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EXAMPLES OF INDIVIDUAL ANALYSES

INTESTINAL MICROBIOTA

Microbiota in the animal or human intestine has evolved together with the host. Consequently, the gastrointestinal tract could be considered a metacommunity, comprising many local microbial communities in different ecological niches. Each individual gut compartment has its unique physiochemical characteristics and each location is inhabited by a specialised microbial composition.

TYPICAL MICROBIAL TARGETS IN LOW INTESTINE (COLON, CAECUM)



Total eubacteria

Lower intestine is the pivotal site for the bacterial fermentation in GI tract. High bacterial density indicates breakdown of dietary components that are beyond the endogenous digestion system of the host. It is possible that with poorly digestible diets, a considerable proportion of total dietary energy comes from bacterial fermentation.

Family Lachnospiraceae

Lachnospiraceae are one of the most abundant families from the order Clostridiales found in the lower GI tract. It contains bacterial species the exact identities of which are poorly known. Many members of this family are known to produce butyrate. Higher levels of Lachnospiraceae stabilise the intestinal environment by retarding accumulation of lactate. Generally, Lachnospiraceae are associated with the maintenance of gut health as well as improved animal well-being and performance.

Family Ruminococcaceae

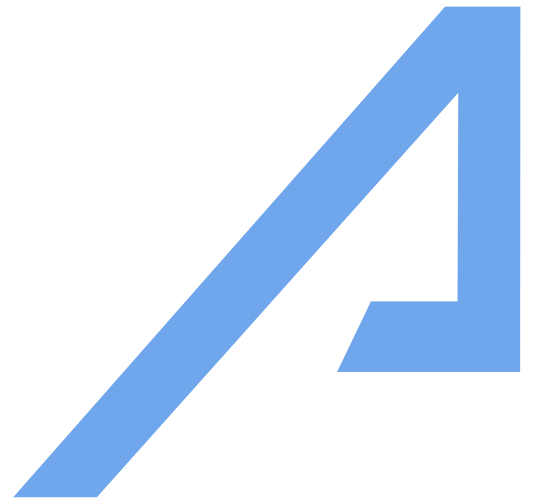
Ruminococcaceae have received attention because they are highly abundant in the lower intestine of warm-blooded animals. For example, the family contains bacteria classified to *Faecalibacterium* and *Subdoligranulum*, which are known to be one of the dominant butyric acid producing genera within intestinal microbiota. In addition, the increase in *Ruminococcaceae* is connected to cellulose-degrading capacity, which facilitates energy capture from complex polysaccharides to promote the intestinal morphological structure.

Bifidobacterium spp

Bifidobacteria are thought to exert a protective role against pathogenic microorganisms such as campylobacter and salmonella via production of antimicrobial agents as well as preventing pathogen colonisation and enhancing the host immune response. On the other hand, these bacteria are saccharolytic and therefore, their high abundance indicates the presence of metabolisable carbohydrates in the lower intestine. This may be an indication of deprived feed-derived energy absorption in small-intestine.

Lactobacillus spp

Like bifidobacteria, also lactobacilli are saccharolytic. Their abundance in the healthy gut is typically low, but in connection to some nutritional disorders may represent tens of percentages of the total bacteria. Whether or not these bacteria directly constitute a health problem, their accumulation in lower intestine appears to serve as a good indicator of digestive disorders.



<i>Bacteroides spp</i>	The abundance of this genus in the low intestine can range from less than one percent to tens of percentages. They have been characterised as short-chain fatty acid producers and are among the most effective degraders of indigestible carbohydrates, including resistant starch and cellulose. Characteristics of the bacterial species belonging to the genus <i>Bacteroides</i> vary. Certain species are known to produce harmful putrefactive compounds. Moreover, <i>Bacteroides fragilis</i> is considered an opportunistic pathogen, which can participate in intra-abdominal infections in the events the mucosal wall of the intestine being disrupted.
<i>Clostridium perfringens</i>	<i>Clostridium perfringens</i> is a bacterium nearly always present in the intestine of chickens at low numbers. However, partly due to unknown factors its numbers can occasionally increase several orders of magnitude and it can start producing toxins that cause significant tissue damage. When the outbreak is serious enough it leads to necrotic enteritis and mortality.
<i>Escherichia coli</i>	<i>Escherichia coli</i> is an adaptive species, which is a commensal resident of normal intestinal microbiota in all warm-blooded animals. However, there are many virulent strains within the species causing enteric infections that seriously risk the health of the production animals as well as the consumers of the end-products. Since the conditions favouring the growth of the harmless <i>E. coli</i> hardly differ from those of the virulent ones, it is justified to use the total numbers of <i>E. coli</i> as a potential risk indicator.
<i>Campylobacter jejuni</i>	<i>Campylobacter jejuni</i> generally colonise poultry or swine as commensal organisms. On the contrary, in humans the infection is associated with acute enteritis. It is generally assumed that <i>C. jejuni</i> contaminate poultry or pig meat during processing, surviving throughout the food chain supply to constitute a risk to human health.
<i>Salmonella enterica</i>	Enteric <i>Salmonella</i> infections are the second-most frequently reported zoonotic diseases of humans in the European Union. In foodstuffs, <i>Salmonella</i> is most often detected in fresh broiler, turkey and pig meat. <i>Salmonella enterica</i> is widespread in domestic animals, and commercial poultry flocks are a large reservoir. When flocks become infected on the farm, <i>Salmonella</i> is usually carried asymptotically in the gastrointestinal tract of the birds and may consequently be transferred to processed carcasses via faecal contamination.

