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The impact of microbial deconjugation of bile salts on fat digestion in broiler chickens

Summary

Many bacteria species inhabiting the chicken gut are capable of synthesising bile salt hydrolase (BSH), which catalyses the cleavage (deconjugation) of conjugated bile salts rendering free bile salts with markedly lower detergent properties in the emulsification of fat. Microbial deconjugation of bile salts in the small intestine has been associated with impaired fat digestion and growth depression in chickens. As dietary fat is an important component of broiler feed providing a large proportion of the dietary energy, an efficient fat digestion is evidently important for growth performance. However, limited information is available on the extent of BSH-activity expressed by individual bacteria inhabiting the chicken gut and to which degree this mechanism affects fat digestion and contributes to growth depression in broilers.

The aim of the present thesis was to study the impact of microbial deconjugation of bile salts in the small intestine of broiler chickens on fat digestion. The results obtained are presented in four papers, which are discussed in relation to each other and to the existing knowledge of the research area.

Paper I

The effect of unconjugated bile salts on the degradation of lipids was investigated *in vitro* and *in vivo*. An assay based on automatic titration was established for determination of lipase activity *in vitro*. Using this titrimetric method, the fatty acid release from hydrolysis of glycerol tributyrates by lipase was recorded at different physiological pH-levels (6.6, 6.8, 7.0, 7.5) in response to different ratios of conjugated:unconjugated bile salts (100:0, 75:25, 50:50, 25:75, 0:100). The study demonstrated that the lipase activity was markedly inhibited by a decrease in the ratio of conjugated:unconjugated bile salts. In addition, the magnitude of inhibition was pH-dependent, showing a more pronounced inhibition at low pH. This important influence of unconjugated bile salts on lipase activity at physiological pH was confirmed *in vivo* in a broiler experiment. In the *in vivo* study the effect of dietary supplementation with antibiotics (a combination of avilamycin, 10 mg/kg feed and salinomycin, 40 mg/kg feed) on bacteria count, pH and bile salt concentration in the small intestine of broiler chickens was investigated. This experiment showed numerically and significantly lower counts of lactic acid bacteria and *Clostridium perfringens*, respectively, and a significantly higher pH in antibiotic-fed chickens, suggesting a lower microbial nutrient

fermentation in the small intestine of these birds compared to birds fed the non-supplemented diet. The high pH in the small intestine of antibiotic-fed chickens was accompanied by a significant increase in lipase activity, and coincided with a significantly lower concentration of unconjugated bile salts and a higher ratio of conjugated:unconjugated bile salts.

Paper II

In order to identify the most dominant bacteria with respect to BSH-activity bacteria were isolated from small intestinal content of broiler chickens using non-selective and selective culture media. The isolates were identified according to their cell morphology, fermentation pattern, and by 16S rDNA sequencing. Subsequently, an assay based on high performance liquid chromatography analysis of bile salts and bacterial protein determination was established in order to quantify BSH-activity in the isolates. Within the standard conditions of the BSH-assay established, it was demonstrated that the BSH-activity varied among the isolates (*Lactobacillus salivarius*, *Lactobacillus aviarius*, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus alactolyticus*, *Clostridium perfringens*) according to growth and pre-incubation conditions (medium with or without conjugated bile salts). Only diminutive BSH-activities were found in the two *Lactobacillus* strains, and *Enterococcus faecalis*. *Streptococcus alactolyticus* was completely inhibited in growth at the physiological concentration of bile salts, and hence no activity was detected. Both *Enterococcus faecium* and *Clostridium perfringens* expressed high levels of BSH-activity and were abundant among the bacteria isolated in the present study.

