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Taxonomic and functional diversity within the cellulolytic and xylanolytic communities from the human gut. C. Chassard, C. Robert, A. Bernalier-Donadille (Unité de Microbiologie, INRA, C.R. de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

Plant cell wall polysaccharides are an important source of fibres that have great potential for human heath. However, fermentation of these substrates by the gut microflora often leads to high gas production (H_2, CO_2 and CH_4 in some cases), responsible for digestive discomfort. The correlation between plant fibre ingestion and the genesis of digestive troubles suggests a major role of the fibrolytic flora in H_2 production. The structure and activity of the microflora involved in cellulose and xylan degradation of the main plant cell wall polysaccharides, were investigated. The cellulolytic flora was shown to be very diversified, its structure being different in methane- (CH_4^+) and non-methane-excreting (CH_4^-) individuals. The cellulolytic community from CH_4^+ subjects was mainly composed of new *Ruminococcus* and *Enterococcus* species while this population was essentially represented by new *Bacteroides* species in CH_4^- ones. Furthermore, the cellulolytic species isolated from CH_4^+ subjects degraded crystalline cellulose and were high H_2-producers whereas those from CH_4^- subjects hydrolysed only less crystalline cellulose and did not produce H_2 from this fermentation. By contrast, the xylanolytic community was detected in all faecal samples, independently of the methane status of the volunteers. The structure of this xylanolytic population was also diversified, both Gram positive and Gram negative strains being isolated. In order to prevent digestive troubles associated with plant fibre ingestion, *Bacteroides* sp. nov., the non-H_2-producing cellulolytic species, appears interesting to promote in the human colon. In this context, the nature of the fibre ingested (vegetables, fruit, cereals) would be an important factor to consider since it greatly influences in vitro growth and metabolism of the different cellulolytic species isolated.

Analysis of planktonic and biofilm bacterial communities in a three-stage continuous culture model of the large intestine, and their interactions with HT29 colonic epithelial cells. M.W. Child, A. Kennedy, G.T. Macfarlane (University of Dundee, MRC Microbiology and Gut Biology Group, Level 6, Ninewells Hospital Medical School, Dundee, DD1 9SY, UK).

In order to assess the pattern of colonisation of human faecal bacteria in a three-stage continuous culture system (CCS), twenty different 16S rRNA oligonucleotide probes of varying specificities were used. Bacteria belonging to the genera *Bacteroides, Bifidobacterium*, together with the *Eubacterium rectale / Clostridium coccoides* group, *Atopobium / Coriobacteriaceae* group, *Faecalibacterium prausnitzii*, *Eubacterium cylindroides* group, and lactic acid bacteria were all present in high numbers in the CCS. Other groups and species such as ruminococci, enterobacteria and veillonella also persisted in the CCS, though not always at levels that allowed reliable quanification. Different patterns of colonisation were seen in each of the three vessels and changing the retention time also changed the bacterial composition of the CCS. For example, it was found that members of the enterobacteriaceae preferred to colonise vessel one and that members of the Clostridium cluster XIVa were selected for faster dilution rates. In order to assess if planktonic bacteria are able to colonise the mucosa, in vitro studies investigating faecal bacterial colonisation of mucus surfaces were carried out. Bacteria readily formed homogenous microcolonies on exposed mucus surfaces after only a few hours, and after 2–5 days complex heterogenous biofilms were observed. The interactions between HT29 human epithelial cells and bacteria from each of the vessels of the CCS were also investigated. Certain groups of bacteria such as the atopobia, bifidobacteria and members of *Clostridium* cluster XIVa displayed higher affinity for binding to the apical surface of HT29 cells than other members of the CCS microflora.

The use of a macroarray in the surveillance of antibiotic resistance across Europe. A.J. Patterson, H.J. Flint, K.P. Scott (Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK).

The emergence and dissemination of antibiotic resistance in non-clinical environments is a growing public health concern. Overuse of antibiotics in farming as growth promoters and prophylactics, in addition to disease treatment, may be

partly responsible for the increase in antibiotic resistance within the human population. This study was aimed at determining the relative number of resistance genes present in the gastrointestinal tract of both humans and animals, and also in farm and garden soil samples collected from different locations across Europe. A macroarray containing the resistance genes of two widely used groups of antibiotics, the tetracyclines and macrolides, was used to identify specific genes responsible for resistance at each location. This approach will enable us to monitor the incidence of specific genes and ascertain whether certain genes are more prevalent in the soil compared to animal sources. It is hoped that knowledge of the prevalence of specific genes in different countries will allow a model for the routes of dissemination of antibiotic resistance genes to be developed.

A comparative study of intestinal microbial diversity from birds, pigs and rabbits by restriction fragment length polymorphism analysis. A.M. Pérez de Rozas[a], M. Roca[a], R. Carabaño[b], C. de Blas[b], M. Francesch[c], J. Brufau[c], S.M. Martín-Orúe[d], J. Gasa[d], S. Campoy[a], J. Barbé[a], I. Badiola[a] ([a] CReSA (UAB-IRTA) Bellaterra, Barcelona, Spain; [b] Departamento de Producción Animal E.T.S.I. Agrónomos Madrid, Spain; [c] Departament de Nutrició Animal, IRTA Reus, Tarragona, Spain; [d] Departament de Ciència Animal i dels Aliments, UAB Bellaterra, Barcelona, Spain).

The study of complex microbial communities by culture-independent techniques has improved the global knowledge of microbial ecology. Differences in the position of cleavage sites for restriction enzymes in a universal gene, such as 16S rDNA, are powerful tools for the analysis of the microbial ecology of complex microbiota, as the gut system is, and a good method to analyse bacteria that are difficult to culture. The aim of the present study was to compare the intestinal bacterial communities present in the contents of different intestinal sections of different species of farm animals (rabbits, pigs and chickens) by restriction fragment length polymorphism (RFLP). DNA was extracted from the intestinal contents by the QIAamp DNA Stool Mini Kit (QIAGEN), with some modifications, and it was amplified by PCR with CTACGGGAGGCAGCAGT and CCGTCWATTCMTTTGAGTTT primers and digested with four different restriction enzymes (*AluI, RsaI, HpaII, CfoI*), The biodiversity degree and the frequency of detection of certain bacterial genera were determined from RFLP profiles, and dendograms with the degree of similarity between RFLP profiles were constructed, Important differences in the RFLP profiles were observed by comparing the ileal or caecal samples of rabbits, chickens and pigs, and, as expected, the intestinal microbial components among these three species were highly different. Furthermore, the caeca of pigs and chickens had a higher biodiversity than the ileum, whereas in rabbits the relationship was inverted. Additionally, RFLP profiles were related with the age and maternal origin of the animals.

Hydrogenotrophic methanogens and homoacetogens in termite guts: Competition or coexistence? M. Pester, A. Brune (Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, 35043 Marburg, Germany).

It is generally assumed that hydrogenotrophic methanogens outcompete homoacetogens due to their higher affinity for the common substrate. Nevertheless, both metabolic groups occur simultaneously in many environments. In this study, we compared the situation in the hindgut of lower termites from three different families. In *Reticulitermes* spp., where methanogens are mostly restricted to the gut periphery, in situ rates of reductive acetogenesis considerably surpassed those of methanogenesis. In *Zootermopsis angusticollis*, where methanogens are located inside the gut protozoa, methanogenesis appeared to be the major hydrogenotrophic process. *Cryptotermes secundus*, however, does not contain any methanogens, and homoacetogens seemed to be solely responsible for the hydrogenotrophic activities. Using microsensors, radiotracers, and fluorescence in situ hybridization, we are currently investigating the intestinal hydrogen partial pressures as well as the in situ activities and spatial organization of the two metabolic groups. The aim is to gain a better understanding of the ecological factors governing competition and co-existence in the three model systems.

Evaluation of the location of different gut microbiota in both the lumen of the cecum and in the proximal colon of the guinea-pig by 16S ribosomal DNA based-PCR and denaturing gradient gel electrophoresis. T. Takahashi, S. Karita, M.S. Yahaya, M. Goto (Faculty of Bioresources, Mie University, Kamihama-cho 1515, Tsu 514-8507, Japan).

It is thought that the digesta in the cecum and proximal colon are well distributed both along and across the intestine and are thus homogenous in their bacterial distribution. Accordingly, there are few studies concerning possible local variations of bacterial species in the digesta on either the longitudinal or transversal axis of the cecum. We therefore investigated bacterial species variation in digesta along and across the cecum and proximal colon in guinea pigs. The cecum and proximal colons from the animal subjects were frozen in liquid nitrogen and cut transversely with a scalpel. Digesta of between 100–200 mg were then sampled by punching with an iron pipe of 3 mm in diameter and the DNA of each sample was extracted using a QIAamp DNA Stool mini kit (Qiagen). A portion of the 16S ribosomal DNA was then amplified by PCR using GC357f and 517r primers and was subsequently used for denaturing gradient gel electrophoresis (DGGE). Following electrophoresis, the DGGE bands of the highest intensity were extracted and analyzed by sequencing. These sequenced fragments were then compared with bacterial databases and we found a local variation of *Ruminococcus* and *Clostridum polysaccharolyticum* strains in luminal contents across the cecum and of the *Spirochete* species across the proximal colon. In addition, the levels of both *Ruminococcus* and *Clostridum polysaccharolyticum* in the cecum were different from the quantities found in the proximal colon. These results suggest that different environmental niches exist for bacteria across the cecum, proximal colon and also along the large intestine. It is likely, therefore, that dietary residues may be utilized by different microbiota inhabiting different local areas of the digesta of the cecum and proximal colon.

Temporal stability analysis of the microbiota in human feces by denaturing gradient gel electrophoresis using universal and group-specific 16S rRNA gene primers. T. Vanhoutte[a], G. Huys[a], E. De Brandt[a], J. Swings[a,b] ([a] Laboratory of Microbiology; [b] BCCM^{TM}/LMG Bacteria Collection, Ghent University, Ghent, Belgium).

According to the current insights, the predominant bacterial community in human feces is considered to be stable and unique for each individual over a prolonged period of time. Many of the factors influencing the establishment and consistence of gut bacterial communities are still poorly understood. The objective of the present study was to monitor the temporal stability of both the predominant population and a number of specific subpopulations of the fecal microbiota of four healthy volunteers for 6–12 weeks. For this purpose, a combination of different universal (V_3 and V_6-V_8) and genus- or group-specific (targeting the *Bacteroides fragilis* subgroup, the genera *Bifidobacterium* and *Enterococcus* and the *Lactobacillus* group, which also comprises the genera *Leuconostoc*, *Pediococcus* and *Weisella*) 16S rRNA gene primers was used. Denaturing gradient gel electrophoresis (DGGE) was used to analyze the 16S rRNA gene amplicons generating population fingerprints which were compared visually and by numerical analysis. DGGE profiles generated by universal primers were relatively stable over a 3-month period and these profiles grouped by numerical analysis in subject-specific clusters. In contrast, the genus- and group-specific primers yielded profiles with varying degrees of temporal stability. The *Bacteroides fragilis* subgroup and *Bifidobacterium* populations remained relatively stable which was also reflected by subject-specific profile clustering. The *Lactobacillus* group showed considerable variation even within a two-week period and resulted in the complete loss of subject-grouping. The *Enterococcus* population was detectable by DGGE analysis in only half of the samples. In conclusion, numerical analysis of 16S rRNA gene-DGGE profiles clearly indicates that the predominant fecal microbiota is host-specific and relatively stable over a prolonged time period. However, in-depth analysis of some subpopulations tend to show strong temporal variations (e.g. the *Lactobacillus* group) whereas other autochthonous groups (e.g. the bifidobacteria and the *Bacteroides fragilis* subgroup) do not undergo major population shifts in time.

Stability and activity of an *Enterobacter aerogenes* specific bacteriophage under simulated gastro-intestinal conditions. K. Verthé[a], S. Possemiers[a], N. Boon[a], M. Vaneechoutte[b], W. Verstraete[b] ([a] Lab. Microbial Ecology and Technology, Ghent University, 9000 Ghent, Belgium; [b] Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, 9000 Ghent, Belgium).

There is a renewed interest in the use of bacteriophages, viruses with a bacterial host, in the treatment of bacterial infections, especially those (multi)resistant to antibiotics. Still, little is known about the activity of bacteriophages against their host in the human intestinal tract. The objective of the present study was to develop an in vitro model to investigate bacteriophage stability and activity under gastro-intestinal conditions. The model consisted of a bacteriophage (UZ1) and its host, a *gfp* marked clinical isolate of *E. aerogenes*. Different aspects affecting bacteriophage stability during transit through the gastro-intestinal tract were evaluated, i.e. resistance to stomach acidity, bile and pancreatin. It was found that the use of an antacid prevented the inactivation of the bacteriophage by gastric acidity. Persistence in an intestinal microbial ecosystem was determined using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). A pulse administration of bacteriophage UZ1 to reactor 3, which simulates the ascending colon, showed that in the absence of the host, bacteriophage UZ1 persisted for 13 days in the simulated colon while the theoretical washout was calculated at 16 days. To assess its lytic activity in an intestinal microbial ecosystem, *gfp* specific primers were designed in order to quantify the host strain using real-time PCR. It was observed that the bacteriophage UZ1 was able to replicate and showed lytic activity against *E. aerogenes*BE1/*gfp* in an intestinal microbial ecosystem.

Pure culture and PCR analyses of sulfate-reducing bacteria from swine feces and stored swine manure. T.R. Whitehead, K. Cook, M.A. Cotta (USDA/ARS, National Center for Agricultural Utilization Research, Peoria, Illinois 61604, USA).

Storage of swine manure from large-scale swine facilities results in the microbiological production of a variety of odorous compounds, including ammonia, organic acids and alcohols, and sulfides. Such odors can create a nuisance to local populace, and may contribute to health problems for swine facility workers and animals. Production of hydrogen sulfide, a particularly odorous compound, is associated with the presence of sulfate-reducing bacteria (SRB), but little is known about the population of SRB in the swine intestingal tract and stored swine manure. Swine feces and stored swine manure from a local facility were used as a potential source of SRB. Serial dilutions from the feces and manure using Postgate medium was used to enrich for SRB, then pure cultures were isolated by streaking on Postgate-agar medium. Total DNA was isolated from the fecal suspension, swine manure slurry, enrichment cultures and isolated pure cultures for use in direct PCR of the dissimilatory sulfite reductase A (*dsr*A) gene for phylogenetic analyses, as well as 16S rDNA sequence analyses of pure cultures. Pure culture isolates from feces and manure were presumptively identified as *Desulfovibrio* species by both 16S rDNA and *dsr*A sequence analyses. However, direct PCR and sequencing of the *dsr*A gene from total DNA isolated from the manure slurry also indicated the presence of *Desulfobulbus*-like SRB as well as *Desulfovibiro*-like SRB. These results suggest that molecular methods could be developed to monitor different populations of SRB in manure following alterations in the swine diet as well as variations in manure handling operations.

DNA extraction methods of rumen bacteria: quality and diversity. A.L. Trebbin[a], S. Denman[b], J. Padmanabha[b], D.O. Krause[b], P.T. Scott[c], R.A.M. Al Jassim[a], C.S. McSweeney[b] ([a] School of Animal Studies, University of Queensland, Gatton, 4343, Australia; [b] CSIRO Livestock Industries, St. Lucia, 4067, Australia; [c] Agricultural Molecular Biotechnology Laboratory, University of Queensland, Gatton Qld 4343, Australia).

The inability to culture the majority of microorgansims from environmental samples is limiting our understanding of microbial ecology and diversity. The advent of new methods being adopted to study microbial communities such as ribosomal DNA (rDNA) analyses or more recently, BAC (bacterial artificial chromosome)

based technologies, are dependent on reliable DNA extraction methods. To guarantee complete coverage of the predominant members of the community, the method of DNA extraction employed must be extensive enough to recover material across a wide variety of bacteria without causing loss, degradation or damage to the DNA. DNA extraction protocols vary in many aspects from in situ lysis, physical disruption through to gentle enzymatic lysis. Whilst a number of extraction protocols have been applied to rumen samples, little information regarding the influence of the extraction method on diversity of the DNA has been mentioned. Thus the aim of this study was to evaluate the effect of cell lysis methods on the bacterial DNA diversity and quality from rumen samples. Several techniques that involved either enzymatic lysis, mechanical lysis or the cells being immobilised in agarose plugs before lysis, were investigated. Bacterial diversity comparisons and quality of DNA, were evaluated by the use of Denaturing Gradient Gel Electrophoresis (DGGE) and Pulsed Field Gel Electrophoresis (PFGE) analysis.

Construction and preliminary characterization of a large high molecular weight rumen metagenomic library. R.M. Teather, L.J. Paterson, R.J. Forster (Lethbridge Research Centre, Agriculture and Agri-Food Canada, PO Box 3000, Lethbridge, AB, Canada T1J 4B1).

Many natural microbial communities remain poorly characterized and their genetic resources largely inaccessible due to our failure to cultivate and identify the majority of the community members. Metagenomic approaches, which apply genomic methods to the microbial community as a whole, offer the possibility of gaining ordered access to a community's genetic resources without the need to isolate and cultivate the community members. Successful application of this approach to gain ordered access to the genetic resources of uncultivated organisms from a complex microbial community, where most species probably constitute less than 1% of the population, depends on the ability to prepare representative high molecular weight genomic DNA. We developed an effective protocol for the preparation of representative rumen microbial community DNA with a molecular weight > 500 Kb. Complete rumen samples were processed by mechanical grinding under liquid N_2, treated with protease K and SDS at elevated temperatures, and then mixed with agar and cooled. All subsequent steps (purification, size selection, digestion, and ligation) were carried out in the agar matrix or on a membrane surface. A BAC library of 60 000 clones with an average molecular weight > 200 Kb was generated using this DNA.

Gut microbiome composition revealed by a modified version of serial analysis of ribosomal sequence tags (mSARST). Z. Yu, M. Morrison (The MAPLE Research Initiative, Department of Animal Sciences, The Ohio State University, Columbus, OH 43210, USA).

Our perspectives of gut microbiomes have been greatly altered by the application of cultivation-independent techniques that focus on ribosomal RNAs and (or) the genes encoding these molecules. Despite the new insights into microbial diversity arising from these studies, their comprehensiveness has been limited by costs and the available biological and computational technologies. Serial analysis of ribosomal sequence tags (SARST) is an innovative method of examining microbial diversity that is enabled by improved cloning procedures and high throughput sequencing technology. We developed a modified version of SARST (mSARST) that greatly improves the efficacy of the approach, and used it to unveil the microbial diversity present in community DNA recovered from microbes adherent to ruminal digesta particles. A total of 1024 RSTs were recovered from the library, with an average of 5 RSTs per clone, and more than 350 unique RSTs were identified. Rarefaction analysis showed that the accumulation curve reached a plateau, indicating most of the bacterial species present in the community were accounted for. The most abundant RST is 96.4% identical to a previously sequenced ruminal *rrs* clone, which is affiliated with the *Clostridium leptum* subgroup. Other abundant RSTs in the library were similar to *Prevotella ruminicola*, *Clostridium thermocellum*, *Bacteroides acidofaciens*, *Butyrivibrio fibriosolvens*, *Succiniclasticum ruminis*, and *Ruminococcus flavefaciens*. However, a large number of RSTs were not closely related to any

known bacterial species. The results show that mSARST provides a meaningful assessment of microbial diversity that is more instructive, rapid, and cost-effective than other widely used methods of community analysis.

Molecular techniques for the structural analysis and quantification of an in vitro cultured intestinal microbial community. S. Possemiers, K. Verthé, S. Uyttendaele, S. Bolca, T. Van de Wiele, N. Boon, W. Verstraete (Laboratory of Microbial Ecology and Technology, UGent, 9000 Gent, Belgium).

The importance of studying the intestinal microbial community is evidenced by their significant role in human health. Although in vivo data on intestinal microbiota are usually derived from faecal samples, the latter do not contain a representative microbial composition for the entire colon. Here we describe an in vitro analysis of the microbial community from the proximal to distal colon, using a Simulator of the Human Intestinal Microbial Ecosystem (SHIME). Since more than 50% of the intestinal microbiota are non-culturable, conventional plating techniques typically gave biased results. Therefore we introduced 16S rDNA and rRNA based molecular techniques to allow a complete analysis of the in vitro intestinal microbial community. Firstly, the extraction based methods, PCR-DGGE and Real Time PCR, allowed to monitor the structure and qualitative and quantitative stability over time of the SHIME intestinal ecosystem. Using group specific primers, the effects of inulin and antibiotics on specific bacterial groups such as *Bifidobacterium* sp. could be visualised whereas this was not possible with bacterial primers. A moving window correlation was successfully applied to follow up community stability over time. Secondly, we investigated the structural integrity and activity of bacterial cells by Live/Dead and FISH analysis using Flow Cytometry and by RNA based Real Time PCR. Thirdly, *gfp*-specific Real Time PCR and Flow Cytometry were used to track the behaviour of *gfp*-labelled bacteria in the complexity of an intestinal suspension. In conclusion, we show the usefulness of SHIME to characterise the microbial community from the entire colon by advanced molecular techniques.

Rumen bacterial diversity revealed by ribosomal intergenic spacer analyses and 16S ribosomal DNA sequencing. M. Mitsumori[a,b], S. Denman[b], J. Padmanabha[b], C.S. McSweeney[b] ([a] National Institute of Livestock and Grassland Science, 2 Ikenodai, Kukizaki, Ibaraki, 305-0901 Japan; [b] CSIRO Livestock Industries, Queensland Biosciences Precinct, Carmody Rd, St Lucia, Brisbane, Australia).

Ribosomal RNA (rRNA) intergenic spacer analysis (RISA) which utilizes the intergenic spacer region (ISR) between the small (16S) and large (23S) subunit in the rRNA operon displays significant heterogeneity in both length and nucleotide sequence. This sequence heterogeneity has been used as a marker to distinguish between species and strains. Therefore, in the present study, ISR of rumen bacteria were investigated together with sequences of their 16S rDNA. Various lengths of DNA fragments were amplified from ruminal extracted DNA using universal primers targeting loci in 16S rDNA and 23S rDNA and then cloned and sequenced. From the 43 clones obtained, the lengths of ISR ranged from 136 bp to 677 bp compared with the associated 16S rDNA sequences which ranged from 619 bp to 645 bp. Based on the 16S rDNA sequences, the 43 clones were divided into 8 clusters (A-H). Cluster F belonged to the *Cytophaga-Flexibacter-Bacteroides* (CFB), while the other 7 clusters belonged to the low G+C Gram-positive bacteria (LGCGPB). Except for a minor number of clones, the clusters formed by phylogenetic analysis based on the sequences of ISR corresponded to the same clusters in the 16S rDNA analysis. Therefore, ISR of rumen bacteria would be useful in the analysis of the rumen microbial community at the species and strain level.

Quantification of the gastrointestinal bacterial population in early-weaned pigs by real-time PCR. M. Castillo[a], E.G. Manzanilla[a], M. Martín[b], I. Badiola[c], S.M. Martín-Orúe[a] ([a] Dept. Ciència Animal i dels Aliments; [b] Dept. Sanitat i Anatomia Animal, CreSA, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, 08193, Barcelona, Spain; [c] CReSA, Centre de Recerca en Sanitat Animal, Bellaterra, 08193, Barcelona, Spain).

An experiment was designed with the aim to evaluate the effect of different additives on the total bacteria population, enterobacteria and lactobacilli using real-time PCR (qPCR) and Sybr® Green dye, based on universal and specific regions of the 16S rRNA gene. Thirty-two early weaned pigs were randomly distributed into 4 treatments: a pre-starter diet (Control (CT)) or this basal diet with 0.04% avilamycin (AB), 0.3% butyric acid (AC), or 0.03% of a plant extract mixture (XT). Total bacteria were quantified in samples from the stomach, jejunum and caecum. To asses the results obtained, direct microscopy (DAPI staining) was used as a reference method in jejunum samples. Lactobacilli and enterobacteria were also quantified by qPCR in samples of the jejunum and caecum. No statistical differences in the total bacterial population were found between the different diets. The results obtained by qPCR were higher (11.1 ± 0.88 $\log_{10}$ copies 16Sr DNA·g^{-1} FM) than the results obtained by microscopy (7.8 ± 0.37 $\log_{10}$ bacteria/g FM) in the jejunum contents. However, the correlation between both methods was significant ($P < 0.001$, $r = 0.46$). The mean values obtained by PCR for total bacteria increased along the gastrointestinal tract: 8.0 ± 1.16, 11.1 ± 0.88, and 12.4 ± 0.13 ($\log_{10}$ copies 16S rDNA·g^{-1} FM) for the stomach, jejunum content and caecum respectively. The counts obtained for lactobacilli and enterobacteria in the jejunum and caecum contents were 10.8 ± 1.66 and 13.0 ± 0.39 for lactobacilli and 8.4 ± 0.56 and 12.4 ± 0.14 for enterobacteria respectively. Enterobacteria tended to be lower in the jejunum ($P = 0.11$) only with XT.

Minkowski Metrics –an easy approach for the molecular ecologist. N.R. McEwan (Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB Scotland, UK).

Minkowski Metrics is a family of mathematical equations that are used to study the similarity or difference between populations or samples. They are now becoming a useful tool for the analysis of banding patterns on gels arising from molecular ecological studies. In general, three different Minkowski Metrics have a role to play in molecular analysis; Hamming Distances (HD), Manhattan Distances (MD) and Euclidean Distances (ED). Both MD and ED allow quantitative analysis, whereas HD allows only qualitative analysis. This paper presents a user-friendly approach to the use of Minkowski Metrics. Raw data can be prepared either by scoring bands on a gel as being present or absent (effectively binary scoring) for HD, or by quantitative scoring of bands by densitometry scanning for MD or ED. These scores are entered into a spreadsheet and a pair wise comparison between individual lanes is performed. These data are tabulated as a matrix in the format that can be used as the input file in the NEIGHBOR program within the PHYLIP suite of programs. The output tree file is viewed and used to assess which profiles on the original gel are most similar. The use of Minkowski Metrics can be applied to a range of analytical techniques, including those generated by Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE) and Single Strand Conformation Polymorphisms (SSCP).

The so-far-uncultivated bacterial candidate division "Termite Gro up 1"consists of cytoplasmic symbionts of termite gut flagellates. U. Stingl[a], R. Radek[b], A. Brune[a,c] ([a] LS Microbial Ecology, University of Konstanz, 78457 Konstanz, Germany; [b] AG Protozoology, FU Berlin, 14195 Berlin, Germany; [c] Dept. of Biogeochemistry, MPI for Terrestrial Microbiology, 35043 Marburg, Germany).

In the guts of wood-feeding lower termites, lignocellulose is digested by a specialized symbiotic system that includes specific protozoa and bacteria, mostly being restricted to this habitat. In earlier studies, the group of Ohkuma had shown that the gut of *Reticulitermes speratus* contains prokaryotes whose 16S rRNA genes are only distantly related to those of other bacteria; they were preliminarily classified in a candidate division called "Termite Group 1" (TG-1). By combining a cloning analysis of the bacterial 16S rRNA genes in flagellates separated with microcapillaries from gut suspensions of *Reticulitermes santonensis* and fluorescence in situ hybridization with specifically designed oligonucleotide probes, we showed that TG-1 bacteria are endosymbionts occurring in high numbers in two species of gut flagellates: *Trichonympha agilis* (Hypermastigida) and *Pyrsonympha vertens* (Oxymonadida). Transmission electron

microscopy of ultra-thin sections of gut flagellates revealed that the not yet cultivated TG-1 bacteria are small gram-negative rods with an average length of 0.6 μm and an average diameter of 0.3 μm located within the cytoplasm of their hosts. In the case of *Trichonympha*, these endosymbionts possess extraordinary extensions of the outer membrane. A PCR-based survey with TG-1 specific primers revealed their presence in the guts of all lower termites investigated so far. The results of a detailed phylogenetic analysis support the coevolution between TG-1 bacteria and their flagellate hosts.

Characterization of the microbial diversity of pig intestinal tract by restriction fragment length polymorphism. A.M. Pérez de Rozas[a], M. Roca[a], S.M. Martín-Orúe[b], J.F. Pérez[b], S. Campoy[a], J. Barbé[a], I. Badiola[a] ([a] CReSA (UAB-IRTA) Bellaterra, Barcelona, Spain; [b] Departament de Ciència Animal i dels Aliments UAB Bellaterra, Barcelona, Spain).

Because only a small portion of the intestinal bacteria is cultivable, the development of molecular techniques, such as restriction fragment length polymorphism (RFLP), allows to acquire a better knowledge of bacterial diversity with no culture requirements. Thirty-two animals received a basal diet with different additives (none, antibiotic, acidifier and plant-extract), and the contents of different intestinal sections were collected. A segment of 16S-rDNA was amplified by PCR and digested with four restriction enzymes (*AluI, RsaI, HpaII, CfoI*). The fragments were analysed by gel electrophoresis. The degree of microbial biodiversity (measured as the number of different bacterial species that are compatible with the theoretical RFLP profile obtained from the Ribosomal Database Project using the same primers and restriction enzymes), and the frequency of detection of certain bacterial genera was determined. The degree of biodiversity was lower in proximal sections than in distal sections. No significant differences were observed between the different sections of the small intestine (proximal jejunum, distal jejunum and ileum) or between the sections of the hindgut (caecum, proximal and distal colon). A greater microbial biodiversity was observed in the group that received a diet with the acidifier. Concerning the frequency of detection of some bacterial genera and species, the following was observed: *Bacillus*, *Lactobacillus* and *Enterococcus* were detected in all gut sections of all animals, whereas *Bacteroides, Ruminococcus* and *Clostridium* were only detected in some gut sections of some animals. The different treatments had a significant effect on the detection rate of *Campylobacter*, *Desulfitobacterium* or *Peptostreptococcus*.

High stability of the human faecal microbiota over time revealed by temporal temperature gradient-gel electrophoresis analysis. K. Saunier, C. Lay, P. Lepage, L. Rigottier-Gois, M. Sutren, J. Doré (Unité d'Écologie et de Physiologie du Système Digestif, INRA, Jouy-en-Josas, France).

The stability of the human faecal microbiota over time has to date been described for a limited number of subjects (3 individuals in 2 studies). The aim of the present study was to confirm the reported stability using a larger cohort of individuals. Two faecal samples from 21 healthy adults (12 women-9 men, from 25 to 45 years old with a mean age of 33) were collected 3 months apart (t0 and t1) for each donor. After DNA extraction and amplification of the V6-V8 region of 16S rDNA for each faecal sample, Temporal Temperature Gradient gel Electrophoresis (TTGE) was done to evaluate dominant bacterial species diversity. GelCompar software (version 2.0 Applied Maths, Belgium) was used to assess the degree of similarity between the TTGE profiles. Methodological variability was evaluated by comparison of 3 repeats for 6 faecal samples. Variations in dominant microbiota were assessed for each individual over time and between individuals. The average similarity percentage due to methodological variation was 95.8 ± 2.6% [range: 91.3 to 98.8]. Intra-individual similarity of the dominant faecal microbiota between t0 and t1 was 87.9 ± 6.7% on average [70.9 to 96.2]. Inter-individual similarities were 66 ± 12.3% [32.2 to 90.3] for t0 and 68.2 ± 11% [32 to 93.4] for t1. No sex and age effects were observed. With a methodological reproducibility of 96% for TTGE, we conclude that dominant species diversity of the human faecal microbiota

remains highly stable over 3 months. Nevertheless, changes in intensities of a few bands were seen over time in most of the subjects (18/21), indicative of species level modulations.

Development of solution phase hybridisation PCR-ELISA for the quantification of *Pediococcus pentosaceus* in Nurmi-type cultures. S.M. Waters[a], S. Doyle[b], K.A. Horgan[a], R.A. Murphy[a], R.F.G. Power[c] ([a] Alltech Biosciences Centre, Dunboyne, Co. Meath, Ireland; [b] National Institute of Cellular Biotechnology, Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland; [c] Alltech Inc., North American Bioscience Center, 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA).

Nurmi-type cultures (NTCs), derived from the fermentation of caecal contents of specifically pathogen-free (SPF) birds, have been successfully used for decades to prevent salmonella colonisation in chicks. The quantification of specific bacterial species in these complex ill-defined cultures has mainly been performed until now by culture-dependent techniques such as the determination of colony forming units on selective media. However, limitations of this approach include its low sensitivity and reproducibility, lack of selectivity due to the nature of the accompanying microflora and its time consuming aspect. Furthermore, all culture media fail to identify organisms that are in a physiological state not conducive to growth. The purpose of this study was to develop a sensitive, reliable and reproducible PCR-ELISA procedure for the detection and quantification of a constituent probiotic species, *Pediococcus pentosaceus*, in NTCs. In this technique, biotin-labelled primers were designed to amplify a species-specific fragment of the single copy *Pediococcus pentosaceus* glutamate racemase gene. Resulting amplicons were hybridised with a dinitrophenol (DNP)-labelled oligonucleotide probe in solution and were subsequently captured on a streptavidin coated microtitre plate. The degree of binding was determined by the addition of IgG [anti-DNP]-horseradish peroxidase conjugate, visualised using a chromogenic substrate, tetramethylbenzidine. This novel quantitative method proved to be species-specific, sensitive and reproducible, detecting *P. pentosaceus* at levels as low as 5 CFU per PCR reaction. This methodology has tremendous application in monitoring alterations in the concentration of specific strains in gut microflora with changes in age, diet and over time.

***Desulfovibrio*-like bacteria comprise a significant fraction of the bacterial community at the hindgut wall of European cockchafer larvae (*Melolontha melolontha*).** M. Egert[a], U. Stingl[b], L.D. Bruun[a], B. Wagner[a], A. Brune[a,b], M.W. Friedrich[a] ([a] MPI for Terrestrial Microbiology, 35043 Marburg, Germany; [b] Microbial Ecology, Dept. of Biology, University of Konstanz, 78457 Konstanz, Germany).

The dense gut microbiota of terrestrial insects is generally thought to play an important role in the digestion of soil organic matter, however, both the diversity and function of the intestinal community is still poorly understood. We investigated the physicochemical conditions and microbial diversity in the midgut, hindgut, and their subcompartmental fractions (gut wall and lumen) of the phytophagous larva of the European cockchafer (*Melolontha melolontha*). Steep axial profiles and radial gradients of the redox potential and oxygen partial pressure were accompanied by marked differences between the bacterial community in the midgut and hindgut, as well as the hindgut wall and lumen. While the midgut compartment lacked a stable microbial community, the bacterial community at the hindgut wall was very similar among individual larvae. Based on a clonal analysis of 16S rRNA genes, *Actinobacteria*, *Bacillales*, *Lactobacillales* and γ-*Proteobacteria* were restricted to the hindgut lumen, whereas β- and δ-*Proteobacteria* occurred exclusively at the hindgut wall. T-RFLP and cloning analysis consistently revealed that the *Desulfovibrio* species comprised 10–15% of the bacterial community at the hindgut wall, which was confirmed by FISH with group-specific oligonucleotide probes. Moreover, T-RFLP profiles and the presence of the adenosine-5´-phosphosulphate reductase gene *apsA* in gut DNA extracts of larvae from other European populations suggest that sulphate reducers are regularly colonising the hindgut wall of *M. melolontha* larvae. Our findings represent clear indications for the topology of intestinal sulphate-reducing populations, present also in many other insects.

Bacterial diversity in the caecum of the rabbit. L. Abecia[a,b], N.R. McEwan[b], C.J. Newbold[c], M. Fondevila[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Spain; [b] Rowett Research Institute, Aberdeen, AB21 9SB, Scotland; [c] Institute of Rural Studies, University of Wales, Aberystwyth, Wales, UK).

Unlike most other commercially important animals, relatively little is known about the microbial diversity found in the gut of the rabbit. Previous studies on the composition of the caecum have concentrated on studying organisms which can be grown in vitro, the use of DNA probes to identify candidate organisms or the investigation of metabolic activities where the primary source of the activity cannot be identified. This work allows studies to be expanded to organisms which have not yet been isolated, or whose function is not yet determined. Total digesta was removed from the caecum of a rabbit and the DNA was extracted from it. Fragments of the range of 16S *rDNA* genes were amplified by PCR using "universal" bacterial primers. The resulting amplicons were cloned into the TA Vector (Invitrogen) and clones were selected at random for sequencing. DNA sequences were analysed by BLAST searching and "best hits" for all sequences were used to construct a tree to investigate the diversity of the organisms present. Tree construction was performed using the PHYLIP suite of programmes (DNAdist and Neighbor). The validity of the topography of the tree was determined by repeating the process using 1000 bootstraps. As with most similar studies, the majority of the sequences isolated from the caecal contents showed greatest similarity to sequences from uncultured organisms from other gut environments. Greatest similarity to organisms where the species was already documented included members of the *Clostridium* / *Eubacterium* cluster and species from the genus *Ruminococcus*.

Microbial counts in different sites of the gastrointestinal tract of the horse. D. Yañez[a], M. Moore-Colyer[a], A. Longland[b], C. Walsh[a], E. Bakewell[b], A. Kirk[c], E. Grime[a], A. Northover[a], D.R. Davies[b], G. Griffith[c], C.J. Newbold[a] ([a] Institute of Rural Science, University of Wales, Aberystwyth, UK; [b] Institute of Grassland and Environmental Research, Aberystwyth, UK; [c] Institute of Biological Science, University of Wales, Aberystwyth, UK).

Equines can gain between 60–70% of their energy from short chain fatty acids formed during microbial fermentation in their gastrointestinal tract. As part of an ongoing investigation of microbial activity in the equine gut we have determined microbial numbers and diversity in various compartments of the gut. Samples from 9 sites (stomach, small intestine, caecum, left and right ventral colon, left and right dorsal colon, rectal faeces and external faeces) of the gastrointestinal tract were taken from three freshly slaughtered grass-fed Welsh mountain ponies. Total and cellulolytic bacteria and anaerobic fungi were estimated by the most-probable-numbers method. Lactic acid bacteria numbers were estimated using MRS agar. Total bacteria were detected in every sampling site, while cellulolytic bacteria were not detected in the stomach or small intestine of the animals. Both populations tended to gradually increase their numbers from the beginning to the end of the gastrointestinal tract (24, 4, 16, 44, 21, 25, 36, 43 and $25 \times 10^7 \cdot g^{-1}$ digesta SED 11.2 for the total cultivable count and 0,0,5,31,14,13,27,10 and $29 \times 10^5 \cdot g^{-1}$ digesta SED 4.29 for the cellulolytic count respectively. Lactic acid bacteria were detected in the small intestine and all colon samples at circa 10^4–10^5 per g of digesta, no attempt was made to enumerate them in the other gut compartments. Conversely anaerobic fungal counts and distribution showed a high variability between animals with no clear pattern discernible between sampling sites.

