Understanding the gut microbiome: intervention towards total gut integrity

The gastrointestinal (GI) tract of chicken harbours a complex plethora of micro-organisms. The diversity of microbiota increases with the growth of the host until it reaches a stable yet dynamic state. This ranges from more than 5 phyla and 17 genera of bacteria as reported in several studies.

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Firmicutes are the predominant phylum throughout the chicken GI tract, representing up to 98% of the bacterial community in the crop, gizzard, ileum and caeca. Actinobacteria are the only phylum generally identified in the crop with an abundance of 1%. Proteobacteria are the second most abundant phylum, whereas Bacteroidetes become dominant in the caeca of older chickens.

Within the phylum Firmicutes, Lactobacillaceae is the most abundant family in the crop (94-98%), gizzard (59-86%), and ileum. In contrast to the crop, gizzard and ileum, where Lactobacillaceae are dominant, Ruminococcaceae and Lachnospiraceae, belonging to the phylum Firmicutes, and Rikenellaceae (phylum Bacteroidetes) are most abundant in the caecum.

The abundance of Lachnospiraceae, Lactobacillaceae, and Enterobacteriaceae shifts significantly in favour of Rikenellaceae and Clostridiaceae as chickens grow older.

These micro-organisms come into contact with the GI tract immediately after hatch where the surface microbiota of hatching eggs constitutes the first inoculum to the naive chicken gut. Several studies showed a dynamic heterogeneity of the gut microbiota in relation to the different GI segments (crop, gizzard, ileum, and caecum), along with the development of bacterial diversity in growing chickens. Differences in bacterial composition between different GI segments are reported to be related to differences in gut morphology, pH, oxygen concentration/ tension, nutrient availability, and the presence of bile acids and digestive enzymes.

The chicken gut microbiome

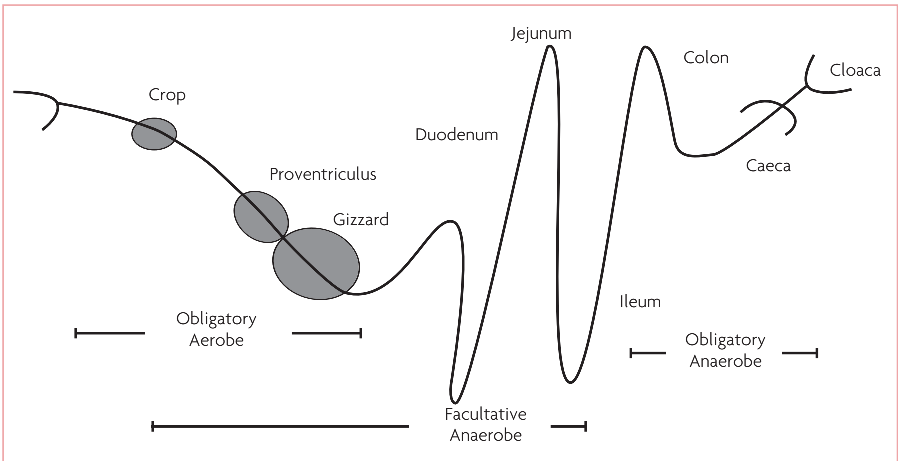
Today's scientifically managed classes of poultry (breeders, layers and broilers) have a defined set of feed formulation. Moreover, the differential anatomy and physiology leads to spatial and temporal differences in digestion and absorption of various nutrients.

Consequently, the dietary interactions with individual micro-organisms and the host's endogenous secretion leads to varying oxygen and carbon dioxide tension throughout the gut. The oxygen and carbon dioxide tension becomes a critical factor in determining the characteristic of the microbiome and becomes distinct by day 12-15 of the chick's life.

The chicken gut microbiome can therefore be compartmentalised into three distinct microbiome (Fig. 1.)

- Crop to gizzard – rich in oxygen tension favouring aerobes.
- Duodenum to jejunum – balanced oxygen and carbon dioxide tension favouring obligatory anaerobes.
- Ileum to cloaca – rich in carbon dioxide tension favouring obligatory anaerobes.

Fig. 1. Schematic diagram of the gastrointestinal tract in poultry highlighting segments of distinct microbiome.



