



Henk Enting

Curriculum

Henk Enting es líder sénior en tecnología avícola para los negocios de nutrición y salud animal de Cargill a nivel mundial. En este cargo, es responsable de desarrollar e implementar tecnología avícola dentro de Cargill Animal Nutrition and Health, y contribuye a la cartera de proyectos de innovación.

Henk se graduó con distinción en 1991 de la Universidad de Wageningen en los Países Bajos, con un enfoque principal en la cría de animales, la nutrición animal y la economía agrícola. De 1991 a 2005, trabajó como investigador de nutrición avícola y coordinador de grupos avícolas en Schothorst Feed Research en Lelystad, Países Bajos. En 2005, obtuvo un doctorado de la Universidad de Wageningen sobre el efecto de las dietas de baja densidad en reproductoras de pollos de engorde. En el mismo año, comenzó como investigador avícola en el Centro de Investigación Avícola de Nutreco en Casarrubios del Monte, España. Regresó a los Países Bajos en 2008 y trabajó para

Resumen de la ponencia

Microbiota and its effect on performance of broiler chickens

Henk Enting^{1}, Anne Goderis¹, Jean de Oliveira² and Malhar Khambete³
Cargill Animal Nutrition and Health, ¹Velddriel, The Netherlands; ²Vilvoorde, Belgium; ³Minneapolis, United States;
e-mail: henk_enting@cargill.com

Introduction

The reduction of the use of antibiotics in drinking water and in feed (anti-microbial growth promoters; AGP) has resulted in an increasing interest in how microbiota is affecting bird performance, as the intestinal microbiota is believed to play an important role in bird performance and health (Sekirov et al., 2010; Pedroso et al., 2015; Kogut, 2019). However, the relationship between microbiota composition and bird performance is not that clear and trial results have been contradictory and non-conclusive (Diaz Carrasco et al., 2019). Furthermore, it has been difficult to identify specific microbiota populations that can be linked to performance and to understand how to help change the microbiota in a direction that is associated with good performance. The microbiota is very complex (Sekirov et al., 2010) and the complexity of the microbiota composition and the potential non-linear relationships between bacteria can be one of the main reasons that it has been difficult to establish clear links between microbiota composition and broiler performance. In order to get a better understanding of the potential relation between microbiota composition and bird performance, a more simplified approach was taken by developing a microbiota analysis platform that was based on a fluorescence microarray, which can be applied in both research trials and in the field. This paper provides examples of work that has been done to link microbiota composition to bird performance.

Development of microbiota platform for routine use in R&D trials and field conditions

A microbiota analysis platform based on a fluorescence assay was developed that included microbiota biomarkers that were selected based on performance differences between individual chickens in R&D trials and based on food safety risks. The construction of the microarray followed the methodology as was described by Ladirat et al. (2013) for a human intestinal microarray. The marker probes on the array included different bacteria at family, genus and individual species level that are present in the intestinal tract of broiler chickens. The probes were validated in trials in research centers and farms in the field by using advanced non-linear statistics and artificial intelligence-based models. The advanced data analysis approach made it possible to identify relationships between microbiota composition and performance, pathogen risk, and other parameters. Initially this was done based on samples taken from caeca, but over time cloaca swab samples were added to the database as well and relationships were established between cloaca swab microbiota data and bird performance. This was important to facilitate widespread use of the platform and remove limitation to the number of samples taken. The number of probes were updated over time with an increasing number of research trials and farm collections in order to improve correlations between microbiota composition to performance and other parameters. Based on comparisons with 16S rRNA gene sequences and shotgun metagenomics sequences this platform is continuously evaluated and markers can be changed in order to improve correlations to key research and field relevant topics.

