincubation time was adequate to generate a sufficient amount of oligosaccharides. Next, there is the observation that hydrogen peroxide breaks down xylan under physiological conditions, thereby releasing smaller

Table 1: Bacterial growth of piglet digesta in media supplemented with arabinoxylan preincubated with the monoenzyme or the multienzyme [OD690nm]1

	Stomach		Jejunum		Colon	
Enzyme	Enzyme	Buffer	Enzym	Buffer	Enzym	Buffer
Multi	0.02 (±0,01)	0.07 (± 0.01)	0.41 (±0.2)	0.31 (±0.07)	0.25 (±0.06)	0.17 (±0.14)
Mono	0	0.03 (±0.05)	0.18 (±0)	0.30 (±0.02)	0.02 (±0.05)	0.10 (±0.07)

1 = 1mg/ml final concentration

Table 2: Bacterial growth of piglet digesta in media supplemented with lichenan preincubated with the monoenzyme or the multienzyme [OD690nm]1

	Stomach		Jejunum		Colon	
Enzyme	Enzyme	Buffer	Enzym	Buffer	Enzym	Buffer
Multi	0.95 (± 0.04)	0.67 (± 0.01)	0.91 (±0.1)	n.d.	0.55 (±0.02)	0.23 (±0.06)
Mono	0	n.d.	0.2 (±0.06)	0.20 (±0.05)	0.13 (±0.02)	0.14 (±0.01)

1 = 1mg/ml final concentration; n.d. = not determined

fragments with reducing ends (Miller, 1986). Bacterial growth in stomach contents is largely determined by lactic acid bacteria, more specifically by lactobacilli as the dominant bacterial population of the piglet stomach. Lactobacilli produce hydrogen peroxide as metabolic waste product, but are incapable of detoxification. Thus, the hydrogen peroxide concentration inevitably increases in closed systems such as the in vitro assay used in this study. Using Lactobacillus crispatus or L. paracasei as inoculum, production of hydrogen peroxide was as high as 5 mmol H2O2 per 108 cells/ ml broth in a study by Thomas et al, 2003. Song et al, 1999 determined L. crispatus, L reuteri and L. gasseri among the most active H2O2 - producers in the vaginal microbiota. Since Miller showed breakdown of xylan with concentrations as low as 0.1 mmol H2O2 and cell numbers in the stomach of weaned piglets are in the range of 108 cells/ ml stomach content, it is reasonable to expect this effect to occur in vivo. For this in vitro study, it could mean that due to the activity of the arabinoxylanase the scavenging reaction of xylan with H2O2 was partly inhibited and H2O2 accumulated with consequent growth inhibiting effects on lactobacilli. Furthermore, enzymatic production of oligosaccharides may inhibit bacterial enzyme production due to product inhibition.

1,3 – 1,4 ßâ-glucan

Compared to the control, no growth differences were observed for incubations with the 1,3 – 1,4 ß-glucan medium and added monoenzyme (table 2). This was expected, as the enzyme only attacks arabinoxylan bonds. Increased bacterial growth was observed in incubations with stomach and colon contents and the multienzyme preparation. This enzyme preparation contains significant amounts of 1,3 – 1,4 ß-glucanases as well as exo-acting enzymes that are able to produce oligosaccharides and sugar monomers. Since such substrates accumulate in an in vitro system, it is reasonable to assume that they were used for bacterial growth. Even without enzyme supplementation, soluble mixed linked â-glucans are readily fermented by the porcine small intestinal microbiota and digestibilities may reach up to 90% (Bach-Knudsen and Hansen, 1991). Thus, the small intestinal microbiota in pigs is adapted to the breakdown of soluble mixed linked â-glucans. In this in vitro study, the controls for stomach contents displayed the highest growth, while jejunum and colon contents showed gradually less growth potential on the mixed linked â-glucan substrate. It is highly likely, that lactic acid bacteria were responsible for the high growth in stomach contents, as they are the dominant bacterial group in the piglets stomach. However, enzymes for the hydrolysis of mixed linked 1,4-â-glucans have not been characterized in lactobacilli yet and there is only one report on an endo – acting mixed linked â-glucanase from an Enterococcus faecium strain (Beckmann et al., 2000).