Finding a favourable environment

Probiotics administered through poultry feed exhibits two phases of survival – vegetative cells and spores. Vegetative forms are expressed in a favourable environment, while spores are strategic measures adopted by these bacteria in a harsh environment.

Spore forming probiotics germinate in the chicken gut in a favourable environment, colonise and undergo proliferation. Optimum proliferation in the chicken gut from day one ensures pathogens to be competitively excluded and resulting in healthy microbiota. Hence, probiotics should find a favourable environment in the GI segment shared by pathogens to confer competitive exclusion in the chick's early life.

Critical analysis

- Bacillus subtilis is a robust spore forming bacteria that survives wide pH variation and the harsh environment of bile acid in the chicken gut.
- They are considered to be obligatory aerobes but a recent report supports proliferation capability in anaerobic

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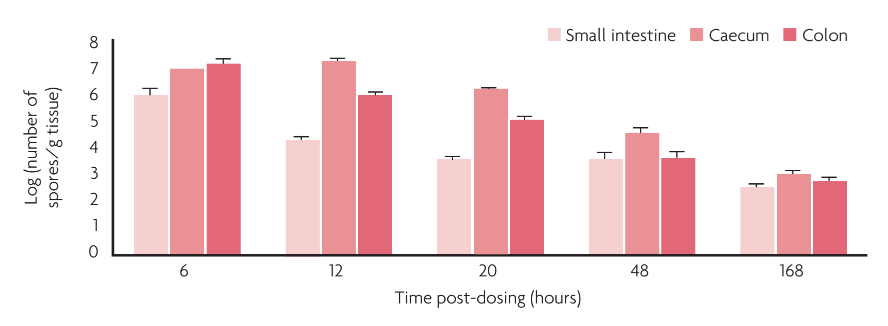

Fig. 2. Sporulation preference of Bacillus subtilis in the chicken gut. Tissue samples were taken post-mortem at 6, 12, 20, 48, and 168 hours after 10^9 spores had been administered to each chick (Cartman et al, 2008).

conditions. In anaerobic conditions, Bacillus subtilis can use nitrate as an electron acceptor to respire in the presence of pyruvate as a facilitator. In the absence of oxygen and nitrate, they are known to derive energy for survival through fermentation viz, butanediol fermentation.

- With the establishment of a distinct microbiome based on oxygen and carbon dioxide tension by day 12-15 in the chicken gut, Bacillus subtilis, being an aerobe, will germinate and colonise rapidly in the upper gastrointestinal tract (crop to duodenum).
- Studies have shown that Bacillus subtilis undergoes two to three cycles of germination and sporulation throughout the poultry GI tract. The sporulation frequency increases as it moves towards the hind gut (Fig. 2). In other words, Bacillus subtilis exists in spore form in ileum to cloaca and thereby undergoes feed passage.
- Clostridium perfringens is a normal commensal of the chicken hind gut (ileum to cloaca) where up to 10^4cfu/ml gut content is found in a healthy flock. They are endospore forming obligatory anaerobes and are known to proliferate in strict anoxic conditions. Furthermore, these bacteria can utilise undigested protein more efficiently in strict anaerobic conditions without any requirement of facilitator relative to Bacillus subtilis.
- Based on the colonisation preference of aerobes and anaerobes, it is obvious that Bacillus subtilis fails to competitively exclude Clostridium perfringens from day one leading to an unaddressed threat.

Conclusion

Distinct microbiome based on oxygen and carbon dioxide tension by day 12-15 in the chicken gut selects for bacteria proliferating in a niche environment. It is very important that probiotics administered through drinking water and feed is capable of proliferating in GI segments shared by pathogens like Clostridium perfringens.

Although, Bacillus subtilis can counteract Clostridium perfringens in the small intestine during dysbacteriosis, the probability of getting the former outrun in a high challenge area is more. Bacillus subtilis as a DFM alone cannot competitively exclude these pathogens in the hind gut. Hence, strategic intervention could be a combination of aerobes and anaerobes capable of competitively excluding Clostridium perfringens from day one, leveraging total gut integrity. ■

References are available from the author on request