Based on experience, it was defined that at least 20 birds per treatment are need for research trials. For on farm collections, 24 samples per age per house (1 sample per bird) were gathered. The samples were stored in tubes that contained a solution that killed all organisms but preserved the nucleic acids. Sample analysis included DNA extraction, quality control, fluorescence labeling, and standard procedures for microarray analysis (Druyan et al., 2008; De Oliveira et al., 2013, Van der Hoeven et al., 2013). Results of microbiota analyses from R&D trials and on farm collections were stored in one database that include effects of different raw materials, nutrient levels, feed additives, farm management conditions, farm performance levels on the same feed, disinfection procedures, heat stress conditions, and parent stock. The database includes over 39,000 samples from 145 different events, and it is used to identify patterns in microbiota composition and microbiota development that affect broiler performance. From this database, recommendations can be derived on how to change the feed composition or management conditions in order to obtain a microbiota composition and maturation profile that is associated with good broiler performance or other parameters like the incidence of specific pathogens.

Farm performance and microbiota composition

In figure 1, examples are provided of the microbiota composition of farms with differences in performance level. The left plot in figure 1 shows differences in microbiota of broiler chickens on underperforming (bad) and good performing farms at 35 days of bird age with all birds receiving the same feed and having similar housing conditions. There was a difference of 410 g in final body weight between the two farm performance classes. Underperforming farms showed significantly higher *Enterococcus hirae*, *Parabacteroides* and *Lactobacillus crispatus* signals, while good performing farms were significantly higher in *Lachnospiraceae* spp.

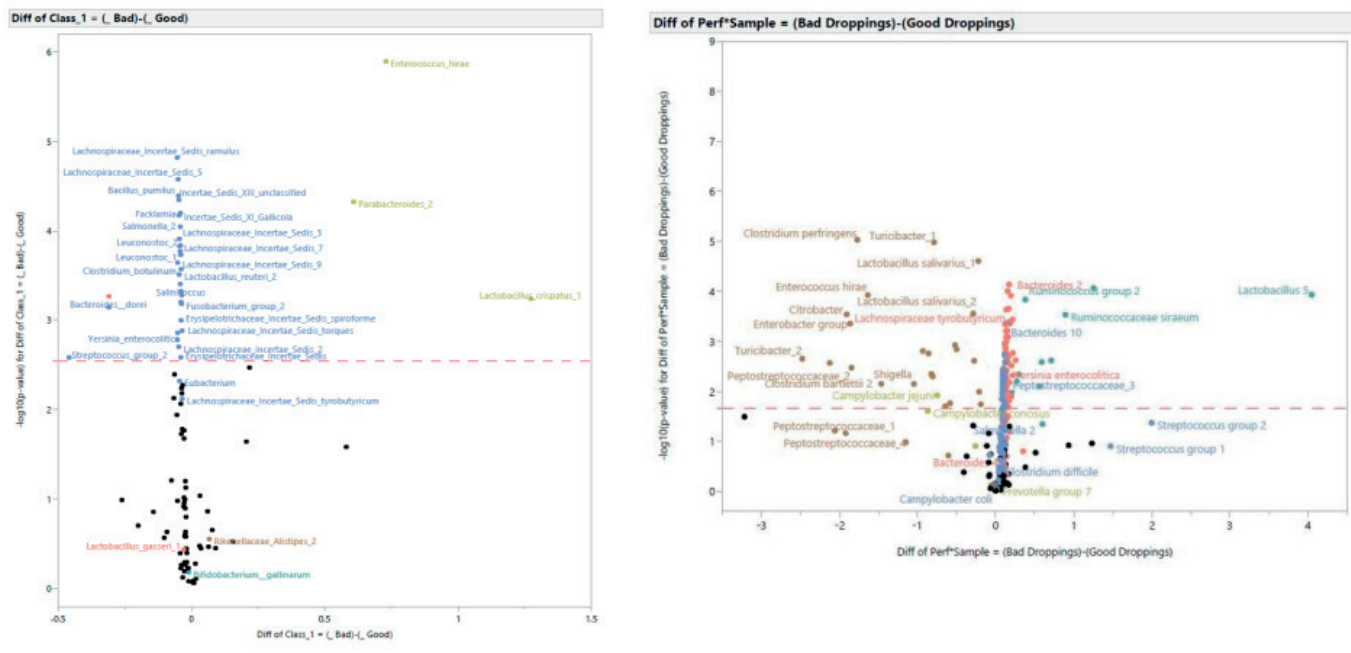


Figure 1. Volcano plot of microbiota differences between broiler chickens at 35 days of age (left plot) and between turkeys at 11 weeks of age (right plot) between good and underperforming (bad) farms having the same feed and similar housing conditions; differences above the dotted line are statistically significant.