Paper III

The effect of dietary fat source (soya oil or a mixture of lard and tallow) and subtherapeutic levels of antibiotics (a combination of avilamycin, 10 mg/kg feed and salinomycin, 40 mg/kg feed) on the bacterial community in the ileum of broiler chickens at various ages (7-, 14-, 21-, 35 days) was studied using PCR-denaturing gradient gel electrophoresis (DGGE) analysis in combination with bacteriological culture. The bacterial origins of the DGGE-fragments were identified by sequencing. Bacterial enumeration results together with DGGE profiles showed that the composition of the microflora was age-dependent and was influenced by both dietary fat source and antibiotic supplementation. The incidence of streptococci, *Clostridium perfringens* and enterobacteria increased with age. Lactobacilli (*L. johnsonii*, *L. crispatus*, *L. reuteri*) predominated in the ileum during the entire growth period. *Clostridium perfringens* was the bacterial group most affected by the dietary treatment, where *Clostridium perfringens* was clearly reduced in birds fed soya oil compared to birds fed the mixture of lard and tallow. In addition, the incidence of *Clostridium perfringens* was considerably lower in birds fed the antibiotic-supplemented diets than in birds fed the non-supplemented diets. Further, different strains of *Clostridium perfringens* type A were detected in response to age and dietary treatment.

Paper IV

The influence of dietary fat source (soya oil or a mixture of lard and tallow) and antibiotic supplementation (a combination of avilamycin, 10 mg/kg feed and salinomycin, 40 mg/kg

feed) on fat digestion in broiler chickens at various ages (7-, 14-, 21-, 35 days) was investigated with special reference to microbial deconjugation of bile salts. Bird performance, digestibility of fatty acids, pancreatic lipase activity, and biliary bile salt concentration were measured. In content from the small intestine pH, counts of lactic acid bacteria and *Clostridium perfringens*, bile salt concentrations, viscosity, and lipase activity were determined. Age-related changes in fatty acid digestibility corresponded temporally with changes in biliary and intestinal concentrations of bile salts and to a less extent with changes in intestinal lipase activity. The digestibility of individual and total fatty acids was higher in birds fed soya oil as compared to birds fed a mixture of lard and tallow, where the greatest improvement was shown in young birds. Compared to birds fed the mixture of animal fat, lower counts of lactic acid bacteria and *Clostridium perfringens*, higher pH, and higher concentrations of total bile salts were demonstrated in the small intestine of birds fed soya oil. Lower counts of lactic acid bacteria and *Clostridium perfringens* in the small intestine of broilers fed antibiotic-supplemented diets coincided with a higher intestinal pH, a lower concentration of unconjugated bile salts, a higher intestinal lipase activity and a higher digestibility of fatty acids compared to birds fed the non-supplemented diets. In particular in older birds, where the number of bacteria and the concentration of unconjugated bile salts were highest, dietary supplementation with antibiotics showed beneficial effects on intestinal lipase activity, fat digestion, and body weight.

In conclusion, the present thesis demonstrated the existence of a pH-dependent inhibition of lipase activity by unconjugated bile salts *in vitro* and *in vivo*. Although, it can not be excluded that some *Lactobacillus* species play an important role in BSH-activity in the small intestine, the present thesis demonstrated that among common bacteria isolated from the small intestine of chickens, *Enterococcus faecium* and *Clostridium perfringens* are dominant with respect to BSH-activity. Both PCR/DGGE and bacterial counts showed that the composition of the microflora changes in relation to age and dietary fat source and antibiotic supplementation. In particular, the incidence of *Clostridium perfringens* increases with age and is markedly influenced by fat source and antibiotics. Furthermore, the present thesis provides evidence that the dietary fat source has a great impact on fat digestion in the beginning of the growth period, where the physiological capacity of the chicken, in terms of adequate bile salt concentration, is limited. The effect of antibiotics on fat digestion is in contrast more pronounced later in the growth period, where the number of BSH-active bacteria such as *Clostridium perfringens* is high. Finally, this thesis emphasises that an impaired fat digestion and hence growth depression in broiler chickens are associated with a deficiency in bile salts, which can be ascribed partly to a limited capacity of the bird in the beginning of the growth period to synthesise and recycle bile salts and partly to a high microbial deconjugation of bile salts later in the growth period.