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Yakult Symposium

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O-1 **Functional analysis of the GI-tract microbiota.** C.C.G.M. Booijink[a,b], E.G. Zoetendal[a,b], H. Smidt[a,b], M. Kleerebezem[a], W.M. De Vos[a,b] ([a] Wageningen Centre for Food Science (WCFS), The Netherlands, [b] Laboratory of Microbiology, Wageningen University, The Netherlands).

The human gastrointestinal (GI) tract provides home to a complex microbial community, collectively termed microbiota. Though major effort has been put into describing the diversity and stability of the GI tract microbiota, its functionality has only been investigated at the isolate level. In this study, cDNA Amplified Fragment Length Polymorphism (cDNA-AFLP; Bachem et al., 1996 Plant J. 9: 745–753) was used as an RNA fingerprinting technique to investigate the dominant transcripts expressed by the microbiota. In short, cDNA-AFLP visualizes transcript diversity after selective amplification of specific restriction fragments obtained from cDNA fragments synthesized from total RNA. Since no prior sequence knowledge about the genomic diversity is necessary for application of this method, we hypothesized that it should also be applicable to complex consortia of mostly uncultured micro-organisms, such as those found in the human GI tract. cDNA-AFLP was used to compare RNA and MicrobExpress enriched mRNA extracted from fecal samples collected at different time points. Subsequently, sequence analysis was performed to trace the corresponding expressed genes. This resulted in the identification of cDNAs that were characterized by sequence analysis. This revealed that 35% of them could encode prokaryotic proteins belonging to several classes that will be discussed. A minor fraction was assigned with eukaryotic origin. Furthermore, comparison of the temporal profiles revealed a relatively variable microbial transcriptome, while 16S rRNA gene-targeted PCR-DGGE profiles remained stable during this period. This study is the first to show that cDNA-AFLP can be applied for functional analysis of complex microbial ecosystems.

O-2 **Prokaryotic community of the mucosal fraction of human ileum: a metagenomic approach.** M.C. Leclerc[a], P. Robe[b], P. Marteau[c], R. Nalin[b], M. Gelfand[d], J. Doré[a] ([a] INRA, Jouy-en-Josas, France, [b] Libragen, Toulouse, France, [c] HEGP, Paris, France, [d] ITTP, Moscow, Russia).

A fosmid library of 20000 clones was constructed from an ileum segment obtained from a 50 years old individual. The sample was checked for its adequate sampling and representation of a "healthy" individual. To determine whether our metagenomic library adequately represents the dominant bacteria in the mucosal ecosystem and to compare this approach with the PCR based techniques, the same DNA was used to do 16S rDNA PCR-based molecular inventories, for both Eubacteria and Archaea. Ninety-six clones from the metagenomic library were randomly chosen, sub-cloned and full inserts were sequenced at CNS (French National Sequencing Center, Evry, France). Phylogenetic assignment of 84 fosmid inserts, with an average insert size of 40 kb, showed that 50 were part of the BPP (*Bacteroides-Prevotella-Porphyromonas*) phylum and 34 were members of the Firmicutes. Such composition compared well with the 16S rDNA diversity of the molecular inventory performed on the same DNA. However, the proportion of the phyla slightly differed between the PCR and the metagenomic approach, suggesting bias of the PCR or/and large insert cloning procedure. Annotation of 96 × 40 kb-fosmid inserts has been providing preliminary data on dominant genes of the ileum mucosa ecosystem. Among this set of putative 4 000 genes, 700 proteins have been so far identified. The presence of 30 genes coding for fibre-degrading enzymes or its regulation were detected within fosmid inserts belonging to BPP and Firmicutes phyla. This work demonstrates that the metagenomic approach can provide information on the functions of cultured and uncultured prokaryotic organisms of the ileum mucosa. A few examples of post-genomic strategies for studying the distribution and expression of these enzymes will be discussed.

O-3 **Identification DNA-microarray for the monitoring of intestinal bacteria during gastroenteritis.** K. Knösche[a], T. Hain[b], T. Chakraborty[b], E. Domann[b], T.T. Bachmann[a] ([a] Institute for Technical Biochemistry, University of Stuttgart, Allmandring 31, 70569 Stuttgart, [b] Institute for Medical Microbiology, University of Giessen, Frankfurter Str. 107, 35392 Giessen, Germany).

Bacterial gastroenteritis is one of the major reported infectious diseases in Germany, and internationally three to five billion cases of acute diarrhea occur yearly. Diarrheal diseases can quickly reach epidemic proportions, especially in cases of food-borne pathogens. Due to this fact enteritic pathogens have to be reported to the public health departments. Microarrays enable a parallel and fast identification of the particular pathogen and do not rely on cultivation of the organisms, which is often problematic in the case of intestinal bacteria. In the frame of the German national project PathoGenoMik, we developed an oligonucleotide microarray to identify 18 bacterial species and 13 genera comprising the most prevalent gastroenteritis-causing bacteria. Additionally, the array includes identification probes for the most important resident intestinal bacteria and common probiotic strains used for medical treatment. Therefore, the microarray enables the monitoring of the aetiopathology of inflammatory bowel diseases and the assessment of the therapeutical success with probiotic strains, which were shown in practical applications to be effective therapeutics. Following the multiple probe concept, we designed specific probes for the 16S and 23S rRNA genes applying the ARB software environment. For the validation of the array, clinical isolates were used and the results were confirmed by sequencing. The future impact of the microarray will be greatly enhanced by the combination with other DNA-microarray modules developed in PathoGenoMik (ESBL, quinolone resistance, pathoadaptive mutations, and parasitic protozoa) and the extension to a quantitative detection system to monitor population dynamics and spread of antibiotic resistance.

O-4 **Distribution of the main functional groups of microorganisms in the gut of IBS subjects.** C. Chassard[a], P. Marquet[a], C. Del'homme[a], C. Dubray[b], K.P. Scott[c], H.J. Flint[c], M. Dapoigny[d], G. Bommelaer[d], A. Bernalier-Donadille[a] ([a] INRA, Unité de Microbiologie, 63122 Saint-Genès-Champanelle, France, [b] Département de Pharmacologie clinique, CHU, Place Henri Dunant, 63000 Clermont-Ferrand, France, [c] Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK, [d] Service d'Hépato-Gastroentérologie, Hôtel-Dieu, 63000 Clermont-Ferrand, France).

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder characterized by abdominal pain and disturbances in bowel function. The causes of IBS are poorly known. Many factors have been proposed as possible reasons behind IBS, but recent studies suggest that the intestinal microbiota may play an important role in this pathology. An alteration in faecal flora of IBS patients has thus to be considered. In this context, we investigated the distribution of the main functional groups of micro-organisms in the gut of IBS subjects compared to that in healthy volunteers. All IBS patients selected for the study fulfilled the Rome II criteria for IBS. Both IBS and asymptomatic subjects ate an occidental diet including 10–15 g of dietary fibres. Faecal samples from both groups of volunteers were collected and processed within a few hours for analyses based on microbial cultivation. Extensive variation was observed in the gastrointestinal microbiota of IBS subjects compared to asymptomatic subjects. IBS patients showed important disturbances in microbial communities implicated in H_2 and lactate metabolism. In particular, the number of sulphate-reducing bacteria was significantly higher in IBS subjects compared to healthy ones ($P < 0.05$). Furthermore, the population levels of lactic acid bacteria (bifidobacteria, lactobacilli) as well as lactate-utilizing bacteria were significantly lower in IBS than in healthy volunteers. Disruption affecting functionally important groups of micro-organisms was therefore evident in the gut of IBS subjects. These observations will be completed by studying the distribution of the predominant groups of micro-organisms identified by molecular methods in faecal samples from IBS and healthy subjects.

O-5 **Culture-independent analyses of the temporal variation of the dominant faecal microbiota, *Clostridium* spp., *Bacteroides* spp., in Crohn's disease and colon cancer.** P.D. Scanlan, F. Shanahan, J.R. Marchesi (Alimentary Pharmabiotic Centre/Department of Microbiology, University College Cork, Cork, Ireland).

The human gastrointestinal microbiota shows host-specific diversity, temporal stability and significantly contributes to maintenance of a healthy gut. However, in inflammatory bowel disease (IBD) and colon cancer this microbiota

has been implicated as a contributory factor to the illness. The aim of the present study was to compare bacterial dynamics in Crohn's disease (CD) and colon cancer patients with a control group. This aim was achieved using a culture independent method to assess the temporal stability, relative diversity and similarity of the dominant fecal microbiota, *Clostridium coccoides* group, *Bacteroides* spp. and *Bifidobacterium* spp. in all individuals. Fecal samples were collected from CD and colon cancer individuals over several time points. DNA from the fecal samples was isolated and denaturing gradient gel electrophoresis (DGGE) profiles were generated for the different microbial groups by specifically targeting different regions of the 16S rRNA gene. The species profiles were compared on the basis of similarity (Sorenson's pair-wise similarity coefficient) and also various indices of diversity. Temporal stability and diversity was variable for both disease states when compared to the control group. Significant alterations observed in group-specific profiles for two functionally important mutualistic groups of bacteria in the gut indicate that the changes in these microbiota have implications for the host's gut health through loss of functionality, especially butyrate production.

O-6 **Serine protease autotransporters (SPATE) and autoaggregative adhesins from *Escherichia coli* are potential virulence factors in inflammatory bowel disease.** D.O. Krause, C.N. Bernstein, S. Sepehri, R. Kotlowski (University of Manitoba, Winnipeg, Manitoba R3Y 2N2, Canada).

Inflammatory bowel disease is a chronic digestive tract disease in humans made up of Crohn's disease and ulcerative colitis. The root of the disease is thought to lie at the intersect of a microbial antigen, a susceptible human genome, and a dysfunctional immune system. Culture independent methods can be used to identify bacteria that are uniquely associated with inflammatory bowel disease, and serve as a prelude to targeted cultivation. Isolation of bacteria is important, because ultimately the virulence factors associated with disease must be studied. We utilized 84 biopsies from 46 controls and IBD patients drawn from a unique population-based case-control study. Ribosomal intergenic spacer analysis (RISA) was conducted to identify unique species in biopsy tissue, and PCR analysis was used to identify virulence factors. RISA analysis identified unique bands in IBD biopsies which were identified as *Escherichia coli*. Targeted culture revealed a significantly higher number (4 logs) of *Enterobacteriacea* in IBD biopsies ($P < 0.05$). There were also significantly ($P < 0.05$) more serine protease autotransporters (SPATE) and adhesions in the *E. coli* from IBD biopsies. Together, the autoaggregative properties of the adhesions and the proteolytic and mucolytic activities of the SPATE's might explain the varied phenotypes of IBD. This is the first study in which SPATE's have been associated with IBD.

O-7 **Exploration of the human intestinal microbiota during antibiotic administration using microbiomics approaches.** J.K. Jansson[a], C. Jernberg[b], S. Löfmark[b], C. Edlund[b] ([a] Department of Microbiology, Swedish University of Agricultural Sciences, 750 07, Uppsala, Sweden, [b] Department of Laboratory Medicine, Karolinska University Hospital, 141 86, Stockholm, Sweden).

The use of molecular tools to characterize complex microbial communities, microbiomics, has great potential to increase our understanding of the microbial ecology of the human gut and the influence of diet, disease, antibiotics and probiotics on the gut microbiota. We aimed to study the impact of clindamycin administration for 7 days on the human intestinal microbiota using microbiomics approaches. In a preliminary study, the composition of the intestinal microbiota of healthy subjects returned more rapidly to pre-treatment levels in those that simultaneously ingested a probiotic as assessed by terminal restriction fragment length polymorphism (T-RFLP) analysis of DNA extracted from fecal samples. In a long-term study, disturbances in the composition of the human fecal microbiota of 4 healthy subjects, compared to 4 control subjects, were observed after 7 day clindamycin exposure for up to 2 years as assessed by T-RFLP using both general and *Bacteroides* specific primers. Although the total bacterial community only exhibited a transient change during clindamycin administration, there was a severe disruption of the specific *Bacteroides*

community. Even after 2 years post-treatment there was no sign of a return of the *Bacteroides* community to its original composition prior to clindamycin administration. Some *Bacteroides* clones that were typed by rep-PCR were shown to become resistant after treatment. In addition, increases in several antibiotic resistance genes, such as *ermF*, were found by real-time PCR in DNA extracted from the fecal samples of treated individuals. The results of these studies demonstrate that a combination of microbiomics approaches can define changes in the composition of the gut microbiota during antibiotic treatment. In the future, molecular fingerprinting tools may be used as a means to type the intestinal microbiota of individuals in order to design individually optimized drug treatments and diets to maintain a healthy gut environment.

O-8 **Murine gut microbiome: nature and nurture.** M.K. Friswell, P. Gilbert, D.G. Allison, I.J. Stratford, B.A. Telfer, A.J. McBain (School of Pharmacy and Pharmaceutical Sciences, University of Manchester, M13 9PL, UK).

Little is known of the impact of immune status or individual genotype on microbiome composition. We have used molecular ecological approaches to characterise the gut microbiome of various mouse strains, bred and housed in a common environment, to test the hypothesis that genotype and immune status dictate the eubacterial community faecal flora. Faecal samples were collected from different mouse strains, identically fed and housed and inbred within the same facility. Mouse strains (3–7 individuals) comprised: C3H (control strain); MF1 (T cell deficient); C57 (geriatric); CD1 (immunodeficient); GFEC (immunocompetent). C3H and MF1 strain mice were moved after weaning (4 weeks old), and as adults (8 weeks) to a different common environment and faecal flora was monitored. All samples were analysed by PCR-DGGE, utilising primers specific for the V2-V3 region of eubacterial 16S rDNA. Hierarchical dendrograms (cluster analysis) based on DNA sequence analysis were constructed to objectively compare microbial community fingerprints. Dominant DGGE bands were excised and sequenced for identity. Each mouse strain had a unique eubacterial faecal finger print, clustering individuals within hierarchical dendrograms (80–100% homology), whereas different strains converged at 60–65%. Sequence analysis showed dominant microflora to include *Bacteroides*, *Eubacterium* and *Campylobacter*. Faecal flora was conserved within strains from adulthood regardless of environment, yet animals relocated to different environments at weaning showed temporary dysbiosis leading to a new, stable microflora at eight weeks. These modified floras were again strain specific. Results clearly indicate that murine genotype and immune status, together with the microbial challenge from their environment during maturation, affect the adult microbiome.

P-1 **Sequence variation among α-toxin of *Clostridium perfringens* strains isolated from diseased and healthy chickens.** L. Abildgaard[a], R.M. Engberg[a], K. Pedersen[b], A. Schramm[c], O. Højberg[a] ([a] Danish Institute of Agricultural Sciences, Department of Animal Health, Welfare and Nutrition, PO Box 50, 8830 Tjele, Denmark, [b] Danish Institute for Food and Veterinary Research, Denmark, [c] University of Aarhus, Institute of Biological Sciences, Department of Microbiology, Denmark).

Necrotic enteritis is a severe gastrointestinal disease in broiler chickens caused by *C. perfringens* producing α-toxin (phospholipase C); a key virulence factor in the pathogenesis of gas gangrene in humans as well. The gene sequence and structure of the α-toxin may influence the pathogenesis. The α-toxin was recently reported to be highly conserved in C. *perfringens* isolated from diseased chickens. Variations were observed in only nine out of the 398 amino acid (aa) positions. We sequenced the α-toxin gene from further 60 strains of *C. perfringens*, isolated from diseased as well as healthy chickens. Overall the sequences showed close similarity (> 98% on the amino acid level) to the formerly reported chicken isolate sequences. Variations were observed in 23 aa positions, leading to 26 different α-toxin sequence types among the 60 isolates. Moreover, the gene sequence from three isolates had an insertion of 834 nucleotides at the 5' end corresponding to the N-terminal domain of the enzyme encompassing the catalytic site. The three strains were all isolated from healthy birds, but were different strains (different pulsed-field gel electrophoresis profiles). Studies are

being carried out to reveal whether these isolates produce α-toxins with phospholipase activity. In conclusion, a higher degree of variation in the aa sequences of α-toxin than reported earlier from chicken studies was found. The variation could not be connected to an outbreak of disease and it remains to be clarified, if phospholipase activity of the toxin is influenced by the observed variations.

P-2 **The bacterial community of the horse stomach.** R.A.M. Al Jassim[a], S.E. Denman[b], J.D. Hernandez[a], C.M. McGowan[a], F.M. Andrews[c], C.S. McSweeney[b] ([a] School of Animal Studies, Faculty of Natural Resources, Agriculture, and Veterinary Science, The University of Queensland, Gatton Campus QLD 4343, Australia, [b] CSIRO Livestock Industries, St. Lucia, 4067 Australia, [c] College of Veterinary Medicine, The University of Tennessee, USA).

Culture dependent and independent techniques were used to study the bacterial diversity of the horse stomach. Samples of stomach contents and lining were obtained from twenty horses post mortem and all samples were processed by: (1) culturing onto a pre-reduced modified agar MRS medium for enumeration and isolation of lactic acid producing bacteria and (2) extraction of genomic DNA and analysis by denaturing gradient gel electrophoresis (DGGE). Plugs were taken from DGGE bands for cloning and further DNA analysis and sequencing. The numbers of colony forming units (CFU) in stomach contents cultured on MRS-agar medium averaged 2.3×10^7. With the exception of *Lactobacillus salivarius*, which was detected in both techniques, other isolates belonging to the genus *Lactobacillus* grown on MRS medium were different from those belonging to the same genus in the clone library. Cultured bacteria clustered with bacterial species belonging to the genera *Lactobacillus, Streptococcus* and *Clostridium*, while DGGE clones belonged to the genera *Propionibacterium, Clostridium, Lactobacillus, Prevotella, Pasteurella*, and *Pseudomonas*. Other fewer clones were closely related to *Escherichia, Actinobacillus, Moraxella, Rhodococcus, Veillonella, Legionella* and *Eubacterium*. Results of this study demonstrated that lactic acid producing and spore forming bacteria are capable of surviving the acidic conditions of the stomach and are associated with the contents and the lining of both the healthy and ulcerated stomachs. This is the first report on the bacterial diversity of the stomach of the horse. Work is in progress to identify bacterial changes associated with changes of diet and management of thoroughbred horses and the possible involvement of bacteria and their products in the pathogenesis of stomach ulceration in horses.

P-3 **Detection and activity of a bacteriocin produced by *Lactobacillus* strains.** F.N. Allouche, A. Hellal, A. Laraba (Laboratoire Bioénergie et Environnement, Centre de développement des Énergies Renouvelables, BP 62, route de l'Observatoire Village Céleste, Bouzareah, Algeria).

Ten strains of *Lactobacillus* isolated from raw cow milk and commercial dairy starter were shown to be antagonistic towards several foodborne pathogens. These strains produced and excreted an antibacterial substance in MRS broth which inhibited the growth of *Bacillus subtilis, Staphylococcus, Escherichia coli* and *Pseudomonas aeruginosa.* The study of growth kinetics and excretion permitted to choose only one strain of *Lactobacillus acidophilus* as a model for production and purification of bacteriocin. The bacteriocin of *Lactobacillus acidophilus* Lba1 was isolated and purified by acid extraction and reversed-high-performance liquid chromatography. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) indicated a homogeneous protein with an estimated molecular weight of 30 000 Da. A preliminary characterisation showed that the inhibitory substance was heat-labile; activity was detected at pH 5. Bacteriocinogenic activity was destroyed by autoclaving at 121 °C for 20 min. Bacteriocin was stable at 45 °C and over the pH range 5 to 6. It was sensitive to proteolytic enzymes, indicating that the active moiety of the inhibitor was proteinaceous in nature. These properties suggested that the inhibitory substance could be considered as a bacteriocin-like substance.

P-4 **Quantification of protozoal concentrations in the rumen and duodenum of growing lambs using real-time PCR: effect of age.** A.

Belanche[a], G. De la Fuente[a], L. Calleja[a], D.R. Yañez-Ruiz[b], C.J. Newbold[b], J. Balcells[a], M. Fondevila[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Spain, [b] Institute of Rural Sciences, University of Wales, Aberystwyth, UK).

Although protozoa contribute 50% of the microbial mass in the rumen, the contribution of protozoa to microbial flow to the duodenum remains unknown. The presence of protozoa in rumen contents and their contribution to microbial N in duodenal chymus was studied in 3 groups of 5 Rasa Aragonesa lambs that were slaughtered at 30 (only milk-fed lambs), 45 (weaning lambs, being fed on milk, concentrates and straw) and 90 (end of fattening period, given concentrates and straw) days of age. Bacterial and protozoal standards were extracted from rumen contents by differential centrifugation and sedimentation-filtration, and their purity was 97.92 ± 1.23 and 61.48 ± 9.89%, respectively. Genes encoding bacterial 16S rDNA and protozoal 18S rDNA subunits were quantified by real-time PCR. Total rumen bacterial DNA was 16.05 ± 6.02, 22.52 ± 3.70 and 19.11 ± 2.65 ng DNA/g FM for 30, 45 and 90 d lambs and similar among groups for the chymus samples (5.32 ± 6.88 ng DNA/g FM). Protozoal DNA appeared in the rumen of 2 milk-fed lambs (3.03 ± 0.81 ng DNA/g FM), was almost nil at weaning and could only be detected in the rumen of 2 out of 5 of the fattened lambs (11.82 ± 1.73 ng DNA/g FM), showing that optimal environmental conditions for protozoa are slowly acquired after weaning with a high individual variability. Estimations by real-time PCR were validated by microscopy counts. Protozoal DNA in the chymus was in concordance to rumen values. Estimated contribution of protozoa DNA to duodenum was only significant in 90 days faunated lambs (3.53 ± 0.93 ng DNA/g FM).

P-5 **Identification of antibiotic resistant genes in *Streptococcus thermophilus* using microarray.** G. Berruti[a], A.H.A.M. van Hoek[b], H.J.M. Aarts[b], L. Tosi[a], L. Morelli[a], M. Callegari[a] ([a] UCSC, Istituto di Microbiologia, Piacenza via Emilia Parmense 84, 29110 Piacenza, Italy, [b] RIKILT, Institute of Food Safety Wageningen University, Wageningen, The Netherlands).

The increase of antibiotic resistant (AR) populations in the food chain community is now well recognized as a source of antimicrobial resistance determinants for the gut flora and is generally caused by horizontal gene transfer. In order to increase the understanding of the molecular biology and ecology of AR determinants and their hosts, our interest should not only be limited to pathogenic bacteria but also take account of bacteria coming from food-related environments, like *S. thermophilus*, for which very few data are available. These bacteria might not represent a clinical risk in themselves, but they can be vehicles of AR genes. Here we report the assessment of a 50 and 60-mer oligonucleotide DNA-based microarray for the identification of AR genes in a significant number of *S. thermophilus* strains. The AR genes represented by the oligonucleotides on the microarray belong to: aminoglycoside, extended spectrum β-lactamase (ESBL), chloramphenicol, macrolide lincosamides and streptogramin (MLS) group, sulfonamide, tetracycline, trimethoprim and vancomycin. All strains were obtained from the UCSC culture collection, except for 11 strains that were isolated from raw milk during the study, and all originated from dairy products. To validate the results obtained by microarray analysis all isolates were subjected to PCR and MIC testing on Iso-sensitest medium (with lactose 1% w/v) based on a microdilution method. *tetS* and *ermB* genes were found and sequenced in different *S. thermophilus* isolates. Although for streptomycin very high MIC values were observed no streptomycin related genes were found by microarray analysis.

P-6 **Optimizing growth conditions for ruminal ciliates in the in vitro system Rusitec.** C.P.J. Bonnet[a], L. Meile[b], M. Kreuzer[a], C.R. Soliva[a] ([a] Institute of Animal Science, Animal Nutrition, Switzerland, [b] Institute of Food Science and Nutrition, Laboratory of Food Biotechnology, ETH Zurich, 8092 Zurich, Switzerland).

The Rumen Simulation Technique (Rusitec) was shown to maintain ruminal ciliates during ≥ 15 days of in vitro incubation. In order to guarantee high and stable ciliate numbers, there is still a need to improve Rusitec conditions and operation protocol. Therefore, two Rusitec trials were conducted investigating the effect of different modifications on ciliate growth. In the

first trial, carried out with bovine ruminal fluid and lasting for 15 days, three modifications were tested; (i) polyurethane sponge was bedded as a matrix into the fermenters to protect the ciliates from being washed out of the system, (ii) the pore size of the feed bags was increased from 100 to 200 µm, and (iii) the buffer flow rate was halved while keeping the buffering capacity constant. In the second trial, lasting for 10 days, the effect of two incubation media on the growth of isolated or cryo preserved/revitalized *Entodinium* spp. was tested in the presence of live ruminal bacteria. One medium consisted of defaunated bovine ruminal fluid, the other one of the ciliate cultivation medium of Dehority, commonly used in batch cultures. No conclusive effects on ciliate growth of sponge addition, varied feed bag pore size or reduced buffer flow rate were observed. The larger bag pore size, however, resulted in an increased apparent feed degradation and higher fermentation gas formation. Additionally, an increased bacterial population size was found with the larger bag pore size. *Entodinium* spp. were successfully cultivated in both media, but growth conditions were more favorable in the Dehority medium than with defaunated ruminal fluid.

P-7 **Isolation of bacteriophage active against *E. coli* O157:H7 and *Salmonella* spp. from cattle and swine.** T.R. Callaway[a], T.S. Edrington[a], A.D. Brabban[b], E.M. Kutter[b], R.C. Anderson[a], R.B. Harvey[b], D.J. Nisbet[a] ([a] USDA/ARS, Food and Feed Safety Research Unit, College Station, TX, USA, [b] Evergreen State College, Olympia, WA, USA).

Bacteriophage are fairly common members of the gastrointestinal microbial ecology. However, the incidence of bacteriophage in mammals has not been examined. Bacteriophage have been proposed as potential strategies to reduce foodborne pathogenic bacteria, especially *E. coli* O157:H7 and *Salmonella* spp. If phage are to be used in this fashion, we must understand what role they play in the microbial ecology of the gut. From a regulatory aspect, the incidence of phage is necessary information prior to the approval process. Therefore the current study was designed to determine the incidence of phage active against *E. coli* O157:H7 and *Salmonella* in the feces of commercial feedlot cattle and commercial finishing swine in the United States. Fecal samples ($n = 60$) were collected from each of four feedlots in two Great Plains states and from each of four commercial swine finishing operations in one state. Samples ($n = 6$) were collected from 10 randomly selected pens throughout the operation. The total number of isolates collected from each species was $n = 240$. *Salmonella* and *E. coli* O157:H7 were found in 3.8% and 11.7% of the cattle fecal samples, respectively. Bacteriophage active against *E. coli* O157:H7 were isolated from 15% of the individual cattle fecal samples in 55% of the cattle pens. Swine results were similar for *Salmonella* spp. and phage active against *Salmonella* spp. Results indicate that bacteriophage are fairly widespread across commercial production facilities in both cattle and swine, indicating that they could potentially be used as a food safety intervention strategy.

P-8 **An animal model that reproduces microbial disruption observed in the gut of IBS subjects: a new animal model for IBS?** C. Chassard[a], C. Del'homme[a], P. Marquet[a], M. Dapoigny[b], G. Bommelaer[b], A. Bernalier-Donadille[a] ([a] INRA, Unité de Microbiologie, 63 122 Saint-Genès-Champanelle, France, [b] Service d'Hépato-Gastroentérologie, Hôtel-Dieu, 63000 Clermont-Ferrand, France).

Irritable bowel syndrome (IBS) is the most common chronic gastrointestinal disorder. However, the origins of IBS remain not clearly understood. Many factors have been proposed as possible reasons behind IBS, recent studies suggesting that intestinal microbiota may play an important role in this pathology. We recently evidenced important disruption of different functional groups of microorganisms in the gut of IBS subjects. Further investigations on mechanisms involved in the physio-pathology of IBS, as well as those concerning new IBS therapy, would need suitable in vivo models. Our objective was to develop an animal model able to reproduce the microbial disruption observed in the intestinal flora of IBS subjects and to analyse the effect of this flora on the host. Germ-free rats were inoculated with faecal microflora from either IBS patients fulfilling the Rome II criteria or healthy subjects. Distribution of the main functional groups of microorganisms establishing in the gut

of human-flora associated (HFA) rats and total gas excretion by these animals were analysed and compared for each, IBS and healthy HFA rats. Similar disruptions in functional groups of microorganisms were shown in IBS-HFA flora rats and in IBS subjects, disturbances in microbial communities involved in H_2 and lactate metabolism being evidenced in the gut of the model as well as IBS subjects. In addition, an extensive increase in H_2-excretion of IBS-HFA rats was observed compared to healthy-HFA rats. IBS-HFA rats could thus be considered as an animal model reproducing microbial disruptions characterizing IBS gut flora. Further studies are underway to evaluate the impact of this IBS gut flora on the animal host.

P-9 **A comparison of *Clostridium difficile* carriage in inflammatory bowel disease and irritable bowel syndrome out-patients.** E.M. Clayton[a,b,c], M.C. Rea[a,b], F. Shanahan[b,d], E.M. Quigley[b,d], C. Hill[b,c], R.P. Ross[a,b] ([a] Moorepark Food Research Centre, Teagasc, Fermoy, Co. Cork, Ireland, [b] Alimentary Pharmabiotic Centre, National University of Ireland, Cork, Ireland, [c] Department of Microbiology, National University of Ireland, Cork, Ireland, [d] Department of Medicine, Cork University Hospital, Cork, Ireland).

Irritable bowel syndrome (IBS) is a common gastrointestinal (GI) disorder characterized by abdominal pain and disturbances in bowel function. The causes of IBS are poorly known and symptoms vary from constipation to diarrhoea. The condition is diagnosed in the absence of detectable clinical pathology. Crohn's disease and ulcerative colitis are idiopathic inflammatory disorders known collectively as inflammatory bowel disease (IBD). Both disorders may present conditions ranging from excessive bowel movements to vomiting and weight loss. Toxigenic *Clostridium difficile* is the leading identified cause of diarrhoea and colitis associated with antibiotic therapy. It has been well documented that the organism spreads nosocomially and causes hospital outbreaks of *C. difficile*-associated diarrhoea (CDAD). Certain individuals can become asymptomatic carriers of toxigenic *C. difficile*, whilst others develop severe colitis and multiple relapses depending on the speed of the immune response. The aim of this study was to determine, whether patients suffering from IBD and IBS have a higher carriage rate of *C. difficile* than asymptomatic healthy volunteers. *C. difficile* was selected from stool samples on Cycloserine Cefoxitin Egg Yolk (CCEY) agar and phenotypic isolates were confirmed by 16S rDNA sequencing. The genetic relatedness of *C. difficile* at a strain level was characterized by genomic fingerprinting (PFGE). Toxicity testing of the resultant isolates demonstrated that up to 53% of *C. difficile* from IBS and IBD patients were toxigenic compared to only 10% of those from healthy people.

P-10 **Activity spectrum of reuterin, a broad spectrum antimicrobial produced by *Lactobacillus reuteri* from glycerol, on intestinal bacteria.** V. Cleusix, G. Le Blay, S. Vollenweider, C. Lacroix (ETH-Zentrum, 8092 Zurich, Switzerland).

Reuterin produced from glycerol by *Lactobacillus reuteri,* a normal inhabitant of the human intestine, is a broad spectrum antimicrobial agent. Reuterin is active against intestinal pathogens, yeasts, fungi, protozoa and viruses, but its effect upon the commensal intestinal bacteria is unknown. The aim of this study was to test the activity of reuterin against a representative panel of intestinal bacteria. Reuterin was produced with cells of *L. reuteri* in pure glycerol solution (85% yield). After cell elimination by centrifugation, the supernatant was filter-sterilized, lyophilized and reuterin was purified with acetone/ethyl acetate on a silica gel column. Purity of reuterin was checked with the tryptophane-HCl assay, using acrolein as standard. Minimal inhibition (MIC) and minimal bactericidal concentrations (MBC) of reuterin were determined for different strains belonging to *Bifidobacterium* (6), *Bacteroides* (3), *Lactobacillus* (6), *Eubacterium* (2) Gram-positive cocci (2), *Clostridium coccoides* (4)*, Escherichia coli* and *Listeria* (2), using a twofold serial dilution microplate assay under anaerobic conditions. A supplemented BHI broth, reduced with titanium citrate and allowing a high growth for all tested bacteria, was used for the activity test. The most resistant bacteria to reuterin were the 2 strains of *L. reuteri* (MICs 30–50 mM) followed by the other lactobacilli and *Clostridium clostridioforme* (MICs 15–40 mM). *E. coli*, *Ruminococcus productus, Bifidobacterium breve* and *Listeria innocua* exhibited intermediary sensitivities

(MICs: 7.5–15 mM), followed by the other *Bifidobacterium* spp., *Streptococcus salivarius* and 2 of the 3 *Bacteroides* tested (MICs 1.9–3.8 mM). The most sensitive strains to reuterin were *Bacteroides vulgatus* and *Clostridium difficile* (MICs 1.9 mM). Our results suggest that reuterin could be used to selectively inhibit a number of intestinal pathogens such as *Clostridium difficile*, while preserving health-promoting commensal bacteria of the human gastrointestinal tract.

P-11 ***Lactobacillus reuteri*** **decreases *E. coli* concentration in the presence of glycerol in an in vitro model of colonic fermentation.** V. Cleusix, G. Le Blay, S. Vollenweider, C. Lacroix (ETH-Zentrum, 8092 Zurich, Switzerland).

Lactobacillus reuteri is a well-known probiotic culture that produces, in the presence of glycerol, a potent broad spectrum antimicrobial substance named reuterin, active against many intestinal pathogens in vitro. Moreover, *L. reuteri* has been shown to prevent intestinal infections in vivo. However, the mechanism of action and the potential implication of reuterin are not known. In this study, we tested the effect of *L. reuteri* on the gut microbiota and its capacity to secrete reuterin in situ using a novel in vitro colon model mimicking conditions of the ascending colon. A fecal sample collected from a healthy person was immobilized in polysaccharide gel beads under anaerobic conditions. Beads were inoculated in two reactors, run in parallel for 60 days and continuously fed with a nutritive medium simulating the adult ileal chime. The effects of not adding or adding daily 10^8 cfu·mL^{-1} of *L. reuteri* without and with different glycerol concentrations (0, 10 and 100 mM) were tested on the major intestinal bacteria populations in effluent samples with fluorescence in situ hybridization. Short chain fatty acids, 1,3-propanediol and reuterin concentrations were quantified by HPLC. The adjunction of 100 mM glycerol induced an increase in 1,3-propanediol concentrations in the presence (15.6 ± 4.2 vs 1.9 ± 0.5 mM) or absence (37.0 ± 4.3 vs 2.1 ± 0.7 mM) of *L. reuteri*, whereas no reuterin was ever detected. Moreover, glycerol induced a decrease in *E. coli* populations in the presence of *L. reuteri*, whereas the adjunction of *L. reuteri* alone had no effect. These data suggest that the inhibition of *E. coli* by *L. reuteri* in the presence of glycerol could derive from the in situ production of reuterin despite its absence of detection in the fermented medium. Indeed, reuterin is a very reactive compound that is usually not detectable in complex media.

P-12 **Characterization of *Lactobacillus* microbiota from the duodenum of organically farmed pigs.** P.S. Cocconcelli[a,b], A. Gentili[a], M.L. Callegari[a,b] ([a] Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore, Via Milano 24, 26100, Cremona, Italy, [b] Istituto di Microbiologia, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy).

The *Lactobacillus* population of intestinal scraped samples collected from three organically farmed pigs was studied using molecular biology techniques, including cultivation independent DGGE analysis, and an isolation based approach, where strains were taxonomically identified by species-specific PCR, ARDRA and 16S rDNA sequencing. The dominant species detected by DGGE analysis were *L. reuteri*, *L. crispatus*, *L. vaginalis* and *L. fermentum*, while *L. johnsonii*, *L. reuteri* and *L. mucosae* were identified using cultivation-based approaches. RAPD fingerprint demonstrated that each subject harboured a unique collection of lactobacilli. Strains were analysed by physiological assays in order to evaluate their probiotic capacity. The resistance of strains to synthetic gastric juice and the ability to adhere to the intestinal epithelium was also evaluated. A hydrophobicity test and the presence of genetic determinants coding for important adhesion factors were assessed to determine potential colonisation ability. A susceptibility test performed on isolated lactobacilli to antimicrobials of clinical and veterinary importance revealed that 31% of the analysed strains were sensitive to all 11 antibiotics tested.

P-13 **Monitoring major ruminal bacteria in sheep during adaptation to *Neotyphodium coenophialum*-infected tall fescue straw with real-time PCR.** M.J.M. De Lorme, S.L. Lodge-Ivey, A.M. Craig (College of Veterinarian Medicine, Oregon State University, Corvallis, OR 97330, USA).

Tall fescue toxicosis is a disease caused by ergot alkaloids produced by grass/straw-infesting fungal epiphytes. Sheep have a tolerance above that of other grazing animals to fescue toxicosis. This increased tolerance supports the belief that there are unique microorganisms or groups of microorganisms in the rumen of sheep capable of detoxifying alkaloids, primarily ergovaline, produced by the fungal endophyte, *Neotyphodium coenophialum*. Six major sheep ruminal bacteria were monitored using real time PCR and six group specific primer sets as well as a universal bacterial primer set during the feeding and adaptation to *Neotyphodium coenophialum*-infected tall fescue straw. Serum prolactin levels were monitored as indicators of toxicosis. Serum prolactin was decreased by approximately 72% in sheep exposed to 500 ppb of ergovaline as compared to the control group, indicating clinical fescue toxicosis. *Prevotella bryantii* $B_1$4 and the *Streptococcus* group (*S. bovis, S. caprinus, S. equines)* were detected in low levels through the entire feeding period and were not different between treatment groups. *Selenomonas ruminantium-Mitsuokella multiacida* JCM6582 was the most abundant organism found in the samples and *Eubacterium ruminantium* (ATCC 17233) was undetectable in most samples over all sampling times. *Ruminococcus flavefaciens* (ATCC 19208T) sequence was detected in moderate levels through the entire feeding period and levels were approximately the same between treatment groups. *Ruminococcus albus* was detected in low levels through the entire feeding period and was not affected by treatment. These results shows no change in the bacterial populations monitored, indicating that either the detoxification does not cause a population shift or the organism(s) responsible for the detoxification has yet to be identified.

P-14 **Phylogenetic analysis of TNT degrading enrichments isolated from ovine rumen fluid.** M.J.M. De Lorme, A.M. Craig (College of Veterinarian Medicine, Oregon State University, Corvallis, OR 97330, USA).

The clean up and degradation of munitions wastes is a growing concern as environmental restrictions increase. One of the major munitions wastes in the United States is 2,4,6-trinitrotoluene (TNT). Anaerobic microbes found in the rumen are well equipped with reductive enzymes that are often responsible for the rapid reduction of TNT in bioremediation systems. Previous work has shown that whole bovine rumen fluid is capable of rapidly degrading TNT in vitro. Two TNT-degrading enrichments were cultivated from ovine rumen fluid in order to assess the TNT degradation potential of ovine ruminal bacteria. Each enrichment was capable of degrading 100 ppm TNT in less than 24 h. Full 16S rDNA was amplified from the enrichments using universal eubacterial primers, then cloned and sequenced in order to identify individual members. In one enrichment designated 3–1, 91% of clones had high sequence similarity (99%) to *Enterococcus faecium* and *E. durans*. The remaining clones had high similarity to *Lactobacillus* species with the strongest association to *L. mucosae* (99%). The other enrichment, designated 4–1, produced only one sequence type with a 99% similarity to *Streptococcus bovis*. These results indicated that more than one species of bacteria from the rumen is capable of degrading TNT. We feel it may be possible to use sheep as bioreactors for the bioremediation of munitions wastes. However, in vivo studies and assessments of toxicity in the host animal are still to be addressed.

P-15 **Stx2 synthesis in pathogenic *Escherichia coli*: transcriptional analysis, secretion and activity.** T. De Sablet, Y. Bertin, A.P. Saint-Gobert, J.P. Girardeau, C. Martin (INRA Clermont-Ferrand-Theix, Unité de Microbiologie, 63122 Saint-Genès-Champanelle, France).

Shiga-toxin producing *Escherichia coli* (STEC) are associated with food-borne infections causing human diseases, ranging from uncomplicated diarrhoea to hemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS). Cattle and other ruminants are the main reservoir of STEC strains. Shiga-toxins (Stx) are the most critical virulence factors responsible for the principal manifestations of HUS and HC, and it has been suggested that the varied capacity of STEC strains to cause serious disease in humans is associated with the type and/or amount of Stx produced. We determined the *stx2* alleles and cytotoxicity of a total of 42 Stx2-producing STEC obtained from patients or from healthy cattle. Nine strains belong to the seropathotype

A, containing O157:H7 LEE-positive strains, which are the most frequently associated with severe outbreaks in the world. The remaining strains belong to seropathotype C that include mainly non-O157:H7, LEE-negative strains frequently associated with sporadic cases of HUS. Then, a subset of 24 strains was analyzed for *stx2* transcription and Stx2 secretion, with and without mitomycin C induction. The *stx2* allele was mainly associated with the most pathogenic human strains belonging to the O157:H7 serotype. In contrast, *stx2-*$_{vha}$ and *stx2-*$_{vhb}$ were mainly associated with strains belonging to the less virulent seropathotype C. In addition, our results suggest that the higher virulence of strains producing the Stx2 variant could be related to the higher expression of the gene and to the higher secretion of its product, in basal as well as in induced conditions.

P-16 **Enumeration in cattle of verocytotoxigenic *E. coli* containing *ehxA* and *eaeA* virulence genes.** S.E. Denman, R.A. Gilbert, J. Padmanabha, C.S. McSweeney (CSIRO Livestock Industries, Queensland Bioscience Precinct, Queensland 4067, Australia).

In order to enumerate putative enterohaemorrhagic *E. coli* populations present in bovine samples, a method based on DNA hybridisation and hydrophobic grid membrane filter (HGMF) technology was developed. In contrast to previously published HGMF methods, which use single DNA probes to detect virulence factors such as the shiga toxin genes, this method utilises several DNA probes and colour detection, allowing for the identification of multiple virulence genes within bacterial isolates on replicate HGMF membranes. Molecular probes were developed for EHEC virulence factors encoded by the genes for enterohemolysin (*ehxA*), intimin (*eaeA*), as well as the shiga toxin genes, stx_1 and stx_2. In this way, it was possible to identify *E. coli* strains incorporating any combination of these virulence genes, thus enabling the specific enumeration of potential EHEC strains, containing a full genetic complement for enterohaemolysin, intimin and at least one shiga toxin gene. HGMF analysis of these populations also indicated that diet did have an effect on the prevalence of these *E. coli* subpopulations, however the incidence and overall concentrations of faecal populations were low (< 3 $\log_{10}$ MPN per g faeces). Within the detection limits of HGMF analysis, results also indicated that the hide and carcase of animals found to shed putative EHEC in their faeces, were not subsequently contaminated with these organisms.

P-17 **The impact of lifestyle on the human child intestinal microbiota.** J. Dicksved[a], H. Flöistrup[b], A. Scheynius[c], J.K. Jansson[a] ([a] Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden, [b] Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden, [c] Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute and University Hospital, Stockholm, Sweden).

The use of molecular tools has recently enabled detailed exploration of the composition of the human intestinal microbiota. To date, however, few studies have taken the known large inter-individual variation into consideration and most studies are based on few analyzed individuals. In addition, factors that contribute to the microbial composition in the human intestine are still unknown. In this study we used the molecular profiling approach, terminal-restriction fragment length polymorphism (T-RFLP), in combination with multivariate statistics, to assess the microbial community structure in fecal samples of 90 children from three European countries. In particular, we studied how certain lifestyles impact on the microbial community composition. We found significant differences in the diversity of the fecal microbiota in children from families with an anthroposophic lifestyle compared to children living on farms. In addition, we observed that several of the characteristics associated to the anthroposophic lifestyle, such as a restricted use of antibiotics and a diet mainly based on biodynamically or organically produced food items, also correlated with a higher microbial diversity. Lactic acid bacteria and related species were also specifically monitored by T-RFLP. The lactic acid bacterial communities in the gut could be divided into two groups depending on the presence or absence of specific dominant populations. However, the functional significance of these dominant populations is currently not known. In conclusion, the ecology of the intestinal tract requires more exploration

to understand the mechanisms that drive the microbial composition and to couple composition to function.

P-18 **Development and application of RNA and DNA based DGGE to study the bacterial colonisation of fresh perennial ryegrass in the rumen.** J.E. Edwards, S.A. Huws, E.J. Kim, A.H. Kingston-Smith, N.D. Scollan (Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK).

Currently, the temporal sequence by which rumen bacteria colonise fresh plant material is poorly understood. Analysis of population changes by molecular techniques has been limited by dominant amplification of chloroplast-derived 16S rDNA. Development of a new primer pair for profiling bacterial populations by Denaturing Gradient Gel Electrophoresis (DGGE) has resolved this, enabling investigation of bacterial colonisation of fresh perennial ryegrass in the rumen, using both RNA and DNA as molecular markers. Sequence analysis of the dominant bands in DGGE profiles of fresh plant material confirmed that the new primers detected 16S rDNA from epiphytic bacteria, not chloroplasts. The methodology was applied to perennial ryegrass previously incubated *in sacco* in the rumens of two grazing, cannulated cows, and machine washed. Duplicate polyester bags gave consistent DGGE profiles. The plant epiphytic samples formed a distinct group from the rumen incubated samples, although RNA and DNA based profiles clustered differently within this. Rumen incubated samples clustered together regardless of which molecular marker was used, and no differences between the colonising bacterial populations were observed at the 5, 10, 15 or 30 min time points. These results demonstrate that the process of bacterial colonisation of fresh forage in the rumen can be studied using either DNA or RNA. While population changes relating to colonisation of perennial ryegrass by rumen bacteria occurred in the first 5 min, population diversity was then consistent until 30 min of ruminal incubation. It is possible that changes in population sizes were also occurring over this period of apparent stability.

P-19 **A comparison of colon and faeces microbial diversities in horses using biomolecular techniques.** C. Faubladier[a], V. Julliand[a], L. Veiga[a,b], F. Chaucheyras-Durand[b,c] ([a] ENESAD, Dijon, [b] Lallemand Animal Nutrition, Blagnac, [c] Unité de Microbiologie, INRA, Saint-Genès-Champanelle, France).

Some limitations in the knowledge of the microbial diversity in the equine digestive ecosystem are linked to the difficulty in studying non cultivable micro organisms. New techniques based on molecular analysis could be used to describe the variety of microbial populations and to study their evolution depending on the different compartment. Our objective was to evaluate the usefulness of TTGE (Temporal Temperature Gel Electrophoresis) and RISA (Ribosomal Intergenic Spacer Analysis) to compare colonic and faecal microbial diversities in horses. Four mature geldings, fitted with a right ventral colon cannula, received a morning meal providing a minimum of 200 g/100 kg BW of starch. After fourteen days of adaptation to the diet, faecal and colonic contents were collected five hours after the morning meal in order to perform biomolecular analysis (RISA and TTGE). DNA was extracted, amplified by PCR and separated on adapted gels. Similarity analysis of the electrophoresis gels was performed using the program Diversity database BIO RAD. Preliminary data highlighted strong individual differences in microbial profiles. Similarity indexes from comparison of banding patterns have been determined for two animals. The microbial profiles were almost identical between faecal and colonic content for one horse (95–100% similarities with RISA; 77–95% with TTGE) but were different for the other one (23% with RISA; 41% with TTGE). More analyses are in progress. The results obtained in this study will demonstrate if faeces are representative of the colon microbial diversity. Furthermore, using RISA and TTGE, it will be possible to compare the impact of different factors on the microbial diversity in both colonic and faecal ecosystems.

P-20 **Molecular phylogeny of the Neocallimasticales (Mycota) based on ribosomal RNA genes: exploring the root of the fungal kingdom.** K. Fliegerová[a], K. Voigt[b] ([a] Institute of Animal Physiology and Genetics Academy of Sciences of Czech Republic, Prague, Czech

Republic, [b] Institute of Microbiology, Friedrich-Schiller University, Jena, Germany).

Anaerobic fungi inhabit the gastrointestinal tract of herbivores, especially ruminants, and make a significant contribution to rumen metabolism, particularly to the digestion of plant structural material. Anaerobic fungi exhibit two basic forms, with nuclear migration throughout the hyphal mass for polycentric species (*Anaeromyces*, *Orpinomyces*) and with concentration of nuclear material in a zoosporangium for monocentric species (*Neocallimastix, Piromyces, Caecomyces*). All genera of rumen fungi have been assigned to the family Neocallimasticaceae of the class Chytridiomycetes. However, gut fungi differ significantly from other chytrids in many aspects, and the relationship between rumen anerobic fungi and aerobic chytrids is not answered satisfactorily. Also chytridiomycete fungi are well known to be direct descendants from the common ancestor of all true fungi and are therefore at the root of recent fungi. We are going to present a comparative phylogenetic analysis of rumen anaerobic Chytridiomycetes (Neocallimasticales) with special emphasis on polycentric genera in comparison to aerobic chytridiomycetous and zygomycetous fungi based on all available 18S and 28S rDNA sequence data. We have investigated partial SSU rDNA fragments using primer pair NS1 and NS2 and partial LSU rDNA fragments using the primer pair NL1 and NL4. Sequence analysis of these spacers has been applied to thirteen strains of gut anaerobic fungi (*Orpinomyces*, *Anaeromyces*, *Neocallimastix*) and eleven strains of aerobic chytrids and zygomycetes (*Mucor, Hyphochytrium, Allomyces, Blastocladiella, Catenaria, Phlyctochytrium, Nowakowskiella, Monoblepharis*). Since chytridomycetes are believed to be the most basal lineage of the kingdom Mycota, our investigations aim at the phylogenetic reconstruction of the root of the Fungi. At the same time we own a reliable tool for molecular taxonomical identification of obligate biotrophic anaerobic fungi, for which cultivation in artificial culture is rather tedious.

P-21 **Effect of two energy and two protein sources in sugar cane based diets on the population of rumen ciliate protozoa in water buffalo (*Bubalus bubalis*) and zebu cattle (*Bos taurus indicus*).** R. Franzolin, F.P. Rosales, W.V.B. Soares (Universidade de Sao Paulo, FZEA, Pirassununga-SP, Brasil).

The true role of the protozoa population in the rumen still is not clear, since wide differences occur among ruminant animal species, feeding systems and environmental conditions around the world. These organisms have survived in the rumen for thousands of years and knowledge of their function may provide a key to improving animal production and preservation of the environment. The effect of diet upon both rumen protozoa concentrations and generic distribution is obvious. In general, *Entodinium* species represent more than 80% of the population for most domestic ruminants even under grazing conditions. However, our previous studies have shown that a different population is generally found in water buffalo. Four rumen fistulated buffaloes and four zebu cattle, in two 4 × 4 Latin Square design experiments, were fed with chopped sugar cane (60% DM) and a 2 × 2 factorial schedule of energy sources (corn grain vs. citrus pulp) and protein (urea vs. soybean meal). Zebu cattle had higher rumen protozoa concentrations than buffalo. Generic composition was significantly different between buffalo and cattle; average percentage compositions were *Entodinium* (61.0% vs. 84.9%), subfamily Diplodiniinae (28.6% vs. 1.4%), *Epidinium* (5.3% vs. 0.0%) and *Dasytricha* (2.6% vs. 11.5%), respectively. Percentages of *Entodinium* and Diplodiniinae species were higher in cattle fed ground corn as the energy source, whereas the same species were higher in buffalo when urea was the protein source. Sugar cane based diets maintained much higher percentages of *Dasytricha* in the rumen of cattle. Comparing the concentration and composition of rumen ciliate populations in these two animal species confirms the previous observations that the fauna of water buffalo differs considerably from that of other ruminants under similar feeding and environmental conditions.

P-22 **Bacterial diversity and antibiotic resistance in colon contents from hooded seals (*Cystophora cristata*) in the Greenland Sea.** T. Glad[a], K.M. Nielsen[a], L. Nordgård[b], M.A. Sundset[c] ([a] Department of Pharmacology and Institute of Pharmacy, University of Tromsø,

Tromsø, Norway, [b] Norwegian Institute of Gene Ecology, Tromsø, Norway, [c] Department of Arctic Biology and Institute of Medical Biology, University of Tromsø, Tromsø, Norway).

Hooded seals are abundant in the arctic/subarctic regions of the North Atlantic Ocean. They prey on a variety of pelagic fish, squid and to some extent crustaceans. Once a year they gather at particular breeding sites, one of them being the pack ice of the Greenland Sea, to give birth and mate. In this study, colon content was collected from six female hooded seals harvested during a three weeks expedition to the Greenland Sea in March–April 2004. Samples were processed within 15 min of sampling. Both aerobic and anaerobic bacteria in the colon content of the six animals were enumerated. Numbers of anaerobic bacteria ranged between 8.1 (± 1.1) × 10^8 and 5.2 (± 1.2) × 10^9 cfu·mL^{-1}, and numbers of aerobic bacteria between 1.2 (± 0.3) × 10^8 and 3.1 (± 1.1) × 10^9 cfu·mL^{-1}. Further, we searched for aerobic and anaerobic bacteria with tetracycline and ampicillin resistance determinants. No anaerobic tet^r and amp^r isolates were detected after 72 h growth (detection limit > 20). Aerobic tet^r isolates were found in all samples, the numbers ranging from 4.8 (± 3.7) × 10^2 to 1.8 (± 1.5) × 10^3 cfu·mL^{-1}. Aerobic amp^r isolates were found in three of the samples, the numbers ranging between 1.4 (± 1.7) × 10^1 and 1.6 (± 0.3) × 10^4 cfu·mL^{-1}. A selection of phenotypical amp^r isolates (n = 208), as well as community DNA isolated directly from the colon contents, were investigated for the presence of ampicillin resistance gene bla_{TEM} by PCR. No bla_{TEM} genes were detected. We are currently making 16S rDNA clone libraries based on colon contents from three seals. A number of clones will be analysed by DNA sequencing in order to describe the bacterial diversity in the colon contents of hooded seals.

P-23 **Quantification and visualization of the uncultured bacterial groups U2 and U3 from the rumen.** H. Goto, H. Yabuki, T. Shinkai, Y. Kobayashi (Graduate School of Agriculture, Hokkaido University, Japan).

Recent studies on phylogeny of fiber-attaching rumen bacteria have revealed the presence of several groups of uncultured bacteria. We developed real-time PCR assays and a fluorescence in situ hybridization (FISH) protocol for quantification and visualization of two of such uncultured groups (U2 and U3, Koike et al., FEMS Microbiol. Lett. 229: 23–30, 2003) in the rumen. The PCR primers were designed to amplify partial 16S rDNA of U2 and U3, and specificity of the PCR was confirmed by sequencing the amplicons. The real-time PCR assays for these groups were developed and tested for their accuracy and sensitivity. The quantifiable range of U2 and U3 of these newly developed assays was 10^2 to 10^9 copies of 16S rDNA at pure culture level. The assay values were highly reproducible with minimum intra- and inter- assay variations (< 14%). A DNA probe was designed for U2 and a FISH protocol was developed for detecting U2 attaching to fibrous materials. The copy number of 16S rDNA of U2 was higher in the solid phase than in the liquid phase of rumen digesta both from sheep and cattle ($P < 0.05$). Proportions of U2 and *F. succinogenes* of total bacteria were high enough to be representatives in the rumen (0.6–1.9 and 4.0–6.1%, respectively). Ruminally incubated milled orchardgrass hay stem was used for the quantitation and visualization of U2. U2 showed the maximum at 12 h (× 10^9/g) and then decreased gradually. The same trend was observed by FISH detection. *F. succinogenes* showed the maximum population size at 24 h, generally exceeding the population size of U2. These results suggest that U2 could be one of the major members of the fibrolytic consortium in the rumen.

P-24 **Molecular characterisation of the colonic mucosal associated flora.** G.L. Green[a], J.D. Sanderson[a], J. Brostoff[a], B.N. Hudspith[a], D.S. Rampton[b], K.D. Bruce[a] ([a] Life Sciences, King's College London, London, UK, [b] Queen Mary School of Medicine and Dentistry, Institute of Cellular and Molecular Science, London, UK).

Despite the significant roles the bacterial community of the large intestine plays in health and disease, the mechanisms involved remain poorly understood. We have used Denaturing Gradient Gel Electrophoresis (DGGE) of phylogenetically-informative 16S rRNA genes amplified from DNA extracted directly from colonic biopsies to profile mucosa associated bacterial flora.

We focused on eighteen patients who showed no signs of gastrointestinal disease at colonoscopy. Analysis showed that the bacterial communities present at adjacent sites (2–5 cm apart) within individuals were highly similar both in terms of the presence and absence of bands (Mean Similarity Coefficient (MSC) 99%, Standard Deviation (SD) 1.9%), and in the relative band intensities. Two hundred and seven matched bands were determined within the profiles from adjacent biopsies, the mean difference in the relative intensities was 1.5% (SD 1.9%). The number of bands detected in the profiles from each individual ranged from 9 to 23 and MSC for the communities studied was 40% (SD 14%), indicating that the majority of species differed between individuals. Subsequently, it was found that the bacteria associated with the mucosa sampled from different regions of the large intestine (rectum, sigmoid, transverse and descending colon and caecum) was highly similar within an individual (MSC 98%, SD 1.4%). This work strongly suggests that the dominant bacterial species present throughout the large intestine vary little between different colonic locations. Studies such as these are important in order to provide additional insights into the bacterial populations that are involved in the complex interplay between these micro-organisms and the gastrointestinal tract.

P-25 **Antimicrobial resistance of *Escherichia coli* and *Enterococcus* spp. from an integrated, semi-closed, swine and human population.** R.B. Harvey[a], H.M. Scott[b], W.Q. Alali[b], L.D. Highfield[b], T.R. Callaway[a], T.L. Poole[a], R.C. Anderson[a], D.J. Nisbet[a] ([a] Food and Feed Safety Research Unit, Agricultural Research Service, USDA, College Station, TX 77845, USA, [b] Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA).

Abstract withdrawn.

P-26 **Enteral feeding, compared to parenteral feeding, enhances bacterial gut colonization in neonatal pigs.** R.B. Harvey[a], K. Andrews[a], R.E. Droleskey[a], K.V. Kansagra[b], B. Stoll[b], D.G. Burrin[b], T.R. Callaway[a], R.C. Anderson[a], D.J. Nisbet[a] ([a] FFSRU, ARS, USDA, College Station, TX 77845, USA, [b] Children's Nutrition Center, ARS, Houston, TX 77845, USA).