Novel propionate producing bacteria are abundant along the porcine gastrointestinal tract. G. Skene[a], O. Højberg[b], B.B. Jensen[b], H.J. Flint[a] ([a] Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK; [b] Danish Institute of Agricultural Sciences, 8830 Tjele, Denmark).

The porcine gut contains a diverse population of commensal bacteria, which have a major impact on gastrointestinal (GI) function and health of the host. The major short chain fatty acids

(SCFA) in the porcine colon are acetate, propionate and butyrate. Propionate is considered important in preventing pathogen invasion, serves as an energy source for colonocytes and is gluconeogenic. Currently, there is a lack of information on propionate producers in the porcine GI tract. Our studies on 19 pigs resulted in the isolation of more than 58 propionate producing isolates, many of these from the proximal and distal wall of the ileum and colon. Analysis of 16S rDNA sequences indicated that these fall into three subclusters within the *Sporomusa* subgroup of cluster IX, and they showed less than 95% similarity to any database sequence of cultured bacterial species. All three subclusters had similar phenotypes. One of the three subclusters closest known relative was *Anaerovibrio lipolyticus*, but they differ from this organism in being able to hydrolyse starches, but not lipids. The two other subclusters showed closest similarity to *Selenomonas ruminantium* and the *Mitsoukella* species. All three subclusters showed good growth on lactate and on glycerol. Fermentation patterns revealed that all isolates are major propionate producers, depending on the substrate used. This work shows that major groups of so far unidentified bacteria can be cultured and characterised. These bacteria may be an important part of the microbiota involved in stabilising a healthy gut.

Culture-dependent and culture-independent analysis of the microbiota associated with *Ceratitis capitata*, the Mediterranean fruit fly reveal that *Enterobacteriaceae* constitute the dominant populations of the fly's gut. A. Behar[a,b], B. Yuval[a], E. Jurkevitch[b] ([a] Dept. of Entomology; [b] Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, 76100 Rehovot, Israel).

Many species of fruit flies (Diptera: Tephritidae) are infamous for their proclivity to oviposit in fruit, and hence cause serious damage to agricultural crops. The Mediterranean fruit fly *Ceratitis capitata*, is a polyphagous, cosmopolitan, ubiquitous, and invasive species. Throughout their lives, these flies have constant contact with a multitude of micro-organisms. However, the microbial populations associated with the medfly, their functions, and their roles in its life are still very poorly known. Such knowledge may lead the way to improved control of the pest – through direct targeting of the insect, or through improved rearing of sterile males released in the wild. We initiated a systematic study of the microflora of the medfly's gut, using both culture-dependent and culture-independent approaches. Fingerprinting using PCR-denaturating gradient gel electrophoresis (DGGE) analysis of 16S rDNA amplicons of pooled culturable populations and of directly amplified DNA purified from the guts, followed by the sequencing of isolated bands, revealed that *Klebsiella oxytoca*, *K. pneumoniae*, and *Enterobacter agglomerans* are always present in wild flies (male, female, larvae, adults, during the whole season) and constitute major populations. Also, differences in bacterial population profiles were seen between male and female flies during part of the active season (spring). In sterile males reared for release and F1 flies from the wild raised in the laboratory, only very few bacterial species were found in emerging adults, however diversity increased during the first days of adult life. Moreover, the fingerprints differed according to the type of diet provided (sugar, protein, or mixed diets).

Effect of feeding silage differing in water-soluble carbohydrate content on numbers and diversity of rumen micro-organisms. D. Yañez[a], N. Scollan[b], D.R. Davies[b], R. Dewhurst[b], R.J. Merry[b], R. Evans[b], C.J. Newbold[a] ([a] Institute of Rural Science, University of Wales, Aberystwyth, UK; [b] Institute of Grassland and Environmental Research, Aberystwyth, UK).

Grasses of high water soluble carbohydrate (WSC) content have been shown to increase animal performance as a result of improved rumen function. Previous studies carried out using the rumen simulating fermentor RUSITEC found that high WSC grasses and silages stimulated the fermentation and suggested shifts in the bacterial and protozoal populations. To assess the effects of feeding silages prepared from grass of differing WSC content on rumen microbial population, six rumen cannulated Hereford × Friesian steers were fed diets of a control silage prepared from perennial ryegrass (cv. Fennema; CGS) and a silage with higher residual sugar

content prepared from a high WSC perennial ryegrass (cv. Ba11353; HSGS) in a two period changeover experimental design. Liquid phase rumen samples were collected 2 h after feeding. Total and cellulolytic bacteria were estimated using a most-probable-numbers method and protozoa by direct counting. Bacterial and protozoal diversity in the samples was estimated by PCR-DGGE using, respectively, 16S and 18S ribosomal DNA specific primers. Cellulolytic bacteria numbers were lower (7.42 vs. 35.7 × $10^7 \cdot mL^{-1}$ respectively, $P = 0.004$) and total protozoa higher (13.2 vs. $8.12 \times 10^5 \cdot mL^{-1}$, $P = 0.025$) in the rumen fluid of animals fed the HSGS diet, however total bacteria were not significantly affected by the offered diet (3.71 vs. 2.41 × $10^9 \cdot mL^{-1}$). As expected the bacterial community displayed more diversity than the protozoal community as indicated by DGGE. Cluster analysis of DGGE band polymorphism showed that the experimental period explained most of the similarity in both bacteria and protozoa. However, within experimental periods, samples of amplified bacterial rDNA taken from animals fed the same diet shared the highest level of similarity, while no clear pattern was observed for rumen protozoa. Our results suggested that differences in silage WSC content can cause significant shifts in microbial community structure.

Effect of molasses diets on population profiles of rumen bacteria. M.X. Tolosa[a,b], T. Dinh Van[a], A.V. Klieve[b], D. Ouwerkerk[b], D.P. Poppi[a], S.R. McLennan[b] ([a] Schools of Animal Studies and Veterinary Science, University of Queensland, St Lucia QLD 4072, Australia; [b] Department of Primary Industries, Animal Research Institute, Yeerongpilly QLD 4105, Australia).

The objectives of this study were to obtain profiles of the predominant rumen bacterial species of beef cattle fed diets containing varying proportions of low-quality Pangola grass-hay and molasses. Four rumen-cannulated Brahman-cross steers were fed molasses as 0, 25, 50 and 75% of the diet once daily. Urea comprised 3% of the molasses. Steers were allocated to one of four diets in a 4 × 4 Latin square design with periods of 28 days. Following a 3-week adaptation period, rumen fluid samples were taken immediately prior to feeding and 8 h after feeding over two consecutive days. Total genomic DNA was extracted from the rumen fluid samples by bead beating. Subsequently, 16S rDNA was amplified by polymerase chain reaction with primers specific for the fragment between variable regions 2 and 3 (V2-V3) of the gene. The amplified V2-V3 products were analysed using DGGE. A comparison of two sets of four DNA samples, each set representing the two extreme dietary conditions (diets containing 0 and 75% molasses) belonging to two different animals, revealed the presence of dominant species in the rumen samples from the steer fed the highest molasses treatment that were not present in the samples obtained from the control steer. Furthermore, there were no differences in this steer between the DGGE banding pattern derived from the morning and afternoon, and between two consecutive days. Therefore, it can be concluded that predominant species of the rumen bacterial community appear to be particularly stable throughout the day and over at least a two-day period in animals fed a given diet. Further studies are being undertaken to identify those predominant species. A longer-term objective of this research is to investigate linkages between diet, rumen microbial populations and the efficiency of microbial protein production.

Differences in rumen microbial population induced by the quality of dietary forage. M. Fondevila[a], G. Muñoz[a], G. de la Fuente[a], M. Pérez-Quintana[b], J. Balcells[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain; [b] Departamento de Química y Biología, Universidad de Matanzas, Matanzas, Cuba).

Previous studies showed that the lower the quality of dietary forage, the higher the degradation of poor quality roughages. Therefore, rumen bacterial and protozoal populations of sheep given alfalfa hay (AH), untreated (US) or ammonia-treated (TS) straw were studied. Four rumen cannulated sheep were adapted to diets for 14 days in three consecutive periods. Samples for bacterial and protozoal counts were taken just before the morning feed. In addition, pH and volatile fatty acid concentration (VFA) were determined 0, 4 and 8 h after feeding. Average pH ranked US > TS > AH (6.87; 6.60 and 6.33; $P < 0.05$). The proportion of acetate was lower

and propionate was higher with the AH diet ($P < 0.05$). There were no differences ($P > 0.10$) among AH, US and TS in total (8.89; 8.82 and 9.17), cellulolytic (7.89; 7.79 and 7.90) or xylanolytic (8.86; 9.13 and 8.97) bacterial counts ($\log_{10}$·mL^{-1}). The protozoal concentration ($\log_{10}$·mL^{-1}) tended ($P = 0.08$) to be higher with AH than US (5.25; 4.91 and 5.04 for AH, US and TS). The proportions (%) of *Entodinium* were lower (69.4; 87.1 and 87.2), and *Dasytricha* higher (13.5; 3.8 and 3.6) in AH than in US and TS, whereas no differences were observed in the *Diplodiniinae* subfamily. *Ophryoscollex* (5.5%) and *Epidinium* (< 1%) protozoa were only observed with AH, whereas *Isotricha* (< 1%) appeared in AH and TS diets. No quantitative or qualitative differences in microbial populations were observed between the straw diets. Although the bacterial population did not differ among the diets, AH promoted a higher protozoal diversity.

Denaturating gradient gel electrophoresis used to investigate alternative strategies to in-feed antibiotics in weaned piglets. F. Duffes, C. Perrier, P.L. Lallès, B. Sève (INRA-UMRVP-Rennes, Domaine de la Prise, 35590 St-Gilles, France). Supported by the European project Healthypigut (contract No. QLK5-CT2000-00522).

The newborn sterile gut is rapidly colonised by maternal and environmental microbes. This colonisation starts with lactic acid bacteria, enterobacteria and streptococci. After the introduction of solid feed, such as at the time of weaning in pigs, obligate anaerobes increase in number and diversity until an adult-type pattern is achieved. Gut microflora participate in health maintenance by forming a barrier preventing gut invasion by pathogenic bacteria, a phenomenon known as colonisation resistance. Fibre-rich diets are occasionally recommended in poor sanitary situations because they regulate feed intake and enhance the gastro-intestinal tract development and the installation of barrier flora. Therefore gut health can be potentially improved or restored by dietary manipulation. Studies on gut microbiology during the period of weaning in pigs are rare and deal with classical culture techniques. Novel molecular techniques provide a unique opportunity for investigating bacterial diversity, including non-cultivable genera/species, along the gut. A combination of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) can be used to study the microbial diversity in weaning piglets that were fed diets containing low (wheat and barley) or high (pea) digestible proteins and soluble (beet pulp) or insoluble fibres (bran). The results indicate that dietary fibres could positively modify gut microflora in weaned piglets.

Quantification of cellulolytic bacteria in the rumen using real-time PCR. F. Rieu-Lesme, P. Mosoni, E. Forano (Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

Real-time PCR technology was applied to the quantification of cellulolytic bacteria in the rumen of cows. The aim of this study was to examine the effect of two different diets on the distribution of the three main fibrolytic species: *Fibrobacter succinognenes, Ruminococcus albus* and *Ruminococus flavefaciens.* Rumen contents were collected from eight cows, four cows were fed with hay and concentrate and the four others were grazing cows (intensive vs extensive rearing). Four protocols for genomic DNA isolation were compared to give the best quality of extracts with good yields. Species-specific primers targeting a 445 bp fragment from the 16S rDNA of *F. succinogenes* and a 175 bp fragment from *R. albus* were used in real-time polymerase chain reaction assays conjugated with the fluorescent SYBR Green I dye. Serial dilutions of the 16S rDNA gene (10^2 to 10^8 copies) of the reference strain of each species were used to construct the calibration curves. These curves showed linearity between the log values of 16S rDNA copy number of each species and real time PCR threshold cycles. The PCR efficiencies were between 96.2 and 100% ($r = 0,99$). The results are expressed as 16S rDNA copy number of the target species/µg total DNA. Statistical analysis run on SAS 8.1 software (GLM procedure) showed that the number of *F. succinogenes* was significantly higher ($P < 0,0001$, $F_{diet} = 40,51$, 1df, $n = 15$) in the rumen of cows fed with grass compared to the rumen of cows fed with hay and concentrate, while the number of *R. albus* was not affected by the diet ($F_{diet} = 1,67$). Quantification of *R. flavefaciens* is currently under study.

Detection of Crenarchaeota in the rumen of cows. F. Rieu-Lesme (Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

The purpose of this study was to determine if Crenarchaeota are present in the rumen, an environment in which they have not been previously observed. Real-time polymerase chain reaction amplification on the LightCycler technology was performed with new primers specific for non-thermophilic Crenarchaeota 16S rDNA on the rumen of eight cows fed under two different dietary regimes (concentrate vs grass). Crenarchaeota were detected in all samples. A specific fragment (220bp) for 16S rDNA of group1 Crenarchaeota was amplified from total genomic DNA of the microorganisms present in the rumen content of the eight animals. Further experiments are planned to quantify these Crenarchaeota in the rumen ecosystem. This study represents the first report showing the presence of Crenarchaeota in the rumen and supports the view that these microrganisms are cosmopolitan. Indeed mesophile Crenarchaeota have not been isolated yet. Therefore, we are trying to find experimental conditions to purify these organisms from the rumen, in order to study their physiology, biochemistry, and ecological role. The detection of Crenarchaeota 16S rDNA sequences raises questions regarding their functional significance and what role they may play in environments such as the digestive tract.

Comparison of the microbiota attached to insoluble food substrates with the planktonic population from in vitro human colon simulations. E.C. Leitch, A. Walker, S.H. Duncan, H.J. Flint (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Food particles that reach the colon consist predominantly of insoluble fractions recalcitrant to digestion by human enzymes. In the colon, these particles are fermented by the resident microbiota. Although molecular approaches have been successfully used to identify the major bacterial species that make up the resident gut microbiota, little is known about the microbial species that colonise insoluble food particles within the colon. We sought to determine the major bacterial species that attach to insoluble substrates and to determine their comparative significance within the planktonic population as a whole. The insoluble fractions of various food substrates were incubated in colon simulators inoculated with faecal samples. The attached and planktonic populations were analysed using fluorescent in situ hybridisation (FISH) and denaturing gradient gel electrophoresis (DGGE). Amplified total community small subunit rDNA was used to generate clone libraries and the sequences obtained allowed species identification. Overall, the major species that attached to the insoluble substrates comprised a subsection of the species found in the general bacterial population. This suggests that the attached population is a specialised community.

Rumen microbial population dynamics in response to photoperiod. N.R. McEwan, L. Abecia, M. Regensbogenova, C.L. Adam, P.A. Findlay, C.J. Newbold (Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB, Scotland, UK).

The Soay sheep breed is native to the Scottish Hebridean island. They are highly inbred, thereby reducing the potential for host animal genetic variability. They also show high periodicity, with their dietary intake being greater in the summer, relative to that seen in the winter. Soay sheep were housed under long day or short day (16 or 8 h light per day respectively) conditions. The animals were housed under these conditions for 12 weeks. During this time, the animals were given unrestricted access to food, with daily dietary intake being monitored. At the end of 12 weeks, the animals were weighed prior to being euthenised. Rumen digesta was collected and frozen immediately. DNA were isolated from the digesta and fragments of both the protozoal and bacterial small sub-unit RNA genes were amplified for DGGE analysis. DGGE gels were analysed by using pairwise Hamming Distance analysis. The animals housed under long day conditions were shown to have a significantly higher dietary intake [1277 g DM per day (SEM = 71.8 g), as opposed to 729 g DM (SEM = 36.3 g)], which is reflected by a higher body weight [43.0 kg (SEM = 1.14 kg) and 38.8 kg (SEM = 1.37 kg)]. Hamming Distance analysis of the DGGE gels with protozoal 18S genes showed no difference between the animals

housed under different light conditions. However, the bacterial DGGE profiles showed a split between the profiles from short day and long day housed animals. We conclude that there is a shift in the major bacterial population, but not the protozoal population, in the rumen as a result of intake levels, despite the composition of the diets being identical.

Daidzein increased the density but not the composition of the *Lactobacillus* community in piglet digesta during in vitro fermentation as revealed by DGGE and dilution PCR. W. Yao[a,b,c], W.Y. Zhu[a], Z.K. Han[a], B.A. Williams[c], S. Tamminga[c], A.D.L. Akkermans[b] ([a] Laboratory of Gastrointestinal Microbiology, Nanjing Agricultural University, 210095 Nanjing, PR China; [b] Laboratory of Microbiology, Wageningen University, The Netherlands; [c] Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands).

To investigate the effect of daidzein on *Lactobacillus* community change during in vitro fermentation, digesta from 12 conventionally raised piglets of the same litter with three on each slaughtering day were used as inocula in in vitro fermentation treatments: (a) VanSoest medium with 0.5 g of glucose and 50 $mg \cdot kg^{-1}$ of daidzein; (b) VanSoest medium with 0.5 g of glucose; (c) VanSoest medium only. After 48 h fermentation, DNA was isolated from culture pellets and *Lactobacillus* specific PCR primers, *Lab0677r* and *Bact0011f*, were used to selectively amplify 16S rDNA. V1-V3 regions of the lactobacilli 16S rDNA were further amplified using nested PCR with primers Univ0515rGC and Lab159f. PCR amplicons were analyzed using denaturing gradient gel electrophoresis (DGGE). All samples showed similar change in DGGE pattern after fermentation; some dominant bands disappeared and the density of some dominant bands obviously increased, suggesting that some species may be enriched while others may not be able to grow in the culture. DGGE patterns were different between digesta from different GI compartments either before or after fermentation, but no difference was observed between the treatments for each digesta sample. Dilution PCR with primers *Lab0677r* and *Bact0011f* was then applied to semi-quantify lactobacilli and showed that daidzein significantly increased the number of lactobacilli after 48h fermentation. A similar effect was observed with the plate counting method. In conclusion, daidzein did not affect *Lactobacillus* composition, but significantly enriched some *Lactobacillus* species, suggesting that daidzein may have the potential for use as a prebiotic substance in animal feed.

Lactic acid producing bacteria from the gastrointestinal tract of different host species, conserved and diverse microbial populations. R.A.M. Al Jassim[a], P.T. Scott[b] ([a] School of Animal Studies, The University of Queensland, Gatton 4343 Australia; [b] School of Agronomy and Horticulture, The University of Queensland, Gatton 4343 Australia).

The relationship between host animals and the microorganisms that inhabit the gastrointestinal tract is a product of evolution that has occurred over millions of years. The anatomical and physiological characteristics of the gastrointestinal (GI) tract of these animals and their feeding behaviour determine the types of microorganisms and their respective numbers. Despite the fact that many bacterial species inhabit a wide range of animal species, there is a clear host preference found with lactic acid producing bacteria (LAB). In a series of experiments using cattle, sheep, camels, horses, and pigs, the contents from different segments of the GI tract were cultured in modified semi-selective MRS agar medium and the isolates were identified by DNA sequences analysis of the 16S rDNA, confirming the broad host range of some LAB but demonstrating the more narrow host range for other isolates, particularly some true lactobacilli. Dietary changes alter microbial populations but maintain bacterial diversity as for normal conditions. The major LAB in ruminants are *Streptococcus bovis*, *Selenomonas ruminantium*, and *Lactobacillus vitulinus*. In horses, in addition to *S. bovis*, and *S. equinus*, other species including *Lactobacillus salivarius*, *Lactobacillus mucosae*, *Lactobacillus delbrueckii*, and *Mitsuokella jalaludinii* are found to be the key true lactobacilli. In pigs, *Lactobacillus ruminis*, *Enterococcus faecium* and *Mitsuokella multiacidus* are the dominant LAB. The results of these studies clearly show that true lactobacilli are specific to simple stomached animals while streptococcal bacteria are the predominant LAB in ruminants.

Changes of bacterial population connected with bowel diseases. J. Kopečný[a], J. Hajer[b], J. Mrázek[a] ([a] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídeňská 1083, Prague 4, Krč, Czech Republic; [b] IInd Clinic of Internal Medicine, University Hospital Královské Vinohrady, Charles University, Prague 10, Šrobárova 50, Czech Republic).

The bacterial microflora in the human intestinal tract represent an extremely complex yet relatively stable ecological community. The traditional methods for identifying bacteria are able to recover between 20 to 40% of bacterial species. In contrast, molecular biology techniques allow a rapid and specific detection of a wide range of bacterial species and have become a key procedure for identifying microorganisms in similar complex ecosystems. DNA from fecal samples were isolated with mechanical bead beating or chemical extraction methods. Specific PCR primers were selected in order to detect the following bacterial species in the feces of healthy persons and in the stools of patients with indicated irritable bowel syndrome or a different form of inflammatory bowel diseases: *Faecalibacterium prausnitzii, Eubacterium limosum,* cellulolytic species *Ruminococcus albus and R. callidus* and groups of anaerobic bacteria *Prevotella, Bifidobacterium, Butyrivibrio, Pseudobutyrivibrio, Bacteroides fragilis, Clostridium coccoides,* sulfate reducing bacteria, methanogenic. Simultaneously, the bacterial spectrum in fecal samples was observed with DGGE using primers for 16S DNA of prokaryotes, bifidobacteria and methanogens. Gradient electrophoresis gave more complex results about the bacterial population shifts in the patient's fecal samples. The aim of the study was to prove the changes in the microbial population observed by classical methods. In healthy persons, most of the selected groups of bacteria were detected. Unfortunately, not all fecal samples harbored cellulolytic species. The patient's digesta showed much higher variability in their colonic bacterial population and in almost all cases the cellulolytic bacteria were missing.

Evaluation of PCR detection methods for *E. coli* O157 in environmental samples. D. Al-Ajmi[a,b], J. Padmanabha[b], S. Denman[b], R.A.M. Al Jassim[a], C.S. McSweeney[b] ([a] School of Animal Studies, University of Queensland, Gatton, 4343, Australia; [b] CSIRO Livestock Industries, St. Lucia, 4067, Australia).

Enterohaemorrhagic *E. coli* (EHEC) serotype O157:H7 is implicated in causing outbreaks and sporadic cases of food and waterborne diarrheal diseases associated with hemorrhagic colitis and haemolytic uraemic syndrome in humans. In Australia, contamination of meat for human consumption with these pathogenic serotypes of *E. coli* is an issue of increasing concern to both meat processors and the livestock production community. Assessment of the extent to which carcasses are at risk of being contaminated with pathogenic *E. coli* populations while on various diets, may enable the livestock industry to devise feeding systems thereby reducing the contamination of the carcasses destined for human consumption. As a result, a specific, sensitive, economical and rapid method of screening for *E. coli* O157:H7 on the environmental samples will be required. Therefore, an experiment was conducted to establish a multiplex PCR method to simultaneously detect 5 different genes known to be specific for *E. coli* O157:H7 from a faecal sample. The genes selected were eaeA, gatD, rfbE and uidA. The 5 pairs of primers that specifically amplify the genes above, were tested to detect O157:H7 in the environmental sample using various extraction procedures. This multiplex PCR assay should provide a convenient method of screening for *E. coli* O157:H7 from a large number of environmental samples.

The influence of different types of fibre on the abundance of the major bacterial groups in the pig rectum measured by FISH. M. Castillo[a], G. Skene[b], S.H. Duncan[b], H.J. Flint[b], S.M. Martín-Orúe[a] ([a] Dept. Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Bellaterra, 08193, Barcelona, Spain; [b] Microbial Genetics Group, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, UK).

The establishment and maintenance of a stable microbiota is crucial for the good health of pigs since young animals are often susceptible to gastrointestinal tract infections. An experiment was designed to quantify bacterial shifts in the major bacterial groups in the rectum of growing pigs

after the administration and comparison of 4 different diets. Thirty-two growing pigs were randomly distributed into four treatments. The animals were fed ad libitum for 6 weeks with a commercial diet (15% barley, 28% soya 44, 54% corn and vegetable oil (basal diet, A). Fine-sized corn of basal diet (2.5 mm) was changed to 4 mm corn (B treatment), and sugar beet pulp (8%) or bran (10%) were added to the basal diet in treatment C and D respectively. After the experimental period, the animals were slaughtered and the GI tract was sampled. Digesta samples were diluted and fixed with formaldehyde prior to analysis. Fluorescent in situ hybridisation (FISH) was used to quantify bacterial populations by using five probes: EUB338, EREC482, RFLA729 plus RBRO730 and FPRA645 to quantify total bacteria and the predominant groups of low G+C Gram positive bacteria belonging to clusters IV and XIVa in samples of rectum digesta. Total bacteria were 1.98E11 ± 6.63E10 bacteria·g^{-1} fresh matter and cluster XIVa and ruminococci were the predominant bacterial groups in all treatments. *Faecalibacterium* spp. represented 1.3 ± 0.3% of the total bacterial population. The administration of these diets did not significantly change the bacterial populations of the pigs and each diet maintained high proportions of the cluster IV and XIVa groups.

Effect of dietary changes on the bacteriophage population in the rumen of sheep. R.A. Gilbert[a], J. Shepherd[b], A.V. Klieve[c], J.V. Nolan[d], C.J. Newbold[b], R.J. Wallace[b] ([a] CSIRO Livestock Industries, Queensland Bioscience Precinct, St Lucia, Qld 4067 Australia; [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [c] Animal Research Institute, Queensland Department of Primary Industries, Moorooka, Queensland 4105, Australia; [d] University of New England, Armidale NSW 2351, Australia).

The effect of alternating the diet from a hay-based to a silage-based diet on the rumen phage population of four cannulated sheep was examined in a 2 × 2 factorial design experiment. Two animals were fed each diet during two 20 d feeding periods, each consisting of an initial 14 d feed adaptation period and 6 d monitoring period, in which rumen fluid samples were collected on days 15 and 20, immediately before feeding and again at 2 h and 6 h after feeding. Phage DNA extracted from rumen fluid samples was used to determine rumen phage concentration, and phage population diversity was determined by pulsed field gel electrophoresis separation of phage DNA. In addition, rumen bacteria and protozoa were enumerated and pH, and concentrations of volatile fatty acids, ammonia and L-lactate, were determined. No significant differences occurred in either the size or diversity of the rumen phage population between sheep fed silage or hay-based diets. These findings contrast with previous studies that have shown the size of the rumen phage population to vary dramatically between different dietary regimes. Other parameters of fermentation and microbial composition suggested that the rumen microbial population may not have altered significantly between the two diets and therefore did not cause major changes in the size and relative composition of the rumen phage population.

Functional and ecological characterization of newly isolated *Fibrobacter succinogenes* strains in relation to their phylogeny. T. Shinkai, N. Matsumoto, Y. Kobayashi (Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan).

Fibrobacter succinogenes strains were newly isolated from the rumen of sheep receiving orchardgrass hay, orchardgrass pasturage, alfalfa hay or rice straw. Then, rumen solid, liquid and ruminally incubated forage stems were the bacterial sources. Thirty-three strains in total were obtained mainly from ruminally incubated forage stems. Most of these (29 of 33) were affiliated with group 1, based on their partial 16S rDNA sequences. Four strains were classified into group 2, and then two of these formed a novel sub-group, the branching of which was supported by an 82% bootstrap value. Only these two strains produced yellow pigments when cultured with Avicel. Group 1 strains showed faster growth to a higher extent than group 2 strains when cultured with Avicel. Although there were variations in number and molecular size of CMCases that were detected on SDS-polyacrylamide gel by activity staining, group 2 strains possessed 112kD CMCase as a common enzyme. Meanwhile, strains belonging to groups 1 and 2, when grown on Avicel, showed no particular

difference in specific activity of CMCase and in adherence to Avicel and three natural substrates (orchardgrass, alfalfa and rice straw). The ruminal distribution of groups 1 to 3, calculated based on PCR-RFLP analysis, showed that group 1 is dominant (57–75%) irrespective of the diet given, followed by groups 3 (18–26%) and 2 (7–17%). From these functional and ecological data on *F. succinogenes* strains, it is thought that group 1 could contribute to ruminal fiber digestion more than the other groups.

H_2/CO_2 metabolism in the human gut: In vitro study of the relationships between methanogens and reductive acetogens. C. Del'Homme, C. Chassard, A. Bernalier-Donadille (Unité de Microbiologie, INRA, C.R. de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

In humans, dietary fibres are transformed by the intestinal microflora in fermentative metabolites, mainly short chain fatty acids (acetate, propionate and butyrate) and gases (H_2,CO_2 and in some cases CH_4). Gases are eliminated from the colonic ecosystem through excretion in breath and flatus. However, H_2 and CO_2 are mainly eliminated in situ by hydrogenotrophic microorganisms represented by methanogenic archaea and reductive acetogenic bacteria. Methanogens are known to outcompete acetogens for H_2/CO_2 utilisation in diverse ecosystems. In methane-excreting individuals, the acetogen population was indeed detected at very low levels in the faeces, but specific inhibition of methanogenesis revealed the presence of an active H_2-utilising acetogenesis. The objective of our study was therefore to clarify the nature of the interaction existing between these two hydrogenotrophic microflora in the colon. The associations of *M. smithii*, the predominant methanogen and *R. hydrogenotrophicus*, acetogen isolated from human faeces, were performed in vitro in batch culture with H_2/CO_2 in the gas phase. The effect of different physico-chemical parameters [H_2/CO_2 partial pressure (PP), presence of glucose], on the expression of this microbial interaction was investigated. The results showed that the two hydrogenotrophic microorganisms both maintained their growth in the cocultures, demonstrating that *M. smithii* did not outcompete the acetogen in vitro whatever the culture conditions applied (gas PP or presence of glucose). However, H_2/CO_2 metabolism by *R. hydrogenotrophicus* seemed to be less effective in all the co-cultures than in its monoculture while CH_4 production by *M. smithii* appeared similar in co- and mono-cultures of this strain. The mixotrophic ability of the acetogen did not seem to confer a sufficient energetic advantage to this strain for H_2/CO_2 competition with methanogens. FISH analysis of the two microbial populations in mono- and cocultures should provide complementary information on this microbial interaction.

Genome sequencing of *Clostridium proteoclasticum*. G. Attwood, A. Cookson, B. Kelly (Rumen Microbial Functional Genomics, Nutrition and Behaviour Group, AgResearch, Grasslands, Palmerston North, New Zealand).

Our group is sequencing the genome of *Clostridium proteoclasticum* a gram positive, butyrate-forming rumen bacterium, closely related to *Butyrivibrio hungatei*. This organism is common in New Zealand ruminants and is involved in ruminal protein and xylan degradation. Large and small insert libraries were generated from *C. proteoclasticum* DNA and clones were sequenced to give 9× coverage of the genome. After the high throughput sequencing stage, the sequence was assembled into 547 contigs ranging in size from < 2 up to 370 kb. Primer-walking along clones with inserts greater than 2 kb was carried out and assembly of this sequence reduced the number of contigs greater than 2 kb to 146. Closing of the sequence is continuing. The available sequence suggests the genome to be 3.99 mb in size which was verified by pulsed-field gel electrophoresis of genomic DNA digests. Pulsed-field gels of genomic DNA also identified 3 large extrachromosomal elements, possibly megaplasmids, of approximately 360, 320 and 190 kb. A large contig (360 kb) in the sequence probably represents the complete sequence of the largest putative megaplasmid. The analysis of the genome sequence found 3 653 open reading frames, of which 52.3% have been assigned tentative functions; the remainder are either conserved hypothetical proteins, or hypothetical proteins of unknown function.

pS86/pEF47 related plasmids are frequently encountered in Gram-positive cocci. A. Sprincova, V. Stovcik, P. Javorsky, P. Pristas (Institute of Animal Physiology, Slovak Academy of Sciences, Šoltesovej 4–6, 04001 Košice, Slovak Republic).

The usual ecological niche for *Enterococcus* species is the intestine of humans and other animals, but *enterococci* are ubiquitous and can be found free-living in the soil, on plants, or in dairy products. The appearance of strains resistant to many antibiotic therapies has become an important public health concern. *Enterococci* are infamous for their ability to transfer their resistance genes to other *Enterococci* and even other genera via plasmid transfer. However little is known about plasmid contents of enterococci. Plasmid pEF47 was isolated from a rumen isolate of *E. faecalis* and analysed. Restriction mapping and partial nucleotide sequence comparisons indicated that the pEF47 plasmid is closely related to the pS86 plasmid, previously characterised from a non-related human isolate of *E. faecalis*. PCR analysis targeted at the pEF47/pS86 replication protein confirmed the similarity of plasmids as well as the wide occurrence of related plasmids in Gram-positive *Enterococci*. Tetracycline resistance (*tet*L) encoding plasmid pAMalfa1 belongs to this family as well. Three hundred Gram-positive cocci isolated from 3 different sheep (either from the rumen or faeces) were tested for tetracycline resistance. The rate of resistance to tetracycline was up to 30% in both rumen and faeces isolates. Isolates harboured *tet*L and *tet*M, respectively. All *tet*L positive isolates were then tested for the presence of the pS86 replicon but no correlation between the occurrence of the *tet*L tetracycline resistance determinant and the presence of the pS86 replicon was observed.

Occurrence of restriction-modification systems in *Butyrivibrio* and *Pseudobutyrivibrio.* J. Mrázek[a], M. Piknová[b,c], P. Pristaš[b], J. Kopečný[a] ([a] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídeňská 1083, 14220, Prague 4, Czech Republic; [b] Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4–6, 04001 Košice, Slovak Republic; [c] Institute of Chemistry, Faculty of Science, P. J. Šafárik University, Moyzesova 11, 04001 Košice, Slovak Republic).

Motile and butyrate producing anaerobic bacteria are one of the most abundant bacteria in the rumen, and are represented mainly by *Butyrivibrio* and *Pseudobutyrivibrio* strains. Because of a possible and easy transformation of these bacteria with plasmid vectors, there is a need to characterise their DNA protection system. Thirty-seven strains of ruminal bacteria belonging to the *Butyrivibrio* and *Pseudobutyrivibrio* species were screened for the presence of site-specific restriction endonuclease and modification methyltransferase activities. Seven strains possessed endonuclease activities detectable in crude cell extracts. The recognition sequences and optimal reaction conditions for seven of them were determined. Five enzymes were found to be isoschizomers of type II endonucleases (EcoRV, NsiI, AseI (2×) and SauI), one was type IIS (FokI) and one remained unknown. The optimal reaction was found to be in low ionic strength buffer and all enzymes possessed sufficient activities at 39 °C. The presence of a DNA modification system among all the strains was also determined. In most of the isolates the high methylation activities correlated with the presence of restriction nuclease. Only a few strains possessed unaccompanied modification methyltransferases.

Characterisation of adaptive resistance in *Prevotella bryantii* to the growth promoting antibiotic flavomycin. J.E. Edwards, R.J. Wallace, N.R. McEwan (Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, Scotland, UK).

Flavomycin is a bacteriostatic antibiotic, which acts by inhibiting the transglycosylation reaction during peptidoglycan biosynthesis. Preliminary observations in vitro demonstrated that several species of rumen bacteria were able to increase their tolerance to this antibiotic with *Prevotella bryantii* displaying the largest change, with a four-fold increase in resistance. The basis of this phenotypic observation was investigated further. Growth of *P. bryantii* in the presence of flavomycin was characterised by a concentration dependent increase in the length of the lag phase, which could be decreased by prior exposure to

flavomycin. This adaptation was reversible after prolonged growth in the absence of the antibiotic. Growth of a clonal line of *P. bryantii* on agar, containing a flavomycin concentration gradient, was characterised by an advancement of the growth zone along the gradient with time. Investigation of the cell viability in the presence of the antibiotic confirmed that a proportion of the cells present were less sensitive to flavomycin, suggesting that a difference in the growth or metabolic state of the cells conferred tolerance. Flavomycin tolerance was not the result of a general stress response since increased tolerance was only observed for antibiotics that also targeted peptidoglycan synthesis, although this increase was marginal compared to that of flavomycin. Comparison of the proteome of flavomycin adapted *P. bryantii* with unadapted and heat shocked cells demonstrated that three protein spots specifically increased in intensity as a result of flavomycin adaptation. The potential role of these proteins within the adaptive resistance mechanism is discussed.

Horizontal gene transfer from soil to rumen bacteria. K. Tóthová, K. Nigutová, R. Malík, P. Pristaš, P. Javorský (Institute of Animal Physiology Slovak Academy of Sciences, Šoltésovej 4–6, 04001 Košice, Slovakia).

Ruminants can be exposed to toxic concentrations of different environmental pollutants, including heavy metals, by consumption of contaminated feed and water. The ingested toxic elements can be inhibitory to both the fermentative activity and growth of the microbes, thereby changing the physiological steady-state of rumen fermentation. Some of the ruminal bacteria, e.g. *Butyrivibrio fibrisolvens*, *Megasphaera elsdenii* and *Selenomonas ruminantium* exhibit intermediate sensitivity to heavy metals, while e.g. *Streptococcus bovis* is very refractory mainly to mercury. Microbes may modify the toxicity of the elements to the animal by decreasing the toxicity e.g. sulphide production resulting in the precipitation of heavy metals. An alternative mechanism of a relatively high resistance of ruminal strains to mercury could be by decreasing the permeability for mercury transport into the cells or by the presence of enzymes that reduce Hg^{2+} to metallic mercury (Hg^0) and thereby decrease its toxicity. Sheep on a poor pasture consume soil, and cattle can eat soil when suffering from nutritional deficiencies e.g. phosphorus deficiency. Bacteria that contain different heavy metal resistance genes are probably also consumed together with soil material, especially in contaminated areas. All the data encouraged us to formulate a working hypothesis that the heavy metal resistance genes of soil origin transferred into the ruminal bacteria, may play a significant role in the elimination of environmental stress within the rumen microbial ecosystem and in this way contribute to the adaptation of herbivores to the actual environment. The verification of this hypothesis is a main goal of our work.

Characterisation of the novel conjugative transposon Tn*B1230* which is involved in transfer of the tetracycline resistance gene *tet*(W) from the rumen anaerobe *Butyrivibrio fibrisolvens*. K.A. Kazimierczak, C.M. Melville, H.J. Flint, K.P. Scott (Microbial Genetic Group, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB).

A new ribosome-protection-type tetracycline resistance gene *tet*(W) was first identified in the Gram-positive rumen anaerobe *Butyrivibrio fibrisolvens* 1.230. *tet*(W) is one of the most widespread tetracycline resistance genes in environmental samples. It was found in other isolates of *B. fibrisolvens*, *Selenomonas* spp., *Mitsuokella* spp., *Fusobacterium prausnitzii* and *Bifidobacterium longum* from bovine and sheep rumen as well as porcine and human faeces. The *tet*(W) gene in *B. fibrisolvens* 1.230 is highly mobile and its transfer is associated with the movement of the transposable chromosomal element Tn*B1230*. This novel conjugative transposon is 40–45kb in size and sequence analysis of internal 21kb identified 14 open reading frames, ten of which showed significant similarity (up to 72%) to proteins encoded by the *Enterococcus faecalis* conjugative transposon Tn*1549*, which carries VanB-type vancomycin resistance. The organisation of 12kb of Tn*B1230* sequence closely resembles that between *orf16* and *orf23* in Tn*1549*, however the remaining 9kb shows rearrangements in the orientation and order of ORF. The open reading frames (*orf11*) at the end of the current sequence encodes protein with 32% similarity to a protein encoded on another genetic element found in VanG-type

resistant *E. faecalis*. Therefore Tn*B1230* seems to be related to several conjugative genetic elements described previously. The aim of this work was to decipher the genetic organisation of Tn*B1230* and investigate its involvement in the transfer of *tet*(W).

