Scientific Developments in the Fields of Eubiotic and Phytogenic Ingredients

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Presentation

1. Introduction
2. Challenges
3. Current state/latest developments
4. Summary
Introduction
Main reasons for interest:

1. Increasing global human population and demand for animal protein.
2. Resultant resource pressures require improved production efficiency.
3. Consumers demand more ‘natural’ production methods, which includes both reduced antibiotic use and risk of foodborne illness/pathogens.

Definitions

Eubiosis (eubiotic) – (promotes) optimum microbial balance in the intestine.

Phytogenic - derived from plants.
Importance of the intestine

Home to major populations of bacteria, viruses, fungi and bacteriophages – more cells and genes than host!

Major immune organ – home to 70% of immune cells.

Nutrient digestion and absorption.

Gastrointestinal disorders are very costly to the animal production industry, for example:

- Necrotic enteritis = $6 billion
- Coccidiosis = $3 billion
- Dysb(acter)iosis = $2 billion?
Gut microbiome is the centre of the.....body!

- Immune/vaccine responses
- Metabolism/body composition
- Behaviour/psychology
- Muscle myopathies
- Autoimmune & metabolic diseases
- Cancer

(Young, 2017)
Gut microbiota simplified

(Diagram taken from Bailey, 2010; Stanley et al., 2013. Oakley et al., 2014)
Gut microbiota establishment and stability

(Taken from Pourabedin and Zhao, 2015)
Eubiosis involves multiple components

External agent(s)
(e.g. nutrients, drugs, environment/bedding material, invading microbes, mycotoxins)

Microbiota

Gut barrier/Immune system

Due to interactions, factors that contribute to each component can help achieve (or not) eubiosis.

Gut health - definition
‘the ability of the gut to perform normal physiological functions and to maintain homeostasis, thereby supporting its ability to withstand infections and non-infectious stressors’
(Kogut et al., 2017)
Challenges
What to target?
What is an optimal gut microbiota?

Gut microbiota is complex (still much not well identified/characterised).
➢ Who is really ‘good’ or ‘bad’?

Studies highlight diverse microbiota among ‘similar’ individuals.
➢ significant inter-individual variation.

However, despite this variation, metabolic function appears largely conserved.

(e.g. Stanley et al., 2013; Oakley et al., 2014)
What to target?
E.g. sub-(MIC*) inhibitory effects

MIC often considered lowest concentration of an antimicrobial with any effect on bacteria.

MIC defined by lowest concentration that prevents visible growth, thus ignoring effects on:

➢ adherence
➢ host defence susceptibility
➢ virulence expression
➢ growth dynamics

What more can we learn from AGPs**? Seek ‘immuno-cooperation’?

(*minimum inhibitory concentration; **antimicrobial growth promoters)
What to target?  
E.g. inflammation?

Inflammation is an essential immune response to protect and repair.

Inflammatory responses are nutrient demanding.

However, studies show increased pro-inflammatory responses better protect against an array of pig and poultry pathogens.

Interest in promoting anti-inflammatory processes, which favour Th2 (extracellular) responses at the expense of Th1 (intracellular).

What’s really the optimum in commercial production?

Seek ‘immuno-cooperation’?

(Broom and Kogut, accepted)
Where to target?
E.g. antimicrobial effects of organic acids

<pH 5.5
Largely undissociated*.
>Microbial penetration?
Less pH reduction potential

(*Higher pKa = less dissociated at given pH)

<pH 6.5
All OAs effectively dissociated.
Microbial penetration?
Effective pH reduction?

(Diagram taken from Bailey, 2010; Broom, 2015)
Gut microbiota/health manipulation challenges

Complexity of gut microbiome (still unknown community members of unknown influence) and interaction with immune system/responses.

Knowing what to influence, when and where?

➢ Earliest possible intervention seems most effective.

Overcoming inter-individual variation.

Technologies.

➢ Evolving techniques (e.g. high throughput sequencing) & resultant knowledge will be invaluable.

Still much to learn!
Current state/latest developments
## Eubiotics:

<table>
<thead>
<tr>
<th>Conventional</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotics</td>
<td>Bacteriophages</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Bacteriocins</td>
</tr>
<tr>
<td>Fatty acids (varying chain length)</td>
<td>Antibodies</td>
</tr>
<tr>
<td>Enzymes (Essential oils)</td>
<td>Toll-like receptor agonists</td>
</tr>
<tr>
<td>(Essential oils)</td>
<td>Minerals (e.g. Zn, Cu)</td>
</tr>
<tr>
<td></td>
<td>Vaccines (mucosal)</td>
</tr>
</tbody>
</table>

...anything that influences microbial and (thus) intestinal balance.

Too many for individual consideration here!

## Phytogenic:

Various parts of plants (sometimes purified) in various forms, notably essential oils.
1. Better appreciation of the importance of (strain) specificity.....

7 *Lactobacillus casei* strains given once daily ($10^8$ CFU/day/mouse) for 7 days by oral gavage – caecal microbiota.

Clustered treatments not different ($p \leq 0.05$). Genera comprising >5% of the total microbiota in at least one treatment are presented.

