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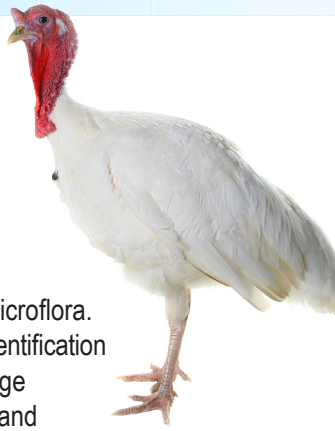
QTI™ ANIMAL HEALTH & NUTRITION

Effects of Non-Pathogenic Bacteria on the Turkey Microflora



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Key Points:

- New bacterial genetic identification technology has been vital in detecting and documenting changes in microflora populations.
- Dramatic differences in bacteria are noted in higher-performing turkeys compared to lower performing birds.
- New molecular tools enhance and improve DNA sequencing, potentially helping future DFM development.

The first weeks of a commercial bird's life are a critical time for the establishment of a mature and beneficial intestinal microflora. A 2004 study by Apajalahti et al. established that the bacterial community within a single intestinal location of a bird changes rapidly and then stabilizes. Numerous researchers have documented that the chicken's intestinal microbiota stabilization occurs between 4 and 25 days of age. Turkeys are likely different, and our current estimates indicate that microbiota maturation extends to at least 6 weeks of age. However, these are only estimates, and variables such as bird type, environment, nutritional plan, and anatomical location will influence the maturation process. Because of the variations and differences in microflora establishment, direct-fed microbials (DFMs) are a common approach for populating the avian intestinal tract.

The ileum (the lower 1/3 of the turkey's small intestine) is the primary site of nutrient absorption and immune function in the gut. Our research group's interest lies in increasing overall bird development and performance, thus the ileal microbiota has been a major focus of our investigations. The use and effectiveness of a DFM in this anatomical area is considered to be instrumental. The impact of a DFM on the ileal microflora is dependent on the following criteria: 1) the bacterial species composition of the DFM and its ability to adapt to host specificities; 2) timing of application of the DFM; 3) the ability of the DFM to change the resident microflora, which requires an understanding of the baseline microbiota within the turkey gut and how it changes over time; and finally, 4) an understanding of the anatomical region of the intestine, which in this case is the ileum.

Technology has been vital regarding the investigation of the complexities of turkey microflora. Recent advancements in bacterial genetic identification technologies have allowed us to analyze large communities of bacteria in the turkey ileum and how they relate to daily weight gain. It has also enabled us to rapidly identify previously non-cultivable species based on the genetic make-up of the bacteria. Finally, it has facilitated the management and distillation of large amounts of data, in order to detect and document changes in the microflora populations. These approaches would have been cumbersome, if not impossible with previous technologies.

Our research group (Danzeisen et al. 2013) has utilized high throughput DNA sequencing capabilities to identify differences in the ileal microflora of higher-performing flocks, compared to lower-performing flocks. Over the course of 12 weeks we followed the daily weight gain and ileal bacterial community composition of multiple commercial and research flocks. We demonstrated that there is a clear and orderly microflora succession of bacterial species as the birds matured, irrespective of the flock examined. However, the timing of this bacterial succession differed in respect to the productivity of the animals. Specifically, uniquely-identified species of ileal microflora appeared earlier in the higher-performing flocks than it did in the lower-performing flocks. Graph B illustrates the change in average proportions of classes of bacteria in comparison to average daily weight gain. Dramatic differences in bacterial classifications and percent of composition are noted in the higher-performing turkeys (RF) than the lower performing birds (CF).

The identification of these unique microflora components yielded several candidate bacterial species that were significantly associated with enhanced bird growth under commercial conditions. These bacteria included a candidate, non-cultivable species known as Candidatus division Arthromitus. Graph B illustrates the early appearance and higher population levels (red-colored group) of Candidatus Arthromitus in higher-performing flocks. These are bacteria that have been known for over 100 years, but only recently

cultured. They have been described as segmented, filamentous bacteria because of their unique morphology in the animal gut (Illustration C). Interestingly, in mice these bacteria have been shown to promote the development of white blood cell populations within the tissue structure of the intestine. They have also demonstrated important and beneficial functions in the development of other components of the localized intestinal immune system.

