Advances in Leveraging Gut Microbe-Host Interactions for Performance Benefits

Elizabeth Galbraith
AFMA Forum 2016 Sun City, South Africa
What I will cover today:

1. Background
   - The challenge: meeting increasing global food demands
   - Balancing gut health and efficient production

2. Development of the Avian Gastrointestinal Microbiota
   - Evidence of vertical transmission
   - Microbiota in the growing bird

3. Impact of Microbiota on Performance
   - Gut structure and integrity
   - Digestion and nutrient utilization
   - Development of host immune function

4. Conclusions and Take Home points
Meeting the needs of a growing population

150,000

The world’s population will grow by 150,000 people daily for the next 40 years. To feed them, we’ll need 70% more safe, nutritious food.
Importance of the poultry market to South Africa

- More poultry products consumed annually in South Africa than all other animal-protein sources combined
- Poultry industry provides 65.5% of locally produced animal protein consumed on a volume (kg) basis

Sources: SAPA Industry Profile, 2012
Rising to meet increased demand

- 66% increase in broiler body weight at 42 days
- 32% improvement in feed conversion ratio*

Globally, average productivity of farm animals is still estimated to be 30-40% below genetic potential. WHY?

*Amount of feed required for 1Kg weight gain

Based on Rauw 1998 and Ross recommendations
Why is feeding the world with safe, nutritious food still a challenge?

- Broad range of issues – all have a link to gut health
- Gastrointestinal microbiota play a key role in gut health
Commercial production conditions can make birds more susceptible to clinical & sub-clinical disease

Post hatch/week one
- Mortality
- Colibacillosis

2 weeks onwards
- Necrotic Enteritis (NE)
- Footpad dermatitis (Pododermatitis)
- Coccidiosis

3 weeks
- *Campylobacter*
- Dysbacteriosis

4-6 weeks
- Enterococcal spondylitis
- Endemic colibacillosis

- Antibiotic Growth Promoters (AGPs) are going away.
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The first hours and days immediately after hatch are important in GIT development and long term performance. Density of $10^8$-$10^{10}$ cells/g of digesta by day one post-hatch.

- The GIT is quickly colonised by environmental bacteria.
- Colonisation is driven by:
  - Exposure to microorganisms
  - Favourable conditions for colonisation
- The microbiota is more easily influenced during this period

Apajalahti et al., 2004
The importance of the intestinal microbiota in broiler performance

- The synergistic relationship between the avian host and resident microbiota has co-evolved to offer benefits to both host and microbe
- The avian intestinal tract is colonised with hundreds of different bacterial species

- Digestion & nutrient utilisation
- Intestinal immune development
- An environment conducive to beneficial bacteria
- Gut development structure & integrity
The gut microbiome is not a static entity

Desired situation

Undesired situation

Performance

 Beneficial populations

Opportunistic populations

Non-beneficial populations

Vaccination
Handling
Litter

Rodents
Antibiotics
Insects

Gizzard
Development
Heat
Ventilation

Gut motility
Density
Age
Diet

Growth rate

Beneficial populations

Opportunistic populations

Non-beneficial populations
Avian Pathogenic *E. coli* (APEC)

- *Escherichia coli* is present in the normal intestinal microflora of birds. So why is it a big deal in poultry gut health?

Complications associated with avian pathogenic *E. coli* (APEC) together are considered one of the leading causes of economic loss in the poultry industry worldwide.

Only some strains with specific virulence attributes, are designated as avian pathogenic *E. coli* (APEC).

APEC-associated conditions: acute colisepticaemia, fibrinopurulent polyserositis, aerosacculitis, pericarditis, salpingitis, synovitis, omphalitis, yolk sac infection, swollen head syndrome, coligranuloma, and cellulitis.

Zanella *et al.*, 2000 and Giovanardi *et al.*, 2005
APEC: Growing evidence for vertical transmission

- APEC has been recovered from birds of all ages

- Increasing scientific evidence suggests that vertical transmission is a potential route of infection in young birds

APEC isolates from visceral organs of broiler chicks with colibacillosis and corresponding breeders have been shown to have similar pathotypes and genotypes

Giovanardi et al., 2005
Objective: Determine the genetic similarity and virulotype of APEC isolated from breeders and corresponding day-old chicks from multiple farms and producers in South Africa.

