Bacillus based growth promoter improves growth performance in broilers

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It is widely recognised that the intestinal microflora has a great impact on avian growth and health through its effect on gut morphology, nutrition, pathogenesis of intestinal diseases and immune responses.

It is also well known that the intestinal bacterial composition can be manipulated through dietary changes and by a variety of feed additives. The most established approach to control the gut flora and enhance animal growth has been the feeding of sub-therapeutic doses of antibiotics (antibiotic growth promoters; AGP). The reduction, and for European countries the total removal, of AGP from broiler feed has resulted in a need for alternative growth promoters to keep up high production performance as well as intestinal health.

Complex microflora

In chickens the alimentary tract is sterile at hatching, but within a few hours microorganisms gain access and the gut microbiota develops into a more complex and diverse microflora as the bird ages.

In this context, older birds harbour a microbiota more diverse and complex and are less susceptible towards gut-related infections and diseases compared to younger birds. Studying the intestinal microflora using traditional plate counting causes difficulties due to the high diversity, high concentration and because many of the bacteria in the GIT are so-called non-countable.

A culture-independent approach such as PCR-denaturing gradient gel electrophoresis (DGGE) has been demonstrated to be a powerful tool for studying microbial compositions in response to, for example, age, diet components, and feed additives.

The use of Bacillus spore formers as growth promoters is not a new concept. Bacillus subtilis is one of the five Bacillus strains on the American GRAS list of direct feed microbials. B. subtilis added to broiler feed has shown improvements in nutrient utilisation by increasing metabolisable energy and nitrogen retention.

However, the underlying mechanisms behind the effects, which very likely are species and even strain specific, are not well understood and are getting an increasing level of scientific interest. Thus, PCR-DGGE was applied in order to investigate changes in the dominant bacterial communities following the introduction of GalliPro (Bacillus subtilis) in the feed for broiler chickens.

The aim was to elucidate whether a growth enhancing effect of feeding GalliPro was mediated through its effect on the ileal microbiota.

GalliPro Bacillus subtilis (g/t)	0	500
Start weight, day 1 (g)	42.8	42.8
End weight, day 35 (g)	1906[a]	1936[b]
Feed conversion, day 1-35 (g/g)	1.887	1.881

[a,b]Means with different superscripts differ significantly (P<0.05)

Table 1. Bird performance.

Production trial

A production trial involving two dietary treatments; one control standard basal diet and the basal diet supplemented with GalliPro (500g/t feed) corresponding to 8 x 10^5 spores/g feed were used for this investigation. Commercial northern European diets (wheat/soy based diet) without coccidiostats were fed ad libitum during the 35 day experimental period.

Chicken live weight and feed intake were recorded on a pen basis on days 1, 14, 28 and 35. Feed conversion and growth rate were calculated on a pen basis, where each pen represented one replicate.

At the end of the production period, three birds per pen were randomly selected and killed. Immediately after killing, the gut was removed.

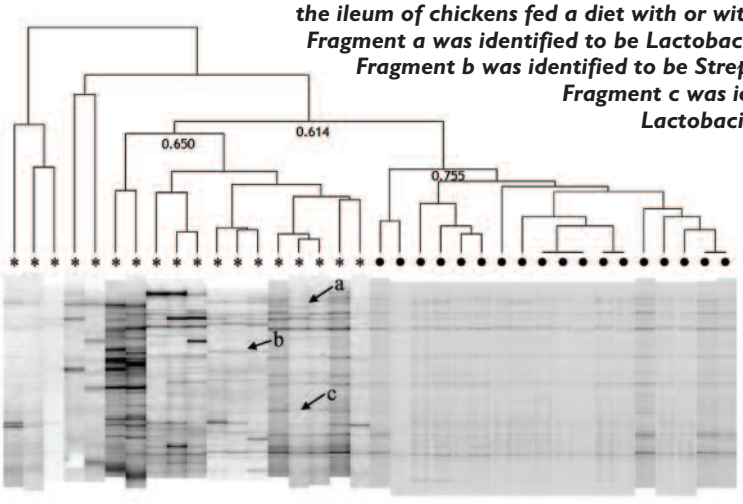
After excision, the small intestine was placed in a plastic bag and stored at -20°C until DGGE analyses. Digesta from the ileum was used for DNA extraction, followed by PCR amplification and denaturing gradient gel electrophoresis analyses (DGGE).