Total parenteral nutrition (TPN) has been associated with mucosal atrophy, impaired gut barrier function, and translocation of luminal bacteria with resultant sepsis in preterm human infants. Currently, we examined the effects of enteral (ENT) or TPN treatments on translocation events in neonatal pigs and on colonization and composition of microbiota in the neonatal gut. Newborn, colostrum-deprived pigs (< 24 h old) were fitted with intravenous catheters and were fed either ENT (n = 13) or TPN (n = 13) for 7 d. After 7 d of treatment, pigs were euthanized and samples were collected for bacterial culture from the blood, intestinal tract and organs. ENT pigs had an increased number of bacterial genera isolated, higher concentrations of bacteria (cfu·mL^{-1}), and increased colonization of all segments of the intestinal tract compared to the TPN pigs. Translocation of bacteria from the intestinal tract to tissues or blood was similar (8 of 13) for both groups. The ENT group had 1/13 positive for *Clostrium difficile* toxin A whereas the TPN group had 5/13. We concluded that ENT favored increased bacterial concentrations comprised of more speciation in the gastrointestinal tract compared to TPN, and that TPN-treated piglets were at higher risk of colonization by toxin-expressing strains of *C. difficile*.

P-27 **Plasmid occurrence in local population of *Selenomonas ruminantium* is not affected by the presence of restriction and modification systems.** J. Ivan, P. Javorský, P. Pristaš (Institute of Animal Physiology of Slovak Academy of Sciences, Šoltésovej 4-6, Košice 04001, Slovakia).

Restriction and modification systems (RMS) in bacteria primarily act to protect the organism from foreign DNA invading the cell. However, only few RMS were tested for the protection in vivo, and there is limited data on the effect of RMS on the horizontal gene transfer frequency. In our previous work, a complex population of RMS systems was detected in local population of the rumen anaerobe *Selenomonas ruminantium*, when at least 12 different RMS phenotypes were observed. Simultaneously, in more than

60% of studied strains, multiple plasmid DNAs were detected in size ranging from 1.4 kb to more than 40 kb. The aim of this work was to analyze the correlation between RMS occurrence and the presence of small plasmids pSRD191 (1.4 kb), pSRD192 (2.3 kb) and pSRD194 (4.1 kb) in *S. ruminantium*. The three plasmids were cloned into *Escherichia coli* plasmids and analyzed for the presence of restriction sites. No significant correlation was found between the occurrence of plasmids and the presence of RMS in *S. ruminatium*, indicating that the distribution (spreading) of plasmids is not affected by the presence of RMS.

P-28 **Construction and function based screening of metagenomic libraries of the human gut microbiota.** B.V. Jones, J.R. Marchesi (Alimentary Pharmabiotic Center, University College Cork, Cork, Ireland).

A highly diverse and complex microbial ecosystem resides within human gastro-intestinal (GI) tract. This community has been found to consist of > 1000 species of bacteria. It is estimated that 70–80% of these are uncultured. The uncultured fraction of the gut microbiota is likely to contain novel metabolic capabilities with the potential to influence human health. To access the metabolic capabilities of the human gut microbiota, large-insert metagenomic libraries were constructed, and high through-put function driven screening implemented. 12 functional screens have been optimised to detect clones producing protease, lipase and esterase, DNase, cellulase, bile salt hydrolase, bacteriocins, quorum-sensing signal molecules, low pH tolerance, oxalate degradation, siderophores, antibacterial and antifungal activity. To date a total of 517 000 clones have been examined using 8 of these screens, and clones positive for production of protease, lipolytic enzymes, cellulase, DNase, oxalate degradation, and tolerance of low pH have been identified. In total 882 positive clones were isolated. End sequencing data from protease positive and oxalate degrading clones indicated that these clones originated from a wide range of bacterial groups present in the gut. Further characterisation of clones exhibiting protease production indicated that the optimum pH varied between pH 6 and pH 7, while no activity was observed at pH 8. Ability to degrade host derived proteins was assessed but no activity against fibrin, elastin, or collagen was detected. These results suggest that the human gut metagenome is a rich source of novel enzymes and bioactive agents, which can be accessed though functional screening of metagenomic libraries.

P-29 **Use of Ranikhet-inactivated vaccine combined with V4 HR and BCRDV for the prevention of viscerotropic velogenic Newcastle disease in backyard poultry.** M.A. Kafi, M.M. Amin, M.B. Rahman (Department of Microbiology and Hygiene, Faculty of Veterinary Medicine, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh).

The performance of V4HR (asymptomatic strain) of Newcastle disease Virus after primary and secondary vaccination as well as its efficacy administered with mesogenic (M) live and Ranikhet-inactivated vaccine were investigated. Similarly comparison of F strain vaccine known popularly as BCRDV in Bangladesh was also accomplished. To perform this, 180 day-old chicks were divided into six groups, A, B, C, D, E and F, that were vaccinated with different schedule except group F, which served as unvaccinated control. The birds were reared separately with proper hygienic management and feed adlibitum. ND vaccinations were carried out at 7 (1 week) 28 (4 weeks), 63 (9 weeks) and 126 (18 weeks) days of age via ED with V4HR and BCRDV while in case of mesogenic (M) live and Ranikhet-inactivated vaccine i/m route was followed. The HI titre manifested a protective potentiality against ND when interpreted to probable value of serum neutralization (SN). It was also observed that the birds of group A administered repeatedly 4 times with V4HR exhibited a HI titre of 40 to 80 with a GMT of 49.25 in the last occasion. It was observed that group B, where the birds had three successive inoculations of V4HR followed by mesogenic (M) live, the HI titres in most cases were at 40 to 80 with a GMT of 42.87. In case of group C, the HI titres were found to be 160 to 320 with a GMT of 242.51 of sera samples collected after vaccination with Ranikhet inactivated vaccine preceded by three successive administration of V4HR. The birds of group D, which had three successive inoculations of BCRDV followed by mesogenic (M) live vaccine, the HI titre of sera

samples collected on last occasion varied from 80 to 160, of which the GMT was 98.49. In case of group E, the vaccine used was Ranikhet-inactivated vaccine where sera samples exhibited HI titre of 80 to 160 with a GMT of 113.14. The unvaccinated control group manifested no such measurable both for the HI titre and GMT on 142 days age of birds. From this study it may be concluded that the vaccination schedule consisting of either V4HR or BCRDV followed by mesogenic live vaccine or Ranikhet inactivated vaccine provided protective potentiality of HI titres. The V4HR when administered alone for maximum of three successive administrations produced HI presence of protective potentiality. The mesogenic (M) live vaccine when administered after three successive inoculation of BCRDV had a comparatively higher level of HI antibody than similar schedule with V4HR. Vaccination schedule consisting of three successive inoculation of V4HR followed by Ranikhet Inactivated vaccine elucidated highest than similar schedule with BCRDV.

P-30 **Swedish allergic infants have less diverse intestinal microbiota compared to healthy infants, at their age of one week.** C. Karlsson[a], M. Wang[a], C. Olsson[b], G. Molin[a], A Wold[c], B. Hesselmar[d], R. Saalman[d], I-L. Strannegård[d], N. Åberg[d], I. Adlerberth[c], S. Ahrné[a] ([a] Laboratory of Food Hygiene, Department of Food Technology, Engineering and Nutrition, PO Box 124, Lund 22100, Sweden, [b] Department of Surgery, Universitetssjukhuset MAS, 20502, Malmö, Sweden, [c] Department of Clinical Bacteriology, Gothenburg University, 41346 Göteborg, Sweden, [d] Department of Paediatrics, Gothenburg University, 41685 Göteborg, Sweden).

The prevalence of allergy is rising, especially in developed countries. The intestinal microflora is considered important for a correct maturation of the immune system in children. Thus it is of great interest to investigate how the composition of the flora differs between healthy individuals and patients with immunological diseases. The aim of the present study was to analyse differences in the composition of the dominating intestinal microbiota in Swedish allergic (diagnosed at 18 months) and healthy infants, at their age of one week. DNA was extracted from faecal samples and the 16S rRNA genes were amplified with universal primers. The samples were analysed with Terminal Restriction Fragment Length Polymorphism (T-RFLP). In this study we showed that the microbiota of healthy infants generated a statistically significant higher number of peaks compared to allergic infants, when the *Alu*I enzyme was used ($P = 0.003$). Also the Shannon and Weiner diversity index was significantly higher in healthy infants ($P = 0.005$). Furthermore, T-RFLP was proved to be a suitable method for analysing the diversity of the intestinal microbiota in infants. Results from this study showed that a high bacterial diversity seems to be one of the important parameters for not developing allergy.

P-31 **Effects of mastication on temporal bacterial colonisation and degradation of fresh perennial ryegrass in the rumen.** E.J. Kim, J.E. Edwards, R. Sanderson, A.H. Kingston-Smith, N.D. Scollan, M.K. Theodorou (Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK).

Understanding of interactions between fresh forage and microbes during microbial colonisation and degradation of masticated feed boli in the rumen is limited. Our objective was to examine temporal changes in bacterial colonisation and degradation of fresh perennial ryegrass (PRG) in the rumen and the influence mastication has on these processes. Samples of freshly-cut PRG and masticated PRG bolus material were incubated in polyester bags in the rumen of two grazing cows for 0, 0.25, 0.5, 1, 2, 4, 8, 12 or 24 h, then machine-washed and freeze-dried. With bolus material, 20.1% (s.e. 3.05) of the dry matter (DM) was immediately soluble, whilst, there was a lag of 1.3 (s.e. 0.11) h before any net DM loss was observed with intact forage. Total genomic DNA was extracted from the fresh materials and residues, and analysed by 16S rDNA based Denaturing Gradient Gel Electrophoresis (DGGE) to profile the colonised bacterial populations. Cluster analysis of DGGE data showed that plant epiphytic samples clustered distinctly from residues following rumen incubation. Bacterial colonisation was evident within 15 min. Rumen incubated samples grouped together in both animals, however, intact PRG

from early incubations (2 h) clustered consistently within this. The delay in onset of degradation of intact PRG, compared to PRG bolus, shows the contribution of mastication to the process of ruminal degradation of forage. Differences in DGGE banding patterns between intact and bolus material indicated that mastication was associated with a difference in the colonising bacterial population. Further analysis of the colonising protozoa and anaerobic fungi is in progress.

P-32 **Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater impacted by swine production facilities.** S. Koike[a], I.G. Krapac[b], H.D. Oliver[a], J.C. Chee-Sanford[c], R.I. Aminov[d], R.I. Mackie[a] ([a] Department of Animal Sciences, University of Illinois, Urbana-Champaign, USA, [b] Illinois State Geological Survey, Champaign, IL 61821, USA, [c] USDA Agricultural Research Service, USA, [d] Rowett Research Institute, Aberdeen AB21 9SB, UK).

Antibiotics are routinely used in the livestock industry and a concern is that farm-generated resistant bacteria can easily migrate into soil, ground- and surface- waters via seepage from waste handling lagoons and from land application of manure. To monitor the dissemination of resistance genes into the environment, we determined the occurrence of tetracycline resistance genes (Tc^r) in groundwater underlying two swine confinement operations, by establishing a well network around the lagoons at each of two facilities. Groundwater and lagoon samples were collected at six sampling times from 2000 through 2003. Total DNA was extracted and PCR was used to detect seven Tc^r [*tet*(M), *tet*(O), *tet*(Q), *tet*(W), *tet*(C), *tet*(H) and *tet*(Z)]. The concentration of Tc^r was quantified by real-time qPCR. To confirm the *tet* gene source in groundwater, comparative analysis of *tet*(W) gene sequences was performed on groundwater and lagoon samples. All seven Tc^r persisted in groundwater during the three-year monitoring period at both sites. At Site A, the level of detection frequency and concentration for Tc^r was correlated with other inorganic contaminants associated with animal waste, confirming that seepage from the lagoon influenced the distribution of Tc^r in groundwater. Comparative analysis of *tet*(W) sequences revealed that the impacted groundwater contained almost identical gene sequences (99.8% identity) with that found in the lagoon. This result supports the dissemination of Tc^r from the lagoon into groundwater. However, novel sequence clusters of resistance genes were also found. This supports the hypothesis that the source of resistance genes is multifactorial and is not limited to swine manure.

P-33 **The effect of celiac diet on microbes in the colon.** J. Kopečný, J. Mrázek, K. Fliegerová (Laboratory of Anaerobic Microbiology, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídeňská 1083, 142 20, Czech Republic).

Celiac disease is a digestive disorder, caused by an immune reaction in the small intestine, resulting in damage to the surface of the small intestine and an inability to absorb several grain proteins. The allergens are grain glutens (prolamins) – glutein and gliadin from wheat, barley, rye and oat. Some speculate that celiac disease has been around since humankind switched from a foraging diet of meat and nuts to a cultivated diet including grains such as wheat or barley. Celiac disease can be cured with a gluten-free diet. The direct effect of celiac diet on the intestinal microbes was not described. In healthy individuals, we studied the effect of celiac diet and celiac diet combined with chitosan. Based on DGGE analysis of digesta, it was found that celiac diet significantly influences the structure of the gut bacterial population. Microbial metabolism was changed as well as short chain fatty acids were increased and pH decreased. Application of celiac diet with chitosan (3 g per day) changed the structure of the bacterial population and their metabolism even more. Beside that, chitosan increased total counts of fecal chitinolytic bacteria and decreased the body weight of the treated persons. We described the effect of celiac diet in indicated childhood celiac disease patients. The changes in SCFA, pH and microbial population correlated with the trends obtained with healthy individuals. We have sequenced DNA fragments of bacteria present in the lumen as well as the in the adhered population to intestinal biopsy samples. Under in vitro conditions, we have observed that isolated

prolamins inhibited the growth of some anaerobic bacteria. Degradability of prolamins was also tested.

P-34 **The incidence of *Listeria monocytogenes* in meat and meat products, Zagreb area, Croatia.** I. Kovaček[a], N. Knežević-Jonjić[a], D. Puntarić[a], J. Bošnir[a], B. Matica[a], D. Kovaček[b], N. Uršulin-Trstenjak[c] ([a] Zagreb Institute of Public Health, Mirogojska 16, Zagreb, Croatia, [b] Biotechnical Faculty, University of Bihać, Bosnia and Herzegovina, [c] High Medicine School, Mlinarska 38, Zagreb, Croatia).

Listeria monocytogenes is a pathogen involved in several outbreaks and sporadic cases of foodborne illness associated with the consumption of contaminated foods. It can be isolated from dairy products, meat and meat products and other foods. Its wide-spread existence in different foods is related to its ability to grow and survive in unfavourable conditions. This study examined the incidence of *L. monocytogenes* in meat and meat products in the Zagreb area, Croatia. In meat products, *L. monocytogenes* is often affected by several types of stress caused by the processing treatment, like heating, freezing and drying. Recovering stressed *L. monocytogenes* from food is of great importance, because sublethally injured bacteria can recover and regain their pathogenicity. A total of 202 samples were examined, from stores, butcher shops and restaurants. *L. monocytogenes* was isolated from approximately 23% of raw meat and thermally treated products, meaning the danger of disease is slim, but a good thermal processing is by all means necessary. *L. monocytogenes* was also found in meat by-products for end-use, i.e. in those products which will get no further thermal treatment. One needs to exercise extreme caution with these products, since they are being kept on refrigerator temperature, which allows *L. monocytogenes* to multiply. Therefore, such high-risk food items need to be regularly inspected for the existence of this rare pathogen.

P-35 **Analysis of the temporal stability and diversity of the bacteria in the human gastrointestinal tracts of healthy individuals using DGGE and RAPD PCR.** S.F. Lawton, J.R. Marchesi (Alimentary Pharmabiotic Centre, Department of Microbiology, University College Cork, Ireland).

The microbial diversity of the human gastrointestinal (GI) tract is generally thought to be constant over time, but the bacteria that are metabolically active may vary considerably with time. The aim of this project is to analyse these microbes using culture independent approaches such as Denaturing Gradient Gel Electrophoresis (DGGE) and Random Amplified Polymorphic DNA (RAPD), using RNA as an indicator of metabolic activity and DNA as an indicator of diversity. Samples were collected from four individuals over a six month period. Nucleic acids were isolated and cDNA synthesised from the RNA, and both gDNA and cDNA were used as a template for PCR. DGGE was used to compare gDNA and cDNA using both universal and group specific primers for the 16S rDNA gene, in order to analyse the temporal stability and diversity of microbes in the GI tract of healthy individuals. Initial DGGE results displayed differences between an individual's gDNA and cDNA, thus indicating that microbial diversity and microbial metabolic activity may be dissimilar. Genomic DNA was used for RAPD analysis. In the RAPD approach there were notable differences in diversity between individuals, but also within these individuals, over time.

P-36 **The antimicrobial peptide Pediocin PA-1 has no effect on common intestinal bacteria compared to nisin and antibiotics.** G. Le Blay, A. Zihler, C. Lacroix, I. Fliss (ETH-Zentrum, 8092 Zurich, Switzerland).

Bacteriocins are anti-microbial peptides naturally produced by many micro-organisms, including lactic acid bacteria (LAB). They include a wide variety of peptides and proteins in terms of size, microbial targets, and mechanisms of action. Although for some broad-spectrum bacteriocins such as nisin and pediocin, the antimicrobial activity against human and animal pathogens has been largely studied, the inhibitory effects toward intestinal bacteria have never been reported. The aim of this study was to test the sensitivity of the most representative intestinal bacteria to broad-spectrum LAB bacteriocins and to compare data with antimicrobial effects of common antibiotics. Twenty-one

common intestinal bacteria were grown in supplemented brain heart infusion (BHI) medium and their sensitivities to nisin Z (102 $\mu g \cdot mL^{-1}$), nisin A (102 $\mu g \cdot mL^{-1}$), and pediocin PA-1 (205 $\mu g \cdot mL^{-1}$) were evaluated using the agar diffusion test. The antibiotic sensitivity of intestinal strains was tested using disc assays. Nisin A and nisin Z exhibited very similar antimicrobial spectra, inhibiting at different levels all Gram-positive intestinal bacteria tested, except *Streptococcus salivarius*. In contrast, pediocin PA-1 exhibited high activity against *Listeria* but showed no detectable effect on intestinal bacteria. Most intestinal bacteria were sensitive to penicillin (10 µg), ampicillin (10 µg), chloramphenicol (30 µg) and tetracycline (30 µg) (19 of 21), whereas high resistance was observed against oxacillin (1 µg) and streptomycin (10 µg). In contrast to antibiotics and nisins that exhibited a large spectrum of activity, pediocin PA-1 had a much narrower spectrum, suggesting its potential utility in the treatment of intestinal infections.

P-37 **Pediocin PA-1 is active against *Listeria innocua* in an in vitro model for the terminal ileum.** G. Le Blay, I. Fliss, C. Lacroix (ETH-Zentrum, 8092 Zurich, Switzerland).

Listeria monocytogenes is responsible for severe food-born infections, which can be life-threatening especially for infants and elderly populations. The emergence of antibiotic-resistant pathogens has stimulated the search for new strategies, such as the use of bacteriocins, to prevent or cure food-born infectious diseases in the intestine. In this study, we evaluated the efficacy of partially purified pediocin PA-1 from *Pediococcus acidilactici* to inhibit *Listeria innocua* (used as a model for the pathogen, *L. monocytogenes*) under physiological conditions of the terminal ileum during a continuous in vitro fermentation. A fecal sample collected from a healthy person was immobilized in gel beads and cultivated for 30 days in a continuous stirred tank reactor, fed with a nutritive medium simulating the ileal chime (pH 7.5). After reaching a pseudo-steady state (day 19), the reactor was inoculated with *L. innocua* at a final concentration of 10^7 $cfu \cdot mL^{-1}$. The concentration of *L. innocua* in the reactor was followed during 8 h by plating on selective medium. Commensal populations were monitored with fluorescence in situ hybridization and short chain fatty acid concentrations by HPLC. Three bacteriocin concentrations (2, 3 and 5 MIC) were tested on the disappearance kinetics of *L. innocua* in the continuous reactor. Our data showed a dose-dependent effect of pediocin PA-1. The adjunction of pediocin at 5 MIC induced a disappearance of listeria below 2.5 Log $cfu \cdot mL^{-1}$ in 5 h, whereas the adjunction of 3 and 2 MIC induced listeria concentrations of respectively 3.2 and 3.7 Log $cfu \cdot mL^{-1}$ after 8 h, compared to 4.5 Log $cfu \cdot mL^{-1}$ with the control listeria without adjunction of pediocin. In addition, pediocin-PA1 had minimal effects on the balance of commensal bacteria. Our study suggests that pediocin PA-1 could be a potential candidate to prevent or treat listeria infection, as an alternative or complement to antibiotherapy.

P-38 **Assessing the methanogen diversity of the human intestinal flora by targeting the *mcrA* gene.** A. Mihajlovski, M. Alric, J.F. Brugere (ERT-CIDAM, Université d'Auvergne, Clermont-Ferrand, France).

The human colon includes a wide variety of micro-organisms which perform an intense metabolic activity. Hydrolytic and fermentative bacteria produce important quantities of H_2, reutilized by 3 groups of hydrogenotrophs: the Sulphate-Reducing Prokaryotes (SRP), the acetogenic bacteria and the methanogenic microorganisms, exclusively members of the archaea domain. Up until now, colonic methanogens are represented by *Methanobrevibacter smithii* (CH_4 production from H_2 and CO_2), and also sometimes by *Methanosphaera stadtmanae* (CH_4 production from H_2 and methanol), both belonging to the class Methanobacteriaceae. They are known to be quantitatively variable between people, in an age- and nutrition-dependent manner, some people being non-carriers. To date, their presence is mainly determined by a breath-test (CH_4 is detected in breath when there are at least 10^7 methanogens per gram of faeces) and/or by culture-dependent techniques (which probably do not recover all the micro-organisms present in samples). Molecular tools targeting the SSU rRNA (or gene) can avoid these drawbacks. An alternative way for the characterisation of metabolically-defined species is to target a specific molecular marker involved in the metabolic pathway of interest. In various ecosystems, this approach has led to a successful

detection of methanogens using the *mcrA* gene. It encodes the α sub-unit of the methanogen-specific methyl-CoM reductase, a α2β2γ2 enzyme catalysing the last step of methanogenesis. Stools have been collected from 6 healthy volunteers and primers designed from an alignment of known *mcrA* sequences. PCR amplifications from stools were positive for 5 of the 6 samples. Cloning is now being performed, followed by RFLP studies. The sequencing of each RFLP pattern will permit to better identify the diversity of the colonic methanogenic flora.

P-39 **Evaluation of three techniques for the extraction of DNA from rumen fluid.** S. Muetzel, D. Paillard, R.J. Wallace (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

A critical step in microbial community analysis is the choice of an accurate way to extract DNA from environmental samples. Indeed, there is an increasing number of DNA extraction kits available for various types of environmental samples. In this study, two commercial DNA extraction kits (Qiagen Stool kit and Qbiogene FastDNA Soil kit) and a phenol/chloroform-based extraction modified from a previously published study (Whitford et al., 1998, Anaerobe 4: 153–163) were compared and modified to optimise yield and purity of the DNA extracted. Quantitative PCR was subsequently used to evaluate the extraction efficiency for DNA from three recalcitrant organisms commonly present in the rumen: *Butyrivibrio* sp., *Ruminococcus albus* and *Streptococcus bovis*. The Qiagen Stool kit is the only method that relies only on a chemical lysis. Although the yield of DNA appeared to be high, the lysis of small Gram-positive bacteria was poor. The Soil kit yielded very good quality DNA but comparatively low amounts of DNA. A three-fold increase of the mechanical lysis time improved the DNA recovery by 250%. The phenol/chloroform technique initially resulted in poor quality DNA that had to be further purified on a column. Quantitative PCR specific for three Gram-positive bacteria of the rumen demonstrate that the Stool kit was not appropriate to extract DNA from ruminal cocci. The phenol/chloroform technique was more appropriate for *Butyrivibrio* spp., but not for the two other species. These experiments demonstrate that a mechanical lysis is needed in order to extract DNA from cocci, but do not suggest a single method for all organisms.

P-40 **Effects of anti-*Helicobacter pylori* treatment and probiotic supplementation on intestinal microbiota.** E. Myllyluoma[a,b], T. Ahlroos[c], L. Veijola[d], H. Rautelin[e,f], S. Tynkkynen[b], R. Korpela[a,b,c] ([a] Institute of Biomedicine, Pharmacology, University of Helsinki, Helsinki, Finland, [b] Foundation for Nutrition Research, Helsinki, Finland, [c] Valio Ltd, Research and Development, Helsinki, Finland, [d] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland and Herttoniemi Hospital, Helsinki, Finland, [e] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland, [f] HUSLAB, Helsinki University Central Hospital Laboratory, Helsinki, Finland).

Antimicrobials may disrupt the balance of the microbiota by causing quantitative and qualitative changes such as decrease in colonization resistance against pathogens. The aims of this study were to evaluate the effect of recommended antimicrobial treatment of *H. pylori* infection, consisting of clarithromycin, amoxicillin and lansoprazole, on intestinal microbiota and the ability of probiotic combination administration to prevent treatment-induced alterations in the intestinal microbiota. Faecal samples were obtained from 39 *H. pylori* infected patients randomized into two treatment groups receiving treatment with placebo or treatment with probiotics. Nineteen *H. pylori* negative volunteers were also included into the study as a control group. Samples were collected before, during and after the treatment, and the microbiota was analyzed by fluorescent in situ hybridisation, quantitative PCR and culturing. The quantities of predominant bacterial groups were altered significantly after the antimicrobial treatment in both groups and disturbances were found even after 9 weeks of the treatment ($P < 0.001$). Probiotics slightly counteracted the effects of anti-*H. pylori* treatment, monitored as significantly less alterations in the total numbers of aerobes ($P < 0.001$) and lactobacilli/enterococci ($P < 0.05$). At the baseline, the composition of the microbiota between *H. pylori* positive vs. negative individuals was different as far as

clostridia and the total number of aerobes were considered ($P < 0.05$). This study suggests that the recommended treatment for *H. pylori* infection induces significant disturbances in the intestinal microbiota. The probiotic supplementation could have some potential in inhibiting the microbiota alterations.

P-41 **Multiple bacteriocin structural genes are present in animal gut cocci.** K. Nigutova, P. Javorský, P. Pristaš (Institute of Animal Physiology, Slovak Academy of Science, Košice, Slovakia).

Bacteriocins are antimicrobial peptides and proteins that inhibit or eliminate the growth of the target, usually phenotypically close organisms. In recent years there has been considerable interest in bacteriocins produced by lactic acid bacteria (LAB), especially by *Streptococcus* and *Enterococcus* strains. Enterococcal and streptococcal isolates obtained from the gastrointestinal tract of different animals were analysed for bacteriocin-like activity (BLA) production and resistance by overlay test. The production of BLA was tested by a simple method on several indicator microorganisms. About 40% of the tested strains showed BLA production in this test. The occurrence of bacteriocin-like activities among the rumen bacteria suggests that bacteriocin production and resistance of ruminal bacteria to bacteriocins may play an important role in general ecology of the rumen ecosystem. The occurrence of selected bacteriocin structural genes encoding enterocin A, enterocin B, enterocin P, enterolysin A, and cytolysin was analysed by PCR analysis and compared with data on bacteriocin production and sensitivity. Every tested strain showed the presence of at least a single bacteriocin structural gene, and up to four structural genes were found in a single isolate. Cytolysin and enterocin B homologues were the most frequently detected, while enterocin A was found to be the least common. The bacteriocin genes in Gram-positive bacteria are located on plasmids, but the location on chromosomal DNA has been reported for several other bacteriocins. The high frequency of bacteriocin structural genes in the tested strains indicated the possibility of horizontal gene transfer of bacteriocin structural genes. The results of this study should broaden the knowledge of bacteriocin-like activity production and resistance among Gram-positive bacteria.

P-42 **Molecular identification of methanogens from sheep from Venezuela.** N. Obispo[a], X. Ma[b], A.-D.G. Wright[b] ([a] Instituto Nacional de Investigaciones Agrícolas (INIA) Edificio 3 área universitaria de la UCV, Maracay Apartado de Correo 4653, Aragua, Venezuela, [b] CSIRO Livestock Industries, Private Bag 5, Wembley, WA 6025 Australia).

The diversity of rumen methanogens in sheep from Venezuela was investigated at the molecular level by using individual 16S rRNA gene libraries. These libraries were prepared from the rumen contents obtained from 10 sheep. A total of 76 clones were examined by using *Hae*III and *Bcn*I digestion to create riboprints. The riboprint patterns have revealed 16 phylotypes, five of which represent existing strains and 11 were new. Among the new strains, three belong to the genus *Methanobrevibacter* and were detected by the *Bcn*I digestion. Denaturing gradient gel electrophoresis (DGGE) analysis was also applied to the same samples. The results of DGGE profile indicated that variations of methanogen at the molecular level exist between individual sheep. Of the 14 DGGE bands detected, four sheep had nine prominent bands, one sheep had only four prominent bands, and the remaining five sheep had between four to nine bands. A detailed study of the comparison of the methods of individual 16S rRNA gene libraries and the use of DGGE analysis in methanogen molecular diversity is under way.

P-43 **High prevalence of oral tetracycline resistance genes in Bolivian Amerindians.** I. Pantoja[a], M. Mojica[b], G. Vargas-Pinto[b], K.P. Scott[c], A. Patterson[c], H.J. Flint[c], M. Blaser[d], M.G. Dominguez-Bello[a] ([a] University of Puerto Rico, San Juan, Puerto Rico, [b] Hospital Boliviano Japones, Sucre, Bolivia, [c] Rowett Research Institute, Aberdeen, UK, [d] New York University, New York, NY, USA).

Indigenous bacteria are an important reservoir of antibiotic resistance genes. The potential for conjugal transfer to pathogens limits the utility

of antibiotics in treating infections. The level of circulation of Tetracycline resistance (tet^R) genes in third world countries with relatively low antibiotic usage has not been assessed. In this study, we aimed to assess the presence of ribosomal protection gene *tet*(M) in patients and healthy rural subjects in Bolivia. We obtained oral swabs from 58 Bolivian Amerindians; 40 were symptomatic patients consulting the Gastroenterology service and 18 were asymptomatic from rural communities. All volunteers provided informed consent of participation. DNA was extracted and subjected to amplification of 16S rRNA genes and *tet*(M) genes. Bacterial DNA was present in 51 of 58 oral samples, illustrated by amplification of 16S rDNA. *Tet*(M) amplified in 81% and 70% of symptomatic and asymptomatic subjects, respectively ($P = 0.413$). Oral tet^R genes were present in a high proportion of subjects from a rural community in Bolivia, suggesting there might be a selective factor other than antibiotics favouring the persistence of resistance determinants in oral bacteria.

P-44 **Detection of novel mosaic tetracycline resistance genes.** A.J. Patterson, M.T. Rincon, H.J. Flint, K.P. Scott (Rowett Research Institute, Bucksburn, Aberdeen, UK).

Tetracycline resistance (Tc^r) has become the most common type of bacterial antibiotic resistance, mainly as a consequence of the intense selection pressure exerted by the excessive use of tetracycline, not only in human and veterinary medicine but also in animal feeding practices. Approximately 40 Tc^R genes conferring resistance by four different mechanisms, ribosomal protection (RP), efflux, enzymatic inactivation and modification of the rRNA target, have been described. Recently, various forms of mosaic genes, presumably arising by recombination between wild-type ribosome protection type genes, have been discovered. In this study, we amplified Tc^R genes in a wide range of human saliva, human faecal, and animal faecal samples, using full-length *tet*(O) primers. Sequence analysis of these products illustrated that mosaic genes represented 78% and 46% of sequences, in pig and human faecal samples, respectively. The genes identified included novel combinations of *tet*(O), *tet*(W) and *tet*(32). It appears from this study that constant antibiotic exposure drives the evolution of these genes, selects for the bacteria carrying them, and results in the predominance of mosaic genes in certain environments. We are investigating the evolutionary advantages of mosaic Tc^R genes over their wild-type counterparts, but there is evidence that they confer increased levels of antibiotic resistance to their bacterial hosts.

P-45 **Use of rifampicin-resistant *Clostridium perfringens* strains for infection studies on necrotic enteritis in broiler chickens.** K. Pedersen, L. Bjerrum (Danish Institute for Food and Veterinary Research, Department of Poultry, Fish and Fur Animals, Hangøvej 2, 8200 Aarhus N, Denmark).

Abstract withdrawn.

P-46 **Mercury-induced shift in rumen protozoan *Entodinium caudatum* associated bacterial populations**. M. Piknova[a], J. Mrázek[b], S. Kisidayova[a], Z. Varadyova[a], T. Tothova[a], P. Javorský[a], P. Pristaš[a] ([a] Institute of Animal Physiology of Slovak Academy of Sciences, Košice, Slovakia, [b] Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Praha, Czech Republic).

The rumen protozoal population is characterized by multiple complex protozoa-bacteria interactions. Heavy metals, including mercury, can be inhibitory to the growth of both protozoa and bacteria present in the rumen. The aim of the present study was to elucidate the role of bacteria-protozoa interactions in response to stress evoked by an increased mercury level. During long term in vitro cultivation of *Entodinium caudatum* with an increased dose of mercury, the mercury resistance of the *E. caudatum* culture increased from an initial level of less than 0.5 µg per mL to more than 10 µg per mL. No changes of the chemical status of mercury were detected in the protozoal culture, indicating the absence of detoxification – mercury reductase – activities. DGGE analysis of associated bacterial populations revealed a clear shift of the eubacterial population structure towards hydrogen sulphide producing bacteria. Very limited changes were observed in the archaebacterial population structure. The data obtained indicated that free living

bacteria protect protozoal cells and their archaebacterial endosymbionts by eliminating mercury into its insoluble form.

P-47 **Development of a molecular technique to study the equilibrium of poultry gut microbiota.** C. Pissavin[a], I. Gabriel[b], C. Burel[a], S. Mallet[b], R. Maurice[a], M. Lessire[b], P. Fravalo[a] ([a] French Agency of Food Safety, BP 53, 22440 Ploufragan, France, [b] National Institute of Agronomic Research, 37380 Nouzilly, France).

The recent changes in the European rules lead to modifications in the feeding and rearing conditions of poultry. Little is known about the ecology of the poultry gut. The standard bacteriological methods enable only the study of a minority of the gut microbiota. Since a decade, several molecular methods, all based on the amplification of the 16S rDNA gene, were applied to the study of different ecological niches. The aim of this study was to estimate the relevance of the CE-SSCP to be used as an epidemiological tool. Among several tested DNA regions, we retained the V3 region as the PCR reaction target. Our results showed that this method gives reproducible data. The fingerprints of caeca, ileum and stool samples exhibited from about 25 to 40 bands. The pattern of the ileum is closer to that of the cloacal stool than that of the caeca. The variability between individuals was weak. However, fingerprint of pooled samples may be a better indicator of the animal health inside a husbandry. In order to estimate the variability inside and between housings, we compared molecular gut patterns of animals coming from the same hatchery and reared under the same conditions (zootechnical parameters control, feeding), in parallel in two separate husbandries. The numeration of specific flora (total aerobic bacteria, coliforms, lactic bacteria) was performed by using conventional methods. Only a slight difference was observed for the coliform counts comparing the caeca and the cloacal stools. The fingerprint of the total flora revealed very slight variabilities inside the two housings. However, more differences were detected when comparing one housing to the other. In consequence, this method gives gut flora fingerprints that may be useful to analyse the effect of various treatments on the equilibrium of gut flora.

P-48 **Characterization of the 16S-23S rRNA gene intergenic spacer region to develop probes for tracking probiotics in the reindeer rumen.** K.E. Præsteng[a,b], R.I. Mackie[b], I.K.O. Cann[b], S.D. Mathiesen[c], M.A. Sundset[a] ([a] Department of Arctic Biology, University of Tromsø, Norway, [b] Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA, [c] Department of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, Norway and Nordic Saami Institute, Guovdageaidnu, Norway).

Reindeer in northern Norway are free-ranging and experience large seasonal variations in feed quality and abundance. Warming of the Arctic region with periods of subsequent thawing and freezing of snow in winter occasionally expose reindeer to starvation, resulting in a reduced number of rumen bacteria and death. Supplementary feeding has become more common in the Saami reindeer husbandry to alleviate the effects of starvation and weight loss. Use of ruminal probiotics during these periods may help prevent digestive disorders and increase animal welfare. Phenotypic screening and subsequent 16S rDNA sequencing of 51 rumen bacterial isolates from reindeer revealed that 7 of these were novel (< 97% sequence identity to previously described strains). Three fibrolytic bacterial isolates (strain 8/94-32 and 8/9293-9 from Norwegian reindeer, and strain E14 from Svalbard reindeer) were selected for a probiotic to increase plant cell wall digestibility. These were both CMC-active and cellulolytic. No inhibition was found between the strains when grown in co-culture. The 16S rDNA sequence of strain 8/94-32 confirmed a 98% identity to *Ruminococcus flavefaciens*, and 8/9293-9 and E14 98–99% identity to *Butyrivibrio fibrisolvens.* Specific oligonucleotide probes to detect and quantify the probiotic strains are being developed based on 16S-23S rDNA intergenic spacer region (ITS) sequences (489–626 bp). Analysis of the ITS region of *B. fibrisolvens* strains (n = 10) isolated from reindeer, strain E14, *R. flavefaciens* FD-1 and *R. albus* Ra8, showed heterogeneity between the strains. Heterogeneity was also found among ITS sequences from the same isolates, indicating that *B. fibrisolvens* and *R. flavefaciens* have multiple ITS regions. Probe specificity will be evaluated based on sequence comparison with the database, as well as hybridization with a range of

rumen bacterial isolates. Real-time qPCR primers will be developed and validated for use in feeding trials to track the probiotic strains and evaluate their ability to establish in the rumen.

P-49 **Comparative molecular analysis of an ovine ruminal enrichment culture using two different DNA separation techniques.** R.M. Rattray, A.M. Craig (Oregon State University, Corvallis, Oregon, USA).

Abstract withdrawn.

P-50 ***Helicobacter pylori*** **status in an urban population is inversely associated with asthma.** J. Reibman, M. Marmor, M.-E. Fernandez-Beros, L. Rogers, G.I. Perez-Perez, M.J. Blaser (Departments of Medicine and Environmental Medicine, NYU School of Medicine, and VA Medical Center, New York, NY 10016, USA).

Reduced exposures to gastrointestinal microbiota may provide one explanation for the increase in asthma prevalence, consistent with the "hygiene hypothesis." Since the prevalence of the gastric colonizer *Helicobacter pylori* is diminishing in human populations, and the presence of these bacteria in a host is inversely related to gastroesophageal reflux disease (GERD), we hypothesized that gastric colonization with *H. pylori* is inversely related to the presence of asthma. Adult asthma cases (n = 347) and controls (n = 214) were identified from an ongoing asthma registry in New York City and serum IgG antibodies to whole cell antigen (*H. pylori*$^+$) or the immunodominant CagA antigen (CagA$^+$) were measured. Seropositivity to the *H. pylori* or CagA antigens was present in 47.0% of the study population. After adjustment for potential confounding, asthma was associated with atopy, as expected, and inversely associated with CagA seropositivity (OR = 0.65, 95% CI = 0.42–1.0). Age of onset of asthma was significantly older (median = 21 years) among individuals with *cagA*$^+$ strains compared to *H. pylori* individuals (median = 10 years). These data are consistent with the hypothesis that *H. pylori* colonization, especially with *cagA*$^+$ strains, is protective against asthma presentation in this population. One possible mechanism may involve *cagA*$^+$ *H. pylori* induction of atrophic gastritis, which protects against GERD by decreasing gastric acidity, and thus might protect against one of its potential consequences.

P-51 **A broad host range integration vector for bioluminescent in vivo imaging of bacteria.** C.U. Riedel, S.C. Corr, I.R. Monk, C.G.M. Gahan, C. Hill (Alimentary Pharmabiotic Centre, University College Cork, College Road, Cork, Ireland).

Bioluminescent imaging of bacterial infections and contaminations has recently been applied to a range of different organisms. However, the molecular tools to label different bacteria have been highly specific. Here, we report the construction of a broad host range integration vector for bioluminescent labeling of both Gram positive and Gram negative bacteria. A constitutive and highly active promotor was synthetised based on the *L. lactis* consensus sequence. To further enhance promotor activity the 5' untranslated region of the *hly* promotor of *L. monocytogenes* was introduced. This hybrid synthetic promotor was cloned upstream of the promotorless synthetic *lux*ABCDE operon of *P. luminescence*. After functionality was shown in vitro in *E. coli* and *L. monocytogenes* the *luxABCDE* operon was cloned together with the synthetic promotor into a broad host range integration vector based on the temperature sensitive replicon of pGh9::ISS1. The resulting vector was then used to generate integration mutants of a range of bacterial genera as diverse as *Escherichia*, *Salmonella*, *Listeria*, and *Lactobacillus*. Suitable integrants were analysed in vitro for any defects in growth and virulence and the site of integration was identified. Suitable bioluminescent strains were then used to study infection or colonisation of murine models as well as contamination of food products. Bioluminescence was detected in intact animals, disected organs, and food products and was shown to correlate closely with colony forming units in infected organs. In conclusion, a new tool for bioluminescent labeling was developed suitable for the non-invasive monitoring of a wide range of bacteria.

P-52 **Identification of novel tetracycline resistance genes in the metagenome of the**

human colon-associated bacterial ecosystem. M.T. Rincon, K. Kazimierczak, P. Young, D. Henderson, K.P. Scott, H.J. Flint (Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK).

Tetracycline resistance (Tc^R) genes are widespread in nature due to the extensive use of tetracyclines as therapeutic antimicrobial agents in veterinary and human medicine and as feed additives in farm animals. Tc^R genes are often associated with chromosomal mobile elements or plasmids that greatly enhance their probability of transfer between different bacterial hosts. In this work we report the use of a metagenomic approach for the analysis of Tc^R genes in a human colon-associated bacterial ecosystem. A metagenome library containing the collective genome of bacteria isolated from a faecal sample of a volunteer who had been on long-term tetracycline therapy was constructed using a fosmid vector. Approximately 4000 clones with an average DNA insert size of 35 kb, equivalent to approximately 35 bacterial genomes, were screened for the occurrence of Tc^R genes. A total of 88 clones exhibiting resistance to tetracycline at 10 $\mu g \cdot mL^{-1}$ were isolated for further analysis. PCR-based analysis carried out using gene-specific primer sets revealed the *tet*(O/32/O) mosaic gene to be the most abundant known Tc^R gene, followed by *tet*(W), *tet*(O) and *tet*(Q). Many clones appeared to contain multiple Tc^R genes, with *tet*(O/32/O) and *tet*(W) the most common combination. Sequencing of selected clones also revealed the existence of a further new Tc^R gene closely linked to *tet*(O/32/O). Computer analysis indicates that the newly identified gene belongs to the efflux pump type of Tc^R genes, similar to *tet*(A). Extensive analysis of one insert revealed that the tandem *tet*(O/32/O) and new efflux pump Tc^R genes are located in a mobile element similar to a previously characterized transposon in *Enterococcus faecalis* that carries a cluster of genes implicated in resistance to vancomycin, although vancomycin resistance genes were not detected in this case. In conclusion, the metagenome approach provided a powerful method for gaining information on both known and previously unknown Tc^R genes.

P-53 **Prevalence of *Helicobacter pullorum* in broiler chickens reared in northern Italy.** M. Rossi[a], V. Sanguinetti[a], R. Gavioli[a], P. Lozito[a], G. Manfreda[b], R.G. Zanoni[a] ([a] Department of Veterinary Public Health and Animal Pathology, University of Bologna, Italy, [b] Department of Food Science, University of Bologna, Italy).

During 2005, in order to establish the prevalence of *Helicobacter pullorum* in broiler chickens, a total of 155 caecum contents collected from animals intensively reared in 31 poultry farms (5 animals per farm) were sampled at the slaughterhouse. A modified Steele-McDermott membrane filter method was used to isolate *H. pullorum*. Each fresh sample was diluted 1:4 in a mixture containing 25 mL Brain Heart Infusion, 75 mL horse serum and 7.5 g glucose. Three hundred microlitres of this suspension were inoculated on a membrane filter (0.65 µm) previously applied on the surface of a Brucella Agar 5% sheep blood plate. After one hour of incubation at 37 °C in increased H_2-microaerophilic atmosphere, the filter was removed and the plate was incubated up to 7 days. Small, white-greyish colonies of gram-negative, slightly curved rod bacteria were preliminary identified as *H. pullorum* by a PCR assay based on 16S rRNA, and then subjected to a RFLP-PCR assay to distinguish *H. pullorum* from *H. canadensis*. According to the PCR and PCR-RFLP results, 127 out of 155 animals examined were positive for *H. pullorum* (82%) and 100% of farms resulted infected. All positive samples showed a high number of colonies (> 50 cfu/plate) phenotypically consistent with *H. pullorum* in the media of first isolation, which suggests that this microorganism, when present, colonizes the broiler chicken caecum at an elevated load.

P-54 **Study of spirillar enteric flora of dogs and cats in Italy.** M. Rossi[a], V. Sanguinetti[a], D. Giacomucci[a], P. Acutis[b], R.G. Zanoni[a] ([a] Department of Veterinary Public Health and Animal Pathology, University of Bologna, Ozzano Emilia, Bologna, Italy, [b] State Veterinary Institute of Piedmont, Liguria and Aosta Valley, Torino, Italy).

During 2002, in order to define the prevalence of *Campylobacter* spp, enteric *Helicobacter* spp. and *Anaerobiospirillum* spp., a survey of 190 dogs and 84 cats, either healthy or affected

by gastroenteric disease, was carried out. Eighty strains of *Campylobacter*, 92 strains of *Helicobacter* and 30 strains of *Anaerobiospirillum* were isolated using different selective media such as Skirrow, Blaser-Wang and CCDA. The identification of the genus was carried out by specific PCR. *Campylobacter* was isolated from 53 dogs and from 27 cats, *Helicobacter* from 65 dogs and 18 cats and *Anaerobiospirillum* from 27 dogs and 3 cats. A polyphasic approach was used to identify the species of both *Campylobacter* and *Helicobacter.* Seventeen *C. jejuni,* 33 *C. upsaliensis,* 2 *C. helveticus* and one *C. lari* from dogs and 7 *C. jejuni,* 6 *C. upsaliensis* and 14 *C. helveticus* from cats were identified using phenotypic tests and species-specific PCR. Whole cell protein profile analysis, phenotypic tests, 16S rDNA PCR-RFLP and a phylogenetic study of partial *groEL* sequences were used to identify 22 *H. canis* and 14 *H. cinaedi* in dogs and 12 *H. canis* and 2 *H. cinaedi* in cats. "*Helicobacter rappini*" with a typical flexispira morphology in TEM was found in 37 dogs and 5 cats. The protein profiles of these isolates were similar to that of the strain "*Flexispira rappini*" ATCC 49308, forming together a distinct cluster at the 82% similarity level. There was no statistically significant correlation found between the isolation of *Campylobacter* or *Helicobacter* and the presence of gastroenteric symptoms in both dogs and cats.

P-55 **Influence of diet and sampling site on the diversity of the bacterial community attached to the rumen epithelium in lambs.** S. Sadet, C. Martin, B. Meunier, D.P. Morgavi (INRA, Theix, 63122 Saint-Genès-Champanelle, France).

The bacteria attached to the rumen epithelium (bacterial epimural community, BEC) are a ubiquitous component of the rumen microbial ecosystem, still their biological role is not well known. In order to better understand the functions of BEC, the effect of diet and sampling site on its diversity was studied using PCR-DGGE with universal eubacterial 16S rDNA primers. Eight lambs were allocated into two groups fed forage or a concentrate-rich diet. After slaughter, 5 different sites of the rumen epithelium (dorsal (D1, D2), ventral (V), caudal (C) and lateral (LA) area) were sampled, as well as rumen content of the liquid (LP) and solid (SP) phases. Clustering analysis of DGGE profiles showed that neither diet type nor sampling site had any influence on BEC structure. For diet type, a confounding animal effect cannot be discarded due to the experimental design. However, for both LP and SP samples separate clusters were obtained for each diet. This result suggests that the bacterial population from the rumen contents was more affected by diet than BEC. Furthermore, diversity indexes based on DGGE operational taxonomic units showed no differences between diets or among sampling sites, except for V, which tended to be more diverse than D1 ($P = 0.1$) for the Shannon index (H). BEC diversity was, irrespective of sampling site, equally distributed within the rumen of individual lambs. However, BEC structure differed among animals and seems to be influenced by the host. Future work will look at assessing the diversity of BEC functional groups and link them up with their physiological activity under different feeding conditions.

P-56 **Microbial community changes in the intestine of the pre-adolescent turkey.** A.J. Scupham (National Animal Disease Center, USDA, Agricultural Research Service, Ames, IA 50010, USA).

Colonization of the intestine by opportunistic pathogens can be inhibited by the native flora, but factors such as antibiotic use and age can disrupt the equilibrium, providing a susceptible environment. Thus, identifying periods of innate susceptibility in the poultry intestine is important for both animal health and food safety concerns. To monitor development of intestinal communities, cecal droppings from individual male broad-breasted turkeys ($n = 5$ and 6 birds per trial) were sampled weekly (from day of hatch through 18 weeks of age). Automated Ribosomal Intergenic Spacer Analysis (ARISA) community profiles identified a continual increase in both bacterial and fungal species richness with a slight decrease during weeks 16–18. Sorensen's analysis of the fingerprint data and MEGA 3.0 dendrograms describing community similarity identified a period of community transition at week 12 in the two trials. Bacterial libraries representing weeks 9, 11, 12 and 14 were sequenced. While both ARISA and

LIBSHUFF analyses indicated a significant difference in the species represented in the two trials, a predominance of *Clostridia*-like species at week 9 was replaced by a predominance of *Bacteroides*-like species in weeks 11, 12 and 14. *B. uniformis* prevalence in week 11 libraries (85% and 65%) of both trials provides compelling evidence that it may act as a vanguard, preparing the gut environment for colonization by other *Bacteroides* species. The dynamics of intestinal communities in maturing poultry suggest a continuous susceptibility to colonization by pathogens, with a period of exceptional potential around week twelve. *Bacteroides uniformis* has been identified as a potentially important microbe associated with maturation of the intestinal community and protection from colonization by opportunistic pathogens.

P-57 **Type A *Clostridium perfringens* subtypes are differentiated between necrotic enteritis and healthy broiler intestinal isolates and are distributed among and between hosts.** G.R. Siragusa[a], M.G. Wise[b] ([a] Agricultural Research Service, United States Department of Agriculture, Russell Research Center, 950 College Station Road, Athens, Georgia, 30605, USA, [b] Current address: Bacterial Barcodes, Inc., Athens, Georgia, 30605, USA).

We have consistently observed a differential rep-PCR pattern distribution between gut isolates of type A *Clostridium perfringens* from a necrotic enteritis disease-state origin and those of healthy flock mates of commercially reared drug-free (antibiotic growth promotant free) broiler chickens. From a drug free broiler isolate library, we previously found and reported five major rep subtypes of *Cl. perfringens* amongst broiler gut isolates as well as environmental isolates obtained from darkling beetle adult and larvae and spent litter. Those findings indicated a subtype of *Cl. perfringens* which was dominated by disease-state derived *Cl. perfringens* strains. Here, a library of *Cl. perfringens* gut isolates obtained from healthy and necrotic broiler chickens from 3 different drug-free farms (fed no ionophoric anticoccidials or antibiotic growth promotants yet receiving an anti-coccidial vaccine) were subtyped using rep-PCR and analyzed with DiversiLab v 3.1 analytical software. We present data which suggest that within a single broiler chicken intestinal tract can be found not only disease state linked *Cl. perfringens* isolates but healthy-state isolates. Disease state isolates from these three farms were highly related (>90% similarity) based on rep-PCR patterning. Overall, our data suggest that strains exist which in some manner might contribute to the opportunistic mechanism of necrotic enteritis in drug-free broilers. The individual or combined pathogenic potential of these isolates remains to be tested in an appropriate broiler chicken necrotic enteritis disease model.

P-58 **Engineering improved tolerance to stresses encountered in the gastrointestinal tract.** R.D. Sleator, C. Hill (Department of Microbiology and Alimentary Pharmabiotic Centre University College Cork, Ireland).

The objective of this study was to engineer *Listeria monocytogenes* strains with a significantly improved ability to tolerate stresses encountered in the external environment and during gastrointestinal transit, thus, improving *Listeria's* efficacy as a potential vaccine and drug delivery platform. Using a directed evolution approach, based on a random mutagenesis strategy involving the *E. coli* XL1-Red mutator strain, we generated a mutant variant of the listerial *betL* gene (designated *betL**), encoding a secondary betaine uptake system. The mutant *betL** promotes a dramatic increase in resistance to a number of biologically relevant stresses when expressed in a variety of different surrogate hosts. Using a luciferase (Lux) reporter system in combination with the IVIS Imager System (Xenogen Corporation, Alameda, CA), we tracked *betL** expression, in real time, both in vitro under various environmental stresses and in vivo in animal models of infection. In each case strains expressing *betL** demonstrated a marked improvement over those expressing wild type *betL*, both in terms of gene expression and bacterial growth. Sequence analysis of the mutated gene revealed a single nucleotide deletion in the spacer region between the –10 and –35 promoter elements upstream of the *betL* coding region. This deletion presumably introduces a conformational 'twist' in the putative promoter, thereby increasing its transcriptional output. Furthermore, the *betL** mutation appears to counter the heretofore unreported 'twisted'

cell morphology observed using scanning electron microscopy of *L. monocytogenes* grown at elevated osmolarities. It is possible to selectively improve genes required for bacterial stress survival both inside and outside of the host. Such mutated genes systems may ultimately be used for the construction of more physiologically robust bacterial based vaccine and drug delivery platforms.

P-59 **Antibiotic resistance, resistance genes and transfer in animal gut cocci.** V. Stovcik, P. Javorský, P. Pristaš (Institute of Animal Physiology of Slovak Academy of Sciences, Šoltésovej 4–6, Košice 04001, Slovakia).

Enterococci have emerged as important pathogens responsible for various infections, particularly of respiratory infections not only in humans. Recent surveys have found widespread resistance to various antimicrobial agents, especially macrolides, tetracyclines, and in recent years also to beta-lactam antibiotics. The aim of the present study was to analyze antibiotic resistance, resistance genes and transfer in animal gut cocci. Three hundred rumen or faecal isolates were tested for tetracycline, erythromycin and ampicillin resistance. High resistance frequency was observed for tetracycline and erythromycin (40% of isolates). Eight (4%) isolates showed resistance to ampicillin due to lactamase production. Rumen isolates showed different resistance profiles compared to faecal isolates. *Tet*M and *Tet*L determinants were found to be predominant in tetracycline resistant isolates, whereas all erythromycin resistant isolates possessed *erm*B determinant. No known *bla* gene was detected in ampicillin resistant isolates. All resistance determinants were found to be transmissible with relatively high frequency. BOX-PCR fingerprinting indicated that both clonal spread of resistant strain(s) and horizontal gene transfer are responsible for resistance transfer in animal gut cocci. The gastrointestinal tract of animals and the enterococci inhabiting it could serve as a reservoir of mobile resistance determinants available for resistance spreading.

P-60 **The prevalence of mobile gene cassettes in indigenous gut microbiota and dairy starter bacteria.** J. Štšepetova, E. Sepp, K. Truusalu, K. Lõivukene, P. Hütt, E. Songisepp, M. Mikelsaar (University of Tartu, Department of Microbiology, Ravila 19, 50411, Tartu, Finland).

The integrons are mobile gene cassettes that contain the determinants of site-specific recombination systems to capture genes. Up to now, these genes are associated with transferable antimicrobial resistance. However, there are few data on the prevalence of integrons in indigenous gut microbiota and dairy starter strains. The aim of the present study was to estimate the prevalence of the *Int1* gene in indigenous *E. coli*, *Lactobacillus* and *Bifidobacterium* isolates and in starter lactic acid strains. A total of 30 indigenous *E. coli* isolates were collected from stool of 10 antibiotic naïve infants (age < 1 y, born in 1998) and another 30 isolates from stool of 9 elderly people (age > 65 y, born before 1940). Additionally, 11 *Lactobacillus* and *Bifidobacterium* sp. of human origin and 7 food starter lactic acid strains were included. Microbial DNA was extracted using the QIAamp DNA Mini Kit. *Int1* was detected by PCR with primers *Int1*-F and *Int1*-R according to Leverstein-van Hal. The prevalence of the *Int1* gene was significantly higher in the samples of infants as compared to elderly people (16/30 vs. 5/30, $P < 0.01$). PCR products of *Int1* were detected also in 3/7 starter lactic acid bacteria and in 3/11 lactobacilli and bifidobacteria. The integrons of tested bacteria varied in the size of the PCR amplicons. The prevalence of *IntI* genes in indigenous *E. coli* isolates was higher in late birth cohorts. At the same time, *IntI* genes can be found also in different food starter lactic acid bacteria and among indigenous *Lactobacillus*/ *Bifidobacterium* sp. isolates. Their role in carriage and transfer of antibiotic resistance or some other genes needs elucidation.

P-61 **Resistance of *Methanothermobacter thermautotrophicus* to therapeutics and antimicrobial substances.** S. Šurín[a], L. Čuboňová[a], A. Majerník[a], P. McDermott[b], J.P.J. Chong[b], P. Šmigáň[a] ([a] Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 90028 Ivanka pri Dunaji, Slovakia, [b] Department of Biology, the University of York, York, YO10 5WY, UK).

Methanoarchaea are found as microbial commensals in the guts of insects to humans. However, a role for these commensals in the human digestive tract and their potential for the generation and dispersal of drug resistance is only poorly understood. *Methanothermobacter thermautotrophicus* strain ΔH is relatively easy to culture in the laboratory, has been extensively studied and is a very close relative of *Methanosphaera stadmanae* and *Methanobrevibacter smithii*, two known human methanogenic commensals. We have used *M. thermautotrophicus* to screen for genetic mutants resistant to different therapeutics such as the antibiotic neomycin, the uncoupler TCS or the potassium-retaining diuretic amiloride. Application of amiloride resulted in the isolation of colonies of spontaneous mutants at a frequency of 10^{-6}, a rate similar to that observed for neomycin resistance. Strains resistant to both these drugs have been stably cultivated in selective and non-selective growth medium for several months. The growth rate of resistant strains was lower than that of the wild type. However, methanogenesis was elevated in both resistant strains. Moreover, the resistant phenotypes display similar changes in their protein spectra when compared to those of the wild type. Interestingly, the phenotypes of both resistant strains indicate the presence of defects in their bioenergetic processes. In particular, ATP synthesis and ion transport were affected by mutations. Here we propose a direct mechanism of amiloride resistance based on biochemical data. This study reveals how different, commonly used therapeutics can cause specific resistant phenotypes in methanogens, which can generate increased amounts of methane compared to wild type.