The second plot in figure 1 provides differences in microbiota in excreta droppings for underperforming (bad) and good performing turkeys at 11 weeks of age (the body weight difference at 12 weeks of age was 381 g between the two farm performance classes), receiving the same feeds and with similar housing conditions. As for broiler chickens, clear differences were observed between underperforming and good performing birds. Turkeys on underperforming farms had higher signals for *Bacteroides*, *Yersinia enterocolitica*, *Ruminococcus* and *Lactobacillus_5* (5 was used as strain identifier). Good farms showed more *Turicibacter*, *Lactobacillus salivarius*, *Clostridium perfringens*, *Shigella* and *Campylobacter jejuni*. Both performance classes had higher signals for *Peptostreptococcus* but for different probes. This may indicate an excess of undigested protein.

From the two examples it appears it was possible to find clear differences between conditions such as performance differences in both broiler chickens and turkeys although biomarkers involved may be different. We learned that other factors like disinfection procedures, breed, age, antibiotic use, and climate play an important role as well. However, across all comparisons between good and underperforming birds we have observed similarities, where a less good maturation of the microbiota is associated with lower performance; for example, overgrowth of *Lactobacilli* spp. after the starter period is also linked to impaired bird performance. In general, the differences in microbiota composition match with those reported by Diaz Carrasco et al. (2019) for microbial taxa linked to good and underperforming broiler chickens.

Raw materials, nutrients, and feed additives

Besides differences in microbiota between birds on good and underperforming farms, differences can be found when using different raw materials and nutrient levels in feeds and when using different feed additives (Van der Hoeven et al., 2013; Gao et al., 2017). Figure 2 gives an example of differences in microbiota using different main raw materials in the feed. The results in figure 3 indicate that clear differences can exist in the microbiota composition when using different starch rich raw materials, where rice and corn grouped together and wheat and wheat + medium chain fatty acids (MCFA) grouped together in another quadrant. Protein rich raw materials had a microbiota profile that was different from that of starch rich raw materials (bottom right quadrant), and signals for *E. coli*, *Salmonella*, *Campylobacter* were higher for these raw materials (data not shown). As for raw materials, significant differences in microbiota composition can be observed when different feed additives are used (Torok et al., 2011; Granstad et al., 2020). Depending on the composition and the mode of action of the feed additives, these can help to improve the microbiota maturation, may enhance specific strains or clusters like butyrate producers and *Lactobacilli* spp., or can help to control the presence of pathogenic bacteria and proteolytic microbiota

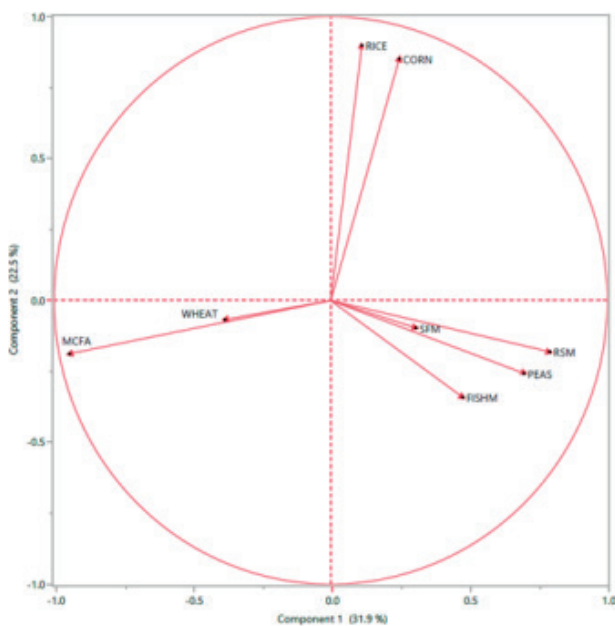


Figure 2. Principle component analysis of microbiota of broiler chickens at 21 days of age receiving diets with different main raw materials; SFM: sunflower seed meal; RSM: rapeseed meal, FISHM: fish meal

Changing microbiota in order to improve bird performance and health

Information about the effect of feed composition, feed additives, and management conditions on microbiota can be used to design intervention strategies to change microbiota on farm, especially when microbiota is different from the composition that is associated with good performance. Figure 3 provides an example of the effect of a change in feed composition that was made based on an assessment of the microbiota at 14, 21 and 35 days of age of underperforming and good performing broiler chickens. The change in feed composition included a reduction in dietary crude protein levels in the pre-starter and starter feeds and a combination of prebiotics, essential oils, butyrate and MCFA.

