INFLUENCE OF NUTRITION AND THE CHARACTERISTICS OF FEED ON THE MICROBIOLOGICAL PROFILE OF THE GASTROINTESTINAL TRACT IN CHICKEN AND PIGS

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SUMMARY

The intestinal microbiota has an extremely complex composition, including bacteria, archaea, viruses, yeasts and moulds. Microbes contribute to the digestive process, bacterial fermentation of non-digestible carbohydrates and dietary fibre helps to generate energy for the host. Thus, microbial fermentation can be considered as trophic factor especially when high fibre ingredients and by-products are used. Protein fermentation yields a spectrum of undesirable metabolic products such as ammonia that can have negative effects on animal health and performance.

Due to significant methodological progress in recent years it was possible to elucidate the effects of dietary factors on the composition of the microbiota and their metabolic activity. It was found that the host development is strongly influenced by a functioning microbiota in newborn or newly hatched animals. The microbiota has a protective function and prevents colonization by pathogenic bacteria. Furthermore, it affects the morphology of the gastrointestinal tract, the mucus formation and in particular the gastrointestinal immune system. The composition of the microbiota is characterized in chickens and pig by a quite intensive early life development. The concentrations of the microbiota increase in the course of the digestive tract, the highest bacterial counts are found in the cecum and colon. That results also in characteristic fermentation patterns: While in the upper digestive tract lactic acid dominates, mainly short chain fatty acids are formed in the hind gut. Levels are clearly subjected to dietary factors. Thereby, it is possible to identify nutritional effects and to optimize the microbiota in order to achieve a
Influence of nutrition and the characteristics of feed on the microbiological profile of the gastrointestinal tract in chicken and pigs

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Outline

• Symbiotic benefits
  - Digestion – tolerance - health
• Intestinal microbiota in chicken and pigs
  - Composition
  - Metabolic activity
  - Specific role in digestion
• Interacting nutritional factors
  - Feed composition
  - Feed additives
Definition

• Intestinal microbiota
  - Community of microorganisms
  - living in the digestive tract

  - Bacteria
  - Archaebacteria
  - Viruses
  - Yeasts, moulds
  - (Proto-/Metazoa…)

Relevance of gut microbiota for digestion

• Nutritional benefits of the host-microbe relationship

  1. Breakdown
     - Non-digestible carbohydrates and others
  2. Synthesis
     - Vitamins K, B₁₂, folic acid, SCFA
  3. Immunological tolerance
     - Proteins, peptides
Relevance of gut microbiota for digestion

• Symbiotic benefits of the host-microbe relationship

1. Normal post-hatch/-natal development
2. Protective function - colonisation resistance
3. Morphology of the GIT: intestinal angiogenesis, villus architecture
4. Function of the GIT: mucus secretion
5. Development of the immune system

Immune system

• Gut = biggest immune organ
• External surface
  - Microbiota
  - Feed antigens
  - Environment
  - Pathogens

Ferrara 2012
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Microbiota in the crop

- Mainly gram positive bacteria
- Facultative anaerobes
- Lactobacilli ↑
- Microbial metabolites
  - Acetic acid: 7 – 25 µmol/g
  - Lactic acid: 43 - 80 µmol/g
  - Low degree of acidification
  - „First barrier“ against pathogens
Microbiota in the small intestine

**Small intestine**

- Increasing bacterial concentrations
- Firmicutes dominate
- Anaerobes increase in the digesta
- Lactobacilli, *E. coli* + coliforms, Enterococci

**Moderate metabolic activity**

- Lactic acid dominates
- Short chain fatty acids: very variable in both species
- Mainly acetate
- Butyrate, propionate ↓
Microbiota in the caecum and colon

- Highest bacterial density
- Mainly anaerobes
- Lactate ↓
- SCFA ↑

“Microbiome profiles“

Predict performance and health impact

Optimized diets

Bacteria phyla distributions using V3 amplicon sequencing (n = Top 100 OTUs). For each timepoint (Day 7, 14, 21 and 42) and part of intestine (I = ilea, C = caeca)