Temperature and pH characteristics of a lysozyme gene cloned in *E. coli* and lactic acid bacteria. A.S. Akinalp[a], M. Asan[a], N. Ozcan[a], M.S. Ekinci[b], E. Ozkose[b] ([a] Cukurova University, Animal Science Department, 01330, Adana, Turkey; [b] Kahramanmaras University, Animal Science Department, 46060, Kahramanmaras, Turkey).

Lysozyme is one of the antimicrobial proteins produced by a wide range of organisms to improve their immune system. A shuttle vector (pL2) carrying the lysozyme gene (pBluescript + lysozyme gene (pL1) and pTRW10 replication origin) was constructed. The pL2 vector DNA contains a *Hind*III cut DNA fragment in the LacZ region encoding the *Streptococcus* replication origin of the *E. coli-Streptococcus* shuttle vector pTRW10. The pL2 vector was transferred to *E. coli* DH5α, *Streptococcus thermophilus* FI8976 and *Lactococcus lactis* IL1403 by electroporation and the lysozyme enzyme was actively produced by all these bacteria. All these bacteria expressing the lysozyme gene were sensitive to osmotic shock in a hypotonic media such as water, and the lysozyme produced was very active and thereby prevented the growth of *Micrococcus luteus* cells. A 15 kDa lysozyme protein expressed by *S. thermophilus* was not denaturated and was observed on SDS-PAGE gels after heating the enzyme at 70 °C for 15 min. The enzyme was also shown to be active on *M. luteus* cells by preventing their growth and yielding clear non-bacterial zones. The optimum pH of the enzyme was between 6 and 9 and the thermostability of the enzyme expressed by *S. thermophilus* was increased to 70 °C for 15 min. However, the lysozymes produced by *E. coli* and *L. lactis* were denaturated at 70 °C for 15 min. The results are discussed in the context of both health and food safety particularly for dairy products.

The neonatal gut flora and vitamin K. G. Cooke, J. Behan, Dr M. Costello (Department of Applied Science, Institute of Technology Tallaght, Old Blessington Road, Tallaght, Dublin 24, Ireland).

The occurrence of late haemorrhagic disease, particularly in breast fed neonates, has been associated with low levels of vitamin K. Vitamin K, an essential component of the blood clotting mechanism, is provided in the diet (phylloquinones) or produced by intestinal bacteria (menaquinones). Prophylactic treatment for late haemorrhagic disease has involved the administration of vitamin K either intramuscularly or orally using various regimes. Specific bacteria within the gut flora contribute significantly to individual vitamin K requirements. The neonate initially has a sterile gut. This project looked at the establishment of the selected representative bacterial groups of the gut flora at three time points in the 0–6 week range, differentiating between breast fed and formula fed babies. Microbiological methods were used to specifically enumerate *Lactobacilli* sp., *Bifidobacteria* sp., *Enterococci* sp., *Staphylococci* sp., *Bacteroides* sp., *Clostridia* sp. and coliforms present in the gut. *Bifidobacteria* sp. and *Lactobacilli* sp. were more prevalent in the gut flora of breast fed neonates whereas *E.coli* and *Enterococci* sp. were more prevalent in the gut flora of formula fed neonates. Selected isolates were subsequently analysed for vitamin K production. No suitable standards exist to positively identify the extracted vitamin K; however, LC-MS analysis was used to confirm TLC and HPLC analysis of the extracted vitamin. Vitamin K has been isolated from several bacteria. Current studies involve looking at the absorption of the various forms of vitamin K in human intestinal cells and the toxicity of the different forms on the intestinal cells.

Changes in bacterial community structure in the rumen of sheep after the switch from a high-grain diet to a forage diet using a PCR-SSCP method. D. Macheboeuf, J. Deffaud, C. Martin (INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France).

The rumen is a complex ecosystem in which the host, the microrganisms, and the food components have an important role in its functioning. It has been shown that cereal supplementation of

a forage diet decreased the rumen bacterial diversity, but the bacterial changes after the switch from a high-grain diet to forage diet has been less studied. We used 4 ruminally cannulated sheep fed, on a two feeding period design, a high-wheat diet (60% wheat + 40% hay, W) and a forage diet (100% hay, H) without transition. Ruminal content samples were collected on 2 consecutive days for each animal and each period. The biodiversity of the sample was estimated by the number of peaks in the profile obtained from single strand conformation polymorphism (SSCP) analysis after PCR amplification with universal bacteria 16S rDNA primer. The changes in the bacterial community structure were evaluated through the variation of relative peak sizes in the profile. The method detected a maximum of 35 different peaks representative of one or more bacterial strains. There were no differences among animals on the total number of peaks (mean 19) in the profile but the peaks were not the same indicating that the bacterial communities were significantly different. The forage diet significantly increased ($P < 0.001$) the number of major peaks in the profile as compared to the wheat diet (8 and 10 for the W and H diets respectively). Twenty-one of the 35 peaks were affected ($P < 0.01$) by the diet change. The peaks that contain strains known as amylolytic and lactate users (*S. bovis, M. elsdenii, S. ruminatium*) decreased ($P < 0.01$) while the peaks that contain strains known as cellulolytic did not change (*R. albus, R. flavefaciens, F. succinogenes*) or increased (*E. cellulosolvens,* $P < 0.001$). Nine unknown peaks increased largely with a significant animal × diet interaction effect ($P < 0.001$) indicating a different adaptation reaction of the bacterial community to diet change.

PCR-SSCP comparison of 16S rDNA sequence diversity of the ruminal ecosystem using four extraction methods. D. Macheboeuf, J. Deffaud, C. Martin (INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France).

The purity of the DNA extracted from the ruminal ecosystem is a key issue that affects the sensitivity of biodiversity analysis methods such as PCR- single strand conformation polymorphism (SSCP). Four DNA extraction methods from freeze-dried ruminal contents of 4 sheep were tested: (A) an enzymatic method (invitrogen kit); (B) a classic phenol/chlorophorm extraction; (C) an extraction with polyvinylpolypyrrolidone (PVPP); (D) an extraction method on membrane adsorption (dneasy plant, Qiagen kit). After extraction, absorbances were measured at 230, 260 and 280 nm. The experimental responses were the extraction yield, protein (OD_{260}/OD_{280} ratio) and humic acids (OD_{260}/OD_{230} ratio) contamination indexes. The biodiversity of the sample was estimated by the number of peaks in the profile obtained from SSCP after PCR amplification with universal bacteria 16S rDNA primer. The DNA extraction yield was higher ($P < 0.05$) for methods A and B than for methods C and D (3.17 and 3.77 vs. 1.55 and 0.55 $\mu g \cdot mg^{-1}$, respectively). The protein contamination index was not significantly different between the methods (1.88–1.56). The humic acids contamination index was better ($P < 0.05$) with A (1.79) as compared to the others (1.32 in mean for B,C and D). The PCR amplification was clearly more efficient with DNA from C and D (a smaller inhibitory effect). The SSCP profile of each DNA extract was similar for the 4 methods (7–8 major peaks). However, the number of minor peaks was higher (15) with C than with the other methods (6, 8, 4 for A, B and D respectively). There were distinct differences in the biodiversity representation among extraction methods and the results showed that greater DNA yield is not synonymous with higher sequence diversity. Although C was more time consuming than the others, it decreased the inhibitory effect on PCR and displayed the largest diversity profile.

Different strategies used by ruminal lactate-utilizing bacteria to overcome bacteriophage infections. M. Piknova[a,b], P. Pristas[b], P. Javorsky[b] ([a] P.J. Safarik University, Faculty of Science, Institute of Chemistry, Moyzesova 11, 04001 Košice, Slovak Republic; [b] Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4–6, 04001 Košice, Slovak Republic).

Restriction-modification (R-M) systems are generally believed to protect bacterial cells against invading foreign DNA molecules, particularly bacteriophages. The most abundant are type II R-M systems, which comprise two separate

sequence-specific enzymes: (1) a restriction endonuclease that specifically cleaves (phage) DNA within the recognition sequence and (2) a modification methyltransferase that specifically methylates DNA. It has been shown that a large number of R-M systems can be found in natural bacterial populations. The widespread presence of R-M systems in bacteria would be a consequence of the selective advantage of having a defense tool against phage infection. The analysis of R-M activities in seven phenotypically different rumen strains of *Megasphaera elsdenii* revealed the presence of GATC-specific restriction-modification systems (MboI isoschizomers) in all of the strains tested. A complete lack of other restriction and/or modification enzymes previously characterized in closely related *Selenomonas ruminantium* was confirmed by a methylation protection assay. Based on the results of our experiments, it could be assumed that *M. elsdenii,* as compared to *S. ruminantium,* uses a strategy different from R-M systems for bacteriophage protection.

Diversity of rumen methanogens from sheep in Western Australia and Queensland identified by 16S clone libraries. C. Pimm, A.F. Toovey, A.J. Williams, B. Winder, S. Rodgers, K. Smith, A.-D.G. Wright (CSIRO Livestock Industries, Private Bag 5 Wembley WA 6913, Australia).

Individual 16S clone libraries were prepared from the rumen contents of 17 Merino sheep in Western Australian fed 3 different diets (grazing pasture vs. oaten hay vs. lucerne hay). Amongst the 733 clones examined, 65 phylotypes were found to be similar to cultivated methanogens of the order *Methanobacteriales*. The diversity of rumen methanogens was the greatest in the sheep grazing pasture. *Methanobrevibacter* strains SM9, M6 and NT7 accounted for over 90% of the 733 clones examined, with M6 being more prevalent in grazing sheep and SM9 more prevalent in sheep fed the lucerne-based diet. Five new species were identified, two of which have very little sequence similarity to any cultivated methanogens. In contrast, an investigation into the molecular diversity of rumen methanogens from pooled rumen fluid contents from five Merino sheep in Queensland, Australia revealed that only eight of the 34 phylotypes from 89 clones were similar to methanogens belonging to the order *Methanobacteriales*. The remaining 26 phylotypes represented a new taxonomic order of methanogens, atypical for the rumen environment, some 15–20% dissimilar to *Methanobacteriales*.

Implications for disease of colonic bacterial diversity: A culture independent analysis of the microbial community from the colon of individuals with IBD and colon cancer. P. Scanlan, F. Shanahan, J. Marchesi (Dept. of Microbiology, University College Cork, Cork, Eire).

The large intestine is the most heavily colonised part of the gastrointestinal (GI) tract with numbers reaching 10^{12} bacteria per gram of luminal contents. Micro-organisms are essential to maintaining the normal functioning of the gut, and as such have a profound effect on the health status of their host from an immunological, dietary and physiological perspective. Thus, the potential role bacteria hold in various disease states of the GI tract is widely appreciated. In this study the total bacterial diversity and the diversity of specific bacterial genera of colon cancer ($n = 40$), polyp ($n = 40$) and inflammatory bowel disease (IBD) patients, on specific probiotic trials, were analysed. Molecular techniques (ribosomal intergenic spacer analysis and denaturing gradient gel electrophoresis) widely used in the microbial ecology field were employed to investigate the bacterial populations at given time points. The results obtained were compared to those of normal subjects and are discussed here. The methods employed illustrated not only considerable variation between individuals but also intra-individual variation in certain sub-groupings of the trials.

Fe-hydrogenases from bovine rumen: a metagenomic approach. E. Severing, A. Ederveen, G.W.M. van der Staay, S.Y. Moon-van der Staay, R.M. de Graaf, Th.A. van Alen, N. McEwan*, C.J. Newbold*, J.-P. Jouany#, T. Michałowski*, P. Pristas*, J. Fried*, G. Ricard*, M.A. Huynen*, J.H.P. Hackstein (Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands and the EU projects # ERCULE/*CIMES).

The evolution of eukaryotic Fe-hydrogenases is still poorly understood. It has remained unclear until now as to whether these hydrogenases represent an old eukaryotic heritage or whether they were acquired by bacterial-to-eukaryote gene transfer. Here we describe the recovery of a set of DNA sequences encoding the H-cluster of Fe-hydrogenases from rumen ciliates. The rumen ciliates were isolated from the rumen fluid of a cow by electromigration. The DNA of the total rumen ciliate population was purified and used to amplify the H-clusters of Fe-hydrogenases by PCR with degenerated primers. For the identification of the corresponding ciliates, PCR was performed on DNA from type-strain ciliates. Phylogenetic studies revealed the presence of a monophyletic group of eukaryotic Fe-hydrogenases in the bovine rumen. Supported by the EU Contract QLRI-CT-2000-01455 "ERCULE" and Contract QLK3-2002-02151 "CIMES".

Study of two simple techniques for cryopreservation of rumen ciliate protozoa. E. Nsabimana[a], D. Macheboeuf[a], C.J. Newbold[b], J.-P. Jouany[a] ([a] INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France; [b] University of Wales, Aberystwyth, UK).

Rumen ciliates are difficult to cryopreserve. The two-step freezing technique, which has been applied successfully (Nsabimana et al. 2003, Appl. Environ. Microbiol., 69, 3826–3832), is complex and needs a special equipment to be performed. Two simple techniques using Bicell® and M.Frosty® freezing cells and allowing the respective cooling rates of 0.5 °C/min and 1 °C/min, were tested for their ability to cryopreserve the following ciliate species: *Dasytricha ruminantium, Entodinium caudatum, Epidinium ecaudatum caudatum, Eudiplodinium maggii, Isotricha intestinalis, Isotricha prostoma, Metadinium medium*. The ciliates were isolated from sheep monofaunated with each species. Centrifuged fresh rumen fluid was used as the freezing and thawing media. Equilibration with DMSO (0.56 M) was set at 25 °C or 30 °C for 5 or 10 min before freezing in the two tested freezing cells. Tubes containing the ciliates were plunged into liquid nitrogen when the freezing cells reached the temperature –80 °C. The survival rate (SR) of ciliates was determined from their motility. After two weeks of storage in liquid nitrogen, the highest SR were obtained with *D. ruminantium* (100%), *I. prostoma* (100%), *I. intestinalis* (100%), *E. ecaudatum caudatum* (87%) for the two freezing cells. Bicell® allowed a higher SR for *M. medium* (87 vs. 80%) and for *E. maggii* (94 vs. 76%), while M.Frosty® gave better results for *E. caudatum* (64 vs. 40%). In conclusion, the two simple freezing techniques Bicell and M.Frosty can be applied to cryopreserve the rumen ciliates with an acceptable rate of survival. This project was supported by EU infrastructure grant ERCULE (QLRI-CT-2000-01455) www.ercule.com.

The effect of temperature on the in vitro viability of the mixed rumen protozoal population. G. de la Fuente[a], M. Pérez-Quintana[b], J.A. Cebrián[c], M. Fondevila[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos y, Universidad de Zaragoza, Spain; [b] Departamento de Química y Biología, Universidad de Matanzas, Matanzas, Cuba; [c] Departamento de Bioquímica y Biología Molecular y Celular; Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain).

Survival of rumen protozoa is reduced at low temperatures, making their storage in refrigeration difficult and hampering their study. A mixed rumen protozoal population (type A) from sheep was anaerobically diluted 1:100 in culture medium and kept at 38, 15 and 5 °C for 2, 4 or 6 h to determine the effect of temperature on cell viability, according to an estimation of membrane damage by a double-stain fluorescence method (5 tubes per treatment). Temperature was reduced from 38 °C to 15 °C at 3 °C·min^{-1} and from 15 to 5 °C at 0.6 °C·min^{-1}. Apparently, all genera responded to both stains, and *Ophryoscolex* showed an effect of temperature (80.3, 55.4 and 54.5% viability at 38, 15 and 5 °C; $P < 0.01$). However, only *Entodinium* cells were in a number high enough to be strictly compared. The viability of the *Entodinium* species in the rumen inoculum was 93.8 ± 2.69, and remained between 90.5 and 79.8% when cultivated at 38 °C for 2 to 6 h. The proportion of non damaged cells after 2 h at 15 or 5 °C did not diminish (87.3 and 86.2%; $P > 0.05$). However, viability was reduced ($P < 0.05$) to 61.3 and 59.9 % after 6 h at 15 °C and 5 °C (s.e.m. = 3.78). The viability of *Entodinium* was reduced at refrigeration temperatures; around 60% of the

cells remained undamaged even after 6 h. Later cultivation of the residues for 72–96 h rendered lower protozoal numbers than expected. However, both techniques are not directly comparable, and some species do not grow well in vitro.

Do rumen protozoa have an absolute requirement for live bacteria? B.A. Dehority, R.A. Patterson (Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA).

In vitro cultivation of rumen protozoa in the presence of antibiotics has led to the suggestion that they have an absolute requirement for live bacteria. In our lab, attempts to inhibit bacterial overgrowth in protozoal cultures by the addition of penicillin and streptomycin appeared to be successful, in that several days substrate could be added at the same time and overcame the necessity of adding substrate daily. However, the cultures slowly died out over the following several weeks. This led to a series of studies on the relationship between the presence of live bacteria and viability of rumen protozoa in vitro. The protozoal species studied were *Epidinium caudatum, Entodinium caudatum, Entodinium exiguum* and *Metadinium affine*. Half of the culture was transferred to an equal volume of fresh media at intervals ranging from 2 to 4 days and counts were made at each transfer. For all species, exposure to the antibiotics for 24 h followed by washing to give at least a 200-fold dilution of the antibiotics, resulted in a gradual decline in their concentration, generally noticeable at the first transfer. Concentrations fell to near zero in two weeks. Addition of protozoa-free supernatant from thriving cultures had no obvious effect on viability. Bacterial concentrations, measured by MPN assay, were determined at various time intervals and were similar in cultures inoculated with antibiotic-treated or untreated protozoa. These data suggest the possibility that the antibiotics have a protozoacidal effect which is not related to the presence of live bacteria.

Identification of mitochondrial-type chaperonin 60 (HSP 60) proteins in the anaerobic ciliate *Nyctotherus ovalis*. E. Engels, G.W.M. van der Staay, S.Y. Moon-van der Staay, J.H.P. Hackstein (Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL-6525ED Nijmegen, The Netherlands).

Two genes, both encoding a mitochondrial-type chaperonin 60 protein (HSP 60), were identified in a macronulcear gDNA library of the hydrogenosome-bearing, anaerobic ciliate *Nyctotherus ovalis* from the hindgut of the cockroach *Blaberus spec.* var. Amsterdam. We were able to reinforce the presence of two genes encoding HSP60 on (macronuclear) gene-sized chromosomes using PCR on total DNA from *Blaberus spec.* var. Amsterdam. This means that these genes are an integral part of the *N. ovalis* genome. Since aerobic, HSP 60 proteins of mitochondriate organisms are involved in mitochondrial import and protein folding,;also these genes argue for a (ciliate) mitochondrial ancestry of the hydrogenosomes of *N. ovalis*. Phylogenetic analysis confirmed previous evidence that the hydrogenosomes of *N. ovalis* are anaerobic mitochondria that produce hydrogen. Supported by the EU Contract QLK3-2002-02151 "CIMES".

Identification of a PP_i-dependent phosphofructokinase from the anaerobic ciliate *Nyctotherus ovalis*. E. Engels[a], G.W.M. van der Staay[a], S.Y. Moon-van der Staay[a], M.A. Huynen[b,c], J.H.P. Hackstein[a] ([a] Dept. Evolutionary Microbiology, Fac. Sci.; [b] Nijmegen Centre of Molecular Life Sciences (NCMLS); [c] CMBI, University of Nijmegen, Toernooiveld 1, NL-6525ED Nijmegen, The Netherlands).

One of the key regulatory steps in the glycolytic pathway is an irreversible, committing step catalysed by an ATP-dependent phosphofructokinase (PFK) (ATP-PFK; EC 2.7.1.11). In certain organisms (i.e. bacteria, a few anaerobic eukaryotes, and plants) this step is catalysed by a PP_i-dependent PFK (PP_i-PFK; EC 2.7.1.90), which uses PP_i instead of ATP as a phosphoryl donor, conserving ATP and rendering the reaction reversible under physiological conditions. We identified two genes encoding PP_i-dependent PFK in a gDNA library of *N. ovalis*, which is made up exclusively from macronuclear gene-sized chromosomes. This is the first report of a PP_i-dependent PFK in ciliates. Notably, phylogenetic analysis indicates a close relationship with plant PFK. One might speculate as to

whether this gene has been acquired by lateral gene transfer from plants or by an endosymbiotic (organelle) gene transfer to the nucleus from a cryptic, (secondary) ancestral plastid. Supported by the EU Contract QLK3-2002-02151 "CIMES".

Experiences with transport of deep freezing samples of rumen protozoa in dry ice. S. Kišidayová[a], Z. Váradyová[a], E. Nsabimana[b], J.-P. Jouany[b], C.J. Newbold[c] ([a] Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic; [b] INRA-URH/DVA, Centre de Clermont/Theix, 63122 St-Genès-Champanelle, France; [c] Institute of Rural Science, University of Wales, Aberstwyth, UK).

Ciliate protozoa were transported between Slovakia and France on six occasions. The rumen ciliates were frozen by a two-step freezing protocol and were kept in liquid nitrogen (LN). The frozen samples were kept in dry ice in a polystyrene box during transport by air mail. After transport, they were immediately immersed into LN. The survival rate was determined after at least 24 h storage in LN. The successful transport depended on the mass of dry ice, duration of transport, cell concentration and their original viability after deep freezing. The optimal mass of dry ice was 10 kg. The optimal transport time was 24–78 h when cryosamples were still frozen and residual dry ice was present in the box. The winter months were preferred for transport. The best survival rates were observed with ciliates taken from monofaunated animals, with high cell concentrations and when a viability greater than 60% was reached after deep freezing. The decrease of recovery after transport was between 17 and 57%. This project was supported by EU infrastructure grant QLRI-CT-2000-01455: www.ercule.com

View on cryopreservation of rumen ciliate isolated from in vitro cultures. S. Kišidayová[a], Z. Váradyová[a], A. Marcin[b], E. Nsabimana[c], J.-P. Jouany[c], T. Michałowski[d], C.J. Newbold[e] ([a] Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovak Republic; [b] Agroecological Research Institute, Michalovce, Slovak Republic; [c] INRA-URH/DVA, Centre de Clermont/Theix, 63122 St-Genès-Champanelle, France; [d] Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna near Warsaw, Poland; [e] Institute of Rural Science, University of Wales, Aberstwyth, UK).

The cryopreservation of rumen ciliate in vitro cultures has been performed in our laboratory for fourteen years. In total thirteen species of rumen ciliates were treated for deep-freezing in liquid nitrogen and successfully cryopreserved: *Entodinium caudatum, Entodinium furca monolobum, Entodinium simplex, Entodinium exiguum, Entodinium bursa, Eremoplastron bilobum, Epidinium ecaudatum f. caudatum, Eudiplodinium maggii, Diplodinium denticulatum, Diplodinium denticulatum f. anacanthum, Diploplastron affine, Ophryoscolex caudatus f. tricoronatus, Polyplastron multivesiculatum.* A number of parameters influenced the survival rates of the cells. Nevertheless, although optimal freezing protocols were determined on a few of the above species, a more generic approach was also applied to the above-mentioned species from in vitro cultures with good results. The cryopreservation and regeneration of rumen ciliates cultivated in vitro for a prolonged period can be enhanced by long-term supplementation of cultures with osmoactive compounds and other supplements. Such an established cryobank serves as a stable source of cultures for repeated experiments. This project was supported by EU infrastructure grant QLRI-CT-2000-01455: www.ercule.com

Homologous recombination in actin alleles in the rumen ciliate *Entodinium caudatum.* N.R. McEwan[a], N.A. Thomas[a], C.J. Newbold[b], T. Michałowski[c] ([a] Rowett Research Institute, Aberdeen, Scotland, UK; [b] The Institute of Rural Studies, University of Wales, Aberystwyth, Wales, UK; [c] Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland).

The actin genes isolated from a population of *Entodinium caudatum* are already known to have a highly variable codon utilisation pattern, whilst maintaining amino acid identity in the final protein product. However, the previous description concentrated on sequences derived from a population of cells started from about 20 progenitor *E. caudatum* cells. Instead this work makes use of sequences derived from a culture of *E. caudatum* which was established from

a single cell, and had been grown in vitro for around 8 months (approximately 250 cell division generations) before harvesting cells. The cells were harvested from in vitro cultures and fragments of the actin genes amplified by PCR. Amplicons were cloned into the TA cloning kit (Invitrogen) and sequences of inserts were determined. DNA sequences were aligned and areas of nucleotide variation were identified. By examining the codons used to encode each individual amino acid, it was clear that at any specific site, only one of two codons was used in all sequences. In total, four different actin alleles were identified in this population, all of which could be explained by homologous recombination. This suggests that the original progenitor cell used to set up the in vitro culture was heterozygous for this gene, and that there were two sites where homologous recombination events had taken place. In conclusion, it appears that the codon variability described previously was probably a result of the number of morphologically identical founder cells used to establish the population, as opposed to the codon variability being generated following the establishment of the population.

Are *Ophryoscolex caudatus* and *Ophryoscolex purkynjei* two different species of rumen ciliates or only two different forms of the same species? R. Miltko[a], T. Michałowski[a], R. Malik[b], P. Pristas[b], J.H.P. Hackstein[c], P. Javorsky[b] ([a] The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland; [b] Institute of Animal Physiology Slovak Academy of Sciences, Košice, Slovakia; [c] Catholic University of Nijmegen, Nijmegen, The Netherlands).

Actually the genus *Ophryoscolex* includes most complex ruminal ophryoscolecids which have been divided into eight different species. Individuals classified as *Ophryoscolex caudatus* are characterized by the presence of three circles of secondary caudal spines and a long main caudal spine. *Ophryoscolex purkynjei* is also equipped with three circles of secondary spines but the main spine is short. During the long term in vitro culture of ciliates initially exhibiting the features of *O. caudatus,* we observed a decrease in the number of typical "*caudatus*" forms followed by an increase in the protozoa possessing a distinctly reduced caudal spine i.e. "*purkynjei*" forms. Moreover, we periodically observed an increase in the number of "*caudatus*" forms following the transfer of ciliates to a continuous culture system. On the contrary, a replacement of CO_2 used to saturate the culture medium with a mixture of N_2 (95%) + CO_2 (5%) led to the reduction of caudal spines and also to a reduction or simplification of the secondary spines. These observations suggest that ciliates identified as *O. purkynjei* and also *Ophryoscolex spinosus* could in fact only be the morphologically changed *O. caudatus* protozoa. To clarify this question the different forms of these ciliates were picked up from maintained cultures and 18S rDNA RFLP analysis was performed following amplification by the single cell PCR technique but no differences were found. This shows that changes in the protozoa phenotype were not accompanied by the differences in their genotype. (This project was supported by EU grant QLK3-2002-02151-CIMES.)

Gut ciliates from mammals are monophyletic. S.Y. Moon-van der Staay[a], G.W.M. van der Staay[a], C.J. Newbold[b], N.R. McEwan[b], T. Michałowski[b], P. Javorský[b], D. Macheboeuf[a], J.-P. Jouany[b], J.H.P. Hackstein[a] ([a] Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands; [b] ERCULE.COM).

The foreguts and hindguts of many herbivorous mammals host a community of very diverse microorganisms. The eukaryotes are predominantly represented by ciliates. The evolutionary history of these ciliates is still a matter of discussion. Although detailed knowledge about the diversity of the ciliates is the key to address this question, the conventional approach based on morphology has limitations when one tries to assess the ciliate diversity. The limited number of morphological characters, a general problem in the current protist taxonomy, and in particular, difficulties to isolate the ciliates from gut samples for a detailed characterization are the major problems for classical analysis. Here, we initiated a study on the diversity and the origin of gut ciliates using a molecular approach. The protozoa were investigated by sequencing of 18S rDNA libraries from total rumen contents of ruminants (sheep, goats and cows) and feces of

hindgut fermenters (horses and elephants). Phylogenetic analysis revealed that there is an enormous diversity of the ciliates, and that all the gut ciliates of the mammals (plus marsupials) share a common ancestor. This work was supported by the EU infrastructure grant QLRI-CT-2000-01455, ERCULE.

Diversity of rumen ciliates in a red deer assessed from 18S rDNA and morphology. S.Y. Moon-van der Staay[a], G.W.M. van der Staay[a], T. Michałowski[b], D. Macheboeuf[a], J.-P. Jouany[b], C.J. Newbold[b], J.H.P. Hackstein[b] ([a] Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands; [b] ERCULE.COM).

A library of 18S rRNA genes was created from the rumen contents of a red deer (*Cervus elaphus*), and 72 clones were partially sequenced. All sequences were from ciliates. With the aid of the sequences of validated representatives of rumen ciliates retrieved from the culture collection of the ERCULE project (www.ercule.com), we were able to identify the phylogenetic positions of the 51 different ciliate clones found in the rumen. Studying a formaldehyde-fixed aliquot of the same sample using light-microscopy, 22 ciliate species could be distinguished at the morphological level. Several clones clustered with the morphologically identified type species, but they were still significantly different at the 18S rDNA level. Moreover, morphologically very similar groups that have been assigned tentatively to the same genus were found to consist of many phylogenetically distinct lineages at the 18S rDNA level. Thus, ciliates in the rumen of the red deer are likely to be much more diverse than assumed previously on the basis of morphological studies. This work was supported by the EU infrastructure grant QLRI-CT-2000-01455, ERCULE.

Pyruvate: ferredoxin oxidoreductase (PFO) genes from the rumen: protozoal or bacterial origins? E. Severing[a], A. Ederveen[a], G.W.M. van der Staay[a], S.Y. Moon-van der Staay[a], R.M. de Graaf[a], T.A. van Alen[a], N. McEwan[b], C.J. Newbold[b], J.-P. Jouany[c], T. Michałowski[b], P. Pristas[b], J. Fried[b], J.H.P. Hackstein[a] ([a] Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands; and from the EU projects [b] ERCULE and [c] CIMES).

Phylogenetic analysis of eubacterial and eukaryotic PFO genes suggest a complex history for PFO. Here we describe the recovery of novel DNA sequences encoding PFO genes, which were retrieved from a total rumen ciliate population using a metagenomic approach. The rumen ciliates were isolated from the rumen fluid of a grass-fed Holstein-Friesian cow by electromigration. DNA from the total rumen ciliate population was purified and used as a template to amplify 2 kb fragments of potential PFO genes by PCR with degenerated primers. Phylogenetic analysis revealed the presence of several clusters of PFO genes. It remains to be analysed as to whether the recovered PFO sequences are derived from eukaryotes or prokaryotes, since the isolated DNA is contaminated with DNA from rumen bacteria. Moreover, many of the ciliates may host endosymbiotic bacteria that potentially can be the source of the recovered PFO genes. Supported by the EU Contract QLRI-CT-2000-01455 "ERCULE" and Contract QLK3-2002-02151 "CIMES".

Determining if protozoal cells isolated from ruminal fluid represent those passing to the duodenum for a quantitative assay of protozoal N. J.T. Sylvester[a], S.K.R. Karnati[a], Z. Yu[a], C.J. Newbold[b], M. Morrison[a], J.L. Firkins[a] ([a] The Ohio State University, Columbus, Ohio, USA; [b] University of Wales, Llanbadarn Fawr, Aberystwyth, UK).

The measurement of duodenal flow of protozoal N would improve studies evaluating the efficiency of microbial protein synthesis in the rumen. Regardless of the protozoal marker method, N flows are typically calculated using N and marker concentrations generated from the protozoa isolated from ruminal contents as a standard. Predominant protozoal genera present within the rumen, however, might differentially sequester and not accurately represent the genera passing post-ruminally. Denaturing gradient gel electrophoresis (DGGE) was used to test the hypothesis that protozoal cells isolated from ruminal contents are appropriate for our quantitative PCR assay measuring protozoal N flow to

the duodenum. Ruminal and duodenal samples were pooled from two Holstein cows fed either low forage or typical forage diets. Genomic DNA was extracted and purified, and two ciliate-specific primer sets were used to amplify hyper-variable regions within the 18S rRNA gene producing 223- and 297-bp amplicons with a G:C clamp. Denaturing gradients and running conditions were optimized for each type of amplicon. The DGGE banding profiles were markedly similar within rumen and duodenal samples from the same animal within diet but were less similar for the respective sites among the animal. After electrophoresis, the bands were excised, re-amplified, and sequenced. Sequence similarity searches were performed using BLASTn of GenBank. The presumptive identification of the sequences from excised bands from both amplicon types corresponded with predominant generic distributions observed microscopically for each animal. Based on these data, the use of protozoal standards collected from the rumen seems appropriate for a quantitative PCR assay measuring duodenal protozoal N.

Diversity in the length of macronuclear chromosomes in the phylum Ciliophora; rumen ciliates and *Nyctotherus* –a case study. N.A. Thomas[a], M. Regensbogenova[b,c], R.M. de Graaf[d], E. Devillard[a], P. Pristas[b], G.W.M. van der Staay[d], P. Javorsky[b], J.H.P. Hackstein[d], C.J. Newbold[e], N.R. McEwan[a] ([a] Rowett Research Institute, Aberdeen, Scotland, UK; [b] Institute of Animal Physiology, Košice, Slovakia; [c] University U.P.J.S. Department of Molecular and Cell Biology, Košice, Slovakia; [d] Department of Evolutionary Microbiology, University of Nijmegen, Nijmegen, The Netherlands; [e] The Institute of Rural Studies, University of Wales, Aberystwyth, Wales, UK).

Ciliates possess two types of nuclei: the micronucleus and the macronucleus. Micronuclei contain the cell's complete genetic complement and macronuclei contain a sub-population of the DNA present in the micronuclei. Despite comprising a sub-population of the micronuclear DNA complement, the macronucleus is larger, and contains more DNA. This apparent anomaly is because the macronucleus contains hundreds to thousands of copies of each macronuclear, highly processed chromosome. Ciliate cells were harvested, embedded in agarose plugs, and subjected to proteinase K treatment. Plugs were added directly to wells in agarose gels. After electrophoresis, a smear of DNA was observed, which for the rumen ciliates indicated the presence of DNA substantially larger than 10 kb. In the case of *Nyctotherus ovalis*, as with certain hypotrichous/stichotrichous ciliates, the size of the DNA observed was typically 0.5–10 kilobases (kb), suggesting that its macronuclear chromosomes are gene-sized molecules. A more accurate size of the chromosomes from rumen ciliates was determined by pulse field gel electrophoresis (PFGE). Typically the DNA seen following PFGE was around 40–50 kb, demonstrating these cells do not have gene-sized chromosomes. PFGE gels were blotted onto membranes and probed with ciliate genes. These blots produced discrete bands, rather than smears, implying the size of the chromosomes on PFGE was genuine, and not a minimum size resulting from degradation of larger chromosomes. We conclude that both these taxa possess highly processed chromosomes in their macronucleus, but that only *Nyctotherus ovalis* possesses chromosomes which are gene-sized in length. This work was supported by the EU infrastructure grant QLK3-2002-02151: CIMES.

Gene-sized macronuclear chromosomes in the anaerobic ciliate *Nyctotherus ovalis*. A.H.A.M. van Hoek, T.A. van Alen, G.W.M. van der Staay, S.Y. Moon-van der Staay, B. Boxma, J.H.P. Hackstein (Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands).

Ciliates, unicellular protists with a world-wide distribution, are characterised by a nuclear dimorphism. They possess two types of nuclei, i.e. a "germ-line" micronucleus and a "somatic" macronucleus. The macronucleus of all ciliates studied so far contains highly amplified, rearranged chromosomes. In certain ciliates, micronuclear chromosomes are processed in a way that gene-sized DNA molecules are generated in the course of macronuclear development, which contain only a single open reading frame (ORF). This ORF is flanked by short runs of non-coding leader and trailer sequences; of course, these

mini-chromosomes become capped by telomeres after processing. *Nyctotherus ovalis* was the first (anaerobic) heterotrichous ciliate for which such a genome organisation could be demonstrated. Here we describe several of these gene-sized chromosomes, which have been sequenced from telomere to telomere. The presence of potential regulatory sequences is discussed. Supported by the EU Contract QLK3-2002-02151 "CIMES".

The time of transportation in dry ice can affect the survival rate of frozen rumen ciliate protozoa. E. Nsabimana, S. Kisidayova, D. Macheboeuf, J.-P. Jouany (INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France).

One of the major limits for the study of rumen protozoa is the difficulty to get them pure and alive and grow them to get enough biomass. Cryopreservation and exchanges of cells between laboratories is a promising way to stimulate research on these microorganisms. Four species of ciliates were isolated either from monofaunated sheep (*Isotricha prostoma, Epidinium ecaudatum caudatum, Eudiplodinium maggii*) or from an in vitro culture (*Entodinium furca monolobum*). All species were frozen according to the two-step freezing procedure (2SFP) and were then stored in liquid nitrogen for several months. In addition, the cells of *I. prostoma* were frozen according to Bicell (BC) and M. Frosty (MF) procedures and were stored in dry ice for 24 h. All frozen tubes were maintained in dry ice during transportation from France to Slovakia. Two transport times of frozen cells were tested: 48 h for *I. prostoma* and *E. furca monolobum,* or 12 days for *E. ecaudatum caudatum* and *E. maggii*. The survival rates (SR) of the ciliate species in each transport condition were determined from motility tests. Transport of frozen ciliates within 48 h did not alter the survival rates of *I. prostoma* (80%) and *E. furca monolobum* (50%), independently of the freezing technique. Transport of *E. ecaudatum* and *E. maggii* for 12 days decreased the SR by 20% when the 2SFP was applied. The SR of *E. ecaudatum caudatum* decreased by 60% during the 12 day-period when BC and MF procedures were used, while *E. maggii* were less sensitive: there was no decrease or a 40% decrease of SR with the BC and MF techniques, respectively. In conclusion, frozen ciliates can be transferred between laboratories with reasonable SR, especially when the 2SFP is applied. Large differences between ciliate species are underlined when other freezing techniques are used. This work was supported by the EU Infrastructure grant ERCULE (QLRI-CT-2000-01455) www.ercule.com.

Variability in the concentration of sulfate-reducing bacteria in swine manure and feces as determined using a real-time PCR assay. K.L. Cook, M.A. Cotta, T.R. Whitehead (NCAUR, USDA-ARS, Peoria, IL 61614, USA).