As shown in Table 1, supplementation with GalliPro enhanced the final body weight significantly by approximately 2.0% (P<0.05). Additionally, the feed conversion ratio (FCR) was numerically improved by GalliPro showing less feed consumption per kg gain in birds.

The DGGE patterns in Fig. 1 represent the dominant bacterial microflora in the ileal

Continued on page 13

Fig. 1. Dendrogram representing relationships fragment banding patterns from the ileum of chickens fed a diet with or without GalliPro. Fragment a was identified to be Lactobacillus salivarius. Fragment b was identified to be Streptococcus spp. Fragment c was identified to be Lactobacillus paracasei.



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• Control

Continued from page 11
samples of chickens fed either the basal diet or the diet supplemented with GalliPro.

Computer analysis of the DGGE patterns of the ileal profiles from control birds and birds fed GalliPro resulted in a grouping associated with the dietary treatment. The microflora profiles show a separation into two main groups.

This group separation illustrated a shift in the bacterial microflora in the ileum of chickens fed the GalliPro compared to the control birds. The ileal bacterial community profiles were very consistent within the chickens of the control group showing a uniform and simple microbial composition. In contrast, GalliPro clearly increased the microbial diversity as shown by the 1.5-2.0 fold higher average number of DGGE fragments in ileal samples of GalliPro-fed chickens (~13 fragments) as compared to control birds (~8 fragments).

Identification of fragments represented only in the GalliPro fed birds and not in the control birds was performed. Fragment a and c were identified by DNA sequencing as a Lactobacillus salivarius, and a Lactobacillus paracasei group member, respectively.

Fragment b was identified as a member of the genus Streptococcus.

Definite identification of this bacterium was not obtained as the search showed equally high similarity to several species including Streptococcus gallolyticus, S. equinus, and S. bovis. Although, the microbiology of the caeca and of the crop of chickens has received considerable attention, the ileum was chosen in this study as the segment of interest due to being the site of nutrient absorption and being considered the target for the effect of GalliPro on the feed utilisation.

Enhanced performance

Feed supplementation with GalliPro enhanced chick performance as well as enriched the birds with a more diverse and complex bacterial composition in the ileum, which expectedly provides a more robust microbiota less susceptible towards diseases and infections. This may also indicate an earlier maturation of the intestine of the birds fed GalliPro.

As shown by the DGGE profiles and subsequent DNA sequencing, GalliPro favoured growth of lactic acid bacteria, which commonly are considered beneficial due to their health properties such as protecting against pathogen colonisation and stimulating the immune response of the host.

The Lactobacillus salivarius detected in the majority of chickens fed GalliPro has previously been found to appear in a development succession of the avian gut microbiota and is often associated with a more mature bacterial community.

Moreover, Lactobacillus salivarius exhibits strong probiotic characteristics showing a great capability to reduce pathogen colonisation of salmonella and Escherichia coli.

Accordingly, a high persistence rate in the gut and a stimulated immune system of the host are some of the probiotic properties associated with Lactobacillus paracasei.

Manipulating the microbial composition in the gut using either single or multiple probiotic strains have been demonstrated previously. However, a growth enhancing effect of a Bacillus which is reflected by a marked increase in the gut flora diversity has, to our knowledge, never been described previously. Further, considering the low stability of lactic acid bacteria preparations during feed processing and feed storage, the thermo-stabile bacilli spores seem to be an advantageous and effective route to enhance the incidence of beneficial lactic acid bacteria in the animal gut.

Future approach

Finally, the PCR-DGGE technique was proven very applicable and useful in monitoring changes in the microbiota in the chicken gut in response to feeding GalliPro. However, to obtain more insight into the ecological significance behind the microbial changes in response to Bacillus supplementation, the sequencing of more DNA fragments and/or PCR-DGGE analyses using species-specific primers could be a valuable approach in future studies. ■