



# In search of a healthy internal balance

**THE INTESTINAL TRACT CONTAINS MILLIONS OF BACTERIA, BOTH GOOD ONES AND BAD ONES. DESPITE THE GROWING INTEREST IN GUT MICROBES, KNOWLEDGE OF THE INTESTINAL MICROBIOTA IS VERY LIMITED. ACCORDING TO FINNISH RESEARCHERS A BETTER UNDERSTANDING OF THE MICROBIOTA COMPOSITION IS A PREREQUISITE FOR FUTURE GUT HEALTH RESEARCH.**

**M**icrobes of the gastrointestinal tract form a uniquely complex ecosystem, which has fundamental effects on animal's well-being and productivity. Until recent years, intestinal microbes were mainly regarded as harmful for the animal. Microbes misappropriate energy derived from the feed and have traditionally been considered to impede the growth of the animal. Antimicrobial agents have been added to feed for growth promotion and to prevent gastrointestinal diseases, but also to reduce bacterial mass in the gut. However, gradually the point of view has been revised and these days the gut microbes are not considered only harmful. Instead, some microbes are known to have beneficial effects on the host and probiotic bacteria are even added to feeds in order to enhance animal health and productivity. Intestinal bacteria are known to be vital to ruminants, but today the significance of intestinal bacteria has been accepted with other production animals, too. In ruminants the intestinal microbes metabolise nutrients and produce fatty acids essential to the animal. In non-ruminants perhaps the most important function of the bacteria is to mature and stimulate the immune system of the animal. In the alimentary canal the microbes interact closely with the host's immune cells and thus modulate animal health in a comprehensive manner (Hooper and Gordon, 2001). Laboratory studies have shown that intestinal microbes are essential for the development of immunity and the survival of germ free animals is compromised (Umesaki and Setoyama, 2000).

### UNKNOWN MICROBIOTA

Despite the growing interest in gut microbes, knowledge of the intestinal microbiota is very limited. Intestines contain huge amounts of bacteria and the bacterial density is extremely high, even  $10^{10-11}$  bacteria in a gram of gut content (Eckburg *et al.*, 2005). Gut microbiota consists of hundreds of bacterial species, and the composition of the microbiota varies in the different regions of the gut (Conway, 1997). One of the major hindrances in analysis of intestinal samples has been the inadequate analysis methodology. Traditional analysis methods such as bacterial cultures were developed to detect particularly pathogenic bacteria and they are not best suited for analysis of bacteria of normal gut microbiota. During the last decade, the methods based on the detection of nucleic acids of microbes have enabled more comprehensive analysis of microbiota (Zoetendal *et al.*, 2004). When the knowledge increased, some bacterial species showed to be

beneficial for the host (Hooper and Gordon, 2001). Certain bacteria may apparently enhance the immunity and symbiotic bacteria can protect the host against infections by hindering the attachment of pathogenic microbes to the gut epithelium. Favouring of non-pathogenic microbiota with appropriate feeding brings obvious benefits to both animals and farmers. Thus, gut microbes are not our enemies and cooperation with microbes will be an essential part of successful farming in the future.

### THE CYTOMETRIC METHOD

Knowledge of the composition of microbiota in healthy animals living in normal farming conditions is a prerequisite for successful gut health research (Eckburg *et al.*, 2005). The Finnish Research Programme (Suomen Rehu Ltd and CyFlo Ltd) applies increasing data from intestinal microbiota studies to animal sciences and focuses on research and development of modern health promoting feeding and functional feed ingredients. The characterisation of the non-ruminants' gut microbiota has been the first step in the programme. Intestinal samples collected have been analysed with a new cytometric method. Previously, even high-tech analysis methods used for studying intestinal microbiota have been based on the lysis of bacteria, which can distort the data. The cytometric method used in this project detects bacterial cells as whole. Unbroken bacteria are labelled for the detection with DNA-stain and oligonucleotide probes and analysed rapidly in a flow cytometer. The method enables species specific and accurate counting of bacteria.