A second bacterial species significantly associated with enhanced weight gain was *Lactobacillus aviarius*. In 2007, Gong et al. reported that this is a dominant poultry-adapted species and it has been found to be a strictly anaerobic bacterium unlike other *Lactobacillus* species. This species is correlated in multiple studies with improved bird performance. Other ileal bacterial species identified as significantly associated with enhanced weight gain included *Lactobacillus johnsonii, Lactobacillus ingluviei*, and *Clostridium bartlettii*. As depicted in Illustrations D and E, all of these bacteria had predictable patterns of appearance, which is promising from the standpoint of the ability to promote shifts in the microbiome.

The concept of microbiome modulation has been around for years. There are differing opinions on the best means to modulate the gut towards improving bird performance and reducing pathogen load. Whatever the opinion, newer molecular tools which enhance and improve DNA sequencing are changing the way that we can assess baseline, and subsequently examine the ability to modulate baseline microflora communities in an animal. These approaches hold great promise for the refinement of DFMs tailored towards application in specific production animals and management challenges. We hope that this approach in turkeys exemplifies what might serve as a common platform for future DFM development.

References

1. Apajalahti J, Kettunen, A., Graham. H. 2004. Characteristics of the gastrointestinal microbial communities with special reference to the chicken. World's Poult Sci J 60:223-232.
2. Amit-Romach E, Sklan D, and Uni Z. 2004. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. Poult Sci 83:1093-1098.
3. Scupham AJ. 2009. Campylobacter colonization of the Turkey intestine in the context of microbial community development. Appl Environ Microbiol 75:3564-3571.
4. van der Wielen PW, Keuzenkamp DA, Lipman LJ, van Knapen F, and Biesterveld S. 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb Ecol 44:286-293.
5. Danzeisen JL, Calvert AJ, Noll SL, McComb B, Sherwood JS, Logue CM, and Johnson TJ. 2013. Succession of the turkey gastrointestinal bacterial microbiome related to weight gain. PeerJ 1:e237.
6. Klaasen HL, Koopman JP, Poelma FG, and Beynen AC. 1992. Intestinal, segmented, filamentous bacteria. FEMS Microbiol Rev 8:165-180.
7. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV et al. . 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139:485-498.
8. Wu HJ, Ivanov, II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, and Mathis D. 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 32:815-827.
9. Gong J, Si W, Forster RJ, Huang R, Yu H, Yin Y, Yang C, and Han Y. 2007. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. FEMS Microbiol Ecol 59:147-157.
10. Fujisawa T, Shirasaka, S., Watabe, J., Mitsuoka, T. 1984. Lactobacillus aviarius sp. nov.: A new species isolated from the intestine of chickens. Syst Appl Microbiol 5:414-420.
11. Czerwinski J, Hojberg O, Smulikowska S, Engberg RM, and Mieczkowska A. 2012. Effects of sodium butyrate and salinomycin upon intestinal microbiota, mucosal morphology and performance of broiler chickens. Arch Anim Nutr 66:102-116.
12. Feng Y, Gong J, Yu H, Jin Y, Zhu J, and Han Y. 2010. Identification of changes in the composition of ileal bacterial microbiota of broiler chickens infected with Clostridium perfringens. Vet Microbiol 140:116-121.
13. Torok VA, Hughes RJ, Mikkelsen LL, Perez-Maldonado R, Balding K, MacAlpine R, Percy NJ, and Ophel-Keller K. 2011b. Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. Appl Environ Microbiol 77:5868-5878.

Figure 1:

A) Average daily weights of Light versus Heavy flocks. Flocks 5, 6, and 8 were of significantly higher weights at all time points than flocks 1-4 and 7.
B) Class-level average proportions of bacteria analyzed from a single commercial flock of lower average daily weight gain (CF), a single 48,000-bird research flock of higher average daily weight gain (RF), and pooled samples from Light (L) or Heavy (H) flocks in Minnesota. Note the red-colored group indicating Candidatus Arthromitus that appears earlier in high-performing flocks.
C) Microscopy of segmented filamentous bacteria (SFBs) that were significantly associated with Heavy flocks and appeared earlier in research flocks.
D) Log10 heatmap (white-yellow-orange-red-dark red) depicting the top 50 bacterial species in the flocks examined.
E) Expanded view of the abundance of dominant bacterial species (Y axis) in the turkey ileum of birds from CF, RF, L, and H groups at different time points (W=weeks of age). Overall community composition was used in QIIME to define three phases of microbiome development, separated by green and blue dashed lines. Note that microbiome succession occurs two weeks earlier in research vs. commercial flocks, correlating with heavier birds at these time points.