Mohd Shaufi et al. Gut Pathogens 2015 7:4
Relevance of gut microbiota for digestion

- **Caecal microbial communities from male broilers**
  - a) by diet: ▲, diet A; ▼, diet B; ■, diet F; ●, diet G
  - b) by birds
    - Improved performance (▲) or poor performance (▼)

![Diagram](image)

Torok et al. 2011

Chicken caecal microbial profiles arranged according to the FCR of the farm from which they originated

![Graphs](image)

Rinttilä and Apajalahti, 2013
Chicken caecal microbial profiles of individually caged birds arranged according to diet metabolizability

**Low performance**

**High performance**

![Graph showing caecal bacterial profiles of 8 birds with ME:GE 0.5 - 0.6 and 0.8 - 0.9](image)

Rinttilä and Apajalahti, 2013

Microbiota identifies an ecosystem structure linked with growth traits

**ARTICLE**

*Enterotypes of the human gut microbiome*

Manimegalingam Arumugam*, Jensen Raes,3,6, Eric Le Paslier,3,6, Denis Le Pelley,3,6, Takul Yamada,6, Daniel R. Mendes,6, Gabriela R. Fernandez,3,6, Julien Tap,1,2, Thomas Bruls,1,2,3, Jean-Michel Botto,1,2, Marcelo Bertalan,6, Natalia Bornfeldt,6, Francesc Casellas,7, Leyden Fernandez,7, Laurent Gautier,7, Torben Hansen,1,2, Masahira Hattori,8, Tetsuya Hayashi,9, Michel Kleerebezem,6, Ken Kurokawa,6, Marion Leclerc,6, Florence Levenez,6, Chayavanh Manichanh,6, H. Bjorn Nielsen,6, Trine Nielsen,6, Nicolas Pons,2, Julie Poulsen,6, Anjie Qin,6, Thomas Schönitzer-Porten,1,2, Sebastian Sims,6, David Torrents,1,2, Edgardo Ugarte,6, Erwin G. Zoetendal,1,2, Jun Wang,1,2, Francisco Guarnier,7, Olof Pedersen,1,2,20,21, Willem M. de Vos,6,24, Soren Brunak,6, Joel Dore,6, MetaHit Consortium,1, Jean Weissenbach,1,2,3, S. Durko Ehrlich* & Peer Bork1,6,

Our knowledge of species and functional composition of the human gut microbiome is rapidly increasing, but it is still based on very few cohorts and little is known about variation across the world. By combining 22 newly sequenced faecal metagenomes of individuals from four countries with previously published data sets, here we identify three robust clusters (referred to as enterotypes hereafter) that are not nation or continent specific. We also confirmed the enterotypes in two published, larger cohorts, indicating that intestinal microbiota variation is generally stratified, not continuous. This indicates further the existence of a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet and drug intake. The enterotypes are mostly driven by species composition, but abundant molecular functions are not necessarily provided by abundant species, highlighting the importance of a functional analysis to understand microbial communities. Although individual host properties such as body mass index, age, or gender cannot explain the observed enterotypes, data-driven marker genes or functional modules can be identified for each of these host properties. For example, twelve genes significantly correlate with age and three functional modules with the body mass index, hinting at a diagnostic potential of microbial markers.