To characterise the composition of intestinal microbiota in production animals, faecal samples were collected from pigs ( $n = 242$ ) and piglets ( $n = 180$ ) and cecal samples from turkeys ( $n = 133$ ), broilers ( $n = 96$ ) and layers ( $n = 15$ ). All samples were from animals living in normal farming conditions. The animals were having several kinds of feed and thus the samples collected can be considered to represent microbiota of each animal species in general. In laboratory, the bacterial cells were separated from the sample, fixed and stained according to a protocol published previously (Vaahtovuori *et al.*, 2005). Briefly, faecal sample was suspended in phosphate-buffered saline (PBS) and the bacteria were separated with centrifugations. The bacterial cells were fixed with paraformaldehyde, washed several times with PBS and re-suspended in 50% ethanol-PBS. The fixed bacteria were stored at  $-20^{\circ}\text{C}$  until analysed with flow cytometry. For cytometric analysis the bacteria were hybridised with



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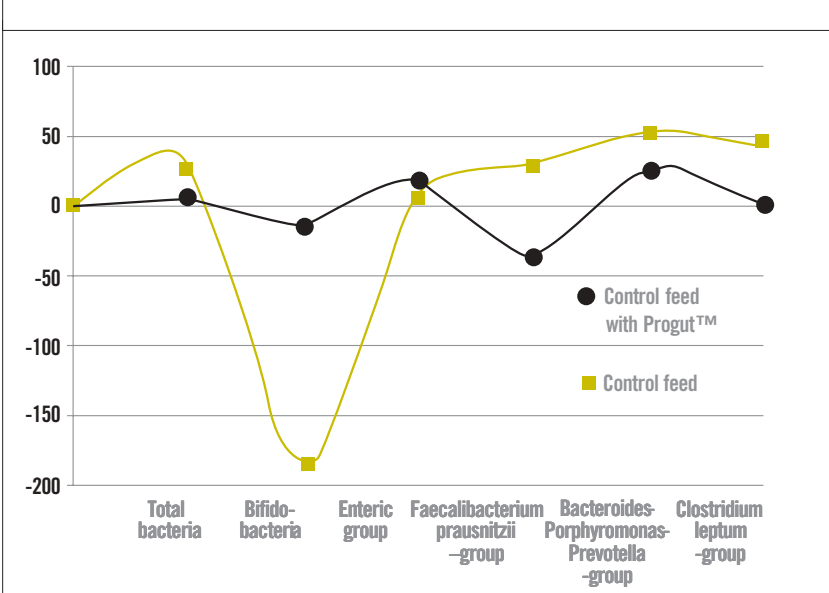
**Ms. Eveliina Munukka, B.Sc.**

TABLE 1-THE TOTAL BACTERIAL COUNTS AND THE COUNTS OF DIFFERENT BACTERIAL GROUPS.

Bacteria	Animal sample				
	Piglet faeces	Pig faeces	Turkey cecum	Broiler cecum	Layer cecum
Total bacteria	$1.3 \times 10^{11}$	$1.3 \times 10^{11}$	$3.6 \times 10^{11}$	$2.5 \times 10^{11}$	$2.5 \times 10^{11}$
Bacteroides-					
Porphyromonas-	$3.7 \times 10^{10}$	$2.7 \times 10^{10}$	$2.5 \times 10^{10}$	$1.8 \times 10^9$	ND
Prevotella group					
Bifidobacterium group	$5.4 \times 10^9$	$4.0 \times 10^9$	$3.7 \times 10^{10}$	$4.7 \times 10^9$	$8.5 \times 10^9$
Enteric group	$8.5 \times 10^9$	$9.7 \times 10^9$	$2.8 \times 10^9$	$2.4 \times 10^9$	$5.1 \times 10^9$
Faecalibacterium					
prausnitzii- group	$5.4 \times 10^9$	$5.3 \times 10^9$	$1.0 \times 10^{11}$	$2.3 \times 10^{10}$	ND
Clostridium leptum-					
group	$4.5 \times 10^9$	$3.0 \times 10^9$	ND	$3.0 \times 10^9$	ND

ND= Not Done

FIGURE 1- BACTERIAL COUNTS FROM THE FINNISH STUDY.

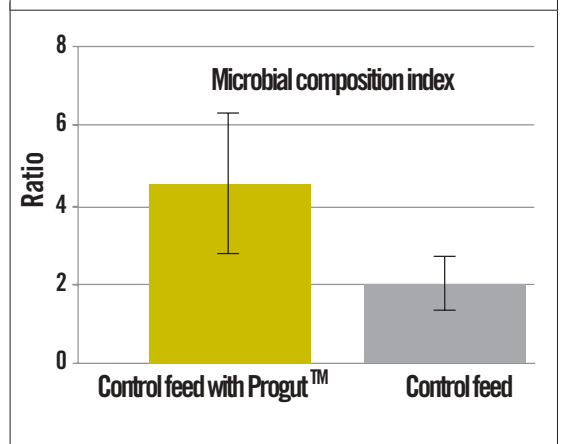


oligonucleotide probes and stained with DNA-stain. Five different probes hybridising *Bacteroides-Porphyromonas-Prevotella* -group bacteria, *Bifidobacterium*-group bacteria, enteric group bacteria (e.g. *Escherichia coli*), *Faecalibacterium prausnitzii* -group bacteria and *Clostridium leptum* -group bacteria were used. With cytometry, the total bacterial counts (i.e. counts of DNA-stained bacteria) and the amount of the hybridised target bacteria were counted. The results are presented in Table 1.