P-62 **Control of *Campylobacter jejuni* colonization in chickens treated with probiotics or bacteriocins.** E.A. Svetoch[a], B.V. Eruslanov[a], V.D. Pokhilenko[a], Y.N. Kovalev[a], L.I. Volodina[a], V.V. Perelygin[a], E.V. Mitsevich[a], I.P. Mitsevich[a], V.N. Borzenkov[a], V.P. Levchuk[a], O.E. Svetoch[a], T.Y. Kudriavtseva[a], N.J. Stern[b] ([a] State Research Center for Applied Microbiology, Obolensk, Russia, [b] USDA, ARS, RRC, PMSRU, Athens, GA, USA).

Chicken intestinal material was screened for diverse bacterial genera, and isolates were assessed for antagonism (zones of inhibition) against *Campylobacter jejuni*, a bacterial commensal in poultry. Isolates from these genera were used to treat chickens in an attempt to control colonization by *Campylobacter*. Individual and groups of antagonistic isolates (probiotics) were gavaged into naïve chicks to protect against *Campylobacter* challenge. Only when *Campylobacter* challenges approached a chick CD 50%, were prophylactic probiotic treatments effective. Unless the treatment was 100% effective, as birds aged, *Campylobacter* numbers approached untreated control birds' levels. Commercial flocks may be exposed to *Campylobacter* at various levels anytime during production. To understand the mechanism enabling competitive exclusion, two of our most promising antagonists, *Lactobacillus salivarius* NRRL B-30514 and *Paenibacillus polymyxa* NRRL B-30509, were further studied. We characterized distinct bacteriocins (antimicrobial peptides) produced by each of the isolates. When bacteriocins were purified and fed to colonized chickens, the subject birds became free of *Campylobacter*, or manifested at least a one-million fold reduction in levels when compared to the untreated control animals. Whereas treatments with probiotics were at best only modestly effective in controlling *Campylobacter* colonization, orally administered bacteriocins provided substantial reductions of *Campylobacter*. We believe that in vivo bacteriocin activity provides the central explanation for competitive exclusion within the intestinal tract.

P-63 **Comparing gut microflora in pigs by using an integrated probing system.** N. Thanantong, A. Anderson, S. Edwards, O.A.E. Sparagano (School of Agriculture, Food and Rural Development, University of Newcastle upon Tyne, NE1 7RU, UK).

The gastrointestinal tract of mammals harbours a complex microbial community in which many species still have not been characterized. The microflora in a gut is in constant evolution but needs to stay in equilibrium with the other species, including autochthonous and non-autochthonous species. Changes in diet and farrowing events were monitored in sows while weaning times and feeds were followed in piglets. A macro-array-based method was developed to simultaneously screen *Lactobacillus, Streptococcus* and *Bifidobacterium* species in different

parts of the pig gut (ileum, caecum and colon), rectal samples were also collected. The methodology is based on a 16S-based PCR coupled with a reverse line blot hybridisation technique using several species-specific biotinylated probes, which have been shown to be highly specific. Chemiluminescence was used as the detection method. No differences in the microflora population targets were observed between boars and gilts or between replicated experiments but changes in the diet had some impact on the presence of *Lactobacillus* and *Streptococcus* species, while samples collected during farrowing times showed a higher number of species in the gut of all processed sows. The presence or not of fructo-oligosaccharides (FOS), used as dietary additives to improve enteric health in pigs also showed a shift between *Lactobacillus acidophilus* and *Streptococcus hyointestinalis* populations.

P-64 **In vitro evolution of the equine gastric microflora.** M. Varloud[a], V. Julliand[b] ([a] EVIALIS, 56250 Saint-Nolff, France, [b] ENESAD, 21000 Dijon, France).

Microbial concentrations in the gastric contents of conscious horses have been shown to increase from 5.7 to 7.2 $\log_{10}$ cfu·mL^{-1} during the first postprandial hour (Varloud et al., 2004). This experiment was set to bring explicative data of this phenomenon through an in vitro evaluation of the postprandial evolution of the equine gastric microflora. Four mature geldings (429 kg BW) were adapted to a hay and pellets based diet during 3 weeks. Gastric contents collected before (C1) and 1 h after the meal (C2) were diluted before stomacher treatment and filtration. This inoculum (23 mL) was added to ground pelleted feed (0.7 g, 20% starch) beforehand hydrated with dilution medium (47 mL) in hermetic flasks. Blanks without inoculum or without substrate were tested. After incubation at 38 °C in a shaking water bath, fermentations were stopped after 60, 120 and 210, and 60 and 210 min, for C1 and C2, respectively. The pH was measured immediately. The concentrations of total anaerobes, lacticolytic bacteria, Streptococci and Lactobacilli were investigated in the raw gastric contents and after in vitro incubation. Lactate and volatile fatty acid concentrations were evaluated at the end of the incubation. Between 60 and 210 min of incubation, the total anaerobic population increased from 5.1 to 6.1 $\log_{10}$ cfu·mL^{-1} with C1 and from 5.8 to 7.1 $\log_{10}$ cfu·mL^{-1} with C2. In the same time, the lacticolytic population increased from 3.9 to 5.9 $\log_{10}$ cfu·mL^{-1}. The concentrations of Lactobacilli and Streptococci increased more slowly during the incubation. This in vitro development of the population was not equivalent to that previously observed in vivo. Multiplication of bacteria within the chyme may not explain entirely the in vivo increase of bacterial concentration.

P-65 **Prevalence of two *Lactobacillus* and *Bifidobacterium* probiotic strains in the neonatal ileum.** R. Wall[a,b,c], G.F. Fitzgerald[b,c], S.G. Hussey[d,e], C.A. Ryan[d], M. O'Neill[e], R.P. Ross[a,b], C. Stanton[a,b] ([a] Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland, [b] Alimentary Pharmabiotic Centre, Cork, Ireland, [c] Department of Microbiology and [d] Department of Paediatrics and Child Health, Erinville Hospital, University College, Cork, Ireland, [e] Mayo General Hospital, Castlebar, Mayo, Ireland).

The purpose of this study was to investigate the colonization of the ileum with lactobacilli and bifidobacteria using samples obtained from two infants. Initially, ileostomy samples were examined from a preterm infant at 50 and 74 days old by plating its caecal contents on *Lactobacillus* and *Bifidobacterium* selective agar. Twenty of the resulting *Lactobacillus* isolates were genomically fingerprinted by pulsed field gel electrophoresis and found to be a single strain. 16S rDNA sequencing identified this strain as *Lactobacillus paracasei*, which was recovered at both time points. Indeed, this strain dominated the small intestine throughout the study period (24 days). In contrast, culturable bifidobacteria were absent. In the other ileostomy case, two cecal samples were taken from a term infant at 24 and 31 weeks old and a fecal sample at 44 weeks following ileostomy reversal. In this case, lactobacilli were not detected in any of the samples, however, bifidobacteria were obtained when the ileostomy had been reversed. 16S rDNA sequencing revealed two different strains; *Bifidobacterium animalis* subsp. *lactis* and *B. scardovi*. Interestingly, the *B. animalis* subsp. *lactis* strain exhibited the same PFGE pattern as

the well-defined probiotic strain *B. animalis* subsp. *lactis* BB12, while the *L. paracasei* strain isolated in the first study was identical to *L. paracasei* NFBC 338 probiotic strain. This study demonstrates the prevalence of two different probiotic strains in the upper intestinal tract at an early stage of human life.

P-66 **Enrichment in a complex medium of a novel bacterial group from the rumen of wild sika deer as confirmed by real-time PCR assays and FISH detection.** H. Yamano, K. Miyawaki, Y. Sawabe, Y. Kobayashi (Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan).

Phylogenetic analysis of rumen microbiota in wild sika deer, and quantitation of some bacterial groups were previously attempted. Through phylogenetic analysis, unknown bacterial group A was found in the CFB (*Cytophaga Flavobacter Bacteroides*) phylum. According to real-time PCR quantitation, the population size of group A showed a 17-fold increase from summer to winter. This result suggested that in the rumen of wild sika deer, the unknown bacterial group A may have important roles in digestion of fibrous diet (mainly bark) in winter. The present study was aimed at enriching this novel uncultured bacterial group in a complex medium for future isolation and physiological analysis. As the first step, we screened a carbon source suitable for enrichment of this group. Also, deer rumen fluid was assessed as a supplement to a complex medium. The enrichment was evaluated by real-time PCR assays and FISH detection. Of 13 carbon sources tested, powdered bark of Japanese oak was most effective in enrichment of the group A. Supplementation of deer rumen fluid also successfully stimulated the growth of this group. After 5 days incubation in a complex medium, population size of the unknown group A drastically increased ($\times 10^{4-5}$). Relative proportion of the group A in total bacteria also increased from 0.9% to 4.1% during the incubation. The enriched group A was confirmed by sequencing of the products of group-specific PCR. In addition, FISH detection confirmed the growth of this group. These results suggest that the yet uncultured group A is cultivable in the complex liquid medium containing habitat-stimulating components from the original deer rumen digesta.

P-67 **Sequencing and annotation of pCY360, a megaplasmid from *Clostridium proteoclasticum* B316^T.** C. Yeoman[a,b], W.J. Kelly[a], J. Rakonjac[b], G.T. Attwood[a] ([a] Metabolism and Microbial Genomics, Food and Health Group, AgResearch Ltd., Grasslands Research Centre, PB 11008, Palmerston North, New Zealand, [b] Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand).

The genome of *Clostridium proteoclasticum* B316^T consists of 4 replicons; a ~ 3.2 Mb chromosome and three megaplasmids of 360, 290 and 190 kb. The largest megaplasmid, designated pCY360, has been completely sequenced and annotated. GLIMMER analysis identified 400 open reading frames (ORFs), but only 15% of these show significant sequence similarity to previously described genes of known function. A putative origin of replication (oriR) was identified containing a replication initiation protein, RepB, a plasmid partitioning protein, ParA, and two large inverted repeats. ORFs related to conjugative transfer functions and type IV pilus formation, including members of both the mating pair formation (mpf) complex and the relaxosome, as well as the coupling protein TraG, have been identified and most are clustered together in a 35 kb region. pCY360 also contains nine genes described in Koonin's minimal gene set, fulfilling Ochmans definition of a miniature chromosome, however, all nine genes are also found on the *C. proteoclasticum* chromosome. Ten transposase genes are present (7 belong to the IS4, IS200 or IS605 transposase families) and some share homology with transposases on both the chromosome and the co-resident 290 kb megaplasmid, suggesting that transposon-mediated gene shuttling has occurred. The function of pCY360 is unknown, but it appears to contribute to proteins expressed at the cell membrane/surface, as 19% of its ORFs are predicted to contain one or more transmembrane regions. Analysis of codon usage indicates that pCY360 is similar to the host chromosome, indicating that it has resided within *C. proteoclasticum* for a significant period of time.

P-68 **Genetic diversity of the microbial community in ileostomy patients.** E.G. Zoetendal[a,b], C.C.G.M. Booijink[a,b], S. El Aidy[a,b], H. Smidt[a,b], M. Kleerebezem[b], W.M. De Vos[a,b] ([a] Laboratory of Microbiology, Wageningen University, The Netherlands, [b] Wageningen Centre for Food Sciences, Wageningen, The Netherlands).

The microbial biomass in our gastrointestinal (GI) tract consists of a myriad of microbes that are pivotal to health and disease. In recent years, 16S ribosomal RNA (rRNA)-based approaches provided a phylogenetic framework of more than 1 200 microbial species inhabiting our GI tract and gave insight into the temporal, spatial and inter-individual microbial diversity (Zoetendal et al., 2006). Nevertheless, most studies have focused on the colon, while insight into structure, dynamics and functionality of the small intestinal microbiota remains limited. To access the small intestinal microbiota without using interfering procedures, effluent samples from eight ileostomy patients were collected. Since these individuals have their colon removed, the intestinal content leaves their body at the end of the ileum via a stoma, providing non-invasive access to small intestinal content. PCR-DGGE of 16S rRNA genes indicated that the bacterial community structure in these effluent samples is less complex and less stable in time compared to colonic samples. Moreover, differences between morning and afternoon communities were often observed. From four effluent samples of one individual a metagenomic library consisting of 25000 fosmids was constructed. 16S rRNA approaches indicated that this library was representing the actual microbial diversity reasonably well. In addition, database hits with bacterial genera that are often described as inhabitants of the small intestine were frequently encountered when analyzing end-sequences of randomly selected fosmids. The details of this analysis will be discussed at the conference.

P-69 **Long-chain fatty acids and fatty aldehydes in rumen bacterial genera *Butyrivibrio* and *Pseudobutyrivibrio.*** M. Zorec, R. Marinšek-Logar (University of Ljubljana, Biotechnical Faculty, Zootechnical Department, Domzale, Slovenia).

The analysis of long-chain fatty acids and fatty aldehydes is a frequently used chemotaxonomic method in bacteria and it was applied to evaluate heterogeneity in the group of rumen bacteria phylogenetically related to the type strains *Butyrivibrio fibrisolvens*, *B. hungatei*, *Clostridium proteoclasticum*, *Pseudobutyrivibrio ruminis* and *P. xylanivorans*. Long-chain fatty acids and fatty aldehydes of 62 strains were transesterified with HCl in methanol and their methyl esters analysed by gas chromatography using non-polar column Equity-1 (Supelco). According to the long-chain fatty acid and fatty aldehyde profiles strains formed 4 similarity groups, but to the type strain *C. proteoclasticum* no similar strain was found. The major fatty acids and fatty aldehydes were C16:0, DMA C18:1 c11, DMA C16:0 and C18:1 c11 in *B. fibrisolvens* group, C16:0, DMA C14:0, DMA C16:0, C14:0 and DMA i-C15:0 in *P. ruminis* group, a-C17:0, C16:0, i-C16:0, DMA i-C16:0, DMA a-C15:0, DMA a-C17:0 and DMA C16:0 in *B. hungatei* group, and C16:0, DMA C18:1 c11, DMA C16:1 c9 and DMA C16:0 in *P. xylanivorans* group. The *P. ruminis* group was characterized by a high proportion of straight saturated fatty acids and fatty aldehydes and the *B. hungatei* group by a high proportion of branched fatty acids and fatty aldehydes. Strains within the groups showed similarity also in the type and quantity of fermentation products. This study defines the characteristic profiles of long-chain fatty acids and fatty aldehydes for *Butyrivibrio* and *Pseudobutyrivibrio* species, enabling rapid, accurate and simple identification of new isolates and a more proper delineation of bacterial species.

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O-9 **Microarray gene expression profiling of *Ruminococcus flavefaciens* FD-1 grown on cellobiose or cellulose.** M.E. Berg[a], D.A. Antonopoulos[a], M.T. Rincon[b], M. Band[a], A. Bari[a], T. Akraiko[a], A. Hernandez[a], R. Kim[a], L. Liu[a], J. Thimmapuram[a], I. Borovok[c], S. Jindou[c], R. Lamed[c], H.J. Flint[b], E.A. Bayer[d], B.A. White[a] ([a] University of Illinois, Urbana, IL 61801, USA, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK, [c] Tel Aviv University, Ramat Aviv 69978 Israel, [d] The Weizmann Institute of Science, Rehovot 76100, Israel).

The 4× draft genome of the *Ruminococcus flavefaciens* FD-1 sequencing project has 1143 assembled contigs averaging 3686 bp (ranging from 205 to 31132 bp). *In silico* analysis of the cellulosomal organization and components revealed a cluster of ORFs for the putative scaffoldin proteins, ScaA, ScaB and ScaC that are similar to those of strain 17, except for the number of cohesins in ScaA and ScaB. In addition, an unprecedented number (~ 190) of putative dockerin motif-containing ORFs (four major phylogenetic clusters) are associated with protein families, including xylanases, cellulases, proteinases, serpins, surface-anchoring proteins and unknown activities. An interesting contig (4.67 kb) appears to encode a modular protein termed "superzyme", which has two GH_11 domains, a GH_10 domain, a CBM22 module, a dockerin, and a polysaccharide deacetylase separated by glutamine/asparagine-rich linkers. Microarray expression profiling allows us to analyze which of the strain FD-1 cellulosome-associated genes are expressed in response to growth on either cellobiose or cellulose. EndB-type dockerins seem to be the most common in the up-regulated contigs. The multi-domain gene families of the dockerin, containing ORFs or contigs with the highest up-regulation (3.5 to 23-fold) were superzyme (10 to 23-fold), CE_3/CBM_22/CesA, Ricin B-like lectin CBM/CBM_6/lipase GDSL, alpha-glucosidase, GH_30/CBM_22/CE_3/Ricin-like CBM/PL_11, and ScaC/ScaA/ScaB (3.5-fold). Multi-domain cellulosomal components with a dockerin sequence are highly correlated with up-regulation in response to cellulose.

O-10 **Analysis of *Methanobrevibacter ruminantium* genome sequence.** W.J. Kelly, G.T. Attwood (Metabolism and Microbial Genomics, Food and Health Group, AgResearch Ltd, Grasslands Research Centre, Palmerston North, and Pastoral Greenhouse Gas Research Consortium, New Zealand).

The genome of *Methanobrevibacter ruminantium*, a prominent methanogen in New Zealand ruminants, is being sequenced as part of a Pastoral Greenhouse Gas Research Consortium-funded project to mitigate greenhouse gases. The genome is approximately 3.0 Mb with a G+C content of 33.62%. At least 60 genes are involved in methanogenesis and hydrogen transfer. Comparison of the genome sequence with *Methanobacterium thermoautotrophicum* and *Methanosphaera stadtmanae* indicates methanogenesis gene organisation is conserved within the *Methanobacteriales*. Unlike *M. stadtmanae*, *M. ruminantium* has genes for citrate synthase and isocitrate dehydrogenase, indicating that the oxaloacetate to oxoglutarate pathway can operate via citrate, aconitate and isocitrate, as well as via malate, fumarate and succinate. The *M. ruminantium* genome appears to encode many large surface proteins that contain repeat sequences also found in *M. stadtmanae* proteins. The characteristics of these proteins indicate that they may mediate association with other rumen microbes. More than 50 genes are involved in the synthesis and export of exopolysaccharides, which is in agreement with previous observations that this organism produces a capsule. Approximately 50% of the ORFs identified by GLIMMER analysis have no known function. Determining the function of these new genes will improve our understanding of the biology of this organism and help define its role in methane formation in the rumen.

O-11 **Post-genomics approaches to study the functionality of bifidobacteria in infant faeces.** E.S. Klaassens[a], R. Boesten[b], F.H.J. Schuren[b], E.E. Vaughan[a], W.M. De Vos[a] ([a] Laboratory of Microbiology, Wageningen University, The Netherlands; [b] TNO, Zeist, The Netherlands).

Following birth, the human gastrointestinal tract is rapidly colonised by microorganisms that play a role in the nutrition, physiology and health of the host. Bifidobacteria are especially predominant in the infant colon. In this study,

we have applied post-genomic technologies, including metaproteomics and bifidobacterial community transcript profiling, to obtain understanding of the activity of commensal bifidobacteria in infant faecal microbiota. The metaproteomes of infant faecal microbiota were determined by extraction and subjection of the proteins to two dimensional (2D) gel electrophoresis. These 2D gels revealed changes of the number and intensity of protein spots in time, but the patterns remained similar for each individual. In-gel digestion of protein-spots and sequencing of the peptides revealed a protein which shares high similarity to bifidobacterial transaldolases, testifying for the activity of bifidobacteria. In addition, transcript profiling of the infant faecal microbiota was performed. Total RNA was hybridised to a DNA microarray comprising clones covering the genomes of several bifidobacterial species. Significantly hybridising clones were sequenced and compared with those in the public databases. While some sequences were found to be ribosomal RNA, the majority showed similarity to protein-encoding genes predicted to be involved in vitamin production, stress response, sugar transport and metabolism, and housekeeping functions. Remarkably, similarity was observed to an operon involved in the utilisation of milk oligosaccharides and mucin sugars, indicating the functionality of bifidobacteria. To the best of our knowledge these are the first metaproteomes from human faecal bacteria, as well as community transcriptomes of commensal bifidobacteria from the human intestinal faecal ecosystem.

O-12 **Impact of reducing dietary carbohydrate intake upon the faecal microbial community and metabolites in human volunteers.** S.H. Duncan[a], A.M. Johnstone[a], G.E. Lobley[a], G. Holtrop[b], A. Belenguer[a], D. Bremner[a], M. Hrdinova[c], H.J. Flint[a] ([a] Rowett Research Institute and [b] BioSS, Bucksburn, Aberdeen AB21 9SB, UK, [c] Institute of Animal Physiology, Prague, Czech Republic).

Ketogenic ("Atkins-type") diets are popular strategies for weight loss and involve very low intakes of carbohydrates (< 20 $g \cdot d^{-1}$). Reasonable intakes of fermentable dietary carbohydrates, however, are considered beneficial to gut health through microbial production of butyrate, a major energy source for colonocytes that may also protect against cancer and other bowel diseases. The objective of this study was to investigate the impact of two high protein diets, differing in carbohydrate supply (20 $g \cdot d^{-1}$ and 170 $g \cdot d^{-1}$), on colonic bacterial communities and their fermentation products. Male, obese volunteers (17) consumed a maintenance diet for 3 days followed by 4 weeks on each of the experimental diets. Faecal samples were collected at the end of each dietary regime. Total faecal SCFA concentrations decreased significantly ($P < 0.001$) with reduced carbohydrate intake. This decrease was particularly marked for butyrate, which declined as a proportion of total faecal SCFA ($P < 0.001$). The composition of the faecal microbiota, studied using a panel of ten 16S rRNA-targeted FISH probes, also changed with carbohydrate intake. Notably, the proportion of bacteria belonging to the *Roseburia/Eubacterium rectale* group of butyrate-producing bacteria decreased approximately fourfold between the maintenance diet and the diet with the lowest carbohydrate intake ($P < 0.001$). It can be concluded that this group is particularly dependent on fermentable carbohydrates to occupy a nutritional niche in the colon, and is also probably the main contributor to butyrate formation. Interestingly, clostridial cluster XIVa bacteria other than *Roseburia/E. rectale* increased as the carbohydrate supply decreased ($P < 0.025$). Although a sufficient supply of butyrate is generally considered to be necessary to maintain gut health, it has not been established whether changes of the duration and magnitude shown here are likely to have significant consequences for gut health.

O-13 **Contribution of ruminal protozoa to duodenal flow of N, CLA and TVA in steers fed silage differing in water-soluble carbohydrates.** D.R. Yáñez-Ruiz[a], N.D. Scollan[b], R.J. Merry[b], C.J. Newbold[a] ([a] Institute of Rural Sciences, University of Wales Aberystwyth, Llanbadarn Campus, SY233AL, Aberystwyth, UK, [b] Institute of Grassland and Environmental Research, Plas Gogerddan, SY233ED, Aberystwyth, UK).

There is currently increasing interest in the fatty acid composition of ruminant products such as meat and milk, particularly the content of conjugated linoleic acid (*cis*-9, *trans*-11 CLA). CLA is derived from CLA produced in the

rumen and synthesis in meat or milk from the ruminally derived precursor trans-vaccenic acid (C18:1-*trans*-11; TVA). To date the production of CLA and TVA within the rumen has been considered solely from the perspective of bacterial metabolism and it has been suggested that protozoa are of minor importance. This experiment was designed to estimate the quantitative contribution of rumen protozoa to the total N, CLA and TVA to the duodenum in steers fed two silage diets (CS, control silage and HS, silage high in water soluble carbohydrates). Protozoal duodenal flows were estimated by using 18S rDNA determined by real-time PCR as a marker for protozoal biomass. By Denaturing Gradient Gel Electrophoresis we established that rumen protozoa populations were similar to those flowing to the duodenum. Estimated duodenal flow of protozoa represented 12 and 15% of the total N flowing to the duodenum. Protozoa accounted for 49.0 and 63.6% of the cis-9, trans-11 CLA, on the CS and HS diets, respectively, while 39 and 40% of the total TVA reaching the duodenum was also of protozoal origin. These results demonstrate the important contribution of protozoa in the flow of CLA and TVA to the duodenum and suggest that the role of protozoa in ruminal biohydrogenation merits further investigation.

O-14 **Metabolism of linoleic acid by bacterial species isolated from the human gut: biosynthesis of conjugated linoleic and hydroxy fatty acids.** E. Devillard, F.M. McIntosh, S.H. Duncan, H.J. Flint, R.J. Wallace (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Conjugated linoleic acids (CLA) confer a broad range of health-promoting properties. The main dietary sources of CLA in the human diet are ruminant products. CLA, mainly the *cis*-9, *trans*-11 isomer, are formed by ruminal bacteria as intermediates in the biohydrogenation of linoleic acid (LA). LA is isomerised to CLA, which is then hydrogenated to *trans*-vaccenic acid (TVA), then to stearic acid (SA). We recently confirmed that the human faecal flora also uses LA to form CLA, TVA and SA. In this study, we investigated the role of 30 major colonic bacterial species (low G+C content cluster IV and XIVa and high G+C content cluster) in LA metabolism. When the bacteria were cultivated in a medium containing LA, and the total fatty acids were extracted and analysed by gas chromatography, it emerged that 26 strains metabolised LA. Measurement of the linoleate isomerase activity of the bacteria indicated that only 9 strains converted LA to CLA. In culture, the same 9 strains produced CLA and/or TVA as final metabolites. Among the other 17 LA-metabolising strains, 11 of them produced a 10-hydroxy fatty acid, while the remaining 6 strains produced various C18:1 fatty acids. This study suggests that some human gut bacteria produce *cis*-9, *trans*-11-CLA and TVA from LA by a biohydrogenation mechanism similar to the one described for ruminal bacteria. However, most of the strains studied here seemed to metabolise LA to form other products, such as hydroxy fatty acids. It would be interesting to study the metabolism of these hydroxy fatty acids, which could be precursors of the multitude of CLA isomers produced when mixed faecal flora is incubated with LA. This is particularly important since it has been recently shown that specific CLA isomers have potential local effects on colon health.

P-70 **Effect of polyethylene glycol on in vitro gas production and digestibility of tannin-rich feedstuffs from North Africa arid zone.** R. Arhab[a], M. Rira[b], D. Macheboeuf[a], H. Bousseboua[b] ([a] Unité de Recherches sur les Herbivores, INRA, 63122 Saint-Genès-Champanelle, France, [b] Laboratoire de Génie Microbiologique et Applications, Université Mentouri, 25000 Constantine, Algeria).

The aim of the present study was to investigate the effect of polyethylene glycol on in vitro gas production, estimated in vitro organic matter digestibility (OMD) and metabolisable energy (ME) of four forages and two date palm by-products widely utilized by North African farmers. The feedstuffs evaluated include two families of plants: Gramineae (*Aristida plumosa* and *Danthonia froskalaii*) and Leguminoseae (*Genista saharae* and *Astragalus gombiformis*), and two date palm by-products: racemes and leaves. Phytochemical analysis showed that all feedstuffs contained antinutritional factors such as steroids, saponins and tannins. Date palm leaves had the highest concentration of extractable phenols and of total and condensed tannins; 61.8, 49.1 and 36.2 $g \cdot kg^{-1}$ DM, respectively. *A. plumosa* was the forage with the lowest concentration of phenols and total tannins, 5.4 and 3.2 $g \cdot kg^{-1}$ DM,

respectively, whereas *G. saharae* had the lowest concentration of condensed tannins, 0.7 g·kg^{-1} DM. Addition of polyethylene glycol (PEG) increased gas production (20.87–25.03 mL/200 mg DM), OMD (47.25–50.83 g/100 g DM) and ME (8.96–9.62 Mj·kg^{-1} DM), without and with PEG respectively ($P < 0.05$). The best improvement for gas production (16.87, 28.76 mL/200 mg DM), OMD (44.69, 54.32%) and ME (8.73, 10.21 MJ·kg^{-1} DM), with and without PEG, respectively, was recorded for *Aristida plumosa.* The lowest increment values of gas production and ME were noted for *Astragalus gombiformis,* whereas the lower increment of OMD was observed for *Danthonia froskalaii*. These results indicate that the positive effect of PEG on the degradation of these substrates was more pronounced in forages with low tannin content than in those with high tannin concentration.

P-71 **In vitro ruminal fermentation of some deserts browse species and date palm by-products.** R. Arhab[a], D. Macheboeuf[a], R. Bergeault[a], H. Bousseboua[b] ([a] Unité de Recherches sur les Herbivores, INRA, 63122 Saint-Genès-Champanelle, France, [b] Laboratoire de Génie Microbiologique et Applications, Université Mentouri, 25000 Constantine, Algeria).

The present study was carried out to assess the in vitro rumen fermentation and production of fermentation end-products of four perennial browse species: *Aristida plumose*, *Danthonia froskalaii, Genista saharae* and *Astragalus gombiformis*, and two date by-products: racemes and leaves, comparatively to vetch-oat hay. The samples were fermented for 72 h in a batch fermenter with ruminal fluid of sheep using the pressure transducer technique. At the end of fermentation, pH, volatile fatty acids and ammonia production were measured. There were wide variations among forages in crude protein, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin. Crude protein content varied from 25 g·kg^{-1} DM in racemes to 125 g·kg^{-1} DM for *Astragalus gombiformis*. The NDF and lignin ranged from 586.1 to 824.4 g·kg^{-1} DM and 43.6 to 142.4 g·kg^{-1} DM, respectively. Polyphenolic content varied between forages and families. The by-products had the highest values for total extractable phenols, total and condensed tannins. However, the Gramineae forages had the lowest values ($P < 0.001$). The forages produced a lower gas volume than vetch-oat hay ($P < 0.001$). The highest value observed in forages was for *Danthonia froshkalaii* (6.08 mmol·g^{-1} DM) and the lowest for date palm leaves (2.08 mmol·g^{-1} DM). The molar proportion of total volatile fatty acids was influenced by the forage species ($P < 0.001$). The acetate to propionate ratio was highest for *Astragalus gombiformis* (3.69) and lowest for racemes (2.01). For ammonia production, the highest value was observed for *Astragalus gombiformis* and the lowest value for vetch-oat hay. However, the mean ammonia production for date palm by-products was negative. This result could indicate the formation of complexes between tannins and protein.

P-72 **The diversity and prevalence of conjugated linoleic acid-producing *Bifidobacterium* species in the human gastrointestinal tract.** E. Barrett[a,b], R.P. Ross[a,b], G.F. Fitzgerald[b,c], F. Shanahan[b,d], C. Stanton[a,b] ([a] Teagasc Biotechnology Centre, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland, [b] Alimentary Pharmabiotic Centre, Cork, Ireland, [c] Department of Microbiology and [d] Department of Medicine, University College Cork, Ireland).

The aim of this study was to identify intestinally derived culturable bifidobacteria strains with ability to bioconvert free linoleic acid to conjugated linoleic acid (CLA) and to determine their prevalence and diversity among human populations. Samples were collected from 28 healthy neonates, 10 healthy adults and 20 elderly adults infected with *Clostridium difficile*, and plated on cys-MRS containing mupirocin to pre-select for bifidobacteria. The isolates were screened for CLA biosynthesis, spectrophotometrically at a wavelength of 233 nm, following incubation in the presence of free linoleic acid. In total, 18 genetically distinct, based on pulsed field gel electrophoresis, CLA producing strains were isolated and identified as bifidobacteria based on the enzyme fructose-6-phosphate phosphoketolase, their partial 16S ribosomal DNA or HSP60 heat shock protein DNA sequences and by *Bifidobacterium* species specific PCR reactions. The CLA producing bifidobacteria strains were identified as either *B. breve, B. longum, B. infantis, B. dentium* or *B. catenulatum*, with CLA conversion efficiencies varying from 2.6–76%, as

determined by gas liquid chromatography. The predominant CLA isomer produced by these bifidobacteria was *cis*-9, *trans*-11 $C_{18:2}$, with the most efficient producers belonging to the species *B. breve* and *B. longum*. Patients infected with *C. difficile* were the best source of CLA producing bifidobacteria, in terms of both production efficiency and prevalence. The results show that 1 in 4 subjects harboured CLA producing bifidobacteria. This diversity and prevalence of CLA producing bifidobacteria among subjects of varying ages may be a further potential health promoting property of the genus.

P-73 **Alternative mechanisms of metabolic cross-feeding between human gut bacteria.** A. Belenguer, S.H. Duncan, G. Calder, G. Holtrop, P. Louis, G.E. Lobley, H.J. Flint (Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB UK).

Carbohydrates that reach the human colon have a major influence on fermentative metabolism by gut bacteria. Resistant starch and fructo-oligosaccharides (FOS) have been considered to promote *Bifidobacterium* strains, but their influence on metabolism and microbial competition is likely to be complex. Bifidobacteria form acetate and lactate as major fermentation products, but these may be further metabolised by other species via cross-feeding. Furthermore, a wide variety of gut bacteria can be expected to compete for starch and FOS breakdown products. The aim of this study was to investigate possible mechanisms of metabolic cross-feeding, particularly in relation to the formation of butyrate, which plays an important role in gut health. Out of twelve *Bifidobacterium* strains tested, four grew on starch and nine on FOS in pure culture. Metabolic cross-feeding was investigated in co-cultures between strains of *Bifidobacterium adolescentis* able to grow on starch or FOS, and strains of butyrate-producing bacteria that alone are unable to utilise starch or FOS for growth. Stable isotopically labelled lactate and acetate were used to study their utilization. ^{13}C-acetate or ^{13}C-lactate labelling confirmed that *Eubacterium hallii* was able to convert acetate and lactate into butyrate in the co-cultures. Separately, a non lactate-utilising *Roseburia* strain, however, also produced butyrate in co-cultures with *B. adolescentis*, implicating a second mechanism of cross-feeding. We conclude that there are two distinct mechanisms of cross-feeding between *Bifidobacterium* and butyrate-producing strains, one due to consumption of lactate and acetate and the other due to cross feeding of partial breakdown products from complex carbohydrates.

P-74 **The ability of the rumen ciliate *Diploplastron affine* to digest and use 1,3-β-D-glucans.** G. Bełżecki, R. Miltko, T. Michałowski (The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna near Warsaw, Poland).

It is well known that 1,3-β-D-glucans are present in cereals and in the fungal cell wall. Cereals are commonly used in ruminant nutrition while some species of fungi inhabit the rumen. Rumen ciliates engulf and digest small particles of the ground cereals and fungal zoospores. However, little is known about the digestion and role of the mentioned polysaccharides in nutrition of rumen protozoa. The aim of the present study was to determine the ability of the rumen ciliate *Diploplastron affine* to digest and use 1,3-β-D-polysaccharides for growth. In cultivation experiments the ciliates were grown in vitro. They were fed a diet consisting of powdered hay (0.3 $mg \cdot mL^{-1}$ culture$\cdot d^{-1}$), and wheat gluten (0.08 $mg \cdot mL \cdot d^{-1}$) while experimental cultures were also supplemented with 1,3-β-D-glucan from *Euglena gracilis* (0.015, 0.03, 0.06 and 0.12 $mg \cdot mL \cdot d^{-1}$). No positive effect of the added polysaccharide on the growth of ciliates was found as the number of ciliates in the control cultures was about 2300 mL^{-1}, while in experimental cultures there were 2400, 2300, 1800 and 1200 individuals, respectively. In biochemical experiments the degradation rate of 1,3-β-D-glucan (lichenan) and laminarinobiose (1,3-β-D-disaccharide) from *Laminarina digitata* was measured by crude enzyme preparation of bacteria-free *Diploplastron affine*. The degradation rate of lichenan and laminarinobiose was 33.5 and 81 μM glucose released mg^{-1} protein h^{-1}, respectively, while pH optima of these reactions were 5.0 and 5.5. Non-denaturing polyacrylamide gel electrophoresis combined with zymogram technique revealed the presence of two protein bands active against lichenan. End products of the digestion of this polysaccharide were identified by thin layer chromatography (TLC). Glucose was the main product of substrate hydrolysis, however, traces of longer oligosaccharides were also present.

P-75 **Interaction of buckwheat seed extracts and intestinal microflora regarding changes of bacterial profile of intestinal microflora and antioxidant status of extracts.** E. Biedrzycka, L. Markiewicz, M. Bielecka, R. Amarowicz (Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland).

Buckwheat (*Fagopyrum esculentum*) contains a variety of valuable antioxidant compounds such as flavonoids, tocopherols and phenolic acids, but little is known so far about their relation with intestinal microflora (IM). The aim of the study was, therefore, to define interaction of: (1) the monocultures of pure strains of IM representatives, including *Bifidobacterium animalis* 30, *Lactobacillus plantarum* IB, *Escherichia coli* 94 and *Enterococcus faecium* T-208, (2) their co-culture, and (3) human faecal microflora (FM, 3 volunteers), and acetone buckwheat seed extracts (whole grain WGBE or shelled SBE; 2.5 mg·mL^{-1} of medium). DPPH and ABTS scavenging, pH and IM quantitative (culturing methods) and qualitative (PCR-DGGE) profiles were determined in 24-h cultures at 37 °C under anaerobic conditions. The controls were: medium without bacteria and cultures in the medium without extracts. The buckwheat extracts stimulated growth and acidifying activity of *B. animalis* 30, and slightly decreased that of *L. plantarum* IB and *E. coli* 94 (WGBE) monocultures. In the co-culture, intensified growth of mainly *B. animalis* 30 and *L. plantarum* IB was observed. The growth of FM bacterial populations was differentiated and reflected host FM-specific responses (inhibition or stimulation of growth) with lactobacilli reduced to the greatest extent. No significant qualitative alterations were observed in DGGE profiles of eubacteria, *Bifidobacterium*, *Lactobacillus* and *Clostridium coccoides*-group, whereas the respective extract-induced quantitative changes were confirmed. IM generally decreased scavenging activity of both extracts, similarly for the pure strain co-culture and FM. The share of WGBE soluble compounds in DPPH scavenging activity (43–50%) lowered to a higher extent (by 43%) in the IM co-culture than upon FM (only by 2–19%). The high share (64–76%) of soluble compounds in ABTS scavenging activity of WGBE was significantly reduced by both co-culture and faecal microflora (by 79–89%).

P-76 **Accumulation of rumen biohydrogenation intermediates through grazing of botanically diverse pastures is associated with changes in the rumen protozoal population.** C. Boeckaert[a,b], N. Boon[a], M. Lourenço[b], W. Verstraete[a], V. Fievez[b] ([a] Laboratory of Microbial Ecology and Technology, Ghent University, Ghent, Belgium, [b] Laboratory for Animal Nutrition and Animal Product Quality, Ghent University, Ghent, Belgium).

In an attempt to study the effect of variation in the botanical composition of pastures on the fatty acid metabolism of grazing ruminants, 21 lambs were assigned for 12 weeks to either an intensive ryegrass, clover or herbage rich pasture and the fatty acid profile of rumen, abomasum, intramuscular and subcutaneous fat, sampled at slaughter from non-fasted animals, was determined. Concentrations of hydrogenation intermediates (C18:1 t10 + t11, C18:2 t11c15, CLA c9t11, CLA t10c12) in the rumen of lambs grazing the herbage rich pasture were 54% higher compared to lambs grazing intensive ryegrass or clover pasture while the abomasum fatty acid profile showed no significant differences in the amount of hydrogenation intermediates between lambs grazing the different pastures. The disappearance of these significant differences in the abomasum, subcutaneous and intramuscular fatty acid profile suggest they might have been associated with rumen protozoa, as the latter mainly sequestrate in the rumen. Indeed, molecular fingerprinting of the ciliates by PCR-DGGE analysis revealed that the protozoal communities evolved differently according to the grazed pasture, provoking the appearance of an extra DGGE band in rumen samples of herbage rich grazing animals. Identification of the latter, after excising and sequencing, showed 98% similarity to *Diplodinium dentatum*. Overall, DGGE patterns of rumen ciliates in animals grazing the herbage rich pasture mutually clustered and were separated from those grazing other pastures. In addition, quantitative Real Time-PCR of rumen ciliates showed a significantly lower amount of protozoa in association with the herbage rich pasture.

P-77 **Rapid screening of bacterial genes coding histidine and tyrosine decarboxylase using PCR.** R. Burdychova[a], A. Spanova[b], B. Rittich[b], T. Komprda[a] ([a] Mendel University

of Agriculture and Forestry in Brno, Faculty of Agronomy, Department of Food technology, Czech Republic, [b] Masaryk University, Faculty of Science, Institute of Experimental Biology, Brno, Czech Republic).

Biogenic amines (BA) are the low-molecular organic bases which are formed due to the amino acid decarboxylase activities of microorganisms in fermented foods. These compounds have been studied due to the toxicological effects derived from their vasoactive and psychoactive properties The formation of histamine or tyramine in foods depends on the presence of free histamine and/or tyramine in samples and the presence of microorganisms having histidine or tyrosine decarboxylase activity. The aim of this work was to use a recently published PCR procedure (Coton et al., 2005, J Microbiol Meth 63: 296–304) for the identification of microorganisms with tyrosine and histidine decarboxylase genes. The microorganisms isolated from cheese (3 strains), lactic acid bacteria (LAB) of the genus *Lactobacillus* obtained from the Czech Collection of Microorganisms (CCM, Brno) (19 strains), the Culture Collection of Dairy Microorganisms (CCDM, Tabor, Czech Republic) (7 strains) and newly isolated from human faecal samples (20 strains), were used for the analysis. Altogether 10 ng of genomic DNA isolated using a phenol extraction procedure was used as template in PCR. All DNAs were shown to be amplificable using a primer set targeting the 16S rRNA gene as an internal PCR control. PCR products were detected using agarose gel electrophoresis. It was shown that PCR products of specific length were amplified using a primer set for the tyrosine decarboxylase gene and a primer set for the histidine decarboxylase gene with DNA of 3 strains isolated from cheese, 1 strain collected in CCM, 1 strain collected in CCDM and 1 strain of human origin. The results presented in this report show that PCR is a suitable and quick method for the rapid screening of bacterial genes encoding histidine and tyrosine decarboxylase.

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P-78 **Identification and characterisation of xylan-degrading bacteria from the human colon.** C. Chassard[a], M. Adams-Leclerc[b], A. Bernalier-Donadille[a] ([a] Unité de Microbiologie, INRA, 63122 Saint-Genès-Champanelle, France, [b] Unité d'Écologie Physiologie et du Système Digestif, INRA, Domaine de Vilvert, 78 332 Jouy-en-Josas, France).

In humans, plant cell wall polysaccharides (mainly cellulose and hemicelluloses) represent an important source of dietary fibres that are digested by gut microorganisms. Despite the extensive degradation of xylan in the colon, the population structure and the taxonomy of the predominant bacteria involved in degradation of this polysaccharide have not been extensively explored. Xylanolytic isolates were first obtained from faecal samples, using xylan growth medium. Phylogenetic analysis of these strains showed that they belonged to *Roseburia* and *Bacteroides* genera. Isolates related to *Bacteroides* corresponded to four new species of this genus, whereas *Roseburia* strains could be assigned to the species *R. intestinalis*. The xylanolytic *Roseburia* and *Bacteroides* species degraded and efficiently fermented xylan from different botanic origins. End-products of xylan fermentation by *Bacteroides* isolates were mainly acetate, succinate and propionate, with some lactate being also produced to a lesser extent, depending on the species considered. *Roseburia* isolates fermented xylan into butyrate, formate, lactate and H_2. The xylanase activity of *Bacteroides* and *Roseburia* isolates was quantified and compared with that of xylanolytic bacteria already isolated from the human gut and closely related to our strains. Most of our xylanolytic isolates showed higher xylanase activity than that of *Roseburia intestinalis* (L1-82) and *Bacteroides ovatus* (0038). In addition, zymogram analyses revealed common characteristics between *Roseburia* isolates and *R. intestinalis,* while the endoxylanase system of *Bacteroides* isolates appeared specific to each species and very different from that of *B. ovatus*. Our results provide new information on the diversity of the xylanolytic flora in the human colon and demonstrate that unidentified xylanolytic bacteria exist in the human gut.

P-79 **Molecular quantification of the human gut acetogen, *R. hydrogenotrophicus,* in faecal samples using real-time quantitative PCR.** C. Del'homme[a], E. Delmas[a], Y. Leblond[a], A.

Bernalier-Donadille[b] ([a] Laboratoires Mayoly Spindler, 78400 Chatou, France, [b] Unité de Microbiologie, INRA, 63122 Saint-Genès-Champanelle, France).

Gas production associated with the fermentative process in the human gut can be responsible for digestive troubles (abdominal pain and bloating and/or flatus). H_2, whose production is higher in patients with these digestive disorders, must be removed from the colon in order to prevent gas-associated symptoms. Among the three microbial H_2-consuming mechanisms existing in the colon, reductive acetogenesis is of particular interest, since acetate formed from CO_2 reduction by H_2 is non gaseous, non toxic and an energy source for eukaryotic cells. Utilisation of the hydrogenotrophic potential of acetogenic bacteria was thus suggested as new strategy to prevent gas-associated troubles through enhancement of H_2 consumption in the gut (patent 0006009). In this context, the effect of daily administration of *R. hydrogenotrophicus*, an acetogenic species isolated from the human gut, to decrease H_2 production was investigated in an animal model. A method allowing specific identification and quantification of the added *R. hydrogenotrophicus* in the gastrointestinal (GI) tract was first developed. Real-time PCR with SYBR Green I was used for the detection of the acetogenic species from faecal DNA, using specific primers. With this molecular approach, the distribution of this acetogenic species in the human population was found to be close to 12%, including faecal samples from children. The correlation between the important decrease in H_2 excreted by human-flora associated (HFA) rats receiving *R. hydrogenotrophicus* and the presence of the acetogenic species in the GI tract of these animals was further evidenced. Finally, excretion kinetics and survival of *R. hydrogenotrophicus* was evaluated using real-time-PCR after its inoculation to HFA rats.

P-80 **Role of ciliate protozoa in the metabolism of unsaturated fatty acids in the rumen.** E. Devillard[a], F.M. McIntosh[a], C.J. Newbold[b], K. Young[a], M. Barbier[a], R.J. Wallace[a] ([a] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK, [b] University of Wales, Aberystwyth, Wales, UK).

Conjugated linoleic acids (CLA) have been shown to improve human health. CLA are mainly found in milk and beef. They are intermediates in the ruminal biohydrogenation of dietary linoleic acid (LA). It seems that most of the biohydrogenation of LA occurring in the rumen is carried out by bacteria. The role of rumen protozoa in this process remains equivocal. In this study, analysis of cellular lipids indicated that protozoa contain proportionally more CLA and *trans*-vaccenic acid (TVA), which is a precursor of CLA in animal tissues, than ruminal bacteria. In incubations with different rumen content fractions (strained rumen fluid SRF, bacterial fraction BAC, protozoal fraction PRO) with LA, the rate of LA metabolism was very similar in SRF and BAC and was much higher than in PRO. The products formed in SRF and BAC were identified as CLA and TVA, and were not present in the incubations with PRO. A possible alternative route for formation of CLA and TVA was that protozoa could carry out desaturation of stearate. Incubations of SRF, BAC or PRO with ^{14}C-stearate showed that desaturation of stearate does not occur in any of the rumen content fractions. Furthermore, using PCR-based methods, no homologous genes to fatty acid desaturases were found in DNA extracted from ruminal protozoa. Thus, although protozoa are rich in CLA and TVA, they do not form these two fatty acids. The most likely explanation for these high concentrations is that protozoa incorporate CLA and TVA formed by bacteria. This work also suggests that the flow of unsaturated fatty acids, including CLA and TVA, from the rumen could be higher in protozoa than in bacteria.

P-81 **Differential export of two *Ruminococcus albus* cellulases by the Tat and Sec translocation pathways of *Escherichia coli*.** J. Esbelin, C. Martin, E. Forano, P. Mosoni (Unité de Microbiologie, INRA, Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France).

Ruminococcus albus is a Gram-positive bacterium that degrades plant cell walls in the rumen of herbivores. In order to ensure this function of degradation, *R. albus* synthesizes a high number of glycoside hydrolases, which are active after they have been exported and, some of them, anchored, at the cell-surface. In bacteria, the proteins destined to cross the cytoplasmic membrane are synthesized as precursors and possess

a signal sequence (SS) directing them to the "Sec" or "Tat" translocation pathways. The composition of the signal sequence of the two major cellulases of *R. albus* strain 20, Cel9D and Cel48B, suggests that each enzyme translocates using one of the two pathways. In order to confirm this hypothesis, the translocation of the two enzymes was studied by expressing SS-GFP fusions in *E. coli*. The cytoplasmic or periplasmic localization of the recombinant proteins was monitored by Western blot and fluorescence microscopy in a wild type *E. coli* strain and in Sec and Tat isogenic mutants. This study shows that the SS of Cel9D directs the pre-protein to the "Tat" translocation pathway, while the GFP fused to the SS of Cel48B is exported through the "Sec" machinery. These observations suggest that genes encoding components homologous to the Tat and Sec systems exist in *R. albus* and should be looked for once the genome sequence of *R. albus* strain 8 is complete.

P-82 **Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose.** G. Falony, A. Vlachou, K. Verbrugghe, L. De Vuyst (Research Group of Industrial Microbiology and Food Biotechnology, Department of Applied Biological Sciences and Engineering, Vrije Universiteit Brussel, Brussels, Belgium).

Cross-feeding between colon bacteria is thought to be the link between the bifidogenic and the butyrogenic effect caused by the addition of oligofructose to the diet. Acetate and lactate produced by bifidobacteria may form the substrates for butyrate-producing colon bacteria that are reported to account for 2–3% of the human gut microbiota. In this study, cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting colon bacteria was studied through in vitro mono- and coculture fermentations. Growth of *B. longum* BB536 on fructose or oligofructose led to the production of acetic acid and, in lesser amounts, formic acid, lactic acid, ethanol, and succinic acid. *Anaerostipes caccae* DSM 14662 produced only butyric acid and gasses when growing on fructose; no growth on oligofructose was observed. In a coculture with *B. longum* BB536, production of butyric acid by *A. caccae* DSM 14662 was detected, attributed to growth of the latter strain on the acetic acid, lactic acid, and fructose formed during the degradation of oligofructose by *B. longum* BB536. *Roseburia intestinalis* DSM 14610 grew on both fructose and oligofructose, but showed an absolute requirement for acetate. Only butyric acid and gases were formed. A coculture of *R. intestinalis* DSM 14610 with *B. longum* BB536 on oligofructose showed initial growth and acetate production by the *Bifidobacterium* strain, followed by oligofructose degradation and acetate conversion by *R. intestinalis* DSM 14610, leading to the production of butyrate. Two distinct types of cross-feeding between *B. longum* BB536 and acetate-converting colon bacteria were observed, both leading to the production of butyrate from oligofructose.

P-83 **Bile tolerance mechanisms of *Listeria monocytogenes*: implications for survival in the host GI tract.** C.G. Gahan, R.D. Sleator, M. Begley, C. Hill (Department of Microbiology and Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland).

Listeria monocytogenes is an important foodborne pathogen that is related to the low G/C lactic acid bacteria. In order to colonise the host GI tract prior to invasion, the pathogen must survive a number of sub-optimal microenvironments, including regions of elevated osmolarity and the presence of bile salts within the small intestine. Since these same challenges face the colonization of related probiotic bacteria, we propose that *L. monocytogenes* represents a useful, genetically tractable model with which to study microbial adaptation to conditions in the GI tract. We have used functional genomic analysis of the *L. monocytogenes* genome to examine a number of loci responsible for bile tolerance in this organism. This work demonstrated a significant role for bile salt hydrolase (BSH) and bile acid dehydratase in both bile tolerance and colonization of the murine GI tract. Furthermore, it was demonstrated that *bsh* expression is tightly regulated by the alternative sigma factor Sigma B and the major regulator of virulence gene expression, PrfA. Examination of *bilE*, a locus with significant homology to osmolyte uptake systems, demonstrated that this system is essential for bile tolerance in *L. monocytogenes.* We determined that BilE functions as a bile exclusion system that prevents intracellular accumulation of bile acids. Mutants in *bilE* are incapable

of effective colonization of the GI tract and subsequent infection in the murine model of disease. Finally, we employed a transposon mutagenesis approach to determine novel bile tolerance loci in *L. monocytogenes*. This approach uncovered 12 loci that are required for bile tolerance in this organism, including *zurR, lytB* and a homologue of the *capA* locus required for capsule formation in *Bacillus anthracis*. Interestingly the majority of loci are located within a 46-gene region of the *L. monocytogenes* genome that contains genes responsible for stress resistance and homeostasis. Overall this work represents a characterization of a number of genetic loci that are essential for microbial adaptation to conditions in the upper GI tract.

P-84 **Comparison of microbial pellets for estimation of microbial protein synthesis in continuous-culture fermenters.** A.I.M. García[a], M.J. Ranilla[b], E.M. Alcaide[a], M.D. Carro[b] ([a] Unidad de Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain, [b] Departamento de Producción Animal I, Universidad de León, 24071 León, Spain).

Measurement of ruminal microbial protein synthesis relies on the isolation of a microbial pellet representative of those microbes leaving the rumen. The aim of this study was to compare the estimates of microbial protein synthesis in continuous-culture fermenters fed two diets with different forage:concentrate ratio (80:20 [F80] and 20:80 [F20]) obtained by using microbial pellets isolated from effluent (EMP) or from fermenters content (FMP). A 14-day incubation run was carried out, each diet was fed to four fermenters and ^{15}N was used as a microbial marker. On days 12 and 13, total effluent was collected for analyses of non-ammonia N and ^{15}N enrichment, and for isolation of EMP after incubating about 500 g of effluent at 38 °C for 15 min with a saline solution containing 0.1% methylcellulose. On the last day, the content of fermenters was treated with methylcellulose solution before isolating FMP. Microbial protein synthesis was estimated from ^{15}N enrichment in both effluent and microbial pellets. For both diets, FMP presented a greater ($P < 0.02$) ^{15}N enrichment than EMP. Compared to FMP, the use of EMP overestimated microbial protein synthesis by 4.4 and 9.2% for diets F80 (365 vs. 381 mg N/d) and F20 (427 vs. 465 mg N/d), respectively, but differences were not significant ($P = 0.66$ and $P = 0.18$). These results indicate that, under the conditions of the present experiment, both FMP and EMP can be used as a reference pellet for estimating microbial protein synthesis in continuous-culture fermenters.

P-85 **Antioxidant activity and total polyphenol content studies in selected products using an in vitro digestion tract model with human faecal flora.** M. Gumienna, K. Goderska, Z. Czarnecki (Institute of Food Technology of Plant Origin, Department of Fermentation and Biosynthesis, The August Cieszkowski Agricultural University of Poznań, Poland).

Intestinal microflora is the main component of the digestion tract and plays an important role in digestive functions. The aim of this research was to analyse changes in microbial composition and interactions of the microflora with biologically active compounds. Products from leguminous plants (Red Kidney bean instant flour) and aronia (fruit juice) were selected for our investigations. The digestion tract model, which comprises 3 compartments: stomach, small intestine and large intestine, was used for controlling and regulating the environment in which digestion processes occurred. In each compartment of the model the total amount of phenolic compounds (Folin-Ciocalteu Reagent), antioxidant $ABTS^{\bullet+}$ (mg Trolox) activity and changes in microbial composition were investigated during the digestion. Faecal flora was isolated from feces of 3 human volunteers. The intestinal model was inoculated with bacterial flora at a concentration of 10^6 cfu·mL^{-1}. Growth of *Lactobacillus, Bifidobacterium, Enterococcus* and Enterobacteriaceae was enumerated on selective media. Growth of investigated bacteria, especially of the *Enterococcus* species, was affected by the digested aronia juice. The amount of bacteria after digestion in the large intestine was maintained at 10^8 cfu·mL^{-1} and for *Enterococcus* at 10^6 cfu·mL^{-1}. The instant flour from red Kidney beans also stimulated bacterial growth. In effect, the amount of bacteria detected in the large intestine was 10^{11} cfu·mL^{-1}. The highest antioxidant activity as well as amount of phenolic compounds after the digestion process was determined for the instant flour, while for aronia juice

a decrease in the antioxidant activity and total amount of phenolic compounds was observed.

P-86 **Effect of defaunating the rumen on microbial protein synthesis in native Iranian sheep.** K.K. Jafari[a], M. Rezaeian[b], M. Zahedifar[c], A. Mirhadi[c] ([a] Islamic Azad University, Branch of Savadkooh, Iran, [b] Faculty of Veterinary Medicine, University of Tehran, Iran, [c]Animal Science Institute, Karaj, Iran).

The rumen microbial ecosystem mainly consists of bacteria, protozoa and anaerobic fungi, which have an important function on the rumen fermentation process. In this study, the contribution of protozoa on the amount of microbial protein synthesis was determined. Four Iranian castrated male lambs (breed of shal) in a latin square design were used. Defaunating the rumen was done by using an emptying and washing method (Jouany and Senaud, 1979). The amount of microbial protein synthesis was measured using the Chen procedure (1992). The basis of this method is determining the amount of purine derivatives excreted via urine. The results of this experiment indicated that defaunating the rumen of sheep increased microbial nitrogen synthesis (11.8 vs. 7.73 g·day^{-1}), and a significant difference was observed ($P < 0.05$).

P-87 **Strain-specific architecture in cellulosome assembly in *Ruminococcus flavefaciens*.** S. Jindou[a], I. Borovok[a], M. Rincon[b], H.J. Flint[b], M. Berg[c], B.A. White[c], E.A. Bayer[d], R. Lamed[a] ([a] Tel Aviv University, Ramat Aviv 69978, Israel, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK, [c] University of Illinois, Urbana, IL 61801, USA, [d] The Weizmann Institute of Science, Rehovot 76100, Israel).

Ruminococcus flavefaciens FD-1 is a Gram-positive, anaerobic, cellulolytic bacterium, which commonly inhabits the digestive tracts of ruminants. Shotgun and complementary manual sequencing of the genome has revealed about six cellulosome-organising proteins (scaffoldins) and circa 190 dockerins, representing at least four different phylogenetic types. The architecture of the cellulosome system in strain FD-1 was assessed by bioinformatic analysis and by studying the interactions among overexpressed cellulosomal protein modules. Cellulosome organisation in strain FD-1 is generally similar to that of strain 17 but also exhibits some very novel properties. Surprisingly, the major scaffoldin, ScaB, comprises two different types of cohesins in strain FD-1, five of which are related to the cohesin sequences of ScaB in strain 17 and four others are similar to those of ScaA. The hybrid nature of ScaB scaffoldin is reflected in its ability to bind both ScaA and ScaC dockerins as well as several dockerin-containing enzymes. Scaffoldin ScaA of strain FD-1 has three cohesin modules, whose N-terminal cohesin is very divergent from the others but still appears to recognize weakly the latter type of dockerin. Moreover, the cohesin of the FD-1 ScaC adaptor protein binds certain enzyme-borne dockerins, in contrast to the corresponding cohesin of strain 17, which apparently fails to recognise the corresponding dockerins. Finally we also demonstrate that the cohesin of the FD-1 anchoring ScaE binds to a novel class of dockerins (e.g., from ScaB, ScaF and ScaH), but only limited cross-strain cohesin-dockerin interactions have been observed.

P-88 **Monitoring of rumen bacteria attached to ruminally incubated rice straw quantified by real-time PCR.** S. Koike, H. Yabuki, Y. Kobayashi (Creative Research Initiative Sousei, Hokkaido University, Sapporo 001-0021, Japan).