TYPICAL MICROBIAL TARGETS IN SMALL-INTESTINE (JEJUNUM, ILEUM)



Total bacteria The small-intestine, which consists of the duodenum, jejunum and ileum, is the GI tract compartment where most of the digestion and absorption of nutrients occurs. Bacteria in the small-intestine use and compete directly for the same readily fermentable diet-derived nutrients that are being utilised by the host. Hence, alterations in these bacterial populations may reduce nutrient capture of the host thus impairing body weight gain and feed conversion ratio. On the other hand, the host can recover part of the energy lost to microbes by absorbing and metabolising bacterial fermentation products such as lactic and volatile fatty acids.

Lactobacillus spp Lactobacillus is the dominating bacterial genus in the small intestine of warm-blooded animals, showing either homo- or heterofermentative lactic acid metabolism. Certain *Lactobacillus* species have been shown to excite bacterial bile salt hydrolysis, generating impaired lipid absorption and consequent dietary energy losses.

Enterococcus spp The metabolism of enterococci is like that of the homofermentative lactobacilli. A characteristic feature of enterococci is that they tend to possess and transfer antibiotic resistance genes and are thus considered potentially risky group of bacteria if therapeutic or prophylactic antibiotics are being or have been used.

Escherichia coli *Escherichia coli* is an adaptive species, which is a commensal resident of normal intestinal microbiota in all warm-blooded animals. However, there are many virulent strains within the species causing enteric infections that seriously risk the health of the production animals as well as the consumers of the end-products. Since the conditions favouring the growth of the harmless *E. coli* hardly differ from those of the virulent ones, it is justified to use the total numbers of *E. coli* as a potential risk indicator.

Salmonella enterica Enteric *Salmonella* infections are the second-most frequently reported zoonotic diseases of humans in the European Union. In foodstuffs, *Salmonella* is most often detected in fresh broiler, turkey and pig meat. *Salmonella enterica* is widespread in domestic animals, and commercial poultry flocks are a large reservoir. When flocks become infected on the farm, *Salmonella* is usually carried asymptotically in the gastrointestinal tract of the birds and may consequently be transferred to processed carcasses via faecal contamination.

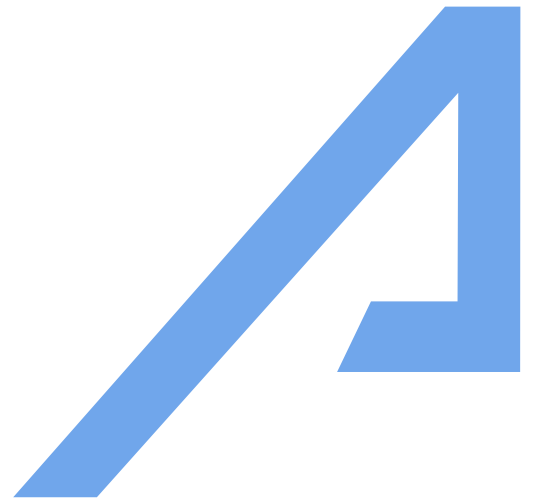
Eimeria spp Coccidiosis of chickens is an intestinal disease caused by parasitic protozoa of the genus *Eimeria*. Transmission occurs via the ingestion of infective oocysts that have sporulated over several days following their excretion in the faeces as discussed above. The disease is extremely contagious and infective oocysts in the environment are resistant to most disinfectants making its eradication difficult.

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MICROBIAL METABOLITES IN INTESTINAL TRACT

The analyses described above indicate the composition of bacterial communities in the intestinal tract. In addition to the composition, it is also important to know the type and intensity of bacterial fermentation that takes place in different gut compartments.

SCFAs – VOLATILE FATTY ACIDS & LACTIC ACID



The principal end products of intestinal microbial fermentation are short-chain fatty acids (SCFA; VFAs), such as acetate, butyrate, propionate and lactate. It has been shown that up to 95% of the SCFA produced during carbohydrate fermentation are used by the host. In addition to energy yielding activity, SCFA formation reduces pH of the intestinal environment, which may inhibit acid-sensitive pathogenic bacteria.

Example of intestinal SCFA panel:

- Volatile fatty acids: acetic, propionic, butyric, valeric acid
- Branched-chain fatty acids: iso-butyric, 2- Methyl-butyric, Iso-valeric acids
- Non-volatile fatty acids: lactic acid

PROTEIN FERMENTATION END-PRODUCTS

Once carbohydrates are exhausted in the low intestine of the host, sources of protein material are fermented and metabolised to salvage energy. Although proteins and amino acids provide a less significant energy source in the lower intestine, their importance lies mainly in the formation of potential systemic toxins and carcinogens because of protein or amino acid fermentation by putrefactive bacteria.

In addition to being toxic to the host, these end products of protein fermentation tend to increase the pH of the intestinal contents. The general assumption is that low pH is beneficial for gut health, as the growth of the acid-sensitive pathogenic microorganisms is suppressed in such conditions.

Example of intestinal protein fermentation panel:

- Phenol, indole & skatole
(a result of anaerobic fermentation of the aromatic amino acids)
- Biogenic amines
(a result of amino acid decarboxylation)
- Ammonia
(a result of oxidative or reductive deamination of amino acids)