Ramoay-Caldas et al. 2016
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The chicken gastrointestinal microbiome

• Diet is a major factor in influencing microbial composition + activity
Gut health

- “Dysbiosis”
- Necrotizing enteritis ↑
  - Non-starch polysaccharides → increased viscosity of the intestinal contents
  - High-protein diets, especially with animal protein sources (fish meal)
  - Link between coccidia and increased activity of toxin-producing strains of *Clostridium perfringens*

Relevance of gut microbiota for digestion

- Active proteolytic microbial community in the hindgut
  - *Bacteroides, Clostridium, Fusobacterium, Streptococcus, and Enterococcus* spp.
    - Ammonia (Oxidative or reductive deamination of amino acids)
    - Phenols and indole (Tyrosine and tryptophan)
    - Amines (Amino acid decarboxylation)
Gut health

- **Digesta viscosity**

  - Effects on *Cl. perfringens* and lesions by Necrotic Enteritis

    - Scores increase with wheat > maize (Branton et al. 1997)
    - Carboxymethylcellulose (2-8 g/kg): Viscosity ↑, no effect on the disease scores (Waldenstedt et al. 2000)

- **Protein-/Amino acid intake**

  - Protein ↑ → *Cl. perfringens* ↑
    (Drew et al. 2004, Fishmeal, 40 % XP; Wu et al. 2014)
  - Glycine ↑ → *Cl. perfringens* ↑
    (Dahiya et al., 2005, 2007; Wilkie et al., 2005)
  - Methionine (0.2-0.8 %, 24.5 % Cp) ↑ → Reduction of *Cl. perfringens* (Dahia et al. 2007)

![Figure 1. Mean necrotic enteritis (NE) lesion scores in experiments 1 and 2 in 28-d-old broiler chickens given experimental diets (0 to 28), containing different levels or sources of Met. Bars represent mean ± SEM, n = 12. Lesion score scored on a 0 to 4 scale, where 0 = no intestinal lesion (apparently normal); 1 = shyly congested serosa and mesenteric engorged with blood; 2 = thick-walled and fulctile intestines with small red patches (≤5); 2 = focal necrotic lesions; 3 = patches of necrosis (1 to 2 cm long); and 4 = diffused necrosis typical of field cases.

Dahia et al. 2007
Necrotic enteritis

- *Cl. perfringens* α-Toxin reduces intestinal glucose transport

![Graph showing glucose transport](image)

Rehman et al. 2006

Nutrition and microbiota

- **Macronutrients**
  - Protein
  - Carbohydrates
  - Fibre
  - Fat

  **Fermentation**

  **Shift of the intestinal microbiota**

  - Less diarrhea in piglets with low protein diets
  - Effects of enterotoxigenic Escherichia coli more severe with high protein diets
    - (Opapeju et al. 2010, Kim et al. 2011 Heo et al. 2010)
Microbiota

- **Macronutrients**
  - Protein
  - Carbohydrates
  - Fibre
  - Fat
  - Fermentable carbohydrates
    - Fructose
    - Lactobacilli, Bifidobacteria
    - Amylose-rich Maize starch
    - Bifidobacteria
  - Soluble fibre increasing viscosity
    - May increase digestive problems
  - Unsoluble fibre
    - Protective effects

- **Endotoxemia, Inflammation?**
- **Immune effects?**

Probiotics: indications

- **Nutrition and health in pigs**
  - Early piglet losses > 15 %
  - Underdeveloped piglets
  - Post Weaning Diarrhoea
  - High need for AB treatment
  - Increasing debate on AB resistance
Mode of action of probiotics

- **Growth stimulation**
  - Bifidobacteria
  - Lactobacilli

- **Lactate**
  - pH ↓
  - Epithelial cell growth ↑
  - Colonic blood flow ↑
  - Motility modification
  - Absorption of water, minerals ↑
  - Mucus production ↑

- **Adherence inhibition**
  - Growth inhibition

- **Immune modulation**
  - Pathogens
  - Harmful bacteria

- **Adherence inhibition**
  - Growth inhibition

- **Lactate utilizing bacteria**

- **Probiotic**

Experimental design

**Test group**
Start of feed supplementation in the test group (B. cereus)

28 days ante partum.

Sows a.p.

Weaning

Age of the piglets 0 (days)

Time after infection (days)

Piglets

Oral infection with Salmonella

Animal sections
(6 piglets per group and time point)

**Control group**
no supplementation of B. cereus, also infected with S. Typhimurium

Mod. OHASHI and USHIDA 2009
Salmonella and diarrhea scores

![Graph showing control animals with diarrhea, Bacillus treated animals with diarrhea, and number of animals in each group over time post infection (Days).]