Real time quantitative PCR was used to target sulfate-reducing bacteria (SRB) in order to evaluate their concentration in stored swine manure and swine feces. Real-time quantitative PCR permits rapid, sensitive detection of target organisms using fluorescently labeled probes. As the PCR product is produced, fluorescence signal from the probe increases in proportion to the starting concentration of the target sequence. In this case, SRB were targeted using a common probe and three different sets of primers designed to specifically amplify the *dsr*A gene sequences of: *Desulfobulbus*-like (Group 1) or *Desulfovibrio*-like (Group 2) SRB matching slurry clones or *Desulfovibrio*-like (Group 3) SRB matching slurry enrichment culture clones and isolates. Results indicate that Group 1 SRB were more common in pit slurry (concentrations ranging from 1.0×10^4 to 2.0×10^8 *dsr*A copies·mL^{-1} slurry). Concentrations of Group 2 (4.5×10^3 to 1.0×10^6 *dsr*A copies·mL^{-1} slurry) and Group 3 ($< 1 \times 10^3$ to 4.3×10^6 *dsr*A copies·mL^{-1} slurry) SRB were lower. Group 3 SRB were greater than 65% of the total population in the enrichment cultures. However, the SRB population was less than one percent of the total slurry population as determined by 16S rDNA analysis. Results suggest that this real-time PCR assay is sensitive and specific for detection of SRB in stored swine manure.

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Role of protozoa and lactate-metabolizing bacteria during a ruminal butyric rather than lactic latent acidosis of sheep. L. Brossard[a,b], C. Martin[a], F. Chaucheyras-Durand[b,c], B. Michalet-Doreau[a] ([a] Unité de Recherches sur les Herbivores, INRA, Centre de Recherche de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France; [b] Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France; [c] Laboratoire de Microbiologie, INRA, Centre de Recherche de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France).

We used six ruminal cannulated Texel wethers to study the relative role of protozoa and lactate-metabolizing bacteria in ruminal fermentative patterns during an induced latent acidosis. Sheep were consecutively limit-fed a control diet H (100% hay) for one week and, after a short transition period (one week), an acidotic diet W (60% wheat + 40% hay) for another week. Ruminal pH, VFA, lactate and NH_3 concentrations, protozoa and lactate-utilizing bacteria counts, relative 16S rRNA proportions of three main bacterial species implicated in lactate metabolism (a lactate-producing species, *Streptococcus bovis*, and two lactate-utilizing species, *Selenomonas ruminantium*, and *Megasphaera elsdenii*) using specific oligonucleotide probes, and lactate dehydrogenase (LDH) activity were determined for both diets. The pH parameters (mean, minimum, maximum, time and areas under pH 6.0 and 5.5) measured with the W diet indicated a latent (i.e., subacute and maintained) acidosis. However, we observed in this study a butyric rather than lactic acidosis. Ruminal lactate concentration remained at low levels with the acidotic diet (< 4 $mmol \cdot L^{-1}$), while VFA composition was oriented towards butyrate at the expense of acetate, with propionate remaining constant. This may be explained by a proliferation of Entodiniomorphs with the W diet, whereas lactate-metabolizing bacterial populations remained fairly constant in numbers, rRNA proportions and LDH activity. It was suggested that protozoa may control pH decrease and lactate accumulation by substrate competition. An experiment with defaunated and faunated sheep could provide further evidence that protozoa undertake a controlling task in the rumen during latent acidosis.

Effect of a specific blend of essential oils on the colonization of substrates by rumen microorganisms. S.M. Duval[a], C.J. Newbold[a], N.R. McEwan[b], R.C Graham[b], R.J. Wallace[b] ([a] The Institute of Rural Science, University of Wales, Aberystwyth, SY23-3AL, UK; [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Previous studies found that essential oils were able to affect ruminal fermentation and to decrease both the rate of deamination of amino acids and the degradation of protein supplements in Dacron bags. It was suggested that this last effect may be due to the essential oils decreasing the colonization of substrates by rumen bacteria. In the present study DNA extracted from samples of soyabean meal, peas and rapeseed meal incubated in the rumen of sheep receiving a diet of grass silage plus a high or low protein concentrate, plus or minus a commercial essential oils mixture (EO, CRINA RUMINANTS fed to supply 110 mg per sheep per day) was used as the template for 16S rDNA PCR-DGGE. Cluster analysis of DGGE band polymorphism showed that the host animal explained most of the similarity in the attached bacteria. However, within animal amplified bacteria rDNA extracted from the same substrate shared the highest level of similarity, while no clear pattern was observed due to supplementation with essential oils or the concentrate fed. When bands were excised from the gels and the resultant sequences matched against databases all the sequences were found to be from, or related to, ruminal microorganisms and it was apparent that the attachment of some bacteria to some substrates might be effected by the specific blend of essential oils. Study sponsored by CRINA S.A., 15 chemin de la Combe, Gland, CH-1196, Switzerland.

Effect of time after defaunation on methane production in vitro. M.J. Ranilla[a], D.P. Morgavi[b], J.-P. Jouany[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).

Protozoa-associated methanogens produce a large proportion of the methane generated by

ruminants. The elimination of rumen protozoa has been proposed as a way to reduce the emission of this greenhouse gas. However, little is known about the long-term efficacy of this practice. The effect of time after defaunation on ruminal fermentations was studied in vitro using protozoa-free rumen inocula obtained from faunated sheep in which protozoa were eliminated by centrifugation, sheep defaunated for 6 weeks, and sheep defaunated for more than one year; Short-, Medium-, and Long-term defaunation, respectively. Defaunated inocula were also centrifuged. Incubations were done on alfalfa hay (300 mg) for 24 h. Methane production was higher ($P < 0.001$) for Long, with no differences between Short and Medium (409, 358 and 347 μmol, respectively). Total VFA production was similar ($P > 0.05$) for the three inocula (mean 1324 mmol) but the fermentation pattern differed between them, with lower acetate and butyrate (0.67, 0.70 and 0.70, and 0.04, 0.06 and 0.06; $P < 0.01$) and higher propionate (0.24, 0.20 and 0.18; $P < 0.001$) molar proportions for Short than for Medium and Long, respectively. In agreement with methane production, acetate to propionate ratio was the highest for Long (3.73, $P < 0.001$), intermediate for Medium (3.47, $P < 0.01$) and lowest for Short (2.82). These results suggest that adaptation of the microbial ecosystem to defaunation and its effect on methanogenesis takes a long time to occur. The increase in methane production in long-term defaunations, if confirmed in vivo, would negate the benefit of eliminating protozoa to reduce emissions.

Does maternal microbiota act as an intermediate agent for probiotic action in suckling piglets? D. Taras[a], W. Vahjen[a], M. Macha[a], L. Scharek[b], K. Tedin[c], L. Wieler[c], M.F.G. Schmidt[b], O. Simon[a] ([a] Institute of Animal Nutrition; [b] Institute of Immunology and Molecular Biology; [c] Institute of Microbiology and Animal Epidemic Diseases, Free University of Berlin, Germany).

Since the EU-decision to ban antibiotics as feed additives, many have tried to transfer the probiotic concept to animal nutrition. Because of lacking detailed knowledge on the influence of probiotics on microbial-host interaction, an interdisciplinary approach was conducted to evaluate the effects of a selected probiotic on microbiology, immunology, gut morphology, physiology and animal performance. Two groups of sows and their respective litters were studied, either receiving or not receiving *Enterococcus faecium NCIMB 10415*. Overall body weight and feed conversion ratio were not persistently influenced, although the apparent praecaecal digestibility of almost all studied amino acids improved. Probiotic fed weaned piglets showed a significant reduced incidence of watery feces, compared to the controls (40% vs. 60%). DGGE profiles representing the dominant colon microflora indicated a decrease in microbial richness and evenness in suckling piglets of probiotic fed sows. Suckling, 14 days old piglets exhibited less intraepithelial CD8-positive lymphocytes than control piglets. Also, the occurrence of *E. coli* serogroup O141 was reduced in the probiotic-treated piglets. All these results may suggest a reduced immunological burden, which is most probably the result of an already modified maternal fecal composition. Supporting observations are differences in bacterial metabolite concentrations, in probiotic cell counts, bacterial rRNA content and DGGE profiles between suckling piglets of both treatment groups. In addition, DGGE profiles derived of feces from pregnant sows before and two months after continuous probiotic supplementation are less similar to each other than comparable samples of control sows, indicating a probiotic influence on maternal microbiota.

Composition of intestinal microflora can explain the difference in the probiotic effect. K. Ushida[a], M. Tokunaga[a], Y. Ohashi[a], K. Hashizume[a], T. Tsukahara[a,b] ([a] Kyoto Prefectural University, Shimogamo, Kyoto 606-8522, Japan; [b] KYODOKEN Institute, Shimoitabashi, Kyoto 612-8073, Japan).

The effect of a probiotic is substantially varied by the subject, although the same effect is anticipated for the majority of subjects. We found the evidence that explains such individual variation. Three caecectomized female pigs were used. Caecal digesta was collected and anaerobically incubated in vitro with two probiotic bacteria, *Lactobacillus acidophilus* and *Megasphaera elsdenii*. Butyrate was the major SCFA produced in the digesta from two pigs, while propionate

was the major SCFA produced in the digesta from one pig. The difference in the SCFA production was suggested to be due to the individual difference in caecal microflora. When *L. casei*-fermented milk was fed to other three caecectomized pigs, caecal butyrate concentration was increased in two pigs. The remaining pig did not react with *L. casei*-fermented milk. This latter pig showed relatively higher propionate concentration in the caecum independent of *L. casei*-fermented milk. The caecal microflora of these pigs was analyzed by the 16S rDNA profile using temperature gradient gel electrophoresis (TGGE). Hierarchical clustering analysis on the TGGE band profiles indicated that caecal microflora of two pigs resembled each other and that that of the remaining pig was distinct. Unique TGGE bands for this particular pig were excised and sequenced. Two *Succinivibrio dextrinosolvens*-like partial 16S rDNA sequences were identified as unique bands for this pig that did not react with *L. casei*-fermented milk. Colonic microflora characterized in propionate production might not respond well to probiotics that are anticipated to promote butyrate production in the large intestine.

Prebiotic effects of fructooligosaccharides in the simulator of the human intestinal microbial ecosystem. T. Van de Wiele[a], N. Boon[a], H. Jacobs[b], W. Verstraete[a] ([a] Laboratory Microbial Ecology and Technology, Ghent University, 9000 Gent, Belgium; [b] Cosucra SA, 7740 Warcoing, Belgium).

Functional foods such as probiotics and prebiotics which modulate the colon microbial community towards a more beneficial composition for human health, are the subject of many research efforts. Of all possible prebiotics, inulin type fructooligosaccharides (FOS) have been investigated the most. Using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), we monitored the influence of a specific inulin formulation on the microbial community from the colon ascendens to colon descendens, both in terms of metabolic activity as the community structure. Inulin was supplemented to the SHIME reactor during a 5 week treatment period. Short chain fatty acid production by colon microbiota increased with 45%, 24% and 31% in the colon acendens, transversum and descendens, with a shift towards propionic and butyric acid, which is beneficial seen the positive influence of these compounds towards colonocytes. Realtime PCR analysis with bifidobacteria specific primers revealed a significant increase with 1 to 1.5 log CFU·mL^{-1} for Bifidobacteria populations from the proximal to distal colon, showing the selective effect of inulin towards these bacteria. This is the first report of quantitative PCR on in vitro cultured microbiota from different parts of the colon. DGGE analysis showed a significant shift in bifidobacteria and lactobacilli populations due to inulin supplementation, whereas no remarkable shifts for Eubacteria populations were observed. We conclude that the SHIME model for the human gut in combination with a broad range of molecular analysis tools is very useful for studying the prebiotic effects of inulin in different parts of the gastrointestinal tract.

Dosage dependent effects of *Moringa oleifera* seed meal on proteolytic activity and community composition of rumen microbes in vitro. E.M. Hoffmann, N. Selje, S. Mützel, K. Becker (Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim, 70593 Stuttgart, Germany).

Moringa oleifera is a small, pan-tropical tree with multiple uses for virtually all parts of the plant. A water extract from Moringa seeds has been shown to inhibit ruminal protein degradation in vitro. This activity was investigated further, using rumen fluid from fistulated Holstein cows and a protein rich substrate formulation with the Reading Pressure Technique (RPT). Various dosages of defatted Moringa seed meal (MSM; 0 to 2.0 mg dry matter·mL^{-1} incubation volume) were added and 3 µM monensin served as the control. The kinetics of protein degradation was monitored by dot blot and PAGE. Proteolysis was retarded by inclusion levels greater than 0.5 mg·mL^{-1} MSM in a dosage dependent manner. The protein concentrations after 12 h of incubation were 68 and 95% of the initial concentration with 1.5 and 2 mg MSM·mL^{-1}, whereas protein in the control was almost completely degraded. The effect exceeded that of monensin (43%). Ammonia release was reduced for the two highest concentrations of MSM. Negative side effects appeared on gas and SCFA production. Changes of the microbial community structure

were monitored by specific PCR for fragments of the SSU rRNA gene of low GC gram positive bacteria (LGC) and the Prevotella- Bacteroides-cluster, and subsequent separation by DGGE. The most striking effects were detected within the LGC over the incubation time as well as between the treatments. This is in congruence with the present concept that most of the known proteolytic bacteria belong to LGC and are affected by monensin.

Effect of selenium on the antioxidant enzyme activities of ruminal bacteria. K. Holovská Jr.[a], A. Sobeková[a], K. Holovská[a], V. Lenártová[a], P. Javorský[b] ([a] Department of Chemistry, Biology and Biochemistry, University of Veterinary medicine, 04181 Košice, Slovakia; [b] Institute of Animal Physiology, Slovak Academy of Science, Košice, Slovakia).

The effect of various Se levels on the activity of antioxidant enzymes: glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) of the ruminal bacteria *Streptococcus bovis 4/1* and *Selenomonas ruminantium E32* isolated from sheep rumen, as well as on mixed bacteria isolated from rumen fluid was investigated. Bacteria were grown anaerobically in medium containing 0 or 5 $\mu g \cdot mL^{-1}$ $HgCl_2$ and different concentrations of Se in the form of seleno-L-methionine (0.1, 0.2, 0.3 ppm). In *S. bovis* the addition of Se significantly increased both GSHPx and SOD activities. At the highest Se concentration (0.3 ppm), SOD activity, the first line of defense against superoxide radicals, increased 2.9 times as compared to the control. This points to the possible pro-oxidative effect of Se. When *S. bovis* was grown in the presence of mercury, a known prooxidant, both SOD activity and TBARS contents, the marker of lipid peroxidation, increased significantly. The addition of Se again decreased SOD activity and TBARS contents. Mercury had no effect on GSHPx activity. In *S. ruminantium* only GSHPx activity was detected. This activity was not influenced by Se. On the contrary to *S. bovis,* mercury decreased GSHPx activity (1.3 times). The addition of Se had no antioxidative influence. The effect of Se was different on mixed bacteria isolated from the rumen fluid of sheep fed a diet supplemented with 0.3 ppm Se. SOD activity was almost completely inhibited and GSHPx activity decreased 3.8 times as compared to control sheep.

Influence of dietary zinc oxide (ZnO) and copper sulphate ($CuSO_4$) on the gastrointestinal ecosystem in newly weaned piglets. O. Højberg, N. Canibe, B.B. Jensen (Danish Institute of Agricultural Sciences, PO Box 50, 8830 Tjele, Denmark).

Dietary ZnO and $CuSO_4$ have been observed to promote piglet growth and alleviate problems of post-weaning diarrhoea, however the mechanisms are only sparsely described. In the present study, dietary doses of 2500 ppm ZnO-Zn reduced the number of lactobacilli, throughout the gastrointestinal tract of weaned piglets, whereas coliforms and enterococci were found in higher numbers. Dietary doses of 175 ppm $CuSO_4$-Cu also reduced the lactobacilli in the stomach, while coliforms were reduced in the caecum and colon of these animals. Concentrations of lactate and succinate were lower in the digesta from the stomach and distal small intestine of the pigs receiving the high ZnO dose. On the contrary, lactate and succinate accumulated in the caecum and colon of these animals, concomitant with a reduction in concentrations of short-chain fatty acids (acetate, butyrate and propionate). The inhibition of lactobacilli in the stomach and ileum of ZnO treated animals probably led to a higher flow of readily fermentable substrates to the caecum and colon, promoting the temporary accumulation of lactate and succinate. Dietary $CuSO_4$ did not affect the organic acid content. The influence of ZnO on the gastrointestinal microbiota resembles the suggested working mechanism of some of the growth promoting antibiotics, namely suppressing gram-positive commensals rather than potentially pathogenic gram-negatives. $CuSO_4$ seems to inhibit the coliforms as well, however, the growth promoting effect of high dietary doses of ZnO and, to a lesser degree, $CuSO_4$ may be due to reduced fermentation of digestible nutrients in the proximal part of the gastrointestinal tract rendering more energy available for the host animal.

Use of odd-chain fatty acids as markers of the microbial colonisation of freshly-ingested herbage in the rumen. E.J. Kim, J.K.S. Tweed, R.J. Merry, R.J. Dewhurst (Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK).

Three in situ studies were conducted to examine the use of odd-chain fatty acid profiles to study microbial colonisation of freshly ingested herbage in the rumen. In study 1, fresh perennial ryegrass was incubated in dacron bags in the rumen for 2, 8 and 24 h. After removal of the bags from the rumen four different washing methods were applied; (1) manual squeezing; (2) soaking and gently shaking in a sink full of water; (3) washing under running tap water; and (4) use of a washing machine. In study 2, fresh perennial ryegrass was subjected to different physical treatments: (i) no processing: grass was gently folded and placed into dacron bags; (ii) chopping into approximately 1 cm lengths using scissors; (iii) crushing with a metal roller, but not chopping; (iv) chopping and crushing; (v) mechanical chopping; (vi) ingested boli (boli retrieved at the oesophageal orifice after swallowing); and (vii) freeze-drying and grinding. Samples were incubated in the rumen for 2 and 7 h. In study 3, the boli from two contrasting forages (ryegrass and white clover) were collected and incubated in the rumen (0, 1, 2, 4, 8 and 24 h). Fatty acid methyl esters were extracted from washed residues and determined by GC using tricosanoic acid as the internal standard. Two odd-chain fatty acids (iso C15:0 and iso C17:0) found in the rumen bacteria that are not detected, or found in negligible concentrations in fresh forages, can be used as markers of microbial colonisation. The concentration of these and other odd-chain fatty acids increased with incubation time in all three in situ studies. The results indicate rapid and continued microbial colonisation of freshly ingested forages. Differences in odd-chain fatty acid profiles suggest changes in populations of micro-organisms during the colonisation progress. Further analysis using molecular techniques is in progress.

A method to screen the dose-response effects of essential oils on the activity of rumen microbial population. D. Macheboeuf[a], Y. Papon[a], M. Arturo-Schaan[b], A. Mercier[b], J.-L. Mousset[c], J.-P. Jouany[a] ([a] INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France; [b] CCPA ZA Nord-Est du Bois-de-Teillay, 35150 Janzé, France; [c] TECHNA Les Landes-de-Bauches BP 10 Route de St-Etienne-de-Montluc 44220 Couëron, France).

Since 1999, several antibiotics used in animal feeding have been banned in the European Union and all of them will be removed from the market in 2006. So, there is a need for efficient natural additives to substitute the chemical additives banned. Many essential oils are known for their bactericidal effect and thus could be used to control the digestive microbial ecosystem. However, there is scarce information available on the dose that can be effectively used in ruminants. The aim of this study was to screen the dose effect of 8 essential oils (EO) on ruminal fermentation patterns estimated in an in vitro batch system after a 16 h-period of fermentation with a high concentrate substrate. The response of the microbial ecosystem was evaluated through the modelling of the production curves of volatile fatty acid (VFA) and gas plotted against the dose of EO. Three profiles were determined for the curves. On profile 1, the additive has a linear, negative effect on the end products of fermentation (observed with one EO). On profile 2, observed with 2 of the 8 EO tested, a negative sigmoid shape appeared at the lower doses, followed by a slight decreased plateau level for the highest doses. In that case, VFA and gas productions were lowered by 40–50% ($P < 0.01$) when compared to controls (fermenters without EO). The loss of the methanogenesis function was a feature of the sigmoid point transition. On profile 3, for 5 of the tested EO, a drastic drop in VFA and gas production was observed after a threshold dose after that all fermentative activities were completely stopped. Curve profiling of VFA or gas production allowed a fast screening of EOs, with determination of the optimal and inhibitory doses, the latter being harmful to feed digestion.

Comparison of dose-response effects of thymol and thyme essential oils on in vitro rumen fermentations. D. Macheboeuf[a], Y. Papon[a], M. Arturo-Schaan[b], A. Mercier[b], J.-L. Mousset[c], J.-P. Jouany[a] ([a] INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France; [b] CCPA ZA Nord-Est du Bois-de-Teillay, 35150 Janzé, France; [c] TECHNA Les Landes-de-Bauches BP 10 Route de St-Etienne-de-Montluc 44220 Couëron, France).

Plants contain various secondary compounds that could be used as natural additives to

improve feed utilization in ruminants. Among these compounds, essential oils (EO), e.g. thyme EO, have been shown to have anti-microbial activity and could be used to manipulate rumen fermentation. The biological activity of thyme EO is mainly attributed to thymol. However, some authors noted that natural EO are more effective than pure components. This could be due to synergistic effects among different components present in natural oils. The aim of this study was to compare the dose effect of thymol and thyme EO on the fermentation pattern of mixed ruminal microbes estimated in an in vitro batch system after a 16 h-period of fermentation with a high concentrate substrate. The response of the microbial ecosystem was evaluated through the modelling of the production curves of volatile fatty acids, gas and NH_3 plotted against the dose of thymol supplementation coming from thyme EO (composition 47% thymol, 20% terpinene, 20% *p*-cymene) or the dose of pure thymol. For all end-products, the sigmoid curves obtained from pure thymol or thyme EO had the same profile. However, the necessary dose to obtain the same inhibitory effect was lower for thyme EO than for pure thymol indicating that the natural EO had a stronger effect. When we corrected the thyme EO dose with the presence of *p*-cymene, a phenolic constituent close to thymol. The corrected profiles of thyme EO were exactly superimposed with pure thymol profiles for propionate, butyrate and very close for gas, NH_3 and acetate productions. The results showed that no synergistic interaction occurred in thyme EO between thymol and the other constituents but only an additive effect between thymol and p-cymene.

In vitro characterisation of the bactericidal capacities of dietary sphingosine in the intestinal tract. S. Possemiers, S. Bolca, J. Van Camp, W. Verstraete ([a] Laboratory of Microbial Ecology and Technology, UGent, 9000 Gent, Belgium; [b] Department of Food Technology and Nutrition, UGent, 9000 Gent, Belgium).

Dietary sphingolipids have gained increasing attention as functional food constituents because of their possible positive impact on human health. Animal studies have shown that food sphingomyelin and its metabolites ceramide and sphingosine inhibit colon carcinogenesis and positively alter cholesterol uptake. Sphingosin also has a putative bactericidal effect towards food pathogens. We further characterised the sphingosine bactericidal activity to evaluate whether it acts selectively against pathogens and does not negatively influence the indigenous intestinal microbial community. Firstly, Live/Dead analysis was applied using Flow Cytometry to evaluate the susceptibility of selected pure cultures of intestinal species to sphingosine and the results were compared with plating results. Secondly, the sphingosine bactericidal activity was measured in vitro by simulating intestinal conditions in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME). The role of pH, aerobisation, temperature and medium composition were monitored to elucidate the mechanism of this antibacterial activity and its possible cellular targets. We found that 25 $\mu g \cdot L^{-1}$ sphingosine had negligible effects on *Lactobacillus casei* and *Bifidobacterium breve* in saline buffer whereas *Escherichia coli*, *Enterobacter aerogenes* and other intestinal pathogens like *Campylobacter jejuni* and *Listeria monocytogenes* were strongly inhibited. This implies a possible selective effect of sphingosine towards pathogens. However, in the complex matrix of an intestinal suspension, the bactericidal activity was strongly influenced by environmental conditions, with a higher activity at a lower pH.

Enhanced expression of lignolytic activity by the basidiomycete *Trametes versicolor* can improve degradation of bisphenol A. M. Takamiya[a,b], N. Magan[b], P.J. Warner[b] ([a] National Institute of Technology and Evaluation, 2-49-10, Nishihara, Shibuya-ku, Tokyo, 151-0066, Japan; [b] Cranfield University, Silsoe, Bedfordshire, MK45 4DT, UK).

Bisphenol A (4, 4'-isopropylidenediphenol) is an endocrine disrupter which affects the vertebrate/ invertebrate reproduction system and thus has a serious impact on animals in industrial regions world-wide. We utilized the white rot basidiomycete, *Trametes versicolor* to examine the potential for over-expression of lignolytic enzymes and quantified enzyme production levels in relation to different concentrations of bisphenol A. Our studies identified that the growth of *T.versicolor* was tolerant of up to 300 ppm of biphenol A with an LD_{50} value of 200 ppm. The

production of laccase, manganese peroxidase and lignin peroxidase were stimulated in the presence of up to 300 ppm of the analyte. The maximum concentrations of these enzymes were 0.06, 0.75 and 0.42 $U \cdot mL^{-1}$ in 300 ppm bisphenol A, respectively, after 14 days of incubation. Gene expression of laccase and lignin peroxidase was demonstrated by RT-PCR. Gene products were identified whose expression was correlated with the concentrations of biphenol A used. To date a putative PCR product has been amplified using primer sequences from another *Trametes* spp. which has 55% similarity with the laccase gene (lccK) of *Pleurotus ostreatus*, 57% with the manganese peroxidase isozyme (MPGI) of *T. versicolor*. This work suggests that a potential exists for utilizing fungal inoculants like *T. versicolor* in environments contaminated with endocrine disrupters for the prevention of their entry into human and animal food chains. By manipulation of the environment in which the fungus is grown it may be possible to optimize expression of the degradative enzymes involved.

Effects of saponin-containing methanol extract of *Sapindus rarak* on ruminal flora and fermentation characteristics in vivo. E. Wina[a], S. Muetzel[b], E.M. Hoffmann[b], K. Becker[b] ([a] Research Institute for Animal Production, PO Box 221 Bogor, 16002 Indonesia; [b] Institute for Animal Production in the Tropics and Subtropics (480b), University of Hohenheim, Fruwirthstrasse 12, D-70599, Stuttgart, Germany).

Previous in vitro experiments have shown that a methanol extract of pericarps of *Sapindus rarak* which contains saponins, reduces protozoal numbers. An in vivo feeding trial was, therefore, conducted using 28 sheep, all fed with a mixture of dried sugar cane top and wheat pollard (75:25) at the level of 4% body weight. Methanol extract of *S. rarak* was mixed with wheat pollard and was offered twice a day at the level 0, 4, 8 and 12 $g \cdot day^{-1}$ to groups of 7 sheep each for 105 days. Rumen liquor was taken at day 98 and 100 of the experiment. The methanol extract of *S. rarak* changed several rumen parameters: pH slightly increased from 6.64 to 6.83 ($P < 0.05$) and ammonia content tended to decline from 0.33 $mg \cdot mL^{-1}$ to 0.27 $mg \cdot mL^{-1}$ ($P = 0.06$). The total SCFA did not change but the molar proportion of propionate increased while butyrate was significantly lower ($P < 0.05$). Xylanase and CMC-ase activities were depressed when protozoal counts decreased dramatically ($P < 0.001$). Using18S rRNA as a marker, the concentration of eukaryotes decreased with an increasing level of methanol extract, however, the concentration of eukaryotes in the control group was surprisingly low. Long term feeding (100 days) of methanol extract of *S. rarak* showed a permanent effect of defaunation. Currently, several fibre-degrading microorganisms are being quantified by a hybridisation technique.

Effects of formate and hydrogen on the inhibitory activity of 2-nitropropanol and nitroethane on ruminal methane production in vitro. R.C. Anderson, T.R. Callaway, R.B. Harvey, Y.S. Jung, K.J. Genovese, T.S. Edrington, J.L. McReynolds, D.J. Nisbet (U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX 77845, USA).

Ruminal methanogenesis is an inefficient process resulting in losses of 2 to12% of gross energy intake. Presently, we report the effects of two inhibitors on ruminal methane production in vitro. Mixed populations of ruminal microbes collected from a cow grazing rye grass were incubated (39 °C) in duplicate for 24 h under CO_2 or H_2 (100%) with or without 60 mM added formate. The cultures were supplemented with or without 2-nitropropanol or nitroethane (5 mM). Methane accumulations were higher ($P < 0.05$) in control cultures (containing no added nitrocompound) incubated with added formate (35.5 ± 16.0 and 36.6 ± 3.4 $\mu mol \cdot mL^{-1}$ when under CO_2 and H_2, respectively) than in controls incubated without added formate (17.4 ± 0.8 and 20.5 ± 0.3 $\mu mol \cdot mL^{-1}$ when under CO_2 and H_2, respectively). In the absence of added formate, methane accumulations in cultures incubated with 2-nitropropanol were numerically decreased by 33 and 8% when under CO_2 and H_2, respectively. When incubated with added formate; however, methane accumulations were reduced ($P < 0.05$) 87 and 64% when under CO_2 and H_2, respectively. In contrast, methane accumulations were reduced more than 95% in cultures incubated with nitroethane, regardless of format addition.

Accumulations of H_2 in the 2-nitropropanol supplemented cultures were higher ($P < 0.05$) than in controls only when incubated under H_2, with or without added formate, but were higher in the nitroethane cultures only when incubated with both H_2 and formate. Further research with these nitrocompounds may yield strategies to reduce costs associated with ruminal methane production.

Liveweight responses by sheep to vaccination against ruminal methanogens. S.K. Baker[a], P.E. Holloway[a,b], N.J. Edwards[a,c], V.S. Whiffen[a,d], T.L. Smith[a,d] ([a] CSIRO Livestock Industries, Floreat Park Research Laboratory, 6014, Western Australia; [b] Currently: Tasmanian Police, Hobart, 7000, Tasmania; [c] SARDI, Naracoorte, 5271, South Australia; [d] Murdoch University, Murdoch, 6150, Western Australia).

Ruminants have low titres in plasma and saliva of antibodies against ruminal micro-organisms, and titres are increased with vaccines where ruminal micro-organisms are antigens. Production responses include increases in digestible dry matter intake and efficiency of wool growth (Baker, 1994; PCT AU94/00633). Here liveweight responses were measured, and the antigen composition of the vaccine against ruminal methanogens was essentially as before (Baker, 1994). The adjuvant comprised mineral oil (Montanide ISA50®, 1.2mL) and saponin (Quil A (Superfos), 0.5mg). The vaccine (2 mL per sheep) was given subcutaneously to two groups (A ($n = 8$), B ($n = 9$)) and again 15 weeks later to B. The adjuvant alone was given subcutaneously to the 'control' sheep (C ($n = 6$) and D ($n = 9$)) and again 15 weeks later to D. The sheep, Merino wethers, six-month-old (live weight 28.5 ± 2.5 kg), consumed *ad libitum* oaten hay (*Avena sativa*), lupin seed (*Lupinus angustifolius*) and minerals (Siromin®) (0.85:0.10:0.05). This diet maintains live weight of mature sheep, but gives young sheep little opportunity for growth. Between 26.5 and 36 weeks after initial vaccination a supplement (40 $g \cdot d^{-1}$ canola-meal, the residue after oil-extraction of *Brassica napus* seed) was given. Vaccinated sheep grew faster ($g \cdot d^{-1}$) (mean, sem) (38.2, 4.27 (A), 44.6, 4.03 (B)) than the 'control' sheep (23.2, 4.93 (C), 28.9, 4.03 (D)) ($P = 0.003$). These growth rates are modest and before this they were similar between groups ($P = 0.700$). They were associated with increased titres in plasma and rumen fluid of immunoglobulin G specific to the antigens, and might be attributed to reduced methanogen activity coupled with additional dietary protein.

In vitro rumen microbial growth as affected by the addition of malate and fumarate. R.G. Martínez, M.J. Ranilla, M.L. Tejido, M.D. Carro (Dept. Producción Animal I, Universidad de León, 24071 León, Spain).

Batch cultures of mixed rumen microorganisms were used to study the effects of disodium fumarate and disodium malate on microbial growth. The diets consisted of forage (alfalfa hay:corn silage, 50:50) and concentrate (corn:barley:soybean meal, 35:50:15) in the proportions of 80:20 (C20 diet) and 20:80 (C80 diet). Samples of each diet were incubated with buffered rumen fluid from sheep for 16 h at 39 °C and ^{15}N was used as a microbial marker. Fumarate and malate were added to the incubation bottles to achieve final concentrations of 0 (CON), 8 mM fumarate (FUM) and 8 mM malate (MAL). For diet C20, the addition of malate and fumarate increased ($P < 0.05$) microbial N production (15.4, 16.5 and 18.0 mg microbial N for CON, FUM and MAL, respectively), but the efficiency of microbial growth was only improved ($P < 0.05$) by malate (68.0, 70.1 and 75.9 mg microbial $N \cdot g^{-1}$ organic matter disappearance (OMD)). In contrast, for diet C80 fumarate and malate did not affect ($P > 0.05$) microbial growth nor its efficiency (17.7, 17.9 and 18.1 mg microbial N and 54.7, 53.7 and 56.4 mg microbial $N \cdot g^{-1}$ OMD for CON, FUM and MAL, respectively). For both diets, fumarate and malate increased volatile fatty acid production by 15.3 and 16.2% (means for both diets for FUM and MAL, respectively) and decreased methane production by 4.4 and 5.5%, with no differences ($P > 0.05$) between malate and fumarate being detected. The results show that effects of fumarate and malate on in vitro microbial growth can be influenced by the incubated diet.

Screening the activity of medicinal plants and spices for decreasing ruminal methane production in vitro. R. García-González, S. López, M. Fernández, A.B. Rodríguez, J.S. González (Departamento de Producción Animal I, Universidad de León, 24071 León, Spain).

Recently, plants and plant extracts have been suggested as alternative additives to be used in animal feeding. Different medicinal plants, herbs and spices (up to 167) were tested in a screening trial to assess their potential to modify ruminal fermentation in vitro, in particular to evaluate their effectiveness for decreasing methane production. All the plants used were obtained from different herbal shops and phytotherapy companies. In vitro incubations were carried out in serum bottles using 450 mg of diet (50% lucerne hay, 20% grass hay, 30% barley), 70 mg of each plant additive, and 50 Ll of buffered rumen fluid. After 24 h of incubation at 39 °C, gas production and pH were recorded and samples were taken for gas and VFA analyses. Incubation residues were determined by filtration. Methane production and the acetate to propionate ratio were considered as the main criteria for plant selection. In the screening trial, most of the plants tested did not cause significant effects on those parameters. Amongst the plants causing a noteworthy change in rumen fermentation, three seemed to be the most effective in shifting the key fermentation parameters: *Rheum officinale* (root), *Rhamnus frangula* (bark) and *Allium sativum* (bulb). Incubations were repeated with these plants to confirm the results observed. DM degradation was affected by the plant addition, decreasing with *R. officinale* (–4%) and *R. frangula* (–6%) and increasing with *A. sativum* (+4%).The three plants reduced methane production in comparison with control cultures, by 74%, 44% and 15%, respectively. As expected, this decrease was accompanied by a lower acetate to propionate ratio.

Manipulation of rumen microbial populations by dietary lauric and myristic acid mixtures supplied as feed additives to control ruminal methanogenesis in vitro. C.R. Soliva[a], L. Meile[b], M. Kreuzer[a], A. Machmüller[a,c] ([a] Institute of Animal Science, Animal Nutrition, Zurich, Switzerland; [b] Institute of Food Science and Nutrition, Laboratory of Food Biotechnology, Swiss Federal Institute of Technology Zurich, 8092 Zurich, Switzerland; [c] Current address: School of Rural Science and Agriculture, University of New England, Armidale NSW 2351, Australia).

Dietary lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acid mixtures were shown to suppress ruminal formation of the ecologically detrimental methane in vitro. However, there is still little information available about the associated modifications in the rumen microbial community. A long-term in vitro study using the Rumen Simulation Technique (Rusitec) was carried out to reveal whether the methane-suppressing effect of $C_{12:0}/C_{14:0}$ mixtures is caused by a direct toxic effect on methanogens or indirectly through affecting the bacterial and protozoal hydrogen suppliers. Changes in cell counts of total rumen Archaea and single methanogenic orders, therefore, were opposed to shifts in protozoal and bacterial counts. Seven different $C_{12:0}/C_{14:0}$ mixtures were supplemented (5% of dietary DM) to a concentrate-based basal diet. The unsupplemented basal diet served as the control. The experimental runs lasted for 15 to 25 days. Protozoa were eliminated by some of the $C_{12:0}/C_{14:0}$ mixtures investigated. Unexpectedly, total bacteria counts did not show any compensatory increase. Nevertheless, hydrogen supply for methanogenesis was never limited since hydrogen accumulated in the Rusitec fermenters. Those $C_{12:0}/C_{14:0}$ mixtures, which significantly diminished methanogenesis, also decreased total archaeal counts and altered the methanogenic order composition. In some cases a strong decrease in methane formation occurred but only a weak decline in archaeal counts was observed. Possible explanations for this inconsistency are either changes in the archaeal population composition or in the metabolic activities of methanogens or both. Since the methane-suppressing $C_{12:0}/C_{14:0}$ mixtures did not impair ruminal fiber degradation they seem to be suitable as feed additives in controlling rumen methanogenesis.

Methane abatement in sheep by immunization against rumen methanogens. A.-D.G. Wright[a], P. Kennedy[b], C. O'Neill[b], A.F. Toovey[a], S. Popovski[a], S.M. Rea[a], C. Pimm[a], L. Klein[a] ([a] CSIRO Livestock Industries, Private Bag 5 Wembley WA 6913, Australia; [b] CSIRO Livestock Industries, PO Box 5545, Rockhampton Mail Centre, QLD 4702, Australia).

Thirty Merino sheep were randomly allocated to three treatment groups ($n = 10$) to determine if methane emissions from sheep immunized with anti-methanogen vaccines were significantly lower than non-immunized sheep. The control

group received primary and secondary immunizations containing the adjuvant only, one treatment group received primary and secondary immunizations containing a 3-methanogen vaccine formulation (VF3+3), and the other treatment group received a primary immunization with a 7-methanogen mix followed by a secondary immunization with the 3-methanogen mix (VF7+3). For the duration of the experiment, the sheep were offered medium quality Rhodes grass hay at 90% of ad libitum intake. Four weeks post secondary immunization, on a per animal basis there was a significant 13% reduction in methane in the VF3+3 group compared to the controls. When methane emissions were adjusted for dry matter intake (DMI), there was still a significant 8% reduction ($P = 0.051$) in methane production per kg DMI. The methane emissions from sheep immunized with VF7+3 were not significantly different when compared to the sheep in the control group ($P = 0.883$). Both treatment groups had significantly higher plasma and saliva titres (IgG and IgA) at 3 and 6 weeks post-secondary immunization when compared to the control sheep ($P < 0.001$). The average IgG and IgA antibody titres in both plasma and saliva of the VF3+3 immunized sheep were up to 800% higher than those for the VF7+3 immunized sheep ($P < 0.001$) at both 3 and 6 weeks post-secondary immunization.