Real-time PCR assays were developed for the rumen bacteria including 12 representative species and 2 uncultured groups. Untreated- and sodium hydroxide (NaOH) treated rice straw was prepared to evaluate the effect of differential digestibility on the formation of bacterial community. The nylon bags containing respective rice straws were incubated for 10 min to 96 h in the rumen of sheep, and the target bacterial species associated with the straws were quantified by real-time PCR. As expected, the digestibility of NaOH-treated rice straw was higher than that of untreated straw. Irrespective of the treatments, the fiber-associated populations for *S. ruminantium*, *F. succinogenes* and uncultured bacterial group U2 were higher than those of the other bacterial species during the initial stage of incubation (within 6 h), then they maintained their population sizes on the straws. These 3 species/group may play an important role in rice straw degradation in the rumen. The kinetics of

bacterial attachment was different between the treatments, i.e. the higher peak level of attachment for fibrolytic species was detected at the initial stage of incubation on NaOH-treated straw than on untreated straw. On the other hand, some of the non-fibrolytic species showed a slower increase on NaOH-treated rice straw compared to untreated straw. This suggests that the higher digestibility of NaOH-treated straws was achieved under the less synergistic relationship between fibrolytic and non-fibrolytic species. The disappearance of less-digestible fractions and soluble substances from the rice straw by NaOH treatment might provide higher accessibility of easily digestible fiber for fibrolytic species and lower availability of the substrates for the non-fibrolytic bacteria.

P-89 **Molecular identification of carbohydrate-fermenting bacteria in the human colon by RNA-stable isotope probing.** P. Kovatcheva-Datchary, M. Egert, A.A. De Graaf, N.E.P. Deutz, A. Maathuis, H. Smidt, W.M. De Vos, K. Venema (WCFS, PO Box 557, 6700 AN Wageningen, The Netherlands).

Prebiotic carbohydrates are believed to be important promoters of human gut health, mainly through their interactions with the microbial community in the human colon. However, these interactions are far from being fully understood. We used the RNA-stable isotope probing (SIP) technique to identify those microbial populations that are involved in the fermentation of dietary relevant carbohydrates in the human colon. In a pilot study, the microbial community of an in vitro model of the human colon (TIM-2) was supplemented with 40 mM [U-^{13}C]-glucose. Within 2 h, the glucose was completely fermented yielding mostly lactate, acetate and butyrate. Moreover, ^{13}C-labelled bacterial 16S rRNA was isolated already 1 h after incubation with [U-^{13}C]-glucose. Terminal-restriction fragment length polymorphism (T-RFLP) analysis showed five dominant microbial species comprising the bacterial community of the colon model. Phylogenetic analysis of ^{13}C-labelled 16S rRNA linked the most active bacterial glucose-fermenters in the model to *Clostridium perfringens* and *Streptococcus bovis*. In a second experiment, 1 g of [U-^{13}C]-starch isolated from potatoes, grown in the presence of $^{13}CO_2$, was added to the in vitro model. The starch was fermented more slowly than glucose, yielding mainly acetate, butyrate and propionate. After 4 and 8 h of incubation, isotopically labelled RNA could be isolated. T-RFLP fingerprinting of labeled 16S rRNA identified an ~ 70 bp T-RF as representative for primary starch-fermenting bacteria. Further phylogenetic analyses are underway to determine the taxonomic affiliation of these starch consuming bacteria. In conclusion, RNA-SIP combined with metabolic analyses appears promising to identify bacteria actually involved in colon fermentations and link them to metabolic products, not only in the course of in vitro but also of in vivo studies.

P-90 **Evaluation of the effect of tannin-rich plants on rumen microbial fermentation.** C. Longo[a,b,c], J. Hummel[b], J. Liebich[c], R.S. Dias[a], E.F. Nozella[a], S.L.S. Cabral Filho[a], P. Burauel[c], A.L. Abdalla[a], K.-H. Südekum[b] ([a] Centre for Nuclear Energy in Agriculture, SP, Brazil, [b] University of Bonn, Germany, [c] Forschungszentrum Jülich, Germany).

Some plants have been neglected as fodder due to the presence of anti-nutritional factors, such as condensed tannins (CT), affecting microbial activity. Significance of influence of CT can be evaluated via a test for CT, or via in vitro fermentation (with and without PEG). Four legumes, *Styzolobium aterrimum* L (STA), *Styzolobium deeringianum* (STD), *Leucaena leucocephala* (LEU) and *Mimosa caesalpiniaefolia* (MIC), and *Cynodon*x Cynodon grass (CYN) as control were tested in the Hohenheim gas test and for their CT content. Gas production (GP) was recorded at 10 intervals over 96 h of incubation (two runs with three syringes for each plant). GP parameters were determined by an exponential model. CT in the legumes, expressed as leucocyanidin-equivalent, ranged from 20 (STA) to 205 (MIC) $g \cdot kg^{-1}$, and 96-h GP ranged from 98 (MIC) to 215 (LEU) $mL \cdot g^{-1}$. LEU, STA and STD presented similar 96-h GP values, but differed ($P < 0.05$) from CYN and MIC. There was a PEG effect on 24-h GP for all plants except CYN ($P < 0.05$), which lasted until 96-h GP only for MIC. Although STA had less CT than STD and LEU, they expressed a similar PEG effect ($P > 0.05$). MIC had a much higher Lag-phase (2.7 h) than the other plants, which ranged only from 0.07 to 0.64. PEG largely reduced Lag-phase of MIC to 0.20 h. Despite the presence of CT, LEU

and STD were well fermented, showing differences in biological activity of CT, which can best be evaluated by in vitro fermentation tests.

P-91 **Butyrate metabolism in human colonic bacteria: characterisation of a novel CoA-transferase.** P. Louis, C. Charrier, H.J. Flint (Rowett Research Institute, Gut Health Division, Microbial Ecology Group, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK).

Low mol% G+C Gram positive bacteria comprise a major group within the microbial community of the human large intestine. They exhibit a fermentative metabolism and for many strains butyrate is one of the main fermentation acids produced. Butyrate serves an important role in maintaining gut health, as it is the preferred energy source for the colonic wall and has regulatory effects on cell differentiation and apoptosis. We therefore aimed at investigating the pathway leading to butyrate formation in representative strains. The final step of butyrate formation can proceed via two alternative pathways. We have shown previously that the CoA-transferase route is the predominant route for butyrate formation within human colonic bacteria (Louis et al., 2004, J Bacteriol 186: 2099–2106). While all the genes for butyrate formation via the alternative butyrate kinase route have been described in clostridia, the gene for the CoA-transferase reaction remained elusive. We have recently identified a novel CoA-transferase gene from the clostridial cluster XIVa strain *Roseburia* sp. A2-183. Characterization of the gene product showed that butyryl-CoA is the preferred substrate for this enzyme, making it a very strong candidate for the CoA-transferase involved in butyrate formation. Similar genes could be identified in various other human gut bacteria from clostridial cluster IV and XIVa. The identification of this gene will further our understanding of butyrate formation in human gut anaerobes and enable us to study its regulation in response to environmental changes.

P-92 **Retention time of substrate affects fermentation characteristics in Rusitec fermenters.** M.E. Martínez, S. Ramos, M.L. Tejido, M.J. Ranilla, L.A. Giraldo, M.D. Carro (Departamento de Producción Animal I, Universidad de León, 24071 León, Spain).

Previous studies have shown that simulation of rumen fermentation in Rusitec fermenters fed high-concentrate diets is usually unsatisfactory. Since rumen retention time of digesta is one of the factors affecting rumen fermentation in vivo, the objective of this study was to investigate the effects of two retention times of concentrate – 24 h (T24) and 48 h (T48) – on rumen fermentation parameters in Rusitec fermenters. Eight fermenters were fed daily 21 g dry-matter (DM) of concentrate and 9 g DM of alfalfa hay contained in separate nylon bags. Forage retention time was 48 h in all fermenters. After 10 days of adaptation, diet degradability and production of volatile fatty acid (VFA) and methane were measured on four consecutive days. Concentrate retention time did not affect ($P > 0.05$) the dilution rate (mean value of 3.79% h^{-1}) or the rumen pH (mean value of 6.47). Degradability of both DM and fibre was lower ($P < 0.01$) for T24 (66.5 and 18.9%, respectively) than for T48 (71.2 and 26.6%), but no differences between treatments were observed in total VFA production (80.1 vs. 81.6 mmol·d^{-1}; $P = 0.645$). Reducing concentrate retention time from 48 to 24 h decreased methane production (21.7 vs. 18.2 mmol·d^{-1}; $P = 0.005$) and molar proportions of acetate (52.3 vs. 48.6%; $P = 0.008$) and butyrate (24.3 vs. 22.9%; $P = 0.004$), but it increased the proportion of propionate (14.8 vs. 19.5%; $P = 0.020$). As a consequence of these changes, both acetate:propionate and methane:VFA ratios were lower ($P < 0.01$) for T24 (2.52 and 0.23) than for T48 (3.54 and 0.27). These results indicate that changing the substrate retention time is a suitable means to alter rumen fermentation characteristics in Rusitec fermenters.

P-93 **NMR study of plant fibre degradation by *Ruminococcus albus* and *Fibrobacter succinogenes*.** M. Matulova[a], R. Nouaille[b,c], P. Capek[a], M. Péan[d], A.-M. Delort[b], E. Forano[c] ([a] Institute of Chemistry, Slovak Academy of Sciences, Bratislava, 845 38 Slovak Republic, [b] Laboratoire de Synthèse et Étude de Systèmes à Intérêt Biologique, UMR 6504 Université Blaise Pascal-CNRS, 63177 Aubière Cedex, France, [c] Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand-Theix, 63122

Saint-Genès-Champanelle, France, [d] DEVM/ GRAP, CEA Cadarache, France).

Cellulose and wheat straw degradation by *Ruminococcus albus* and *Fibrobacter succinogenes* was monitored using NMR spectroscopy and chemolytic methods. In situ solid state NMR, ^{13}C CP MAS (cross polarisation magic angle spinning) was used to monitor the modification of the composition and the structure of cellulose and ^{13}C-enriched wheat straw during growth of the bacteria on this substrate. There was no preferential degradation either of amorphous regions of cellulose versus crystalline ones, or of cellulose versus hemicelluloses in wheat straw, whatever the bacterial species. Liquid state 2D NMR experiments and chemolytic methods were used to analyse in detail the sugars released in the culture medium, and integration of NMR signals enabled their quantification at various times of culture. The results showed a different mode of attack of the plant fibres by the two bacteria: cellodextrins were accumulated in the culture medium of *R. albus*, but not of *F. succinogenes*, and sequential activity of the esterases on hemicelluloses was also different in the two species. This suggests that the two cellulolytic bacteria have specific roles in plant cell wall degradation in the rumen ecosystem.

P-94 **Variations in intermediates and products of linoleic acid biohydrogenation by two different human mixed colonic flora.** F.M. McIntosh, R.J. Wallace, E. Devillard (Rowett Research Institute, Bucksburn Aberdeen AB21 9SB, UK).

Conjugated linoleic acids (CLA) are considered to be beneficial to human health. They are produced in the rumen as intermediates in the biohydrogenation of linoleic acid and passed into the ruminant products. In this study, we show that CLA can also be produced endogenously in the human gut from linoleic acid, and that this process varies considerably between human subjects. Fresh faecal samples were obtained from two healthy female volunteers (V1 and V2). Faecal samples were incubated anaerobically with linoleic acid (LA) and samples removed at time intervals. LA was metabolised at a similar rate in the samples from both volunteers. However the products of biohydrogenation were different between the 2 samples. The sample from V1 showed low production of CLA, present as mainly one isomer (C18:2, *cis*-9, *trans*-11) and a high production of stearate. In contrast, the sample from V2 produced high amounts of CLA of several isomers. The relation between differences in the ability to produce CLA and microbial diversity was investigated. The biodiversity of the bacterial populations of the samples from the two volunteers was shown using denaturing gradient gel electrophoresis and 16S rDNA libraries. From the analysis of 16S rDNA sequences, we observed that the sample from V1 contained a very high proportion of bacteria close to *Butyrivibrio hungatei* (Cluster XIVa), whereas the sample from V2 showed a large population of bacteria related to *Eubacterium siraeum* (Cluster IV). The endogeneous production of CLA in the human gut varies between subjects, and is probably linked to different colonic flora. This is particularly interesting since it has been recently shown that specific CLA isomers have potential local effects on the colon.

P-95 **The existence and composition of distinctive bacterial communities colonising each of three insoluble dietary substrates in a model of the human colon.** E.C. McWilliam Leitch, A.W. Walker, S.H. Duncan, H.J. Flint (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

The breakdown of insoluble dietary carbohydrates, such as resistant starch and plant polysaccharides, and of endogenously-produced mucin by gut bacteria, has an important impact upon the health of the human colon. While substrate preferences have been determined for a few isolated gut bacteria, the identity and diversity of primary colonisers of a given insoluble substrate in the complete gut community remains largely unknown. Continuous flow fermentors inoculated with faecal samples were used here to model bacterial colonisation of starch, bran and mucin. Attached bacteria were identified by PCR-amplified 16S rRNA sequences and fluorescent in situ hybridisation. Diversity within the resulting attached communities was low. There was evidence for a high degree of substrate-specificity, and 81% of the identified OTU's were recovered from only one substrate. The main starch-adherent species *Ruminococcus bromii*

and *Bifidobacterium adolescentis* were only detected on starch. Likewise, an uncultured group related to *Clostridium hathewayi* was found only on bran, and *B. bifidum* was detected only on mucin. *R. lactaris* and closely related uncultured clones were major colonisers of mucin for samples from three individuals. Inter-individual variation was also apparent. Bifidobacteria were the predominant species found on starch for samples from two individuals, whereas *Eubacterium rectale* and *R. bromii* were the co-dominant colonisers for the other two individuals. In conclusion, this study showed that distinctive bacterial communities form on different insoluble substrates in a model of the human colon. In addition, it appears that the dominant primary colonisers of a given substrate may differ between the gut microbiota of different individuals.

P-96 **Isolation of xylanase genes from two new xylan-degrading species from the human colon, *Bacteroides* sp. and *Roseburia intestinalis*.** C. Mirande, C. Chassard, A. Bernalier-Donadille, E. Forano, C. Béra-Maillet (Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

Fibrolytic bacteria of the human gut ecosystem play a major role in the degradation of plant cell wall polysaccharides of the ingested dietary fibres. However, these microorganisms have not been extensively studied. Hemicellulolytic bacteria were found predominant in this ecosystem compared to the cellulolytic populations. As hemicellulolytic activity was found in the predominant colonic microflora and xylans considered as a preferential substrate for the fibrolytic bacteria, we recently isolated bacterial strains with high xylanolytic activities from the feces of healthy volunteers. These isolates were identified as *Bacteroides* sp. and *Roseburia intestinalis* species. Genomic libraries were then constructed for both *Bacteroides* sp. and *R. intestinalis* isolates and xylanase genes were detected by screening of the xylanolytic activity of the clones. Furthermore, PCR products were generated by using specific primers designed from the sequence of xylanase genes present in the databases, comprising other *Bacteroides* species xylanase genes, and from family 10 and 11 xylanase sequences. Xylanolytic equipment of *Bacteroides* sp. and *R. intestinalis* is further discussed.

P-97 **Effect of red beet juice and crisps from red beet on the growth of intestinal microorganisms and antioxidant stability.** M. Neumann, W. Grajek (Department of Biotechnology and Food Microbiology, Agricultural University, ul.Wojska Polskiego 48, 60-627 Poznań, Poland).

One of the most interesting vegetables, rich in antioxidant compounds, is red beet. It contains red betacyanins and yellow betaxanthins. Antioxidants, taken up with food into the gastrointestinal tract, react with many physiological secretions like HCl, hydrolytic enzymes, bile salts and pancreatic fluids and have contact with intestinal microorganisms. This treatment leads to chemical changes in antioxidant structure and results in changes of antioxidant capacity. The aim of the work was to study the influence of gastrointestinal digestion and microbial fermentation of red beet products on the growth of some groups of intestinal microflora and on the antioxidant capacity. In the experiments, red beet juice and crisps from red beet were used. The product digestion was performed using an in vitro model consisting of a gastric reactor (pepsine, 1 M HCl, pH 2.0, 2 h) and small intestine reactor (pancreatic extract, bile salt, $NaHCO_3$, pH 7.4, 2.5 h). The culture medium was inoculated with a fecal human bacterial suspension at ~10^6 cfu·mL^{-1}. Drawing of samples was done after 2 and 21 h of digestion. In samples, the antioxidant capacity of red beet products and changes in the microorganism cell densities were determined. Bacterial groups were counted using the following agar media: McConkey for *Escherichia,* MRS for *Lactobacillus*, Garche's for *Bifidobacterium* and Kanamycin Esculin Azide for *Enterococcus*. To determine the antioxidant capacity, the samples were centrifuged and the antioxidant capacity was assessed in the supernatant on the basis of the scavenging activity of ABTS and DPPH radicals. The results of our investigation showed a correlation between the type of product digested and the intensity of intestinal microorganism growth, as well as the effect of product digestion on its antioxidant capacity. It was found that crisps from red beet introduced into the culture medium stimulated growth of the studied microorganisms. The

results also indicated decreases of the antioxidant capacity after exposure to the gastrointestinal tract.

P-98 **Binding and biotransformation of the *Fusarium* mycotoxin zearalenone to α-zearalenol by lactic acid bacteria.** V. Niderkorn[a,b], H. Boudra[a], D.P. Morgavi[a] ([a] Herbivore Research Unit, INRA-Theix Research Centre, France, [b] Lallemand S.A.S, 31702 Blagnac, France).

Lactic acid bacteria (LAB) are widely used in the preparation of fermented foods and feeds and as probiotics in human and animal nutrition. Some strains of this group of bacteria have the ability to decrease the bioavailability of mycotoxins present in foods and feeds. The objective of this work was to select LAB able to detoxify zearalenone (ZEN), an estrogenic *Fusarium* mycotoxin frequently found in maize grain and silage. We screened 197 strains of LAB for their ability to remove ZEN by either binding or biotransformation in a model representative of maize silage. Bacteria (5×10^8 cfu·mL^{-1}) were incubated at 25 °C for 24 h in maize infusion acidified to pH 4 and containing 5 μg·mL ZEN. After incubation, the medium was centrifuged and the supernatant was analysed by HPLC. Efficiency of removal by binding was genus specific. While *Lactobacillus*, *Leuconostoc*, *Lactococcus*, and *Pediococcus* bound about 20% of ZEN, the most active genera, *Streptococcus* and *Enterococcus* bound 40 and 35% of ZEN, respectively ($P < 0.05$). In addition, 8 *Lactobacillus* and 3 *Leuconostoc* strains were able to biotransform between 28 and 59% of ZEN to a single metabolite. This compound was identified by LC-MS as α-zearalenol (α-ZOL), which is more estrogenic than ZEN and is thought to be the activated form of the toxin in mammals. The ability of some strains to bind ZEN is a positive technological property. However, the biotransformation of ZEN to α-ZOL, which was identified for the first time in bacteria, cannot be considered as a detoxification.

P-99 **Ability of lactic acid bacteria to bind or/and metabolize N-nitrosodimethylamine (NDMA) connected with growth stages of the bacteria.** A. Nowak, Z. Libudzisz (Institute of Fermentation, Technology and Microbiology, Technical University of Lodz, ul. Wolczanska 171/173, 90-924 Lodz, Poland).

Lactic acid bacteria have been reported to have antimutagenic or anticarcinogenic properties in vitro and in vivo. The purpose of the present investigation was to study the capacity of lactic acid bacteria to decrease the level of carcinogen – NDMA. Bacterial strains tested were: *Lactobacillus plantarum* WL, *Lactobacillus casei* DN 114 001, *Lactobacillus casei/paracasei* KNE1, *Lactobacillus casei* J/III, *Lactobacillus casei* BD. The concentration of NDMA examined was 10 μg·mL^{-1} in a special MRS broth with low nitrogen quantity. Samples were inoculated and incubated for 168 h at 37 °C under anaerobic conditions. Using Koch's plate method, the number of cells was determined at 0 h, 4, 8, 12, 24, 48, 72 (or 96) and at 168 h incubation. Simultaneously, with high performance liquid chromatography (HPLC), the level of NDMA in samples was determined. The addition of NDMA did not affect the growth of bacteria during 168 h incubation. There was a relationship between the level of NDMA in the sample and the stage of growth of bacteria. In all cultures the amount of NDMA was decreased at the beginning (between 0 h and 4 h) from 10 μg·mL^{-1} to 8.6 μg·mL^{-1} (for *L. plantarum* WL) or to 9.5 μg·mL^{-1} (for *L. casei/paracasei* KNE1). This could indicate the initial ability of lactic acid bacteria to adsorb the compound. Along with reaching the logarithmic stage of growth, the resorption of NDMA from broth was observed for all strains. After 24 h incubation (after reaching the stationary phase of growth) the concentration of NDMA was the same as at the "zero" time for all strains (10 μg·mL^{-1}). As of 96 h of incubation, the level of NDMA was decreasing just along with decreasing number of living cells in the samples. The results indicate that lactic acid bacteria can lower NDMA concentration in the culture and it could be either bound or metabolized. The decrease in mutagenic activity of the compound was confirmed in comet assay.

P-100 **Influence of heterocyclic aromatic amines (HCA) on growth and survival of *Lactobacillus* strains.** A. Nowak, Z. Libudzisz (Institute of Fermentation, Technology and Microbiology, Technical University of Lodz, ul. Wolczanska 171/173, 90-924 Lodz, Poland).

The aim of the study was to estimate the ability of intestinal lactobacilli to grow and survive in the presence of 3 HCA: IQ, MelQx and PhIP. Concentrations selected for the research were: 5 $\mu g \cdot mL^{-1}$, 25 $\mu g \cdot mL^{-1}$; additionally 100 $\mu g \cdot mL^{-1}$ for IQ and 75 $\mu g \cdot mL^{-1}$ for MelQx. Bacterial strains tested were: *Lactobacillus plantarum* WL, *L. casei/paracasei* KNE1, *L. casei* J/III, *L. casei* BD and *L. casei* DN 114 001. To define the influence of HCA on growth of lactobacilli, the cells were cultivated 24 h in MRS medium at 37 °C under anaerobic conditions with the addition of final concentrations of IQ, MelQx and PhIP. For all HCA tested, there was no reduction in the number of living bacterial cells as compared to the control. To define the influence of HCA on survival of bacteria, the biomass achieved after 24 h incubation in MRS broth was centrifuged, suspended in phosphate buffer with appropriate concentrations of each compound and incubated for 168 h. All the HCA had some influence on the survival of lactic acid bacteria and it depended on the strain, incubation time, the compound and its dosage. The most significant impact was observed in case of IQ, when the number of living cells was reduced for: *L. casei* DN (at 100 $\mu g \cdot mL^{-1}$) after 72 h and *L. casei* J/III (at 100 $\mu g \cdot mL^{-1}$) after 120 h, *L. casei* BD (at 5, 25 and 100 $\mu g \cdot mL^{-1}$) after 48 h and *L. paracasei/casei* KNE1 (at 100 $\mu g \cdot mL^{-1}$) after 120 h of incubation. MelQx had some impact on survival of the bacteria after 144 h for *L. casei* DN (at 5, 25, 75 $\mu g \cdot mL^{-1}$), *L. paracasei/casei* KNE1 (at 25 and 75 $\mu g \cdot mL^{-1}$) and *L. plantarum* WL (at 75 $\mu g \cdot mL^{-1}$). In case of PhIP, a decreased number of living cells was noticed for *L. casei* DN (at 25 $\mu g \cdot mL^{-1}$) after 96 h, *L. paracasei/casei* KNE1 up to 48 h, and *L. plantarum* WL after 72 h. There was no influence observed from 48 h up to 72 h of incubation compared with the control. The results indicate that intestinal lactic acid bacteria can survive in the presence of all the concentrations of HCA tested up to 72 h with no influence on the number of living cells during the transit time, which lasts from 48 h to 72 h.

P-101 **Activation of pro-estrogens from hops by intestinal bacteria.** S. Possemiers[a], S. Bolca[a,b], A. Heyerick[b], W. Verstraete[a] ([a] Laboratory of Microbial Ecology and Technology, Ghent University, Belgium, [b] Laboratory of Pharmacognosis and Phytochemistry, Ghent University, Belgium).

Hops have been used for centuries in beer brewing, but in the last few years they have gained increasing attention as a source of prenylflavonoids, with 8-prenylnaringenin (8-PN) being the most potent phytoestrogen identified so far. Typically, isoxanthohumol (IX) is the major prenylflavonoid present in beer (≤ 4 $mg \cdot L^{-1}$) and 8-PN concentrations are low (<100 $\mu g \cdot L^{-1}$). Therefore, with the current knowledge, no health effects due to estrogens in beer were to be expected through moderate beer consumption. However, we recently showed that, in parallel with other phytoestrogens, the human intestinal microbiota may crucially influence the activity of hop prenylflavonoids. Upon incubation of fecal samples, an *O*-demethylation of IX into 8-PN was discovered, leading to increased exposure to the strong phytoestrogen. Analysis of 51 intestinal communities revealed interindividual differences in the intestinal transformation potential, separating individuals into high, moderate and slow IX activators. Using the SHIME, a five-step simulator of the intestine, we showed that the production of 8-PN mainly occurs in the distal colon. In vivo, HFA rats were used and an IX-containing supplement was dosed to 50 postmenopausal women, and a clear correlation between intestinal transformations and urinary 8-PN excretion was shown. Finally, a bacterium capable of this *O*-demethylation was selected, which produced 8-PN and which could startup IX conversion in the SHIME and in a pig intervention trial. Therefore, the activity of the intestinal microbiota could easily increase the 8-PN levels tenfold and the final exposure to 8-PN after moderate beer consumption does not depend on the 8-PN concentrations, but rather on a combination of the IX concentrations and the intestinal transformation potential to activate IX into 8-PN.

P-102 **Proteomics-based investigation of major metabolic pathways of a human gut anaerobe, *Fusobacterium varium*.** J. Potrykus[a,b], S.L. Bearne[a], R.L. White[b] ([a] Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Canada, [b] Department of Chemistry, Dalhousie University, Halifax, Nova Scotia B3H 1X5, Canada).

Bacteria comprising the normal flora are often beneficial to the host, making the investigation of their physiology relevant to our health. We describe a proteomics-based exploration of the metabolism of the butyrate-producing gut anaerobe *Fusobacterium varium*. Changes in the proteome of *F. varium* in response to different growth substrates were monitored using two-dimensional polyacrylamide gel electrophoresis. Cells were grown in a chemically defined minimal medium supplemented with glucose, pyruvate, intermediates of the core acetate-butyrate pathway (acetoacetate or crotonate), or amino acids (L- or D-glutamate, L-histidine, L- or D-lysine, L- or D-serine) as major energy sources. In the selected pH isoelectrofocusing range, pH 4–7, approximately 380 protein spots were detected that were common to all investigated growth conditions. Pronounced differences were observed between the proteomes of bacteria thriving on glucose and those fermenting an amino acid as the main energy source. About 90 protein spots were chosen for MS/MS analysis and de novo sequencing. The analysis revealed that some proteins (e.g. specific catabolic enzymes) serve as unique markers for a particular growth substrate, while intracellular concentrations of other proteins (e.g. translation factor EF-Tu) appear to be independent of variations in the identity of the growth substrate.

P-103 **Effects of dilution rate on methane production and fibre degradability in Rusitec fermenters fed a high-concentrate diet.** S. Ramos, M.E. Martínez, M.J. Ranilla, M.L. Tejido, L.A. Giraldo, M.D. Carro (Departamento de Producción Animal I, Universidad de León, 24071 León, Spain).

Increasing dilution rate in rumen fermenters often results in an increase in pH, and therefore effects of both factors on fermentation characteristics are confounded. The aim of this study was to analyse the effects of two dilution rates – low (LDR, 3.8% h^{-1}) and high (HDR; 5.4% h^{-1}) – on fermentation parameters in Rusitec fermenters maintained at a similar pH. Eight fermenters were fed daily 30 g dry-matter (DM) of a 30:70 alfalfa hay:concentrate diet and each experimental treatment was assigned to four fermenters. After 10 days of adaptation, methane and volatile fatty acid (VFA) production and diet degradability after 48 of incubation were measured on four consecutive days. There were no differences ($P > 0.05$) between treatments in ruminal pH (6.55 and 6.47 for LDR and HDR, respectively) or methane production (21.7 and 22.4 $mmol \cdot d^{-1}$). On the contrary, both production of total VFA and fibre digestibility augmented from 81.6 to 90.5 $mmol \cdot d^{-1}$ ($P = 0.028$) and from 29.8 to 36.8% ($P < 0.001$), respectively, by increasing the dilution rate. Molar proportions of acetate and butyrate were decreased ($P < 0.001$) by 4.0 and 10.4%, and those of propionate and other VFA (calculated as the sum of isobutyrate, isovalerate and valerate) were increased by 20.1 and 21.6% when the dilution rate was changed from 3.8 to 5.4% h^{-1}. As a consequence, the acetate:propionate ratio was lower for HDR than for LDR (2.83 vs. 3.54; $P < 0.001$). These results indicate that increasing the dilution rate in Rusitec fermenters can influence rumen fermentation by increasing diet degradability and VFA production and by changing the pattern of VFA production.

P-104 **Metabolism of the pyrrolizidine alkaloid senecionine by an enrichment of ovine ruminal microbes as detected by high-performance liquid chromatography and mass spectrometry.** R.M. Rattray, J.M. Duringer, A.M. Craig (Oregon State University, Corvallis, Oregon, USA).

Pyrrolizidine alkaloids (PAs) produced by the plant tansy ragwort (*Senecio jacobia*) have been shown to act as hepatotoxins when metabolites of these molecules are created by eukaryotic enzymes. Sheep have been shown to have an extremely high in vivo resistance to PAs when compared to cattle and horses fed tansy ragwort. Previous work has demonstrated that this resistance is conveyed by unique ruminal bacteria. A bacterial consortium capable of degrading mixed PAs from tansy has been enriched from ovine rumen fluid and designated L4M2. The disappearance of the PA senecionine from experimental cultures of L4M2 was observed by high performance liquid chromatography (HPLC) analysis within the first four hours of incubation. The detection of metabolites as a result of degradation of senecionine was performed by monitoring absorbance at 220 nm as well as full-spectrum analysis. In addition, mass spectrometry was used to determine the putative chemical configuration of senecionine metabolites. The appearance of metabolites was

detected after about three hours of incubation and persisted through the experimental period. Since animal pathways in the liver have been shown to convert some non-toxic metabolites of senecionine into toxic forms, it is desirable to know the putative structure of metabolites for this compound created by L4M2 in vitro. We can then begin to determine whether the bacterial metabolites produced in the host animal's rumen simply pass through the digestive system or are absorbed, thereby providing the opportunity for toxic conversion and subsequent liver cirrhosis.

P-105 **Novel structural and catalytic elements in the *Ruminococcus flavefaciens* cellulosome revealed by genome analysis.** M. Rincon[a], I. Borovok[b], M.E. Berg[c], D.A. Antonopoulos[c], R. Kim[c], L. Liu[c], J. Thimmapuram[c], S. Jindou[c], R. Lamed[b], H.J. Flint[a], E.A. Bayer[d], B.A. White[c] ([a] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK, [b] Tel Aviv University, Ramat Aviv 69978 Israel, [c] University of Illinois, Urbana, IL 61801, USA, [d] The Weizmann Institute of Science, Rehovot 76100, Israel).

Ruminococcus flavefaciens is one of the major cellulolytic bacteria cultured from the rumen of herbivores. The bacterium specializes in the breakdown of complex polysaccharides from the plant cell wall thanks to the synthesis of a variety of exocellular hydrolytic enzymes. Recent investigations have established that *R. flavefaciens* strain 17 produces a large, multicatalytic, protein complex that is associated with the bacterial cell envelope. This complex shares many characteristics with the cellulosome reported in *Clostridium thermocellum,* but also displays many novel features. Shotgun sequencing of the genome (currently at 4× coverage) from a different strain (*R. flavefaciens* FD1) provided an ideal opportunity to gain a broader perspective on *R. flavefaciens* cellulosome organisation, by extending the functional and sequence analyses performed with *R. flavefaciens* 17. *In silico* analysis revealed the occurrence of about 180 ORFs which contain a dockerin-like motif. Surprisingly, many dockerin-containing sequences contain unusual flanking regions with dissimilar predicted activities, such as cysteine proteases, serine protease inhibitors and other regions of unknown function. Predicted glycosyl hydrolase-coding sequences represent at least 36% of all retrieved dockerin-containing ORF's. Structural proteins and proteases account for 3 and 8% respectively, whereas ORF's with unknown regions account for 53% of the total. In addition to scaffoldin-like homologs of the previously characterized ScaA, ScaB, ScaC and ScaE of strain 17, newly identified cohesin-like domains were also retrieved. Phylogenetic analysis of dockerin motifs and cohesin-like modules revealed a divergence, which is predicted to be linked to different dockerin-cohesin specificities in the cellulosome assembly.

P-106 **Polysaccharidases isolated from the rumen metagenome.** M.T. Rincon, D. Henderson, J.C. Martin, K.P. Scott, H.J. Flint (Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK).

Microbes in the rumen are responsible for pregastric fermentation of feedstuff, from which the volatile fatty acid products provide the host animal with major sources of energy and carbon. The ruminant diet is particularly rich in lignocellulosic material. In order to fully degrade the recalcitrant matrix of complex polysaccharides in the plant cell wall, microbes adapted to live in the rumen ecosystem have evolved a great variety of enzymes that cleave the wide variety of linkages present in ligno-cellulose. The small number of cultured cellulolytic rumen bacteria that is available may not, however, reflect the full range of cellulose degraders that are present in the rumen ecosystem. Metagenome approaches therefore offer the prospect of recovering novel genes and activities. In this work, carried out through the EU FPV GEMINI project, bacterial DNA was extracted from total rumen contents, and libraries were constructed using a bacteriophage lambda vector (average insert size of ~ 5.5 kb). Functional screening of the phage library for polysaccharidase activities was carried out using plate overlays, followed by Congo red staining. Insert DNA was recovered after circularization by in vivo excision, and subjected to sequence analysis. Enzymes belonging to glycosyl hydrolases family 5 (http://afmb.cnrs-mrs.fr/CAZY/acc.html) were the most abundant among those retrieved after screening with carboxymethyl cellulose. However, novel glycosyl hydrolases were retrieved after screening with the substrates xyloglucan and lichenan. Phylogenetic relationships suggested by the sequences indicate that that cloned inserts originate from

relatives of a wide range of anaerobic bacterial genera, including *Prevotella*, *Fibrobacter*, *Bacteroides*, *Clostridium* and *Selenomonas*.

P-107 **Transcriptome analysis of the cluster XIVa human colonic anaerobe *Roseburia inulinivorans*.** K.P. Scott, J. Martin, J. Potrykus, G. Campbell, M.T. Rincon, G. Rucklidge, C.-D. Mayer, H.J. Flint (Rowett Research Institute, Aberdeen, UK).

Roseburia species together with *Eubacterium rectale* represent one of the main groups of butyrate producers in the human colon, and can comprise up to 10% of the total bacterial population in human faeces. *Roseburia inulinivorans* A2-194 is able to utilize a particularly wide range of growth substrates, including inulin, FOS, resistant starch and fucose for growth. Differential gene expression of *R. inulinivorans* during growth on different host-derived and dietary substrates was investigated using a combination of genomic microarray screening and proteomics. Genes induced on inulin encoded a sucrose hydrolase, fructose kinase and PTS transport system proteins specific for fructose uptake. Genes induced on starch included an α-amylase functionally similar to one expressed by the rumen anaerobe *Butyrivibrio fibrisolvens*, an α-glucanotransferase and various ABC transporters. Comparison of 2D gels of the proteome of the bacteria grown on the same substrates indicated fourteen differentially expressed proteins, and further analysis indicated that one of them was identical to the sucrose-6-phosphate hydrolase identified through microarray analysis. A complete switch in metabolism was required to enable the bacterium to grow on fucose and resulted in the formation of propionate and propanol. The genes induced on fucose were primarily directly involved in fucose and propanediol utilisation.

P-108 **Localization of cellulolytic rumen bacteria on the plant fibrous materials determined by a newly developed fluorescent in situ hybridization protocol.** T. Shinkai, Y. Kobayashi (Graduate School of Agriculture, Hokkaido University, Japan).

In this study, we established a new fluorescent in situ hybridization (FISH) protocol to minimize the self-fluorescence of orchardgrass hay as a representative grass material. By using this protocol, we have successfully detected fibrolytic rumen bacteria attaching to stem and leaf sheath sections of orchardgrass hay under a fluorescent microscope. Real-time PCR assays were also employed to quantitatively monitor representative fibrolytic rumen bacteria on the material. *Fibrobacter succinogenes* was estimated to account for 6.9–8.1% of total bacteria both in the stem or the leaf sheath in terms of 16S rDNA copy number. *F. succinogenes* was located as firmly attaching not only to cut edges but also to undamaged plant surfaces. Although *Ruminococcus flavefaciens* was present at only 0.34–0.59%, this bacterium was detected to a greater extent in the leaf sheath than in the stem (according to real-time PCR assay values and frequency of FISH signals). Furthermore, *R. flavefaciens* created a lot of pits on the sheath and many cells of this species were located along edges of the pits. *F. succinogenes* was considered to play the central role in fiber digestion of orchardgrass hay stem, since this bacterium is clearly detectable by FISH and assayable as the largest in population size in the less degradable stem section. Further use of this FISH protocol in combination with quantitative PCR assays may clarify the whole scheme of fiber digestion by a bacterial consortium comprised of the representative fibrolytic bacteria and even functionally unexplored bacteria.

P-109 **Detection and quantification of chitinases in the human fecal chitinolytic bacterium *Clostridium paraputrificum* J4.** J. Šimůnek[a], G. Tishchenko[b], H. Bartoňová[a] ([a] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídeňská, 1083, 140 00 Prague 4, Czech Republic, [b] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovský Sq. 2, 162 06 Prague 6, Czech Republic).

A strictly anaerobic, mesophilic and chitinolytic bacterium, *Clostridium paraputrificum* J4, was isolated from human faces. In response to various types of chitin used as growth substrates, the bacterium produced a complete array of chitinolytic enzymes: endochitinase, exochitinase, N-acetylglucosaminidase, chitosanase and chitin deacetylase. The high activity of endochitinase (256.4 pkat·mL^{-1}), exochitinase (346.5 pkat·mL^{-1})

and N-acetylglucosaminidase (154.4 pkat·mL^{-1}) was induced in the presence of colloidal chitin. The chitinolytic enzymes from the culture filtrate were fractionated by an ultrafiltration process using polymer membranes (Millipore, USA) with cut-off 100, 50, 30 and 10 kDa. The fractions were characterized by enzymatic activities, SDS-PAGE and concentration of proteins. The chitinase variability was confirmed on zymograms of renaturated SDS-PAGE. The enzymes were visualized as fluorescent bands by using 4-methylumbelliferyl derivatives of N-acetyl-β-D-glucosaminide, β-D-N,N'-diacetylchitobioside, or β-D-N,N',N''-triacetylchitotriose for N-acetyl-β-glucosaminidase, chitobiosidase, or endochitinase, resp. The stability of the chitinolytic enzymes within the pH (3–8) and temperature (10–60 °C) range as well as the temperature optimum of the enzyme activity were determined. In the second step the chitinolytic enzymes of *Cl. paraputrificum* J4 were separated from a culture filtrate by an ion exchange chromatography on the carboxylic POLYGRAN-27 sorbent. The adsorbed enzymes were eluted under a stepwise pH gradient (pH 5; 5.5; 6; 6.5; 7; 7.5 and 8) in 0.1 M phosphate buffer. The protein and enzyme recovery reached 90%.

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P-110 **Fermentation activity of intestinal *Lactobacillus* strains and end products in the presence of fructooligosaccharides.** K. Śliżewska (Institute of Fermentation, Technology and Microbiology, Technical University of Lodz, ul. Wolczanska 171/173, 90-924 Lodz, Poland).

Twenty-six intestinal *Lactobacillus* bacteria, belonging to the species: *L. acidophilus*, *L. casei*, *L. crispatus*, *L. gasseri, L. paracasei/casei* and *L. rhamnosus*, were grown in MRS medium containing inulin (Sigma, Raftiline®HP and ST) and oligofructose (Raftilose®P95), as well as monosaccharides (glucose, fructose, galactose) and disaccharides (lactose, sucrose), as a source of carbon. In media containing inulin the biomass was low and ranged from 0.2 to 1.1 g·L^{-1} (average 0.4 g·L^{-1}). In media with oligofructose the biomass ranged from 0.2 to 1.4 g·L^{-1} (0.6 g·L^{-1}). In control media, containing mono- or disaccharides, the biomass was much higher and reached from 0.7 to 2.3 g·L^{-1} (average 1.2 g·L^{-1}). The fermentation of inulin led to the production of acetic acid as a predominant metabolite. The amount of this acid ranged from 2.3 to 6.5 g·L^{-1} (average 4.0 g·L^{-1}), while that of lactic acid ranged from 0.1 to 3.9 g·L^{-1} (average 1.0 g·L^{-1}). As a result of oligofructose fermentation, lactic acid was a predominant metabolite for *L. casei* and *L. paracasei/casei*, while acetic acid was a predominant metabolite for *L. acidophilus*, *L. rhamnosus, L. gasseri* and *L. crispatus*. The amount of lactic acid ranged from 1.7 to 10.9 g·L^{-1} (average 5.2 g·L^{-1}), while acetic acid from 3.0 to 5.7 g·L^{-1} (4.1 g·L^{-1}). As a result of monosaccharide and disaccharide fermentation lactic acid was a predominant metabolite. The amount of this acid ranged from 6.0 to 20.3 g·L^{-1} (9.2 g·L^{-1}), while acetic acid ranged from 2.8 to 6.7 g·L^{-1} (4.2 g·L^{-1}). Moreover, it was found that in the group of the strains tested, a varied amount of succinic acid, acetaldehyde, diacetyl and ethanol were produced, irrespective of the carbon source applied.

P-111 **The influence of non-enzymatically glycosylated potato proteins on the survival and metabolic activity of chosen bacteria.** D. Świątecka[a], K. Marciniak-Darmochwał[a], H. Kostyra[a], A. Świątecki[b] ([a] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland, [b] Faculty of Biology, Department of Microbiology, Warmia and Mazury University, Olsztyn, Poland).

The non-enzymatic glycosylation of proteins is considered to be a very important process occurring in living organisms and food, and modifies the structure and properties of proteins. Thus, it brings about decreases in the nutritional value of food. Additionally, such modified proteins are likely to become the source of biologically active peptides as well as allergens. Consequently, it greatly affects human and prokaryotic organisms, causing changes in their functioning. Regrettably, little is known about the influence of such modified proteins on the intestinal microflora and ensuing health consequences for the host. Hence, the aim of this study was to establish the dependences between potato proteins modified by non-enzymatic glycosylation and bacterial proliferation rate, survival and metabolic activity. The application of various

fluorescent techniques was carried out to assess the mentioned impact exerted on *Enterococcus faecalis*, *Escherichia coli* and *Bacillus subtilis*. The rate of bacterial proliferation in the presence of examined proteins was estimated directly with DAPI, whereas bacterial dehydrogenase activity was indicated with the fluorogenic substrate-tetrazolium chloride (CTC). Subsequently, bacterial survival in the presence of non-enzymatically glycosylated proteins was established with the fluorescent probe (Live/Dead® BacLight™ Bacterial Viability Kit). Glycosylated potato proteins were preferably utilized as a substrate and energy source by probiotic bacteria to unmodified ones, and were used likewise by pathogenic bacteria during the later period of cultivation. The opposing impacts were exerted on the opportunistic bacteria by two different fractions of glycosylated potato proteins. Obtained results confirm the highly significant influence of glycosylated proteins on bacterial physiological and metabolic status.

P-112 **Metabolism of the food associated carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) by human intestinal microbiota.** L. Vanhaecke, T. Van de Wiele, W. Verstraete (Laboratory of Microbial Ecology and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium).

2-Amino-1-methyl-6-phenylimidazo[4, 5-*b*]pyridine (PhIP) is a mutagenic, carcinogenic heterocyclic amine formed from meat and fish during cooking. Although the formation of hazardous PhIP metabolites by mammalian enzymes is well documented, nothing is known about the PhIP transformation potency of human intestinal bacteria. In this study, the in vitro metabolism of PhIP by human faecal samples was investigated. Following anaerobic incubation of PhIP with stools freshly collected from 6 healthy volunteers, we found that PhIP was extensively transformed by the human intestinal bacteria. HPLC analysis showed that the 6 human faecal microbiota transformed PhIP with efficiencies from 55 to 95% after 72 h incubation, resulting in one major derivative. ESI-MS/MS, 1D (^{1}H, ^{13}C, DEPT) and 2D (gCOSY, gTOCSY, gHMBC, gHSQC) NMR and IC analysis elucidated the complete chemical identity of the microbial PhIP metabolite, being 7-hydroxy-5-methyl-3-phenyl-6,7,8,9-tetrahydropyrido[3',2':4,5]imidazo[1,2-*a*]pyrimidin-5-iumchloride. At present, no information is available about the biological activity of this newly discovered bacterial PhIP metabolite. Our findings however suggest that bacteria derived from the human intestine play a key role in the activation or detoxification of PhIP, a digestive fate ignored so far in risk assessments. Moreover, the variation in transformation efficiency between the 6 human microbiota indicates inter-individual differences in the ability to convert PhIP. This may predict individual susceptibility to carcinogenic risk from this suspected dietary carcinogen.

P-113 **Determination of breakdown pathway of tryptophan and indole acetic acid to skatole by *Clostridium scatologenes* and swine manure slurry.** T.R. Whitehead, N. Price, M.A. Cotta (USDA-ARS-National Center for Agricultural Utilization Research, Peoria, IL, 61604 USA).

Production of the odorant chemical skatole (3-methyl-indole) is a problem both within swine (boar taint) and during storage of swine manure, where skatole can contribute to odor production from large-scale swine facilities. Previous research has suggested that skatole is produced from the bacterial breakdown of tryptophan, but definitive proof has not been published. *Clostridium scatologenes* is one of the few isolated bacterial strains reported to produce skatole. Skatole production was initially determined from medium containing increased levels of tryptophan or indole acetic acid (IAA) using gas chromatography-mass spectrometry (GC-MS) following growth of *C. scatologenes*. In order to conclusively determine that skatole was being produced from these substrates, deuterium-labeled tryptophan or IAA were added to the medium and deuterated products were identified by GC-MS. Deuterated skatole was found to be synthesized from both tryptophan and IAA, and deuterated IAA was also formed from tryptophan. When the same deuterium-labeled substrates were incubated with fresh swine manure slurry, deuterated IAA and skatole were again identified, as well as a number of oxidation products. These results demonstrate conclusively that skatole can be derived from tryptophan and/or IAA by *C. scatologenes*, and bacteria are present in stored swine manure that can produce skatole and other products from these substrates.

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O-15 **Comparison of direct-fed microbial and antibiotic supplementation on innate and adaptive immune characteristics of weanling pigs**. M.E. Davis[a], D.C. Brown[b], M.S. Dirain[b], H.D. Dawson[c], C. Maxwell[b], T. Rehberger[a] ([a] Agtech Products, Inc., Waukesha, Wisconsin, USA, [b] University of Arkansas, Fayetteville, Arkansas, USA, [c] USDA/ARS Beltsville Human Nutrition Research Center, Beltsville, MD, USA).

The commensal microflora within the gastrointestinal tract have a profound effect on immune system development. Therefore, strategically selected direct-fed microbials may guide immune system development toward effective, appropriate responses to encountered challenges. Supplementation with *Lactobacillus brevis* to pigs before weaning decreased the expression of several genes within the jejunum involved in the toll-like receptor pathway, specifically TLR4, TLR9, TRAF6, SARM, and TIRP, indicating the influence of this direct-fed microbial on the developing innate immune system of the porcine gastrointestinal tract. *Lactobacillus brevis* in combination with a *Bacillus*-based direct-fed microbial administered after weaning resulted in a decrease in the percentage of phagocytic monocytes during the post-weaning period similar to that observed with antibiotic (Carbadox) supplementation. Furthermore, supplementation with *Lactobacillus brevis*, the *Bacillus*-based direct-fed microbial, or Carbadox resulted in increased proportions of specific T cell populations determined flow cytometrically within the peripheral blood during the post-weaning period that distinctly differed from proportions in unsupplemented pigs. These differing populations expressed cell surface molecules indicative of activated and memory populations of T cells, including major histocompatability complex-II, cells double-positive for CD4 and CD8, the γδ T cell receptor, and the interleukin-2 receptor (CD25). These data demonstrate the effect of direct-fed microbial supplementation on the young pig's developing immune system. In addition, the alteration of the gastrointestinal microbial population by direct-fed microbial supplementation seems to elicit anti-inflammatory effects and immune characteristics similar to those observed with antibiotic administration.

O-16 **Intestinal microbial composition affects the ratio of digestive ileal brush border enzyme activity to gene expression in the gnotobiotic pig.** B.P. Willing, A.G. Van Kessel (Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada).

In early life, colonization of autochthonous microbes induces major morphological and physiological changes in the gastrointestinal tract of the host, including nutrient digestion and absorption. To study microbial influence on intestinal development, two separate gnotobiotic experiments were performed, each with 16 piglets allocated to 4 treatment groups: germ free (GF), mono-association with *Escherichia coli* (EC), mono-association with *Lactobacillus fermentum* (LF) or conventionalisation with sow feces (CV). At day 14 isolated epithelial cells (IEC) and whole tissue were collected at 75% of the small intestinal length. Enzyme activity and gene expression of lactase-phlorizin hydrolase (LPH) and aminopeptidase N (APN) were measured in homogenized ileal tissue and IEC using specific substrates and quantitative PCR (qPCR), respectively. Similar results were obtained for both whole tissue and IEC. CV pigs had reduced APN activity, but had increased gene expression relative to GF, making the specific activity/mRNA (A:G) ratio dramatically lower ($P < 0.05$). Similarly, LPH A:G ratio was significantly reduced ($P < 0.05$) in CV pigs as compared to GF, although gene expression was not up-regulated. LF pigs had A:G ratios that were similar to GF, while the EC pigs were more similar to CV. Co-incubation of LF, EC and fecal bacteria with APN indicate a direct relationship between enzyme inactivation and specific activity in ileal tissue. We hypothesize that enterocyte up-regulation of brush border enzyme expression occurs as either a direct response to microbial colonization or as a feedback mechanism in response to reduced enzyme activity through microbial degradation. This mechanism may play a role in ensuring effective competition of the host with the intestinal microbiota for available nutrients.

O-17 **Interactions between the mucin-degrader *Akkermansia muciniphila* and host cells in the GI tract.** M. Derrien[a], E.G. Zoetendal[a], E. Norin[b], W.M. De Vos[a] ([a] Laboratory of Microbiology, Wageningen University, Hesselink van

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The first level of interactions between host and bacteria starts in the mucus layer that protects epithelial cells from the luminal content. While the mucins (mucus glycoproteins) present in this layer have a protective role, they are also known to serve as carbon and nitrogen source for some intestinal microbes. However, the exact interaction between mucin-degrading microbes and the host is largely unknown. In the current study, we focus on the interaction between the mucin-degrading bacteria and the host using both cultivation and high throughput molecular approaches. In a previous study, *Akkermansia muciniphila* (CIP 107961^{T}, ATTC BAA-835 ^{T}), a novel Gram-negative anaerobic organism, which is capable of utilizing mucin as sole carbon and nitrogen source, was isolated from a healthy volunteer. Fluorescent In situ Hybridization (FISH) combined with flow cytometry using an *Akkermansia*-specific 16S rRNA-targeted probe was used to quantify *Akkermansia*-like bacteria in 15 faecal samples and revealed it to account for approximately 1% of the total faecal cells. In order to determine the in vivo impact of *A. muciniphila* on its host, mono-association of germ-free mice was performed with *A. muciniphila*, and *L. plantarum* WCFS1, which serves as control for a non mucin-degrading bacterium. Biopsy samples were collected from different areas of the intestine 7 days after the inoculation, and subsequently used for transcriptomic analysis using Affymetrix Genechip Mouse genome 430A arrays. The data from this study will be presented and discussed with specific reference to the expression of mucin genes.

O-18 **Engineering of the human commensal *Bacteroides ovatus* for the controlled in situ delivery of immunomodulatory proteins.** Z.Z.R. Hamady[a], M.D. Farrar[a], T. Whitehead[b], K.T. Holland[a], J.P. Lodge[a], S.R. Carding[a] ([a] Institute of Molecular and Cellular Biology and Department of Surgery, University of Leeds, Leeds, UK, [b] Fermentation Biotechnology Research, National Center for Agricultural Utilization Research, Peoria, Illinois, USA).

Soluble growth factors that promote epithelial repair and can suppress inflammation are of clinical interest as therapeutic agents for chronic intestinal inflammation that is characteristic of inflammatory bowel disease. However, when administered orally as recombinant proteins they are unstable and systemic administration increases the risk of unwanted side effects. Alternative means of delivery have been considered of which delivery via live micro organisms has shown real promise. The aim of this work was to genetically engineer the human commensal colonic bacterium *Bacteroides ovatus* to produce and secrete mammalian cytokines under the control of the xylanase promoter. The xylanase promoter was cloned and sequenced using an inverse-PCR approach. The coding sequence of the mature human cytokines TGF-β or KGF was PCR amplified from cDNA and cloned downstream of the xylanase promoter in the *E. coli-Bacteroides* suicide vector pBT2 that was introduced into *B. ovatus* by conjugation. Resulting transconjugants were tested for cytokine gene expression by reverse transcription-PCR and protein production by enzyme-linked immunosorbent assay (ELISA) and bioassay. Recombinant strains of *B. ovatus* secreted high levels of human TGF-β or KGF in a xylan-dependent manner. Cytokine expression was minimal in the absence of xylan. Studies to assess the efficacy of these strains in the treatment and prevention of chemically induced colitis in mice are ongoing.

O-19 **Probiotic bacteria impede *Listeria monocytogenes* infection and modulate the mucosal immune response.** S.C. Corr[a,b], C. Hill[a,b], C.G.M. Gahan[a,b] ([a] Department of Microbiology, University College Cork, Ireland, [b] Alimentary Pharmabiotic Centre, Biosciences Research Institute, University College Cork, Ireland).

Invasion of the gastrointestinal epithelium, translocation across this epithelial barrier and survival of the host immune response are critical to the pathogenesis of *Listeria monocytogenes*. However, despite a thorough understanding of the intracellular pathogenesis of this organism, little is known about how the pathogen interacts with the host microflora at the mucosal surface. This study utilises in vitro culture of the C2bbe1 intestinal cell line to examine the effect of

pre-treating epithelial monolayers with probiotic bacteria on Listerial invasion. We found that probiotic bacteria significantly reduce *L. monocytogenes* invasion of epithelial cells in vitro. We determined that this inhibition is due to a secreted factor directly affecting the epithelial monolayer, thus inhibiting invasion. Furthermore, Enzyme-Linked Immunosorbent assays (ELISA) demonstrated a significant reduction of pro-inflammatory cytokine (IL-8) and increased anti-inflammatory cytokine (IL-10) following probiotic treatment of monolayers and subsequent Listerial challenge. Finally, a novel luciferase reporter system was used to analyse organ-specific infection by *L. monocytogenes* in the A/J mouse model following oral dosing of mice with *Lactobacillus salivarius* UCC118. We have shown for the first time that a probiotic bacterium can significantly reduce *L. monocytogenes* infection in vivo. We observed a dramatic reduction of Listerial infection of livers and spleens following probiotic dosing of mice compared to a placebo control. This study assesses the ability of various well-characterised probiotic bacteria to inhibit the initial step of *L. monocytogenes* host cell infection and the inflammatory response. As not all probiotic bacteria have the same therapeutic effects, understanding the mechanism of action of some of these probiotics will aid selection of strains useful for therapeutic application and treatment of infections.

O-20 **Enterohaemorrhagic *Escherichia coli* inhibit nitric oxide synthesis by activated human intestinal epithelial cells.** M. Vareille, T. De Sablet, A. Durand, C. Martin, A.P. Gobert (Unité de Microbiologie, INRA, Centre de Theix, 63122 Saint-Genès-Champanelle, France).

Enterohaemorrhagic *Escherichia coli* (EHEC) is a food borne pathogen causing human diseases ranging from uncomplicated diarrhea to haemorrhagic colitis and life-threatening complications, such as haemolytic-uremic syndrome (HUS) or thrombotic thrombocytopaenic purpura. The majority of EHEC carry a pathogenicity island called Locus of enterocyte effacement (LEE) which encodes virulence proteins and can alter activated mucosal intestinal cells, the first line of defense against these pathogens. Therefore, the capacity of EHEC to modulate the innate immune response in infected colon may be a critical step in pathogenesis. Inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) is a free radical synthesized by cytokine-stimulated cells, which possesses numerous inflammatory and immunological functions. To determine the effect of EHEC on NO production by human intestinal epithelial cells we used the human epithelial cell lines HcT-8 and Caco-2 which were stimulated for 6 h with cytokines, in the presence or absence of different strains of EHEC. Total RNA was purified and iNOS mRNA was analysed by RT-PCR and real-time PCR. After washing, cells were cultured for 24 h in a fresh medium containing antibiotics; nitrates, the stable products of NO in culture, were then measured in the supernatants. These results showed that iNOS mRNA expression and NO production were induced in cytokine-activated cells when compared to unstimulated cells. These effects were inhibited by adding EHEC to the cultures. The effect on iNOS induction was observed when LEE-positive and LEE-negative strains were used and did not require direct contact with bacteria. Moreover, this inhibition was not observed when a non-pathogenic *E. coli* was used.

IL-1 **Resident intestinal bacteria direct expression of a bactericidal lectin.** H.L. Cash, C.V. Whitham, C.L. Behrendt, L.V. Hooper (Center for Immunology, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA).

The human gut harbors a vast consortium of resident bacteria that make important contributions to human nutrient metabolism and intestinal development. Maintaining the mutually beneficial nature of this host-microbial relationship requires strict corralling of gut bacteria in the intestinal lumen, as microbial incursions across epithelial layers can elicit damaging inflammatory responses. Members of the Reg family of C-type lectins are expressed in intestinal epithelial cells and show increased expression in the mucosa of inflammatory bowel disease patients. We have found that intestinal bacteria induce Paneth cell RegIIIγ expression in mice, that the protein is secreted into the gut lumen, and that it binds preferentially to the surfaces of gram positive bacteria. Further studies have revealed that both RegIIIγ and its human counterpart HIP/PAP bind to bacterial peptidoglycan via its

carbohydrate backbone. Consistent with this binding, both RegIIIγ and HIP/PAP exhibit direct bactericidal activity against a variety of gram positive bacteria. This activity can be inhibited by soluble carbohydrates that mimic the sugar structures found in peptidoglycan, suggesting that peptidoglycan binding is required for microbial killing. Thus, RegIIIγ and HIP/PAP represent a new family of bactericidal proteins that seek out their microbial targets via interactions with bacterial cell wall carbohydrates. These proteins likely play an important role in preventing epithelial penetration by resident gut bacteria, thus preserving a commensal host-microbial relationship. Furthermore, our results suggest that human Reg proteins exhibit increased expression in inflammatory bowel disease mucosa as a compensatory mechanism designed to limit bacterial invasion of mucosal surfaces.

