The effects of non-starch-polysaccharide hydrolyzing enzymes on nutrient metabolism and microbial metabolism products in the gastrointestinal tract of piglets

9 Summary

Corn-based diets for poultry and swine rich in NSP-content were supplemented with NSP-degrading enzymes in order to diminish the anti-nutritive effect of non-starch-polysaccharides, the increased digesta-viscosity and all subsequent related effects.

The aim of this study is to investigate the consequences of using both enzyme preparations *Roxazyme G2* (multienzyme-preparation) and *Ronozyme WX* (monoenzyme-preparation) from DSM Nutritional Products (Grenzach-Wyhlen, Germany) and to compare these to a control-group whose diet is not supplemented. The monoenzyme-preparationcontains only an “endo”-(1,4)-ß-xylanase activity (EC-Nr.:3.2.1.8.) derived from *Thermomyces lanuginosus ssp.*. The multienzyme-preparation performs three major enzyme activities: An “endo”-(1,4)-ß-glucanase (EC 3.2.1.4.), an “endo”-(1,3-1,4)-ß-glucanase (EC 3.2.1.6.) and an “endo”-(1,4)-ß-xylanase (EC 3.2.1.8) all of wich are derived from *Trichoderma longibrachiatum*.

In addition, the hypothesis that the concentration of microbial metabolic products and the microbial metabolism of nutrients change with the use of NSP-degrading enzymes will be tested.

To this end, an *“in-vitro”* study to estimate the growth potential of bacteria from digesta samples of the gastrointestinal tract of a piglet with different NSP-containing substrates was carried out as well as a feeding trial with weaned piglets (race EUROC aged from 21 to 57 days old).

For the statistical analysis of the data, the computer program “Spss for Windows” (version 12.0) was used. Because of the fact that not all parameters showed a normal distribution, a *Kruskal-Wallis* statistical test, which is a distribution-free test, was performed. In the event that trends or any statistical significance appeared, the *Mann-Whitney*-test was conducted as a follow-up test. The underlying probability for error was 5 %.

The feeding trial was conducted with three groups: a control-group fed a basal diet without enzyme supplementation, a *Roxazyme*-supplemented group and a *Ronozyme*-supplemented group. Each group consisted of 20 piglets (ten males and ten females). Performance parameters such as live-weight, live-weight gain, feed consumption, feed conversion ratio and consistency of faeces were recorded over a period of four weeks. Subsequently, after a period of nine days, digesta samples from the stomach, jejunum and colon were taken to determine the ilial digestibility of crude protein, crude fat, crude fibre, acid detergent fibre (ADF),

neutral detergent fibre (NDF), crude ash, starch and amino acids. The ilial digestibility was determined through an indicator method using chromium oxide ($Cr_2O_{3)}$ as a marker. The three diets were supplemented by 0,5 % chromium oxide.

Evaluation of the performance parameters showed the live weight gain in the first four weeks was about 6,8 % higher in the multienzyme-enzyme-preparation group and 7,1 % higher in the mono-enzyme-supplemented group than in the control-group. However, this improvement was not statistically significant. A decrease of the feed conversion ratio in the mono-enzyme group was observed during the 1st to 4th week of the feeding trial. However, this also proved to be insignificant.

In numerical terms, the digestibility of crude fibre, crude ash, ADF, NDF and starch as well as the digestibility of total amino acids of the mono-enzyme preparation group increased marginally compared to the control-group. These observed differences between the enzyme supplemented groups compared to the control group, especially the ileal digestibility of starch, were so slight that it is not possible to confirm if they were due in this case to the often postulated “cage-effect”. This data must also be considered in light of the very high ileal digestibility of the diet used in this study. As a result of this, possible or expected effects of enzyme supplementation on the ileal digestibility when compared to the control-group are not clearly discernible.

The viscosity of digesta samples were determined in order to identify a possible decrease of viscosity resulting from the added enzymes. Compared to the control-group, the viscosity of jejunal digesta samples decreased as a trend in the group receiving a multi-supplemented diet, whereas it decreased significantly under mono-enzyme addition. The digesta samples from the colon only displayed a numerical decrease of the digesta viscosity.

In addition, bacterial metabolites like ammonia, lactate and volatile fatty acids in the digesta samples were determined and the concentration of conjugated and deconjugated bile acids was ascertained in order to make the possibly different effects of both enzyme preparations on the gastrointestinal microbiota visible. The hypothesis that the concentration of microbial metabolic products as well as the microbial metabolism of nutrients is possibly changing under enzyme addition will be examined.

In regard to the bacterial metabolites measured, a comparison of both enzyme supplemented groups with the control group showed no statistically significant differences. The hypothesis that the concentration of microbial metabolic products as well as the microbial metabolism of nutrients is changing with NSP-degrading enzyme supplementation, was confirmed although it was not represented statistically. This is demonstrated in the colon as a numerically

increased concentration of total volatile fatty acids. This could be an indication for an increased bacterial fermentation under enzyme supplementation.

The tendency that the Ronozyme supplemented diet has a decreasing effect on the deconjugation of bile acids could show that a xylanase supplement in piglet's diets leads to a restraining effect on bile acid deconjugating microbiota as it does in poultry.

An agar diffusion assay with digesta samples from the stomach, jejunum and colon serves as evidence of the digesta samples' predominant enzyme activities and with that a comparison of the diet's supplemental enzyme preparations. Here it emerges that the mono-enzyme preparation Ronozyme is more stable when compared to the multi-enzyme preparation Roxazyme and is therefore better able to display its effect further distal in the gastrointestinal tract. The decreased viscosity in digesta samples from colon also demonstrates Ronozyme's greater stability and associated ability to be effective in more distal parts of the gastrointestinal tract. While Roxazyme caused barely any decrease in viscosity in the distal part of the intestine when compared to the control group, the mono-enzyme preparation Ronozyme, however, caused a clear reduction in viscosity.

The *„in-vitro"* study with digesta samples in this dissertation has shown that intestinal bacteria react differently to the presence of NSP-degrading enzymes. Production of multiple NSP fragments by the multienzyme preparation from the (1,3-1,4)-ß-Glucan may enhance bacterial growth in jejunum, while the hydrolysis of (1,4)-ß-arabinoxylans with the (1,4)-ß-arabinoxylanase of the Ronozyme seemed to inhibit bacteria in all tested intestinal segments.