Use of gut flora modulators in animal nutrition

Marisol Castillo – Jornades PATT CRESA 2018

L’impacte del microbioma en la salut dels animals, 31.05.2018
Introduction

- Maintaining a healthy gut is an essential element for efficient, profitable, and sustainable animal production.

- Gut health has a marked impact on production efficiency, animal welfare, environmental protection, and food safety.

- Current restrictions: Antibiotics and other restrictions (minerals)

- Enteritis, especially bacterial enteritis, has been and will continue to be a challenge for modern poultry production globally, especially for antibiotic free production.

- NE- Recent studies have demonstrated more than 80% of positivity all around the world, with Europe being the region with the highest incidence (94% compared to 89% in USA, 75% in South America and 65% in Asia).

- Hyperprolific sows- Weaning is still a challenge for some producers – multifactorial approach
Introduction

DIET

BIOSECURITY

OTHERS

MANAGEMENT

FEED ADDITIVES

Clinical intake over weeks for different pathogens: E. coli, B. hyodysenteriae, B. pilosicoli, L. intracellularis.
Introduction

DIET
- Ingredients, Nutrient, Homogeneity, Digestibility, Palatability

BIOSECURITY
- Vaccines, auto-vaccines, windows, animals, others

MANAGEMENT
- Batches, litter, all in/all out, water and feed

FEED ADDITIVES
- Gut flora modulators, acidifiers, plant extracts, others (enzymes)

OTHERS
Probiotics

- Alive microorganisms that confer a benefit on the host when being fed with them.
- Bacteria (non and sporulated) and yeast
- Mode of action well known – gut flora modification, direct production of some nutrients and metabolites, immune system enhancement, quorum sensing effect
- Competitive exclusion (mixtures of bacteria). Age dependent effect
- Vaccines compatibility need to be checked
Prebiotics

- Non digestible feed ingredients that stimulate the growth or activity of beneficial bacteria and limit the development of pathogens in the GIT. Mainly: MOS, FOS, Inulin, B-glucans

- Physical effect vs. changes in fermentation pattern
  - FOS- fermented by bifidobacteria –indirect effect inhibiting other bacteria
  - MOS- inhibits colonization of enteric pathogens (type-1-fymbriae receptors) by blocking bacterial adhesion to gut mucose
  - B- galactomannans- inhibiting adhesion to gut receptors
Acids

• SCFA and MCFA
  • Propionic, acetic, butiric, benzoic
  • Caproic, caprilic, capric

• Due to their different chemical structures, different acids have different properties. The response to diet/water acidification may be affected by type of acid, inclusion level, diet composition and dietary buffering capacity.

• Main action SCFA upper gastrointestinal tract

• Antibacterial effect species specific

• Down regulation of the expression of invasion genes (butyrate, propionate) antiinflammatory, nutrients for epithelial cells
Plant extracts

- High antibacterial effect
- Several molecules (limonen, timol, carvacrol, cinnamaldehyde)
- Known mode of action – depending on the molecule
- High dependence on dose and purity
- Reduction of membrane potential, pH decrease, synergic effect with other compounds
Others: enzymes

PROTEASE

• Protease is receiving more attention as an effective tool to increase amino acid digestion, improve growth performance, and reduce feed cost in poultry industry.

• Beyond releasing amino acids, gut health benefits of protease have also been recognized.
How can a protease optimize gut health?

- Reduction of putrefaction in the distal GIT
- Reduce harmful metabolites
- Hydrolysis of proteinaceous antinutrients and antigenic proteins
- Change in dynamics of digestion and absorption
- Enhanced gut physiology
- Increased Glu for enterocyte energy metabolism
- Reduce viscosity
- Altered flow of NSP in the intestine
- Improved Ca and P retention
- Enhanced availability of AA for mucin synthesis
- Enterocyte turnover or tight junction integrity
- Stimulate synthesis of digestive enzymes
- Upregulate peptide transporter gene expression
- Reduce pathogenic bacteria attachment to the intestinal mucosa
- Degradation of biofilm
Intestinal microbiota compete with host for AA

- Nutritional requirements of bacteria are species-dependent: “simple” (E.coli) to “complex” (Lactobacilli)

- Some intestinal bacteria are highly dependent on amino acids in easily digestible form

→ Competition with the host!