P-114 **Characterisation of the effects of lactobacilli on the integrity of intestinal epithelial cells.** R.C. Anderson[a], A.L. Cookson[a], R.D. Hurst[a,b,c], L.A. Cassidy[b], W.C. McNabb[a], N.C. Roy[a] ([a] Metabolism and Microbial Genomics Section, Food and Health Group, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand, [b] Meat Quality and Safety Section, Food and Health Group, AgResearch Limited, Ruakura Research Centre, Hamilton, New Zealand, [c] Current address: Human Health and Performance Group, HortResearch Limited, Hamilton, New Zealand).

The gastrointestinal barrier (GIB) is compromised in conditions such as inflammatory bowel diseases. It is hypothesised that commensal bacteria, such as lactobacilli, play a critical role in maintaining the integrity of the GIB. Our objective was to determine the effect of two *Lactobacillus* sp. (*L. plantarum* and *L. acidophilus*) on the integrity of the GIB. The lactobacilli were isolated from a commercial probiotic product and identified using 16S rDNA sequencing. The effects of these bacteria and the probiotic product were tested in an in vitro bioassay that mimics the GIB. This assay consisted of a monolayer of intestinal epithelial cells (Caco-2) grown on a semi-permeable membrane. The trans-epithelial electrical resistance (TEER) across the monolayer was measured using a voltohmmeter to determine the strength of the tight junctions between the individual epithelial cells. The addition of the whole probiotic product increased the TEER by 30%. *L. plantarum* alone, isolated from the product, increased the TEER by 50% compared to the controls (i.e. media with no bacteria added). This is in agreement with the reported literature. Conversely, the *L. acidophilus* strain isolated from the same product decreased the TEER by 30% compared to the controls. These results indicate that *L. plantarum* has the potential to improve gastrointestinal health and wellness and may be a useful organism to evaluate further as a potential additive for novel health-promoting foods.

P-115 **A tool for studying adherence of intestinal pathogens on intestinal mucus – opportunities for screening products for pathogen inhibition in various hosts.** J. Apajalahti, O. Siikanen, L. Purkamo, M. Lauraeus (Alimetrics Ltd, Höyläämötie 14, 00380, Helsinki, Finland).

The pathogenesis of many enteric pathogens is initiated by adherence of the bacteria on the intestinal epithelium lining. Through adherence, bacteria can resist washout with digesta passage and get into the vicinity of the host cells, the ultimate targets of toxins and intracellular pathogens. Prevention of pathogen adherence on the mucus overlying epithelial cells would remove a significant mediator of pathogenesis and make the host less susceptible for the respective enteric diseases. In the method presented here, intestinal mucus covering the epithelium was isolated from an animal species, breed, age, dietary group and intestinal section relevant for the application and the problem of interest. Mucus was then washed and immobilised onto polystyrene wells and used for studying adherence of radiolabeled pathogens representing different bacterial species, serotypes and strains isolated from animal hosts, and even from specific farms with recurrent pathogen outbreaks. The method provides a means to study the mechanisms of adherence and to classify bacterial strains according to their binding potential and specificity. Furthermore, this in vitro method can be applied to the development of health products aimed at prevention of pathogen colonisation in the animal and human intestine. We have found specific representatives of product categories such as direct fed microbes (probiotics) and complex carbohydrates that are especially effective in preventing the adherence of pathogenic

Escherichia coli and *Salmonella enterica* strains. Some products have to be present prior to pathogen introduction to prevent adherence, whereas some actually detach pathogens already adhered. The former case characterises compounds suitable for preventive treatment, whereas the latter could work also in the therapy of pathogenesis.

P-116 **Effect of inoculation with intestinal bacteria on bodyweight and intestinal inflammation in the interleukin-10 knockout mouse.** M.P.G. Barnett[a], S.T. Zhu[b], A. Cookson[a], R. Broadhurst[c], B. Knoch[a], W.C. McNabb[a], N.C. Roy[a] ([a] Metabolism and Microbial Genomics Section, Food and Health Group, AgResearch Limited, Palmerston North, New Zealand, [b] Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand, [c] National Resources Group, AgResearch Limited, Hamilton, New Zealand).

The interleukin-10 (IL-10) knockout (KO) mouse develops Crohn's disease-like colitis when raised under conventional conditions. This colitis is caused in part by an inappropriate response to normal intestinal bacteria. Our aim was to produce a more reliable model of inflammation by orally inoculating IL-10 KO mice with 12 *Enterococcus* strains and complex intestinal flora collected from C57BL/6 mice raised under conventional conditions (EF+CIF). Bodyweight was recorded throughout the trial. At 12 weeks of age, intact intestinal sections were assigned a histological score (HIS) based on inflammatory cell infiltrates and tissue destruction. Bodyweight of IL-10 KO mice was lower ($P < 0.05$) than controls, while the HIS (particularly colon) of IL-10 KO mice inoculated with EF+ CIF was higher ($P < 0.05$) than that of control mice. Inoculation also gave more consistent HIS compared to IL-10 mice which were not inoculated. These results show that IL-10 KO mice inoculated with intestinal bacteria are an improved model of intestinal inflammation. This model will now be used to test how specific dietary components affect gene expression relevant to the development of Crohn's disease-like colitis.

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P-117 **Effect of selected strains of lactic acid bacteria on non-specific cellular and humoral defence mechanisms of healthy and *Salmonella*-infected chickens.** M. Bielecka[a], A.K. Siwicki[b], E. Biedrzycka[a], R. Wójcik[b], W. Smoragiewicz[c], A. Orłowski[a], A. Majkowska[a] ([a] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland, [b] University of Warmia and Mazury, Olsztyn, Poland, [c] University of Québec, Montreal, Canada).

The ability to enhance the immune system is one of the most important probiotic features. The aim of the study was to determine the influence of potentially probiotic strains of *Lactobacillus* and *Bifidobacterium* on selected non-specific cellular and humoral defence mechanisms and protection against salmonellosis in chickens. The experiment was performed on 240 chickens (Ross 308) randomly allocated into 6 groups. All chickens were fed the standard diet (including the control group), those in one group received feed supplemented with the antibiotic avilamycin, and those in four groups were administered with the selected, well-defined single strains of *Lactobacillus salivarius* AWH, *L. acidophilus* BS, *Bifidobacterium longum* KNA1 and *B. animalis* 30 as active cultures in the amount of 10^9–10^{10} cells per chicken per day. On day 14 before the end of the experiment, half of the chickens in each group were separated and infected with *Salmonella* (~ 10^6 cells per chicken every third day). Among the strains tested, *L. acidophilus* BS and *B. animalis* 30 were found to be the most effective. The strains increased phagocytic abilities of blood leukocytes, stimulated lysozyme activity (LSM) and increased the level of gamma-globulin in healthy chickens. In the chickens infected experimentally with *Salmonella,* the selected strains (BS, 30) significantly ($P < 0.05$) activated phagocytic ability of leukocytes and proliferative response of lymphocytes, and increased activity of LSM, the level of gamma-globulin and Ig Y in blood serum in comparison to non-infected chickens. In the groups of chickens receiving avilamycin, immunosupression was observed. The results indicated the possibility to use the carefully selected probiotic strains as one of the alternatives for antibiotics for chicken rearing.

P-118 **Probiotic bacteria in early defence against viral infection.** T. Botič[a], M. Ivec[a], S. Koren[b], M. Jakobsen[c], H. Weingartl[d], A. Cencič[a,e] ([a] University of Maribor, Faculty of Agriculture, Vrbanska c.30, 2000 Maribor, Slovenia, [b] University of Ljubljana, Medical Faculty, 1000 Ljubljana, Slovenia, [c] The Royal Veterinary and Agricultural University, Dept. of Dairy and Food Science, Rolighedsvej 30, 1958 Frederiksberg C, Denmark, [d] Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, 1015, Arlington Street, Winnipeg, Manitoba, R3E 3M4, Canada, [e] University of Maribor, Medical Faculty, Slomskov trg 15, 2000 Maribor, Slovenia).

Probiotics are undoubtedly important in supporting a functional yet balanced immune system. A further application of immunomodulatory bacteria in health care is in the control of microbial pathogens, including various viruses. We investigated if probiotic bacteria can prevent or reduce viral infection. The in vitro system used was composed of the pig small intestinal cell line IPEC-J2 (A. Blikslager, USA), pig macrophage cell line 3D4/21, and Vesicular Stomatitis Virus –VSV, as a model virus. *L. paracasei* F19*, L. paracasei/rhamnosus* Q85 and *B. longum* Q46 were used in the experiment as the probiotic strains. Cell survival and viral inhibition were determined by antiviral assay, NO production and the activity of mitochondrial enzymes in infected versus non-infected cells. Pre-incubation of cell monolayers with probiotic bacteria reduced viral infectivity up to 60%, in the intestinal epithelia and in the macrophages. The highest accumulation of NO in the culture supernatants was observed in macrophages after treatment with *L. paracasei* F19. The MTT-test showed that *L. paracasei* F19 has a positive effect on cell viability in the virus infected and non-infected cells. The results of our study, in a cell culture model, showed that probiotic bacteria exhibit antiviral activity by direct interactions with the virus, triggering the intestinal epithelia and activation of macrophages.

P-119 **Adaptation of adhesion test on model epithelial cells for anaerobic bacteria.** T. Čepeljnik, M. Narat, B. Lah, B. Dolenec, R. Marinšek-Logar (Zootechnical Department, Biotech. Fac., University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia).

Caco-2 cells are often used as model epithelial cells in studies of bacterial adhesion. So far only aerobic or facultatively anaerobic bacteria were used in this kind of assays. In our study the adhesion test was adapted in such a manner that it was suitable for anaerobic bacteria such as e.g. *Pseudobutyrivibrio xylanivorans* Mz5^T, originating from the rumen of a Holstein-Friesian cow. This bacterium has some attributes that make it suitable as probiotic strain (very active hydrolases, bacteriocin and CLA production). One of the criteria for a probiotic candidate is also the ability of the bacterial strain to adhere to epithelial cells. In our case the adhesion test was performed in an anaerobic chamber in standard 96-well plates. A thousand bacterial cells were added per Caco-2 cell at neutral pH for 30 min. During the test optimization it was determined that the best method for separating adhered bacteria from Caco-2 cells was homogenization with an automatic pipette. The next step was the development of a reliable quantification of the adhered bacteria, that are usually too few in number to be determined by microscopy. Therefore our work focused on viable counting methods. After the adhesion test had been carried out the adhered bacterial cells were enumerated by a pour plate method and by a newly developed method, i.e. measurement of their lag phase duration in growth medium M330 in parallel. We have confirmed the adhesion capability of *P. xylanivorans* Mz5^T: 1.04 bacterial cells from the late logarithmic growth phase adhered per Caco-2 cell under the selected assay conditions. We have shown that the adapted adhesion test could be suitable for anaerobic bacteria, too. Further optimisation of the method is planned.

P-120 **Microbial extinctions and modern diseases.** M.G. Domínguez-Bello (Department of Biology, University of Puerto Rico, Rio Piedras, San Juan, Puerto Rico, USA).

Animals evolved in a microbial world. The dynamics of co-evolution of animals with their indigenous microbes – eukaryotic, prokaryotic and viral – has shaped animal's immunity. Selectivity of the immune function – combating

specifically pathogens – is important for the host-microbial balance. This dynamic equilibrium that evolved through millions of years, is suddenly altered in modern humans and their domesticated animals by cultural and technological antimicrobial practices (hygiene, antibiotics), leading to modern diseases involving autoimmune disorders. This paper discusses host microbial function in health, from an example of true digestive mutualism in the rumen, to indigenous microbes in indigenous peoples and microbial related diseases of modernity.

P-121 **Probiotic bacteria alter intestinal absorption and metabolism of aflatoxin B_1 in vitro.** S. Gratz[a,b], H. Mykkänen[a], H. El-Nezami[b] ([a] Department of Public Health and Clinical Nutrition, University of Kuopio, Finland, [b] Food and Health Research Centre, University of Kuopio, Finland).

Probiotic bacteria are known to have numerous beneficial effects, especially on gut health. Recently, probiotics have been shown to bind several food carcinogens, including aflatoxins, to their surface. Until now, extensive in vitro work has identified potential candidate strains such as *Lactobacillus rhamnosus* strain GG (LGG), and there is some evidence that aflatoxin binding by LGG also occurs in vivo in animals and humans. Nevertheless, little is known about the interactions between bacteria, aflatoxin and the intestinal tract. Aflatoxin B_1 (AFB_1) is a low molecular weight lipophilic compound, efficiently absorbed in the intestinal tract by passive diffusion and metabolized into free metabolites, such as aflatoxin M_1 (AFM_1), aflatoxin Q_1 (AFQ_1) and aflatoxicol (AFL), or to a highly reactive epoxide which then forms adducts with DNA and proteins. The metabolism and toxicity of AFB_1 have been studied in human cellular systems derived from liver or respiratory tract, but the impact of the intestinal metabolism is still to be investigated. We therefore wanted to study the uptake and metabolism of AFB_1 by Caco-2 intestinal cells and the impact of LGG on these processes. AFB_1 was mixed into the cell culture medium and AFB_1 and its metabolites were measured by HPLC. Furthermore, LGG was suspended in culture medium and added to cells before adding AFB_1 and metabolites were measured as above. Preliminary results show that Caco-2 cells were able to metabolize AFB_1 into AFM_1 and AFL and production increased with incubation time. In the presence of LGG, less AFM_1 and AFL were detected in culture medium. These results indicate that LGG might reduce the absorption and metabolism of AFB_1 in intestinal epithelium cells.

P-122 **Inhibition of human atopic dermatitis like skin lesion by the administration of *Lactobacillus johnsonii* NCC533 (La1) during weaning period in a model mouse, NC/Nga – the predicted inhibitory mechanism of lesion by La1 from gene expression analyses.** R. Inoue[a], M. Otsuka[a], A. Nishio[a], Y. Nakamura[a], Y. Fukushima[b], K. Ushida[a] ([a] Kyoto Prefectural University, Laboratory of Animal Science, [b] Nestlé, Japan).

We previously found that the oral administration of probiotic *Lactobacillus johnsonii* NCC533 (La1) during a specific time of weaning period significantly inhibited the development of human atopic dermatitis (AD) like skin lesion in a model mouse, NC/Nga. In this study, we propose the predicted inhibitory mechanism of skin lesion by La1 from gene expression analyses. Skin, Peyer's patch (PP) and mesenteric lymph nodes (MLN) were collected from the mice in either control or La1 administrated group after induction of AD and total RNA was extracted from these organs. The expression of various genes, proinflammatory cytokines for instance, was examined by real time RT-PCR. The gene expression of proinflammatory cytokines such as IL-8 was significantly down-regulated in all examined organs of the La1 administrated group compared to those of the control group. Interestingly, significant down-regulation of co-stimulatory molecules, CD80 and CD86, was observed in skin and PP of the La1 administrated group. CD80 and CD86 were known to be expressed in activated APCs and promote T cell activation through its receptor, CD28. Thus this result suggests that both mucosal and systemic APCs in AD condition may be unnecessarily activated by antigens and that they induce immune responses such as inflammation and infiltration of mast cells. The administration of La1 in weaning period seems to work on APCs and eventually inhibit redundant activation of them both mucosally and systemically. As a consequence of this, development of AD-like skin lesion may be inhibited by La1.

P-123 **Rose hip and lactobacilli used in an ischemia-reperfusion model in mice to suppress the intestinal injury.** M. Jakesevic[a], Å. Håkansson[a], D. Adavi[b], S. Ahrné[a], B. Jeppsson[b], G. Molin[a] ([a] Food Hygiene, Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, 221 00 Lund, Sweden, [b] Department of surgery, Malmö University Hospital, Lund University, Malmö, Sweden).

Ischemia-reperfusion (I/R) in the intestines is an inflammatory condition which results in the loss of mucosal barrier function, increased translocation of pathogenic bacteria and elevation of systemic cytokines. During I/R there is activation of leukocytes and reactive oxygen species (ROS) which leads to lipid peroxidation and DNA damage. Polyphenols, such as flavonoids and tannins, act as antioxidants and may prevent lipid peroxidation. Rose hip has a high content of antioxidants and is thought to be effective in prevention of lipid peroxidation. Bioavailability and absorption of polyphenols in the body depend on their metabolism in the small intestine. The non-absorbed fractions of the polyphenols are degraded by colonic microflora to more simple compounds, such as phenolic acids. Tannins form strong complexes with protein and are, in that way, nutritionally undesirable, but they might have positive effects by being anticarcinogenic and antimutagenic. Tannins are quite resistant to microbial attack, but some bacteria, such as *L. plantarum*, *L. pentosus* and *L. paraplantarum,* are capable of degrading tannins to simpler and non-toxic constituents like phenolic acids, which can further be spliced into derivates of phenylpropionic or phenylacetic acids. In this study, nine strains belonging to *L. plantarum*, *L. pentosus* and *L. paraplantarum* together with two different species of rose hip were used in an I/R model in mice. The aim of the study was to investigate, if administration of Lactobacillus strains and/or rose hips can suppress or prevent I/R-induced injury of the intestinal tract.

P-124 **Serological and genetic markers in Crohn's disease patients and their healthy relatives.** M. Joossens, S. Joossens, N. Van Schuerbeek, K. Claes, P. Rutgeerts, S. Vermeire (Division of Gastroenterology, University Hospital Gasthuisberg, Leuven, Belgium).

Crohn's disease (CD) is a chronic inflammatory disease of the gut of unknown aetiology. Both genetic (e.g. CARD15) and microbial (e.g. ASCA for Anti-*Saccharomyces cerevisiae* Antibody) markers have been associated with CD. An exceptionally high frequency of familial CD has been reported in Northern France and Belgium. We hypothesize that a high prevalence of genetic and/or environmental risk factors might explain high disease prevalence in these families. Therefore 21 families (74 patients; 87 healthy relatives, HR) with at least three CD patients per family and 10 matched healthy control families (HC, 58 persons) were studied. ASCA IgG/A/M (Sendid B et al., 1996) and genotypes for CARD8 (C10X), CARD15 (R702W, G908R, 1007fs), TLR4 (A299G) and DLG5 (R30Q) were determined, as well as 4 novel serological markers: anti-outer membrane porin (OMP IgG) (INOVA, USA), anti-mannan (gASCA), anti-chitobioside and anti-laminaribioside carbohydrate (ACCA IgA, ALCA IgG) (all Glycominds, Israel). In the CD families, the genetic variants were equally found in patients and their relatives. The prevalence was higher than previously observed in sporadic CD and HC. The prevalence of ASCA IgG/A/M (71%; 38%; 4%), gASCA (60%; 12%; 6%) and ALCA IgG (44%; 13%; 13%), but not of ACCA IgA (40%; 32%; 24%) and OMP IgG (36%; 27%; 14%) was significantly higher in CD patients compared with their first-degree HR and with HC, respectively. Whereas HR of CD patients showed a higher prevalence of ASCA IgG/A/M compared with HC ($P = 0.001$), no significant difference was found between HR and HC for gASCA and ALCA IgG. In multiple affected CD families, an exceptionally high prevalence of genetic and microbial markers was found. Correlations with faecal microbiota are currently investigated using DGGE.

P-125 **Study of pathogenesis of *Helicobacter pylori* in Iran.** P. Khaki, S.M. Bidhendi, H. Eliasi, M. Alimoradi (Razi Vaccine and Research Institute, Karaj, Iran).

H. pylori is a causative agent of chronic active gastritis, gastric and duodenal ulcers, and gastric adenocarcinoma. The organism is one of the most common human bacterial pathogens, estimated to be infecting at least half of the world's

population. The goal of our study was to evaluate the pathogenesis of *H. pylori* in vitro and in vivo. 250 *H. pylori* strains were isolated from 330 patients who attended at the Gastroenterology Department of Taleghani hospital, Tehran, during May 2003 to August 2005. Three cell lines (BK, Hela and BHK), were used for evaluation of the cytopathic effects of urease and cytotoxicity of *H. pylori* isolates. For study of the pathogenesis of *H. pylori* in vivo: (1) Histopathology slides were prepared from 50 patients with dyspeptic complaints. (2) The pathogenesis of the bacteria was studied in rhesus monkeys. Orally we administered 10^6 cfu·mL^{-1} of the *H. pylori* to 2 monkeys, and one served as negative control. The *H. pylori* isolates were isolated from 144 (97.3%) out of 148 gastritis patients, 43 (86%) out of 50 duodenal ulcers, 10 (50%) out of 20 gastric ulcers, 6 (60%) out of 10 gastric adenocarcinomas, and 48 (47%) out of 102 asymptomatic attenders. We found that *H. pylori* can induce vacuolation, deformation and finally lysis of epithelial cells in vitro and perhaps in vivo, either by direct activity of cytotoxin or by its strong urease activity. The results showed that the cell lines of BK and Hela (epithelial cells) were susceptible to cytotoxin and urease of *H. pylori* isolates. 63.64% of the isolates showed cytotoxin activity of which 98% were from duodenal ulcers, 66.7% from gastritis patients and 33.3% from asymptomatic attenders. The bacterium was isolated from experimentally infected rhesus monkeys 2 months after oral injection. Also the histopathologic slides showed vaculation, presence of polymorphonuclears and inflammation in their stomach, while the control was negative. *H. pylori* infection is extraordinarily common in Iran. The results showed that *H. pylori* is implicated as a key risk factor in gastritis, duodenal ulcer and perhaps gastric adenocarcinoma. This study suggests that the cytotoxicity and urease activity of *H. pylori* are two important virulent factors of the organism. The in vivo observations demonstrate that *H. pylori* infection in rhesus monkey may serve as a model for human infection.

P-126 **Comparison of the influence of propolis and virginiamycin on broiler performance and immune response to ND vaccine.** S.M.M. Kiaei[a], M. Modirsanei[a], H. Bozorgmeherifard[b], B. Mansoori[a], B. Gholamian[b], A. Ghalyanchi[b], M. Rabani[b] ([a] Departement of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [b] Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran).

This study was carried out to compare the effects of propolis and virginiamycine on the performance parameters and on the immune response of broiler chicks to the ND vaccine. Three hundred and sixty male day-old Ross308 broiler chicks were randomly assigned to four treatments (three replicates of 30 chicks). Chicks were fed one of the four dietary treatments: (1) a corn–soy meal as control diet, (2) Control diet with 3000 ppm propolis for starter and 2000 ppm propolis for grower and finisher diets, (3) Control diet with 6000 ppm propolis for starter and 4000 ppm propolis for grower and finisher diets, (4) Control diet with 100 ppm virginiamycine during the whole experimental period. Body weight gain (BWG), feed intake (FI), feed conservation ratio (FCR), survivability and efficiency productive index (EPI) were recorded at 21 and 42 days of age. Prior to administration of ND vaccine (day 17) and 10 days after the vaccination, blood samples were taken from 10 chicks of each treatments for HI test. Data were analysed using ANOVA. Scheffe test was also used to compare means showing significance ($P < 0.05$). Chicks on treatment 4 showed higher BWG, FI and EPI compared with the other treatments, although treatment 2 showed lower FCR. Virginiamycine improved the immune response of the chicks against ND vaccine while no effect was observed by Propolis. It seems that propolis could not have any immunostimulant effects but appeared to be promising as a potential growth promoter in chicks.

P-127 **Time-course of the progression of intestinal inflammation in interleukin-10 knockout mice inoculated with intestinal bacteria.** B. Knoch[a,d], M.P.G. Barnett[a], S.T. Zhu[b], R. Broadhurst[c], A. Cookson[a], W.C. McNabb[a], S.O. Knowles[a], C.E. Ugarte[d], N.C. Roy[a] ([a] Metabolism and Microbial Genomics Section, Food and Health Group, AgResearch Grasslands, Palmerston North, New Zealand, [b] Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand, [c] National Resources Group, AgResearch Ruakura, Hamilton, New Zealand, [d] Institute of Food, Nutrition

and Human Health, Massey University, Palmerston North, New Zealand).

Between 5 and 12 weeks of age the interleukin-10 (IL-10) knockout (KO) mouse develops a Crohn's Disease-like colitis when raised under conventional conditions of housing and feed. Inoculation at 5 weeks with intestinal bacteria may hasten the onset and progression of this intestinal inflammation. Our aim was to determine the optimal tissue sampling time for the IL-10 KO and C57BL/6J (control) mice when inoculated orally with 12 *Enterococcus* strains plus complex intestinal flora collected from other control mice. At 7, 8.5, 10, 12 and 14 weeks, intact intestinal sections were assigned a histological score (HIS) based on inflammatory cell infiltrates and tissue destruction. The HIS of the intestinal tissues in the IL-10 KO mice increased ($P < 0.05$) until 12 weeks with the colon being the most affected tissue. While bodyweight of the control mice continued to increase up to 14 weeks, IL-10 KO mice failed to gain weight beyond 11 weeks. These results show that by 11 weeks IL-10 KO mice inoculated with intestinal bacteria reliably develop sufficient inflammation to be a suitable model of Inflammatory Bowel Disease. Nutrigenomics New Zealand is a collaboration between AgResearch Limited, Crop and Food Research, HortResearch and The University of Auckland and is largely funded by the Foundation of Research, Science and Technology (FRST). MPG Barnett is funded by a FRST Postdoctoral Fellowship.

P-128 **Isolation and partial purification of chicken intestinal mucus for use in the study of attachment of Lactobacilli to the poultry GI tract.** K. McGreeghan-Crosby[a], J.K. Thompson[b], C. Nicolson[b], M.A. Collins[a,b] ([a] Department of Food Science, Queen's University of Belfast, UK, [b] Agriculture, Food and Environmental Science Division, Department of Agriculture and Rural Development for N. Ireland, Newforge Lane, Belfast, BT9 5PX, Northern Ireland, UK).

Intestinal mucus is considered to be the primary site of attachment for probiotic microorganisms. In order to construct a simple yet realistic model, chicken intestinal mucus was extracted and partially purified. The product proved to be a glycoprotein with a carbohydrate content of > 20%. A poultry GI tract strain of *Lactobacillus thermotolerans* was observed to flocculate in the presence of both chicken mucus and commercially produced porcine mucin which may be associated with cell adhesion to the glycoprotein matrix. This model system will be used to study adhesion associated changes in gene expression in *Lb. thermotolerans*.

P-129 **Cloning of the murC gene in order to study its putative role in attachment of Lactobacilli to poultry GI tract epithelium.** K. McGreeghan-Crosby[a], J.K. Thompson[b], M.A. Collins[a,b] ([a] Department of Food Science, Queen's University of Belfast, Agriculture, Food and Environmental Science Division, Belfast, Northern Ireland, UK, [b] Department of Agriculture and Rural Development for N. Ireland, Newforge Lane, Belfast, BT9 5PX, Northern Ireland, UK).

Differential Display experiments have suggested that the *murC* gene in Lactobacilli appears to be upregulated in the presence of mucin. As this glycoprotein gel is normally found as a protective layer coating the GI tract epithelium, this could indicate that *murC* plays a role in the attachment of Lactobacilli to the intestinal epithelium. In order to confirm this hypothesis, the *murC* gene from a poultry GI tract strain of *Lactobacillus thermotolerans* was cloned and its expression studied. Degenerate primers based on known *murC* DNA sequences were used to generate a 700 bp *murC* amplicon. DNA sequencing revealed an identity with a *murC* fragment previously identified by differential display as being upregulated by mucin. Northern analysis, using a model system based on MRS broth containing increasing concentrations of porcine mucin and chicken intestinal mucus, was used to confirm the Differential Display data. A transcript of ~ 3 000 nucleotides in length was observed suggesting the *murC* gene may be part of a polycistron. An attempt was made to identify the flanking regions of the *murC* gene using inverse PCR. Sequence data were consistent with *murC* forming part of a polycistron. Experiments using Differential Display to study changes in gene expression in the presence of intestinal mucin have generated several other amplicons of interest, including proteins with mucus binding motifs.

P-130 **The aerobic/anaerobic cell culture model for studies on bacterial adhesion.** A. Olejnik, M. Lewandowska, W. Grajek (Department of Biotechnology and Food Microbiology, August Cieszkowski Agricultural University of Pozna, ul. Wojska Polskiego 48, 60-627 Poznań, Poland).

Adhesion to the intestinal tract has become generally accepted as an important colonization factor used for the selection of probiotic cultures. Studying bacterial adhesion in vivo is difficult and in vitro models with intestinal cell lines are widely accepted methods for this assessment. The most common model used is the enterocyte-like cell line Caco-2, obtained from an adenocarcinoma of the human colon and showing similarity to the epithelial cells of the gastrointestinal tract. This model is recommended to study probiotic adhesion, invasion of pathogenic bacteria, trans-epithelial transport of nutrients, immune stimulation, and others. In the conventional Caco-2 culture system, the Caco-2 cells are attached to the surface of T-flasks or to a porous membrane as a cell monolayer. The cells are usually fed with the Dulbecco's modified Eagle medium from the apical side of the monolayer. In the new culture system, we used a two-chamber reactor, divided in the middle with a porous polycarbonate membrane. The upper surface of the membrane was colonized with Caco-2 cells and fed with non-aerated, low-nutrient medium to simulate intestinal, anaerobic conditions. The bottom chamber was fed with the Dulbecco's modified Eagle medium, rich in nutrients and well aerated to create aerobic condition. We studied some physiological aspects in the new model of Caco-2 culture, especially the influence of initial inocula and serum addition on the Caco-2 cell differentiation and growth kinetics. Well-developed monolayers of intestinal cells were obtained after 21 days when the initial concentration of Caco-2 cells was $1 \times 10^4 \cdot cm^{-2}$ and at foetal bovine serum supplementation at 20%. In the adhesion study, three probiotic strains were used: *Lactobacillus casei* (ATCC 39539), *Lactobacillus rhamnosus* GG (ATCC 53103) and *Lactobacillus acidophilus* LC1 (ATCC). The yield of bacterial adhesion was strain dependent, and *Lactobacillus rhamnosus* GG exhibited the best adhesion ability.

P-131 **Intestinal epithelial cell responses to *Lactobacillus plantarum*.** S. Pavan[a,b], J.J.M. Van de Sandt[b], K. Venema[a,b], M. Kleerebezem[a,c] ([a] Wageningen Centre for Food Sciences, Wageningen, The Netherlands, [b] TNO Quality of Life, Zeist, The Netherlands, [c] NIZO food research, Ede, The Netherlands).

Intestinal epithelial cells (IEC) constitute the interface between the host and the gut luminal environment and are the first cells sensing the presence of intestinal bacteria, which results in a variety of immunological and physiological responses. The global transcriptional response of differentiated IEC monolayers (Caco-2, HT29-MTX) to *Lactobacillus plantarum* WCFS1, a model of a commensal as well as probiotic microorganism, was studied using combined in vitro and *in silico* approaches. IEC were cultivated alone or in the presence of human blood monocyte-derived dendritic cells. The mono- or co-cultures were stimulated with *L. plantarum*, using either standard laboratory cultures or bacterial suspensions subjected to passage through a dynamic in vitro model of stomach and small intestine. Statistical analyses of the human cDNA microarray data revealed a clear and dose-dependent transcriptional response of IEC to *L. plantarum*, involving modulation of cell cycle and cell signaling functions. Although each of the two IEC lines used in these experiments displayed distinct native gene expression profiles, both specific and common responses to bacterial stimulation were observed. In co-culture experiments, the presence of dendritic cells triggered strong transcriptional responses in Caco-2 cells, while the IEC response to *L. plantarum* stimulation remained limited under these conditions. These results show that IEC are able to sense and react to the presence of harmless gut bacteria and that responses generated after exposure to physiological bacterial doses include modulations of IEC signaling.

P-132 **Daily oral supplementation with *L. paracasei* CNCM I-2116 leads to a down-modulation of milk protein hypersensitivity in mice.** S. Pecquet, S. Chibani-Chennoufi, F. Rochat, A. Mercenier (Nutrition and Health Department, Nestlé Research Center, Vers chez les Blanc, Nestec Ltd, 1000 Lausanne, Switzerland).

Cow's milk allergy (CMA) is one of the leading causes of food allergy in children. Sensitisation to milk proteins occurs early in life and leads to atopic symptoms. Some clinical studies showed that probiotic treatment of "at risk" infants might prevent them from allergic symptoms, in particular from atopic dermatitis. In this study, we used a milk hypersensitivity mouse model to evaluate the beneficial effect of the probiotic *L. paracasei* CNCM I-2116 on the onset of atopic symptoms. C3H/HeJ mice were sensitised 4 times at weekly intervals with whey proteins in combination with cholera toxin. All along the trial, mice received either *L. paracasei* CNCM I-2116 (5×10^8cfu·mL^{-1}) or maltodextrine (matrix control) in drinking water. One week after the last sensitisation, mice were orally challenged with β-lactoglobulin and symptoms (scratching, puffiness and activity loss) were followed for 45 min. At sacrifice, blood and faeces were collected for seric IgE, IgG1, MMCP1, histamine and faecal IgA. Faecal probiotic counts were recurrently controlled during the trial. Mice treated with maltodextrine showed a high symptom score (4.1 ± 1.8) compared to *L. paracasei* CNCM I-2116 treated mice (1.3 ± 0.9). This significant difference could be correlated to a similar trend observed with MMCP1, total IgE and specific IgG1 levels. This preliminary data firstly indicates that this experimental model is suitable to evaluate the preventive effect of probiotics in allergy. Secondly, this study provides the evidence that probiotics can modulate food hypersensitisation in mice. Indeed, we demonstrate that daily administration of *L. paracasei* CNCM I-2116 protects mice from cow's milk protein hypersensitivity.

P-133 **An immunoblot for the determination of antibodies against Lactobacilli.** A-L. Prangli, M. Utt, R. Uibo (Department of Immunology, University of Tartu, Estonia).

The development of specific adaptive immune responses to indigenous *Lactobacillus* spp. has been insufficiently characterised among humans. Partly, this is connected to the fact that no good tools are available for antibody measurement against these microbes. In the present study we have developed an immunoblot assay for the determination of serum antibodies against different lactobacilli. Different lactobacilli were obtained from the culture collection of lactobacilli from the Department of Microbiology, University of Tartu. Our results showed IgG type antibodies in a group of 55 healthy children (age range 1–17 years) most commonly against *L. plantarum* 20 kDa, 28 kDa, 29 kDa, 31 kDa, 47 kDa, and 68 kDa proteins, and against *L. fermentum* and *L. acidophilus* proteins of 38 kDa and 31 kDa, respectively. When results in different patient groups (64 children either with celiac diseases, type I diabetes or *Helicobacter pylori* infection with abdominal complaints; age range 0.7–18) were compared to the healthy children group, we revealed a characteristic pattern of reactivity in a celiac disease group with *L. fermentum* and *L. acidophilus* antigens. Children with type I diabetes and with *H. pylori* infection did not differ from the control group in IgG type antibody frequency. A higher frequency of IgA type antibodies against *L. fermentum* proteins was observed in the celiac disease and *H. pylori* infection groups. Our study showed that immunoblot is a reliable method for the determination of serum antibodies of IgG and IgA types against *Lactobacillus* spp. in children. Whether the revealed reactivity is characteristic for the tested lactobacilli strains needs to be controlled in further studies. However, in the present configuration our assay is usable for the determination and comparison of antibody presence in different clinical associations.

P-134 **Mannan induced changes in cytokine expression and growth of enteropathogenic *E. coli*-challenged broilers.** P. Singboottra[a], F.W. Edens[a], A. Kocher[b] ([a] North Carolina State University, Department of Poultry Science, Raleigh, NC 27695-7635 USA, [b] Alltech Biotechnology Centre, Sarney Summerhill Rd, Dunboyne, Co. Meath, Ireland).

Mannan oligosaccharides (MOS) isolated from the outer cell wall of a specific strain of *Saccharomyces cerevisiae* (Bio-Mos®, Alltech Inc.) is an alternative to the prophylactic use of antibiotics in poultry feeds and has been used to promote enteric health. The mechanism of action of MOS is agglutination of enterobacteria with type-1 fimbriae preventing them from colonizing the GIT. We have shown in vitro that macrophages given a mannan-rich fraction (MRF) found in the commercial product Bio-Mos have

altered abilities too express inflammatory cytokines such as IL-6 and nitric oxide. The altered cytokine expression was mediated through a transitory decrease in the expression of the toll-like receptor 4 (TLR-4), which binds to pathogen-associated molecular patterns stimulating NF-κB translocation from the cytoplasm to the nucleus where it activates genes encoding the inflammatory cytokines. MRF was fed to broiler chickens in four treatments: (1) basal diet-non-challenged, (2) MRF-non-challenged, (3) basal-enteropathogenic *E. coli*-challenged (EPEC), and (4) MRF-EPEC. EPEC (10^6 cfu) was administered by gavage to day old chicks. At three weeks of age, MRF-fed chicks had significantly increased body weights even with EPEC. Abdominal exudate macrophages, collected after Sephadex G50 stimulation, were divided into primary and LPS-stimulated groups and assayed for IL-6 and nitric oxide expression. Both primary and LPS-stimulated macrophages from basal-fed-EPEC and non-challenged chicks had elevated IL-6 activity and nitric oxide levels as compared with MRF-fed-EPEC and non-challenged chicks. These data confirm in vitro observations and suggest that mannan can regulate macrophage activity associated with intestinal health.

P-135 **Interleukin-18 levels in the small bowel after oral infection with *Salmonella.*** A. Splichalova[a], I. Trebichavsky[a], I. Rychlik[b], Y. Muneta[c], Y. Mori[c], I. Splichal[a] ([a] Institute of Microbiology, Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic, [b] Veterinary Research Institute, Brno, Czech Republic, [c] National Institute of Animal Health, Tsukuba, Japan).

IL-18 is a pleiotropic cytokine playing a critical role in intracellular infections as an IFN-γ inducer. It was shown recently that IFN-γ is the major protective factor in protection against *Salmonella.* The effect of IL-18 in *Salmonella* infections is, however, not so unequivocal. We have therefore used a microbiologically defined germ-free model and compared it with a conventional one. One-week-old germ-free (GF) pigs or conventional pigs (CV) from the pigsty were orally challenged with 10^8 cfu of *Salmonella enterica* serotype Typhimurium for 24 h: either with a virulent LT2 strain (causing similar disease in both humans and pigs but fatal for GF pigs) or with a non-pathogenic LT2 *aroA* deletion mutant. IL-18 levels were measured by ELISA in ileal washes (IL-18 was not detected in plasma even after the infection). GF pigs did not contain IL-18 in intestinal washes, whereas CV pigs contained 146 $pg \cdot mL^{-1}$. LT2 infection caused an increase of IL-18 levels, whereas the *aroA*$^-$ mutant did not cause any significant change. These findings show that IL-18 is secreted in the gut in response to the infection. Sterile GF pigs have no IL-18 in the lumen of the small bowel, whereas endogeneous microflora stimulate its production in CV counterparts from the pigsty. The vaccinal mutant did not induce IL-18 contrary to the virulent *Salmonella.* Thus, IL-18 levels in the small bowel correlated with the belligerence of the associated microbe.

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P-136 **The preventive effect of *Enterococcus faecalis* cell preparation (EC-12) against the experimental infection of porcine edema disease.** T. Tsukahara[a,b], N. Nakanishi[c], C. Kameue[a], K. Nakayama[b], N. Matsubara[d], M. Hamasaki[e], K. Ushida[a] ([a] Laboratory of Animal Science, Kyoto Prefectural University, Shimogamo, Kyoto 606-8522, Japan, [b] Kyoto Institute of Nutrition and Pathology, Kyoto 610-0231, Japan, [c] Kyodoken Institute, Kyoto 612-8073, Japan, [d] Combi Corporation, Saitama 338-0832, Japan, [e] Kanematsu Corporation, Tokyo 105-8005, Japan).

Porcine edema disease (ED) is caused by Shiga toxin 2e-producing *Escherichia coli* (STEC). ED has become frequent in pig farms and the use of antimicrobials has resulted in the development of antimicrobial-resistant STEC. Accordingly, the use of materials other than antimicrobials is requested for the prevention of ED. Oral administration of the cell preparation of *Enterococcus faecalis* strain EC-12 (EC-12) to weaning piglets decreased their mortality in a STEC-contaminated farm. Experimentally STEC-infected piglets were used as a model for ED to determine the appropriate dose level of EC-12 to prevent the ED. Fifteen 21 days old pigs were divided into five groups; STEC challenge with the control diet, STEC challenge with dietary EC-12 either at 0.005, 0.01 or 0.05% (w/w), and

no STEC challenge with the control diet. Challenge was carried out at 25, 26 and 27 days old using STEC contained in capsules resistant against gastric digestion. All pigs were euthanized at 32 days old. Daily weight gain, feed efficiency and the palpebral edema was improved by supplementation of 0.05% EC-12. The eosinophil infiltration in the lamina propria of jejunum of the same piglets reduced to 1/2 level of those observed in STEC challenge with the control diet group. Accordingly, 0.05% dietary EC-12 appeared to be effective to improve clinical symptoms in the weaned piglets infected by STEC.

P-137 **Modulating mucin dynamics using functional carbohydrates.** Z. Uni, A. Smirnov (Department of Animal Science, Faculty of Agriculture, Hebrew University, Rehovot, Israel 76100).

The epithelium of the gastrointestinal tract is covered by a mucus layer that acts as a medium for protection, lubrication and transport between the luminal contents and the epithelial cells. The mucus layer is composed predominantly of mucin glycoproteins produced by goblet cells which are believed to be capable of aggregating several bacterial species and preventing the attachment of pathogenic bacteria. The mucus layer is also a component of the innate host response that is regulated in response to inflammation and infection. A number of protective mucus-layer-associated proteins are either co-secreted with mucins or interact with the mucous environment to perform their protective function. Recently it has been proposed that nutritional and microbial factors may affect both mucin biosynthesis and secretion in the small intestine. The use of "functional carbohydrate" such as mannan oligosaccharides (MOS) derived from a specific strain of yeast can change mucin dynamics. It can interact with Galectins, a family of animal lectins. This lectin can regulate cell growth and survival, by interacting with cytoplasmic and nuclear proteins, thereby affecting intracellular signaling pathways. Its presence in the intestine may also lead to changes in the gut microflora by competitive exclusion of selective bacterial species and therefore change the microenvironment of intestinal epithelial cells, including mucin-producing cells. Results from a recent study showed that adding MOS supplementation (Bio-Mos® Alltech Inc. 2 $g \cdot kg^{-1}$) to broiler diets led to increased expression of the chicken intestinal mucin gene, accumulation of the mucin in the goblet cells and thicker mucus adherent layer. These changes may contribute to gut health and may influence gut function by affecting nutrient uptake. The mucin layer is an unstirred layer which contains mucus, IgA, and probably digestible nutrients (dipeptides and di-tri-carbohydrated and fat micelles), it may serve as a medium which incorporates these nutrients and makes them more available for the enterocytes.

P-138 **Oral dose of *Lactobacillus plantarum* and *Megasphaera elsdenii* increased intestinal IgA secretion, colonic butyrate and growth of colonic mucosa in piglets.** K. Ushida[a], R. Inoue[a], N. Shimojo[a], S. Takahashi[a], C. Kameue[a], T. Tsukahara[b] ([a] Kyoto Prefectural University, Shimogamo, Kyoto 606-8522, Japan, [b] Kyoto Institute of Nutrition and Pathology, Kyoto 610-0231, Japan).

Piglets are often vulnerable after weaning to entero-pathogens due to the incomplete immune defense. This is particularly true when they are raised without dietary antimicrobials. Stimulation of IgA, which is the primary immunological defense line in the mucosal immune system, may help the situation above. Lactobacilli may stimulate IgA secretion when orally dosed. The piglets also suffer from non-pathogenic diarrhea due to maladaptation to the diet. Colonic butyrate may help the situation in supporting the growth and function of the colonic mucosa. *Megasphaera elsdenii*, a lactate-utilizing butyrate producer, may help butyrate production particularly when combined with lactobacilli. Weaned piglets (21 days old) were orally dosed once a day either (L) 10^{10} *L. plantarum* LQ80, or (LM) 10^{10} LQ80 with 10^{9} *M. elsdenii* pig isolate. LQ80 was contained in capsules resistant to gastric digestion. *M. elsdenii* was contained in capsules resistant to gastric and intestinal digestion. An untreated control (C) was also prepared. During 2 weeks of test period, fecal IgA was higher in L and LM than C. At the end of the test period, jejunal and ileal IgA tended to be higher in L and LM than in C. Colonic butyrate was higher in LM than the others. Thickness of the colonic

mucosa was greater in LM than the others. As a result, fecal score was improved in LM and daily weight gain during the test period was improved in both LM and L. This study was supported by Secure and healthy livestock farming project of NILGS, Japan.

P-139 **Adhesion of *Bifidobacterium* strains to intestinal mucus and its influence on competitive exclusion of *Escherichia coli* from the intestine.** E. Wasilewska, M. Bielecka (Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10-747 Olsztyn, Tuwima 10, Poland).

Adherence or attachment of some bacteria to eukaryotic cells or tissue surfaces and blocking of epithelial receptors may represent one of the mechanisms for prevention of pathogenic microflora invasion. The aim of the studies was to investigate the ability of live, dead or disrupted bifidobacterial cells to competitively exclude adherent enterohemorrhagic and enterotoxigenic *Escherichia coli* from intestinal mucosa. Commercial preparations (Sigma), as well as mucus freshly isolated from human and laboratory rats, was used in the studies. The results revealed a great diversity of bifidobacteria binding to glycoproteins included in the mucus. Depending on the strain, 100-800000 live bifidobacterial cells attached to 1 mm^2 surface of the microplate covered by the mucus. The source of mucus isolation had no significant influence on the *Bifidobacterium* adherence; however, the strains of human origin adhered slightly better to mucus isolated from humans. Such preferences were not observed in the well-adhering strains of *B. animalis* and *B. animalis/lactis* species – isolated from rats and fermented milks, respectively. The tested bifidobacterial strains exhibited a diverse influence on the adhesion of enterohemorrhagic/enterotoxigenic *Escherichia coli*. Depending on the strain or its physiological state, a significant decrease (0.5–50%) or increase of the number of *E. coli* cells attached (in some cases over twofold) was observed. Live bifidobacterial cells revealed the strongest inhibiting effect towards enterohemorrhagic *E. coli* 94 (serotype O157:H7), which was characterized by the strongest adhesion to the mucus. Blocking the adherence of the remaining strains tested, i.e. *E. coli* DSM 10973 (serotype 06), DSM 30083 (serotype O1:K1:H7) and s20 strain freshly isolated from rat, was somewhat lower. Testing of lactic acid bacteria adhesion to mucus and careful selection of the strains with profitable adherence abilities seems to be very important for a probiotic effect in the intestine.

P-140 **Disappearance of purified plasma IgG from the rumen.** Y.J. Williams, S. Popovski, S. Rea, A.D.G. Wright (CSIRO Livestock Industries, Centre for Environment and Life Sciences, Private Bag 5, Wembley Western Australia 6913).

Abstract withdrawn.

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O-21 **Effects of mannan-oligosaccharide-based yeast cell wall preparations on the prevalence of antibiotic resistant bacteria in axenic culture of intestinal bacteria: are there strategies for decreasing the prevalence of antibiotic resistant bacteria in the intestinal tract?** S.M. Scheuren-Portocarrero, M.M. Newman, K.A. Dawson, C.A. Moran (Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, USA, Alltech Biosciences Center, Nicholasville, KY 40356 , USA).

A series of studies have examined the effects of various yeast cell wall components from the commercial product Bio-Mos® on the prevalence of multi-drug resistant *Salmonella* and *E. coli* in in vitro culture systems. While long-term (24 h) exposure to low levels of mannan-enriched yeast cell wall preparations (0.3%) did not affect the growth rate of any of the bacterial strains studied, the relative proportion of *Salmonella monterido* in a multi-resistant population that were resistant to streptomycin and ampicillin and *E. coli* XL1-blue that were resistant to lincomycin and tetracycline decreased over time. Decreased resistance to streptomycin was associated with the loss of a 2.5 kb plasmid in the *s. enteritidis* strain and the loss of 6 plasmids (1 kb, 1.5 kb, 2 kb, 2.5 kb, 3.5 kb and 7 kb) from the multi-resistant strain of *S. monterido*. However, similar plasmid losses were not seen in genetically engineered strains of *E. coli*. Exposure to the yeast cell wall preparations delayed the formation of plasmid-containing transconjugates when antibiotic-resistant strains of *E. coli* were incubated with antibiotic-sensitive recipient cells in a defined laboratory media and in cultures containing mixed populations of swine fecal bacteria. The results suggest that fractionated yeast cell wall preparations may be useful in strategies for decreasing the prevalence of antibiotic resistant bacteria in the gastrointestinal tract.

O-22 **SAFEWASTES' by-products as potential preventives of intestinal adhesion of bacteria.** P.M. Becker[a], S. Galletti[b], J. Van der Meulen[a] ([a] Animal Sciences Group, Wageningen UR, PO Box 65, 8200 AB Lelystad, The Netherlands, [b] Faculty of Veterinary Medicine, University of Milan, Via Celoria 10, 20133 Milan, Italy).

The recent EU-wide ban on the use of antibiotics as growth promoters in animal feed has prompted a huge demand for substitutes. Within the EU-project SAFEWASTES, specific functional plants and their post-processing derivatives are to be screened both in vitro and in vivo with regard to their potential as alternatives to antibiotics. In general, antimicrobial strategies take advantage of the Achilles' heels or weak spots of bacteria, like their susceptibility to damaging agents, or their ability to attach to alternative adhesion sites. By impeding the colonization of the intestine by enteropathogenic bacteria, not only animal pathogens can be warded off, but also the risk of transmission of human pathogens via the food-chain can be reduced. The SAFEWASTES' products are derived from artichoke pomace, carrot pomace, coneflower, larch sawdust, linseed, mango peel, pumpkin fruit, sunflower, thyme, and willow, amongst other plants, and are provided as raw materials and as water, ethanol, and heptane extracts. To test the adhesion of enteropathogenic bacteria to complex substrates, a new microtitration plate-based assay was developed. First results with different raw materials indicate that artichoke pomace, pumpkin fruit, linseed, sunflower, and coneflower have a binding capability for *E. coli* ATCC 25922, which has an affinity to mannose due to its type 1 fimbriae. The impact of different water soluble plant extracts on the adhesion of *E. coli* K88ac (ETEC, type 4 fimbriae) to brush-border cells of piglets was evaluated microscopically. Of all the products tested, mango inhibited the most efficient brush-border adhesion. The test results indicate that some of the SAFEWASTES' by-products that are at present regarded as waste materials might gain an added value in animal nutrition in the near future.

O-23 **Effect of diet and protozoa on the community composition of feed-adherent bacteria in the rumen.** D.P. Morgavi[a], D. Graviou[a], M.J. Ranilla[b], C. Martin[a] ([a] Herbivore Research Unit, INRA-Theix, 63122 Saint-Genès-Champanelle, France, [b] Departamento de Producción Animal I, Universidad de León, 24071 León, Spain).

The microbial population attached to feed particles is essential for the efficient degradation of complex plant substrates in the rumen. The aim

of this work was to investigate the changes induced by contrasting feeding regimes and protozoa on the structure of the rumen feed-adherent bacterial population. Sheep ($n = 6$) were separated into two groups that received an alfalfa hay or a wheat concentrate:alfalfa hay diet (60:40) in a cross over design with two blocks: faunated and defaunated. The bacterial community was studied by PCR-DGGE on five substrates, 2 cereals and 3 forages that were incubated in sacco for 24 h. Feed degradation was evaluated in sacco (alfalfa hay) and in vitro (concentrate:hay diet, wheat, alfalfa hay, and corn silage). Diversity of the bacterial population for all substrates as measured by Shannon's index (H) was higher for the forage than for the concentrate-rich diet ($P < 0.01$) and when protozoa were absent ($P < 0.01$). This greater diversity was associated with a more efficient in vitro dry matter degradation of forage-fed (67 vs. 59%, mean of all substrates, $P < 0.01$) and protozoa-free (71 vs. 56%, $P < 0.01$) inocula. Degradation of alfalfa hay in sacco showed similar results ($P < 0.05$). An animal variation was observed, variation that was translated into dissimilar DGGE profiles. However, PCA analysis arranged by substrate showed that while most samples from forage diets grouped rather closely, samples from concentrate-rich diets were more dispersed, possibly indicating the ecosystem's instability. Diversity of rumen feed-adherent bacteria was a trait positively associated to substrate degradation and it was affected by a cereal-rich diet and the presence of protozoa.

O-24 **Inulin and *Lactobacillus amylovorus* supplemented to human gut microbiota lower the microbial bioactivation of dietary aromatic contaminants to estrogenic metabolites.** T. Van de Wiele[a], L. Vanhaecke[a], H. Jacobs[b], W. Verstraete[a] ([a] Laboratory Microbial Ecology and Technology, Ghent University, 9000 Gent, Belgium, [b] COSUCRA, 7740 Warcoing, Belgium).

One of the lesser studied mechanisms by which pre- and probiotics exert their health-promoting effects in the gut is through the detoxification of potential toxicants, termed chemopreventive activity. Using a Simulator of the Human Intestinal Microbial Ecosystem (SHIME), we investigated how administration of inulin and *Lactobacillus amylovorus* to an intestinal microbial suspension affected the bioactivation of dietary aromatic contaminants to metabolites with estrogenic properties. The compounds of interest were polycyclic aromatic hydrocarbons (PAH), polybrominated diphenyl ethers (PBDE) and zearalenone. Estrogen bioassay data on PAH-incubated SHIME colon samples revealed, that inulin addition significantly decreased the estrogenicity in the ascending colon (39%) and transverse colon (14%), whereas no chemopreventive effects were seen in the descending colon. Yet, the estrogenic response from descending colon samples, incubated with PAHs, decreased by 20% when *Lactobacillus amylovorus* was supplemented. Interestingly, the colon-specificity of the inulin chemopreventive effect corresponded to the colon-specificity of its prebiotic effects. Conventional culture-based techniques and PCR-DGGE analysis on the SHIME colon suspension revealed the selective effect of inulin treatment towards stimulation of lactic acid bacteria in the ascending colon compartment. Moreover, realtime PCR showed a significant increase in Bifidobacteria, with more than 1 log cells·mL^{-1} from the proximal to distal colon. Our data show that the prebiotic effects of inulin and the probiotic activity of *Lactobacillus amylovorus* may also inhibit the bioactivation of dietary contaminants. Additionally, we infer that the specific conditions in the proximal colon region are most suited to bring about the chemopreventive effects from inulin.

O-25 **Quantification of faecal *Bifidobacterium* and *Lactobacillus* species in infants receiving a prebiotic infant formula.** M. Haarman[a], B. Stahl[b], G. Boehm[b], J. Knol[a] ([a] Numico Research B.V., Wageningen, The Netherlands, [b] Friedrichsdorf, Germany).

A healthy intestinal microbiota, like the microbiota of breast-fed infants, is considered to be important for priming of the infants' mucosal and systemic immune system. Generally *Bifidobacterium* and *Lactobacillus* predominate the microbiota of breast-fed infants. To study the *Bifidobacterium* and *Lactobacillus* composition of infants in more detail, duplex 5' nuclease assays were designed for *Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium*

dentium, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus*. These assays were used to determine the relative amounts of *Bifidobacterium* and *Lactobacillus* species in faecal samples of infants, aged 28 to 90 days, receiving a standard formula (SF; $n = 10$) or a standard formula with 0.8 g/100 mL galacto- and long chain fructo-oligosaccharides (9:1) (OSF; $n = 10$). A breast-fed group (BF; $n = 10$) was studied in parallel as reference. After 6-weeks intervention a significant increase in bifidobacteria and lactobacilli was shown in the OSF-group as well as in the BF-group but not in the SF-group. At the end of the study the *Bifidobacterium* sp. and *Lactobacillus* sp. composition of the OSF-group looked rather similar to that of the BF-group with relatively high levels of *B. infantis*, *B. longum*, *B. breve*, *L. acidophilus*, *L. paracasei* and *L. casei*. Faeces of the SF group contained more *B. adolescentis*, *B. catenulatum* and *L. delbrueckii* and less *L. paracasei* compared to the BF- and OSF-group. In conclusion, the distribution of the different *Bifidobacterium* and *Lactobacillus* species in infants receiving the prebiotic formula mimics that of breast-fed infants whereas that of infants receiving the standard formula does not.

O-26 **PCR-DGGE and real-time PCR analyses of total bacteria, Bifidobacteria and Lactobacilli populations in human subjects consuming calcium gluconate.** K. Anderson[a], J. Chen[a], Z. Yu[a], J. Jenkins[b], P. Courtney[b], M. Morrison[a] ([a] Department of Animal Sciences, Ohio State University, Columbus, USA, [b] Department of Food Science and Technology, Ohio State University, Columbus, OH 43210, USA).

There is a continued interest in the identification and development of prebiotics that selectively increase the abundance of *Bifidobacterium* and *Lactobacillus* in the human gastrointestinal tract. Much attention has been directed towards the use of oligosaccharides of fructose or galactose, but there are also monosaccharides that appear to have similar effects. One of these is gluconic acid, which has been shown in animal trials to be associated with increased numbers of lactic acid bacteria and increased butyrate concentrations; a single human trial showed an increased number of bifidobacteria in response to gluconate supplements. Gluconic acid (or its salts) is widely used as a food additive and it also exists naturally in rice, wine, beer, grape juice, honey, vinegar, and other fermented products. The objective of this study was to examine the impact of a calcium gluconate supplement on the intestinal microbiota of healthy humans, with specific emphasis on bifidobacteria and lactobacilli. Twelve healthy adults consumed a daily calcium carbonate supplement for the first 2 weeks (control period), followed by 2.4 g of calcium gluconate daily for 3 weeks (test period), then reverted back to a daily calcium carbonate supplement for two more weeks (post-test period). Stool samples were collected from each subject once a week and microbiome DNA was extracted from these stool samples and analyzed by V3-based PCR-DGGE and real-time PCR assays. The gross PCR-DGGE profiles for each subject appeared to be unaffected by the treatment schedule. Genus-specific PCR-DGGE profiles also showed that the bifidobacterial populations present in 9 of the 12 subjects were unaffected throughout the study. Conversely, the lactobacilli-specific PCR-DGGE profiles did vary in most of the subjects during the study period. Real-time PCR assays showed there was an appreciable increase in bifidobacterial abundance (0.43 to 1.48 logs) while the calcium gluconate supplement was consumed in only 4 of the 12 subjects, and this stimulatory effect diminished within the two weeks following the termination of calcium gluconate supplementation. Similar observations were also made for the lactobacilli, but they were less pronounced, and collectively, these findings paralleled those obtained from cultivation-based analyses of the same samples. We conclude that gluconate can positively impact bifidobacteria and lactobacilli populations in the human gastrointestinal tract, but the consumption of 2.4 $g \cdot day^{-1}$ is not sufficient in most cases to confer a significant prebiotic effect on these bacteria. Different doses might be required to achieve certain desirable prebiotic effects in different individuals.