Escherichia coli and diarrhea scores

![Graph showing facial score changes over time (DAYS) with E. coli challenge. Score 1 = diarrhea; 5 = hard faeces.]

T1 - T4 indicate different treatment groups.
Conclusion

- Animal performance and microbiota composition seem to be linked
- Animal health is linked with nutrition x microbiota interaction
- Nutrition for animal health and performance
positive effect on the performance and animal health. Recent work clearly shows that the microbiota of highly performing animals has a different composition compared to those with poor performance.

The aim of the manipulation of the intestinal microbiota is not only to influence the performance, but also to achieve better health, especially to reduce the incidence of diarrhea and to combat zoonotic pathogens. Diet is a major factor that affects the microbiota. This begins with the physical nature of the feed, the processing steps, the gross composition but also the use of feed additives. Examples of problems that are caused by a suboptimal microbiota are seen in both, chicken and pigs. Intestinal dysbioses can cause clinical diseases. Examples are an increasing viscosity of the digesta or microbial protein degradation, which have a significant influence on animal health. It was shown that especially the trace elements (copper, zinc), organic acids and their salts, enzymes, probiotics, prebiotics and phytogenic additives have a special impact on the microbes.

In recent years, research was gaining increasing insight in the effects and the mode of action of probiotics. These are nowadays considered as a reasonable alternative for the use of antibiotics in feed. Their effects are based on three pillars: 1. Reduction of pathogenic bacteria; 2. Immunomodulation; and 3. Effects on the intestinal wall. It has been illustrated that the choice of the probiotic has a crucial role for the clinical outcome. Certain probiotics can significantly reduce the occurrence of diarrhea after exposure to diarrheal pathogens in pigs and poultry. It turns out more and more that the performance of animals and health are closely linked to a functioning microbiota. Therefore, an optimized nutrition must include health aspects and not only focus on performance. This touches the important question how to reduce in the future the use of antibiotics in feed for chicken and pigs, in the latter also the use of high amounts of zinc oxide. Zootechnical feed additives play a significant role, in addition to the probiotics and prebiotics also synergistic combinations thereof. Recent years have revealed some interesting developments in the field of trace elements and new bondings and preparations, in organic acids, including protected forms and the phytogenic additives. Table 1 gives an overview on the currently available feed additives and their impact on the intestinal microbiota.

Conclusion: Manipulating the intestinal microbiota is an important aspect for animal performance and health. Improved diagnostics will allow using these options purposefully in future and thus will sustainably improve animal health, welfare and consumer protection.

Key words: microbiota, digestion, fermentation, health, performance
### Table 1.- Feed additives and materials and their impact on the intestinal microbiota

<table>
<thead>
<tr>
<th>Additive</th>
<th>Effect on the microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytogenic substances</td>
<td>Some have antimicrobial activity, but also other effects depending on spectrum of active substances and concentrations</td>
</tr>
<tr>
<td>Organic acids, salts</td>
<td>Preservatives, impact on the microbiota in the upper gastrointestinal tract, can favour lactic acid bacteria, may reduce pathogens</td>
</tr>
<tr>
<td>Zinc, copper</td>
<td>Depending on concentration, can have antimicrobial effects, but act selectively according to latest research with pharmacological levels of zinc oxide</td>
</tr>
<tr>
<td>Probiotics</td>
<td>Bacteria or yeasts, competition with pathogens, regulation of the microbiota composition, strongly depending on the strain</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Fermentable carbohydrates, favour the growth of “beneficial” bacteria; similar effects reported for lactose</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Change substrate availability for bacteria, not yet fully characterized</td>
</tr>
<tr>
<td>Clay</td>
<td>Binding of toxins, not yet clearly characterized</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>May have specific impact on target bacteria</td>
</tr>
</tbody>
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