Sampled 27 breeders and 90 day-old chicks (DOC) originating from 2 South African producers:
- 135 *E. coli* isolates from breeders
- 71 *E. coli* isolates from DOC

Lambrecht et al., 2015
APEC isolate screening methods

- Determined genetic similarity of *E. coli* isolates using RAPD (Random amplified polymorphic DNA) technique
- Screened *E. coli* for 5 virulence associated genes (VGs) using multiplex PCR

<table>
<thead>
<tr>
<th>Virulence Gene</th>
<th>Functional Description</th>
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<tbody>
<tr>
<td><em>iss</em></td>
<td>Increased serum survival</td>
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<tr>
<td><em>iucC</em></td>
<td>Iron sequestering system</td>
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<td><em>tsh</em></td>
<td>Temperature sensitive hemagglutinin</td>
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<tr>
<td><em>cvaC</em></td>
<td>Structural gene for colicin V</td>
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<tr>
<td><em>irp2</em></td>
<td>Iron transport</td>
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*E. coli* isolates containing two or more virulence genes were considered APEC positive (Skyberg, et.al., 2003).
Genetic similarity of APEC isolates from breeders and chicks

- Clustering of RAPD profiles for APEC isolates from breeders and day-old chicks (DOC) sourced from 2 producers

Lambrecht et al., 2015
Greater genetic similarity observed between APEC from breeders and chicks of the same producer suggesting potential vertical transmission.
Comparison of virulotypes for genetically similar APEC

Identical virulotypes: tsh, cvaC, irp2

80% similarity

Source:

Lambrecht et al., 2015
Comparison of virulotypes for genetically similar APEC

Identical virulotype cvaC, irp2

80% similarity

Lambrecht et al., 2015
Common virulotypes differ between chicks and breeders despite RAPD profile similarity

Suggests possible horizontal transfer of virulence genes between APEC strains

*L. coli* isolates containing two or more virulence genes were considered APEC positive

Lambrecht et al., 2015
Virulotype distribution suggests frequent horizontal transfer of virulence genes between genetically similar APEC strains.

<table>
<thead>
<tr>
<th>Virulotype</th>
<th>Farm A/B Breeders</th>
<th>Chicks(A)</th>
<th>Chicks(B)</th>
<th>Farm C Breeders</th>
<th>Chicks</th>
<th>Farm D Breeders</th>
<th>Chicks</th>
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<td>None*</td>
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Lambrecht et al., 2015
Changes in the relative proportions of bacterial populations in the small intestine with age

Day 14
- Bacillus
- Bacteroides
- Blautia
- Brachybacterium
- Brevibacterium
- Butyricicoccus

Day 28
- Candidatus Arthromitus
- Clostridium
- Enterococcus
- Eubacterium
- Faecalibacterium
- Lactobacillus
- Nocardioides
- Oscillibacter

Day 42
- Parabacteroides
- Roseburia
- Ruminococcus
- Staphylococcus
- Streptococcus
- Subdoligranulum
- Turicibacter
- Virgibacillus
- Weissella
- Yaniella
- Other

Neumann et al., 2011
Role of beneficial bacteria, including *Lactobacillus*, in the avian gastrointestinal tract

- Occupy adhesion sites in the gastrointestinal tract preventing the colonisation of non-beneficial populations.
- Compete with other bacterial species for nutrients – nutrient depletion.
- Produce lactic acid (among others) as the main metabolic by-product reducing the micro-environmental pH non-conducive to non-beneficial bacteria.
- Development of the mucosal immune system.
- Probiotics, or direct-fed microbials (DFMs), have been known to help establish and maintain beneficial microbial populations in the host gut.