P-141 **Effect of antibiotics in diets and level of feeding on caecal microbial biodiversity in lactating does as estimated by DGGE.** L. Abecia[a], N.R. McEwan[b], J. Balcells[a], G.E. Lobley[c], M. Fondevila[a] ([a] Departamento de

Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Spain, [b] Institute of Rural Sciences, University of Wales, Aberystwyth, Wales, UK, [c] Rowett Research Institute, Aberdeen, UK).

Studies in growing rabbits highlighted a side-effect of medicated diets on the symbiotic digestive population. Besides, the feeding level influences digestive microbiota. The effect of dietary antibiotics to modify caecal population, and the feeding level on bacterial caecal biodiversity of lactating does was studied. Thirty-six lactating does (6 per treatment) were given a commercial diet alone (no antibiotics, NA) or with zinc bacitracin (BAC) or tiamulin (TIA). Intake was modified by manipulating the litter size at birth to 9 (LS9, high feeding level) or 5 (LS5, low level) pups per doe. Does were slaughtered at day 26 of lactation and their caecal content sampled. DNA was extracted and amplified for DGGE analyses. DNA profiles within a gel were compared using similarity trees from Hamming Distances and presented in pictorial form using UPGMA analysis. The effect of the feeding level was clearly manifested for either NA or BAC diets, whose resulting trees were divided into two different areas for LS5 and LS9. However, this effect was not clear for TIA. When NA and BAC were compared, the antibiotic effect did not manifest and the major factor on diversity was litter size. In contrast, comparison of TIA with NA gave a tree firstly split by antibiotic and then by litter size. Tiamulin has a marked effect on biodiversity, that prevails over that of feeding level, whereas without antibiotics or with those with a low effect on biodiversity, samples cluster mainly according to the feeding level.

P-142 **Microbial biodiversity in the caecum of litters from lactating does given antibiotics in the diet to manipulate their digestive population.** L. Abecia[a], N.R. McEwan[b], J. Balcells[a], E. Solanas[a], G.E. Lobley[c], M. Fondevila[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Spain, [b] Institute of Rural Sciences, University of Wales, Aberystwyth, Wales, UK, [c] Rowett Research Institute, Aberdeen, UK).

Since the microbial digestive population of the offspring is significantly influenced by that of the mother, changes in caecal biodiversity of lactating does induced by antibiotic dietary supply affects the instauration of digestive microbiota in their litters. From parturition, 3 groups of 3 lactating does were given a diet with no antibiotics (NA) or supplemented either with zinc bacitracin (BAC) or tiamulin (TIA). Throughout lactation access of the litter to solid food was avoided to ensure the pups were only milk-fed. After 26 days of lactation, two pups from each litter were slaughtered and their caecal content sampled. After DNA isolation, fragments of 16S rDNA genes were amplified and analysed by DGGE. DNA profiles were compared by pairwise Hamming Distance analysis and presented in pictorial form using UPGMA analysis. The resulting tree of pups from does given either NA or BAC diets was split by litter, with no major effect from the antibiotic. However, the DGGE gel comparing pups belonging to NA and TIA groups primarily showed a split by antibiotic in the diet fed to the mother and then by litter. Accordingly, the tree from bacterial DGGE profiles of pups from the therapeutic diets (BAC and TIA) showed two distinct areas according to the antibiotic used in the maternal diet. It is shown that the bacterial population in the offspring is largely dependent on the microbial biodiversity of their mothers, primarily when this is affected by the dietary inclusion of antibiotics.

P-143 **Rumen microbial population characteristics and detection of transgenic DNA in rumen bacteria from sheep fed Bt176 maize for three years.** G. Acuti[a], M. Trabalza-Marinucci[a], E. Forano[b], P. Mosoni[b], L. Mughetti[a], S. Costarelli[c], C. Rondini[c] ([a] Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, Università degli Studi di Perugia, 06126 Perugia, Italy, [b] INRA, Centre de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France, [c] Istituto Zooprofilattico dell'Umbria e delle Marche, 06126 Perugia, Italy).

The use of genetically modified feeds and the level of public concern are increasing worldwide. One concern is the gene transfer to the rumen bacteria, but there is a lack of studies on this matter. Forty-four Bergamasca × Appenninica ewes were used in this 3-year study. Animals were divided into two groups and received hay ad libitum and either nontransgenic or Bt176 maize. Twelve, 20 and 12 ewes during the first,

second and third year of the trial, respectively, were slaughtered 90 d after lambing and approximately 12 h after feeding. Rumen contents were collected and microbial populations were numerated. The presence of endogenous and transgenic DNA was searched for in DNA extracted from bacterial sub-populations grown for 7 (total and amylolytic) or 14 d (cellulolytic) in vitro. No difference was found between control and Bt176-fed ewes as far as bacterial numbers and protozoal genera distribution were concerned. Nor was the 211 bp fragment from the CryIA(b) transgene or the 226 bp fragment of maize invertase gene detected by PCR in the bacterial cultures. As expected, the rrs bacterial gene (1485 bp), used as a control, was found in all samples. These results show that a long-time exposure to transgenic feed does not affect the rumen microbial populations tested and that the conditions of the rumen environment are likely to prevent bacteria from integrating recombinant DNA.

P-144 **Detection of recombinant maize DNA in rumen contents of dairy cows and in mixed ruminal cultures.** G. Acuti[a], P. Mosoni[b], M. Trabalza-Marinucci[a], C. Antonini[a], E. Forano[b] ([a] Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, Università degli Studi di Perugia, 06126 Perugia, Italy, [b] INRA, Centre de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France).

The fate of transgenic plant DNA fragments in the gastro-intestinal tract of mammals is not completely known. This experiment was designed to compare the persistence of transgenic maize DNA in the bovine rumen and in vitro mixed ruminal cultures. The persistence of maize invertase gene fragment (226 bp) and of CryIA(b) transgene fragment (211 bp) was monitored by PCR in 2 rumen cannulated cows. Animals were fed for 2 weeks a diet containing Bt176 maize (10% of dry matter intake). Samples of whole rumen contents were collected at 0, 30 min, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h after removing the transgenic maize from the diet. An in vitro experiment was performed using rumen fluid from the same cows. Fourteen ml of inoculum (mixed 1:4 with buffer) were added to 175 mg of ground Bt176 maize and incubated at 39 °C in triplicate vials for each sampling time according to the in vivo protocol. Bacterial DNA was detected in all in vivo and in vitro samples. The invertase fragment was detected at all incubation times in the in vitro study and in most in vivo samples. The CryIA(b) fragment was detected up to 2 h in the in vitro trial and found in rumen contents in one case only (at 2 h in one cow). Detection of single-copy genes originating from transgenic feed is difficult in the complex rumen ecosystem. Caution is needed when extrapolating results from in vitro to in vivo studies.

P-145 **A new promising promoter for the production of prebiotic polysaccharides from sucrose or maltose using recombinant bacteria and yeasts.** T. Alamäe, K. Tammus, J. Lashmanova, T. Visnapuu (Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia).

We have isolated the maltase gene promoter of the yeast *Hansenula polymorpha.* The promoter works perfectly in both, bacteria and yeasts. In *Escherichia coli* expression from the promoter is strong and constitutive while in *H. polymorpha* it is induced by maltose and sucrose. Notably, in yeast the promoter is bidirectional, enabling coordinated expression of two proteins. Properties of the promoter suggested its potential application in expression cassettes for the production of prebiotic polysaccharides from either sucrose or maltose in bacteria and yeasts. To test the assumption, two levan sucrase genes were amplified from the genome of a pseudomonad and cloned under the control of the maltase gene promoter in a *E. coli/H. polymorpha* shuttle vector. Bacterial levan sucrases polymerize fructose residues from sucrose into a levan polymer, and some bacterial levans or levan-type polysaccharides have positive health effects as immune stimulators or prebiotics. *E. coli* transformants expressing the levansucrase genes formed slimy colonies on sucrose-containing medium and gained the ability to assimilate sucrose in minimal medium. The best levan producers were selected, respective plasmids were isolated and partially sequenced. The cloned levan sucrases were also functional in yeast, because *H. polymorpha* maltase-disruption mutant transformed with levan sucrase genes regained the ability to metabolize sucrose. Expression conditions of levansucrases in

H. polymorpha will be further optimized to achieve sufficient expression level. Recombinant *E. coli* clones will be used to produce levan sucrase proteins for the study of their biochemical properties. Levan produced from sucrose by recombinant bacteria will be purified and further studied with the final aim to examine its potential prebiotic effect.

P-146 **Effect of oral nitroethane administration on ruminal nitroethane reduction and methane production in cattle.** R.C. Anderson[a], G.E. Carstens[b], E.G. Brown[b], J.L. McReynolds[a], L.J. Slay[b], T.R. Callaway[a], D.J. Nisbet[a] ([a] USDA/ARS, Food and Feed Safety Research Unit, Texas A&M University, USA, [b] Department of Animal Science, Texas A&M University, College Station, TX, USA).

Interventions are sought to reduce economic and environmental costs of ruminant methane emissions. To test the effects of nitroethane on ruminal nitroethane reduction and methane production, we orally administered 0, 1, 2 or 4 g nitroethane·kg^{-1} forage diet·day^{-1} (0, 1×, 2× or 4×, respectively) to Holstein steers (319 ± 6.5 kg). Treatments were administered twice daily and the experiment was replicated twice (3 steers/treatment replicate^{-1}). In the first replicate, in vitro incubation of ruminal fluid collected before and on day 8 of treatment revealed that ruminal nitroethane-reducing activity increased ($P < 0.05$) from 0.022 ± .04 to 0.185 ± 0.12 μmol nitroethane/mL h^{-1} suggesting an in vivo enrichment of nitroethane-reducing microbes. Incubation of ruminal fluid collected before and on day 2, 4 and 8 of treatment revealed methane-producing activity was 25% lower ($P < 0.05$) in 2×- and 4×-treated steers than those measured in fluid from 0×- and 1×-treated steers (7.87 ± 2.14 and 8.00 ± 1.82 μmol CH_4/mL·h^{-1}). In the second replicate, which immediately followed the first, pretreatment ruminal nitroethane-reducing activity (0.408 ± 0.04 μmol nitroethane/mL·h^{-1}) was higher ($P < 0.05$) and methane-producing activity (3.21 ± 0.81 μmol CH_4/mL·h^{-1}) lower ($P < 0.05$) than in the first replicate (0.022 ± 0.04 μmol nitroethane/mL·h^{-1} and 8.60 ± 1.59 μmol CH_4/mL·h^{-1}, respectively). Methane-producing activities measured in ruminal fluid from 1×- and 2×-treated steers were 22 and 26% lower ($P < 0.05$), respectively, compared to 0×-treated steers (3.71 ± 0.67 μmol CH_4/mL·h^{-1}). Methane-producing activity from the 4×-treated steers was 19% lower, but not significantly, than that of the 0×-treated controls. Results demonstrate that nitroethane treatment effectively mitigates methane production for up to 8 days.

P-147 **The microbiological contamination of grains used in the production of probiotics for broiler chickens.** J. Biernasiak, M. Piotrowska, K. Śliżewska, Z. Libudzisz (Institute of Fermentation Technology and Microbiology Technical University of Lodz, 171/173 Wólczańska Street, 90 – 924 Lodz, Poland).

Fodder is prone to contamination with parasites or pests as well as pathogenic bacteria and moulds, with the products of their metabolism – mycotoxins, contributing to changes in nutrition values and influence on animal health. The aim of this study was to determine the level of microbiological contamination and of aflatoxins and ochratoxin A in grains. Additionally, the influence of the fermentation process of probiotic bacteria and yeast (*Lactobacillus paracasei/casei* LOCK 0920, *L. brevis* LOCK 0944, *L. plantarum* LOCK 0945, *Saccharomyces cerevisiae* LOCK 0142*)* on reduction of pathogenic microflora and mycotoxin concentration was evaluated. Three kinds of grains from different regions of Poland were used in the experiment: barley, wheat and corn. The work included identification of selected microorganisms (total number of bacteria, *Enterobacteriaceae*, *Escherichia coli*, *Salmonella*, aerobic and anaerobic bacterial spores, *Staphylococcus*, lactic acid bacteria, yeast and moulds) in the grain. For estimating the level of aflatoxins and ochratoxin A the ELISA test was used. The fermentation was conducted in previously optimized conditions (at 37 °C, during 24 h) for the production of probiotic preparations. The fermentation medium was composed of barley (50%), wheat (45%) and corn (5%) grain mixed with water in 1:1.5 proportions. The most frequently detected microorganisms in grains appeared to be coliforms (present at the range of 10^2–10^4 cfu·g^{-1}), mesophilic aerobic spores (present at the range of 10^5–10^6 cfu·g^{-1}) and moulds (present at the range of 10^2–10^3 cfu·g^{-1}) of which the most frequent were *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria* and *Fusarium*. *Salmonella* was present in 25 g of three samples. *E. coli* and anaerobic bacterial spores were not detected. In

all samples of grain aflatoxins were present and 89% of them were above the UE limit, which is 4 $\mu g \cdot kg^{-1}$. Ochratoxin A was detected in one barley grain and its value was 5 times higher than the UE limit, which is 5 $\mu g \cdot kg^{-1}$. After the fermentation, a decrease in the level of aflatoxins (at about 61%) and ochratoxin A (at about 57%) was observed. Mesophilic aerobic spores and coliforms were not detected in 0.1 g of the sample and *Salmonella* in 25 g of the sample.

P-148 **Survival of probiotics in a dynamic artificial gastrointestinal system.** S. Blanquet[a], S. Denis[a], V. Rousseau[b], F. Paul[b], S. Holowacz[c], G. Garrait[a], G. Hébrard[a], E. Beyssac[a], M. Alric[a] ([a] ERT CIDAM, Faculty of Pharmacy, University of Auvergne, 63000 Clermont-Ferrand, France, [b] GENIBIO, 09190 Lorp Sentarail, France, [c] PiLeGe, 75738 Paris, France).

Probiotic bacteria and yeasts have been suspected to have beneficial effects on health. The survival of ingested microorganisms influences the efficacy of probiotics. Validated models are required to study the mechanisms influencing the behaviour of microorganisms in digestive conditions. Here we present an artificial gastrointestinal system as a powerful tool to predict the survival of probiotics in the stomach and small intestine of human. This dynamic, computer-controlled system has been designed to accept parameters and data obtained from in vivo studies on human volunteers. The main parameters of digestion, such as pH, temperature, peristaltic mixing and transport, digestive secretions and passive absorption of small molecules and water are reproduced as accurately as possible. Gastrointestinal passage and successive conditions are controlled to mimic parameters in humans at different life stages, different food intakes and physiological or pathological conditions. This model offers reproducibility, easy manipulation and sample collection at any level of the digestive tract and at any time during digestion. Survival rates varied from 0.5 to 95% depending on the strains (*Saccharomyces*, *Lactobacillus*, *Bifidobacterium, Lactococcus* and *Streptococcus* spp.) and the simulated conditions (infant or adult conditions, different food intakes). In vitro results were consistent with in vivo data, showing the predictive value of the system.

P-149 **Bioavailability of phytoestrogens from soy and hops.** S. Bolca[a,b], S. Possemiers[a], A. Heyerick[b], D. De Keukeleire[b], H. Depypere[c], M. Bracke[d], I. Huybrechts[e], S. De Henauw[e], W. Verstraete[a] ([a] Laboratory of Microbial Ecology and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium, [b] Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium, [c] Department of Gynaecological Oncology, Ghent University Hospital, Belgium, [d] Departement of Radiotherapy and Clinical Oncology, Ghent University Hospital, Belgium, [e] Departement of Public Health, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium).

Phytoestrogens are plant constituents which possess pro- and antiestrogenic properties and are therefore considered to play a beneficial role in the prevention of hormone related disorders. Soy is the major dietary source of phytoestrogens and hops contain the most potent phytoestrogen. The microbial metabolism is important for the bioactivity of the ingested phytoestrogens. There is a large interindividual variation in the metabolism partially due to the interindividual differences in activity and composition of the gut microbiota. The factors influencing the microbial metabolism are still largely unknown. A randomized intervention study with 150 healthy menopausal women was undertaken. Exclusion criteria were hormone replacement therapy, allergy to soy products and the use of antibiotics up to 3 month before the study. After a 4-day washout period, all participants delivered a fecal sample for incubation purposes and microbiological characterization, a control urine sample, and filled a gas collection bag for methane analysis. Participants ingested a soy or hop extract or 250 mL soymilk 3 times a day for 5 consecutive days. A 24-h urine sample of the fifth day was collected. Subjects consumed their habitual diets but were asked to avoid all products based on soy or hops during the study. A food frequency questionnaire was used to elicit the usual fat and fiber consumption.

P-150 **Effects of a gluco-oligosaccharide on performance and gut microflora in weaned piglets.** M.L. Callegari[a], S. Soldi[a], F. Rossi[a], M. Morlacchini[a], P. Gatti[b], L. Morelli[a] ([a] Università

Cattolica del Sacro Cuore, Piacenza, Italy, [b] Roquette Italia, Alessandria, Italy).

One hundred twenty-eight weaned Duroc piglets (7.2 ± 1.04 kg) were fed for 77 days alternatively with: basal diet (CTR); basal diet supplemented with 2% Nutriose® (GOS); basal diet supplemented with chlortetracycline and spiramycine (1000 and 400 mg·kg^{-1} of feed, respectively), during the first 14 days of trial and then fed with basal diet (CTRM) or GOS diet (GOSM) until the end of the study. At day 0, 14, 35, 56 and 77 animals were weighed and feed conversion ratio (FCR) was determined. Nutriose® reduced the average daily gain in the first 35 d, but from 35th day animals fed GOSM grew faster than animals fed medicated diet. No differences were detected on FCR. The central aim of this study was to evaluate the impact of Nutriose®on the intestinal bacterial community of piglets using microbiological plate counts and a cultivation-independent molecular technique. No effect was detected on bifidobacteria and lactobacilli counts. The PCR-DGGE profiles, obtained with HDA1GC and HDA2 primers, of all piglets were compared and the differences of bacterial community fingerprints were evaluated based on the Dice coefficient. Analysis of profiles considering the presence or absence of bands or their relative intensity demonstrated that there were statistically significant differences between the four groups of animals. The most significant modification in the microflora composition concerned *Escherichia coli*, in fact we detected a quantitative reduction, confirmed by Real time PCR, of this organism in animals fed with prebiotic supplemented diet. These results indicated that a Nutriose® supplementation was able to reduce potential enteropathogenic bacteria.

P-151 **A combination of inulin and type 3 resistant starch alters bacterial fermentation characteristics in the rat cecum and colon.** D.J. Campbell[a], P.R. Heavy[a], F. Brouns[b], A. Adam[b], S. Silvi[c], C. Coudray[d], R.M. Hughes[a], I.R. Rowland[a] ([a] Northern Ireland Centre for food and Health, Centre for Molecular Biosciences, School of Biomedical Sciences, Northern Ireland, [b] Cerestar-Cargill Vilvoorde R&D Centre, Havenstraat 84, 1800 Vilvoorde, Belgium, [c] Dipartimento di Scienze Morfologiche e Biochimiche Comparate, Università degli Studi di Camerino, Camerino, Italy, [d] Centre de Recherche INRA Clermont-Ferrand/Theix, Saint-Genès-Champanelle, France).

Certain dietary resistant starches (RS) and other indigestible carbohydrates, such as fructans, are thought to benefit gut health by acting as substrates for bacterial fermentation leading to the production of short chain fatty acids (SCFA) in the large intestine. These SCFA, particularly butyrate, play an important physiological role in gut epithelial cell energy metabolism and are believed to act as chemoprotectants by suppressing the genesis and growth of tumour cells, in addition to maintaining gut barrier integrity. To date few studies have examined the effects of combining non-digestible carbohydrates on SCFA production in vivo. The purpose of the current study was to elucidate the interactive effects, of a fructan source, inulin (Fibruline Instant® Cosucra) and a type 3 RS, based on retrograded maltodextrins, (Actistar RM®) on SCFA profiles and associated parameters of gut health in the rat caecum and colon. Wistar rats (n = 48) were randomly assigned to one of four dietary regimes for a period of 90 days; a basal maintenance diet, inulin; RS3 or an inulin/ RS3 combination. The experimental diets resulted in higher total SCFA pools in both the rat caecum and colon compared with the control. Caecal pH was lower on the test diets and faecal and caecal bulk and wall weights were higher. Combining the Actistar® RS with inulin appeared to have a synergistic effect on butyrate production in the colon. In animals fed the combination diet, molar proportions of faecal butyrate were comparable with those found in the caecum, the primary site of bacterial fermentation in the rat. These findings suggest that the addition of RS3 to an inulin containing functional food, or Inulin to a diet high in RS, increases the amount of fermentation by butyrate producing bacteria in the colon and may have a beneficial effect on gut health.

P-152 **Fermented liquid feed and fermented cereal grains – microbial composition and effect on the gastrointestinal ecology of piglets.** N. Canibe, O. Højberg, B.B. Jensen (Danish Institute of Agricultural Sciences, Department of Animal Health, Welfare and Nutrition, Research Centre Foulum, PO Box 50, 8830 Tjele, Denmark).

Feeding fermented liquid feed and fermented cereal grains are strategies used to improve the gastrointestinal health of pigs. Fermented liquid feed decreases the counts of coliform bacteria and *Salmonella* along the gastrointestinal tract (GIT), decreases the incidence of swine dysentery, and delays the excretion of *L. intracellularis* in challenged pigs. Lactic acid bacteria are the dominating microorganisms in FLF and fermented grain, but knowledge on microbial composition to genus or species level is scarce. Three dietary treatments were designed: a weaner diet fed on as-is basis (DRY), fermented liquid cereal grain feed (FLG), and fermented liquid feed (FLF). The FLF diet was prepared by storing the feed and water in a closed tank at 20 °C, and the FLG diet by fermenting only the cereals and adding the remaining dietary ingredients immediately before feeding. On day 14 post-weaning, one piglet from each treatment was killed and samples collected from the GIT. Terminal restriction fragment length polymorphism (T-RFLP) analysis of the fermented diets showed a clear dominance of 598 bp fragments, representing e.g. *Lactobacillus pentosus, L. plantarum, L. paraplantarum, Lactococcus* sp., 604–605 bp fragments representing e.g. *L. sakei*, and 606–608 bp fragments representing *Pediococcus pentosaceus*. T-RFLP profiles of digesta from various segments of the GIT indicated that the lactic acid bacteria detected in the diets were also present in the GIT. Further differences between dietary groups in the T-RFLP profiles along the GIT were observed. These results are presented and discussed together with parameters describing the ecology of the GIT of the three dietary groups

P-153 **In vivo and in vitro characterisation of antibiotic safety and competitive exclusion properties of *Lactobacillus salivarius* and *Enterococcus faecium*.** A.J. Carter[a,b,d], R.M. La Ragione[a], V. Perreton[c], M. Adams[b], M.J. Woodward[a] ([a] Department of Food and Environmental Safety, Veterinary Laboratories Agency (Weybridge), New Haw, Addlestone, Surrey, KT15 3NB, UK, [b] School of Biomedical and Molecular Science, Food Safety Research Group, University of Surrey, Guildford Surrey, GU2 7XH, UK, [c] Institute of Veterinary Bacteriology, University of Berne, Langass-Strasse 122, Postfach, 3001 Bern, Switzerland, [d] Probiotics International Ltd, Stoke-sub-Hamdon, Somerset, TA14 6QE, UK).

Probiotic bacteria are thought to act as competitive exclusion agents against food borne pathogens such as *Salmonella enteritidis*. However, the exact mechanisms of action are still to be elucidated. Here we report phenotypic and genotypic characterisation of antibiotic resistance of *Lactobacillus salivarius* using minimum inhibitory concentrations and microarray analysis. In addition preliminary studies on the competitive exclusion of *Salmonella enteritidis* in chickens by a mono-culture of *Lactobacillus salivarius* or a mixed culture of *Enterococcus faecium* and *Lactobacillus salivarius* are discussed. Minimum inhibitory concentrations were used to determine the antibiotic resistance of *Lactobacillus salivarius* to a selection of 18 antibiotics. *Lactobacillus salivarius* was found to be resistant to vancomycin. However, previous reports have indicated that resistance to this antibiotic is intrinsic to *Lactobacillus salivarius* by a non transferable, chromosomal D-Ala:D-Lac ligase. The genome of *Lactobacillus salivarius* was also screened for transferable resistance genes using a Gram positive antibiotic microarray chip containing 90 antibiotic resistance genes. *Lactobacillus salivarius* did not harbour any of the 90 antibiotic resistance genes screened. To test the competitive exclusion properties of the probiotic test organisms, day old White Leghorn SPF (SPAFAS) chicks were inoculated by oral gavage with either ~ 5×10^8 *Lactobacillus salivarius* or ~ 3×10^8 of *Lactobacillus salivarius* and ~ 3×10^8 of *Enterococcus faecium*. On day 2 the birds were challenged with ~ 5×10^5 of *Salmonella enteritidis* by oral gavage. *Salmonella enteritidis* colonisation of the caeca, ileum and colon was evaluated by direct bacterial counts. Interestingly, in the group treated with the dual probiotic culture, colonisation of the caeca, ileum and colon by *Salmonella enteritidis* was reduced by approximately two logs on day 44, compared to the control group. Here we have demonstrated the lack of transferable antibiotic resistance and competitive exclusion properties of *Lactobacillus salivarius*.

P-154 **Fungal colonisation of alkali treated wheat straw in the rumen of sheep.** A.S. Chaudhry (School of Agriculture, Food and Rural Development, University of Newcastle-upon-Tyne, NE1 7RU, UK).

While anaerobic rumen fungi facilitate degradation of fibrous foods in ruminant animals, their efficacy is limited by the presence of cellulose-lignin bonds in foods such as wheat straw. Therefore, it would help if such bonds are modified in order to enhance the efficacy of rumen fungi in degrading and hence utilising wheat straw in sheep. This *in sacco* study examined the effect of alkali treatments (calcium oxide = CaO, sodium hydroxide = NaOH and alkaline hydrogen peroxide = AHP) on the fungal colonisation and hence utilisation of wheat straw when incubated in the rumen of sheep. Comparisons were made between treated and untreated straws to observe colonization microscopically using a trypan blue lactophenol staining technique to reveal the fungal thalli and rhizoids. Structural disintegration of un-incubated and rumen incubated straw particles following alkali treatments was also observed. AHP showed the greatest fragility to straw particles followed by NaOH and CaO treatments. The untreated straw revealed relatively less fungal colonization than the treated straws, which were heavily colonized by variable amounts of rhizoids and thalli. AHP treated straw showed most extensive colonization with the largest network of fungi containing branched and multi-directional rhizoids penetrating deep into the straw tissues, particularly the bundle sheath cells. The thalli of this study resembled mono- and poly-centric genera of anaerobic *Chytridiomycete* fungi. The extent and pattern of fungal colonization compared well with the previous degradability data on treated straws, demonstrating the importance of alkali treatments to improve fibre degradation by rumen micro-organisms, particularly fungi. The efficacy of rumen fungi to utilise fibrous foods can be enhanced if their cellulose-lignin bonds are modified by using alkali pre-treatments.

P-155 **The effect of two different types of arabinoxylanoligosaccharides on the level of human faecal bifidobacteria determined using real-time PCR.** L. Cloetens[a], V. De Preter[a], K. Swennen[b], T. Van de Wiele[c], W. Verstraete[c], W.F. Broekaert[b], C.M. Courtin[b], J.A. Delcour[b], P. Rutgeerts[a], K. Verbeke[a] ([a] Gastrointestinal Research, University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium, [b] Laboratory of Food Chemistry, KU Leuven, Leuven, Belgium, [c] Laboratory Microbial Ecology and Technology, Ghent University, Gent, Belgium).

Arabinoxylanoligosaccharides (AXOS) can be obtained by enzymatic hydrolysis of arabinoxylan and have been shown to exert prebiotic potential in animals. However, their physiological properties in humans have not yet been described. In this study, AXOS with an average degree of polymerisation (avDP) of 15 and an average degree of substitution (avDS) of 0.27 (AXOS-15-0.27), and AXOS with an avDP of 58 and an avDS of 0.58 (AXOS-58-0.58) were compared to investigate their impact on the level of bifidobacteria in human faecal samples. In a randomized cross-over study, 9 healthy volunteers were administered daily either AXOS-15-0.27 or AXOS-58-0.58 in a dose corresponding to 4.88 g arabinoxylan during 2 weeks and switched to the opposite treatment after a washout period of 2 weeks. Faecal samples were collected on the day before (baseline) and the day immediately after each intake period. The amount of bifidobacteria in the faecal samples was measured using real-time PCR (Van de Wiele T et al., 2004, FEMS Microbiol Ecol 51: 143–153). Results were expressed as log bifidobacteria/g faeces. The level of faecal bifidobacteria significantly increased ($P = 0.021$) after a 2-week intake period of AXOS-15-0.27 (7.55 ± 0.35) as compared to baseline (6.86 ± 0.63). In contrast, no differences were observed between baseline (6.94 ± 0.71) and a 2-week intake period of AXOS-58-0.58 (6.95 ± 1.05). These results demonstrate the favourable effects of AXOS-15-0.27 administration on bifidobacteria and indicate its prebiotic potential in humans, whereas AXOS-58-0.58 did not stimulate the bifidobacteria. The results further suggest that the structural conformation of the AXOS molecules influences their prebiotic potential.

P-156 **Molecular and functional characterisation of new *Lactobacillus* isolates from Bulgarian dairy products.** S.T. Danova[a], R.N. Aleksandrova[a], I.N. Iliev[b] ([a] Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. G. Bontchev str, 1113 Sofia, Bulgaria, [b] Plovdiv University, Tzar Assen str, 4000 Plovdiv, Bulgaria).

The new consumer's demand for more natural and minimally processed food has stimulated considerable interest for fermented products, containing living bacteria. Thus, intensive studies on the health promoting properties of Lactic

acid bacteria (LAB) have been established. We characterised LAB microflora of artisanal dairy products from different rural regions of Bulgaria. As a pre-selection procedure 39 strains, determined as mesophilic homo- and hetero-fermentative lactobacilli, were examined for antimicrobial activity. Despite the high percentage of activity against *Listeria*, no positive signal was obtained in Dot hybridization assays with a DIG-labelled DNA probe recognising the anti-*Listeria* motif for bacteriocins of class IIa. Selected active strains did not produce H_2O_2 and were moderate acidifiers in skim milk and MRS broth. They expressed different technologically important characteristics such as resistance to high concentrations of NaCl and to Nisin, along with some probiotic properties and long term viability during cold storage. Molecular characterisation of selected isolates was done according to polyphasic taxonomy. The species affiliation was confirmed by PCR using species-specific primers and the first discrimination was performed by RAPD analysis. Box and rep PCR were used as DNA fingerprinting techniques for the identification of active isolates at strain level. Selection and characterisation of new technologically relevant *Lactobacillus* strains, with potential to guarantee the quality and safety of food, is a necessary step in the development of probiotic foods with impact on consumer acceptance.

P-157 **Effect of dietary intervention with different pre- and probiotics on intestinal bacterial enzyme activities.** V. De Preter, L. Cloetens, E. Houben, P. Rutgeerts, K. Verbeke (Department of Gastrointestinal Research, University Hospital Gasthuisberg, KU Leuven, Herestraat 49, 3000 Leuven, Belgium).

One of the claimed beneficial effects of pre- and probiotics for the human host is, that these substrates are able to reduce the production of toxic and carcinogenic metabolites by suppressing specific enzyme activities in the colon. In the present study, the influence of long-term administration of different pre- and probiotics on the faecal β-glucuronidase and β-glucosidase activity was investigated. The effect was evaluated in a randomized, cross-over study in 50 healthy volunteers. At the start of the study and at the end of each 4-week treatment period the volunteers collected faeces during 72-h. Lactulose and oligofructose-enriched inulin (OF-IN) were chosen as prebiotic substrates, whereas *Lactobacillus casei* Shirota, *Bifidobacterium breve* and *Saccharomyces boulardii* were selected as probiotic strains. Two synbiotic combinations were evaluated as well (*Lactobacillus casei* Shirota + OF-IN and *Saccharomyces boulardii* + lactulose). The enzyme activity was assessed spectrophotometrically and the results were expressed as mg of substrate released/g faeces/h. Lactulose and OF-IN significantly decreased β-glucuronidase activity ($P = 0.001$ and $P < 0.001$, respectively), whereas no effect was observed on β-glucosidase activity. The addition of *L. casei* Shirota and *B. breve* to the diet resulted in a tendency to a decreased faecal bacterial β-glucuronidase activity. To the contrary, *B. breve* significantly increased β-glucosidase levels in the faeces ($P = 0.02$). Supplementation with the synbiotic did not appear to be more beneficial than either compound alone. No influence of *S. boulardii* on bacterial enzyme activity was noted. In conclusion, administration of lactulose, OF-IN, *L. casei* Shirota or *B. breve* resulted in a decrease in β-glucuronidase activity, which is considered beneficial for the host, because this enzyme can produce carcinogenic end-products.

P-158 **Effect of short- and long-term dietary intake of oligofructose-enriched inulin on the colonic fate of NH_3 in healthy volunteers.** V. De Preter, L. Cloetens, P. Rutgeerts, K. Verbeke (Department of Gastrointestinal Research, University Hospital Gasthuisberg, KU Leuven, Herestraat 49, 3000 Leuven, Belgium).

Oligofructose-enriched inulin (OF-IN) is a prebiotic which is able to modify the balance of the intestinal flora, thereby stimulating the growth and/or activity of beneficial bacteria such as bifidobacteria. In the present study, the influence of OF-IN on the absorption of NH_3 was evaluated. The biomarker lactose-[^{15}N, ^{15}N]-ureide (LU) was used as a source of $^{15}NH_3$ to study its metabolic fate (De Preter et al., 2004, Br J Nutr 92: 439–446). Before the start of the study and at the beginning and end of a 4-week treatment period, 19 healthy volunteers consumed a test meal labelled with 75 mg LU. During the study period, they received 10 g OF-IN (b.i.d.). After each test meal, urine (48 h) and faeces (72 h)

were collected and analysed for ^{15}N-content by combustion-IRMS. Results were expressed as % of administered dose. Short-term OF-IN intake resulted in a significant decrease of the urinary ^{15}N content ($P < 0.001$) and a concomitant increase in faecal ^{15}N as compared to baseline ($P < 0.001$). This was probably due to a stimulation of bacterial metabolism, since a significant increase in ^{15}N content in the bacterial fraction was found ($P = 0.001$). After long-term administration of OF-IN, a statistically significant reduction of the urinary ^{15}N content was found ($P < 0.001$) without a significant increase in the faecal ^{15}N content, resulting in an increased retention of the label in the colon. This might be explained by the hypothesis that OF-IN primarily stimulates the growth of mucosa-associated bacteria which might use the $^{15}NH_3$ for their growth or metabolism.

P-159 **Effect of short- and long-term administration of *Lactobacillus casei* Shirota, oligofructose-enriched inulin and their synbiotic combination on the colonic fate of ammonia.** V. De Preter, L. Cloetens, P. Rutgeerts, K. Verbeke (Department of Gastrointestinal Research, University Hospital Gasthuisberg, KU Leuven, Herestraat 49, 3000 Leuven, Belgium).

In the present study the effect of short- and long-term administration of *Lactobacillus casei* Shirota (LacS), oligofructose-enriched inulin (OF-IN) and their synbiotic combination on the colonic NH_3 metabolism was investigated by means of the biomarker lactose-[^{15}N, ^{15}N]-ureide (LU). A reduced urinary ^{15}N-excretion after intake of LU has been shown to reflect an improved removal of the potentially toxic NH_3 from the colonic lumen by stimulation of the bacterial metabolism (De Preter et al. 2004, Br J Nutr 92: 439–446). Therefore, 9 healthy volunteers received consecutively for 1 month 6.5×10^9 LacS (2×/d), 10 g OF-IN (2×/d) and the combination of both. Consecutive intake periods were separated by 2 weeks wash-out. Before the study, on the first day of each intake period (short-term effect) and after each period (long-term), the volunteers consumed a test meal labelled with LU and performed a fractionated 48-h urine collection. The ^{15}N-content of the urine was determined by combustion-IRMS. Both short- and long-term administration of OF-IN resulted in a significantly decreased urinary ^{15}N-excretion: from 51.75 (IQR 41.56–63.12) to 28.24 (IQR 24.01–30.97) ($P = 0.008$) for short-term and to 28.24 (IQR 24.01–30.97) ($P = 0.011$) for long-term intake. For LacS, only short-term administration resulted in a significant effect compared to baseline (51.75 (IQR 41.56–63.12) vs. 47.41 (IQR 27.00–56.05) ($P = 0.038$)), whereas a tendency to a decrease was observed after long-term intake. The synbiotic combination LacS + OF-IN resulted in a significant reduction of the cumulative excretion of the label compared to baseline with $P = 0.008$ for short-term and long-term synbiotic administration. In conclusion, dietary addition of LacS, OF-IN and their synbiotic combination results in a favourable effect on the colonic NH_3 metabolism.

P-160 **Effect of crude linseeds, extruded linseeds or linseed oil on digestion and fatty acid metabolism in dry cows.** M. Doreau[a], E. Aurousseau[a], S. Gachon[a], F. Glasser[a], J. Normand[b], G. Chesneau[c], C. Martin[a] ([a] INRA Theix, 63122 Saint-Genès-Champanelle France, [b] Institut de l'Élevage, 5 rue Hermann Frenkel, 69364 Lyon Cedex 07, France, [c] Valorex 35210 Combourtillé France).

Effects of fatty acids (FA) from linseeds on ruminal digestion and fatty acid metabolism were studied in dry cows fitted with ruminal and duodenal cannulas in a 4×4 Latin square design. Four diets based on maize silage (65%), hay (10%) and concentrates (25%) were compared: control diet (C); diet supplied with 7.5% crude rolled linseeds (RL), diet supplied with 7.5% extruded linseeds (EL); diet supplied with 2.6% linseed oil and 4.9% linseed meal (LO). These four diets did not differ in total OM and fibre digestibility, in OM ruminal and intestinal digestibility, and in duodenal N flow. Microbial N duodenal flow was lower for RL than for C diet. Volatile fatty acid concentration and pattern, and protozoa concentration in the rumen did not vary among diets. Ruminal biohydrogenation of linolenic acid did not significantly differ among diets (average 96.2%) whereas biohydrogenation of linoleic acid was higher for RL (94.5%) than for C (90.7%), EL (91.5%) and LO (92.6%) being intermediate. Trans 18:1 FA duodenal flows were higher for LO and EL than for RL and C diets (93.3, 82.4, 56.1 and 32.1 $g \cdot d^{-1}$,

respectively), this difference being due to *t*10 and/or *t*11 (coeluted) flows: 59.3, 51.6, 24.0, 18.2 $g \cdot d^{-1}$. The same result was observed for other intermediates of biohydrogenation: *c*9*t*11 CLA (0.6, 0.6, 0.2 and 0.2 $g \cdot d^{-1}$) and *t*11*c*15 18:2 (14.0, 12.5, 4.7, 1.5 $g \cdot d^{-1}$). No difference between RL, EL and LO diets was observed for *cis* 18:1 FA and for stearic acid.

P-161 **Effect of essential oils on degradation of starch-rich substrates in a rumen simulating fermenter (Rusitec).** S.M. Duval[a], R.J. Wallace[b], C.J. Newbold[a] ([a] Institute of Rural Sciences, University of Wales, Aberystwyth, SY23AL, UK, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

We have previously shown that a commercial blend of essential oil (EO) compounds was able to decrease the rate of degradation of starch-rich supplements in the rumen. Here we further investigate this phenomenon in the rumen simulating fermenter (Rusitec). Four vessels were used as controls fed daily with 14 g of a basal diet (80% hay, 10% soyabean meal, 10% molasses), 3 g of cut maize and 3 g of rolled barley and 4 were supplemented with 15 mg of EO (CRINA RUMINANTS®) equivalent to 0.02 $mg \cdot mL^{-1}$. To investigate the effect of EO on bacterial attachment, DNA was extracted from barley and maize incubated in the fermentor for 3, 6 and 15 h and used as a template for 16S rDNA PCR-DGGE. EO supplementation had no effect on the number of total, cellulolytic or amylolytic bacteria that could be cultured from the fermentor, nor did it affect VFA production or the degradation of the basal diet. However, pH tended to be higher in vessels supplemented with EO and ammonia production was significantly decreased. Degradation of barley (but not maize), after 48 h incubation, was reduced in the presence of EO and methane production was significantly decreased. Cluster analysis of the DGGE band profile showed that the EO had no detectable effect on attached populations with either substrate. Instead both incubation time and substrate appeared to be major factors affecting the banding pattern. These results support the idea that while EO can have dramatic effects on microbial activity in the rumen, the bacterial groups affected by EO may not be represented by the major bacterial groups as enumerated through culture counts or visualized by DGGE.

Study sponsored by CRINA S.A., 15, rue de la Combe, Gland, CH-1196, Switzerland

P-162 **Fermented feed for laying hens.** R.M. Engberg, N.F. Johansen, B.B. Jensen (Danish Institute of Agricultural Sciences, Research Centre Foulum, PO Box 50, 8830 Tjele, Denmark).

The influence of fermented liquid feed on the activity and composition of the gastrointestinal microflora was studied in an experiment with 480 layers (Babcock, 16 weeks old) housed in floor pens (30 animals/pen). From week 16 to week 38 during the laying period, the birds received an organic feed, which was either fed as dry mash (experimental group 1) or as wet feed (feed:water ratio, 1:1.4) following natural fermentation (experimental group 2). The fermented feed was characterised by a high lactic acid concentration (≈ 260 $mmol \cdot kg^{-1}$ feed) and moderate amounts of acetic acid (20–30 $mmol \cdot kg^{-1}$ feed), high numbers of lactic acid bacteria (10^9–10^{10}/g feed) and a pH of about 4.5. At 38 weeks of age, 5 birds of each floor pen were killed and the contents of crop, gizzard, ileum and caeca were separately collected and pooled. In crop contents, the pH was about 0.2 units lower in birds receiving fermented feed as compared to hens receiving dry feed (4.51 vs. 4.73). In the contents of ileum and caeca, the pH was higher (pH ileum, 7.4 vs. 7.2; pH caeca, 6.5 vs. 6.8), indicating a reduced microbial fermentation in the lower digestive tract following intake of fermented feed. In crop contents, fermented feed increased the number of lactic acid bacteria and reduced the numbers of enterococci. The counts of coliform bacteria were lower in the contents of crop, gizzard and ileum of hens fed with fermented feed. Considering coliform bacteria as indicator organisms for e.g. Salmonella, it can be concluded that fermented feed improves gastrointestinal health due to acidification of the upper digestive tract.

P-163 **Effect of a *Saccharomyces cerevisiae* supplementation on microbial profiles and fermentation patterns in faeces of horses under transportation.** C. Faubladier[a], F. Chaucheyras-Durand[b,c], L. Veiga[a,b], V. Julliand[a] ([a] ENESAD, Dijon, France, [b] Lallemand Animal

Nutrition, Blagnac, France, [c] Unit of Microbiology, INRA, Saint-Genès-Champanelle, France).

Transport is a stressful procedure for horses, which can disturb intestinal microbial communities as well as their environmental parameters and finally provoke digestive disorders like colic. Several studies on *Saccharomyces cerevisiae* have reported that a yeast supplementation could limit the imbalance of the microbial communities in horses, which would provide a potential interest in stressful situations. The aim of our work was to evaluate the effect of a yeast supplementation on the microbial communities and fermentation parameters in faeces of horses under transportation. Four mature geldings received daily a morning meal providing a minimum of 200 g/100 kg BW of starch without or with a supplementation of *S. cerevisiae* (2×10^{10} units), in a Latin square design. After fourteen days of adaptation to the diet, horses were transported at d0. Faecal content was collected at d –1, d0 (after transportation) and at d +3, 4 h after the morning meal, in order to count total anaerobic, cellulolytic, and lactic acid-utilizing bacteria, lactobacilli, and streptococci. Lactic acid, volatile fatty acids and pH were measured on faecal fluid samples collected at the same time. Microbial concentrations were statistically analysed using the GLM procedure of SASv8. The transport had an immediate impact on the concentration of the total anaerobic bacteria, which depended on the supplementation of the yeast. When S. *cerevisiae* was supplemented, the concentration of streptococci increased after the transport and the concentration of lactobacilli at d3 was changed. Further analyses are under process and more data will be reported.

P-164 **Capric acid concomitantly affects rumen methanogenesis and biohydrogenation of linoleic and linolenic acid.** V. Fievez[a], C. Boeckaert[a], G. Bruggeman[b], K. Deschepper[b] ([a] Laboratory of Animal Nutrition and Animal Product Quality, Ghent University, Belgium, [b] Nutrition Sciences n.v., Belgium).

Medium-chain fatty acids (MCFA) are efficient antimicrobial compounds, with lauric and myristic acid being effective in suppressing rumen methane production. Few studies using capric acid (CA), suggest several rumen bacteria also to be susceptible to inhibition by CA. During 24 h batch in vitro incubations with 50 mL buffered rumen fluid, three CA concentrations (0, 20 and 30 mg) were tested in combination with standard dairy concentrate (0.5 g), sunflower (10 mg) and linseed oil (10 mg). Both CA doses inhibited rumen CH_4 production by 85 and 28%, respectively, and equally suppressed rumen short-chain fatty acid (SCFA) production by 23%. Shifts in the rumen SCFA proportions were observed, with 30 mg CA mainly stimulating propionate (+64%) and suppressing butyrate (–50%) proportions, whereas 20 mg CA provoked a significant increase in butyrate (+35%) and decrease in acetate (–9%) proportions. CA significantly reduced apparent biohydrogenation of linoleic and linolenic acid (84.5, 76.6 and 73.8%, SEM 1.0%) and both CA doses equally inhibited further hydrogenation, provoking accumulation of C18:2 t11c15 (+391%) and C18:1t11+t10 (+142%) and reducing C18:0 (–29%). This study further illustrates that supplements inhibiting rumen methanogenesis concomitantly affect hydrogenating bacteria, as has been suggested earlier for fish oil, micro algae and monensin.

P-165 **Monitoring the bacterial population changes in the rumen of the camel associated with change in diet by real-time PCR.** M.B. Ghali[a], P.T. Scott[b], G.A. Alhadrami[c], R.A.M. Al Jassim[a] ([a] School of Animal Studies, The University of Queensland, Gatton, QLD 4343, Australia, [b] ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, St Lucia, QLD 4072, Australia, [c] UAE University, Al-Ain, UAE).

Rumen studies in the past focused on the diversity and characteristics of the different microbes inhabiting the rumen, mainly of cattle and sheep, while very few studies dealt with other ruminants such as camels. In an earlier report we established the presence of common rumen species including *Streptococcus bovis, Lachnospira pectinoschiza, Butyrivibrio fibrisolvens, Prevotella ruminicola* and *Selenomonas ruminantium*. In this study changes in the population of these bacterial species following changes of diet were monitored by real time PCR. Four rumen fistulated camels were fed Rhodes grass hay alone (R) or with barley grain (R+G, 40:60). The

experimental schedule involved 3 consecutive feeding periods each of 3 weeks duration. During the pre-treatment and post-treatment periods all camels were fed the R diet, while during the treatment period they were fed the R+G diet. Genomic DNA was extracted from rumen samples collected during day 8, 11, 18 and 21 at 0, 8 and 16 h after feeding of each period. All the bacterial species, except *S. ruminantium*, decreased in number ($P < 0.01$) following dietary change from R to R+G. *S. bovis, L. pectinoschiza, B. fibrisolvens* and *P. ruminicola* decreased 3, 2.1, 1.9 and 1.7 fold, respectively. In contrast, the population of *S. ruminantium* was increased by 4.2 fold during the same period. The bacterial population decreased further when the camels were shifted back to the R diet. Results indicate that *S. ruminantium*, which is a lactate utiliser, is the only bacterium to increase in number during the grain supplement period. The residual effect of the grain supplement needs further investigation.

P-166 **Post-weaning maturation of rabbit caecal microbial communities: impact of live yeast intake.** T. Gidenne[a], N. Bennegadi-Laurent[a], V. Monteils[b], G. Fonty[c] ([a] INRA Toulouse, SRC, BP 52627, 31326 Castanet-Tolosan, France, [b] ENSA Toulouse, BP 32607, 31326 Castanet-Tolosan, France, [c] INRA, C.R. Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France).

The balance of some caecal microbial populations was assessed in the young healthy rabbit, at weaning (28 d) and two weeks after (42 d), by dot-blot hybridization with phylogenetically targeted 16S rRNA oligonucleotide probes. Animals (doe and litters) were fed ad-libitum a control pelleted diet (group C) or the same diet containing 10^6 cfu of live yeast (Levucell SB®, Lallemand, group SB), from parturition to 70 d of age for youngs. Whatever the age or group, no response was observed with probes targeting rRNA of *E. coli*, anaerobic fungi, *Fibrobacter intestinalis* and *Fibrobacter succinogenes* (detection limit = 25 ng·μL^{-1} hybridised rRNA, i.e. ≈ 10^6 bact·g^{-1}). The total quantity of rRNA decreased from 28 to 42 d of age (394 vs. 241 μg, $P < 0.001$), originating from a decrease in bacterial rRNA (324 vs. 216 μg, $P < 0.001$) and in archaeal rRNA (62 vs. 24 μg, $P < 0.001$), without effect or interaction with dietary treatment. Identified bacteria represented about 50% of the total bacterial community. From 28 to 42 d, bacterial rRNA proportions increased from 84 to 90% ($P < 0.001$) while that of archaea decreased (15 vs. 10%), without an impact of the group effect. The *Flexibacter-Cytophaga-Bacteroides* group was prevalent and remained steady with age (41% of bacterial rRNA), as well as that of *R. flavefaciens* (0.8%), without an effect of yeast addition. The fact that, after weaning, the rRNA proportions of *R. albus* increased more in the SB group (0.3%) than in the C group (0.1%; $P < 0.01$) suggests the existence of a positive interaction between the yeast and this fibrolytic bacterial species.

P-167 **Digestive fate of *Saccharomyces cerevisiae* CBS 493 94, fed at 3 different concentrations to horses**. J. Gobert[a], G. Bertin[b], V. Julliand[a] ([a] ENESAD, 26 bd Dr Petitjean, BP 87 999, 21079 Dijon Cedex, France, [b] Alltech Regulatory Department, 14 place Marie-Jeanne Bassot, 92300 Levallois-Perret, France).

The digestive fate of live yeast, used as microbial additives in herbivores, has been studied in lambs but not in horses. The current recommendation is to supply live yeast each day to horses. This study was undertaken to evaluate and compare the appearance and disappearance kinetics of *Saccharomyces cerevisiae* CBS 493 94 (Sc) (Yea-Sacc 1026®) in the right ventral colon and in the faeces of horses. Eight adult geldings, allotted into homogeneous pairs based on weight, were fed a high fibre diet (35% straw/65% pellets) supplemented daily with Sc at 0 – 4.5×10^8 –9×10^8 or 4.5×10^9 cfu·kg^{-1} DM intake, according to a 4 × 4 Latin square design. Colonic contents and faeces were collected from horses at 0, 3, 6, 9, 12, 24, 36, 48, and 72 h after the first and the last meal supplemented with Sc and yeast concentrations were determined. Sc was detected 3 h and 9 h after the first supplemented meal, in the colon and in faeces, respectively. Peak concentrations were obtained between 3 h and 12 h in the colon (from 5.7×10^3 to 190×10^3 cfu·mL^{-1}) and at 24 h in the faeces (from 8×10^2 to 780×10^2 cfu·g^{-1}). First data for the disappearance kinetics confirm that Sc do not colonize the digestive tract, being no longer detected after 48 h in the colon and 72 h in the

faeces. Knowing the digestive fate of Sc in the colon will explain more precisely their action on the microbial communities. Furthermore, it will contribute to assessing the optimal daily dose of *Saccharomyces cerevisiae* CBS 493.94 for horses.

P-168 **Shifts of rumen microbial population detected by real-time PCR when methanogens are inhibited.** Y.Q. Guo[a], J.X. Liu[a], W.Y. Zhu[b] ([a] Institute of Dairy Science, Zhejiang University, Hangzhou 310029, PR, China, [b] Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, Nanjing 210095, PR China).

Ruminal microbiologists have long sought to develop strategies for the enteric methanogenesis especially within the rumen ecosystem. However, most methane-inhibiting strategies performed different in vivo and in vitro, and the underlying influence on the microbial flora is unclear as well. Real-time PCR was conducted to quantify the rumen microbes including methanogens, total bacteria, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and fungi in the rumen inoculum in vitro when methane synthesis was inhibited. The primer pairs of those microbes and PCR conditions referred to Denman and McSweeney's work. Bromoethanesulfonic acid (BES), a known inhibitor of methanogenesis, was selected as agent. The sodium salt of 2-BES added at a concentration of 5 mM inhibited methane production by 85.7% after 24 h incubation ($P < 0.01$). Total VFA concentration and molar proportions of acetate, propionate and butyrate were significantly affected by BES. Compared with the control, the molar proportion of acetate and the acetate-to-propionate ratio were reduced to 91.8% and 76.1% ($P < 0.01$), and the proportions of propionate and butyrate were increased by 20.9% and 15.0% ($P < 0.01$), respectively. With BES addition, production, utilization and recovery of hydrogen were significantly reduced ($P < 0.01$). The quantity of methanogens in relation to bacteria in BES-added fluid reduced to 10.4% of the value of control fluid ($P < 0.01$). When methanogens were inhibited, the rumen microbial fauna established a new balance to utilize hydrogen. After 24 h incubation, *Fibrobacter succinogenes*, one of the major bacteria for fiber degradation, was 50.0% higher in BES-treated fluid than control ($P < 0.01$). The growth of fungi relative to bacterial growth fell by 61.9% ($P < 0.01$) in comparison with the control. However, the BES did not significantly influence the total number of bacteria and the growth of *Ruminococcus flavefaciens*. In summary, addition of BES could change the rumen microflora and have a different influence on different microbes by inhibiting the methanogens.

P-169 **Effects of urea and controlled release non-protein nitrogen on ruminal fermentation and microbial protein synthesis in rumen-simulating cultures.** G.A. Harrison, J.M. Tricarico, K.A. Dawson (Alltech, Inc., Nicholasville, KY, USA).

Twelve rumen-simulating cultures were used to investigate the effects of two concentrations and two sources of non-protein nitrogen (NPN) on ruminal fermentation and microbial protein synthesis. Sources of NPN were either urea or Optigen$^{II®}$ (Alltech Inc., Nicholasville, KY, USA) and the concentrations tested were equivalent to 50 or 125 g NPN·day^{-1} at a dry matter intake of 22.7 kg·day^{-1}. Cultures were fed a 50:50 forage:concentrate diet twice daily for six days. All diets were isonitrogenous and treatments were applied in premixes. Samples were collected prior to morning feeding during the last 3 days of the experiment for pH, ammonia, VFA, DM disappearance, and microbial protein synthesis determinations. Data were analyzed as a completely randomized design and orthogonal contrasts used to test the effects of NPN source, NPN concentration, and their interaction. Flow rate differed ($P < 0.05$) among treatments and was used as a covariate for all analyses. Culture fluid pH, ammonia, and DM digestibility (apparent or true) were not affected by treatment. The concentration of NPN did not affect bacterial yield or efficiency of microbial protein synthesis. However, feeding Optigen$^{II®}$ increased ($P < 0.10$) bacterial yield (0.213 vs. 0.274 g for 50 g·d^{-1} urea or Optigen$^{II®}$; and 0.149 vs. 0.239 g for 125 g·d^{-1} urea or Optigen$^{II®}$) and improved ($P < 0.10$) the efficiency of microbial protein synthesis (17.2 vs. 20.1 g bacterial N·kg^{-1} DMTD for 50 g·d^{-1} urea or Optigen$^{II®}$; and 13.0 vs. 18.2 g bacterial N·kg^{-1} DMTD for 125 g·d^{-1} urea or Optigen$^{II®}$). Compared to urea, cultures fed diets containing Optigen$^{II®}$ had an average of

42% greater bacterial yield and 27% improved efficiency of microbial protein synthesis.

P-170 **Effect of allicin on fermentation and microbial populations in the rumen simulating fermentor Rusitec.** K.J. Hart, S.E. Girdwood, S. Taylor, D.R. Yáñez-Ruiz, C.J. Newbold (The Institute of Rural Sciences, University of Wales Aberystwyth, Aberystwyth, SY23 3AL, UK).

Garlic (*Allium sativum*) has been used as a medicinal herb in human and animal feeds for many years. However, there is a lack of detailed information on the effects of garlic and its components in such systems. Here an experiment is reported in which the effects of a commercial allicin extract (Neem Biotech), prepared from garlic, on fermentation in the rumen simulation technique (Rusitec) was investigated. Allicin was introduced to each vessel at 0, 3 or 30 mg·d^{-1} over a period of 17 d. Samples were taken for analysis over the last 5 d. There was no significant effect of allicin concentration on daily acetate, propionate, butyrate or ammonia production with mean values of 18, 7, 8 and 44 mmol·d^{-1}, respectively. However, there was a significant ($P < 0.05$) effect on daily methane production with mean values of 4.1, 3.0 and 0.2 mmol·d^{-1} (s.e.d. = 1.16) for 0, 3 and 30 mg allicin·d^{-1}, respectively. Microbial DNA was extracted from residual feed and vessel liquor homogenates, and amplified by qPCR using bacterial universal and methanogen specific primers. There was no significant difference of allicin concentration on the number of cycles to threshold (CT) for total bacteria with a mean value of 14.3 CT. However, there was a significant ($P < 0.05$) difference in CT for methanogenic archaea, with mean values of 22.1, 23.4 and 24.6 CT (s.e.d. = 0.70) for 0, 3 and 30 mg allicin·d^{-1} respectively. Preliminary results from this experiment have shown that allicin had no effect on fermentation parameters with the exception of methane production. It is concluded that allicin had a selective effect on reducing the number of methanogens but had no effect on the total bacterial population.

P-171 **Recovery and phylogenetic analysis of the fumarate reductase gene from the bovine rumen.** K. Hattori, H. Matsui (Faculty of Bioresources, Mie University, Tsu 514-8507, Japan).