High- and low molecular weight wheat extract

Both enzyme preparations showed similar growth inhibition in stomach contents with the high molecular weight wheat extract as for the substrate arabinoxylan (Table 3), but absolute growth in controls was higher than for arabinoxylan. Compared to the control, the monoenzyme led to equivalent growth in jejunum contents. The multi enzyme preparation inhibited growth in jejunum contents, contrary to the growth enhancement observed in incubations with arabinoxylan as substrate. Both enzyme preparation increased growth in colon contents.

Interestingly, the ultrafiltrated fraction of the wheat extract showed only minor differences in growth between controls and enzyme added incubations (Table 4).

Table 3: Bacterial growth of piglet digesta in media supplemented with high molecular wheat extract preincubated with the monoenzyme or the multienzyme [OD690nm][1]

	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzyme	Buffer	Enzyme	Buffer
Multi	0	0.20 (±0.05)	0.69 (±0.01)	0.93 (±0.03)	0.24 (±0.03)	0.08 (±0.06)
Mono	0.03 (±0.01)	0.14 (±0.04)	0.89 (±0.02)	0.91 (±0.05)	0.35 (±0.01)	0.08 (±0.03)

1 = 1mg/ml final concentration

Table 4: Bacterial growth of piglet digesta in media supplemented with ultrafiltrated wheat extract (30 kD cutoff) preincubated with the monoenzyme or the multienzyme [OD690nm]1

	Stomach		Jejunum		Colon	
	Enzyme	Buffer	Enzyme	Buffer	Enzyme	Buffer
Multi	0.01 (±0.01)	0.04 (±0.01)	0.78 (±0.05)	0.69 (±0.06)	0.51 (±0.02)	0.47 (±0.06)
Mono	0.01 (±0.01)	0.03 (±0.01)	0.78 (±0.05)	0.88 (±0.07)	0.43 (±0.01)	0.49 (±0.05)

1 = 1mg/ml final concentration

The bacterial growth in control incubations of stomach contents was as low as with arabinoxylan and much lower than with the high molecular NSP extract. Growth in jejunum contents was also less influenced as with the high molecular NSP extract as substrate. Colon contents displayed only marginal differences between controls and enzyme supplemented incubations.

Without enzyme supplementation very low growth yields were observed in incubations with the high molecular weight extract, while the low molecular weight extract displayed rather high bacterial growth. This leads to the interpretation that endogenous enzyme activity on the high molecular weight extract enhances bacterial growth by the release of readily fermentable oligo- or monosaccharides, which are already present in the low molecular weight extract.

However, arabinoxylan fragments generated by the monoenzyme were not responsible for the promotion of bacterial growth in the colon, as arabinoxylan as sole substrate inhibited growth of bacteria. Due to the activity of bacterial exo-acting enzymes in colon contents, it is probable that the amount of arabinose- and xylose oligomers was sufficient to allow additional growth of bacteria.

CONCLUSIONS

This in vitro study has shown that the activity of endo acting 1,4-ß-arabinoylanases on pure arabinoxylan inhibits growth of intestinal bacteria from stomach, jejunum and colon contents. It is hypothesized that the often observed increased bacterial growth due to exogenous enzyme supplementation in wheat based diets does not originate from enzymatic breakdown of the mayor NSP arabinoxylan. However, breakdown of NSP in wheat based diets may give access to other, more easily fermentable NSP substrates like mixed linked ß-glucans. Thus, the side activities of the multienzyme preparation may directly lead to enhanced bacterial growth, while the monoenzyme preparation may act indirectly by increasing the accessability of fermentable NSP in wheat.

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