- The more the physiological functions of the host (digesta passage, secretion of acids, bile salts and antibodies) limit the growth of microbiota, the larger the proportion of AA available for growth of the host: less competition in proximal part of the intestines

[Table: Amino acid requirements of some small intestine bacteria]

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Lactobacillus spp.</th>
<th>Clostridium perfringens</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Histidine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leucine</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Lysine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methionine</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proline</td>
<td>+</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Serine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Threonine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Growth of the tested strains was dependent on the amino acid.
* Growth of some of the tested strains was dependent on the amino acid.

References:
J. Apajalahti, K. Vienola / Animal Feed Science and Technology 221 (2016) 323–330
Intestinal microbiota compete with host for AA

- Schematic presentation of protein flow in the small intestine
Intestinal microbiota compete with host for AA

- Protein fermentation of ileal bypass protein in the cecum

Ileal bypass protein is subject to fermentation by putrefactive bacteria in the caecum.
→ Toxic compounds
→ Tissue damage, inflammation
Attachment of enterotoxigenic \textit{E. coli} to mucosa surface

Attachment of enterotoxigenic *E. coli* to mucosa surface

- Protease reduced attachment of enterotoxigenic *E. coli* to the intestinal mucosa of rabbits

*Figure 1: Incidence of diarrhoea (combined score 1 to 4) in piglets challenged with K88+ ETEC.*

*Figure 3: Effect of bromelain treatment on weight gain of piglets challenged with K88+ ETEC. *p*<0.05, **p*<0.01.*
Protease upregulates peptide transporter gene expression in pigs

PepT1 gene expression

A. Duodenum

Zuo et al, Animal Nutrition 2015
Degradation biofilm – supporting effect of feed additives

Protease « antibiofilm agent »
Bacteria colonized in biofilm can become accessible to feed additives, thereby altering gut microbiota

Mukherji et al., Enzyme Engineering 2015
Protease Cibenza EP150

- **Cibenza**<sup>EP150</sup>
  - Heat stable protease
  - Produced by natural thermophillic *Bacillus licheniformis* PWD-1
  - Serine protease, endopeptidase, subtilisin family
  - > 600,000 units/gram protease with azocasein as substrate
  - Broad spectrum
    - Casein, collagen, keratin, elastin

1. **Protein digestion by protease**

2. **Probiotic effect of *Bacillus licheniformis* spores**
Proposed Mechanism of Action of EP150 for Gut Health Benefits

↑ protein absorption ->
↓ protein flow to hind gut ->
↓ hind gut protein fermentation

Destroys ANFs
Diet origin – TI
Bacterial origin – protein exotoxin

Accelerates the dynamics of protein digestion in the gut

Beneficial effects of *Bacillus licheniformis* spores
Nutrient Digestion
Improved AA digestibility with magnitude greater for low digestible AAs
Nutrient Digestion

Improvement in AA digestibility more consistent with the presence of trypsin inhibitor

Improvement in SID with Cibenza EP150 (%)

Internal Novus Research, B06IFY013020
Nutrient Digestion

Increased serum coloration in US type diet

Serum coloration

- 1320 day-old male broilers, 12 replicate pens of 22 birds
- orally gavaged with a coccidiosis vaccine at 5X the recommended dose on d 15.
- Corn, SBM diet, CIBENZA EP150 500g/t
- day 30: 3 birds/pen for yellowness measurement

Yan et al. IPPE 2017 - Novus R&D B00IFY016026


Probiotic effect

1. Reduction inflammatory response
   - Lowers α-acid glycoprotein serum levels

2. Improvement gut morphology
   - Supports higher villi/crypt ratio

3. Improvement gut flora balance
   - Reduces *E. coli* bacterial load
   - Enhances growth of Lactobacilli
   - Decreases nr of *Clostridium perfringens*
Gut flora modification

- Reduced ileal C. perfringens in high protein diet

228 Ross broilers
floor pen, 2x2 factorial design
9 replicate pens of 8 birds each,
0-29 days of age, coccidia challenge at day 7: 3x vacc. dose high protein diet with poultry by product meal.