At the underperforming farms, the feed change caused a shift from Proteobacteria to Ruminococcus and Lachnospiraceae at 35 days of age. This indicates a positive development of the microbiota. In the good performing farms, also a change from proteobacteria to bacteria that are found in well-developed caeca such as Lachnospiraceae, Bacteroides and Streptococcus was observed. However, Enterococcus also increased after the change in feed composition. The shift in microbiota was associated with an improvement in body weight of 256 g and in feed conversion ratio of 0.063 on average. The hypothesis that performance would improve more on the underperforming farms compared to the good performing farms was not confirmed; the rate of improvement was similar for both performance classes. The microbiota on the good performing farms had a high abundance of Proteobacteria and was different from the profile that we in general see on good performing farms, which may have contributed to the improvement in performance on the good performing flocks as well.

The results indicated that it is possible to change the microbiota of broiler chickens in the field by a feed intervention, using information from the current situation and the microbiota database in order to find out what improvements can be made.

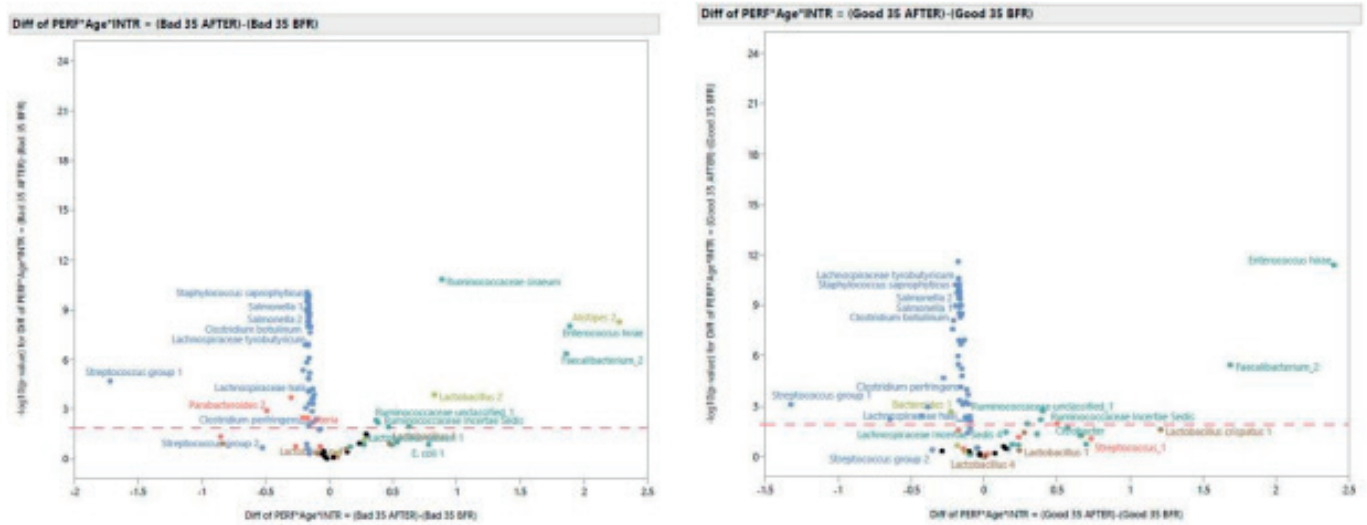


Figure 3. Volcano plot of microbiota differences between broiler chickens at 35 days of age before and after the change in feed composition; the left plot shows differences on underperforming farms and the right plot on good performing farms; differences above the dotted line are statistically significant.

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