Supplementation of fumarate is one of the ways to reduce methane emission from the rumen. Fumarate reducing bacteria utilize hydrogen to reduce fumarate and compete for hydrogen with methanogens in the rumen. Therefore, it is important to gain knowledge about fumarate reducing bacteria to enhance fumarate reduction. However, knowledge on the ecology of fumarate reducing bacteria in the rumen is limited. The fumarate reductase gene (*frdA*) can be a marker gene for fumarate reducing bacteria. In this study, we newly developed a set of specific primers that amplify the *frdA* fragment and examined the diversity of *frdA* in the rumen. A specific primer set for the fumarate reductase gene (*frdA*) was newly developed from selected *frdA* sequences. By using the primer set, a clone library was constructed from DNA extracted from cow rumen and phylogenetically analyzed. Thirty-one clones were obtained and analyzed for DNA sequences. Deduced amino acid sequences were subjected to homology search and phylogenetic analysis. Twenty sequences showed the highest homology to *frdA* of *Pasteurella multocida* at 78% identity and were grouped into a cluster on a phylogenetic tree. Seven sequences showed the highest homology to *Proteus vulgaris frdA* at 85% identity and were grouped into a cluster. The rest of the sequences showed homology to succinate dehydrogenase (*sdh*) and were clustered with *sdh* sequences of various bacteria. All *frdA*-like sequences obtained were distantly related to *frdA* sequences from known bacterial species. These results suggest the presence of uncultured fumarate reducing bacteria in the rumen.

P-172 ***Bellis perennis* tested as new antiprotozoal feed additive in vivo achieved a persistent partial defaunation in sheep.** E.M. Hoffmann[a], N. Selje[a], R.J. Wallace[b], K. Becker[a] ([a] University of Hohenheim, 70599 Stuttgart, Germany, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

The EU project Rumen-up aimed to identify natural plant additives which may replace growth promoting antibiotics in ruminant nutrition. In this context an antiprotozoal activity was demonstrated in vitro for *Bellis perennis* (daisy). To validate this observation in vivo, eight fistulated sheep were fed a control and a treatment diet

with 5% inclusion of grass meal or *Bellis perennis*, respectively, in a cross-over design over a total period of 60 days. Rumen fluid samples were withdrawn regularly and analysed for general fermentation parameters and protozoa counts. Effects observed in response to the additive were an increase in branched SCFA concentration (1.40 vs. 1.50 mM, $P = 0.024$) and a trend to a higher concentration of soluble protein (0.35 vs. 0.40 mg·mL^{-1}, $P = 0.098$). Total SCFA concentration (97.1 vs. 94.9 mM) and ammonium concentration (14.3 vs. 15.2 mM) were not different ($P > 0.1$). Protozoal numbers showed a daily fluctuation pattern in response to feeding and high variation within and between the animals. Nevertheless, *Bellis perennis* significantly reduced the numbers of protozoa (2.20 vs. 1.76 × 10^6·mL^{-1}, $P = 0.031$), irrespective of the sequence in which the diets were fed. This corresponds to a partial defaunation of 20%. As daisy was shown to contain saponins, these are likely to be the defaunating agent. In contrast to other published reports, where antiprotozoal effects of plant derived saponins were only transitory, the daisy effect persisted in vivo for more than 8 weeks.

P-173 **Coarse structured feed stimulates members of the genera *Lactobacillus* and *Mitsuokella* as well as propionate and butyrate producers in the pig stomach.** O. Højberg, L.L. Mikkelsen, B.B. Jensen (Danish Institute of Agricultural Sciences, Research Centre Foulum, 8830 Tjele, Denmark).

Feeding coarse structured diets has been shown to stimulate bacterial production of lactate, butyrate and propionate in the pig stomach and thereby reinforce the stomach as a barrier against spreading of pathogens and zoonoses to distal parts of the gastrointestinal tract. However, detailed information on the influence on the microbial community is still scarce. Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis involving multiple restriction enzymes was used to investigate the effect of diet on bacterial community structure in gastrointestinal samples collected from twenty-four crossbreed Danish Landrace × Yorkshire pigs fed either a fine non-pelleted (FNP), fine pelleted (FP), coarse non-pelleted (CNP) or coarse pelleted (CP) diet. A strong dietary influence on bacterial community structure was observed in the pig stomachs, whereas the effect was less pronounced in the ileum, cecum and colon. Pigs fed the CNP diet had the highest microbial diversity in the stomach as revealed by a higher number of terminal-restriction fragments (T-RFs). By comparison to 16S rRNA gene sequences obtained from a pig bacteria culture collection and GenBank, the phylogenetic inference of the T-RFLP analysis suggested that phylotypes tentatively identified as *Lactobacillus delbrueckii*, *L. mucosae*, *L. reuteri*, *L. amylovorus*, *Mitsuokella jalaludinii*, *Megasphera elsdenii* (butyrate producer) and *Selenomonas ruminantium* (propionate producer) were stimulated in the stomach of pigs fed the CNP diet. T-RFLP analysis of isolates from the pig bacteria culture collection showed good agreement between predicted and observed T-RFs. These results further verified the tentative identifications of the T-RFs from stomach and demonstrated that dominating members of the microbiota could be distinguished by using multiple restriction enzymes.

P-174 **Fatty acid composition of rumen protozoa: a link with chloroplast ingestion?** S.A. Huws, E.J. Kim, M.R.F. Lee, A.K. Kingston-Smith, N.D. Scollan (Institute of Grassland and Environmental Research (IGER), Plas Gogerddan, Aberystwyth SY23 3EB, UK).

Recent data show that rumen protozoa are rich in beneficial polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), compared to rumen bacteria. Evidence suggests that certain rumen protozoa are apt at ingesting PUFA-rich plant chloroplasts, and we hypothesise that this may contribute towards their higher PUFA content. To evaluate this hypothesis steers fitted with rumen cannulae were offered either hay (low chloroplast content) or fresh grass (high chloroplast content) for 2 week periods, before collecting liquid and solid associated bacteria (LAB and SAB, respectively) and protozoal (LAP) fractions at various time intervals pre- and post-feeding. Fatty acid data revealed that the protozoa contained *c.* × 2–3 more C18:0 (stearic acid), C18:3 *n*-3 (α-linolenic acid), and C18:1 *trans*-11 (TVA) than LAB and SAB populations at all time intervals and under both feeding regimes. The protozoa also contained *c.* × 2–3 more α-linolenic acid at all time intervals when fed fresh grass as opposed to hay. Protozoal

chlorophyll content was also *c.* × 2–3 higher, at all time intervals, on the grass compared to hay diet. Fresh grass is known to contain ca. 55% of total fatty acid in the form of α-linolenic acid, thus it is unsurprising that the main increases in protozoal PUFA content under a fresh grass diet occurred with α-linolenic acid. The increases in α-linolenic acid along with concomitant increases in protozoal chlorophyll content provides evidence that rumen protozoa may be rich in PUFA due to the ingestion of chloroplasts. Confocal microscopy also revealed that *Epidinium* spp. were saturated with seemingly intact, intracellular chloroplasts as opposed to the other species which contained very few (< 5). This evidence supports the hypothesis that ingestion of chloroplasts by protozoa increases their PUFA content. The co-interactions of the rumen ciliates with chloroplast are now the focus of further investigation.

P-175 **Effect of forage type and level of fish oil inclusion on bacterial diversity in the rumen.** S.A. Huws[a], M.R.F. Lee[a], S. Muetzel[b], R.J. Wallace[b], N.D. Scollan[a] ([a] Institute of Grassland and Environmental Research (IGER), Plas Gogerddan, Aberystwyth SY23 3EB, UK, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

The fatty acid composition of ruminant products is of particular concern due to the link between fatty acid composition of dietary fat and cardiovascular disease. Ruminant products such as milk and meat are often criticised for high proportions of saturated fatty acids (SFA) compared to polyunsaturated fatty acids (PUFA). This largely reflects the extensive biohydrogenation of dietary PUFA in the rumen by specific bacteria. Much attention is now focused on enhancing our understanding of the microbial species involved in biohydrogenation, with a view to establishing methods of reducing this process. Feeding red clover and fish oil in the ruminant diet has been demonstrated to decrease biohydrogenation in the rumen. It is uncertain if this relates to changes in the bacterial community. Eight steers prepared with ruminal and duodenal cannulae were offered either red clover or grass silage with 0, 1, 2, and 3% fish oil in a Latin square 3-period changeover design. Following 2 weeks on each diet, ruminal digesta samples were collected and processed into liquid and solid associated fractions (LAB and SAB, respectively). Bacterial PCR-DGGE was then performed on 16S rRNA sequences amplified from these samples. Dendograms revealed that the profiles for both LAB and SAB clustered distinctly due to the type of silage fed, and that, within these diet-dependent clusters, sub-clusters were evident according to the fish oil concentration. It is evident that feeding red clover caused differences in the rumen bacterial populations in comparison to feeding grass silage. Fish oil inclusion also caused a shift in the rumen bacterial diversity. We now intend to quantify the bacteria that we currently know are involved in the biohydrogenation pathway using qPCR.

P-176 **Monitoring of bioactive peptides isolated from artisanal Balkan yogurts.** I. Ivanova[a], I. Tzvetkova[a], N. Tzekova[a], M. Dalgalarrondo[b], T. Haertle[b] ([a] Biological Faculty, Sofia University, 8 Dr Tzankov, bd 1164 Sofia, Bulgaria, [b] INRA-LEIMA, 44316 Nantes Cedex 3, France).

The bioactivities of peptides encrypted in major milk proteins are latent until released and activated by enzymatic proteolysis, e.g. during gastrointestinal digestion or food processing. 21 LAB strains isolated from the three different types of Balkan artisanal yogurts cow, sheep and buffalo, were used. Strains isolated from artisanal fermented dairy products represent particular interest as a source of new potential starters for application in the food industry. The present work quantifies the various products of LAB fermentations relative to the inhibition of *St. aureus* and *E. coli* and evaluated the interaction between the isolated LAB and food pathogens in spoiled milk products. The proteolytic activity was evaluated for α-caseins and whey proteins by reverse phase chromatography, PAGE and anti-microbial assays. A food derived bioactive peptide obtained upon the action of an artisanal strain of *Lb. delbrueckii* on α-casein is claimed to be a health-enhancing component which could be used for functional foods and pharmaceutical preparations.

P-177 **Characterization and possible probiotic influence of *Bacillus smithii* strain LTN074 in a murine model.** E. Jõgi, I. Suitso, E. Talpsep, P. Naaber, A. Nurk (Institute of

Technology University of Tartu Nooruse St 1, 50411 Tartu, Estonia, United Laboratories of Tartu, University Clinics, Puusepa 1a, 50406, Tartu, Estonia).

Bacillus sp. spores based probiotics are widespread, especially in South East Asia, but the prophylactic effect of this kind of probiotics is still not very clear. The purpose of the present study was to test survival of *Bacillus smithii* strain LTN074 in the gastrointestinal environment of mice and hamsters as well as its influence on infection caused by invasive *Salmonella enterica* serotype Enteritidis and *Clostridium difficile*. Our strain *Bacillus smithii* LTN074 was isolated from human faeces. Typical *Bacillus smithii* strains are thermophilic lactic acid bacteria usually found as contaminants in the food industry. Due to its flexible metabolism (thermophilic and aerotolerant growth ability) it is quite easy to detect and differentiate this strain from the other gut habitants. We have tested the influence of this strain against two enteropathogens, *Clostridium difficile* and *Salmonella enterica* serotype Enteritidis, in a murine model. We have also combined antibiotics and other metabolically active substances to test the probiotic influence of our strain. We used the mouse and hamster model to determine the colonisation rate of *B. smithii* and possible prevention of pseudomembranous colitis and salmonellosis. *B. smithii* LTN074 was shown to persist in the gastrointestinal tract of mouse and hamster.

P-178 **Changes in rumen pH and population density of *Streptococcus bovis* in sheep during dietary transition.** F.M. Jones[a], N.D. Costa[a], K.R. Guest[b], P.E. Vercoe[b], R.H. Jacob[c], J.M. Snowden[c], A-D.G. Wright[d] ([a] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia, [b] The University of Western Australia, Nedlands, WA, 6009, Australia, [c] Department of Agriculture, South Perth, WA 6151, Australia, [d] CSIRO Livestock Industries, Floreat, WA 6014, Australia).

Changes in pH and the relative population density of lactic acid producing bacteria, such as *Streptococcus bovis*, have not been well documented for ruminants during transition onto grain-based diets. Real time quantitative PCR (RT qPCR) was used to quantify the relative proportion of *S. bovis* in rumen samples from sheep fitted with rumen cannulas. These sheep were fed diets based entirely on either lucerne hay (*Medicago sativa*), yellow lupins (*Lupinus luteus*), soy beans (*Glycine hispida*) or white lupins (*Lupinus angustifolius*) at maintenance (M) ME or at 3 × M. The change in proportion of *S. bovis* (as cells·mL^{-1}) after the first 24 h on feed was 3900-fold in sheep fed 3 × M, 1300-fold in sheep fed soybeans, but only 50-fold on the lucerne diet. At these times, rumen pH decreased to 6.05 in sheep fed 3 × M white lupins, and to 6.3 on the lucerne diet, but rumen pH in the sheep fed soybeans barely changed to 7.02. Thus the changes in the proportion of *S. bovis* were not related to the pH in the rumen environment. Moreover, lupins contain β-glucans, and are thought to decrease the predisposition to acidosis associated with the rapidly fermentable starch in cereal grains. These findings call into question the sequence of events leading to large increases in *S. bovis*, and the precise relationship between increases in *S. bovis* and changes in pH.

P-179 **Determination of the effective dose of *Saccharomyces cerevisiae* CBS 493.94 used as microbial additive for horses.** J.-P. Jouany[a], B. Medina[b,c], V. Julliand[b], G. Bertin[c] ([a] INRA, Centre de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France, [b] ENESAD, 26 bd Dr Petijean, 21000 Dijon, France, [c] Alltech Regulatory Department, 14 place Marie-Jeanne Bassot, 92300 Levallois-Perret, France).

Live yeasts, which are actually recommended as microbial additive for herbivores such as ruminants and horses, do not grow in the digestive tract and have to be supplied continuously to the host. The objective of the present work was to assess the optimal daily dose of *Saccharomyces cerevisiae* CBS 493.94 (Sc) (Yea-Sacc® 1026) for horses. Eight adult geldings weighing 383 ± 9 kg were fed twice a day 670 g DM 100 kg^{-1} BW of a pelleted diet of dehydrated lucerne (55%), wheat bran (25.5%), barley (13%), molasses (2.5%) and a mineral-vitamin mixture (3.5%). Long wheat straw was given on a daily basis of 340 g DM 100 kg^{-1} BW in one meal. Animals, allotted into homogeneous pairs based on weight, were assigned to a 4 × 4 Latin square design, fed twice a day with 4 doses of Sc (0;

4.1×10^9; 8.3×10^9; 1.65×10^{10} cfu·kg^{-1} feed DM) top-dressed on the diet. The experiment lasted 170 days planned over 4 periods of adaptation (21 days), measurements (7 days) and wash out period (15 days). Digestibility of DM, OM and fibre was determined based on a total feces collection. The supplementation of Sc regardless of the dose had no significant effect ($P > 0.10$) on feed intake, animal weight or feces dry matter. Positive and significant effects were observed on digestibilities of dry (+5%) and organic matter (+3.5%) ($P < 0.10$), and on digestibilities of NDF (+8%) and ADF (+12%) ($P < 0.05$) for the two doses of 8.3×10^9 and 1.65×10^{10} cfu·kg^{-1} feed DM, which were not different. No effect was noted when Sc were fed at the dose 4.1×10^9 cfu·kg^{-1} feed DM. These results indicate that the minimum effective dose of *Saccharomyces cerevisiae* CBS 493.94 for horses weighing less than 400 kg is 8.3×10^9 cfu·kg^{-1} feed DM. Doubling of the minimal dose had no effect on the measured digestive parameters.

P-180 **Dietary essential oil supplementation enhanced intestinal immunocompetence in young broiler chicks.** H. Kettunen[a], A. Ouwehand[a], H. Schulze[b], N. Rautonen[a] ([a] Enteromix® Research, Danisco Innovation, Kantvik, Finland, [b] Danisco Animal Nutrition, Leiden, The Netherlands).

Essential oils (EOs) may act as antimicrobials, antioxidants, and immune enhancers in animals. Thus, EOs may support gut health of farm animals in antibiotic-free nutrition programs. The concentration of immunoglobulin A (IgA) in the gut lumen can be used as a measure of the intestinal immunocompetence. We studied the effect of EOs on the performance and intestinal IgA in male Ross 508 broiler chicks. Newly hatched chicks were allocated to six feeding treatments (12 pens per treatment; 30 chicks per pen). Wheat-based mash diet without antibiotics, coccidiostats, or enzymes was supplemented with five different EO blends. Chick performance was measured for days 1–21 and 21–42. On days 21 and 42, we sampled the ileal and caecal contents (six replicate pools of two chicks per diet). Ileal and caecal IgA was measured from the control and two EO blend treatments ("low EO level" and "high EO level") by ELISA. Compared with the control group, the "low EO level" diet significantly improved weight gain and FCR, but did not affect the ileal or caecal IgA at either time point. The "high EO level" diet increased the IgA concentration on day 21 ($P = 0.009$ for caecum, $P = 0.051$ for ileum), and showed a non-significant trend for improved performance at both time points. The IgA concentrations increased from day 21 to 42. On day 42, all the dietary treatments showed similar IgA concentrations. The results suggest that dietary essential oil supplementation may improve intestinal immunocompetence and performance of young broiler chicks, and that the effect is likely dependent on the quality and quantity of essential oils.

P-181 **Probiotics in treatment of patients with Crohn's disease and ulcerative colitis.** P. Kohout[a], D. Tuèek[b], V. Zboøil[c], Z. Beneš[a] ([a] IInd Department Internal Medicine, Teaching Thomayer's Hospital, Prague, Czech Republic, [b] Department of Metabolic Care, Charles University Hospital, Hradec Králové, Czech Republic, [c] IIIth Department Internal Medicine, Teaching Hospital Brno, Czech Republic).

Abstract withdrawn.

P-182 **The influence of dairy season on the safety of sheep milk.** M. Ladyková (Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Slovak Republic).

The aim of this research was to determine the influence of the dairy season on sheep milk safety. Bulk samples of sheep milk were collected from May to September 2004 from the middle part of Slovak Republic. The analysis involved eight samples from each factory in various stages of the dairy season. Milk analyses included determination of composition, technological quality, hygienic and microbial quality. The average value of somatic cell counts (SCC) was ranging from 67 750 mL^{-1} at the beginning of the dairy season to 416 125 mL^{-1} at the end of the dairy season, and the difference was significant ($P < 0.01$). The total bacteria count (TBC) in milk samples ranged from 1.47×10^6 cfu·mL^{-1} (beginning of dairy season) to 9.55×10^5 cfu·mL^{-1} (end of dairy season). A

similar tendency of lower numbers was found for coliform bacterial counts, with an average value at the beginning of dairy season of 1.01×10^6 cfu·mL^{-1} and at the end of dairy season 1.72×10^4 cfu·mL^{-1}. The average value for psychrotrophic bacteria was from 9.18×10^5 cfu·mL^{-1} (beginning of dairy season) to 8.23×10^6 cfu·mL^{-1} (end of dairy season).

P-183 **Diet modulates resilience of the gut ecosystem in human microbiota associated rats.** F. Levenez, V. Rochet, M. Sutren, S. Droineau, A. Foussié, P. Guillaume, S. Rabot, M. Leclerc, J. Doré (INRA-UEPSD, 78350 Jouy-en-Josas, France).

Human gut microbiota appears essentially unique, specific to the host and stable over time at the level of dominant species. It is resilient upon antibiotic treatment in human volunteers. In the present study we investigated the impact of minor perturbations and diet on resilience of the gut ecosystem. Sixteen human microbiota associated rats received a stable diet (basal or inulin 10%) throughout the study (84 d) or a diet with minor perturbations (basal versus inulin diet alternated every 2 d) followed by stable diet after the major perturbation (single dose of 200 mg·kg^{-1} amoxicillin and clavulanic acid, administered to each animal on day 32). Dominant diversity of microbiota was followed using TTGE and its composition using FISH. The microbiota was affected by antibiotic treatment within one day and tended to return to its initial structure within 1–2 d (dominant diversity) to up to 24 d (composition). Enterobacteria were dramatically increased one day after antibiotic treatment, and the increase was statistically significant for 3 d. Firmicutes (*C. coccoides* group) were markedly decreased for 10 d while *Bacteroides* were initially decreased and thereafter increased until day 24. In the absence of minor perturbations, resilience was not affected by the diet. With minor perturbations before antibiotic treatment, resilience was affected by the diet such that inulin promoted a significantly faster return to initial dominant diversity (1 d) and to Enterobacteria initial levels. Effects of dietary perturbations observed on microbiota composition using FISH depended on diet and microbial group. Our results indicate that inulin favors resilience of the gut microbiota after antibiotic treatment. Functional foods should be further investigated in their ability to modulate resilience of the gut ecosystem upon stress.

P-184 **Dietary carbohydrate source determines molecular fingerprints of the rat fecal microbiota.** T.R. Licht, M. Hansen, M. Poulsen, L.O. Dragsted (Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, 2860 Søborg, Denmark).

A study was designed to elucidate effects of selected carbohydrates with different digestibility on the intestinal physiology and microbiota. Five groups of eight rats were fed a western type diet containing cornstarch (reference group), sucrose, potato starch, Raftiline® (a long-chained fructan) or Raftilose® (a short-chained fructan). The animals fed the diets containing potato starch, Raftiline and Raftilose had a significantly higher caecal weight and lower caecal pH when compared to the reference group, indicating increased fermentation. Selective cultivation from faeces revealed a higher amount of *Lactobacillus* spp. in these animals. Additionally, the fructan-fed groups had a lower amount of coliform bacteria in faeces. In the Raftiline- and Raftilose-fed groups, higher levels of butyrate and propionate, respectively, were measured. Principal Component Analysis of profiles of the fecal microbiota obtained by Denaturing Gradient Gel Electrophoresis (DGGE) of PCR amplified bacterial 16S rRNA genes as well as of Reverse Transcriptase-PCR amplified bacterial rRNA revealed a different microbiota in each of the five animal groups. Comparison of DNA-based and RNA-based profiles revealed that 2 species within the phylum Bacteroidetes had a particularly high ribosome content in the animals fed with Raftiline, indicating that growth of these species was specifically stimulated by this particular fructan.

P-185 **Effect of gas pressure and buffer composition on in vitro ruminal fermentation.** D. Macheboeuf, B. Lassalas, R. Bergeault, D.P. Morgavi (INRA CR de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France).

In vitro fermentation is a rapid and efficient way to evaluate ruminant's feedstuffs. However, comparison among published works is difficult because of variations in the methodology. The

most common variations are buffer composition and whether the produced gas is vented or not. The aim of this study was to assess the effect of a buffer developed for protozoa and the effect of gas pressure build-up in an in vitro batch system. The buffers tested were: (protozoa) Simplex medium (SIM) (carbonate/phosphate ratio 1.52 mol·mol^{-1}) from Coleman and Goering-Van Soest medium (GVS, carbonate/phosphate ratio 5.45 mol·mol^{-1}). Fermentation vials contained 25 mL buffer, 15 mL rumen fluid and 400 mg of substrate composed of corn, soja bean meal, and alfalfa hay. Incubation was for 16 h at 39 °C under N_2 gas. At 5h-incubation, after measuring gas production, half of the bottles were depressurized to atmospheric pressure. Depressurization had no significant effect on fermentation parameters and there was no interaction between pressure and media, although hydrogen utilization was slightly higher with high pressure. GVS incubations produced more gas (10.7 vs. 9.7 mmol·g^{-1} DM, $P < 0.01$) and methane (3.0 vs. 2.78 mmol·g^{-1} DM, $P < 0.05$) than SIM, while no differences were observed on fatty acid production. In addition, the chemical CO_2 production and hydrogen utilization were lower ($P < 0.01$) with SIM. These differences could be explained by a more efficient hydrogen fixation in SIM due to its higher phosphate concentration. The dry matter digestibility was higher with GVS (+3.5 points, $P = 0.011$) and the pH lower (6.18 vs. 6.30, $P < 0.01$). GVS buffer seems more appropriate than SIM for routine evaluation of feedstuffs for ruminants.

P-186 **Dose-response effect of diallyl disulfide on ruminal fermentation and methane production in vitro.** D. Macheboeuf[a], B. Lassalas[a], M.J. Ranilla[b], M.D. Carro[b], D.P. Morgavi[a] ([a] INRA-Theix, 63122 Saint-Genès-Champanelle, France, [b] Producción Animal I, Universidad de León, 24071 León, Spain).

Ruminal methanogenesis is an inefficient process resulting in losses of energy intake. The aim of this study was to assess in vitro the dose effect of diallyl disulfide, a compound present in garlic essential oil, on ruminal fermentation, with an emphasis on methane production. Ruminal contents collected from sheep fed concentrate:alfalfa hay (40:60) diet were incubated at 39 °C in buffer for 16 h with a mixed substrate. Diallyl was added at concentrations of 0, 0.5, 1, 1.5, 2, 2.5, and 4 mM in the batch mixture. Treatments were in triplicate. Diallyl, at the lower dose used, drastically decreased the amount of methane produced by 90% as compared to 0 control ($P < 0.001$). Methane proportion of the total gas produced fell from 28% in control to 3.4% with a dose of 0.5 mM. This was associated with an increase in the hydrogen ratio in the gas phase from 0 to 15.3% ($P < 0.001$). Total gas production, although also significantly reduced, was less affected by the additive (from –18 to –31% compared to control). Total VFA production was also linearly reduced from –33 down to –55% for the lower and higher dose, respectively ($P < 0.001$). Acetate was the most affected, with a fall of 50% at 0.5 mM, while butyrate decreased from –8 up to –17% for the 0.5 and 2.5 mM dose, respectively. In contrast, propionate production was not different from the control up to the 2.5 mM concentration. Interestingly, dry matter digestibility was not affected with 0.5 mM and then decreased with dose from 7 to 12% ($P < 0.01$). Diallyl ability to reduce methane production merits further studies.

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P-187 **Combination of feed additives to meet production and environmental targets in ruminants – in vitro optimization.** D. Macheboeuf[a], M.J. Ranilla[b], M.D. Carro[b], M.L. Tejido[b], B. Lassalas[a], D.P. Morgavi[a] ([a] INRA-Theix, 63122 Saint-Genès-Champanelle, France, [b] Producción Animal I, Universidad de León, 24071 León, Spain).

Feed additives for ruminants, normally used to improve production, could also serve to reduce the emission of environmental pollutants like methane and ammonia. However, the mode of action of individual additives often limits their effect to a particular objective. The aim of this study was to optimize a mixture of additives that act concurrently on several fermentation parameters. Three essential oils (diallyl disulfide (DIA), cinnamaldehyde (CIN), carvacrol (CAR)) and 2 organic acids (disodium malate (MAL) and fumarate (FUM)) were tested alone or in combination using a simplex centroid experimental design. The vertices of simplex were one compound alone (coded as i) at the maximum dose (coded as 1) and the other points in the design

were blends of compounds ($1 < i \leq 5$, $0 < \text{dose}(i) < 1$, $\Sigma\text{dose}(i) = 1$). The additive mixtures were added to buffered rumen fluid from sheep containing a 50:50 forage:concentrate substrate and incubated in vitro for 16 h with 3 repetitions in time. Fermentation variables were compared to control without additives and fitted to quadratic or special cubic models; R^2 for methane, ammonia, and volatile fatty acids (VFA) were 0.91, 0.54, and 0.58, respectively. When specifications were targeted to one parameter, like reducing methane, ammonia, or maximizing VFA production, the major compound of the blend was DIA, CIN, and MAL-FUM, respectively. When specifications were to keep dry matter digestibility and VFA production at control levels, but reducing methane and ammonia, the model suggested a mixture (0.60 MAL, 0.32 CIN, 0.02 CAR, 0.07 DIA) that decreased methane (–20%), ammonia (–15%) and the C2/C3-ratio (–30%). Selected combinations of additives can be tailored to a specific type of production or ration as well as to reduce pollutant emissions.

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P-188 **The influence of chestnut tannins on growth and enzymatic activities of selected rumen bacteria.** R. Marinšek-Logar, T. Čepeljnik (University of Ljubljana, Biotechnical Faculty, Zootechnical Department, Domzale, Slovenia).

During the last years there has been a general interest recognised in tannin use in animal nutrition, including ruminants. Tannins can improve the general performance of ruminants through their influence on rumen microflora. They interact with rumen bacteria in different ways: they inhibit bacterial growth, enzymatic activities and attachment to substrates, and inhibit certain pathogenic bacteria and methanogenesis in the rumen. As tannins are a broad group of chemically diverse polyphenolic plant compounds, and their influences are strongly dependent on the nature of the compound and the nature of bacterial carbohydrates and proteins in interaction with it, research is needed on defined bacterial species and defined tannin products. The aim of this study was to follow the effects of chestnut hydrolysable tannins of the commercial product Farmatan® on four selected rumen bacterial species: highly active fiber degrader *Pseudobutyrivibrio xylanivorans* Mz5 and very abundant starch and protein degrading rumen bacteria *Prevotella bryantii* $B_1 4$, *Streptococcus bovis* DSMO 4551 and *Selenomonas ruminantium* DSMO 2150 – the two bacteria that are involved in rumen acidosis. The effects of different concentrations of Farmatan® in the anaerobic bacterial growth medium (0.00, 0.05, 0.25 and 1.00 $g \cdot L^{-1}$) on bacterial growth and enzymatic activities (xylanolytic, amilolytic, cellulolytic and proteolytic) compared to negative controls were followed after 48 h incubation at 37 °C. Statistically significant effects of chestnut tannins were detected at concentrations higher than 0.25 g tannins$\cdot L^{-1}$, which is the concentration of tannins in the cow rumen when applied at recommended doses. The growth of all four investigated bacteria is slightly inhibited at concentrations higher than 0.25. The most profound effect of tannins was found on the amylolytic activity in *P. bryantii* and *S. bovis*. The results suggest that the application of chestnut tannins does not effect the normal ruminal bacteria too much, but it does hinder the fermentation of starch in *S. bovis*, which may be useful in mitigation or prevention of rumen acidosis.

P-189 **Effect of probiotics, prebiotics and synbiotics as functional food supplements on gut microflora of rats.** L. Markiewicz, A. Majkowska, M. Bielecka (Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, ul. Tuwima 10, 10-747 Olsztyn, Poland).

The impact of functional food supplements on the complex intestinal microecosystem is still not enough recognised. The aim of the study was to assess quantitative and qualitative changes in the selected groups of colonic microflora of rats administered with probiotics, prebiotic and synbiotics. The research was carried out on Wistar rats (8 groups, 8 rats in each) administered with: potentially probiotic strains of *Lactobacillus acidophilus* BS and/or *Bifidobacterium animalis* 30, commercial preparation of Raftilose Synergy 1 (OF) (ORAFTI, Belgium) as a prebiotic, and their combinations as synbiotics. The active bacterial cultures were given per os ~ 10^9 cells/rat$\cdot$day^{-1}; 5% OF was added to the diet. The control group was fed with standard diet. After 35 days, selected groups of bacteria were

examined quantitatively and qualitatively using selective cultivation and PCR-DGGE methods, respectively. In the groups of rats receiving *Lactobacillus* and/or *Bifidobacterium* strains, population counts of these genera increased. The respective DGGE patterns showed the presence of both administered strains in the rat colon, with *B. animalis* 30 as a dominant strain. Probiotics neither affected the eubacteria and *Clostridium coccoides* group DGGE patterns nor the population numbers of other colonic bacteria determined. The microflora of animals administered with prebiotic and synbiotics was characterised by significantly higher counts and a more diverse pattern of the *Bifidobacterium* population, whilst the *Lactobacillus* count was stable in spite of inhibition of some of its subpopulation. Moreover, as DGGE profiles showed, prebiotic and synbiotics stimulated some subgroups of eubacteria and *C. coccoides* group, simultaneously inhibiting other subgroups of *C. coccoides*. The impact of synbiotics on the *Lactobacillus* population was rather individual-dependent. The further studies on combined supplements will be continued, as their impact on the microflora may be a sum of their separate effects.

P-190 **Effects of non-antibiotic additives on the microbial equilibrium of broiler chicken intestine.** B. Massias[a], M. Arturo-Schaan[b], A.-M. Elie[a], K. Bebin[b], V. Hocde[c], M. Denayrolles[a], M.C. Urdaci[a] ([a] Enitab, LMBA 1, Cours du Général de Gaulle, 33170 Gradignan, France, [b] CCPA, Z.A. Nord Est du Bois de Teillay, 35150 Janzé, France, [c] Deltavit, France).

Sub-therapeutic doses of antibiotics have been used systematically in the poultry industry in order to promote growth and fight avian pathogens, thus improving productivity. A rise in bacterial resistance has constrained European authorities to ban this practice. The search for substitutes has, therefore, become a priority, because animal health and development is strictly dependent on gastrointestinal microflora. The influence of three feed additives on broiler chicken intestinal microbiota were investigated: Fructooligosaccharidic (sc FOS) prebiotic (additive I) and two plant aromatic essential-oil extracts (additives II and III). Thirty subjects per treatment were sacrificed at 21 and 35 days and samples were collected from intestinal and caecal contents. Samples were cultured by classical methods in MRS medium for LAB isolation and colonies were subsequently sequenced for identification of 16S rDNA. In parallel, a DGGE fingerprinting method was applied to characterize the predominant bacterial populations. Electrophoresis of amplified fragments of 16S rDNA using *Lactobacillus* genus-specific or universal eubacterial primers produced feed additive-specific patterns. Dendrometric analyses of gel profiles and sequencing of DGGE bands permitted identification of bacteria whose presence was modulated by the different additives. Approximately twenty different bacteria in four principal genera were identified: *Bacteroides, Clostridium, Lactobacillus,* and *Ruminococcus.* Variations in bacteria were observed according to age and intestinal compartment. Additives I and II gave the greatest variations especially within the *Lactobacillus* population. Compared to FOS, the tested essential-oil extracts also modulated the gut microflora and so may be interesting as growth promoters in poultry.

P-191 **Role of protozoa and antiprotozoal agents on CLA biohydrogenation in mixed ruminal microorganisms.** N. McKain, R.J. Wallace (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Conjugated linoleic acids (CLA) are health-promoting 18:2 fatty acids which are formed as intermediates during the biohydrogenation of dietary linoleic acid (C18:2, *cis*-9, *cis*-12) to stearic acid (C18:0) in the rumen. A range of materials was investigated for their ability to inhibit CLA breakdown and thereby enhance the flow of CLA from the rumen and into ruminant products, including meat and dairy products. Foliage from *Sesbania sesban*, a leguminous subtropical forage tree, was the most potent inhibitor of CLA breakdown. As *S. sesban* had been previously shown to be antiprotozoal by virtue of its saponin content, other antiprotozoal plants and saponins were investigated for their ability to inhibit CLA biohydrogenation. *Lonicera japonica* (Japanese honeysuckle) decreased the rate of CLA metabolism by 50% in a 3-h incubation with strained ruminal digesta. The inhibition fell to only 20% if the protozoa were removed by slow speed centrifugation. *L. japonica* had little effect on CLA metabolism when the experiments were repeated with digesta from defaunated sheep. The experiments

were repeated with the saponin, glycyrrhizic acid (GA), and its corresponding sapogenin, glycyrrhetinic acid (GTA). In faunated sheep, GA decreased the rate of CLA metabolism by 50% in a 3-h incubation with strained ruminal digesta, whereas the GTA, which as a sapogenin has no antiprotozoal activity, had no effect. There was no effect of GA or GTA if the protozoa were removed by centrifugation. No significant inhibition occurred in digesta from defaunated sheep. Thus, the interaction between antiprotozoal agents and ciliate protozoa leads to an inhibition of biohydrogenation of CLA in ruminal digesta, possibly via materials released from protozoa by lysis.

P-192 **Survivability of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in the human gastrointestinal tract.** M.L. Michaylova, Zh.P. Dimitrov, L. Vlachkova, P. Petrova, (LB Bulgaricum R&D Center, 12A Malashevska Str., Sofia 1202, Bulgaria).

Twelve healthy volunteers consumed over ten days their usual diet supplemented with 250 g buffalo's yogurt. The yogurt was administrated twice a day (2 × 125 g). *L. bulgaricus* and *S. thermophilus* had been isolated from *Crepis biennis linei,* showing high β-galactosidase activity and demonstrating a positive influence on the fecal microflora and metabolites in above human volunteer's tests. Survivability of *L. bulgaricus* (5.9 ± 0.7 log cfu·g^{-1} fecal samples) was registered. The strain identity was confirmed by strain specific oligonucleotide DNA marker. Survivability of *S. thermophilus* was not registered. This result supports our observations from preliminary studies to determine the effect of milk base (sheep's or buffalo's or cow's) and the strain specificity for the survivability of yogurt bacteria in the gastrointestinal tract.

P-193 **Effects of probiotics on body weight gain, body height, diarrhea occurrence and health condition of newborn calves.** M.R. Mokhber-Dezfouli, P. Tajik (Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, PO Box 14155-6453, Tehran, Iran).

The effects of probiotic administration were studied on 120 newborn calves. Sixty calves were randomly assigned as treatment group and the probiotic was added in their daily milk intake. Each received 0.25 g probiotics until 90 d of age. After the first week, all calves (including control group) received starter ration containing 21.0% crude protein and 3.0% crude fat. Body weight gain, body height and general health condition of all calves were observed at d 30, 60 and 90. The condition of feces was also examined daily and the occurrence of diarrhea was recorded throughout the experiment. Mean values of weight gain during three successive months for treatment and control groups were 57.52 and 50.58 kg, respectively. Body weight gained was not significantly different for the first and second month between treatment and control groups (16.9 and 33.87 vs. 14.49 and 33.07 for first and second month in treatment and control groups, respectively). However, these values were significantly different ($P < 0.001$) between treatment (57.52) and control (50.58) groups at three months of age. Diarrhea was observed in 35 calves of the control group, which was higher than 11 cases in calves treated with probiotic ($P < 0.001$). The body height values of control and treatment groups in three successive months were 5.49, 10.82 and 15.00 cm for the control and 5.44, 9.25 and 15.75 cm for the treatment group in the first, second and third month respectively, which showed no significant difference between the two groups during this study. The results of this study indicate that the probiotic compound has beneficial effects, especially on the third month of age in rearing calves.

P-194 **Behaviour of live yeast Biosaf® Sc 47 during digestive transit in dairy cows.** V. Monteils[a], L. Cauquil[b], E. Auclair[c], C. Bayourthe[a] ([a] ENSAT Ave de l'Agrobiopole, BP 32067, 31326 Castanet Tolosan cedex, France, [b] INRA Toulouse, SRC, BP 52627, 31326 Castanet-Tolosan, France, [c] LFA, 1 rue du Haut Touquet, 59520 Marquette-Lez-Lille, France).

The knowledge of the behaviour of live yeasts (LY) in the ruminant digestive tract (DT) was assessed by counting the number of viable LY in the rumen, the ileum and the faeces of fistulated dry cows after a single dose (20 g containing 8×10^9 cfu·g^{-1}) along with their morning meal or after repeated daily doses along with the meal for 3 consecutive days. The transit of LY

through the DT was monitored for 4 and 5 days, respectively. The number of viable LY in digesta was determined by the method for counting the cfu on YM agar containing 1% chloramphenicol. LY appeared at a high level of 1.4×10^5 cfu per mL rumen fluid, 5.3×10^5 cfu per mL ileal content and 1.4×10^6 cfu per g in faeces at 2, 4 and 16 h after administration of a single dose, i.e. 82, 91 and 98% of the dose initially distributed. LY were then gradually eliminated. The number decreased more slowly in the faeces than in the rumen to a negligible amount of 10^2 cells per g after 96 h. When supplementation was repeated daily, LY persisted over 24 h in the rumen at a level equal to 68% of the dose administered. In the faeces, a concentration of 12×10^2 cfu per g was found after 128 h. The results indicate that ingested Biosaf® Sc 47 cannot colonize the rumen and are not entirely killed under the specific conditions of the DT.

P-195 **Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates.** P. Mosoni, F. Chaucheyras-Durand, C. Béra-Maillet, E. Forano (Unité de Microbiologie, INRA Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France).

The supplementation of forage diets with readily fermentable carbohydrates is known to depress ruminal fiber degradation. The most likely explanation for this observation is a decrease in the number of the cellulolytic flora and/or of their fibrolytic activity. The aim of this study was to examine the effect of concentrate supplementation to a forage diet on the number of three rumen cellulolytic bacterial species. Rumen contents were collected from six sheep one hour before feeding with hay and one hour before and three hours after feeding with a hay plus concentrate (50/50) diet. The pH of the rumen contents was monitored on both diets. *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* were quantified by real-time PCR (SYBR Green, LightCycler) from total DNA extracted from the rumen contents using species-specific primers. External standard curves were performed with the 16S rDNA gene of each species (PCR amplified and serial diluted from 10^2 to 10^8 copies) and used for absolute quantification. The addition of 50% concentrate in the diet of sheep resulted in a decrease (1 Log) of the three cellulolytic species just after feeding concommittent with rumen acidification, but this effect was erased at the pre-prandial state with rumen contents close to neutral pH and a number of cellulolytic bacteria similar to that observed on a hay diet.

P-196 **Molecular analysis of the intestinal microbiota of weaning piglets fed with diets supplemented with xylo-oligosaccharides.** P. Moura[a], F. Simões[a], S. Marques[a], L. Alves[a], F. Gírio[a], J.P.B. Freire[b], M.P. Esteves[a] ([a] INETI, Department Biotechnology, Estrada Paço Lumiar, 22, 1649-038 Lisboa, Portugal, [b] ISA, Department Agricultural and Animal Production, Tapada da Ajuda, 1349-017 Lisboa, Portugal).

The effects of xylo-oligosaccharides (XOS) obtained by autohydrolysis of corn cobs, on the modulation of piglet intestinal microbiota were investigated. Experimental diets supplemented with 2% of XOS-rich hydrolysates, with a commercial probiotic, or with both XOS and probiotic, were fed for 4 weeks to 24 weaning piglets (Exp. I) and for 2 weeks to 24 piglets submitted to ileo-rectal anastomosis (Exp. II). Samples from the ileum, caecum and distal colon (Exp. I) and jejunum and ileum (Exp. II) were used for characterisation of the intestinal microbiota by ERIC PCR fingerprinting and analysis of the *Lactobacillus* population using group-specific primers. ERIC PCR profiles from Experiment I and from piglets after weaning (t_0) were compared with ERIC PCR fingerprints of reference strains. The dendrogram obtained revealed a major cluster grouping all the samples from t_0, bifidobacteria, lactobacilli, and most of the samples from RB+XOS and RB+XOS+probiotic. In partial comparisons from each experimental diet, the similarity level between the cluster of bifidobacteria and lactobacilli and the cluster grouping most of the samples from RB, RB+XOS, RB+probiotic and RB+XOS+probiotic was 28, 71, 43 and 54%, respectively. With *Lactobacillus* group-specific primers, the highest detection limits were obtained with samples from the colon of animals fed with XOS. In Experiment II, the diets including XOS produced an increased positive PCR signal in samples from the ileum, as determined by the use of

Lactobacillus group-specific primers and serial dilutions of total DNA. ERIC PCR profiles from Experiment II originated three main clusters, but only one sample from the ileum of a RB+XOS fed piglet was clustered within the group of bifidobacteria and lactobacilli.

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P-197 **Evaluation of a natural dietary strategy for controlling the severity of pathogenic protozoal infections in the gastrointestinal tract.** L. Nollet, R. Murphy (Alltech European Biosciences Centre, Dunboyne, Ireland).

Recent legislation in Europe has led to restrictions on the use of certain pharmaceutical products for the control of protozoal diseases in poultry feeds. As a result, there is an increased emphasis on new nutritional strategies that can address the production problems associated with such diseases. A series of studies evaluated the effects of new feeding strategies that use a natural blended yeast feed ingredient, Natustat^{TM}, on two protozoal diseases in poultry. In *Eimeria* challenge studies in broilers, the weight gain and feed conversion in birds fed Natustat^{TM} (1.925 kg·T^{-1}) was greater (4% and 5%, respectively) than that of the birds receiving an unsupplemented control diet. This performance was comparable to that of birds receiving a Salinomycin (66 ppm) supplemented diet. Birds receiving Natustat^{TM} also had less severe lesions in upper and caecal regions. A trial comparing benefits of Natustat^{TM} supplementation on the improved efficacy of a coccidiosis vaccination (Coccivac B) demonstrated that birds fed Natustat^{TM} had significantly better FCR and lower gut lesion scores compared to the challenged control. In turkey trials using a mild histomoniasis ("Blackhead") challenge, the benefits of Natustat^{TM} at 1.925 kg·T^{-1} was compared to an unchallenged control group and a group of birds receiving the antiprotozoal drug, Histostat® (0.1875 kg·T^{-1}). The results indicate that turkey poults receiving Natustat^{TM} had weight gains and feed conversion ratios which were not significantly different from the unchallenged control birds while caecal and liver lesion were the least severe of all challenged birds. The results of these studies suggest that Natustat^{TM} may be useful as part of a non-pharmaceutical strategy to reduce the risk and severity of intestinal protozoal diseases in poultry.

P-198 **DNA stability and transformability of feed derived DNA in the gastrointestinal tract of rats.** L. Nordgård[a,b], T. Traavik[a], K.M. Nielsen[a,b] ([a] Norwegian Institute of Gene Ecology, Science Park, N9294 Tromsø, Norway, [b] Department of Pharmacy, Faculty of Medicine, University of Tromsø, N9037 Tromsø, Norway).

Considerable amounts of foreign DNA are introduced into the gastrointestinal (GI) tract with food ingestion. Studies have shown that minor proportions of this DNA survive the passage through the GI tract and that it can be absorbed to reach white blood cells, the spleen and the liver, or be present in feces (Schubbert et al., 1994). Furthermore, several bacterial species that are present in the GI tract are known to be competent for natural transformation (Lorenz and Wackernagel, 1994), suggesting that some bacteria can take up food-ingested DNA through natural transformation. The use of antibiotic resistance genes as marker genes in genetically modified organisms has put forward the question whether such antibiotic resistance genes can transform bacteria present in the GI tract in vivo. The aims of this study were to describe the degradation dynamics of food-ingested DNA (chromosomal DNA and DNA present in bacterial lysates) in the GI tract of rats and to determine if this ingested DNA still remains able to transform the naturally competent bacterium *Acinetobacter baylyi* strain BD413 (*AcBD413*). We detected that the rat feed given does not degrade DNA completely, and that this DNA still remains biologically active after being exposed to the rat feed for up to 72 h. We are currently characterizing the degradation dynamics and biological activity of foreign DNA through the GI tract of rats using molecular methods such as PCR, hybridizations and natural transformation assays and the possibility of in vivo transformation of *AcBD413* to occur in gnotobiotic mice.

P-199 **The effect of *L. plantarum* and *L. fermentum* on the microflora in the digestive system of calves.** V. Oberauskas, A. Sederevicius,

R. Zelvyte, R. Sutkeviciene, I. Monkeviciene (Lithuanian Veterinary Academy, Department of Anatomy and Physiology, Tilzes 18, 47181 Kaunas, Lithuania).

High productivity of animals greatly depends on calves' digestive system and its development during the first month after birth. Neonate calves during the period of micro-ecological system formation tend to be especially sensitive to various factors. Therefore, it seems especially important to control the microbiological composition in the calves. Probiotic preparations seem to be a promising means in this case. The preventive dose of a lyophilized preparation containing *L. plantarum* U-14 no less than 1×10^9 cfu·g^{-1}, and *L. fermentum* U-5 – no less than 1×10^8 cfu·g^{-1}, for calves is 4 g daily when given with colostrum or milk. The preventive dose of lyophilized preparation increased the daily weight gain of calves by 16.2% and decreased the signs of diarrhea by 50%. According to the results of one-way analysis of variance the lyophilized preparation effected the total count of *Lactobacillus* in calves' faeces by 7%, by 13.2% for *L. plantarum* and by 11.4% for *L. fermentum,* but had no effect on the number of *L. salivarius* and *L. acidophilus*. The lyophilized preparation had no effect on the total count of enterobacteria. The probiotic preparation was produced from local *Lactobacillus plantarum* U-14 and *Lactobacillus fermentum* U-5 strains and it is intended for the prevention of the digestive diseases of neonate calves, correction of the digestive system microflora and calves weight gain.

P-200 **Ruminal acetogens in dairy cows: dietary effects and quantification of *E. limosum*.** K. Olsson, P. Evans, K.N. Joblin (Rumen Biotechnology, Grasslands Research Institute, AgResearch, PB 11008, Palmerston North, New Zealand).

The ruminal acetogenic bacteria with their capacity to convert H_2 and CO_2 into acetate have the potential to act as H_2 sinks in the rumen. Management of H_2 is a key to controlling ruminant methane emissions. To determine whether ruminal acetogens in grazing livestock can be influenced by diet or diet change, rumen contents from rumen-fistulated dairy cows on three diets were collected and acetogen populations quantified. The successive diet changes involved grazing ryegrass/clover pasture, feeding lucerne (*Medicago sativa*) silage, feeding silage containing 25% grain, and a return to grazing ryegrass/clover pasture. Acetogen population densities in freshly-collected samples were measured by anaerobic culturing under a H_2/CO_2 headspace in a rumen-fluid medium containing BES to inhibit methanogens. Acetogen-positive cultures were identified as those showing H_2 utilisation. The mean ruminal acetogen density found in grazing animals did not change significantly when the diet changed to silage. However, acetogen density increased markedly (3-fold) when the diet contained grain. To obtain further information from samples, a real-time PCR method for quantifying *Eubacterium limosum*, a common ruminal acetogen, was developed. The method is based upon the 16S rRNA gene of *E. limosum* and a self–quenched fluorogenic (LUX^{TM}) primer set. LUX primers were designed, PCR optimised and primers successfully tested for specificity against genomic DNA prepared from 3 strains of *E. limosum*, 5 species of non-target ruminal acetogens, and a range of ruminal bacteria, methanogens, and fungi. At the time of writing, the method has been tested on one of the enumerated samples and showed that *E. limosum* is a predominant acetogen.

P-201 **The effect of yeast on *Listeria innocua* survival in the digestive tract of sheep.** A.M. Olvera-Ramírez[a], N.R. McEwan[a], F.M. McIntosh[b], C.J. Newbold[a] ([a] Institute of Rural Sciences, University of Wales, Llanbadarn Campus, Aberystwyth SY23 3AL UK, [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

The use of yeast to stimulate ruminant production is well established, with increases in productivity reported in both growing and lactating animals. Previously, based on in vitro experiments, we have suggested yeast might also help control pathogenic bacteria within the digestive tract of ruminants. Now we present results showing in sheep supplemented with 5 g·d^{-1} of Procreatin 7 and challenged with the pathogen *Listeria innocua,* that the flow of pathogen from the rumen decreased by 55% (4.0 vs. 1.7×10^8/d). This decreased flow of pathogens was associated with more than doubling of the total bacterial population (8.2 vs. 20.8×10^8·mL^{-1}) and an even greater increase in the numbers of

cellulolytic bacteria (1.5 vs. $9.5 \times 10^7 \cdot mL^{-1}$) that could be recovered from the rumen of sheep fed yeast. The distribution of bacterial species as indicated by 16S rDNA PCR-DGGE also shifted in response to yeast addition. However, despite a decrease in the flow of pathogens leaving the rumen, yeast had no effect on pathogen shedding in faeces (1.5 v 2.4×10^7/d).

Study sponsored by Lesaffre Feed Additives, Km. 57.5 Carretera Mexico-Toluca, CP 50200 Toluca, Edo. de Mexico, Mexico.

P-202 **In vitro activity of essential oils towards intestinal microbes.** A.C. Ouwehand[a], H. Kettunen[a], H. Schulze[b], N. Rautonen[a] ([a] Enteromix® Research, Danisco Innovation, 02460 Kantvik, Finland, [b] Danisco Animal Nutrition, 2300 AE Leiden, NL).

Essential oils have long been known to exert antimicrobial activity and have been suggested to be a reason why certain spices contribute to the preservation of foods. Hence, their effect on food pathogens has been widely investigated. However, the influence essential oils have on members of the intestinal microbiota has received much less attention. We investigated the effect of 10 nature-identical essential oils and 3 natural extracts on the in vitro growth of members of the intestinal microbiota. The essential oils were tested at three different concentrations; 5, 50 and 500 $\mu g \cdot mL^{-1}$. Growth was followed under anaerobic conditions at 37 ºC by automated measurement of absorbance at 600 nm. The effects of nature-identical essential oils on inhibiting growth of *E. coli*, *Clostridium perfringens* or *Salmonella* varied with source and inclusion level. Beneficial microbes such as lactobacilli and bifidobacteria were found to be less sensitive towards nature identical essential oils; inhibition occurred only at levels of 500 $\mu g \cdot mL^{-1}$. The studied natural extracts were found to be highly antimicrobial. *Salmonella*, *E. coli* and *C. perfringens* were sensitive at levels of 5–50 $\mu g \cdot mL^{-1}$, while lactobacilli and bifidobacteria were less sensitive at levels of 50–500 $\mu g \cdot mL^{-1}$. All tested strains, except *Salmonella*, were sensitive to the tested antibiotic, avilomycin. The observations indicate that selected nature-identical essential oils and natural extracts can exert a strong antimicrobial effect against potential pathogenic members of the intestinal microbiota. Due to their lower sensitivity, beneficial members of the microbiota will be spared, giving an opportunity to improve the composition of the intestinal microbiota. This would be a major benefit over antibiotics that often negatively affect the beneficial members of the intestinal microbiota.

P-203 **Functionality of the pig gastrointestinal microbiota in response to alternatives for in-feed antimicrobials.** O. Pérez, W. Pellikaan, M. Verstegen, H. Smidt, W.M. De Vos (Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT, Wageningen, The Netherlands).

Throughout Europe it is normal commercial practice that pigs are weaned at an earlier age than under natural conditions. This causes gastrointestinal disturbances and an increased susceptibility to infection, resulting in economic losses to the pig industry. Currently applied control measures mostly rely on prophylactic antibiotics in feeds. This practice, however, is going to be either banned or significantly reduced due to the risk of antibiotic resistance for both animal and human health. As a result, interest is focusing on alternative ways to stimulate and stabilize the autochthonous gastro-intestinal microbiota. In the framework of the EU-funded project "FeedForPigHealth", the current study aims to detect, localize and quantify key microbial species, their metabolic activity and interaction with the host, as affected by PENS (plant extracts and other natural substances) supplemented to diets, in health and disease. To this end, integrated cultivation-based and direct molecular approaches, targeting phylogenetic as well as functional catabolic marker sequences, are used, and combined with analyses of the digesta for microbial metabolites. Faecal and ileal digesta samples from piglets receiving a standardized reference diet were evaluated by molecular fingerprinting of the overall bacterial and *Lactobacillus* communities, focusing on the effect of a large variety of plant-derived additives on community dynamics during in vitro fermentation assays. In general, incubation with the different substrates (i.e. SBP, chyme and starch) lead to substrate-specific shifts in community profiles, while most of the non-fermentable additives had no significant effect on microbiota composition. Further studies are currently underway to assess the

effect of selected PENS on microbiota composition and activity in vivo.

P-204 **Yeast cell wall preparations prevent the attachment of enteropathogenic *Escherichia coli* on broiler gut mucus.** S. Peuranen[a], A. Kocher[b], K.A. Dawson[b] ([a] Alimetrics Ltd, Höyläämötie 14, FIN-00380 Helsinki, Finland, [b] Alltech Biotechnology Centre, Sarney, Summerhill Road, Dunboyne, Co. Meath, Ireland).

The association of *E. coli* with mucus is considered to initiate bacterial overgrowth in the intestine, leading in the worst case to intestinal or even systemic infection. The specific adherence reaction is mediated by adhesins on the surface of *E. coli* and receptors on the mucus surface of the host intestine. In theory, the adherence can then be prevented by providing adhesin or receptor analogues in feed. An in vitro attachment model with isolated broiler mucus was used to study the efficacy of a commercial yeast cell wall preparation Bio-Mos® (BM, Alltech Inc.) to prevent bacterial attachment. Three different enteropathogenic isolates of *Escherichia coli* were allowed to attach to broiler mucus, and the attachment with and without BM (1 or 2 $g \cdot kg^{-1}$) was quantitatively measured after labelling the bacterial cells with radioactive thymidine. The incubation order of bacteria and BM in the mucus-coated microtiter wells was varied to illustrate the interactions between mucus, bacteria and yeast cell walls. Despite the fact that bird age and gut section affected the efficacy of BM, and that the three bacterial strains had different absolute attachment efficiency to broiler mucus, Bio-Mos showed consistent effect against the attachment of *E. coli*. Its efficacy was as good as the positive control, mannose (1 $g \cdot kg^{-1}$). In most cases the higher inclusion level of BM resulted in better attachment inhibition. At the higher inclusion level, BM was capable of detaching some of the previously attached bacteria from mucus. These observations substantiate further studies on the mode of action of yeast cell wall carbohydrates in the interaction between bacteria and gut mucins.

P-205 **Effect of human milk on the growth of bifidobacteria: stimulation of *Bifidobacterium bifidum,* inhibition of *Bifidobacterium animalis.*** V. Rada, J. Nevoral, E. Vlková, I. Trojanová, J. Killer Jiří (Department of Microbiology, Nutrition and Dietetics, Czech University of Agriculture Prague, Pediatric Clinic of Charles University, Prague – Motol, Czech Republic).

The growth of four strains of bifidobacteria in five different samples of human milk was tested. Two strains of *B. bifidum* were isolated from infant faeces. Two strains of *B. animalis* were isolated from fermented milk products. All strains were identified using biochemical tests followed by species-specific PCR. The faecal flora of infants of milk donors was also analysed using cultivation methods and FISH. Three infants had high numbers of bifidobacteria (>10 log $cfu \cdot g^{-1}$) in their faeces. The remaining two infants did not contain detectable amounts of bifidobacteria in their faecal samples. Good growth of *B. bifidum* (both strains) in human milk was accompanied with a decrease of pH (up to 4.4) and production of lactic acid (up to 2 $g \cdot L^{-1}$). On the other hand, numbers of viable cells of *B. animalis* decreased from 6 log $cfu \cdot mL^{-1}$ to 3 log $cfu \cdot mL^{-1}$ after incubation in human milk. There were significant differences ($P < 0.05$) between bacterial counts of *B. bifidum* and *B. animalis* in all human milk samples tested. Our results suggest that *B. bifidum* is a suitable species for probiotic treatment of fully breast-fed infants, which are free of bifidobacteria. On the other hand, *B. animalis* seems not to be an effective probiotic bacterium for infants.