### COUNT RESULTS

Faecal samples from pigs and piglets contained over one hundred billion bacteria per gram of dry matter. The bacteria of *Bacteroides-Porphyromonas-Prevotella* -group were the most common. The counts were almost 40 billion per gram, which represents over 25 percent of all faecal bacteria. Counts of *Bifidobacterium*-group and

FIGURE 2- IN A DANISH STUDY, THE RATIO OF POTENTIALLY BENEFICIAL BIFIDOBACTERIA AND BUTYRATE PRODUCTION RELATED BACTERIA TO POTENTIALLY HARMFUL ENTERIC GROUP BACTERIA (CONTAINING E.G. E. COLI) WAS CALCULATED.



*Faecalibacterium prausnitzii* -group bacteria were over five billion per gram. With poultry, samples were collected from cecum. Cecal bacterial counts were very high, from 250 billion bacteria per gram in broilers and layers to 360 billion bacteria per gram in turkeys. Of bacterial groups studied, *Bifidobacterium*- and *Faecalibacterium prausnitzii* -groups were the most common both in broilers and turkeys. In pigs and piglets, the five bacterial groups analysed accounted for from 31 to 41 per cent of all bacteria. In turkeys approximately half of the all bacteria present in the samples were detected species or group specifically, while in broilers and layers the percentage of the bacteria detected was smaller. Considering the general complexity and diversity of the intestinal samples, the proportion of the bacteria detected species or group specifically with oligonucleotide probes was fairly high.

### MODULATING THE MICROBIOTA

The new restrictions and the reduction in the use of antibiotic growth promoters (AGPs) has been a challenge for animal production (Dibner and Richards, 2005). AGPs have been considered to improve animal growth by suppressing normal microbiota and increasing nutrient utilisation (Gaskins *et al.*, 2002). Modulation of the microbiota with new feed materials, ingredients or additives towards host-protecting function thus replacing AGPs is a topical issue in animal sciences, and creates fascinating possibilities to support animal health. Preparations containing yeast cells or processed yeast are perhaps the most promising group of products intended to replace traditional AGPs and to support gut health. In the current study, the effects of a hydrolyzed brewery yeast product (Progut™, Suomen Rehu Ltd) were used as an example of the possibility to modulate intestinal microbiota without AGPs. In a Finnish feeding trial performed with piglets living in normal farming conditions, the addition of this

yeast product into piglet feed helped to stabilise intestinal microbiota during weaning and to keep proportions of different faecal bacterial species more stable. In the Finnish piglet study, fecal samples ( $n = 48$ ) were collected at weaning and three weeks later. Bacterial counts from the second samples are compared to those from the first samples and the changes in the counts are presented in per cents. The changes of the composition of microbiota in piglets having feed added with the hydrolysed yeast are less pronounced during three-week period indicating the stabilising effect of the product (*Figure 1*).

Piglets fed with the hydrolyzed yeast product also gained significantly better growth than the control group (daily weight gain 63g higher) and the feed conversion ratio was improved. The results are in harmony with the hypothesis that a stable microbiota during critical growth period can reduce animal's stress and support good growth. In a piglet trial ( $n = 90$ ) conducted at Foulum research centre of Danish Institute of Agricultural Sciences (DIAS) the feed containing the hydrolysed yeast helped to modulate the intestinal microbial composition index (*Figure 2*).

In the same study, the reduction of the occurrence of weaning diarrhoea was statistically significant and the consistency of feces less watery ( $p = 0.001$ ) in piglets having feed added with hydrolyzed yeast product. These examples demonstrate the possibilities to modulate intestinal microbiota favourably.

## INTERNAL BALANCE

The results presented strengthen the conception of intestinal microbiota as a diverse ecosystem that, despite its complexity, is prone to retain its internal balance. Intestinal samples analysed during the current study were taken from fairly heterogeneous animal populations living in normal farming conditions and having different kinds of feeds. Thus the results represent the composition of intestinal microbiota on a general level, and they can be used as a reference material in future feeding and animal health studies. With modern techniques developed the proportions of microbial species and groups of interest can be measured rapidly and accurately. Microbial composition index is an example of new tools that can be used to monitor the changes of gastrointestinal microbiota in different situations. In the near future, parameters describing the composition of microbiota and gut status comprehensively will be used as an indicator of animal well-being. There is no doubt that while the knowledge increases, the importance of the composition of the intestinal microbiota will be emphasised in animal farming. <-

*References are available on request*