Effects of bacteria used for biopreservation of food on the composition of intestinal bacteria. N. Bernbom, P. Saadbye, T.R. Licht, B. Norrung (Department of Microbiological Food Safety, Danish Institute for Food and Veterinary Research, Morkhoj Bygade 19, 2860 Soborg, Denmark).

There is an increasing demand for food products subjected only to gentle preservation methods but with long shelf lives. At the same time, consumers request increased food safety and microbiological quality, but are concerned about conventional chemical preservation methods. These are some of the reasons for the increased focus on bio- preservation, which imply the application of selected microorganisms (frequently lactic acid bacteria) for the control of pathogenic and other unwanted microorganisms in food. The purpose of the present project was to identify putative risks associated with bio-preservation through investigations of the effects of bacteriocins and bacteriocin-producing lactic acid bacteria on the intestinal micro flora. The approaches were studies of basic anti-microbial characteristics of bacteriocin producing bacteria and investigations of the effect of such organisms on the fecal flora of human-flora associated rats. The methods used for the detection of changes in the gut flora were T-RFLP (Terminal Restriction Fragment Length Polymorphism) and traditional selective plating. The results indicate that ingestion of bacteriocin-producing *Lactobacillus plantarum* causes a reduction of the total number of *Lactobacilli* in the intestine.

Efficiency and dose effect of the ruminant feed additive Levucell® SC to improve ruminal pH in induced acidotic sheep. L. Brossard[a,b], C. Martin[a], F. Chaucheyras-Durand[b,c], B. Michalet-Doreau[a] ([a] Unité de Recherches sur les Herbivores, INRA, Centre de Recherche de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France; [b] Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France; [c] Laboratoire de Microbiologie, INRA, Centre de Recherche de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France).

We used two experimental designs to investigate the dose effect of the microbial feed additive Levucell SC (*Saccharomyces cerevisiae*, CNCM I-1077) on ruminal pH during induced latent acidosis in sheep. In Exp.1, twelve ruminal cannulated sheep were fed twice daily a hay diet (100%) for 3 weeks and, following a one-week transition period, an acidotic diet (60% wheat + 40% hay) for two more weeks. The animals were allocated into three groups: control group (L0; $n = 5$); 0.2 g·day^{-1} of Levucell SC (L1, 4.10^9 CFU·d^{-1}; $n = 3$), and 2 g·day^{-1} of Levucell SC (L10, 4.10^{10} CFU·d^{-1}; $n = 4$). Yeasts were added intraruminally once daily before the morning feeding. Ruminal pH was measured continuously by indwelling probes. Daily mean ruminal pH and area under pH 6 were calculated. In Exp.2, six ruminal cannulated sheep were used in a 3 × 3 latin square design. The diets, treatments and measurements were the same as in Exp.1. Compared to the control group, supplementation with yeast reduced ($P < 0.001$) the severity of acidosis in Exp.1 by increasing mean

pH (5.65 vs. 5.83) and decreasing the area under pH 6 (10.5 vs. 6.8 pH unit·h); the L1 dose tended to be more efficient than the L10 dose ($P < 0.1$). In Exp.2, neither the L1 nor L10 dose had an effect on the severity of acidosis. However, 4 sheep among 6 had higher mean ruminal pH during the yeast supplementation periods than during the control periods. It can be suggested that repetitive inductions of acidosis, in a latin square design, and/or too short recovery time could mask the effects of SC. According to these results, it is questionable whether a latin square design is an appropriate methodology for assessing yeasts' and more generally the microbial additives' impact on ruminal fermentations.

Does the probiotic *Lactobacillus reuteri* affect the gut microflora profiles of HIV infected and non-infected children with diarrhoea? I.C. Schloss[a], L. Dell[a], M. Acker[a], V. Abratt[b], S. Reid[b], G. Hussey[c] ([a] Division of Nutrition and Dietetics; [b] Department of Microbiology; [c] Institute of Child Health, University of Cape Town, South Africa).

Oral administration of live *Lactobacillus reuteri* is associated with a reduced duration of diarrhoea in children. This study compared changes in faecal *Lactobacillus* and *Bifidobacterium* in children with diarrhoea following the administration of this probiotic. Infants with acute diarrhoea recruited from a Red Cross Hospital were randomised into either a probiotic (1×10^{10} cfu·mL^{-1} *L. reuteri*) group ($n = 43$, 15 HIV positive) or a placebo group ($n = 38$, 9 HIV positive). A comparative group ($n = 22$) without diarrhoeal disease was matched for age, sex and ethnicity. Baseline colony counts of *Lactobacillus* and *Bifidobacterium* were determined on admission and at 24 h post-intervention. *Bifidobacterium* counts for females was greater than males (7.3 ± 6.1 vs. 6.4 ± 4.9; $P < 0.04$). Baseline *Lactobacillus* counts were higher in the non-diarrhea group (8.1 {7.4–8.4}) vs the diarrhoea group 7.1 {6.2–8.2} $P < 0.05$) independent of HIV or antibiotic status. Infants receiving antibiotics in the placebo group ($n = 20$) showed a greater reduction in *Lactobacillus* counts compared to the probiotic group ($n = 15$) ($P = 0.07$). However, Lactobacillus counts in HIV *positive* children *decreased* following probiotic administration although this did not reach significance. Mean *Lactobacillus* and *Bifidobacteria* counts decreased in the placebo groups and in HIV *negative* children after 24 h but this was not significant. *Lactobacillus reuteri* was lowered in the children with diarrhoeal disease. Oral administration of *L. reuteri* is unlikely to show significant differences in the stool after only 24 h. Probiotics limited the decrease of faecal lactobacillus in infants receiving antibiotic treatment and more research is warranted to determine the true effect of *L. reuteri* in HIV positive infants.

An overview of the Alimentary Pharmabiotic Centre (APC), University College Cork – towards an understanding of the intestinal flora in health and disease. C.G. Gahan[a,b], J.R. Marchesi[a,b] ([a] Alimentary Pharmabiotic Centre; [b] Department of Microbiology, University College Cork, Ireland).

The APC is a multidisciplinary research centre involving clinicians, microbiologists, physiologists, veterinary scientists and cell biologists dedicated to investigate the means by which intestinal bacteria influence health and disease. The Centre is funded through the Science Foundation of Ireland, Centres for Science Engineering and Technology (CSET) scheme and represents an alliance between academic researchers and local and international biotechnology companies. Research at the APC has included study of *Lactobacillus salivarius* and *Bifidobacterium infantis* strains with enhanced probiotic potential. Both *L. salivarius* and *B. infantis* strains demonstrated an ability to reduce chronic inflammation in the IL-10 knock-out mouse model and limit inflammatory cytokine production in tissue culture models. In addition, probiotic commensal strains were capable of reducing host cell infection with *Salmonella* Typhimurium in both in vivo and in vitro model systems. The APC's aim is to examine the molecular mechanisms that underpin the efficacy of probiotic strains. In order to achieve this aim, six collaborative research programmes to examine aspects of microbe-host and microbe-microbe interactions within the GI tract have been initiated. These include a Bioinformatics Programme to sequence and analyse the genome sequences of selected strains, and a bacterial Metabolism Programme for metagenomic analysis of the genetic and functional diversity that exists within the gut

ecosystem. Further research programmes are involved in the analysis of local host responses and systemic physiological responses influenced by the flora of the GI tract, cell-cell signalling between the gut microflora and the ability of probiotic commensals to inhibit pathogenesis of gut pathogens. An overview of these diverse research programmes will be presented.

Characterization of the S-protein of probiotic strain R0052. K.E. Hagen[a,b], T.A. Tompkins[b], G.E. Allison[a] ([a] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5; [b] Institut Rosell Inc., 6100 Royalmount Ave., Montreal, QC, Canada H4P 2R2).

Lactobacilli are commensals found in the GIT of humans and other animals. R0052 is a highly unique strain of the *Lactobacillus acidophilus* group A complex with very interesting probiotic properties. It has been previously demonstrated that R0052 adheres to human epithelial cells, blocks adhesion of EPEC and EHEC and modulates the immune system. These properties are influenced by growth conditions. The objective of this study was to investigate the S-layer proteins of R0052 and analyze their expression under laboratory and industrial growth conditions. S-layers, crystalline proteins that coat the extracellular surface of bacterial cells, are thought to mediate adherence of lactobacilli to the gut epithelium. It was observed that R0052 produces a 46 kDa S-layer protein under both laboratory and industrial growth conditions. The gene encoding the S-protein was cloned and the protein was characterized to further understand the ability of this strain to adhere to human tissues.

The effect of *Saccharomyces cerevisiae* CBS 493.94 on fibrolytic activity of the equine intestinal ecosystem in vitro. V. Julliand[a], B. Medina, G. Bertin[b] ([a] ENESAD, 21 079 Dijon, France; [b] Alltech France, 2–4 avenue du 6 juin 1944, 95190 Goussainville, France).

The addition of yeast to the equine diet could act on fibrolytic activity. To better understand the mechanism, an in vitro technique was performed to evaluate the effect of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc®1026) on the activity of the intestinal ecosystem. Eight horses fitted with intestinal cannulas were fed 4 treatments: HF (high fibre), HF + SC (top-dressed *Saccharomyces cerevisiae* 6.2×10^9 CFU·kg^{-1} DM), HS (high starch), HS + SC, in a latin square design. Intestinal contents were collected, homogenised and filtered (100 µm) anaerobically. Seven mL were inoculated in 63 mL medium plus 350 mg wheat straw (0.8 mm) and incubated at 39 °C. Series of 1 control and 6 repetition-flasks were prepared per *inoculum*. Pressure and gas volume were measured at 3, 6, 9, 15, 21, 30, 42, 54, 72 and 100 h incubation. Cumulated volumes, A (asymptotic value of gas production, mL), L (lag time, h) and T_{50} (half-time reaction, h) were modelled. pH, dry matter disappearance (DMD) and production of microbial biomass (mg) were estimated at 21, 72 and 100 h. The addition of *Saccharomyces cerevisiae* CBS 493.94 to HF enhanced DMD at 21 and 100 h (43.0 vs. 39.8% and 61.2 vs. 55.2%) in the caecum and at 72 h in the caecum (58.6 vs. 54.2%) and colon (59.0 vs. 51.8%). Caecal fibre degradation started earlier (gas production at 9 h was 1.73 vs. 1.34 mL·h^{-1}) and was more efficient (caecal asymptotic values was 62.6 vs. 59.1 mL). When supplemented to HS, *Saccharomyces cerevisiae* decreased the caecal lag-time (2.1 vs. 3.1 h) and increased A values in the colon (73.6 vs. 64.1 mL). No statistical changes were noticed in T_{50}, pH and biomass with yeast. Our results clearly underline that feeding yeast to horses increases the in vitro fibrolytic activity of their intestinal ecosystem, in agreement with previous data.

Benefit on aging and heart health parameters of probiotics. A. Liu[a], E. Duan[b], Y. Duan[c], J. Wu[a], X. Shi[a], Y.H. Gao[d], Q. Shu[e] ([a] Mt Albert Health Research Laboratory, Auckland, New Zealand; [b] Chinese Academy Animal Research Institute, Beijing, China; [c] Nanshan Health & Anti-epidemic Institute, Shenzhen, China; [d] Institute of Food, Nutrition and Human Health, Massey University, Auckland, New Zealand; [e] New Zealand Institute for Crop & Food Research Limited, Auckland, New Zealand).

Our previous report showed that the probiotic strains Drapac™-16 (also known as DP16), Drapac™-28 (DP28), Drapac™-38 (DP38), Drapac™-66 (DP66), and Drapac™-88 (DP88)

had immune-enhancing and anti-infection properties. In the present work, the effects of these strains in promoting antioxidant and free radical scavenging activities were demonstrated using established animal models. Rats (20-month-old for antioxidant activity, 4-month-old for free radical scavenging activity test) were housed under a 12 h light/dark cycle at a controlled temperature and relative humidity with free access to water and food. After acclimatization, the animals were randomised into different treatment groups on the basis of live weight. Different treatment groups were fed with different probiotic strains. Non-probiotic strains were given to the controls. After four months, the rats were humanely killed, and samples were taken for the analysis of relevant parameters. All the treatment groups fed with the probiotic strains had significantly higher antioxidant and free radical scavenging activities, in comparison with the non-probiotic controls. In addition, the experiments were conducted using established methods to test the effects of the probiotic strains on cholesterol and blood lipid levels. The results demonstrated that the probiotic strains lowered cholesterol and blood lipid levels. These results suggest that these probiotic strains have potential health benefits for humans including anti-aging and improved heart health.

Effects of *Saccharomyces cerevisiae* supplements on the rumen microbial population in male Holstein calves receiving diets with different levels of concentration. M. Rezaee[a], M. Rezaeian[b], P. Gamei[c], M. Moradi[c], A. Mirhadi[d] ([a] Research Centre of natural ressources and animal affairs of Tehran, Iran; [b] Department of Animal Health and Nutrition, University of Tehran, Iran; [c] Department of Animal Science, University of Tehran, Iran; [d] Department of Biotechnology, Animal Science Institute, Iran).

The experiment was designed to investigate the effects of *Saccharomyces cerevisiae* (SC 47) supplementation on the rumen microbial population in male Holstein calves. Thirty-six male Holstein calves (average body weight 175.89 ± 20.15 kg) were divided into six groups in a 2 × 3 factorial arrangement in which each group received a diet containing 60, 70 or 80 percent concentrate with or without yeast culture. Rumen contents were collected from each animal by oesophagus tubing at 0, 3 and 6 h after feeding and used for the assessment of the rumen microbial population (protozoa, total bacteria and lactate utilizing bacteria) after pH measurement. Protozoal populations were counted microscopically and bacterial populations by using the most probable number technique. Results showed that yeast supplementation increased the population of lactate utilizing bacteria and rumen protozoa in all of the treatments significantly ($P < 0.05$). A small increase in the total number of ruminal bacteria was also found. The pH of rumen fluid in the 80% concentrate diet was increased significantly ($P < 0.05$). It can be concluded that the inclusion of *Saccharomyces cerevisiae* could have a beneficial effect on the rumen microbial populations in animals receiving high concentrate diets and may result in an improvement in rumen fermentation.

Effect of yeast on pathogen survival in the rumen simulating fermentor Rusitec. A. Olvera-Ramírez[a,c], F.M. McIntosh[b], C.J. Newbold[a], F. Garcia-Garcia[c] ([a] The Institute of Rural Science, University of Wales, Aberystwyth, SY23 3AL, UK; [b] Rowett Research Institute, Aberdeen, Scotland, UK; [c] Saf-Agri México, Km 57.5 Carretera México-Toluca, CP 50200 Toluca, Edo. de México, México).

Yeast preparations based on *Saccharomyces cerevisiae* (SC) have been shown to stimulate microbial activity within the rumen. Here we investigated if such preparations could influence the survival of *E. coli* O157:H7 (EC) and *L. monocytogenes* (LM) in a rumen simulating fermentor (RUSITEC). Fermentations were established in 15 Rusitec vessels using ruminal fluid with a normal fauna, the vessels volume was 750 mL with a dilution rate of 26.5 mL·h^{-1}. Each vessel was fed with 20 g·d^{-1} of a mixed hay concentrate diet, incubated at 39 °C and the treatments, control (no addition of SC) and 500 mg·d^{-1} of 4 different strains of SC ($n = 3$), were added for 17 d. On the 15 d EC (3.12×10^{10} CFU) and LM (1.6×10^{9} CFU) were added to the vessels. The survival of the added pathogens was estimated at 0, 3, 6, 9, 12 and 24 h after inoculation by plating onto selective medium. As in previous experiments, SC tended to stimulate both total and cellulolytic bacteria numbers although again as previously observed there were differences between the different strains of SC (4.4 vs. 10.2, 6.2, 9.9 and 6.5×10^{8}·mL^{-1} total culturable bacteria

for the control and yeast strains 1 through 4 respectively, SED 1.6; 3.8 vs. 4.7, 8.5, 7.2 and 15 × 10^7·mL^{-1} cellulolytic bacteria respectively, SED 2.35). The SC strains 3 and 4 prevented an increase in LM numbers after 3 h (6.8 vs. 6.2, 6.5, 3 and 3.6 × 10^5·mL^{-1}, SED 0.54, respectively) and reduced EC numbers at the same time point (3.0 vs. 2.8, 2.2 1.8 and 2.1 × 10^7·mL^{-1}, SED 0.28, respectively). Thus, it appears that it should be possible to select strains of SC capable of reducing the pathogen load in the rumen.

Characterization of probiotic human *Lactobacillus* spp. strains. S. Pietronave[a], P. Malfa[b], M.G. Martinotti[a] ([a] Università del Piemonte Orientale, DISCAFF, Via G. Bovio, 6, 28100 Novara, Italy; [b] PROGE FARM s.r.l., Via G. Bovio 6, 28100 Novara, Italy).

The aim of the study was the characterization of four probiotic *Lactobacillus* spp. strains (*L. plantarum* P17630, *L. crispatus* P17631, *L. gasseri* P18137, isolated from human vaginal secretions and *L. gasseri* P17632, isolated from the human gut). A growth-inhibitory effect of all the *Lactobacillus* strains against Gram positive, Gram negative and the yeast *C. albicans* was observed. Lactobacilli were examined for the expression of auto-aggregation properties. The results showed that only *L. gasseri* P17632 and P18137 strains were able to self-aggregate and the process was influenced by pH, and enzymatic treatments. An in vitro method which mimics human upper gastro-intestinal transit (G-I) in vivo was applied. *L. gasseri* P17632, showed appreciable survival during simulated G-I transit and was considered intrinsically resistant, moreover this strain was also resistant to bile salts. In addition, the in vitro adherence capacity of *L. gasseri* P17632 using a human intestinal cell model (HT29 cell-line) was analysed. The strain showed a strong adherence capacity similar to that of the positive control *L. acidophilus* NCFM. The results suggest an exploitation of *L. gasseri* P17632 as a bio-therapeutic agent.

The effect of a lactic acid bacterial strain on liquid pig feed in actual farm conditions. C. Plumed-Ferrer, I. Kivelä, P. Hyvönen, A.V. Wright (Institute of Applied Biotechnology, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland).

This study was conducted to investigate the effect of a specific lactic acid bacterium on the fermentation process of pigs' liquid feed. The pigs were divided into two groups and fed with a control diet (non-fermented liquid feed NFLF) or a fermented diet (fermented liquid feed FLF). A rifampicin resistant mutant of a commercial feed starter strain (LAB^{rif}) was used to ferment the liquid diet. Total lactic acid bacteria, LAB^{rif}, yeasts, enterobacteria and pH were measured for liquid feed using standard microbiological techniques. The results showed a high lactic acid population, stable from the first days, in FLF, in contrast with NFLF, which had a lower lactic acid population and it stabilized just after the 9th day. Enterobacteria had low numbers in general, although in NFLF, they were more unstable. The average pH in NFLF was around 4,5 while it remained lower in FLF, approximately 4. In conclusion, the inoculation of LAB^{rif} once every one or two weeks did not avoid the growth of the natural lactic population in the feed but it regulated the fermentation process, providing a lactic population over 9 log cfu·mL^{-1}, a pH stable around 4, and a population of enterobacteria almost inexistent.

Novel health-promoting prebiotics and probiotics. Q. Shu[a], A. Liu[b], E. Duan[c], Y. Duan[d], J. Wu[b], X. Shi[b], Y.H. Gao[e], G. Lu[e], M. Ahmed[e], J. Youssef[a], C. Osborne[a], G. Fletcher[a] ([a] New Zealand Institute for Crop & Food Research Limited, Auckland, New Zealand; [b] Mt Albert Health Research Laboratory, Auckland, New Zealand; [c] Chinese Academy Animal Research Institute, Beijing, China; [d] Nanshan Health & Anti-epidemic Institute, Shenzhen, China; [e] Instititute of Food, Nutrition and Human Health, Massey University, Auckland, New Zealand).

Consumption of prebiotics and probiotics has been demonstrated to promote the health of the human host. Our studies have identified that consumption of food ingredients (colostrum powder, whey powder, whey protein, cartilage extract, or thymus extract) in combination with lactic acid bacteria (LAB) probiotic strains Drapac^{TM}-16 (also known as DP16), Drapac^{TM}-28 (DP28), Drapac^{TM}-38 (DP38), Drapac^{TM}-66 (DP66), or Drapac^{TM}-88 (DP88) can confer health benefits through their immune-enhancing and anti-infection properties. Enhanced immune

function was demonstrated by the activity of phagocytes and natural killer cells, lymphocyte proliferation, and systemic as well as mucosal antibody responses. Anti-infection properties were demonstrated by enhanced resistance to pathogenic bacteria (*Salmonella typhimurium, Escherichia coli* O157:H7, *Staphylococcus aureus*) and viruses (rotavirus) in animal models. Additional health benefits were also observed including lowering cholesterol and blood lipid levels, and enhanced antioxidant and free radical scavenging activities. Some combinations of the ingredients and probiotic strains showed better health promoting properties than individual ingredients or probiotic strains. The prebiotic activities and other health benefits of a range of seafood extracts and non-LAB probiotic strains are also under investigation. Probiotic action against seafood pathogens (*Listeria moncytogenes*) has been observed in recent experiments. Our studies suggest that the consumption of colostrum powder, whey powder, whey protein, cartilage extract, or thymus extract in combination with LAB probiotic strains can promote human health. Research to develop novel seafood-based prebiotics and non-LAB probiotic strains with health-promoting properties shows promising results.

***Enterococcus faecalis* cell preparation (EC-12) significantly alleviating diarrhea diseases in nursing piglets may replace antimicrobials.** T. Tsukahara[a,b], N. Nakanishi[b], A. Shiga[c], N. Matsubara[d], K. Ushida[a] ([a] Kyoto Prefectural University, Shimogamo Kyoto 606-8522, Japan; [b] KYODOKEN Institute, Shimoitabashi Kyoto 612-8073, Japan; [c] SHIGA SWINE CLINIC, Takanabe, Miyazaki 884-0006, Japan; [d] Combi Corporation, Saitama 338-0832, Japan).

"EC-12" is autoclaved and spray-dried cell pellets of *Enterococcus faecalis* strain EC-12. As a dry powdered material, a teaspoonful of EC-12 contains 10^{12} cells which is a substantially higher density than those of the usual probiotics, viable bacterial preparations. Therefore, much stronger reactions from the host, particularly the immune system, are anticipated as compared to usual probiotics. EC-12 stimulates mucosal immune responses that may affect the establishment of pathogens in the intestine. Two field studies were conducted to know if EC-12 alleviates pathogenic diarrhea such as colibacillosis and/or clostridial infections in nursing and weaning piglets. One commercial pig farm suffering from a high death rate caused by colibacillosis and the resultant edema disease was subjected to this study. It is noteworthy that chemotherapies have no potential due to the antibiotic resistant enterotoxemic *Escherichia coli*. Dietary EC-12 significantly reduced the death rate of nursing and weaning piglets at a dose level of 500 $mg \cdot kg^{-1}$ feed. The preventive effect of EC-12 against pathogenic diarrhea was determined in the other pig farm under the antibiotic-free condition. The same dose level of dietary EC-12 significantly reduced the risk of enteropathogenic diarrhea. Indeed no need of antibiotic therapy in EC-12 fed piglets was evident compared to the control litter. These results suggest that dead whole cell preparation of *E. faecalis* strain EC-12 has a potential to replace dietary antimicrobials for nursing and weaning piglets. Since the product consists of dead bacterial cells, there is no risk to spread antibiotic resistant genes out among enterococci.

Effect of ruminal pH on survival of rumen protozoa ciliate in steers. R. Franzolin[a], B.A. Dehority[b] ([a] Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de Sao Paulo, 13635-900, Pirassununga-SP, Brazil; [b] Ohio Agricultural Research and Development Center, The Ohio State University, Wooster-OH, USA).

A contribution to the understanding of the pH factor involved in the survival of the rumen protozoa ciliate population was performed in an experiment designed with four rumen fistulated steers fed high concentrate diets over a 17-week period. They were fed daily on a low level over the first 6 wk with 50% of whole shelled corn (WC) plus 50% of alfalfa pellets (AP), during the following 4 wk with 65% of WC and 35% of corn pellets (CP), the next 3 wk WC alone, and the last 4 weeks WC at a higher level. Rumen population protozoa were monitored weekly and re-inoculation was done in the rumen, protozoa free from other steer inoculum. Diurnal curves of ruminal pH were measured at 2-wk intervals with samples taken at 2, 4, 8, 12 and 24 h after feeding. Turnover rate was measured in all steers on the WC diet at low and higher feeding levels and were shown to be similar for both feeding

levels (mean 0.045·h^{-1}). There were differences ($P < 0.01$) in the average rumen pH values between faunated and defaunated animals in all times after feeding, being respectively: 2 (6.27 and 5.72), 4 (5.73 and 5.31), 8 (5.85 and 5.37) and 24 (5.98 and 5.52). The regression was pH = 5,64 + 0,0268X for faunated ($P < 0.01$; $r = 0.54$) and pH = 5.231 + 0,0210X for defaunated ($P < 0.01$; $r = 0,50$) steers. There was clearly an individual rumen environment behavior about the survival rumen protozoa ciliate. The low ruminal pH is critical on maintaining the rumen protozoa but the level of critical pH seems to vary between individual animals.

Ciliates as a factor increasing outflow of α-D-glucose polymers from the reticulo-rumen to omasum of sheep. T. Michałowski, G. Bełżecki, E. Kwiatkowska (The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland).

The role of ciliates in influencing the outflow of starch and microbial reserve polysaccharides from the reticulo-rumen to the duodenum is controversial. This study was performed in order to examine the effect of the presence of ophryoscolesid ciliates on the contents of 1,4;1,6-α-D-glucans in the rumen and on the pool size of carbohydrates leaving the reticulum in two sheep. The animals were defaunated and then refaunated either with *Eudiplodinium maggii* or *Eudiplodinium maggii* and *Entodinium caudatum*. The weight of the rumen contents and α-D-glucose polymers just before feeding varied in the range of 10.9–18.3 kg and 8.1–20.8 g/rumen in relation to the presence of ciliates and the animal, respectively ($P < 0.05$). The volume of reticular effluent was 10-161·12 h^{-1}, while the concentration of 1,4;1,6-α-D-glucans in the effluent varied from 6 to 138 mg·g^{-1} DM. It increased markedly during the first 2 h after feeding and then decreased. The establishment of *Eudiplodinium maggii* in the rumen of sheep increased the contents of these carbohydrates in the effluent ($P < 0.05$) and the appearance of *Entodinium caudatum* was followed by a further increase. Pool size of 1,4;1,6-α-D-glucans leaving for the reticulum of defaunated sheep was 5.4–5.9 g·12 h^{-1}. It increased to 10.5–14.7 g after the establishment of *Eudiplodinium maggii* ($P < 0.01$) and 15.7–17.4 g after the appearance of *Entodinium caudatum*. The numbers of *Eudiplodinium maggii* in the rumen contents and reticular effluent was 13.5–41.6 × 10^3·g^{-1} and 1.6–9.1 × 10^3·mL^{-1}, respectively. The number of *Entodinium caudatum* was 181.2–253.9 and 98.9–207.7 × 10^3·g^{-1} or mL^{-1}, respectively. Two regulatory mechanisms of the 1.4;1.6-α-D-glucans outflow are proposed.

A preliminary study of chemistry and toxicology of a natural antiprotozoal agent. R. Ningrat[a,b], R.G. Gerdes[a] ([a] The University of New England, Armidale, New South Wales 2351 Australia; [b] Current address: Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

A preliminary study on the chemistry and toxicology of the active agent present in the extract of *Enterolobium cyclocarpum* leaf were carried out. Assuming that the active agent is a saponin, the active agent was isolated by maceration of the water-soluble compounds from the dried leaves, followed by partitioning between water and the n-butanol layer with the butanol layer containing the active fraction. A further fractionation process of the leaf extracts was guided by two bioassays, haemolytic assay and antiprotozoal assay. A haemolytic assay using sheep erythrocytes was developed to test the presence of saponins. It appears that the purified fractions showed strong haemolytic activity, however, the haemolytic activity may not reflect a parallel ability to lyse rumen protozoa. An antiprotozoal assay using rumen protozoa was developed. In vitro results suggest that those purified fractions exhibiting high immobilising activity tend to produce high activity to lyse the cells. A study on the synergistic interaction effects among components in the mixture indicated that their activities were not affected after fractionation. It appears that the results are consistent within the experiment but not between the experiments. GLC analysis of TMS derivatives of monosaccharides liberated by acid hydrolysis was employed to determine the structure of the active agents. The proposed structures of the active agents are reported. It appears that the structure of the active agent is unlikely to be substituted with more than one sugar. Glucose seems to be predominant followed by rhamnose, arabinose and xylose, while galactose is not present in all fractions.

Effect of *Entodinium caudatum* and *Eudiplodinium maggii* on ruminal fermentation and methane production in vitro. M.J. Ranilla[a], D.P. Morgavi[b], J.-P. Jouany[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).

Mixed rumen protozoa are positively associated with increases in feed degradation and methane production. However, the contribution of individual protozoal species is less known. The fermentation characteristic and methane production of two metabolically different protozoa, *Entodinium caudatum* and *Eudiplodinium maggii*, were evaluated in vitro. In a first experiment, rumen fluid from monofaunated and defaunated (control) animals was used to ferment alfalfa hay or maize grain. In a second experiment, protozoa were collected by centrifugation, resuspended in centrifuged rumen fluid from defaunated animals and used to ferment an alfalfa:maize (70:30) mixed feed. The presence of *E. caudatum* or *E. maggii* in in vitro rumen fermentations did not increase the degradation of alfalfa hay and maize grain substrates as compared to controls without protozoa. In experiment 1, VFA production was higher in *E. caudatum* and the presence of protozoa increased the proportion of butyrate ($P < 0.05$). *E. caudatum* increased branched VFA ($P < 0.05$), which suggests higher protein degradation. In experiment 2, however, *E. maggii* incubations tended to produce more VFA due to increased production of acetate ($P < 0.05$). Independently of inocula processing, methane production was consistently higher in *E. caudatum* fermentations ($P < 0.05$), an effect that could reduce the amount of energy available to the animal.

VFA production of selected carbohydrate-rich feed additives at different concentrations fermented with chyme in vitro. E. Bauer[a,b], B.A. Williams[b], C. Voigt[a,b], R. Mosenthin[a], M.W.A. Verstegen[b] ([a] Institute of Animal Nutrition, University of Hohenheim, 70599 Stuttgart, Germany; [b] Animal Nutrition Group, Wageningen University, 6709 Wageningen, The Netherlands).

VFA production of chyme from pigs, alone and in combination with carbohydrate-rich feed additives at different concentrations, was assessed in vitro, to determine which additive might benefit large intestinal fermentation. Virginiamycin was added to chyme to investigate the effect of an antibiotic on VFA production. Test substrates were transgalacto-oligosaccharide (TOS), arabic gum (AG), sugarbeet pulp pectin (SBP), and virginiamycin (ANT). Ileal chyme from cannulated pigs was used as the basal substrate in combination with the test substrates. The test substrates were added at concentrations of 10, 30 and 50%, to 0.5 g of chyme, except for the antibiotic, which was added at concentrations of 5, 25 and 50 ppm. In vitro fermentation was performed using the cumulative gas production technique. The end products measured were total gas, NH_3, total VFA, acetic, propionic and butyric acids. The branched chain ratio (BCR) was calculated. The addition of AG at a concentration of 50% resulted in a decrease in total VFA, acetic and butyric acids, whereas propionic acid production was increased. Addition of TOS led to a higher production of acetic acid at any concentration, and resulted in more propionic acid at a concentration of 50%, than fermentation of chyme alone. Except for the antibiotic, for all the additives, the BCR decreased with increasing concentration. Stimulation of fermentation in the large intestine due to supplementation with dietary carbohydrates may cause a change in the fermentation pattern with less fermentation of protein (as suggested by the decreased BCR) which could benefit gut health.

In vitro study of the hindgut fermentation of potential roughages for pregnant sows. P.M. Becker, A.H. van Gelder, P.G. van Wikselaar, J.W. Cone (Animal Sciences Group, Wageningen UR, Nutrition & Food, PO Box 65, 8200 AB Lelystad, The Netherlands).

Ileal non-digestible, but fermentable compounds in roughage are mainly metabolised to short-chain fatty acids (SCFA), bacterial biomass, and gases such as CO_2 and CH_4 during hindgut fermentation in monogastrics. The SCFA are absorbed by the mucosa of the large intestine and serve as an energy source to the body. Because hindgut fermentation takes much longer than ileal digestion, it is thought that in particular roughages that are metabolised to substantial quantities of SCFA are able to reduce both obesity and

the hunger feeling in pregnant sows and thus support their well-being. After pre-treatment with digestive enzymes, 8 different fibrous whole-plant products and 14 by-products from the food-processing industry were incubated anaerobically with sow faeces as the inoculum. Gas and SCFA production were measured as response parameters for bacterial fermentation. Sugar beet pulp and potato pulp brought about the highest SCFA yields, and together with raw potato chips, the highest amounts of gas. Compared to the pulp products, whole plant materials like grass were fermented much slower and gave larger portions of unfermented remnants. With the exception of liquid yeast feed and raw potato chips, fermentation of all roughages resulted in more than 55% acetic acid among the SCFA after 72 h of incubation. Liquid yeast feed brought about the highest percentage of propionic acid, namely 41%, and raw potato chips the highest percentage of butyric acid, i.e. 25%. The findings might indicate a possibility to control hindgut nutrition and probably health by supplying selected roughages to the diet of pregnant sows.

Does dietary manipulation change the diversity of methanogens and protozoa that interact within the rumen? C.T. Christophersen[a], A-D.G. Wright[b] , P.E. Vercoe[a] ([a] University of Western Australia, Faculty of Natural and Agricultural Sciences, Australia; [b] CSIRO Livestock Industries, Centre for Environment and Life Sciences, WA Australia).

Methanogenic archaea have an important ecological role in rumen fermentation because they use hydrogen and maintain a low hydrogen partial pressure. However, methanogenesis constitutes a loss of energy and carbon for ruminant livestock and in addition methane is a potent greenhouse gas. It is well established that there is a large diversity of methanogens in the rumen, which is influenced by the diet. The interaction between methanogens and rumen protozoa is also well documented, but only a few studies have focused on the diversity of methanogens that associate with protozoa. We used DGGE to examine the type and diversity of methanogens and protozoa that interact in the rumen under different dietary conditions. Samples were collected from fistulated sheep fed three maintenance level diets; a hay diet (control) and two diets with increased grain content (35% and 70%). The control animals were maintained on a hay diet. The samples were divided by a series of centrifugation and washing procedures into free-living methanogens, methanogens that were attached to the surface of protozoa at the time of sampling, and methanogens living inside the protozoa (endosymbionts). Total DNA was extracted from the samples and the 16S or 18S rRNA gene was used to investigate the population structure of methanogens and protozoa respectively. DGGE profiles obtained from different samples collected from animals on different diets will be presented.

Manipulation of the intestinal microflora using manno-oligosaccharides (Bio-Mos). A. Kocher, L. Tucker (Alltech Biotechnology Center, Sarney, Summerhill Rd. Dunboyne, Co. Meath, Ireland).

Due to increased consumer pressure, the livestock industry is focusing on the use of natural growth promoters as an alternative to antibiotics. The effects of phosphorylated manno oligosaccharides (Bio-Mos) are well documented: Bio-Mos can reduce unwanted enteric pathogens such as *salmonella* and *E. coli* in the intestine by blocking Type-1 fimbrae. It can also improve intestinal integrity and can stimulate immunity thus improving overall intestinal health. Experimental data from Portugal has indicated that the addition of Bio-Mos ($1 kg \cdot t^{-1}$) to rabbit diets significantly increased the concentration of acetic acid in the ceca and significantly reduced the pH, indicating an increase in overall fermentation. Similarly, it has been shown that the addition of Bio-Mos to broiler diets improved fecal fiber digestibility from 6.23% (no MOS) to 15.43% (Bio-Mos 2 $kg \cdot t^{-1}$). Furthermore, the inclusion of Bio-Mos significantly reduced the total VFA concentration in the jejunum of turkeys compared to the control group, indicating reduced bacterial fermentation in the upper digestive tract and improved nutrient availability to the host. Comparisons of data from broiler, turkey and nursery pig trials showed that changes in the microflora by adding Bio-Mos resulted in significant improvement in FCR of 1.99% (broiler), 2% (turkey) and 2.4% (nursery pigs) compared to a negative control, whereas the results from

commercial trials in the UK suggested similar growth performance and reduced mortality with Bio-Mos compared to an antibiotic. It is evident that the addition of Bio-Mos can improve fiber digestibility and alter intestinal fermentation patterns, leading to improved animal performance. Hence, Bio-Mos is a viable alternative to antibiotic growth promoters.

Factors influencing the microbial community in commercial broilers. S. Peuranen[a], J. Apajalahti[a], A. Gavin[b], H. Graham[b] ([a] Danisco Innovation, Sokeritehtaantie 20, FIN-02460 Kantvik, Finland; [b] Danisco Animal Nutrition, Box 777, Marlborough, Wilts SN8 1XN, England).

An experiment was designed to determine the effect of Farm, Sampling Time and Diet on the intestinal parameters and performance in commercial broilers. Broilers on two commercial farms each with two houses were fed two commercial wheat-based diets in a cross-over design with sampling at two times. Ileal and caecal digesta samples collected at day 21 were analysed for immunoglobulin A (IgA), prostaglandin E (PGE), total microbial numbers, microbial community profile using % G + C fractionation and *Clostridium perfringens* using Q-PCR. Caecal microbial numbers averaged 1.3×10^{11} per g, and were not influenced ($P > 0.05$) by Farm, Diet or Sampling Time. There were differences ($P < 0.01$) between Farms in the caecal digesta % G + C profiles in the 42–53% and 61–64% regions, but no substantial effect of Diet. Sampling Time also influenced ($P < 0.05$) caecal digesta % G + C in the 53–61% and 68–75% regions. *C. perfringens* was present in half the caecal digesta samples at a level around 10^5 per gram. Ileal and caecal digesta IgA levels ranged from 0.3–1.0 and 1.0–3.3 $mg \cdot g^{-1}$ digesta, respectively, with Sampling Time affecting ($P < 0.02$) caecal IgA. Ileal and caecal PGE levels were 2–6 and 3.5–11.5 $\mu g \cdot g^{-1}$ digesta, respectively, with an effect ($P < 0.04$) of Sampling Time. Bird final weight was correlated ($P < 0.04$) with 45–49% caecal digesta G + C, feed efficiency with 50–54% caecal digesta G + C and mortality to 65–69% caecal digesta G + C. This trial demonstrates that while microbial numbers in the intestine of commercial broilers are relatively constant, the microbial community can change considerably and this may influence bird performance.