P-206 **The effects of organic acids and essential oils on methane production in vitro.** M.J. Ranilla[a], M.D. Carro[a], M.L. Tejido[a], D. Macheboeuf[b], B. Lassalas[c], D.P. Morgavi[b] ([a] Departamento de Producción Animal I, Universidad de León, 24071 León, Spain, [b] INRA, Clermont-Ferrand-Theix Research Centre, Herbivore Research Unit, 63122 Saint-Genès-Champanelle, France).

Organic acids and essentials oils have recently attracted much attention as possible manipulators of ruminal fermentation, but whilst these substances may have some beneficial effect on animal production, their mechanisms of action vary. The effects of two organic acids (disodium malate (MAL) and fumarate (FUM); 8 mM) and

three essential oils (cinnamaldehyde, 4 mM (CIN); carvacrol, 2 mM (CAR); and diallyl sulphide, 0.5 mM (DIA)) on ruminal methane production and fermentation characteristics were evaluated in vitro. Samples (400 mg) of a substrate consisting of concentrate and alfalfa hay (75:25) were incubated with 40 mL of buffered rumen fluid from sheep for 16 h at 39 °C, with one of the 5 treatments or without additive (control; CON). Methane production was measured at 5 and 16 h and the production of VFA was determined at the end of incubation. At 5 h, the methane produced was similar ($P > 0.05$) for all treatments. However, total methane production over the 16 h period was lower ($P < 0.05$) for DIA, CIN and CAR (872, 1039 and 1086 μmol, respectively) compared to CON (1289 μmol), while total VFA production was similar ($P > 0.05$) for all treatments. FUM treatment produced a lower ($P < 0.05$) acetate:propionate ratio (2.63 vs. 3.77, FUM vs. CON, respectively). The results show that under the conditions tested essential oils inhibited methane production in vitro without negatively affecting ruminal fermentation.

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P-207 **Antiprotozoal properties of some plants from Iran.** M. Rezaeian[a], R. Ningrat[b], R.J. Wallace[c] ([a] Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran, [b] Faculty of Animal Husbandry, Andalas University, Limau Manis, Padang 25163, West Sumatra, Indonesia, [c] Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK).

The aim of this study was to investigate five different plant samples from Iran for their effects on rumen protozoal activity in vitro. Plant samples were *Artemisia siberi, Acroptilon repens*, *Rubia tinctorum*, *Crocus sativa* and *Pegamola harmula*. Samples were harvested in April, dried at 100 °C and milled to pass through a 1-mm screen. *Selenomonas ruminantium* was grown in the presence of L-[^{14}C]leucine in medium M2 for 24 h. The incorporated bacteria were added to six Hungate culture tubes (3 sheep in duplicate), each containing 7 mL strained ruminal fluid, 22.5 mg plant sample and 5 mM unlabelled L-leucine, after 1 h of incubation under anaerobic conditions at 39 °C in a shaking water bath. Samples (1 mL) were taken from each culture tube at 0, 1, 2, and 3 h and prepared for the measurement of trichloroacetic acid-soluble radioactivity by liquid scintillation spectrometry. The extent of antiprotozoal activity was determined from the net release of ^{14}C into acid-soluble material. All tested plants showed antiprotozoal characteristics compared with the control samples that were without plant samples. The lowest inhibition was obtained with *Rubia tinctorum* and the highest value with *Artemisia*. Further studies are necessary to find whether their properties will be the same in vivo and to identify the probable chemical component(s) of each plant, which may be responsible for the suppression of the bacteriolytic activity of ruminal protozoa.

P-208 **Growth of *Butyrivibrio fibrisolvens* JW11 and *Fusocillus* P-18 in the presence of fish oil, fatty acids and their derivatives.** A.J. Richardson, R.J. Wallace (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Biohydrogenation of dietary unsaturated fatty acids in the rumen is a major factor which affects the quantities of health-promoting polyunsaturated fatty acids in ruminant meats and dairy products. Two bacteria involved in this process are *Butyrivibrio fibrisolvens* and '*Fusocillus*'. Both species biohydrogenate linoleic acid (LA, *cis*-9,*cis*-12-18:2) producing conjugated linoleic acid (CLA; *cis*-9,*trans*-11-18:2) as an intermediate and, in the case of *Fusocillus*, stearic acid (18:0) as an end product. In dairy cattle, the addition of fish oil to the diet results in an increase in the CLA content of milk. The aim of the experiments reported here was to determine the influence of fish oil and its components on biohydrogenation by *B. fibrisolvens* JW11 and *Fusocillus* P-18. Fish oil, the two main fatty acids of fish oil, eicosapentaenoic acid (EPA,C20:5 (n-3)), docosahexaenoic acid (DHA,C22:6(n-3)), linoleic acid, linolenic acid (LNA), linoleyl and oleyl methyl esters, trilinolein, linoleyl, linolenyl and oleyl alcohols were added to an anaerobic, ruminal fluid-containing medium and the growth of the two bacterial strains was followed turbidimetrically. Fish oil, up to a concentration of 10 mg·mL^{-1}, had no effect on the growth of either *B. fibrisolvens* JW11 or *Fusocillus* P-18, but EPA and DHA inhibited the growth of both

strains at 50 μg·mL^{-1}. LA caused a 12-h lag phase with *B. fibrisolvens* JW11 and in combination with fish oil the effect was exactly the same. Growth of P-18 was inhibited completely by LA and LNA. JW11 was also unable to grow in the presence of LNA. Esterified fatty acids and their alcohols were much less toxic than free fatty acids. It was concluded that the effects of fish oil on biohydrogenation require the release of free EPA and DHA by lipolysis.

P-209 **Recovery of *Lactobacillus casei* DN-114 001^{Rif} in the intestinal tract of healthy humans after consumption of fermented milk.** V. Rochet[a], L. Rigottier-Gois[a], F. Levenez[a], J. Cadiou[a], A. Mogenet[b], J-L. Bresson[b], N. Goupil-Feuillerat[c], J. Doré[a] ([a] UEPSD, INRA, 78352 Jouy-en-Josas, France, [b] CIC, Hôpital Necker, 75015 Paris, France, [c] Danone Vitapole, 91767 Palaiseau Cedex, France).

Milk fermented with *Lactobacillus casei* DN-114 001 has health benefits in humans possibly due to its effects on the intestinal microbiota. We studied the recovery of *L. casei* DN-114 001^{Rif} in the terminal ileum and in faeces of 10 healthy humans after ingestion as a fermented milk. The recovery of *L. casei* DN-114 001^{Rif} was analysed in the terminal ileum for the 8 h following ingestion of 300 mL of the test product, and in the faeces after 300 mL daily consumption of the product for 8 days. Real time PCR was performed using primers specific for the *L. casei/paracasei* group. PCR-TTGE was done for analysis of *Lactobacillus* spp. dynamics. FISH using ribosomal RNA targeted probes was used to measure relative proportions of dominant bacteria in faecal samples. Following consumption of *L. casei* DN-114 001^{Rif}, increases in bacteria equivalents of the *L. casei/paracasei* group were observed in all samples. These numbers reverted to initial levels after the end of the intake. PCR-TTGE profiles showed a band characteristic of *L. casei* DN-114 001^{Rif} during consumption of the test product for all individuals. This band disappeared after the end of consumption. FISH showed no significant differences in the proportions of the phylogenetic groups analysed between the different steps of the study. Consumption of a fermented milk containing *L. casei* DN-114 001^{Rif} induced a marked and transient increase of this probiotic in intestinal samples of healthy subjects. This is associated with stability in the composition of dominant phylogenetic groups of the gut microbiota.

P-210 **In vitro growth inhibition of *Helicobacter pylori* and *Campylobacter jejuni* by *Lactobacillus salivarius*.** K.A. Ryan, Y. Li, P.W. O'Toole (Department of Microbiology and Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland).

H. pylori is one of the most common infections worldwide. It colonises the human gastric mucosa and is a causative agent of gastritis, peptic ulcer disease and gastric adenocarcinoma. The related organism *C. jejuni* is regarded as a commensal in poultry but has emerged as the leading cause of human acute enteritis in the Western world. Guillain Barré syndrome has been implicated as a possible sequela to *C. jejuni* infection. With increased antibiotic resistance becoming a problem, we investigated the ability of twenty-eight strains of *Lactobacillus salivarius* from different sources (five human intestine, five human saliva, four human blood, three human other, two pig intestine, one cat, one cheese, five avian and two unknown) to inhibit growth of *H. pylori* and *C. jejuni*. Seven other species of Lactobacilli were included as controls. Lawns of *H. pylori* or *C. jejuni* were spread onto blood agar plates. Either *Lactobacillus* whole cells or cell free supernatants were then dotted onto paper disks which had been placed on the agar plates. Plates were incubated for 48 h, after which time growth inhibition around the disks was measured. Whole cells from 7/28 strains and cell free supernatants from four of these seven strains inhibited growth of *C. jejuni*, while whole cells from 6/28 strains and cell free supernatants from four of these six strains inhibited growth of *H. pylori*. None of the seventeen strains of human origin inhibited either *H. pylori* or *C. jejuni*. In contrast, 4/5 strains of avian origin inhibited growth of both pathogens. The pH of all *Lactobacillus* spent cultures was 4.5 ± 0.5, so the killing effect observed was not simply due to the production of acid by these strains. These data suggest that strains of *Lactobacillus salivarius* of avian origin are a good source for effective probiotics against *H. pylori* and *C. jejuni.*

P-211 **Composition and temporal stability of oral and intestinal microbiota during**

probiotic ingestion. M. Saarela, J. Maukonen, M.-L. Suihko, J. Mättö (VTT, Biotechnology, Espoo, Finland).

The oral cavity and the colon harbour dense and diverse indigenous microbiota consisting of species representing the same phylogenetic clusters. The aim of the study was to compare the diversity and temporal stability of oral and intestinal bacterial populations during probiotic ingestion. Saliva and faecal samples were collected from ten healthy adult volunteers on three sampling occasions (baseline and after one and two weeks ingestion of probiotic capsules) for assessment of the predominant bacteria, bifidobacteria, lactobacilli and the *Eubacterium rectale-Clostridium coccoides* (Erec) -group by PCR-DGGE and, except for the Erec-group, by culturing. Numbers of culturable anaerobes were log 8.4 ± 0.6 cfu·mL^{-1} in saliva and 10.5 ± 0.2 cfu·g^{-1} in faeces, and the corresponding figures for aerobes were 8.0 ± 0.5 and 7.2 ± 0.8. The predominant bacterial population was more diverse in faeces (mean 34.9 amplicons detected by PCR-DGGE) than in saliva (mean 23.6 amplicons), and the dominant population was generally stable in both sampling sites (average similarities in samples obtained from the same individual were 83.3% in faeces and 92.2% in saliva). Similarly, the Erec, *Lactobacillus* and *Bifidobacterium* populations were more diverse in faeces than in saliva, and with the exception of the *Lactobacillus* population in faeces, their populations were generally stable. Only few similarly migrating amplicons were detected in the PCR-DGGE profiles from faecal and saliva samples, which reflects differences in the species composition between the two sites. In conclusion, the microbiota of the oral cavity and intestines was generally stable during short term consumption of probiotic capsules. The microbiota in faeces was more diverse than in saliva, and the species composition varied between the two sampling sites.

P-212 **Cultured milk replacer with prophylactic influence on intestinal diseases.** A. Sarkinas (Food Institute of Kaunas University of Technology, Taikos av. 92, LT 51180 Kaunas, Lithuania).

The purpose of the investigation was to produce an antagonistic activity strain, possessing the property of surviving in the intestinal tract. The strain of *Lactobacillus acidophilus* L16-4-18-500B was selected with moderate activity in lactic acid production in milk and milk products, capable of surviving in the intestinal tract, inhibiting the growth of coliforms in the product and in the intestinal tract. *Lactobacillus acidophilus* L16-4-18-500B can be applied for production of milk replacers. By means of the strain described, feeds for young calves can be produced. Feeding to calves resulted in an increase of calves' average daily liveweight gain when compared with milk replacer produced according to the same prescription but devoid of *Lactobacillus acidophilus.* The described cultured milk replacer decreases the content of conditionally pathogenic microflora in the intestinal tract and reduces calves' morbidity. Investigations were conducted to examine the influence of *Lactobacillus acidophilus* cell survival and titration acidity dynamics on keeping the condensed milk replacer and emulsion. The optimum storage regime of the product was determined based on the exploratory findings. Studies have been carried out on the influence of *Lactobacillus acidophilus* L16-4-18-500B on the development of *Escherichia coli* in cultured milk replacer and emulsion. Storing of samples at 30, 25, 20, 15 and 8 °C temperatures made it possible to estimate the steadiness of the product. It has been established that the development of *Escherichia coli* was inhibited by *Lactobacillus acidophilus* introduced into the product with starter. In the case of *Lactobacillus acidophilus* contamination of milk should not increase significantly during storage.

P-213 **Dietary essential oil supplementation can affect broiler performance and digesta microbial community.** H. Schulze[a], H. Kettunen[b], A.C. Ouwehand[b] and N. Rautonen[b] ([a] Danisco Animal Nutrition, 2300 AE Leiden, NL, Finland, [b] Enteromix® Research, Danisco Innovation, 02460 Kantvik, Finland).

For many centuries, essential oils (EO) have traditionally been used by man for the pleasant odour of the essence, its flavour or its antiseptic and/or preservative properties. The antibacterial effect of EO is well established in vitro. However, information about the effects of EO in livestock animals is scarce. To assess the effect of different blends and dietary inclusion levels of

EO on performance parameters and digesta microbial community, a 42-day broiler study was conducted. The study was conducted with 2160 1-day old male Ross broiler chickens, allocated to 6 treatment groups (12 replicates per treatment, 30 birds per replicate). The birds were fed mash wheat based diets, free of antibiotic growth promoter and coccidiostat, containing 21% and 19% crude protein and 11.7 and 12.6 MJ ME·kg^{-1} DM for the starter (day 1–21) and grower (day 21–42) phase, respectively. Five blends of EO, varying in composition and inclusion level, were studied. Bird performances were measured for the starter (1–21 days), grower (21–42 days) and overall experimental period. On days 21 and 42, ileal and caecal digesta were collected for microbial analyses. Compared with the control group, the dietary addition of EO improved weight gain and feed efficiency of the birds (statistically significant for one of the EO treatment groups). Dietary addition of EO reduced ileal microbial numbers, in particular at day 42. In caecal digesta, biogenic amines were reduced and volatile fatty acids increased by the essential oil supplementation of the diet. In addition, for some of the dietary EO treatments changes of the microbial profile in caecal digesta were observed. The results suggest that dietary EO can improve broiler performance parameters, reduce the microbial population measured in ileal digesta and change caecal microbial activity.

P-214 **Continuous pH measurements in the rumen of sheep to test the anti-acidotic efficacy of nettles (*Urtica dioica*).** N. Selje, E.M. Hoffmann, P. Lawrence, K. Becker (Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim, 70599 Stuttgart, Germany).

U. dioica had proven to exhibit an anti-acidotic effect in vitro (Kliem et al., 2005). This study aimed at validating this impact on rumen pH in vivo. In a crossover feeding trial six cannulated lambs (BW 54 kg) were fed 900 g concentrate and 400 g per day. At a time, three lambs received a control concentrate N0A (5% grass hay) and three sheep were fed concentrate N5A (5% *U. dioica*). After 3 weeks of adaptation, pH was recorded during three consecutive days in 15 min intervals by probes permanently inserted into the rumen. Daily samples were taken throughout the experiment to analyse rumen parameters. Neither ruminal concentrations of lactate, short chain fatty acids, and ammonium, nor body weight gain were significantly different for the diets. With one exception, all sheep exhibited higher H^+ ion concentrations when fed N5A (ave 3.1e^{-6} M) than with N0A (ave 2.3e^{-6} M), depending significantly on diet and on individual animal ($P < 0.0001$), irrespective of which diet was fed first. These results are contrary to those obtained in the in vitro systems with bovine rumen fluid. However, in the in vitro experiments either 100% ground wheat or a mixture of 30% wheat and 70% maize silage served as substrates, whereas in the in vivo trial the diet was based on various grains (16% wheat, 44% barley, 12% maize). Thus, it cannot be excluded that the efficacy of the plant additive is highly dependent on the composition of the diet and/or the animal species investigated.

P-215 **Microbial composition and metabolite concentration in fermented liquid diets for pigs.** A. Shlimon, N. Canibe, O. Højberg, B.B. Jensen (Danish Institute of Agricultural Sciences, Department of Animal Health, Welfare and Nutrition, Research Centre Foulum, PO Box 50, 8830 Tjele, Denmark).

Feeding with fermented liquid feed reduces the number of enteric pathogens along the gastrointestinal tract of pigs. However, negative effects on growth performance have been observed, probably due to microbial degradation of dietary lysine. Fermenting only the cereal grains is being considered as an alternative to fermenting the entire diet. But a fast and substantial pH drop in fermented grain affects the microbial proliferation in the mixture, resulting in too low lactic acid concentrations, thus reducing the bactericidal effect against pathogens compared to fermented liquid feed. Four experimental treatments were designed: fermented liquid feed (FLF), fermented liquid grain (GRAIN), fermented liquid grain amended with soybean (SOY), and fermented liquid grain amended with calcium carbonate and calcium phosphate (CAP). Feed components and water were incubated at 20 °C and samples taken at various time points during incubation. The pH drop was reduced in the SOY and CAP compared to the GRAIN diet. At time 180, the lactic acid concentration was 151, 55, 87, and 101 mmol·kg^{-1}

in the FLF, GRAIN, SOY, and CAP diet, respectively. Preliminary results of partial 16S rDNA sequences of bacteria isolated from de Man, Rogosa and Sharp agar revealed that members of the genus *Weissella* dominated during the initial hours of incubation, while at 180 h, species of the *Pediococcus* and *Lactobacillus* were mainly represented. The results suggest that fermentation of grain cereals amended with soybean or with calcium carbonate and calcium phosphate could be a way of avoiding microbial degradation of lysine in the mixture while keeping relatively high concentrations of lactic acid, and thereby the bactericidal effect.

P-216 **Ultrasonic modification of alginate matrices for the develop of probiotic delivery systems.** A.I. Sidorov[a], O.V. Manaenkov[a], E.M. Sulman[a], L.E. Smirnova[b], V.F. Vinogradov[b] ([a] Tver Technical University, A. Nikitin str., 22, Tver, 170026, Russia, [b] Tver Medical Academy, Sovetskaya str., 4, Tver, 170642, Russia).

In recent years the practical interest to delivery systems on the basis of polymer matrices has increased. They are close to real food objects, have a prolonged effect, and are capable of the selective release of the encapsulate in different parts of the intestine. Thus, controlling the rate of the matrix dissolving, it is possible to control the rate of the probiotic delivery to the organism. The experiments showed that the most effective and simple method to influence the rate of alginate matrix dissolving is ultrasound. Ultrasound causes destruction of sodium alginate macromolecules, the degree of which depends on the time of treatment and intensity of ultrasound. Three groups of gel beads on the basis of sodium alginate with different molecular weight (MW) were prepared. The first group of beads was prepared from the raw sodium alginate solution. The time of ultrasonic treatment of the solution for the second group of beads was 10 min at the intensity of 92 W/sm^2, and for the third group 20 min at 460 W/sm^2. Thus, the MW of alginate macromolecules is 1.5×10^6; 0.938×10^6 and 0.565×10^6, accordingly. The examination of the bead-dissolving process was carried out in environments simulating stomach and intestine conditions. Full dissolving of beads on alginate basis with the molecular weight of 1.5×10^6 occurs by the 50th min, with the molecular weight of 0.938×10^6 – by the 30th min, and with the molecular weight of 0.565×10^6 – by the 20th min. On the basis of the obtained alginate samples with known MW it is possible to develop drug delivery systems with the set properties, able to be dissolved on narrow sites of a gastroenteric path.

P-217 **Influence of *Bifidobacterium bifidum* on mineral release from bread in the process of enzymatic digestion in vitro.** K.A. Skibniewska, B. Nalepa, A. Babuchowski (University of Warmia and Mazury in Olsztyn, Pl. Cieszynski 1, 10-726 Olsztyn, Poland).

Nutrient bioavailability is a key factor when designing appropriate diets. Many factors, such as diet composition, influence the amount of nutrients to be utilized by the human organism. Enzymatic digestion in vitro is used as a first step in the assessment of the part of diet to be utilized by the organism. Bread of wheat, variety Tonacja, was baked with white meal and with addition of 15%, 30% and 45% of bran. Concentration of Ca, Mg, Mn, Cu, and Fe was determined by atomic absorption spectrometry. Breads were next digested in vitro in a process simulating digestion in the gastrointestinal tract without (control) and with addition of *Bifidobacterium bifidum* inoculum at 10^6 cfu·cm^{-3} (experimental). Bifidobacteria influenced the minerals in different ways. Filtrate after experimental white bread digestion contained less minerals than the control one, especially Ca and Fe, probably because of mineral utilization by bacteria. Increase of mineral release was observed in solution after experimental bread with bran addition digestion vs. the same breads without bacteria.

P-218 **Effect of organic acids and monolaurin on *Escherichia coli*, *Salmonella* spp. and *Clostridium perfringens*.** E. Skrivanova[a], M. Marounek[a,b], G. Dlouha[a] ([a] Research Institute of Animal Production, 10401 Prague-Uhrineves, Czech Republic, [b] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, 14220 Prague, Czech Republic).

Antimicrobial activity of fatty acids, monolaurin, citric, succinic, fumaric, malic and lactic

acid against strains of *Escherichia coli*, *Salmonella* spp. and *Clostridium perfringens* was investigated. Antimicrobial activity was expressed as the minimum inhibitory concentration (MIC) that prevented growth and glucose utilization. Caprylic acid was the only acid inhibiting glucose utilization in all cultures. Its MIC varied from 1 to 3 $mg \cdot mL^{-1}$. *E. coli* strains were inhibited also by capric acid at 5 $mg \cdot mL^{-1}$. *Cl. perfringens* strains were inhibited by medium-chain fatty acids (C_8–C_{14}), oleic acid and one strain also by linoleic acid. The lowest MIC were those of lauric and myristic acid (0.1–0.2 $mg \cdot mL^{-1}$). Growth of clostridia was inhibited by monolaurin at 3 $mg \cdot mL^{-1}$ and citric acid at 4 $mg \cdot mL^{-1}$. Inhibitory effects of other acids were not observed. In cultures of *E. coli* treated with caprylic acid, and in cultures of *Cl. perfringens* treated with lauric acid and monolaurin, transmission electron microscopy revealed damage of cytoplasmic structures. Bacteria maintained the integrity of the outer cell membrane. To assess the effect of fatty acids on inner membrane permeability, K^+ efflux from cells of *E. coli* and *Cl. perfringens* treated with caprylic and lauric acid, respectively, was measured by means of a K^+ ion-selective electrode. Cells treated with cetyltrimethylammonium bromide (CTAB) served as a control. Potassium ions were released by action of CTAB, however, no K^+ efflux from cells treated with caprylic and capric acid occurred. Medium-chain fatty acids, thus, do not increase the K^+ permeability of the cytoplasmic membrane, which usually leads to dissipation of membrane potential.

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P-219 **The influence of probiotics on growth of turkeys.** L. Solčianska, D. Vladárová (Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Slovak Republic).

The aim of our work was to monitor and evaluate the influence of probiotic preparations Bio-Mos, Mycosorb, Lactiferm L-5 and Lactiferm L-200+Se on growth indicators, chosen body measures and slaughter quality of turkeys Large White to 84 days old. One day turkeys were divided into six groups. In the first group were 11 turkeys and we used it as a control group. In the second group were 10 turkeys which were fed with feedstuff enriched with 0.2% of probiotic preparation Bio-Mos. In the third group were 11 turkeys which were fed with feedstuff enriched with 0.1% of probiotic preparation Bio-Mos. In the fourth group were 11 turkeys which were fed with feedstuff enriched with 0.1% of probiotic preparations Bio-Mos and Mycosorb. In the fifth group were 10 turkeys which were fed with feedstuff enriched with 0.03% of probiotic preparation Lactiferm L-5. In the sixth group were 10 turkeys and we added probiotic preparation Lactiferm L-200 + Se to drinking water. At the end of feeder at the age of 84 days we found out the highest average live weight in the sixth group with probiotic preparation Lactiferm L-200 + Se and the lowest average live weight in the second group with 0.2% of probiotic preparation Bio-Mos. The highest average body length was found in the sixth group with probiotic preparation Lactiferm L-200 + Se and the highest average breast half-diameter was found also in the sixth group. We found the highest average length of carina in the fourth group with 0.1% of probiotic preparations Bio-Mos and Mycosorb and the highest average leg length also in the fourth group. We found the highest average breast meat yield and the highest slaughter-house yield in the fourth group with 0.1% of probiotic preparations Bio-Mos and Mycosorb. The highest average share of legs was found in the sixth group with probiotic preparation Lactiferm L-200 + Se.

P-220 **Effect of feeding processed karanj (*Pongamia glabra*) meal on the microbial protein synthesis and immune response of growing lambs.** N.M. Soren, V.R.B. Sastry, T.K. Goswami, S.K. Saha (Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh, India).

Abstract withdrawn.

P-221 **Bacterial community change and *Streptococcus suis* disappearance in piglet gut after oral administration of probiotic S1.** Y. Su, W.Y. Zhu, W. Yao (Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, 210095, RP China).

In China, piglets are commonly infected with diseases such as diarrhoea. In 2005, *Streptococcus suis-2,* a pathogen that can infect both pigs

and humans, caused a number of human infections and deaths. Commensal gut bacteria play an important role for the host health, but also contain potentially harmful bacteria. The effects of probiotic S1 on the gut bacterial community in piglets was investigated. Six litters of neonatal piglets were divided randomly into control group and treatment group. At 7, 9 and 11 days of age, piglets in the treatment group orally received probiotic S1 preparation. At 7, 14, 21(weaning), 24 and 35 days of age, one piglet from each litter was slaughtered. Gut samples were collected and total DNA was extracted. The V6-V8 region of 16S rDNA was amplified and analyzed by denaturing gradient gel electrophoresis (DGGE). DGGE profiles showed that high G+C% bacteria in the hindgut of piglets disappeared after weaning and came back gradually on d 35. Sequencing analysis showed that most of these high G+C% bacteria belonged to *Lactobacillus*. Enumeration by plate counting showed similar changes in the number of lactobacilli. S1 had no marked effect on the gut bacterial diversity. However, a special band appeared on d 14 for the treatment group, with its corresponding sequence 95% similar to *Clostridium disporium*. On d 35, a special band appeared in the control group, with its sequence 99% similar to *Streptococcus suis*. In summary, weaning could cause disappearance of high G+C% bacteria (mainly lactobacilli) in the piglet hindgut. *Streptococcus suis* may be commonly present in pig farms in China and probiotic S1 has a potential role in promoting beneficial bacteria and inhibiting potential pathogens.

P-222 **Dynamic single fermenter model for study of survival and growth of probiotics in the human upper gastrointestinal tract.** I. Sumeri[a,b], L. Arike[a,b], S. Adamberg[b], K. Adamberg[a,b], L. Lapp[a,b], T-M. Laht[a,b], T. Paalme[a,b] ([a] Competence Center of Food and Fermentation Technologies Akadeemia tee 15, 12618 Tallinn, Estonia, [b] Tallinn University of Technologies Ehitajate tee 5, 19086 Tallinn, Estonia).

A dynamic in vitro model to simulate physiological conditions characteristic to the human upper gastrointestinal (GI) tract was developed using a single fermenter system. Two hundred mL of model food containing probiotic microorganisms ($10^{6–8}$ cfu·mL^{-1}) was added to 100 mL 0.1 M HCl. Then 1 M HCl was added at the maximum rate of 20 mmol·h^{-1} until pH = 3.0 was obtained, followed by neutralization of the fermenter with 1 M $NaHCO_3$ until pH = 6.5 was reached. Then a 4% solution of bile salts was added during 10 min to achieve a 0.4% bile salt concentration in the fermenter. After 20 min, feeding with a diluted MRS solution was started at dilution rate D = 0.42 h^{-1} for 5.5 h. During this time the pH was kept at 6.5 and bile salts were diluted down to 0.04%. During the whole process the number of cells were determined by: (1) plating out on MRS; (2) counting the cell number in a counting chamber; (3) staining the cells with SYTO9 and propidium iodide and counting green (live) and red (dead) cells using a fluorescence microscope. The model was evaluated using the commercial probiotic *Lactobacillus acidophilus* La-5 (Chr. Hansen A/S, Denmark). It was demonstrated that survival of La-5 depends on both the physiological state of the microorganism (exponential vs. stationary phase) as well as on the food matrix. Results were compared with behaviour of cells in a static in vitro model.

P-223 **Effects of exogenous fibrolytic enzymes on in vitro ruminal fermentation of grass hay and its cell wall.** M.L. Tejido[a], L.A. Giraldo[a], M.D. Carro[a], J.M. Tricarico[b], M.J. Ranilla[a] ([a] Departamento de Producción Animal I, Universidad de León, 24071 León, Spain, [b] Alltech Inc., 3031 Catnip Hill Pike, Nicholasville, KY 40356, USA).

Batch cultures of mixed rumen microorganisms were used to study the effects of a fibrolytic enzyme preparation (Fibrozyme^{TM}, Alltech Inc., Nicholasville, USA) on the in vitro fermentation of grass hay and its isolated cell walls (neutral-detergent fibre, NDF). Samples (500 mg) of each substrate were incubated in 120-mL bottles with 50 mL of buffered rumen fluid from sheep for 24 h at 39 °C. Enzyme was added directly into the bottles at the beginning of the incubation at three levels: 0 (control; CON), 15 (ENZ15) and 30 (ENZ30) xylanase units·g^{-1} substrate DM. Compared to CON, the addition of ENZ30 to grass hay increased ($P < 0.05$) gas production and VFA production after 5, 10 and 24 h of incubation, without affecting ($P > 0.05$) the acetate:propionate ratio. For grass hay NDF, both ENZ15 and ENZ30 treatments increased

($P < 0.05$) gas and VFA productions after 5 and 10 h of incubation, compared to CON, with higher values ($P < 0.05$) for ENZ30 than for ENZ15; after 24 h, however, only ENZ30 increased ($P < 0.05$) these parameters. Dry matter disappearance, NH_3-N concentration and pH values at 24 h of incubation were not affected ($P > 0.05$) by the addition of enzymes for any substrate. The results indicate that both levels of enzyme addition stimulated the in vitro fermentation of grass hay NDF at short incubation times (5 and 10 h), but only the higher level of enzyme addition (ENZ30) stimulated the fermentation of grass hay.

P-224 **Effects of dietary nitrogen source and amino acid composition on intestinal microbiota.** M. Tokura[a], Y. Jojima[a], M. Mori[a], T. Kobayashi[a], Y. Hongoh[b], T. Inoue[b], M. Ohkuma[b] and R. Fudo[a] ([a] Ajinomoto co., Inc., Suzuki-cho 1-1, Kawasaki-ku, Kawasaki-shi, 210-8681 Japan, [b] RIKEN, Hirosawa 2-1, Wako-shi, Saitama, 351-0198 Japan).

Effects of dietary amino acid (AA) composition on intestinal microbiota were analyzed in adult rats. Experimental groups were prepared according to the dietary protein source: (1) Free AA mixture based on casein AA composition (group AA: as control); (2) Casein (group C); (3) AA mixture lacking either arginine, cystine, phenylalanine, or tryptophan (group AMO_{Arg} etc). T-RFLP profiles of intestinal and fecal microbiota among groups were compared by several statistical approaches. Cluster and principal component analyses indicated that the microbial profile of group AA was more stabilized than that of group C. Fixation index (Fst) and Fst-P values indicated that intestinal and fecal microbiota of group AA were significantly different from groups C, AMO_{Phe} and AMO_{Trp}. According to the Wilk's Lambda of discriminant analysis, relative importance of T-RFs was evaluated, and the most important two T-RFs were identified as *Lactobacillus murinus* and *Turicibacter sanguinis* based on comparison with individual 16S rRNA gene clones. Real time PCR demonstrated that *Lactobacilli* in intestinal microbiota were severely decreased in groups AMO_{Phe} and AMO_{Trp}, though the total bacterial number was unchanged. These results suggest that difference in the dietary nitrogen source causes changes in intestinal microbiota and that deficiency in essential amino acids such as Phe and Trp reduces the intestinal *Lactobacillus* population.

P-225 **Relationships between gut microbial species and energy metabolism in broiler chickens.** V.A. Torok[a], K. Ophel-Keller[a], R.J. Hughes[b] ([a] SARDI, Field Crops Pathology, GPO Box 397 Adelaide, SA 5001 Australia, [b] SARDI, Pig and Poultry Production Institute, Roseworthy, SA 5371, Australia).

The role of gut microflora in animal health has become increasingly important, with the use of antibiotics in animal feeds to promote growth being limited due to legislation and consumer pressure. We have developed terminal restriction fragment length polymorphism analysis (T-RFLP) to examine chicken intestinal microflora based on high-throughput, high resolution fingerprinting of bacterial gene regions. This tool is capable of providing a "snap-shot" of the complex bacterial population at any particular time, and combined with advanced multivariate statistical analysis has enabled relationships between gut microflora and bird performance to be investigated for the first time. Changes in microbial communities along the chicken gut in response to addition of a non-starch polysaccharide degrading enzyme product to a barley-based diet were investigated. We found significant differences in the overall microbial communities between dietary treatments within the ileum and caecum, as well as between the ileum and caecum. Several indicator bacterial species contributing to the diet-induced differences in the overall gut microbial communities were identified and found to be different between the ileum and caecum. Classical growth/performance analysis showed chickens fed the barley plus enzyme diet had a significantly higher apparent metabolisable energy (AME) than chickens on the control barley diet. Correlations were observed between changes in gut microflora of chicken fed barley versus barley plus enzyme diets and an increased AME for individual chickens on the barley plus enzyme diet. The presence of specific beneficial bacterial species and/or the absence of specific detrimental bacterial species may be indicators of improved energy metabolism in these chickens.

P-226 **Effects of live yeasts on the fatty acid biohydrogenation by ruminal microflora.** A. Troegeler[a], J.-P. Marden[b], C. Bayourthe[b], R. Moncoulon[b], F. Enjalbert[a] ([a] École Nationale Vétérinaire de Toulouse, Département Élevage et Produits, Laboratoire d'Alimentation, BP 87614, 31076 Toulouse Cedex 03, France. [b] École Nationale Agronomique de Toulouse, Laboratoire de Zootechnie, 31076 Toulouse Cedex 03, France).

Addition of live yeasts in high concentrate diets for ruminants has been shown to help maintaining the ruminal pH above 6, which could enhance the microbial biohydrogenation of unsaturated dietary fatty acids. Moreover, yeasts improve the growth of *Megasphera elsdenii*, a bacterium which favors the *trans*-10 pathway of biohydrogenation. So the objective of this study was to investigate the effects of live yeasts (*Saccharomyces cerevisiae*) on the biohydrogenation in the rumen of dairy cows receiving a high concentrate diet without added fat. Three ruminally fistulated lactating dairy cows were given three diets based on corn silage (control, control plus 0.5 $g \cdot d^{-1}$ or control plus 5.0 $g \cdot d^{-1}$ of *Saccharomyces cerevisiae* NCYC SC47), according to a Latin square design. Ruminal contents were sampled and liquid and solid phases were separated with a 0.25 mm metal sieve. Fatty acids profiles were obtained by gas chromatography. The two doses of yeast resulted in similar effects. Live yeast significantly decreased myristic and stearic acid proportions, and significantly increased oleic and linoleic acid proportions by 16 and 32% in the liquid and the solid phases, respectively. No significant effect was observed for other biohydrogenation intermediates, but the *cis*9,*trans*11-C18:2 tended ($P = 0.154$) to increase with the addition of yeasts, whereas *trans*10-C18:1 numerically decreased ($P = 0.225$). These results suggest that live yeasts affect microbial activity, lowering the extent of biohydrogenation without shifting toward the *trans*-10 isomers pathway.

P-227 **The trial of fermented carrot juice technology development.** M. Trzaskowska, D. Kolozyn-Krajewska (Warsaw Agriculture University, Faculty of Human Nutrition and Consumer Science, ul. Nowoursynowska 159C, 02-776 Warszawa, Poland).

Prebiotics are defined as nondigestible food ingredients that may beneficially affect the host by selectively stimulating the growth and/or the activity of a limited number of bacteria in the colon. Inulin is recognized as a prebiotic. Consumption of food with this prebiotic can be beneficial for human health. The objective of the study was to work out the technology of fermented carrot juice with inulin addition, as a health additive. The strain of *Lactobacillus acidophilus* CH-2 was applied for fermentation. The criterion of optimization were results of sensory assessment, as the sensory attractiveness has a significant effect on consumer motivation in food and drink choosing. Carrot juice with addition of 3% inulin and 5% saccharose was evaluated as the best. The worst was the juice with lower addition of saccharose (3%) ($P < 0.05$). It confirmed the importance of sweetness in food acceptance. Juices with inulin (3%) and saccharose (5%) addition and with only saccharose (5%) addition had the same sensoric quality ($P > 0.05$). It can be concluded that the inulin does not influence sensory quality of carrot juice. The number of bacteria directly after fermentation was at the level of about 9.04 log $cfu \cdot mL^{-1}$. After storage (at 10 °C) the number of bacteria was 9.09 log $cfu \cdot mL^{-1}$. Sensory quality of the juice just after fermentation was evaluated at the level 6.3 ± 1.5 (at the scale from 0 to 10). After 32 days of storage at 10 °C, the sensory quality decreased to the level 3.0 ± 1.4 ($P < 0.05$).

P-228 **New generation probiotics: potential enhancers of rumen function.** N.D. Walker[a], A. Ameilbonne[a], E. Forano[b], F. Chaucheyras-Durand[c], B. Dull[d] ([a] Dansci-Beneflor Inc., France [b] INRA, Clermont-Ferrand-Theix, France, [c]Lallemand Inc., Toulouse, France, [d]Nutriscience Technologies Inc, USA).

Previous research has demonstrated that probiotic yeasts may be used to positively enhance different aspects of ruminal fermentation, animal productivity and health. This is due to improved ruminal pH stabilization, fibre digestion and anaerobiosis, which is reflected by increased dry matter intake, milk yield and live weight gain for the host. However, these positive effects can be strain and diet dependent, with different yeast strains exhibiting different stimulatory effects, depending on diet composition. In

the frame of a new joint venture, Dansci-Beneflor Inc, formed between Lallemand and Nutriscience Technologies, a program has been undertaken to screen many different strains for their potential as new generation probiotics which will target specific areas of ruminal fermentation, health and welfare. Different yeast strains (169) were selected on the basis that they were GRAS organisms, grew rapidly and could be industrially produced. All were from the genus *Saccharomyces* and in the first screening step were tested for their potential to enhance the growth and metabolic activity of *Fibrobacter succinogenes*, an important ruminal fibre-degrader, already known to be positively affected when grown in the presence of probiotic yeasts. The majority of yeasts tested had a positive effect on the growth and metabolic activity of *F. succinogenes*; in some instances causing almost a 2-fold increase in the rate of fibre-breakdown and ammonia utilization when compared with control incubations. It can be concluded that the addition of live yeast cells can stimulate the growth and activity of a fibre-degrading organism like *F. succinogenes,* although the degree of stimulation can be highly strain dependent. These highly-stimulatory yeast strains will be used in further screening tests.

P-229 **Germination and conjugation of *Bacillus thuringiensis* in the gut of gnotobiotic rats.**
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Bacillus thuringiensis (*Bt*) is an entomopathogenic Gram-positive spore forming bacterium used worldwide in the combat of insect pests. In addition to production of insect-specific toxins, *Bt* produces enterotoxins that may cause diarrhoea in humans. Since plant protection agents based on *Bt* are widely used on e.g. tomatoes and cucumbers there is a risk that humans ingesting those vegetables may also ingest *Bt* spores that could germinate in the gut and express enterotoxins causing disease. We used germfree animals to study whether *Bt* spores are able to germinate, express enterotoxins and transfer plasmids in a mammalian gut. To study germination, germfree rats were fed spores of a *Bt* strain harbouring a plasmid encoding Green Fluorescent Protein enabling us to detect germination by flow cytometry. In vivo conjugation was studied by associating germfree rats with a donor strain harbouring the conjugative plasmid pXO16 and an isogenic recipient strain. Both strains were given as spores and transfer was observed from the donor to the recipient strain. Besides the fact that this is the first time that conjugation between *Bt* has been shown in a mammalian tract, this also confirms that the strain is able to germinate in vivo, since conjugation only can happen between vegetative cells. In vivo enterotoxin production was however only detected in one out of six animals, and in this animal only in the ileal sample.

P-230 **Encapsulated fumaric acid as a means of decreasing ruminal methane emissions.**
T.A. Wood[a], R. Wallace[a], A. Rowe[b], J. Price[c], D.R. Yáñez-Ruiz[c], S.P. Williams[c], C.J. Newbold[c] ([a] Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK, [b] Rowett Research Services, Bucksburn, Aberdeen, AB21 9SB, UK, [c] The Institute of Rural Science, University of Wales, Aberystwyth, SY23 3AL, UK).

A decrease in methanogenesis in ruminants would be advantageous to the agricultural industry in terms of energy loss to the animals as well as being beneficial for the environment. Fumaric acid used as a feed supplement has the potential to decrease methane production but the quantity fed has to be restricted because of the risk of acidosis and decrease in fibre breakdown as well as feed intake that may result from a drop in pH. The objective of this study was to determine if encapsulated fumaric acid (EFA) could decrease methane formation in vitro and to assess its usefulness in a lamb feeding trial. Fumaric acid encapsulated in partially hydrogenated vegetable oil (PHVO) did not negatively affect pH or propionate production when added to ruminal fluid in vitro, but retained its suppression of methane formation. Growing lambs on a control diet with ad libitum straw produced 24.6 $L \cdot d^{-1}$ of methane whereas a 10% addition of fumaric acid (FA) or EFA decreased methane production to 9.6 $L \cdot d^{-1}$ and 5.8 $L \cdot d^{-1}$ respectively ($P < 0.001$).

Live weight gain over 43 d was 182, 168 and 202 $g \cdot d^{-1}$ while feed conversion ratios were 108, 119 and 132 g gain·kg^{-1} feed intake for the control, FA and EFA groups respectively. The 75% decrease in methane described in this study is the largest reported in the literature to date and as well as having an impact on the environment whereby greenhouse gas emissions are abated, there are significant implications for the farming industry with increased efficiency of feed conversion.

P-231 **Physiological and microbial status of rats fed a diet containing grapefruit flavonoids and inulin.** M. Wróblewska, E. Biedrzycka (Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, 10 Tuwima Str., 10-747 Olsztyn, Poland).

Phenolic compounds and non digestible saccharides are potential functional supplements of diet. As both substances are intensively metabolised in the large intestine, their potential physiological interaction is possible. The aim of this work was to characterise the physiological effect and caecal bacterial composition of the separate or joint supplementation of rat diet with grapefruit flavonoids and inulin. The studies were conducted on 32 male young Wistar rats. All animals were fed everyday a fresh diet ad libitum with permanent access to distilled water. After 4 weeks of treatment rats were anaesthetised and serum and caecum content were collected. Addition of both tested preparations caused enlargement of caecal tissue and digesta, compared to the control group. The presence of grapefruit flavonoids (0.3%) in the diet caused an increase of digesta pH, and a decrease of dry matter of caecal content, caecal activity of β-glucosidase and β-glucuronidase, total short chain fatty acids and particularly butyric acid concentration. Dietary flavonoids also significantly affected the caecal population of *Bifidobacterium* and *Escherichia coli*. When 5% inulin was added to the diet, the activity of α- and β-galactosidase and the concentration of butyric and propionic acid increased and the pH of the caecum decreased. Inulin addition to the diet reduced the caecal population of *Escherichia coli* and enlarged the *Bifidobacterium* population. The dietary combination of both tested preparations had a greater impact on the activity of galactosidases and butyric acid content and caused more beneficial changes in a bacterial population.

P-232 **Real-time PCR and PCR-DGGE analyses of stool microbiomes from infants fed human breast milk, infant formula, or formula with added fructo-oligosaccharides.** Q. Xia, Z. Yu, T. Premaraj, T. Williams, M. Morrison (Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA).

The colonization and development of microbiomes throughout the infant gastrointestinal tract are affected by the mode of post-natal nutrition (human milk vs. infant formulas). There are hypotheses that these differences may have long-term impact on health and development. Oligosaccharides present in human milk are believed to be the major components in human milk that stimulate the growth of bifidobacteria and lactobacilli. For these reasons, we evaluated infant formulas without and with an added prebiotic, fructooligosacharide (FOS), for its impact on fecal flora relative to a reference group of human milk fed infants. We obtained samples of infant stools to assess the microbiota approximately 30 days after birth and after 27 days of feeding on a milk-based infant formula (FF), FF supplemented with FOS at 2.4 and 3.4 $g \cdot L^{-1}$ (FF-L and FF-H, respectively), or human milk (HM). Sixty-five samples (17 HM, 20 FF-H, 14 FF-L, and 14 FF) were analyzed. The abundance of total bacteria, bifidobacteria, lactobacilli, *Clostridium difficile*, and *E. coli* were estimated by real-time PCR assays. We used PCR-DGGE to assess the impact on total bacteria, as well as intrageneric diversity of bifidobacteria and lactobacilli. The HM group had significantly lower total bacterial abundance than the FOS supplemented formula-fed groups (HM vs. FF-H, $P < 0.02$; HM vs. FF-L, $P < 0.02$, HM vs. FF $P > 0.07$). Though statistically significant, the differences were rather small. The real-time PCR results found that the least square mean values for total bifidobacteria were also not greatly different among the four feeding groups. The least square mean values for the FF-H and FF groups were slightly higher (by less than 0.9 log) than that for the HM and FF-L groups (not statistically significant). In conclusion, FOS supplementation at the described levels did not

appreciably affect the total number of bifidobacteria or lactobacilli in infant stool samples. However, the PCR-DGGE and DNA sequence analyses suggest subtle shifts in the species of bifidobacteria and lactobacilli in infants consuming infant formula when compared to human milk, irrespective of the addition of FOS.

P-233 **Microbial numbers and diversity in the rumen of sheep supplemented with fumaric acid.** D.R. Yáñez-Ruiz[a], G. Bełżecki[b], R.J. Wallace[c], C.J. Newbold[a] ([a] Institute of Rural Sciences, University of Wales, Aberystwyth, SY23 3AL, UK, [b] Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05–110 Jabłonna, Poland, [c] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Organic acids, including malic and fumaric acid, have been shown to decrease methane formation in the rumen, however, their acidic properties restrict the quantity which can be fed. In a recent experiment we have found that dietary fumaric and encapsulated fumaric acid (as a means of avoiding the acidic effect) decreased methane formation by sheep by 50 and 75%, respectively. The present study was designed to determine the influence of adding encapsulated and free fumaric acid, compared to a control diet, on bacterial and protozoal diversities and on the numbers of bacteria, protozoa and methanogens in the rumen. Microbial diversity was assessed by PCR-DGGE using universal primers that amplify specific regions of the 16S and 18S rDNA, respectively, for bacteria and protozoa. Microbial DNA quantification was assessed by real-time PCR using specific primers designed for bacteria, protozoa and ruminal methanogens. Cluster analysis of the banding profiles showed a tendency of samples treated with fumaric acid and encapsulated fumaric acid to be clustered together for bacterial DGGE, while no clear pattern was observed for rumen protozoa. Real-time PCR revealed no differences in numbers of bacterial, protozoa or methanogens between groups, and a large variability within groups in terms of protozoa numbers. Our preliminary results suggest that fumaric acid caused a shift in the type of bacteria in the rumen, but had no effect on protozoal diversity nor on microbial numbers.

P-234 **Daidzein affects the composition of gastrointestinal bacterial community of piglets**. Z.-T. Yu, W.Y. Zhu, W. Yao (Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, 210095, PR China).

Daidzein is one of the estrogenic isoflavonic compounds. Researches have demonstrated that daidzein could affect growth hormones, immunity, and growth performance in animals. Our recent study showed that daidzein could also increase the number of lactobacilli in piglet digesta after in vitro fermentation. Effects of daidzein on the intestinal bacterial community of piglets were investigated using 16S rDNA-based approaches. Six litters of neonatal piglets were allocated into control group and treatment group. The piglets in the treatment orally received 1 mL, 2 mL and 3 mL daidzein (10 $mg \cdot L^{-1}$) on day 7, 9 and 11, respectively. All piglets were weaned on day 21. On day 14, 21, 24 and 35, one piglet of each group, respectively, was slaughtered. The gut digesta were collected for DNA extraction and analysed by PCR/DGGE analysis. Shannon's index showed no apparent difference in microbial diversity between treatment and control groups before weaning. However, after weaning, piglets fed with daidzein showed a higher bacterial diversity in colon and caecum compared with those in the control. This effect was apparent when the piglets were 35 d old. Three DGGE bands existing in the large intestine of the control were absent in the treatment, and their matching clones had 16S rDNA sequences closest to *Clostridium thermocellum*, *Lactobacillus pontis* and *Streptococcus* sp., respectively. Another three bands were present only in treatment piglets, and their corresponding clones had sequences closest to butyrate-producing bacterium SL7/1, *Clostridium butyricum* and *Ruminococcus obeum*, respectively. In summary, daidzein increased the bacterial diversity of the piglet large intestine, but this effect was not apparent until after weaning, two weeks after daidzein treatment.

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IL-2 **Diversity and function of the human gut microbiota.** H.J.M. Harmsen, L.C.M. Wildeboer-Veloo, G.C. Raangs, R.H.J. Tonk, S.P. Van Tongeren, J.E. Degener, G.W. Welling (Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands).

The large number of bacteria present in the intestinal tract of a human is the essential part of an ecosystem that is strongly interacting with the host and plays an important role in health and disease. To investigate the diversity we have used fluorescent in situ hybridisation (FISH) for determination of the number and identity of intestinal bacteria and compared it to the diversity detected by clone library analysis. Furthermore, the FISH methodology was used to study: (i) what is the diversity and composition of the intestinal microbiota and is it related to the age of the human host (ii) and what is the function of all these microorganisms in relation to health and diseases. Health and disease plays an important role in elderly. Frailty parameters can be used to describe the vulnerability to disease and decline of elderly. In our present studies we try to establish a relation between faecal microbiota composition and frailty in elderly (age > 75 years). The results show significant differences between the bacterial composition of faecal samples from high frail and low frail elderly. Culture-independent methods have become available to detect and to quantify bacteria. Analysis of clone libraries from several studies have revealed the diversity in the human gut. However, it is not fully known what the relation of this diversity is with the health of the host or whether this diversity is related to age and/or life style. We aim at studying the function of some hard-to-culture bacteria in this microbial diversity. Probes were designed for groups of bacteria that are detected by clone library analysis, but of which the relevance in terms of quantity and function in the gut is unknown. Applying these probes on faeces of volunteers and the elderly mentioned before showed the presence of *Clostridium viride* group bacteria, *C. propionicum*, *Eggerthella lenta* and the *Roseburia* genus in relation with ageing. Some of these changes may be related to a changing diet with age, for instance the intake of dietary and insoluble fiber. These fibers are believed to play a beneficial role in gut health. Bacterial degradation of these fibers may increase the production of short chain fatty acids such as butyrate. The involvement of different bacterial groups in fiber degradation was demonstrated using FISH on fibers isolated from faeces. We detected the involvement of *Ruminococcus* species, *Eubacterium rectale/C. coccoides, Roseburia* and *Faecalibacterium* species that were associated with the fiber structures. In all of the above-mentioned studies large individual differences were observed. Our general conclusion is that the bacterial composition of the intestinal microbiota is unique in each individual.

IL-3 **From phylogenetics to metagenomics – insight in gut microbial functionality.** J. Doré (INRA, Unité d'Écologie et Physiologie du Système Digestif, 78352 Jouy-en-Josas Cedex, France).

During the past century, recognition of anaerobiosis and the development of anaerobic culture techniques allowed the isolation and characterization of a large bacterial diversity from the dominant human fecal microbiota. It is commonly reported that over 400 species compose this microbiota. Nevertheless, a large fraction of the dominant gut microbes remains unculturable and most recent evaluations converge towards 70% uncultured cells from fecal dilutions. Today, the long awaited culture independent tools that dramatically developed during the past decades have been allowing a complete reassessment of human gut microbial diversity. Molecular tools have their own biases and limitations, yet they allow the recognition of a myriad of species bearing no cultured representative in currently available strain collections. Comparison of 16S rDNA libraries obtained by cloning has proven especially adapted. We will review the information derived from this phylogenetic approach that sets the stage for future perspectives of functional genomics. On a molecular basis, 80% of the phylotypes observed in the fecal microbiota of healthy young adults belong to four phylogenetic groups : the Gram negative *Bacteroides-Porphyromonas-Prevotella* cluster, low-GC Gram positives of the phylum Firmicutes, belonging to the *Eubacterium rectale-Clostridium coccoides* (cluster XIV) phylogenetic group and to the *Fusobacterium prausnitzii-Clostridium leptum* (cluster IV) and high-GC Gram positives of the *Bifidobacterium* and *Collinsella-Atopobium* groups. Other approaches

such as fluorescent in situ hybridization have confirmed this observation. Further, 80% of the phylotypes (60% of the cloned rDNA sequences) derive from microorganisms that have no culturable representative. This is especially true for the Firmicutes. The proportion of yet un-recognized species (present in an individual's gut microbiota) increases from birth to the old age. Accordingly, specificities of the gut microbiota associated with ageing or intestinal disorders such as inflammatory diseases can be outlined. Comparative analysis of several 16S rDNA based molecular inventories indicates that the dominant fecal microbiota is essentially specific to its host at the species level. High throughput methods also inform us of its resistance to modification and its marked resilience following stress. Considering the expected functional homogeneity of the human intestinal ecosystem, it may be speculated that a true functional redundancy will be evidenced between individuals. Yet it is not clear at this stage whether this is to be found at the level of the gut microbiota genome, proteome or metabolites. We will review the preliminary results derived from the investigation of the metagenome of the human faecal microbiota. This has been addressed using a comprehensive metagenomic approach to investigate the full range of intestinal microbial diversity and potential functionalities. Two libraries of genomic DNA isolated directly from fecal samples of six healthy donors and six patients with Crohn's disease (CD) were constructed to this end, each composed of 25 000 clones. Characterization of 16S rDNAs within metagenomic clones identified 125 non-redundant ribotypes mainly represented by the phyla Bacteroidetes and Firmicutes. Species diversity within Firmicutes was lower in CD patients than in healthy subjects (13 vs. 43 distinct ribotypes; $P < 0.025$). This was confirmed by FISH using fecal samples analysed individually ($n = 12$; $P < 0.02$). Metagenomic libraries were also investigated by functional screening. This allows investigation of yet totally unexplored genomic resources from the gut environment, some of which will prove highly relevant to our understanding of the hots bacteria dialogue. The metagenomic approach appears promising to identify most redundant genomic traits of the human intestinal microbiota and thereby identify the functional balance of this organ. It will further contribute to substantiate the concept of a functional core within the intestinal microbiome.

IL-4 **The gut microflora and colorectal cancer risk.** I. Rowland (Northern Ireland Centre for Food and Health, University of Ulster, Coleraine, BT52 1SA, Ireland).

Colorectal cancer is one of the major causes of death from malignant disease in Western Europe, USA, and Australia. There is considerable evidence from laboratory animals and human studies that the colonic microflora is involved in the aetiology of colon cancer. For example, germ-free rats treated with the carcinogen 1,2-dimethylhydrazine have a lower incidence of colon tumours than similarly treated rats having a normal microflora. We have demonstrated potent DNA-damaging activity in faecal extracts from a proportion of healthy human subjects. Furthermore, intestinal bacteria possess a range of xenobiotic metabolizing enzymes that enable them to produce, from dietary components, substances with genotoxic, carcinogenic and tumour-promoting activity. They can synthesize carcinogens such as N-nitroso compounds, activate carcinogens to reactive DNA-damaging metabolites and deconjugate detoxified carcinogens in the colon releasing the active carcinogen. In addition they can produce tumour promoters such as ammonia and bile acids. It follows from the above that dietary regimens that modify the gut microflora may alter colon cancer risk. Probiotics and non-digestible oligosaccharides (prebiotics), which significantly increase bifidobacteria and lactobacilli in the gut, have considerable potential in this regard and there is considerable evidence from in vitro studies and animal experiments that they inhibit DNA damage and suppress pre-cancerous lesions and tumours in the colon. Evidence from epidemiological studies for anticancer effects of probiotics is limited, but recent dietary intervention studies in healthy subjects and in polyp and cancer patients have yielded promising results on the basis of biomarkers of cancer risk. Potential mechanisms for the anticarcinogenic activity of probiotics include binding of carcinogens, induction of apoptosis, stimulation of Phase II enzymes, and modulation of carcinogen formation by gut bacteria.

IL-5 **Microbially-induced inflammation and colon cancer.** E.M. El-Omar (Department of Medicine and Therapeutics, Aberdeen University, Aberdeen AB25 2ZD, Scotland).

Impressive epidemiologic evidence shows a clear association between chronic inflammatory conditions and subsequent malignant transformation in the inflamed tissue, particularly of the GI tract. The stimulus for the inflammation is often an infective agent such as viruses or parasites (e.g. Hepatitis B/C and Schistosomiasis in hepatocellular carcinoma), and bacteria (e.g. *Helicobacter pylori* -associated gastric cancer). Chronic inflammation is also very relevant to the risk of colorectal cancer (CRC). Inflammatory bowel disease is known to substantially increase the risk of CRC and anti-inflammatory medications (e.g. aspirin) are known to reduce it. The role of gut bacteria in CRC has recently received considerable attention. There is now definitive evidence that the commensal gut microbiota plays a crucial role in maintaining the healthy mucosal barrier. Any disruption of this normal homeostasis is associated with colonic inflammation and increased risk of CRC. Our recent work has focussed on assessing the degree and phenotype of inflammation within normal and neoplastic colonic tissue. We have established that colorectal polyps have a significantly increased complement of acute and chronic inflammatory cells compared to their immediately adjacent normal mucosa. Furthermore, there is a progressive increase in chronic inflammatory cells and mediators that match the progressive increase in the size of polyps and their grade of dysplastic change. This suggests that chronic inflammation is the fundamental pathophysiological mechanism underlying CRC. We have proceeded to investigate the aetiology of the inflammation seen in the polyps and our preliminary data indicate that a large proportion of these polyps have a significantly different mucosal microbiota compared to their immediately adjacent normal mucosa. This difference may reflect a disrupted homeostasis within polyps caused by acquisition or loss of crucial bacterial species that are relevant to inflammation and cancer risk. This field is expanding rapidly and the future promises to be very exciting indeed.