Patterson *et al.*, 2003; Lee *et al.*, 2010; Chaucheyras-Durand *et al.*, 2010
Effect of a multi-strain *Bacillus* spp. DFM or a common AGP on Enterobacteriaceae and Lactobacillaceae populations

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**A**

<table>
<thead>
<tr>
<th>Lactobacillaceae</th>
<th>Enterobacteriaceae</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DFM-treated</td>
<td>8.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>6.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DFM-treated</td>
<td>6.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**B**

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<thead>
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<tbody>
<tr>
<td>Control</td>
<td>7.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>7.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>5.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

Lactobacillaceae and Enterobacteriaceae counts (log cfu/g) in the ceca of broilers with or without a 3-strain *Bacillus* spp. DFM (A) or Virginiamycin (B)

Danisco Animal Nutrition internal data, unpublished
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4. Conclusions and Take Home points
Impact of microbiota on gut structure and integrity

Germ-free chickens
- Reduced weight of small intestine and cecum
- Thinner gut walls, decreased barrier function, increased susceptibility to pathogenic challenges
- Reduced villi height and crypt depth, less surface area for nutrient absorption.

Conventional chickens
- Long slender villi for optimal nutrient absorption capacity
- Improved barrier function
- Production of short chain fatty acids and metabolic products increase enterocyte growth and proliferation

Effect of a 3 strain *Bacillus*-species probiotic on intestinal morphology of broilers

Furuse et al., 1994, LeBlay et al., 2000; Amerah et al., 2011
Impact of gut microbiota on digestion and nutrient utilization

- Microbiota in the ceca are essential for polysaccharide metabolism
  - Chickens do not possess a complete metabolic cycle for producing readily absorbable forms of polysaccharides
  - Genes for carbohydrate metabolism occupied about 20% genes in the GIT microbiota metagenome

- Supplementation can complement native microbiota
  - Increases in nitrogen corrected apparent metabolisable energy (AMEn) with additions of a three strain *Bacillus* probiotic and xylanase, amylase and protease enzymes to broilers fed a corn/soy diet containing some fibrous cereal by-products

<table>
<thead>
<tr>
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<th>AMEn (kcal/kg DM)</th>
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<tbody>
<tr>
<td>Control</td>
<td>2960c</td>
</tr>
<tr>
<td>Bacillus</td>
<td>2995c</td>
</tr>
<tr>
<td>XAP</td>
<td>3042b</td>
</tr>
<tr>
<td>Bacillus + XAP</td>
<td>3093a</td>
</tr>
</tbody>
</table>

Choi *et al.*, 2015; Qu *et al.*, 2008; Romero *et al.*, 2013

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Impact of gut microbiota on digestion and nutrient utilization

Effect of supplementation of 108 Arbor Acre broilers fed a corn-soybean-based ration with a 3-strain *Bacillus* spp. DFM on apparent metabolizable energy (AME) measured on days 39-41 (A), and on Feed Conversion Ratio (B)

![Graph A](image)

**AME** (kcal/kg of dry matter, 39-41 days)

- Control: 2864$^a$
- DFM-treated: 3047$^b$

![Graph B](image)

**FCRc**

- Control: 1.86$^a$
- DFM-treated: 1.78$^b$

$^{ab}$ Values without common superscript are significantly different (P<0.05)

*FCRc: corrected 3 points per 100g liveweight different*
Influence of microbiota on development of host immune function

Intestinal inflammation

- Reduced villi height and fusion, decreased surface area for nutrient absorption.
- Diversion of energy towards rebalancing homeostasis and away from growth.
- Increased secretion of mucus hindering nutrient digestion and uptake and modifying resident microbial populations.
- A reduction in epithelial integrity, increasing the gaps between epithelial cells preventing the retention of bacteria in the gut lumen

% Total energy allocation in broiler chickens

- Waste, feces, urine: 28%
- Production: 40%
- Basic metabolism: 25%
- Immune factors: 7%

Patterson et al., 2003; Brisbin et al., 2008
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Conclusions And Take Home Points

• Global demand will continue to place pressure upon the poultry industry to meet needs despite restrictions on use of AGPs

• Gastrointestinal microbiota establish rapidly after hatch
  • May be influenced by vertical transmission from the breeder
  • Comprised of highly diverse species and proportions change with age

• GI microbiota provide a range of fundamental benefits to the growing bird and can have a profound effect on poultry health and performance
  • Gut structure and integrity
  • Digestion and nutrient utilization
  • Development of host immune function

• Enzymes, Probiotics and Enzyme-Probiotic combinations can complement native microbiota and consistently improve performance

• Understanding the role of the gastrointestinal (GI) microbiota in poultry health and performance can help producers develop strategies to achieve consistent growth performance and increased livability while balancing broader considerations
Thank You!