An amylase-based exogenous enzyme supplement for ruminants increases ruminant production by potentially modifying microbial digestion of starch in the rumen. J.M. Tricarico, A.E. Kozenski (Alltech Biotechnology Inc., Nicholasville, KY, USA).

During the last three years, we studied the effects of an amylase-based supplement on ruminant performance and ruminal fermentation. Improvements in milk production obtained with this supplement appear to be related to changes in ruminal starch digestion that result in butyrate-rich fermentations. Experiments were performed to examine the effects of this supplement on growth of pure cultures of ruminal bacteria on starch. *Butyrivibrio fibrisolvens* strains D1, 49, and A38, *Streptococcus bovis* strain S1, *Megasphaera elsdenii* strain T81, and *Selenomonas ruminantium* strain GA192 were inoculated in Medium 10 broth containing 1.0 $g \cdot L^{-1}$ soluble starch in the presence or absence of supplemental amylase at a final concentration of 60 units/L. Microbial growth in each culture was estimated by measuring turbidity over time. Amylase addition increased the growth rates of D1 (0.007 vs. 0.168 $OD*h^{-1}$, $P < 0.05$), GA192 (0.004 vs. 0.085 $OD*h^{-1}$, $P < 0.05$) and T81 (0.012 vs. 0.036 $OD*h^{-1}$, $P < 0.05$). The amylase supplement did not affect the growth rates of S1 or 49 and reduced the growth rate of A38 (0.131 vs. 0.076 $OD*h^{-1}$, $P < 0.10$). The growth of D1 was also greater (0.373 vs. 0.493 OD at 15 h; $P < 0.05$) in the presence of supplemental amylase when the carbon sources were maltodextrins with an average molecular weight of 3600, but not when lower molecular weight maltodextrins were used. We conclude that this amylase-based supplement for ruminants increases the growth of ruminal bacteria that do not grow efficiently on starch and modifies starch fermentation in the rumen, thus leading to improved performance.

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Quantification of *Fibrobacter succinogenes* cellulase and xylanase gene expression in the rumen of a gnotobiotic lamb by real-time RT-PCR. C. Béra-Maillet, A. Kwasiborski, P. Mosoni, E. Forano (Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France).

Cellulolytic bacteria like *Fibrobacter succinogenes* play a major role in the degradation of plant cell wall polysaccharides in the ruminants by producing several glycoside hydrolases (GH). More than 35 different GH genes have been identified in the genome of *F. succinogenes* S85 but the role and importance of each enzyme in the fibrolytic system of this bacterium is still unknown. We have previously shown, using real-time RT-PCR, that several fibrolytic genes are much more expressed in vivo in the rumen content of gnotobiotic lambs, than in vitro in pure cultures. The relative importance of each GH produced by *F. succinogenes* in vivo can be estimated by quantifying and comparing the expression rates of the corresponding GH genes. In this study, the transcript abundance of *celF*, *cel3* and *xynC* (2 cellulase and one xylanase genes) were quantified using RT-PCR in the rumen content of a gnotobiotic lamb fed a lucerne diet and inoculated with S85 *F. succinogenes* as the sole cellulolytic bacterial strain. *tuf* (transcriptional unit factor) mRNA were also quantified to standardize and compare the expression levels of target genes in several total RNA extracts. The results are discussed.

Do rumen butyrivibrio really possess only family 10 xylanases? T. Cepeljnik, R. Marinsek-Logar (Zootechnical Department, Biotech. Fac., University of Ljubljana, Groblje 3, SI–1230 Domzale, Slovenia).

Xylan is one of the major components of plant polysaccharides which represent the main part of the ruminants' forage. For its effective degradation, the cooperation among fibrolytic enzymes with different specificities is crucial. Simbiontic microflora produce a large number of glycosyl-hydrolases, out of which family 10 and 11 xylanases play an important role. The most active xylanolytic rumen bacteria are *Butyrivibrio* and *Pseudobutyrivibrio* strains, that were thought, until recently, to produce only family 10 xylanases, homologues of the genes *xynA* from strain Bu49 and *xynB* from strain H17c. The maximal number of identified xylanase genes was four in the strain A38. It was suspected that high xylanolytic activity could demand the presence of family 11 enzymes, too. More xylanolytic butyrivibria were studied, with a special focus on the strain with the highest xylanolytic activity among rumen bacteria, *P. xylanivorans* type strain Mz5^T. We obtained the N-terminal sequence of the smallest xylanase, XynT, which represented the majority of the activity in the later growth phases. The sequence revealed that XynT belonged to the family 11 of glycosyl-hydrolases. The degenerated primers were designed and three more *xynT* homologue genes from the strains JL, Bu21 and Bu42 were identified. Additionally, several xylanolytic enzymes were identified on the xylanograms from various strains. Several of them (e.g. Mz3, Mz5^T, Nor37, Bu49, JK 623), produced at least seven distinct xylanases in the medium with xylan. Apparently some additional genes are still to be identified. Nevertheless, these bacteria represent a valuable xylanase gene tool for possible improvement of fiber degradation in ruminants and monogastrics.

Metabolism of secoisolariciresinol by human intestinal bacteria. T. Clavel[a,c], C.A. Alpert[a], A. Bernalier-Donadille[b], A. Braune[a], W. Engst[a], J. Doré[c], M. Blaut[a] ([a] German Institute for Human Nutrition, 14558 Potsdam-Nuthetal, Germany; [b] INRA, 63122 St-Genès-Champanelle, France; [c] INRA, 78352 Jouy-en-Josas, France).

Lignans are phytoestrogens that need to be activated by intestinal bacteria to display their biological properties. They occur in a wide range of food items and are therefore considered as natural substances for the prevention of some diseases. Thus, it appears essential to better understand their metabolism. We focused on the microbial activation of secoisolariciresinol, one of the main lignans in our diet, and aimed to identify bacterial species that carry out its conversion. Based on the analyses of 19 samples, we concluded that the bioactivation of secoisolariciresinol is widely distributed among human beings and we propose a value of 7.2×10^8

active CFU·g^{-1} dried faeces ($n = 3$). The kinetics of transformation showed that demethylation occurs before dehydroxylation. We found that *Eubacterium limosum* DSM 20543, and confirmed that *Peptostreptococcus productus* DSM 2950 carry out the demethylation. Both strains are acetogenic bacteria capable of demethylating phenolic acids. Ferulic acid, syringic acid and vanillic acid were tested and demethylation was observed in all the cases with the exception of ferulic acid incubated with *P. productus*. These results imply that the secoisolariciresinol-demethylase has a wide spectrum of substrates. In parallel, an enrichment of bacteria that produced enterodiol was obtained from faeces. It was found to contain members of the following phylogenetic groups: *Clostridium coccoides*, *Lactobacillus-Enterococcus*, *Streptococcus* and *Enterobacteriaceae*. In high dilutions of the mixed culture, the activity was lost and we observed a significant drop in the percentage of the *Clostridium coccoides* cluster to which *P. productus* and other acetogens belong.

Metabolism of 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ) by the human digestive microflora and by bacteria isolated from it. Ch. Humblot[a], B. Combourieu[b], M.-L. Väisänen[a], J.-P. Furet[a], K. Gloux[a], C. Philippe[a], C. Andrieux[a], A.-M. Delort[b], S. Rabot[a] ([a] Unité d'Écologie et de Physiologie du Système Digestif, INRA, 78352 Jouy-en-Josas Cedex, France ; [b] Laboratoire de Synthèse et Étude de Systèmes à Intérêt Biologique, UMR 6504 du CNRS, Université Blaise Pascal, 63177 Aubière Cedex, France).

The aim of this study was to investigate the metabolism of the heterocyclic amine 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ) by the human digestive microflora. IQ is a mutagenic/carcinogenic compound formed from meat and fish during cooking. When ingested, IQ is mainly metabolised in the liver by the xenobiotic metabolizing enzymes, but intestinal bacteria may also contribute to its biotransformation. So far, only 7-OH-IQ has been identified as an IQ derivative of bacterial origin. This compound is highly mutagenic in vitro but not carcinogenic in vivo. Hence its formation is considered as a detoxification pathway. To find out if other metabolites may be produced by intestinal bacteria, we incubated IQ (0.2 mM) with 100-fold dilutions of stools freshly collected from healthy volunteers. We quantified IQ degradation in the bioconversion cultures by HPLC-DAD analysis of residual IQ, and we characterised the production of IQ metabolites using ^{1}H NMR spectroscopy. This method allows to measure a wide range of metabolites in crude biological samples, simultaneously and without a priori hypotheses. In addition, since only two microbial genera, namely *Clostridium* and *Eubacterium*, have been reported to convert IQ into 7-OH-IQ, we looked for IQ biodegradative strains both in the UEPSD collection of gut microbiota and in the cæcal content of human flora-associated rats gavaged with IQ on a regular basis. HPLC-DAD and ^{1}H NMR analyses confirmed that the whole human digestive microflora converts IQ into 7-OH-IQ and definitely showed that this derivative totally accounts for IQ microbial degradation. We isolated 10 new bacterial strains able to perform this reaction, namely *Bacteroides thetaiotaomicron* (ATCC 29148), *Bacteroides ovatus* (93JE), *Escherichia coli* (21JC, 81JA, 81JC and 102JB), *Clostridium clostridiiforme* (51JD1, 92JB, 102JD2), and *Clostridium perfringens* (G22).

Biohydrogenation and biohydration of long-chain polyunsaturated fatty acids by a ruminal bacterium. H. Hussein, K.N. Joblin (Rumen Biotechnology, AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand).

One of the key steps in the conversion of dietary unsaturated fatty acids (UFA) into fats in meat and dairy products is their transformation by microbes in the rumen. During a study on the ruminal biohydrogenation of UFA in grazing sheep, we isolated a bacterium which biohydrogenated linoleic acid and linolenic acid to *trans*-vaccenic acid. Further investigation showed that isolate OV77 also converted linoleic, linolenic and oleic acids, but not *trans*-vaccenic acid, by biohydration into hydroxy-acids. The hydroxy-acid products, 10-hydroxy-12-octadecenoic acid and 13-hydroxy-9-octadecenoic acid, appeared in cultures simultaneously with the *trans*-vaccenic acid. In culture tests, both linoleic acid and linolenic acid had an inhibitory effect on bacterial growth and both biohydrogenation and biohydration occurred after logarithmic growth ceased. The isolate was a Gram-positive coccus

capable of utilising a range of carbohydrate substrates and which grew as pairs or short chains. The 16S rRNA gene was isolated and sequenced. Alignment against database sequences showed that the isolate is closely related (99.7%) to the type strain of *Enterococcus avium*. To the best of our knowledge, this is the first report of biohydrogenation by an *Enterococcus* sp. The properties of *E. avium* OV77 showed that it is different from the un-classified *Fusocillus babrahamensis*, the only previous ruminal bacterium with the capacity to both biohydrate and biohydrogenate UFA and which has been lost from culture collections.

Distribution of alternative pathways for butyrate formation in human colon bacteria. P. Louis, S.H. Duncan, S.I. McCrae, J. Millar, M.S. Jackson, H.J. Flint (Rowett Research Institute, Gut Microbiology & Immunology Division, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK).

The human colon harbors a diverse range of bacteria that form a complex anaerobic ecosystem. A highly abundant group of bacteria within this ecosystem are gram-positive strict anaerobes of low mol % G + C content. These microbes carry out a fermentative metabolism, which leads to the build-up of short chain fatty acids. Recent work has identified a number of new species that produce butyrate in pure culture. Butyrate is considered to exhibit health-promoting effects on the colon. It serves as a major energy source for the colonocytes and has also been claimed to be protective against colon cancer and inflammatory bowel disease via effects on gene expression and cellular development. The pathway for butyrate formation has been examined in bacteria from other ecosystems. Two molecules of acetyl-CoA are converted to butyryl-CoA in a sequence of reactions that resembles the reverse β-oxidation of fatty acids. For the final step of butyrate synthesis from butyryl-CoA two alternative pathways are known: the enzymes phosphotransbutyrylase and butyrate kinase convert butyryl-CoA to butyrate with the intermediate formation of butyryl-phosphate. Alternatively, a butyryl-CoA:acetate CoA-transferase transfers the CoA moiety to external acetate which leads to the formation of acetyl-CoA and butyrate. We investigated the pathways leading to butyrate formation in 38 different human colon bacteria using degenerate PCR and enzymatic assays. Surprisingly, only four of these isolates, forming two groups, possessed the genes and enzymes for the phosphotransbutyrylase/ butyrate kinase pathway. It seems therefore, that the CoA transferase is the major route for butyrate generation in this ecosystem.

Conjugated linoleic acids are formed in a detoxification mechanism which protects *Butyrivibrio fibrisolvens* from the effects of polyunsaturated fatty acids. M. Maia[a], J.M.C. Ramalho-Ribeiro[a], R.J. Wallace[b] ([a] Estação Zootécnica Nacional, Quinta da Fonta Boa, 2000-763 Vale de Santarem, Portugal; [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Conjugated linoleic acids (CLA) are considered to be health-promoting fatty acids via their effects in preventing cancer, atherosclerosis and in promoting immune function. CLA are found mainly in ruminant meats and dairy products, because they are formed during biohydrogenation of polyunsaturated fatty acids in the rumen by *Butyrivibrio fibrisolvens.* The aim of this work was to understand what benefit this activity, which has important implications for human health but which appeared peripheral to *B. fibrisolvens*, confers to *B. fibrisolvens.* When *B. fibrisolvens* was inoculated into medium containing 50 mg/L linoleic acid (LA), a prolonged lag phase ensued, which ended when all LA and CLA formed by isomerization of LA had been hydrogenated to *trans*-vaccenic acid (TVA). Corresponding methyl esters had no effect on growth, whereas the toxicity of linolenic acid was much greater than LA and CLA. Flow cytometry indicated that the C18:3 and C18:2 fatty acids were more potent than C18:1 in disrupting cell integrity, as measured by the exclusion of propidium iodide. *B. fibrisolvens* appears to be more sensitive to polyunsaturated fatty acids than other ruminal bacteria because of its thin cell wall.

Maltodextrin metabolism in *Fibrobacter succinogenes* S85. R. Nouaille[a,b], M. Matulova[a,c], A.-M. Delort[a], E. Forano[b] ([a] Laboratoire de Synthèse et Étude de Systèmes à Intérêt Biologique,

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Fibrobacter succinogenes S85 is a cellulolytic rumen bacterium. Previous work using ^{13}C and ^{1}H NMR spectroscopy analysed in detail glucose metabolism in resting cells of *F. succinogenes* S85, and showed the polymerisation of this sugar into intracellular glycogen and also cellodextrins. We also found that the bacteria metabolising glucose are able to synthesise and release oligosaccharides identified by two dimensional Nuclear Magnetic Resonance (2D-NMR) techniques as maltodextrins (MD), although this strain is known as unable to use starch or maltose. We studied more deeply maltodextrin metabolism in resting cells of *F. succinogenes* S85. We showed by 2D-NMR techniques that maltodextrins up to six glucose units and maltodextrin-1-phosphate were produced in the intracellular medium and excreted out of the cell. Thin Layer Chromatography analysis of extracellular and intracellular media of resting cells incubated with various substrates showed that no maltose was produced nor used by the bacteria although it was transported into the cell, maltotriose was the minimum length required for the synthesis of longer maltodextrins and was thus considered as a "building block". Maltodextrins, particularly maltotriose and maltodextrin-1-phosphate could originate from glycogen. We also found that, although it did not grow on maltose, the strain *F. succinogenes* S85 was able to use maltotriose or longer maltodextrins. Sequences homologous to that of genes of the well known Maltose system and of the glycogen metabolism of *Escherichia coli* were looked for in the incomplete *F. succinogenes* S85 genome. In particular, orthologs of *malQ* (amylomaltase), *malP* (maltodextrin phosphorylase), *malZ* (maltodextrin glucosidase) and *glgP* (glycogen phosphorylase) were found. Finally, a mechanism for maltodextrin synthesis in *F. succinogenes* is proposed and discussed.

Novel interactions in the assembly of the cellulosome complex in *Ruminococcus flavefaciens* 17. M.T. Rincón[a], J.C. Martin[a], V. Aurilia[b], G.J. Rucklidge[a], M.D. Reid[a], R. Lamed[c], E.A. Bayer[d], H.J. Flint[a] ([a] Rowett Research Institute, Aberdeen, UK; [b] Institute of Protein Biochemistry, National Research Council, Naples, Italy; [c] Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv, Israel; [d] Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel).

Ruminococcus flavefaciens is an anaerobic cellulolytic bacterium that has been isolated from the rumen of herbivores and also from the large intestine of various mammals including humans. Its main activity is the depolymerization of complex plant cell wall polysaccharides. *R. flavefaciens* synthesizes various glycoside hydrolase enzymes which exhibit complex domain architecture and are organized in a multicatalytic protein complex, similar to the cellulosome previously described in *Clostridium thermocellum* and other cellulolytic bacteria. Initially, we isolated and characterized two structural cellulosomal proteins in *R. flavefaciens*: ScaA and ScaB. Cohesins in ScaA are able to interact with specialized dockerin modules present in previously characterized multidomain glycoside hydrolases, such as EndB. More recently, a third structural protein, ScaC, was characterized that is encoded by a gene that lies upstream of the *scaA* and *scaB* genes. ScaC possesses only one cohesin and a C-terminal dockerin. The newly described ScaC scaffoldin interacts specifically with a new set of proteins from *R. flavefaciens* cell-surface extracts, and is integrated into the cellulosome via interaction of its dockerin with ScaA. Based on the phylogenetic analysis of the currently known *R. flavefaciens* dockerin domains, the existence of a fourth scaffoldin protein can be predicted. Functional proteomic analysis of *R. flavefaciens* 17 is helping to elucidate the complex assembly of the different components of the cellulosome. The cellulosome architecture in *R. flavefaciens* shows significant differences with the well-characterized model for the cellulosome in *Clostridium* spp.

Conjugated linoleic acid composition of rumen bacterial and protozoal populations. E. Devillard[a], F.M. McIntosh[a], K. Young[a], M. Castet[a], R.J. Wallace[a], C.J. Newbold[b] ([a] Rowett Research

Institute, Bucksburn, Aberdeen, Scotland, UK; [b] University of Wales, Aberystwyth, Wales, UK).

Conjugated linoleic acids (CLA) confer a broad range of health-promoting properties in man, including prevention of cancer and heart diseases, and stimulation of the immune response. The main dietary sources of CLA are ruminant products. Up to now, it has been assumed that CLA formation within the rumen was associated solely with the bacterial population. Here, we present the results of a study on the fatty acid (FA) content of the bacterial and protozoal fractions of the rumen. Variations of these contents were analysed during the postprandial period. Higher concentrations of stearic acid were measured in the bacterial fractions than in the protozoal fractions (2.5 to 5 fold). In contrast, unsaturated fatty acids (C18:1, C18:2) were found in much lower concentrations in the bacterial fractions compared to the protozoal fractions. The differences between the two fractions were more noticeable after feeding, and increased between 4 and 6 h after feeding. CLA were also found in higher quantities in the protozoal fractions than in the bacterial fractions. Most interestingly, C18:2 c9, t11, which has been shown to be the most beneficial isomer of CLA for human health, was much higher in the protozoal fractions. In both fractions, CLA concentrations increased during the postprandial period. In conclusion, this study shows that protozoa may be responsible for a high proportion of unsaturated fatty acids, particularly CLA, leaving the rumen and available for absorption.

Effect of linseed supply on ruminal polyunsaturated fatty acid hydrogenation and bioconversion. M. Doreau, V. Scislowski, S. Gachon, D. Durand, D. Bauchart (INRA, Unité de Recherche sur les Herbivores, Theix, 63122 Saint-Genès-Champanelle, France).

Dietary supplementation with linolenic acid (18:3n-3) in bovines may enhance meat or milk quality, since this fatty acid (FA) is beneficial to human health. Its hydrogenation and bioconversion in other FA by rumen microbes was studied in three 10-month old steers fitted with a duodenal cannula. They received either a control diet (C) made of 45% hay and 55% concentrates, or the same basal diet supplemented with extruded linseeds (L). Diets C and L contained 2.7 and 5.4% of FA in dry matter. Fatty acids (% total FA methyl esters) of the C and L diets contained 20.3 and 12.3% 16:0 (palmitic), 2.8 and 3.2% 18:0 (stearic), 20.5 and 17.4% 18:1c9 (oleic), 37.7 and 25.0% 18:2n-6 (linoleic), 9.9 and 38.8% 18:3n-3. A representative sample of digesta passing through the duodenum was analysed by gas liquid chromatography. For the C and L diets, duodenal FA comprised 16.9 and 10.8% 16:0; 54.9 and 56.2% 18:0; 3.9 and 2.5% 18:1c9 (58 and 44% of total 18:1 *cis* isomers); 4.42 and 1.72% 18:2n-6; 1.16 and 1.41% 18:3n-3. Assuming a duodenal FA flow equal to FA intake, disappearance was 88 and 93% for 18:2n-6 and 88 and 96% for 18:3n-3 with the C and L diets, respectively. This shows that oil from extruded linseeds is rapidly available for microbial attack limiting polyunsaturated FA incorporation in muscle. Conjugated linoleic acids (CLA) amounted to 0.14 and 0.46% duodenal FA, of which 25% was the c9t11 isomer. Total *trans* 18:1 represented 9.0 and 18.8% FA, of which 68% were isomers 10+11, suggesting that muscle CLA arises mostly from ruminal *trans* 18:1.

Kinetics of ruminal fatty acid concentration as a tool to evaluate the rate of production of fatty acid from microbial hydrogenation. M. Doreau, S. Gachon (INRA, Unité de Recherche sur les Herbivores, Theix, 63122 Saint-Genès-Champanelle, France).

Fatty acid (FA) hydrogenation by rumen microbes is often evaluated by in vitro kinetics, but inconsistent results are sometimes observed. The analysis of in vivo FA kinetics following a ruminal FA infusion was thus performed. Two dry cows fitted with a ruminal cannula received a diet based on maize silage and concentrate (70:30) and 0.3 kg linseed oil. Concentrate and oil were mixed and given at 0900 h. Maize silage was given at 0930 h and 1630 h. Liquid and a homogenous sampling of total content were taken at 0900, 1000, 1100, 1300, 1500, 1800, 2200, 0300 and 0900 h. Fatty acid concentration and composition was determined on methyl esters by gas liquid chromatography. Mean concentration of

FA in DM was 67.0 and 67.5 $mg \cdot g^{-1}$ for liquid and total content, but between- and within-cow variations were higher for liquid. Kinetics of FA concentration were similar for both substrates. Microbial hydrolysis, isomerisation and hydrogenation are rapid : the concentrations of linolenic acid (main FA in linseed oil), of an intermediate compound *trans*-11 *cis*-1518:2, and of vaccenic acid (*trans*-11 18:1) peak respectively at 1, 2 and 4 h after linseed oil intake. Stearic acid, did not vary with time, suggesting a low rate of production from 18:1 isomers. Linoleic acid and oleic acid arising from linseed oil intake peak 1 and 2 h after feeding then decrease, suggesting their hydrogenation. Minor *cis* and *trans* 18:1 isomers peak 4 h after feeding, suggesting their rapid production by hydrogenation or by isomerisation.

Characteristic odd and branched-chain fatty acids of solid- and liquid-associated rumen bacteria. C. Dufour[a], B. Vlaeminck[a], T. Van Nespen[a], R.J. Dewhurst[b], V. Fievez[a] ([a] Department of Animal Production, Ghent University, Belgium; [b] Institute of Grassland and Environmental Research, UK).

Six cows were used in a 4-period incomplete change-over design with six different forages, varying in proportions of grass and legume silage. Forage was fed ad libitum and supplemented with 8 $kg \cdot day^{-1}$ of a standard concentrate. After a 3 week adaptation period total rumen content was evacuated and sampled before the morning feeding and 4 h after feeding. The two samples were considered as repetitions in the analysis. Solid- (SAB) and liquid-associated bacteria (LAB) were separated from the rumen contents by differential centrifugation ($1.600 \times g$ for 15 min and $30.000 \times g$ for 25 min), freeze-dried and analysed for fatty acid methyl esters (FAME), using modified Folch extractions. Total FAME ($mg \cdot g^{-1}$ DM) in SAB was 2.7 times higher than in LAB. Odd and branched-chain fatty acids (OBCFA) of LAB were enriched in branched-chain fatty acids (51.2 ± 3.8% vs. 40.2 ± 3.7%, $p < 0.001$), particularly anteiso C15:0 (28.4 ± 4.0% vs. 19.2 ± 2.7%, $p < 0.001$), whereas C15:0 was more abundant in OBCFA of SAB (38.1 ± 3.8% vs. 32.7 ± 2.9%, $p < 0.001$). The clear differences in patterns of OBCFA between SAB and LAB could be useful to identify SAB and LAB.

Reduced microbial bile salt deconjugation enhances the intestinal absorption of fatty acids and α-tocopherol in broilers. R.M. Engberg[a], A. Knarreborg[b], C. Lauriden[a], S.K. Jensen[a] ([a] Danish Institute of Agricultural Sciences, 8830 Tjele, Denmark; [b] Chr. Hansen A/S, Research, Development and Application, 2970 Hoersholm, Denmark).

The influence of intestinal microbial bile salt deconjugation on the absorption of fatty acids and vitamin E (α- and γ-tocopherol) was investigated in a trial with Ross 208 broilers. The birds ($N = 1600$) were assigned to 4 dietary treatments: supplementation or no supplementation with antibiotics (salinomycin, 40 $mg \cdot kg^{-1}$ feed and avilamycin, 10 $mg \cdot kg^{-1}$ feed), and addition of either 10% animal fat or 10% soya oil. At days 7, 14, 21, and 35 of age, the intestinal numbers of bile salt hydrolase active bacteria (lactic acid bacteria and *Clostridium perfringens*), the concentration of bile salts, the ileal absorption of fatty acids and vitamin E, and the blood plasma concentrations of vitamin E were measured. All measured variables were significantly influenced by bird age. Soya oil decreased *Clostridium perfringens* counts and increased bile salt concentrations. Dietary addition of antibiotics reduced the numbers of lactic acid bacteria and *Clostridium perfringens* in the small intestine and reduced the concentration of unconjugated bile salts. The ileal absorption of fatty acids and α-tocopherol, as well as the plasma status of α-tocopherol was improved by antibiotics. Absorption, and plasma concentration of γ-tocopherol was not influenced by antibiotics. On the contrary to γ-tocopherol, which is present as a free alcohol, dietary α-tocopherol is present as α-tocopheryl acetate, which requires a bile-salt dependent enzymatic hydrolysis prior to absorption. In conclusion, proper digestion of lipid-soluble compounds is highly dependent on the adequate concentration of bile salts in the small intestine in order to provide a proper lipid emulsification and activation of lipolytic enzymes.

Kangaroos have unusually high concentrations of TVA in their foregut. C.F. Engelke[a,b],

B.D. Siebert[c], K. Gregg[d], A.-D.G. Wright[b], P.E. Vercoe[a] ([a] Animal Biology, University of Western Australia; [b] CSIRO Livestock Industries, Centre for Environmental and Life Sciences, Wembley, Western Australia; [c] Department of Animal Science, University of Adelaide, Roseworthy Campus, South Australia; [d] Centre for High-Throughput Agricultural Genetic Analysis, Murdoch University, Western Australia).

Meat and milk products from ruminants have high levels of conjugated linoleic acids (CLA) because of biohydrogenation of fatty acids by ruminal microorganisms and endogenous synthesis in tissue. Kangaroos are not true ruminants but they do have a complex microbial community in their forestomachs that ferment forage. The concentrations of CLA and its precursor, *trans* vaccenic acid (TVA), in the tissue of kangaroos were found to be many times higher than in other species. Our aim was to determine the extent to which microbial biohydrogenation in the forestomach and endogenous synthesis contribute to the high levels of CLA found in kangaroo tissues. Our second goal was to identify the bacteria in the kangaroo forestomach that are responsible for the biohydrogenation and to compare them to rumen microorganisms that perform biohydrogenation. Digesta was sampled from multiple sites along the digestive tract of kangaroos to determine the site and extent of biohydrogenation of dietary polyenoic fatty acids. Fatty acid concentration was determined by gas liquid chromatography. The level of mono-unsaturated TVA in the forestomach of kangaroos was more than four times that of sheep, while the concentration of stearic acid was three times lower than in sheep. These opposite trends demonstrate that biohydrogenation is much less in kangaroos than sheep, contributing to relatively high concentrations of TVA and CLA in kangaroo adipose tissue. Fatty acid profiles of digesta from kangaroos and lambs from the same area will be discussed focussing on the change of fatty acids along the digestive tract in both species.

Effect of short-term period (2 months) and long-term period (12 months) of rumen defaunation on CLA synthesis from pure linoleic and linolenic acids. J.-P. Jouany, B. Lassalas (INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France).

The contribution of rumen protozoa to the rumen microbial ecosystem has been extensively studied by comparing the fermentation process before and after the elimination of protozoa (defaunation). However, nothing is known about the time necessary for bacteria to reach a climax state after the protozoal niche has been emptied. Rumen juice (15 mL) sampled from 2 defaunated and 2 faunated sheep fed a mixed diet was incubated for 0.5 h, 2 h, 5 h and 24 h with Coleman buffer (25 mL) and 500 mg of meadow hay + barley (45/55) in control fermenters. The experimental fermenters were supplemented with 25 mg of pure C18:3n-3 or pure C18:2n-6. Fatty acids were extracted according to Folch et al. (1957) before being transmethylated and analysed by GLC on a 100 m × 0.25 mm i.d. CP-Sil88 capillary column. FA metabolism was compared between a faunated period [F], a short-term period of defaunation [SD] (2 months after defaunation), and a long-term period of defaunation [LD] (12 months). No difference on total CLA (2.5% of total FA) was noted between the LD and F periods. On the contrary, CLA production was stimulated during the SD period (11 and 4% of total FA from C18:3 and C18:2 respectively). This effect was due to a significant increase in t11,t13-CLA (10% of total FA) when C18:3 was used as the precursor, and an increase in t10, c12-CLA (2.5% of total FA) when C18:2 was added into the incubators. No effect of defaunation was noted on the synthesis of vaccenic acid, which is used as a precursor of CLA by the mammary gland. The metabolism of PUFA was strongly modified during the short period of defaunation, while no differences were noted with the faunated period when the rumen was defaunated for 12 months. This work was supported by the EU HealthyBeef Project (QLK1-CT-2000-01423).

Biohydrogenation of linoleic acid to stearic acid by rumen bacteria *Butyrivibrio* and *Pseudobutyrivibrio.* I. Koppová, J. Kopečný (Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídenská 1083, 142 20, Prague 4, Czech Republic).

An important property of the rumen microbial system is its ability to biohydrogenate the C18

unsaturated acids to trans-vaccenic and stearic acid. The requirement of the free carboxylic group is necessary for consecutive biohydrogenation. Fatty acids coming to the rumen are incorporated in lipids. Shortly after ingestion, plant lipids are hydrolyzed by a microbial lipase, causing the release of free constituent fatty acids. The main known lipolytic bacteria in the rumen are the following : *Anaerovibrio lipolytica, Butyrivibrio* and *Pseudobutyrivibrio.* Unsaturated free fatty acids have relatively short half-lives in the rumen content since they are rapidly hydrogenated by microbes to more saturated intermediates. It was found, that anaerobic rumen bacteria, relative to *Butyrivibrio fibrisolvens*, carried out the biohydrogenation of linoleic acid in the two-step reaction. The first step is an isomeration of linoleic acid to *cis*-9, *trans*-11-octadecadienoate. In the second step, hydrogenation of the conjugated linoleic acid leads to *trans*-11-octadecanoate. The enzyme CLA reductase catalyzes the reaction. The intermediate of the biohydrogenation, *cis*-9, *trans*-11-octadecadienoate (conjugated linoleic acid = CLA) is a very important compound. In animal and in vitro studies CLA has been shown to have anticarcinogenic effects, to prevent atherosclerosis, to modulate the immune system and to stimulate lipolysis. We measured the lipolytic activity in anaerobic butyrate producing isolates. *B. fibrisolvens* strains showed a low lipolytic activity, *Cl. proteoclasticum* higher and *B. hungatei* the highest one. The increase correlated with their genetic relationship. Beside that, we observed several strains with high CLA reductase activity.

Relation between phylogenetic position and fatty acid metabolism of different *Butyrivibrio* isolates from the rumen. N. McKain[a], L.C. Chaudhary[a], N.D. Walker[a], F. Pizette[a], I. Koppova[b], N.R. McEwan[a], J. Kopečný[b], P.E. Vercoe[c], R.J. Wallace[a] ([a] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [b] Institute of Animal Physiology & Genetics, Prague, Czech Republic; [c] Animal Science Group, University of Western Australia, Crawley, WA 6009, Australia).

Butyrivibrio forms a genetically diverse group of Gram-positive bacteria, isolated mainly from the rumen of cattle and sheep. It participates in several important functions in the rumen, including fibre breakdown and proteolysis. The significance of *B. fibrisolvens* is particularly high in fatty acid biohydrogenation, as it biohydrogenates linoleic and linolenic acids much more rapidly than other ruminal species. Here, we collected 34 different *Butyrivibrio* isolates isolated in different countries, and compared their specific activity in several aspects of their fatty acid metabolism with their position in a phylogenetic tree derived from 16S rDNA sequence data. Two clear sub-groups were evident, both phylogenetically and metabolically. Group A typified most *B. fibrisolvens* isolates, while Group B contained isolates related to *B. hungatei* and *Clostridium proteoclasticum.* All produced butyrate, but Group A bacteria had a butyrate kinase activity < 40 U·mg^{-1} protein, while Group B were all > 600 U·mg^{-1} protein. The butyrate kinase gene was present in all Group B bacteria tested but not in Group A. Lipase activity, measured by tributyrin hydrolysis, was high in Group B and low in Group A, without exception. Linoleate isomerase activity (which produces CLA), however, did not correspond with phylogenetic position. Group A bacteria all grew in the presence of 200 μg·mL^{-1} linoleic acid, while Group B bacteria were inhibited by lower concentrations, some as low as 5 μg·mL^{-1}. Most significantly, Group B bacteria formed abundant stearate under specific growth conditions, whereas Group A did not. Thus, group B bacteria are probably the same *Fusocillus* isolated in Babraham more than 25 years ago – the only ruminal bacteria until now known to hydrogenate linoleic acid all the way to stearic acid.

Inhibition of ruminal biohydrogenation of linoleic acid by fish oil. I. Wąsowska[a], M. Maia[b], M. Czauderna[a], J.M.C. Ramalho-Ribeiro[b], R.J. Wallace[c] ([a] Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jablonna, Poland; [b] Estação Zootécnica Nacional, Quinte da Fonte Boa, 2000-763 Vale de Santarém, Portugal; [c] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Dietary linoleic acid (LA; C18:2, *cis*-9, *cis*-12) is hydrogenated in the rumen firstly by isomerisation to conjugated linoleic acids (CLA; mainly C18:2, *cis*-9, *trans*-11), which are then hydrogenated to C18:1 fatty acids (mainly *trans*-11; *trans*-vaccenic acid; TVA), which in turn are

hydrogenated to stearic acid (C18:0). Unsaturated fatty acids, particularly CLA, in the human diet are considered beneficial to health, so our aim was to slow biohydrogenation in the rumen, to enable more unsaturated fatty acids to be available for absorption by the animal. Fish oil (FO) has been reported to increase CLA in milk, so its effect on biohydrogenation was investigated. Ruminal digesta were removed from four adult rumen-cannulated sheep immediately before the morning feeding. The fluid was strained and incubated at 39 °C under CO_2 with LA (1.67 mg·mL^{-1}), FO (4.17 mg·mL^{-1}) or LA + FO. The rate of loss of LA was unaffected by FO, but CLA accumulated to lower concentrations in the presence of FO. TVA concentrations doubled over the 24-h incubation period. The predominant fatty acids of fish oil, C20:5 and C22:6, were not metabolised significantly over the same period. These acids proved exceptionally toxic to the growth of *Butyrivibrio fibrisolvens* JW11. Since *Butyrivibrio* is the most important group of ruminal bacteria involved in biohydrogenation, it would appear that the effect of FO on fatty acids in milk may be mediated via a toxicity to the ruminal bacteria, specifically *Butyrivibrio*, carrying out biohydrogenation. Consequently, more TVA will reach the mammary gland, where it will be converted to CLA by Δ9-desaturase.

Screening for *Fusocillus*: factors that affect the detection of ruminal bacteria which form stearic acid from linoleic acid. L.C. Chaudhary, N. McKain, A.J. Richardson, M. Barbier, J. Charbonnier, 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. In a survey of 1,000 fresh bacterial isolates from the sheep rumen, many isolates were found which metabolised linoleic acid (C18:2, *cis*-9, *cis*-12). Most of these were butyrate producers characteristic of *Butyrivibrio* spp. They formed mainly conjugated linoleic acid (C18:2, *cis*-9, *trans*-11; CLA) and *trans*-vaccenic acid (C18:1, *trans*-11). Only one isolate was discovered which formed stearic acid (C18:0). This isolate (P-18) was identical in its morphology and physiology to two '*Fusocillus*' isolates obtained in Babraham more than 25 years ago, which have since been lost. Its phylogenetic position based on 16S rDNA sequence fell alongside various *Butyrivibrio* isolates, including *Butyrivibrio hungatei*, and *Clostridium proteoclasticum*. When strain P-18 was inoculated into media containing linoleic acid, the linoleic acid caused a lag phase, the length of which depended on the medium. Further investigation showed that the variability was caused by the concentration of sodium D, L-lactate in the medium: lactate was not itself bacteriostatic, but it greatly increased the bacteriostatic activity of linoleic acid. Culture age was also a factor: stationary-phase P-18 bacteria did not form stearic acid, whereas mid-exponential-phase cells produced stearate abundantly. Adding linoleic acid to the medium also militated against finding *Fusocillus*-like bacteria, since many of the close relatives of isolate P-18 did not grow in the presence of linoleic acid, some inhibited by concentrations as low as 5 μg·mL^{-1}. The adition of very low concentrations to the medium, or at mid-exponential phase, revealed that most of the isolates which clustered around isolate P-18 were capable of producing stearic acid. Thus, stearic acid production is more widespread than previously thought in this group of ruminal bacteria.