P-value  
CP: 0.13  
EP150: 0.11  
CPxEP150: 0.04
Gut flora modification

Increased fecal bacteria diversity in EU type diet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species Richness</th>
<th>Species Evenness</th>
<th>Shannon-Wiener (H’) index (H’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>36.8 B</td>
<td>0.33 B</td>
<td>1.19 B</td>
</tr>
<tr>
<td>NC</td>
<td>48.8 AB</td>
<td>0.39 B</td>
<td>1.50 B</td>
</tr>
<tr>
<td>EP150</td>
<td>59.5 A</td>
<td>0.64 A</td>
<td>2.61 A</td>
</tr>
</tbody>
</table>

Species richness is simply the total number of species within a habitat or community.
Species evenness is the relative contribution of each of these species to the total number of individuals in a community.
Species diversity is a measure of both richness and evenness.
Gut architecture

Improved gut morphometry in high viscous diet

- Pen trial, 0-21d, 384 birds, 48 pens (2 pens=1 replicate, 6 replicates/treatment)
- 6 chicks (1/replicates picked for examination of intestinal morphology:
  mean values of 10 samples of villi and 10 associated crypts

Wang, 2008, Animal Feed Science and Technology
Gut architecture

• Decreased crypt depth in the presence of xylanase in Corn-SBM-Canola meal-rice bran diet

<table>
<thead>
<tr>
<th>Crypt depth (µm)</th>
<th>Control</th>
<th>Xyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>Xyl: 0.20</td>
<td>EP150: 0.12</td>
</tr>
</tbody>
</table>

Study # B06IMM015044
Effect on Interleukines

Serum IL-10 (pg/ml)

- 1320 day-old male broilers, 12 replicate pens of 22 birds
- orally gavaged with a coccidiosis vaccine at 5X the recommended dose on d 15.
- Corn, SBM diet, CIBENZA EP150 500g/t
- day 30: 3 birds/pen for measuring the level of anti-inflammatory cytokine IL-10 in the serum
Reduced jejunal IL-4 mRNA expression in US type of diet

- 1320 day-old male broilers, 12 replicate pens of 22 birds
- orally gavaged with a coccidiosis vaccine at 5X the recommended dose on d 15.
- Corn, SBM diet, CIBENZA EP150 500g/t
- day 30: 3 birds/pen for measuring the level of anti-inflammatory cytokine IL-10 in the serum
Supporting Trials
Supporting trials for EP150 gut health benefits

- Modify gut microbiota, prevent dysbacteriosis
  - Reduce ileal *Clostridium perfringens*
  - Reduce ileum and colon E. coli
    - Taiwan study
  - Increase ceca lactic acid bacterial
    - Taiwan study
  - Decrease ceca VFA
    - Taiwan study

- Increase gut microbiota species diversity
Supporting trials for EP150 gut health benefits

• Improve gut morphology: ↑ V/C ratio; better villus tip integrity

• Improve barrier function, reduce acute phase protein

• Reduce digesta viscosity
Supporting trials for EP150 gut health benefits

• Reduce inflammation - ↓ IL-1β

• Modulate immune response to Eimeria challenge - ↓ IL-10, IL-4
  • B00IFY016026

• Alleviate oxidative stress - reduce serum and ileum MDA and ileum protein carbonyl
  • Effects of Xylanase and Protease on Gut Health and Growth Performance of Newly Hatched Broiler Chickens. HERCHLER, MARISSA M.S. thesis, North Carolina State University

• Decrease excreta and ceca ammonia
  • Taiwan study
Support healthy intestines

- Protein digestion
- Improved gut flora equilibrium
  - ↓ C. perfringens, E. Coli, coliform spp.
  - ↑ Lactobacilli,
  - ↑ fecal bacteria diversity;
- Gut integrity
  - Higher villi/crypt ratio
  - Improved gut barrier
  - Less inflammation/oxidative stress

Risk mitigation

- Batch-to-batch nutrient variability
  - Degrades antinutritional factors (trypsin inhibitor, ß-conglycinin, glycinin, kafirin)

Improvement environmental footprint

- NH3 in ceca and excreta

Feed optimization saving costs

- Total level of protein RMss & ME level of the diet thanks to
  - CP, AA and E contribution

More flexible use of proteinaceous RMss

- Use of less digestible, cheaper protein sources

Completes the diet enzyme program

- Provides additional benefits when combined with a
  - phytase and/or carbohydrase(s)
  - Superdose phytase

Results obtained through multiple research trials with CIBENZA ® EP150
Products not available in all countries.

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