(Taken from Aktas et al., 2016)
1. Better appreciation of the importance of (strain) specificity.....

*Lactobacillus paracasei F8 (☐), Lactobacillus plantarum F44 (■) and Lactobacillus rhamnosusLGG (■) growth in MRS basal broth with prebiotic substrates (1% v/v)

(FOS (fructo-oligosaccharides)
GOS (galacto-oligosaccharides)
IMOS (isomalto-oligosaccharides)
LAC (lactulose)
XOS (xylo-oligosaccharides)
Glu (glucose)
MRS-BB (de Man Rogosa Sharpe basal broth)
Significance **P < 0·01 vs. MRS-BB)

(Taken from Ambalam et al., 2015)
Probiotic (0.250 kg/ton*), prebiotic (MOS product; 2, 1 and 0.5 kg/ton in starter, grower, and finisher phase, respectively) and synbiotic (½ probiotic + ½ prebiotic doses) to broilers fed ad libitum. (*>5 × 10^{10} CFU of both *Bacillus licheniformis* and *Bacillus subtilis* per gram of product. Significance *abed* P < 0·05)

**Others**

1. Combinations within eubiotic ‘class’
   E.g. optimised combinations of probiotic organisms, prebiotics, etc.

2. Combinations across eubiotic ‘classes’
   E.g. OA(s) + phytogenic(s), enzyme(s) + probiotic(s), etc.

Pro, pre & syn reportedly reduced cost per kg gain by 13, 4 and 15%, respectively.

(Abdel-Hafeez et al., 2017)
3. Newer ‘classes’.....e.g. antimicrobial peptides

Antimicrobial peptides (AMP) fed (2.0 g/kg feed) to weaned pigs (24-32 days of age) on 5 commercial farms.

Significance abP < 0.05

(Xiong et al., 2014)
4. Targeted delivery/protection.....

7.2 mg of either encapsulated* or unencapsulated citral mixed in 100 mg of starter feed - oral gavage (into crop) of 8 birds (12 days of age) per timepoint - digesta then incubated with C. perfringens (10⁷ CFU/g digesta).

*soy-derived polymer called soy protein–soy polysaccharide Maillard reaction product (SPPMP)

(Taken from Yang et al., 2016)
5. Endogenous/in-situ stimulation.....

Broiler chickens challenged with a subclinical dose of *E. coli* K88 at 7 days of age and fed formic and propionic acid-based feed additive (1-35 days of age).

Significance $^{ab}P < 0.05$

(Khodambashi Emami et al., 2017; Broom et al., submitted)
6. Early intervention.....

*In-ovo* (day 18) probiotic administration then challenged with *Salmonella enteritidis* (day of hatch).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hatchability</th>
<th>Day 1 BW (g)</th>
<th>Day 3 BW (g)</th>
<th>Day 7 BW (g)</th>
<th>SE incidence ceca–cecal tonsils 24 h PI</th>
<th>Log_{10} SE/g of ceca content 24 h PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>148/150 (98.6%)</td>
<td>49.13 ± 0.30a</td>
<td>62.53 ± 0.81b</td>
<td>132.89 ± 3.06b</td>
<td>20/20 (100%)</td>
<td>7.13 ± 1.01a</td>
</tr>
<tr>
<td>Probiotic</td>
<td>142/150 (94.6%)</td>
<td>49.72 ± 0.36a</td>
<td>65.42 ± 0.77a</td>
<td>144.98 ± 3.02a</td>
<td>9/20 (45%)*</td>
<td>5.45 ± 1.23b</td>
</tr>
</tbody>
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At d 18 eggs were candled and inoculated with either saline or FloraMax®-B11via in ovo injection into the amnion. On d 21, chicks were pulled from the hatchers and were challenged with *Salmonella enteritidis* (SE) on d of hatch ~10^4 cfu/chick. Incidence data are expressed as positive/total chickens (%) at 24 h post inoculation (PI); asterisks indicate significant differences *P* < 0.001, *n* = 20/group. Log_{10} SE/g of ceca content is expressed as mean ± standard error.

*a,b* Superscripts within columns indicate significant differences *P* < 0.05, *n* = 12/group.

(Teague et al., 2017)
Summary
Current and future developments

1. Latest technologies - greater interrogation and understanding of multiple components of ‘gut health’, including influence of less well studied microbiota members (e.g. viruses). Establish most relevant biomarkers!
2. Greater appreciation of individual strain, or substrate compositional, effects.
3. Combinations – potential for more compatible, synergistic effects (e.g. synbiotics, phytogenic + organic acid, enzyme + probiotic, etc.).
4. Targeted delivery/protection – where to release?
5. Optimised dosing/duration.
6. Endogenous/in-situ stimulation (organisms optimised to produce a range of enzymes, antimicrobial compounds, etc.).
7. Early intervention (parent stock, in-ovo, etc.).
8. Extra-intestinal benefits.
9. ‘Immuno-cooperation’.
10. Additives that are responsive to different conditions.
References

Thank you for your attention!

Any questions?

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