Gnotobiotic rats harboring human intestinal microbiota as a model for studying cholesterol-to-coprostanol conversion. P. Gérard, F. Béguet, P. Lepercq, L. Rigottier-Gois, V. Rochet, C. Andrieux, C. Juste (Unité d'Écologie et Physiologie du Système Digestif, INRA, Domaine de Vilvert, 78352 Jouy-en-Josas, France).

The efficiency of microbial reduction of cholesterol to coprostanol in the human gut is highly variable among the population and the mechanisms remain unexplored. In the present study, we investigated whether microbial communities and their cholesterol metabolism characteristics can be transferred to germ-free rats. Two groups of six, initially germ-free rats were associated with two different human microbiota, exhibiting high and low cholesterol-reducing activities. Four months after inoculation, the enumeration of coprostanoligenic bacteria, fecal coprostanol levels and composition of the fecal microbial

communities were studied in gnotobiotic rats and were compared with those of human donors. A combination of culture (most probable number enumeration of active bacteria) and biochemical approaches (extraction followed by gas chromatography of sterols) showed that gnotobiotic rats harbored a coprostanoligenic bacterial population level and exhibited coprostanoligenic activities similar to those of the corresponding human donor. On the contrary, molecular approaches (whole-cell hybridization with fluorescently labeled 16S rRNA-targeted oligonucleotide probes, and temporal temperature gradient gel electrophoresis of bacterial 16S rRNA gene amplicons) demonstrated that gnotobiotic rats reproduced a stable microbial community, close to the human donor microbiota at the group or genus levels but different at the dominant species level. These results suggest that the gnotobiotic rat model can be used to explore the still unknown human intestinal microbiota involved in luminal cholesterol metabolism, including the regulation of expression of its activity and impact on health.

Construction of an isogenic *Escherichia coli* strain inactivated in *uidA* to address the question: does β-glucuronidase produced by gut microbiota contribute to the genotoxic effect of 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ)? Ch. Humblot, D. Beaud, L. Rigottier-Gois, M. Bensaada, S. Rabot, J. Anba (Unité d'Écologie et de Physiologie du Système Digestif, INRA, 78352 Jouy-en-Josas Cedex, France).

This study was aimed at investigating if the β-glucuronidase produced by gut microbiota contributes to the genotoxic effect of 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ), a mutagenic/carcinogenic compound formed from meat and fish during cooking. When ingested, IQ is mainly metabolised in the liver by UDP-glucuronosyl transferases into non-carcinogenic glucuronidated compounds that are mostly excreted in the urine. Nevertheless, these derivatives are partly excreted via the biliary secretions into the digestive lumen, where it is supposed that colonic microbiota hydrolyse them to release carcinogenic secondary derivatives. While the contribution of the bacterial β-glucuronidase to this reactivation process has been shown several times in vitro, direct evidence for its role in vivo is still lacking. To ascertain the involvement of bacterial β-glucuronidase in the digestive fate of IQ, we compared gnotobiotic rats harbouring a "β-glucuronidase +" *Escherichia coli* strain to gnotobiotic counterparts inoculated with an isogenic mutant inactivated for this enzyme. In the literature, *Escherichia coli* strains referred to as "β-glucuronidase –" are mostly derived from an *E. coli* strain carrying a large deletion of 50 kbp including the *uidA* gene encoding β-glucuronidase. We constructed a strain solely inactivated for this enzyme, applying the method of Datsenko and Wanner (2000) with slight modifications to an *E. coli* strain of the UEPSD collection. Sixteen germ-free rats were inoculated either with the wild type "β-glucuronidase +" strain ($n = 8$) or with the "β-glucuronidase –" mutant strain ($n = 8$). Three weeks after inoculation, the rats were orally dosed with IQ (90 $mg \cdot kg^{-1}$) and genetic damages in the colonic mucosa were measured using the single cell gel electrophoresis assay (Comet assay). The results indicate that the absence of bacterial β-glucuronidase in the digestive lumen dramatically reduces the genotoxic effects of IQ. This is the first in vivo demonstration that bacterial β-glucuronidase plays a key role in the genotoxic effects of this dietary carcinogen.

Epimerization of chenodeoxycholic acid to ursodeoxycholic acid by *Clostridium baratii* isolated from human feces. P. Lepercq[a], P. Gérard[a], F. Béguet[a], P. Raibaud[a], J.P. Grill[b], P. Relano[c], C. Cayuela[c], C. Juste[a] ([a] INRA, CR Jouy-en-Josas, 78352 Jouy-en-Josas Cedex, France; [b] Laboratoire des BioSciences de l'Aliment, Faculté des Sciences et Techniques, 54500 Vandoeuvre-lès-Nancy Cedex, France; [c] Danone Vitapole, Nutrivaleur, Groupe Probiotiques et Fonctions Digestives, Route Départementale 128, 91167 Palaiseau Cedex, France).

Ursodeoxycholic acid-producing bacteria are of clinical and industrial interest due to the multiple beneficial effects of this bile acid on human health. This work reports the first isolation of 7-epimerizing bacteria from the feces of a healthy volunteer, on the basis of their capacity to epimerize the primary bile acid, chenodeoxycholic acid, to ursodeoxycholic acid. Five isolates were found to be active starting from unconjugated chenodeoxycholic acid and its

tauro-conjugated homologue, but none of these strains could epimerize the glyco-conjugated form. Biochemical testing and 16S ribosomal DNA sequencing converged to show that all five isolates were closely related to *Clostridium baratii* (99% sequence similarity), suggesting that this bacterial species could be responsible at least partially, for this bioconversion in the human gut.

Ruminal toxicity of individual mycotoxins and of a mixed-toxin extract obtained from *Aspergillus fumigatus*-contaminated feed. D.P. Morgavi, H. Boudra, D. Graviou, D. Alvarez (INRA, Clermont-Ferrand/Theix Research Centre, Herbivore Research Unit, 63122 Saint-Genès-Champanelle, France).

A. fumigatus is a predominant fungus found in poorly conserved animal feeds where it can produce several secondary toxic metabolites with identified toxic effects. The objective of this work was to assess the toxicity of major toxins produced by *A. fumigatus* on rumen fermentation. The effect of an extract from *A. fumigatus* grown on barley grain was also tested. Gliotoxin, verruculogen, fumagillin, and helvolic acid were incubated alone or combined at concentrations of up to 40 $\mu g \cdot mL^{-1}$ in the presence of mixed rumen fluid and alfalfa hay. Gliotoxin and helvolic acid (40 $\mu g \cdot mL^{-1}$), compared to controls, decreased dry matter and NDF degradation by up to six percentage points ($P < 0.05$), while gas and total VFA production decreased by 20 and 15%, respectively. While negative effects of gliotoxin were not observed at lower doses, helvolic acid affected fermentations at concentrations as low as 10 $\mu g \cdot mL^{-1}$. Fumagillin negatively affected DMD ($P < 0.05$) but had no effect on fermentation parameters. Verruculogen, in contrast, did not have any negative effect. The association of all four toxins had a negative ($P < 0.01$) additive and/or synergistic effect on fermentation parameters at concentrations as low as 5 $\mu g \cdot mL^{-1}$. Extracts of *A. fumigatus* from contaminated feed (8.8 µg gliotoxin, 0.9 µg helvolic acid, and 0.5 µg fumagillin$\cdot mL^{-1}$) decreased ($P < 0.01$) DMD, gas and VFA production by 28, 46, and 35%, respectively, confirming the toxicity and possible synergism of metabolites produced by this fungus. Identification of these toxic metabolites and assessment of their rate of passage to the lower intestinal tract is necessary to evaluate the potential risk of these toxins to ruminants.

Reducing agents prevent patulin toxicity in in vitro rumen fermentations. D.P. Morgavi, H. Boudra, J.-P. Jouany, D. Graviou (INRA, Clermont-Ferrand/Theix Research Centre, Herbivore Research Unit, 63122 Saint-Genès-Champanelle, France).

Patulin, a toxic fungal metabolite, is found in silages, barley malt residues, cereal stubbles, and by-products of the apple industry. Patulin is a broad-spectrum antibiotic and it is teratogenic, carcinogenic, and mutagenic. This mycotoxin has been associated with intoxication cases in cattle and negatively affects rumen fermentation. We investigated the use of SH-containing reducing compounds to prevent patulin's negative effects on the rumen microbial ecosystem. The effect of different concentrations of patulin on the fermentation of alfalfa hay was measured in batch cultures with and without reducing agents. The addition of patulin to the rumen fluid mixture negatively affected ($P < 0.05$) fermentation in vitro. The highest concentration of patulin used (100 $\mu g \cdot mL^{-1}$) decreased DMD (dry matter degradation) by 27%, while gas and VFA production decreased by 64 and 48%, respectively. Even at the low concentration of 25 $\mu g \cdot mL^{-1}$ the toxin decreased ($P < 0.05$) DMD and VFA by 12% and gas production by 30%. Sulfhydryl-containing cysteine and glutathione prevented the negative effects of the toxin on DMD, gas and VFA production ($P < 0.01$). However, non sulfhydryl-containing, ascorbic and ferulic acids did not protect against patulin's toxicity ($P > 0.05$). Patulin was unstable in active buffered rumen fluid as the concentration decreased by half after 4 h of incubation. In the presence of sulfhydryl groups, the toxin disappeared rapidly and was not detected after 1 h of incubation. The utilization of sulfhydryl-containing compounds such as cysteine to avert patulin toxicity could have practical implications in ruminant nutrition.

Lactate fermentation by intestinal flora differs between monogastrics. M.-C. Alexandre-Gouabau, A. David, F. Kozlowski, C. Michel (Digestive Functions and Human Nutrition Unit, INRA, 44316 Nantes Cedex 03, France).

We showed that lactate is mainly converted into butyrate by some human intestinal floras. This result disagreed with previous studies using intestinal floras from animals. To assess whether this discrepancy arose from inherent differences between monogastics, we incubated 20% (w/v) faecal slurries from humans, pigs or rats in vitro, at pH 5.7, with or without L-lactate (30 mM) and we measured both lactate and short-chain fatty acids at different incubation times. All floras totally utilized lactate. However the human floras fermented lactate more rapidly than the others : for pig and rat floras, the lactate disappearance percentage increased between the 8th (15.1 ± 3.6% in average) and the 24th h of incubation (90.8 ± 3.3%) whereas 78.1 ± 3.2% of lactate had disappeared at T6h for human floras. Similar kinetical differences were observed for SCFA productions which reached 51.9 ± 2.7 mM at T8h and 57.8 ± 4.1 mM at T24h for human and animals floras respectively, in lactate containing media. The net final SCFA productions were similarly composed, for both animal floras, of equal amounts of acetate (34.8 ± 4.0% of the total SCFA) and propionate (31.7 ± 3.8%) while butyrate only constituted 24.0 ± 3.5%. Conversely, butyrate was the major product of lactate utilization by human floras (86.4 ± 2.3%), which also led to a small proportion of propionate (12.9 ± 2.2%). This study confirmed that butyrate is the main product of lactate fermentation by human fecal floras and emphasized both kinetical and metabolic differences between human and animal intestinal floras. Whether this results from differences in number or composition of the lactate-using bacterial populations harbored by the different monogastrics is now under process.

A new species of the *Clostridium leptum* group from human intestinal flora is involved in butyrate production from lactate. E. Barrat[a], F. Kozlowski[a], S. Rezé[a], R. Bonnet[b], A. David[a], J. Doré[b], C. Michel[a] ([a] UFDNH, INRA, 44316 Nantes Cedex 03, France; [b] UEPSD – INRA, 78352 Jouy-en-Josas Cedex, France).

Butyrate is produced by human intestinal bacteria during fermentation of the dietary compounds which escape digestion in the upper digestive tract. This organic acid is thought to promote the health of the intestinal mucosae. Stimulating its production would be of particular interest but the human intestinal butyrate producers are still incompletely identified. Since we have recently shown that lactate is a precursor for butyrate synthesis in some human floras, we intended to isolate and identify the bacteria which convert lactate into butyrate. Using human faecal floras preincubated with fructo-oligosaccharide – a substrate which stimulates both lactate and butyrate productions –or with lactate, 12 subcultures producing more than 40 mM of short-chain fatty acids with a butyrate ratio higher than 50%, were selected. The more interesting subculture produced 42 ± 7 mM of SCFA of which butyrate represented 65 ± 7%. Eleven bacterial strains were isolated from this subculture using selective broths and/or treatments. From partial sequencing of their 16S rDNA (500 b), five different species were identified among which a new species of the *Clostridium leptum* group (strain 4–80). None of these isolates were able to produce butyrate from the lactate broth whereas their co-culture resulted in 20 mM of butyrate. Co-cultures of all isolates but one showed that strain 4–80 was crucial for this production. Other necessary strains exhibited high homologies with enterobacteria or the *Bacteroides fragilis* group. In the absence of strain 4–80, acetate was the main end-product of the co-culture(s). This study suggests that lactate conversion into butyrate might require a bacterial consortium rather than a particular species.

Characterisation of butyryl-CoA:acetate CoA transferase (CoA T) from *Roseburia* sp.; a major enzyme for butyrate formation by human gut bacteria. C. Charrier, P. Louis, S.H. Duncan, S.I. McCrae, H.J. Flint (Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland, UK).

Human gut bacteria are responsible for many fermentative reactions involved in dietary food degradation. In the colon, carbohydrates such as fibre and resistant starch not digested in the upper gut are fermented by the commensal

anaerobic microflora that results in the production of short chain fatty acids, mainly acetate, propionate and butyrate. There is increasing interest in butyrate in particular as it is a major source of energy for colonocytes as well as having health-promoting effects in inflammatory bowel diseases and colon cancer through effects on host gene expression. In anaerobic bacteria, butyrate is generated by a central metabolic pathway and two alternative pathways from butyryl-CoA to butyrate, involving either a CoA T or a two-step process involving the phosphotransbutyrylase-butyrate kinase mechanism. From enzymatic studies on bacterial isolates from the human gut we know that the CoA T pathway is the main route for butyrate synthesis. However, only the phosphotransbutyrylase-butyrate kinase pathway genes and enzymes have been identified. Characterisation of the partially purified native CoA T from the human gut anaerobes *Roseburia* sp. A2-183 and L1-82 indicates that the preferred substrates are butyryl-CoA and acetate; similar results were obtained with an overexpressed candidate gene product. Kinetic studies and electron spray ionisation mass spectrometry will further the characterisation of this enzyme and will help to resolve the gene sequence of the CoA T, which is uncharacterised so far. This is a major step forward in understanding the butyrate formation by human gut bacteria and should lead to an understanding of the regulatory mechanisms for butyrate production by gut anaerobes.

Lactate utilisation by human gut anaerobes. S.H. Duncan, P. Louis, H.J. Flint (Microbial Genetics Group, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

The microbial community that inhabits the human colon contains many bacterial species; some of these have only recently been isolated while others remain uncharacterised. The major short chain fatty acids (SCFA) that are found in the colon are acetate, propionate and butyrate. Although many colonic bacteria including bifidobacteria and lactobacilli species produce lactic acid, it does not normally accumulate as a product in the gut of healthy individuals. Lactic acid may, however, accumulate in the colon under certain disease states such as ulcerative colitis attaining concentrations of up to 90 mM. Elevated concentrations of lactate can influence nutrient absorption, water absorption, colonic microflora diversity, colonic pH, and in the case of the D-isomer neurological function. We describe here the isolation of several groups of anaerobic bacteria from human faeces that are able to convert lactate into butyrate. Addition of glucose to batch cultures of these isolates prevented lactate utilisation until the glucose became exhausted. When lactate utilising strains were grown in co-culture with a starch-utilising *Bifidobacterium* sp., with starch as the carbohydrate energy source, however, all of the lactate produced by the bifidobacterium strain was converted to butyrate. Such cross-feeding can help to explain the reported butyrogenic effect of dietary resistant starch. The abundance and maintenance of lactate utilising, butyrate producing bacteria in the colonic ecosystem may be an important factor in stabilising metabolic activities in the colon and in preventing excessive lactate accumulation. Two of the newly isolated lactate-utilising strains are related to *Eubacterium hallii,* while four others represent three new species within the Clostridial cluster XIVa. A survey of previously isolated butyrate-producing bacteria confirmed lactate utilisation in another *E. hallii* strain, and in the species *Anaerostipes caccae*, but not in *Roseburia intestinalis*, *E. rectale* or *Faecalibacterium prausnitzii*. Whereas the *E. hallii* and *A. caccae* strains use both lactate stereoisomers, other strains were found to use only the D-form.

The predominant lactic acid producing and utilizing bacteria from the gastrointestinal tract of the dromedary camel. M.B. Ghali[a,b], P.T. Scott[b], R.A.M. Al Jassim[a] ([a] School of Animal Studies; [b] School of Agronomy and Horticulture, The University of Queensland, Gatton 4343 Australia).

The microbial population of the gastrointestinal tract in ruminants, including camels, is well balanced when animals are fed a roughage-based diet. The major roles of these bacteria are to provide the host with essential nutrients, degrade some anti-nutritional factors, and as a result, maintain the health and well being of the animal. Changing the diet from roughage to concentrate will create an environment favoring the proliferation of both lactic acid producing bacteria

(LAPB) and lactic acid utilizing bacteria (LAUB). An abrupt dietary change from roughage to concentrate has been documented to cause acidosis in camels and anecdotal evidence suggests that it could lead to the development of laminitis. This problem is well documented for cattle and horses but there is little information available on the incidence of acidosis and laminitis in camels. The aim of this study was to identify the predominant LAPB and LAUB of the camel rumen. Conventional culturing methods were used to culture, isolate and purify LAPB and LAUB from the gastrointestinal contents of dromedary camels fed both roughage and concentrate diets. Isolates were identified by 16S rDNA sequence analysis. Biochemical and morphological characteristics of the isolates were also recorded. The results up to date revealed a diverse population of LAPB and LAUB with *Streptococcus bovis* being the predominant LAPB while *Selenomonas ruminantium* is among the key LAPB and LAUB in the rumen of the camel. This is the first report of the characterization of any microbial group from the camel rumen. Further experiments will utilize the molecular data from this study to monitor changes in the microbial population with associated changes in the diet.

pH affects butyrate production by human intestinal flora through changes in lactate utilization. C. Michel, C. Gryson, F. Kozlowski, F. Doulay, M.-C. Alexandre-Gouabau, C. Cherbut (Digestive Functions and Human Nutrition Unit, INRA, 44316 Nantes Cedex 03, France).

A slightly acidic environment stimulates butyrate production by human intestinal flora. We have shown that lactate is a precursor for butyrate synthesis in some human floras. This study assessed whether pH affects lactate production or lactate fermentation. First, we incubated 20% (w/v) faecal slurries from humans ($n = 3$), at pH 5.8 or 6.5, with fructooligosaccharides (10 $g \cdot L^{-1}$) to follow up lactate production. Second, we carried out in vitro incubations with lactate (30 mM) at different pH values (5.6 to 6.8) to measure the rates of lactate utilization and SCFA production. The set up pH value did not affect the total production of SCFA from FOS (120.4 ± 8.7 in average, $P = 0.156$) but modified the relative proportions of SCFA : butyrate proportion increased at pH 5.8 comparatively to pH 6.5 (26.3 ± 3.5 vs. 20.2 ± 3.4%, $P = 0.001$). This occurred at the expense of acetate ($P = 0.009$) and propionate ($P =$ 0,035). Lactate transitorily accumulated in the media and the calculated area under the curve was increased at pH 5.8 (35.8 ± 8.8 vs. 27.5 ± 7.0, $P = 0.058$). Similarly, pH did not modify the net SCFA production from lactate (17.7 ± 0.7 mM, $P = 0.465$) but it dramatically affected the butyrate proportion, which increased linearly from 50.7 ± 3.3% at pH 6.6 to 86.0 ± 2.0% at pH 5.8 ($P < 0.0001$, R = 0.82), while the rate of lactate utilization was inversely affected (3.7 ± 0.2 at pH 5.8 to 4.7 ± 0.3 $mM \cdot h^{-1}$ at pH 6.6 ($P < 0.004$, R = 0.52)). This study suggests that the pH effect on butyrate production by the human intestinal flora mainly stems from changes in lactate fermentation rather than in lactate production. Whether pH acts by selecting different species of lactate-utilizing bacteria or by controlling the enzymes involved in lactate fermentation has to be determined.

Production of equol by human intestinal microbiota. K. Decroos, S. Vanhemmens, S. Cattoir, N. Boon, W. Verstraete (LabMET, Ghent University, Coupure Links, 653, 9000 Gent, Belgium).

Isoflavones are a group of polyphenolic compounds present in soy. Through their mild estrogenic activity and anti-oxidative effects they are thought to play a role in the prevention and treatment of chronic disease. The intestinal microbial metabolism after ingestion, which is subjected to a great interindividual variation, is crucial for the bioactivity and bioavailability of isoflavones. Equol, a metabolite of daidzein, which is produced exclusively by intestinal bacteria, is produced by only ca. 35% of the population and is superior to its precursor in estrogenic and antioxidative activity. It is believed that the beneficial effects of soy consumption are linked to the equol producing status of the individual. Very little is known about the bacterial species involved and the influence of intestinal ecology and diet. In this study a transferable mixed microbial culture, efficiently transforming daidzein into equol, was isolated from a human fecal sample through conventional culturing techniques. Denaturing Gradient Gel Electrophoresis (DGGE) revealed the presence of four dominant species, which were identified by sequencing the 16S

rDNA. Two species could be isolated in pure culture, not producing equol. Isolation of the two other species is currently being performed. Further investigation of the consortium showed that hydrogen gas and SCFA, intestinal metabolites of dietary carbohydrates, stimulate equol production. Especially hydrogen gas played an important role, probably as an electron donor.

Metabolism of the soy isoflavonoid daidzein into equol by the microbial community from the human gut. J. Mathey[a,b], V. Lamothe[c], C. Bennetau-Pelissero[c], V. Coxam[b], A. Bernalier-Donadille[a] ([a] Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; [b] Unité des Maladies Métaboliques et Micronutriments, INRA, CR de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; [c] Unité Micronutriments, Reproduction, Santé, ENITA de Bordeaux, 33175 Gradignan Cedex, France).

Isoflavonoids, diphenols found in legumes, are recognised for providing health benefits. These phytoestrogens remain a source for putative new and innovative dietary health intervention to prevent a wide range of hormone-dependent diseases. Ingested isoflavonoids are mainly converted in the colon by the intestinal microflora. This biotransformation is essential for providing highly estrogenic and anti-oxidant metabolites. In this context, daidzein, one of the main isoflavonoids found in soybeans in its glycoside form, daidzin, is known to be metabolised by the gut microflora into the powerful coumpound equol. The ability of the intestinal microflora to produce equol is known to be subjected to important inter-individual variations. However, little is known regarding this microbial status and the bacterial population involved. The objective of our study was to investigate the microbial community responsible for equol production from soybean daidzein in the colon of equol-producing and non-equol-producing women (n = 15). The baseline level of the equol-producing flora (i.e. diet without soybean supplementation) was close to 10^6–$10^7 \cdot g^{-1}$ faeces in equol-producing subjects whereas this population remained undetectable ($< 10^3 \cdot g^{-1}$ faeces) in non-equol-producing ones. Supplementation of the diet with soybean (100 mg·day^{-1}) for 30 and 60 days increased significantly the level of this bacterial population in equol-producing individuals. The effect of soybean supplementation was further amplified by the addition of a prebiotic or a probiotic. Enrichments of the equol-producing community were obtained from different faecal specimens. Microbial and metabolic characterisations were further carried out on one enrichment, maintained in vitro for 2 years. The kinetics of daidzein transformation seemed to be dependent on H_2 transfer. The bacterial community appeared to be composed of a relatively restricted number of species that were further isolated in pure cultures. Some strains were able to transform daidzein into a compound that chemically resembled equol.

Effect of different environmental conditions on fermentation end-products in colon of suckling piglets. A. Awati[a], B.A. Williams[a], M. Bosch[a], B. Miller[b], K. Haverson[b], D. Patel[b], M.W.A. Verstegen[a] ([a] Animal Nutrition Group, Wageningen Institute of Animal Sciences (WIAS), Wageningen, The Netherlands; [b] Department Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, UK).

For an in vivo study, seven litters were selected, after farrowing. Ten piglets from each litter were randomly divided into two groups of five piglets following one day of colostrum suckling. One group was kept in an SPF isolator (ISO). They were fed a milk replacer hourly to mimic normal suckling behaviour. The environmental conditions were controlled. The second group was kept with the sow on the farm (FM), and was therefore potentially exposed to a wide range of pathogenic, dietary, social and environmental influences. Two piglets (one from each group) were slaughtered at 2, 5, 10, 21 and 28 days of age. Samples were collected from the colon contents of all animals for DM, VFA and ammonia analysis. Combining results from all time intervals, the DM of colon contents was higher in FM piglets compared to ISO piglets. Total VFA concentration was higher in FM piglets, though not significantly different. Acetic acid was proportionally higher for ISO piglets (60% of total VFA) compared to that of FM piglets (48%). On the other hand, propionic acid (16%) and butyric acid (9%) for ISO piglets was proportionally lower than that of FM piglets (19% propionic and 14% butyric acids). BCFA (*iso*-butyric and *iso*-valeric), usually considered an end-product

of protein fermentation, were proportionally higher in the FM group. Ammonia concentration was also significantly higher in the FM group. These results suggest that the environmental conditions from the time of birth to weaning may affect the microbial activity of the colonic microbial population.

Characterising the fermentation capabilities of gut microbial populations from cattle and sheep grazing heathland forage using gas production. D.R. Davies, M.D. Fraser, V.J. Theobald, A.E. Brooks (Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK).

A gas production experiment, as part of a larger programme developing management regimes for the regeneration of heather moorland, was conducted to determine the effects of substrate and breed on the rumen fermentation characteristics of cattle and sheep grazing *Calluna* heath. Samples of *Calluna vulgaris* (Heather) and *Nardus-stricta* dominated semi-natural grazing (Grass) were cut as substrate, from an area which two breeds each of sheep and cattle had grazed. Faeces was collected from six animals per type and diluted (1:1 w/v) in medium, and used as inoculum. The fermentation data showed Grass substrate had significantly ($P < 0.001$) shorter lag time and greater total gas pool size and DM loss than Heather substrate. There were significant differences between animal species, but few differences between animal breeds within species. For Heather substrate only DM loss was ($P < 0.001$) different between animal types, with greater digestion for the sheep inocula. All fermentation parameters were significantly different between animal species for the Grass substrate, with the initial and secondary rates and final DM losses significantly ($P < 0.001$) higher for sheep inocula. In conclusion, the data confirm that heath grasses are more digestible than heather. The data indicate that sheep and cattle possessed gut microflora showing different activities, despite being exposed to the same forage. These results alongside corresponding diet selection and intake data will be used to quantify the consequences of different foraging strategies, which will be incorporated into models developing grazing guidelines for heather moorland.

Nutrient utilisation, blood constituents and growth performance of growing lambs fed varying concentrate levels. M.K. Tripathi[a,b], O.H. Chaturvedi[a], B.M. Goyal[a], S.A. Karim[a], V.K. Singh[a] ([a] Central Sheep and Wool Research Institute, Avikanagar (via- Jaipur) Rajasthan, 304 501 India; [b] Present address: UEPSD, Bât. 440, INRA, Jouy-en-Josas 78352, Cedex, France).

High concentrate diets improve growth performance and lower the cell wall degradation in the rumen because of a shift in rumen microbiota from cellulolytic to amylolytic. A lower ruminal pH decreases the protozoa population and subsequently increases bacterial biomass thus improving ruminant energy utilization. Under three feeding regimens comparing 1.5 and 2.5% of live weight concentrate allowances, and ad libitum with free access to roughage (*Zizyphus nummularia* and *Albizia lebback,* leaves 50: 50), the lambs consumed 85 parts of concentrate, when given free access to concentrate and roughage. Interestingly acidosis did not occur and ruminal pH was above 6.0 among the three groups. Nutrient intake, digestibility and growth improved with increased concentrate intake. Cell wall utilization was also higher ($P < 0.001$) in the lambs consuming 85 percent concentrate compared to restricted concentrate feeding. Average daily gain was 77.2, 97.6 and 150.7 g, respectively in lambs fed concentrate 1.5, 2.5% of live weight and ad libitum. ME intake 56.8 MJ for each kg live weight gain was lower in lambs fed a high concentrate than in those given a restricted quantity. Apparent nitrogen retention and absorption was also higher ($P < 0.001$) in lambs having free access to concentrate. Blood metabolites did not differ and were within normal range of variation among the three groups. Higher cell wall utilization was expected under the restricted concentrate feeding regimen, however, we observed poor cell wall utilization. Probably higher *Zizyphus nummularia* leaf intake caused a lower nitrogen pool in the rumen thus restricting rapid rumen microbial turnover. *Zizyphus nummularia* leaves are known for higher tannin content, while *Albizia lebback* leaves contain higher ash that also limits microbial growth and promotes a faster rate of passage. The tannins and ash contents of the roughage source might have regulated the rumen pH under high concentrate intake. Therefore, the nature of roughage feeding is detrimental under a high concentre feeding regimen for fat lamb production.

Fermentation of modified pectins in cultures of the colonic contents of pigs. M. Marounek[a,b], J. Čopíková[c], V. Skřivanová[a], A. Synytsya[c], L. Sihelníková[c] ([a] Research Institute of Animal Production, Prague 10, 104 01, Czech Republic; [b] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague 4, 14220, Czech Republic; [c] Department of Carbohydrate Chemistry and Technology, Institute of Chemical Technology, Prague 6, 166 28, Czech Republic).

Pectin is a well fermentable substrate which is degraded in the proximal colon. Its impact on colonic metabolism and health is thus limited. Chemical derivatization of pectin can decrease its availability for intestinal microorganisms. Modified pectins might be fermented in the whole colon. The following amidated pectins were prepared and tested in cultures of the colonic contents of pigs: N-butylpectinamid, N-hexylpectinamid, N-oktylpectinamid, N-dodecylpectinamid and N-octadecyl pectinamid. Pectinamides with low (6.2–9.1%) and high (24.3–40.5%) degrees of substitution were prepared. Substrates were added to the contents of the proximal colon of pigs and incubated under CO_2 at 39 °C for 24 h. The production of volatile fatty acids (VFA), gas and ammonia was measured. Highly methylated citrus pectin, used for the syntheses of amidated derivatives, served as a control. The production of VFA and gas in cultures with pectin was correlated ($r = 0.92$; $P < 10^{-3}$). Both production of VFA and gas were significantly lower in cultures with modified pectins than in control cultures. The length of the carbon chain of the alkyl substituents had no effect on the fermentability of pectins. The production of VFA and gas, however, was negatively correlated with the degree of substitution of modified pectins ($r = -0.93$ and -0.88, respectively; $P < 10^{-3}$). The degree of substitution around 20% was necessary to decrease the fermentation rate of pectin to one half. (This study was supported by the Czech Science Foundation: project No. 525/03/0358.)

Development of a molecular methodology for monitoring *Lactobacillus, Bifidobacterium* and *Bacteroides* populations from xylooligosaccharides fermentations. P. Moura, S. Carvalho, F. Simões, F. Gírio, M.P. Esteves (INETI, Department of Biotechnology, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal).

The aim of this work was to set up a molecular approach for application in xylooligosaccharide-containing (XOS) batch fermentations, in order to measure the XOS ability to stimulate the growth of specific bacterial groups from young pig intestinal microbiota. Anaerobic fermentation experiments were performed with crude XOS liquors obtained from autohydrolysis of brewery spent grain (BSG) and with XOS mixtures, ranging from 2 to 25 DP (degree of polymerization), obtained from BSG, corn cobs and *Eucalyptus* wood hydrolysates. The intestinal inocula were samples collected from the ileum, caecum and distal colon of a young pig belonging to the Alentejano breed and a Duroc × Landrace piglet. We used a semi-quantitative PCR detection method, involving the use of genus- and group-specific primers to monitor *Lactobacillus, Bifidobacterium* and *Bacteroides* populations. For the *Lactobacillus* populations we designed and tested the specificity of group-specific PCR primers targeting the 16S rDNA and 16-23S rDNA internal spacer region, whereas primers targeting the 16S rDNA of *Bifidobacterium* and *Bacteroides/Prevotella* genera were obtained from the literature. Specific PCR detection was performed with DNA extracted from cells collected at different fermentation times in order to assess the higher dilution still able to produce a positive PCR amplicon. Preliminary results show the fluctuation of bacterial populations depending on the characteristics of each feedstock, that we believe to be closely related to differences in the structural and DP features of XOS mixtures.

Effect of pH, redox potential and H_2 partial pressure on the stoichiometry of rumen fermentation in vitro. A. Offner, L.-P. Broudiscou, D. Sauvant (INRA, INA-PG, Paris, France).

Current models of rumen metabolism, generally controlled by kinetics principles, do not satisfactorily match in vivo observations for the prediction of fermentation end-product outputs. As part of the development of a mechanistic rumen model compliant with thermodynamic principles, an experiment was aimed at assessing the role of four thermodynamic factors – medium pH and redox potential, hydrogen partial pressure and kinetics of glucose supply (as the sole energy source) – in the stoichiometry of carbon

fluxes to short chain fatty acids and gases, in batch cultures of mixed rumen micro-organisms. Six-hour incubations were anaerobically carried out in 72 mL culture tubes inoculated with three fermentation broths taken from dual outflow continuous fermenters and differing in the nature of cereal (maize or wheat) and the proportion of fibre (25% vs. 50% DM) in the substrate. Prior to incubation, the headspaces were filled with adequate mixtures of carbon dioxide and hydrogen (varying from 0 to 1% molar proportion). The different levels of the four factors were combined in a 24-run fractional factorial design in order to estimate possible first and second order interactions in addition to main and quadratic effects. The pH, varying from 6.8 to 5.8, and the redox potential, within a range of variation of 50 mV, were the most determinant factors on the production of VFA and gas, specially hydrogen and valerate whatever the inoculum used. In contrast, a number of significant relations, e.g. on the production of propionate and methane, appeared to be more inoculum-dependent.

In vitro study of the rumen and hindgut fermentation of starch and cellulose. M.J. Ranilla[a], M.D. Carro[a], F.J. Giráldez[b], A.R. Mantecón[b] ([a] Departamento de Producción Animal I, Universidad de León, 24071 León, Spain; [b] EAE-CSIC. Apartado 788, 24080 León, Spain).

The ruminal and caecal contents from seven lambs fed a concentrate diet were used to study in vitro fermentation. Four hour incubations were performed without added substrate or with starch and cellulose. There were differences between ruminal and caecal fermentation for most of the studied parameters. Total VFA production and the Ac/Pr ratio were higher ($P < 0.001$) with caecal than with ruminal inocula in the absence of substrate (721 vs. 261 μmol and 3.65 vs. 2.64, for caecum and rumen, respectively). When starch was incubated with ruminal fluid, total VFA production almost doubled ($P < 0.001$) the one obtained with caecal contents (3048 vs. 1655 μmol, respectively). Fermentation pattern was also different for both inocula, with lower ($P < 0.001$) acetate and butyrate and higher ($P < 0.001$) propionate molar proportions with ruminal fluid when starch was incubated. Methane production was higher ($P < 0.05$) with ruminal than with caecal fluid (259 vs. 164 μmol, respectively) resulting in a higher ($P < 0.01$) hydrogen recovery. For cellulose, total VFA production and Ac/Pr ratio were higher ($P < 0.01$) in the caecal fermentation (697 vs. 320 μmol and 3.73 vs. 1.99, caecum vs. rumen). Molar proportions of the main VFA followed the same trend as with starch. Methane production was higher ($P < 0.01$) when cellulose was incubated with caecal contents (35.6 vs. 14.2 μmol, caecum vs. rumen), but hydrogen recovery was lower ($P < 0.01$) than that calculated for ruminal fluid. The results point to a high difference between rumen and hindgut fermentation when both inocula were used for in vitro incubations, showing that the first cannot serve as a model for the stoichiometry of the second.

Effect of synchronizing energy and nitrogen supply on rumen fermentation and microbial growth in the rumen simulating system Rusitec. M.L. Tejido[a], M.D. Carro[a], M.J. Ranilla[a], C.J. Newbold[b], S. López[a] ([a] Dept. Producción Animal I, Universidad de León, 24071 León, Spain; [b] The Institute of Rural Studies, University of Wales, Aberystwyth, Ceredigion, SY23 3AL, UK).

Although synchronizing energy and nitrogen availability in the rumen has been proposed as a mechanism to increase bacterial growth rates and efficiency of nutrient utilization, the reported results are not concluding. The aim of this study was to investigate the effect of the pattern of energy and nitrogen supply (synchronous –SYN– vs. asynchronous –ASYN–) on ruminal fermentation and microbial growth in the rumen simulating Rusitec system. Eight fermenters were fed daily 16 g DM of rye-grass and ^{15}N was used as a microbial marker. In the SYN treatment, each fermenter received 0.15 g of NH_4Cl and 1.7 g of maltose infused over the 6 h immediately after feeding. In the four fermenters allocated to the ASYN treatment, the NH_4Cl was infused over the 6 h immediately after feeding and the maltose from 6 to 24 h after feeding. Synchronizing energy and nitrogen supply did not significantly affect ($P > 0.05$) volatile fatty acid (47.5 vs. 46.7 mmol·day^{-1} for SYN and ASYN, respectively) or methane (12.8 vs. 12.6 mmol·day^{-1}) production. Ammonia concentration was over 1.7 mg·L^{-1} in all fermenters, and during the first

6 h after feeding was higher ($P < 0.05$) for SYN than for ASYN. Apparent DM disappearance was unaffected ($P > 0.05$) by the treatment (0.640 vs. 0.633 for SYN and ASYN, respectively). Microbial growth was slightly increased in ASYN fermenters (approximately 10% greater than in SYN), but differences between treatments were not significant ($P > 0.05$). These results indicate that under the conditions of the present experiment the synchrony of energy and nitrogen release has only subtle effects on ruminal fermentation.

Postprandial evolution of the microbial community and biochemical composition of stomach contents in equines. M. Varloud[a], E. Jacotot[b], G. Fonty[c], A. Guyonvarch[a], V. Julliand[b] ([a] EVIALIS, 56 250 Saint-Nolff, France; [b] ENESAD, 21 079 Dijon, France; [c] INRA, 63122 Saint-Genès-Champanelle, France).

Our knowledge of the microflora inhabiting the horse stomach is still very limited although some data suggest its important role in this organ. The objective of our work was to investigate the evolution of both microbial and biochemical profiles in this compartment of horse gastro-intestinal tract. Total anaerobic bacteria (TAB), lactate-utilizing bacteria (LUB), lactobacilli (L) and streptococci (S) were numbered with classical cultural methods and biochemical parameters (pH, concentrations of D- and L-lactate, ammonia (NH_3) and volatile fatty acids (VFA)) were measured in chyme (235 mL) collected on four horses by naso-gastric tubing before the meal, 1:00, 2:00 and 3:30 h after. During the first postprandial hour TAB, L, and S populations increased from 5.8×10^5 to 1.7×10^7 CFU·mL^{-1}, from 1.2×10^5 to 2.6×10^6 and from 1.1×10^5 to 1.6×10^6 respectively. Between 1:00 and 3:30 after the meal, TAB, L, S and LUB populations increased up to 3.7×10^8, 3.7×10^7, 1.4×10^7 and 7.7×10^4, respectively. Lactate concentration increased from 0.09 to 0.17, 0.26, 0.72 g·L^{-1}, 1:00, 2:00, 3:30 after the meal, respectively. This increase was mostly due to L-lactate accumulation. During the 3:30 h following the meal, total VFA and NH3 concentration increased from 0.13 to 0.42 g·L^{-1} and from 1.83 to 3.51 mmol·L^{-1} respectively. Acetate represented 60% of the total VFA concentration increase. These in vivo data which are in agreement with preliminary results obtained postmortem provides complementary information on the postprandial microbial and biochemical kinetics in the horse stomach and confirms the abundant microbial colonization of the horse stomach and the originality of the equine digestion. The role of some major stomacal microbial communities is currently under determination in our laboratory.

Microbial activity in the gastrointestinal tract of the horse. C. Walsh[a], M. Moore-Colyer[a], A. Longland[b], C.J. Newbold[a] ([a] Institute of Rural Science, University of Wales, Aberystwyth, UK; [b] Institute of Grassland and Environmental Research, Aberystwyth, UK).

Despite the fact that microbial degradation of plant materials can fulfil 75% of the energy requirements of the horse, there are relatively few studies into the microbial activity along the digestive tract of equines. Digesta samples from three freshly slaughtered grass fed Welsh mountain ponies were taken from 9 sites: stomach, small intestine, caecum, left and right ventral colon, left and right dorsal colon, rectal faeces and external faeces. These were then used as inocula when measuring gas production over 72 h from sugar beet pulp. Rates of gas production were fitted to the model $p = a + b\ (1 - e^{-ct})$, where p = volume of gas after time t; a = the intercept of gas volume curve at t = 0; b = volume of gas produced at the asymptote; c = rate constant of gas production (h^{-1}). There was no difference in the total potential gas production (a + b) between sites (110, 87, 100, 99, 82, 99, 89, 142 and 97 mL respectively SED 20.1), however, the rate of gas production (c) increased from the stomach, through the small intestine and caecum to the colon and remained constant thereafter (0.010, 0.040, 0.054, 0.072, 0.084, 0.075, 0.081, 0.078 and 0.070 h^{-1} respectively, SED 0.0216). The results of this experiment indicate that it may be feasible to use faeces as an inoculum for gas production studies when studying microbial activity in the colon.

In vitro culture and digestion properties of the rumen ciliate *Eremoplastron dilobum*. M. Banach, R. Miltko, K. Wereszka, G. Belzecki,

A. Kasperowicz, T. Michałowski (The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland).

The in vitro cultivation of rumen ciliates is a method to study their biology as well as the method to get the appropriate material for enzymatic experiments. In the performed study we found that ciliates were able to grow for a minimum of 30 d in media with different chemical compositions. The doubling time of the population cultured in the most preferable medium was, however, longer than 24 h. The ciliates were able to survive in the chemically defined medium supplemented with hay (0.3 mg/mL/d). Wheat gluten added to the medium at the rate of 0.08 mg/mL/d did not improve the growth conditions of the ciliates. The increase in their number was observed when cellulose, and starch were added to the culture salt solution. Microcrystalline cellulose increased the population density of the ciliates but no relation between the supplemented dose of this polysaccharide and ciliate number was observed. The relationship between three xylan doses and ciliate number was negative. Crude enzyme preparation obtained from protozoa free of the intracellular bacteria was able to degrade crystalline cellulose, CMC, xylan and starch, while the rates of their degradation were 0.26, 37.7, 420 and 123.5 μM released reducing sugars or glucose/mg protein/h. Zymograms prepared following the electrophoretic separation of protozoal protein using polyacrylamide gel revealed four enzymes exhibiting features of CMC-ase, two bands active against starch and three enzymes catalyzing hydrolysis of birch wood xylan. (This study was supported by EU "CIMES" grant QLK3-2002-02151).

The characterization of amylolitic activity of the rumen ciliate *Eudiplodinium maggii.* G. Bełżecki[a], T. Michałowski[a], F.M. McIntosh[b], N.R. McEwan[b], C.J. Newbold[c] ([a] The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland; [b] Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [c] The Institute of Rural Science University of Wales, Aberystwyth, Ceredigion SY23 3AL, UK).

Dietary starch is digested by microorganisms inhabiting the rumen but amylolytic properties of many species of protozoa are not well known in detail. This study was carried out to search the ability of one of the most common large ciliate species *Eudiplodinium maggii* to digest and metabolize starch in the rumen. We found that the release of reducing sugars from barley starch and dextrin following their incubation with crude enzyme preparation obtained from bacteria free cells of *Eudiplodinium maggii* was equal to 29.5 and 19.4 μM glucose/mg protein/h, respectively. The release of glucose from maltose and isomaltose was of only 0.45 and 0.14 μM/mg protein/h, respectively. Four protein bands able to degrade starch were identified following native polyacrylamide gel electrophoresis of crude enzyme preparation. Maltose followed by glucose and most often maltotriose were the end products of starch hydrolysis catalyzed by the enzymes isolated from the gel. Similar end products were found when these enzymes were replaced with crude enzyme preparation. Rapid hydrolysis of starch and a very low degradation rate of maltose and isomaltose, as well as an end product of starch hydrolysis suggest that depolymerase type enzymes, like α-amylases, were responsible for the intensive degradation of this polysaccharide. A verification of a *Eudiplodinium maggii* cDNA library resulted in the identification of two gene fragments encoding α-amylases. Based on the identified fragments, the sequence of the bases in complete cDNA was identified. The lengths of the complete cDNA encoding the two different α-amylases in *Eudiplodinum maggii* were 1626 and 1519 bp, respectively.

Microbial interactions between cellulolytic species isolated from the human gut. C. Chassard, B. Gaillard-Martinie, A. Bernalier-Donadille (Unité de Microbiologie, INRA, C.R. de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

The cellulolytic microflora involved in plant cell wall degradation in the human gut was previously shown to be composed of new species belonging to different bacterial genus comprising *Ruminococcus*, *Enterococcus* and *Bacteroides*. The physiological and metabolic characteristics of these cellulolytic strains varied

greatly according to the species considered. While *Ruminococcus* sp. and *Enterococcus* sp. degraded and fermented crystalline cellulose with a production of large amounts of H_2, *Bacteroides* sp. hydrolysed more amorphous celluloses and did not form fermentative H_2. Since fibre fermentation in the human gut generates large amounts of gases, in particular H_2, that are responsible for digestive troubles (flatus, abdominal pain and distension…), the presence of non-H_2-producing fibrolytic species is of particular interest. Nutritional strategies favouring the predominance of such species instead of the H_2-producing ones could indeed allow to maintain fibre consumption while limiting digestive discomfort associated with gas production. In this context, interactions between H_2-producing (*Ruminococcus* sp.) and non-H_2-producing (*Bacteroides* sp.) cellulolytic strains were investigated in vitro, using Avicel® cellulose as the sole energy source. A synergism between the two species was observed in the coculture, cellulose degradation and bacterial biomass being largely increased compared to monocultures. FISH analysis of the coculture population showed that *Bacteroides* sp. predominated *Ruminococcus* sp. This could explain the large decrease of H_2 production observed in the coculture. This preliminary work demonstrated that *Bacteroides* sp. could predominate in the ecological niche and allowed efficient degradation of cellulose with limited H_2 production. The factors that could influence these interactions between cellulolytic species are currently under study in our laboratory.

Chitinolytic activity of the anaerobic polycentric rumen fungus *Anaeromyces mucronatus*. K. Fliegerová, B. Hodrová, H. Bartoňová, L. Štrosová (Institute of Animal Physiology and Genetics, Academy of Sciences of Czech Republic, Vídeňská 1083, Prague 4, 10400, Czech Republic).

Chitin occurs in the cell wall of the thallus in all genera of rumen zoosporic fungi. Chemically, it is a homopolymer of β-1, 4-linked N-acetylglucosamine units with a three-dimensional α-helical configuration. The degradation of chitin is performed by a chitinolytic system consisting of endochitinase, exochitinase and N-acetylglucosaminidase. In chitin-containing microorganisms, like anaerobic fungi, the role of chitinases is autolytic and morphogenetic. Until now, chitinolytic activities of anaerobic fungi have been studied in the monocentric genus *Piromyces communis,* only. Cytosolic chitinase of this fungus as well as extracellular chitinase were purified and characterized. The objective of this study was to investigate the occurrence of chitinases in polycentric fungus *Anaeromyces mucronatus* BF1 isolated from the faeces of the European bison. The isolate was cultivated in rumen fluid containing medium M 10 with glucose ($3\ g \cdot L^{-1}$) as the carbon source for 10 days. Its chitinolytic activities in extracellular, cell wall and cell extract fractions were determined. N-acetyl-β-glucosaminidase activity was not detected in any of the fractions tested. Chitinases exerting endo type activity showed a broad optimum temperature at 20–40 °C. The optimum pH was at pH 6.5. The cell wall associated chitinase activity was nearly twice lower than the corresponding activity in the cell extract and extracellular fraction. The secretion of chitinases increased during 48 h of growth and then reached a plateau up to 96 h. After the exhaustion of the growth substrate the production of chitinases, implicated in the autolysis of the fungal cell wall, reached a maximum at 8 days.

Why some strains of rumen treponemes exhibit a limited ability to utilize inulin for growth? A. Kasperowicz, T. Michałowski (The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland).

Three strains of rumen treponemes were investigated on the ability to grow on two different fructose polymers i.e. the Timothy grass fructan and inulin. The specific growth rate of bacteria and the amount of the utilized source of carbon from the medium were measured. The specific growth rate on the Timothy grass fructan and inulin ranged from 0.25 ± 0.068 to 0.41 ± 0.085 and from 0.18 ± 0.031 to 0.35 ± 0.092, respectively, while the optical density of the bacteria cultures growing on the Timothy fructan was about two times higher than on inulin. The Timothy grass fructan added to the medium was utilized up to 82–94%, while inulin was only utilized up to 30–45%. The initial concentration was 0.5%. No improvement in inulin utilization

was observed following the increase in its concentration to 1%. Thin layer chromatography of the post-culture fluid show that bacteria utilized only inulooligosaccharide molecules containing 3 to 5 fructose residues. The worse growth of the bacteria on inulin than on Timothy grass fructan seems to be the result of its lack of ability to synthesize the fructanolytic enzyme degrading inulin to inulooligosaccharides which then could be utilized by the bacteria. Such an enzyme degrading the Timothy grass fructan to fructooligosaccharides was associated with the bacterial cell wall. We concluded that all 3 strains of treponemes were able to survive on inulin due to the utilization of inulooligosaccharides which are present in a commercial product. (This study was supported by EU grant QLK3-2002-02151.)

Cloning, expression and characterisation of a glycosyl hydrolase from the rumen ciliate *Diploplastron affine*. F.M. McIntosh[a], E. Devillard[a], R.J. Wallace[a], N.R. McEwan[a], T. Michałowski[c], C.J. Newbold[b] ([a] Rowett Research Institute, Aberdeen, Scotland, UK; [b] The Institute of Rural Studies, University of Wales, Aberystwyth, Wales, UK; [c] Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland).

Bacterial breakdown in the rumen is a nutritionally wasteful process leading to inefficient nitrogen utilisation by the host. Ciliate protozoa have been shown to contribute to this loss by engulfing and subsequently digesting rumen bacteria. Little is know about the enzymes within the protozoa that are responsible for bacterial digestion. Here we report the isolation of a gene encoding a lysozyme-like enzyme, GHG11, from the rumen ciliate *Diploplastron affine.* We also give the results of the preliminary characterisation of the gene product expressed in *E. coli.* The gene has an open reading frame of 666 bp encoding a 222 amino acid protein with a theoretical molecular weight of 25 kDa. Molecular analysis shows that GHG11 carries a single catalytic domain similar to the glycosyl hydrolase family 25 (PFAM PF01183), comprising enzymes with only one known activity, lysozyme. It has no similarity to lysozyme proteins from other rumen ciliates such as *Entodinium caudatum.* Expression of the gene was confirmed by Western blotting and by the observation of a clear zone after incubation of a sonicated cell extract at 39 °C in a well cored into agar containing *Micrococcus lysodeikticus* cell walls. The supernatant of the sonicated cell extract was used for further enzyme characterisation. Optimum pH was determined in McIlvaine buffer from pH 3 to 8 and was found to be optimally active at acidic pH. Optimum temperature for activity was determined between 4 and 60 °C and was found to be approximately 39 °C. This project was supported by EU infrastructure grant QLRI-CT-2000-01455: www.ercule.com.

Calcium and magnesium requirements for growth of cellulolytic ruminal bacteria. M.S. Morales-Silva, B.A. Dehority (Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA).

Other than the study by Bryant et al. in 1959, working with *Fibrobacter succinogenes* S85, little information is available on the calcium (Ca) and magnesium (Mg) requirements of the predominant rumen cellulolytic bacteria. We investigated the requirements of *Fibrobacter succinogenes,* strains A3c and S85; *Ruminococcus albus,* strains 7 and 8; and *Ruminococcus flavefaciens,* strains B34b and C94, for these two minerals. Bacteria were grown in slants and transferred to a complete media containing one-tenth the normal concentration of either Ca or Mg. After incubation overnight, the cultures were transferred to either a Ca- or Mg-free medium and grown to an optical density (OD) between 0.6 and 0.7. Inoculum was prepared by diluting the culture to an OD of 0.1 with divalent cation-free medium, and 0.1 mL. was used to inoculate each tube. Growth was monitored by OD (660nm). Data were fitted mathematically to determine growth rate as well as concentrations required for minimum and maximum growth. As Ca concentrations increased, only *F. succinogenes* S85 and A3c and *R. albus 8* responded with an increase in both maximum growth and growth rate, whereas maximum growth and growth rate decreased for *R. flavefaciens* C94. The other cellulolytic strains were not affected. Rate of growth appeared to increase with Mg concentration for all strains. Both *R. flavefaciens* strains had an absolute requirement for Mg and growth of the remaining strains was reduced in its absence. Maximum growth of all strains

was observed with Mg concentrations ranging between 2.5 and 10 $\mu g \cdot mL^{-1}$.

The *Ruminococcus albus pil/sec* locus: expression and putative role of two adjacent *pil* genes in pilus formation and bacterial adhesion to cellulose. H. Rakotoarivonina[a], M.A. Larson[b], M. Morrison[c], J.-P. Girardeau[a], B. Gaillard-Martinie[a], E. Forano[a], P. Mosoni[a] ([a] Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; [b] Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, 68198, USA; [c] The MAPLE Research Program, Department of Animal Sciences, The Ohio State University, 2027 Coffey Road, Columbus, OH 43210, USA).

Ruminococcus albus produces fimbrial-like structures that are involved with the bacterium's adhesion to cellulose. The subunit protein has been identified in strain 8 (CbpC) and strain 20 (GP25) and both are type 4 fimbrial (Pil) proteins. We report here the presence of a *pil/sec* locus that is organized similarly in both strains and our initial examination of a second Pil-protein. Downstream of the *cbpC/gp25* gene (hereafter referred to as *pilA1)*, is a second pilin gene *(pilA2)* and two *sec* genes, *secD* and *secF*. Real-time PCR was used to measure *pilA1* and *pilA2* transcript abundance in *R. albus* 20, and its adhesion-defective mutant D5. The relative expression of *pilA1* in the wild-type strain was 73-fold higher than that of *pilA2* following growth with cellobiose. There were no measurable differences between the wild-type and mutant strain in terms of *pilA1* and *pilA2* transcript abundance, indicating that *pilA1* and *pilA2* transcription is not affected in the mutant strain. Western immunoblots showed that PilA2 is localized in the cytoplasmic and membrane fractions of strain 20, and the anti-PilA2 antiserum does not inhibit bacterial adhesion to cellulose. We postulate that the PilA2 protein plays a role in the synthesis and assembly of type 4 fimbrial-like structures by *R. albus,* but its role is restricted to cell associated functions, rather than as part of the externalized fimbrial structure.

Characterization of two glycoside hydrolases, Cel48B and Cel9C from the rumen cellulolytic bacterium *Ruminococcus albus* 20 and their putative role in bacterial adhesion to cellulose. H. Rakotoarivonina[a], C. Béra-Maillet[a], C. Chambon[b], E. Forano[a], P. Mosoni[a] ([a] Unité de Microbiologie; [b] Plateforme Protéomique, INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

This study was aimed at characterizing two major cellulose-binding proteins (CBP) called CBP1 (115 kDa) and CBP2 (90 kDa) previously identified in the Gram positive cellulolytic bacterium *Ruminococcus albus* 20 and at determining if these two CBP may be involved in bacterial adhesion to cellulose. SDS-PAGE analyses of protein fractions of *R. albus* 20 showed that CBP1 and CBP2 are cell-associated proteins and that their synthesis is stimulated in the presence of cellulose. When *R. albus* 20 and its adhesion-defective mutant were grown on cellulose, the mutant produced lower amounts of both CBP than the parent. Western blots performed with the anti-Adh serum – anti-*R. albus* 20 serum adsorbed with the mutant – showed that both CBP were recognized though CBP2 was better probed than CBP1. Proteomic analysis associated to immunodetection with the anti-Adh serum and mass spectrometry were used to characterize CBP1 and CBP2 and identify the corresponding genes. CBP1 and CBP2 were identified as multidomain family 9 and family 48 glycoside hydrolases called Cel9C and Cel48B, respectively. They present the same modular structure as Cel9B and Cel48A identified in *R. albus* 8 and differ from their clostridial cellulosomal homologs by the lack of a dockerin domain. The fact that these newly identified cellulases of *R. albus* 20 (1) are underproduced by the mutant (2) are cell-associated and (3) are major CBP, tend to show that both proteins are involved in adhesion. Additional studies are required to determine if they are components of a multi-enzyme complex or not.

Optimization of bacterial RNA isolation from the rumen content of conventional sheep. Y. Ribot, C. Béra-Maillet, E. Forano (Unité de Microbiologie, INRA, CR de Clermont-Ferrand/Theix, 63122 St-Genès-Champanelle, France).

Fibrolytic bacteria play a major role in the rumen ecosystem of herbivores. To understand their

function in plant cell wall degradation, it is interesting to quantify in vivo mRNA encoding specific enzymatic activities. Isolation of total microbial RNA from rumen contents is the first step to quantify bacterial gene expression. However, total RNA extracted from rumen contents of conventional sheep may contain, in addition to bacterial mRNA, RNA coming from plant cell walls and eucaryotic cells. This RNA dilution constitutes a limitation to the detection and quantification of bacterial specific transcripts. The aim of this study was to optimize total RNA extraction from rumen contents of conventional sheep, in order to obtain significant and representative amounts of non degraded bacterial mRNA that could be quantified by real-time RT-PCR. Rumen samples (half solid and half liquid phases) were collected from conventional adult Texel sheep six h after feeding with natural grassland hay. RNA protect Bacteria Reagent (QIAGEN) was immediately added to the samples that were grinded using a Polytron apparatus and stored at –80 °C. RNA extraction was performed using a RNAzol (guanidine thiocyanate + phenol + sarkosyl NL) procedure. Several steps in RNA extraction were optimized like cell lysis (bead beating, thermic shock), RNA extraction (RNAzol quantity), and RNA recovery (presence of salt). Total RNA were quantified using RNA macroarrays (Agilent technologies) in order to determine the best extraction procedure.

The chitinases of human gut bacterium *Clostridium paraputrificum* J4: activity screening and enzymes purification. J. Šimunek[a], G. Tishchenko[b], H. Bartoňová[a], B. Hodrová[a], J. Kopečný[a], K. Rozhetsky[c] ([a] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Vídeňská 1083, 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; [c] Polygran Ltd., 99 Hahistadrut Ave, Mifraz Haifa, Israel).

The strain J4 was isolated from human feces and utilized chitin and N-acetyl-D-glucosamine (GlcNAc). According to its morphology, physiological characteristics, API tests and 16S rRNA sequence analysis, the taxonomic location of the isolate was determined as *Clostridium paraputrificum*. Cultivation of the isolate with colloidal chitin resulted mainly in the production of acetate, butyrate, formate and ethanol. In response to various types of chitin used as growth substrates, the bacterium produced a broad spectrum 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 chitin degradability was estimated (colloidal > squid pen > crab shell > fungal chitin). The temperature optimum of chitinase activity was determined for an interval of 20 °C to 50 °C. The pH-profile of chitinase activity showed a broad maximum from pH 4.0 to pH 6.0. The chitinase variability was confirmed on zymograms of redenatured SDS-PAGE, where the extracellular chitinolytic complex was visible as 6 isoenzymes with molar mass 44–96 kDa. The chitinolytic enzymes of *C. 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. At pH close to neutral values, the peaks of highly purified isoforms of exo- and endochitinases were isolated. The protein and enzyme recovery reached 90%.

Expression of cellulase and xylanase genes from anaerobes in *Streptococcus bovis* and *Clostridium paraputrificum*. H. Taguchi[a], T. Kikuta[a], K. Morimoto[b], T. Kimura, K. Sakka, K. Ohmiya ([a] Faculty of Bioresources, Mie University, Kamihama-cho 1515, Tsu 514-8507, Japan; [b] Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795, Japan).

The effective expression of recombinant DNA encoding plant fiber-degrading enzymes in rumen bacteria will contribute to the efficiency of fibrous feed digestion in the rumen. Based on an idea, a plasmid isolated from a starch-degrading rumen bacterium *Streptococcus bovis* no.8 was modified as a vector and used to express *R. albus* endoglucanases (EgI and EgVII) in *S. bovis* no.8. The no.8 was transformed with plasmids, pKASE1 and pEldht7, by an electrotransformation method. The resulted transformant harboring pKASE1 revealed a single band of

EgI by zymogram and western blot analyses. Transformation efficiency was around 10^{-3}. Activity staining revealed two EgVII bands in the intracellular fraction of transformants harboring pEldht7. EgVII activity was about 30 times higher than that of EgI in the transformants. When grown on culture containing antibiotics, the *S. bovis* transformant expressing EgVII exhibited a relatively higher growth rate than the wild strain. *Clostridium paraputrificum* M-21 produces hydrogen gas from a carbon source, chitin but not cellulosic materials. The genes of *Clostridium thermocellum xynA* cth and *Clostridium stercorarium xynA* cst were expressed in M-21 by using a modified shuttle vector (pJIR751-hydpro) between *Clostridium perfringens-Escherichia coli*, having the *C. paraputrificum* hydrogenase gene (*hyd*) promoter region (*pro*). Active bands with molecular weights smaller than intact XynAs reacted with antibody of XynA cst, suggesting digestion of foreign proteins by host proteases. *R. albus* endoglucanases were also expressed in M-21 with the vector. Both transformants harboring *egI* and *t-egI* produced respective endoglucanase. The 80% activity of the latter was excreted from the cells without degradation by host proteases.

Xylanolytic activity of the rumen protozoan *Diploplastron affine*. K. Wereszka[a], T. Michałowski[a], C.J. Newbold[b], N.R. McEwan[c], F.M. McIntosh[c] ([a] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jablonna, Poland; [b] The Institute of Rural Science University of Wales, Aberystwyth, Ceredigion SY23 3AL, Wales, UK; [c] Rowett Research Institute, Bucksburn Aberdeen AB21 9SB, Scotland, UK).

Rumen protozoa contribute to fiber digestion but the ability of individual species to degrade cellulose and hemicellulose material is poorly understood. The objective of this study was to examine if the ciliate *Diploplastron affine* possesses a xylanolytic activity and if this activity is of protozoal origin. We found that crude enzyme preparation of bacteria-free ciliates released reducing sugars from birch wood xylan at the rate of 118 µM glucose/mg protein/h, and pH 5.0 was the optimum for this reaction. Two peaks exhibiting xylanolytic activity ware found following ion exchange chromatography of protozoal proteins on DEAE sephadex A 50 bed. A zymogram prepared after electrophoretic separation of native protozoal protein on a polyacrylamide gel revealed three bands which were able to degrade fructan. Enzymes isolated from the gel were analysed to determine their products after xylan hydrolysis. Screening of a *Diploplastron affine* cDNA library resulted in the identification of a gene encoding a xylanase enzyme. The gene was sequenced and the length of the complete insert was 1710 bp, encoding a derived protein of 529 residues. We concluded that the ciliate *Diploplastron affine* is able to synthesize a xylanolytic enzyme and to use it for xylan degradation. (This study was partially supported by EU "CIMES" grant QLK3-2002-02151 and the first author was the recipient of a Marie Curie program fellowship at the Rowett Research Institute.)

Cellulose metabolism in *Fibrobacter succinogenes* S85: Maltodextrin, maltodextrin-1P and cellodextrin synthesis. R. Nouaille[a,b], M. Matulova[a,c], A.-M. Delort[a], E. Forano[b] ([a] Laboratoire de Synthèse et Étude de Systèmes à Intérêt Biologique, UMR 6504 Université Blaise Pascal-CNRS, 63170 Aubière, France; [b] Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; [c] Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 842 38 Bratislava, Slovak Republik).

In a previous work, we showed that *Fibrobacter succinogenes* S85, a cellulolytic rumen bacterium, was able to synthesize and release oligosaccharides identified by 2D-NMR techniques as maltodextrins and maltodextrin-1-phosphate when it was incubated with glucose. We analyzed in detail maltodextrin metabolism in *Fibrobacter succinogenes* S85, and showed that, although this bacterium is not able to use starch or maltose, it possesses a "maltose-like system". These results were striking and prompted us to investigate whether this system functions in bacteria metabolizing their natural substrate, cellulose. Incubations were also carried out with cellobiose which is the main product of cellulolysis, and with a mixture of glucose and cellobiose. Cell extracts and extracellular media were analysed by 2D NMR spectroscopy and thin layer chromatography. In parallel we looked for

the presence of cellodextrins in these various incubations since it is assumed that cellulose degradation by rumen cellulolytic bacteria, including *F. succinogenes* S85, leads to the release of cellodextrins.The first important result of this work is that maltodextrins and maltodextrin-1-phosphate were detected in all the incubations. These results indicate that the "maltose-like" system of *F. succinogenes* S85 is operating whatever the substrate used including polysaccharides. Also we found that a new metabolite (named X), corresponding to a phosphorylated glucose derivative, was produced in the extracellular medium when cells were incubated with cellulose. The second finding is that no cellodextrins are accumulating in extracellular media of cells whatever the substrate, and particularly cellulose, on the contrary to what is generally admitted in the literature.

Ruminal bacteria on an Atkins diet: metabolic properties of *Eubacterium pyruvativorans*. R.J. Wallace, L.C. Chaudhary, N. McKain, N.D. Walker (Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK).

Eubacterium pyruvativorans I-6^T is a non-saccharolytic, ammonia-producing anaerobic bacterium which was isolated as a species involved in amino acid breakdown in the rumen. This study investigates its metabolic properties. It emerges that *E. pyruvativorans* uses protein hydrolysate and fatty acids, but not carbohydrates, to grow. It grew on pancreatic casein hydrolysate (PCH) as the sole C source. No single amino acid supported growth, and peptides were required for growth on free amino acids. Alanine, followed by leucine, serine and proline were used most extensively during growth. Growth on PCH was increased by the addition of amino acids and ammonia, and also by acetate, propionate and butyrate. Growth on pyruvate was stimulated by the former but not the latter additions. Propionate and butyrate concentrations declined during growth, and valerate and caproate concentrations increased. Labelling experiments suggested a metabolic pattern where two C atoms of butyrate, valerate and caproate were derived from amino acids, with the others being formed from acetate, propionate and butyrate. The metabolic strategy of *E. pyruvativorans* in disposing of reducing equivalents therefore resembles that of *Clostridium kluyveri*, which ferments ethanol using acetate, propionate or butyrate, but differs in the substrates it can use to form pyruvate, the precursor of acetyl CoA. Also like *C. kluyveri*, *E. pyruvativorans* grew on vinylacetate, and, to a lesser extent, lactate and crotonate, but the metabolic significance of these properties is likely to be minor.

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Gene expression of *Lactobacillus plantarum* in the human gastro-intestinal tract. M.C. de Vries[a,b], E.E. Vaughan[a,b], M. Kleerebezem[a], W.M. de Vos[a,b] ([a] Wageningen Centre for Food Sciences, Wageningen, The Netherlands; [b] Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands).

The human gastro-intestinal tract represents a dynamic ecosystem harbouring a great variety of micro-organisms. These commensal bacteria amongst others improve colony resistance against pathogens, modulate the activity of the host immune system and contribute to host nutrition. To study the functionality of one of these micro-organisms and their interaction with the host we determined their in vivo messenger-RNA production. We chose *Lactobacillus plantarum* WCFS1 as a model micro-organism that is of human origin, shows considerable survival following oral consumption, and whose complete genome sequence has been determined. Prior to surgery, potential colon cancer patients ingested for one week a product containing *Lactobacillus plantarum* 299v, a probiotic bacterium highly related to *L. plantarum* WCFS1. Total RNA was isolated from the mucosa of the biopsy material and was hybridised to a DNA-array comprising clones covering the *L. plantarum* WCFS1 genome. Different concentrations of RNA were tested to rule out concentration specific effects. Mucosal cells depleted of bacteria, as well as other *Lactobacillus* species were hybridised to confirm the specificity of the hybridisation. A comparison of the gene expression of *L. plantarum* 299v in the large intestine of different persons and the gene expression in the small and large intestine of one person shows metabolic functions and sugar degradation to occur in all samples. This is the first report of the global gene expression analysis in vivo of a commensal or ingested bacterium in association with the human intestinal mucosa.

Dietary induced changes in *E. coli* populations and prevalence of STEC virulence genes in the faeces of cattle. R.A. Gilbert[a], N. Tomkins[b], J. Padmanabha[a], K.A. Bettelheim[c], D.O. Krause[a], C.S. McSweeney[a] ([a] CSIRO Livestock Industries, Queensland Bioscience Precinct, Brisbane, Qld 4067; Cooperative Research Centre for Cattle and Beef Quality, Armidale, NSW 2351, Australia; [b] CSIRO Livestock Industries, Rendel Laboratory, Rockhampton, Qld 4702, Australia; [c] Microbiology Diagnostic Unit, Department of Microbiology and Immunology, University of Melbourne, Parkville, Vic 3010, Australia).

The effect of dietary inclusion of molasses (simple sugars), grain (starch) and roughage (structural carbohydrate) on the shedding of *Escherichia coli* and Enterohaemorrhagic *E. coli* (EHEC) virulence factors [shiga toxin genes, stx_1 and stx_2, accessory virulence factors, intimin (*eaeA*) and plasmid-encoded enterohemolysin (*hlyA*)] in cattle faeces was investigated. In an animal trial, thirty Brahman cross steers (mean LW ± sem) 329 ± 3.2 kg were initially fed a high grain (80%) diet. The cattle were then allocated into 3 groups and fed a high grain (G), Rhodes grass (R), or Rhodes grass + molasses diet (R + M) *ad lib*. Faecal samples were collected from all animals and biochemical and microbiological parameters were determined. Volatile fatty acid patterns were similar in the R and R + M diets whereas increased *E. coli* numbers, decreased pH and enhanced butyrate and lactate fermentation pathways were associated with the grain diet. Analysis of the concentration of EHEC virulence factors in faeces indicated a marked decrease in *hlyA*, *eaeA* and stx_1 genes in the R and R + M diets, this trend remaining at lairage. Cluster analysis of predominant *E. coli* serotypes isolated from faeces from each of the three dietary treatment groups showed that the R and R + M groups were similar, but quite distinctive from populations isolated from grain fed animals. This study indicates that the type of dietary carbohydrate has a significant effect on the size of the *E. coli* population and therefore may determine the level of pathogenic serotypes.

A metaproteomic approach to study proteins involved in the interaction between commensal bifidobacteria and the human infant intestinal tract. E.S. Klaassens, W.M. de Vos, E.E. Vaughan (Wageningen University and Research Centre, Laboratory of Microbiology, Hesseling van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands).

The human gastrointestinal tract is more densely populated with microorganisms than any other organ and is a site where the microflora may have a pronounced impact on our physiology. In the large intestine, bifidobacteria species are dominant during the entire lifespan of almost every human. The acquisition of the normal microbiota starts at birth when the germfree newborn comes in contact with the environment. Bifidobacteria are especially predominant in infants where they can comprise 40 to 90% of the microbiota in human milk-fed babies. Many beneficial effects have been claimed for bifidobacteria, including protection against pathogens, normal development of the immune system and positive nutritional effects for the intestinal cells and the host. However, detailed insight in the activity and function of bifidobacteria in these processes is not currently available. The aim of this study was to use proteomics to study the interaction of bifidobacteria within the human intestin in order to get a better understanding of how these bacteria influence gut health. Optimisation of proteomic techniques for pure culture bifidobacteria species and bacteria in infant faecal samples was first performed. PCR for the total bacterial community and the bifidobacterial population and denaturing gradient gel electrophoresis demonstrated that bifidobacterial species were predominant in the infant faecal samples. Subsequently the maps generated by two dimensional gel electrophoresis used to visualise the total expressed metaproteome in infant faeces over time, are presented.

Differences in the expression of heat shock protein 70 and IL-8 after exposure of crypt-like and villus-like Caco-2 cells to Lactobacilli strains, their fermentation products, butyrate or *Salmonella enteritidis* 857. S. Fajdiga[a], J.F.J.G. Koninkx[b], J.J. Malago[c], P.C.J. Tooten[b], B.B. Matijašič[a], R.M. Logar[a] ([a] Zootechnical Department, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia; [b] Department of Pathobiology, Division of Pathology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, PO Box 80.158, 3508 TD Utrecht, The Netherlands; [c] Sokoine University of Agriculture, PO Box 3203, Chuo Kikuu, Morogoro, Tanzania).

The enterocytes of the intestinal epithelium are regularly exposed to substances of dietary origin, which are potentially beneficial (lactic acid bacteria) or potentially harmful (lectins, pathogenic bacteria). The beneficial bacterial microflora in the intestine is believed to promote (improve) health. These bacteria produce acids (SCFA, lactic acid) through intestinal fermentation and some antimicrobial substances (hydrogen peroxide, diacetyl, bacteriocins). They stimulate the immune system and compete with pathogens for nutrients or adhesion receptors on the epithelium. In contrast, pathogenic bacteria produce toxins and induce high levels of inflammatory cytokines, which may subsequently cause damage to the intestinal epithelium. The expression of heat shock proteins (Hsps) and cytokines is part of a protective mechanism developed by the intestinal cells to deal with bacteria in the intestinal lumen. Hsps are known to protect the intestinal cells against infection and inflammation, whereas the secretion of cytokine IL-8 leads to neutrophil infiltration at the site of infection. Since its secretion culminates into intestinal cell damage, its down-regulation is vitally important. Our data clearly show that exposure of enterocyte-like Caco-2 cells to *Salmonella enteritidis* 857 induces the expression of both Hsp70 and IL-8. Incubation of Caco-2 cells with certain lactobacilli strains, lactic acid or butyrate also induces the expression of Hsp70, but has little effect on the IL-8 levels. A marked decrease in IL-8 secretion was observed when Caco-2 cells were infected with *Salmonella enteritidis* 857 pretreated with the spent cell supernatant containing microbial products of lactobacilli.

Stimulation of proinflammatory response in intestinal epithelial cells and macrophages by probiotic bacteria. I. Zafošnik[a], N. Pipenbaher[a], P.L. Moeller[b], H. Weingartl[c], A. Cencic[a], M. Jakobsen[b] ([a] University of Maribor, Faculty of Agriculture, Vrbanska c.30, 2000 Maribor, Slovenia; [b] The Royal Veterinary and Agricultural University, Dept. of Dairy and Food Science, Rolighedsvej 30, 1958 Frederiksberg C, Denmark; [c] Canadian Food Inspection Agency, National centre for Foreign Animal Disease, 1015, Arlington street, Winnipeg, Manitoba, R3E 3M4, Canada).

Probiotics have a beneficial effect against allergic reactions, but the mechanisms behind this have not yet been studied. Therefore, the aim of

our study was to investigate the proinflammatory immune response in the pig intestinal epithelial cell line IPEC-J2 (Prof. A. Blikslager, USA) and macrophage cell line 3D4/21 upon treatment with a variety of *Lactobacillus* and *Bifidobacteria* strains, mainly isolated from the human intestinal tract. For this purpose we measured: (1) No_x species production and iNOs synthesis, (2) hydrogen peroxide production; and in IPEC-J2 cell line, (3) the effect on viability and physiological status of the cells. Live *L. rhamnosus*. *L. paracasei*, *L. plantarum* and *Bifidobacterium* were capable of stimulation of a proinflammatory response in IPEC-J2 and macrophages. This effect was not due to the cell wall of probiotic bacteria since dead bacteria did not stimulate the proinflammatory response. Only *L. rhamnosus* and *L. paracasei/rhamnosus* showed the ability of hydrogen peroxide production in macrophages. Interestingly, we found out that *L. rhamnosus*, *L. casei L. paracasei*, *L. reuteri* and *L. plantarum,* as well as most bifidobacteria, can produce No_x species from L-arginine themselves. *L. casei* and *L. ruminus* showed beneficial effects on the IPEC-J2 physiological status, *L. rhamnosus* has a slight cytotoxic effect while the others did not affect the IPEC-J2 cells physiological status. Based on the results obtained, we suggest that despite the fact that *L. rhamnosus* is the strongest proinflammatory probiotic bacteria, *L. casei* and *L. paracasei/rhamnosus* have the highest probiotic potential as proinflammatory probiotic bacteria, not only due to the ability of No_x species induction, but also due to beneficial effects on intestinal epithelia.

The incidence of *E.coli* O157:H7 in food samples in the Zagreb area, Croatia. I. Kovaček, N.K. Jonjič, D. Puntarič, J. Bošnir, B. Matica, M. Štefanac (Department of Health, Ecology, Zagreb Institute of Public Health, Mirogojska cesta 16, 10 000 Zagreb, Croatia).

Enterohemorrhagic *Escherichia coli* O157:H7 strains can pose a serious health threat in tainted food. Traditional culture-based methods for assay of this enteric pathogen in food are relatively slow and the results can be ambiguous. To determine the frequency of enterohemorrhagic *E. coli* in food samples in the Zagreb area, Croatia a new detection system, the immunomagnetic separation was developed to detect *Escherichia coli* O157:H7 in food. EHEC detection was introduced in 1999 in our laboratory. This method separated *Escherichia coli* O157:H7 cells from enriched food samples (modified tryptone soya broth with novobiocin) by means of immunomagnetic beads. After magnetic capture, the beads were spread on MacConkey sorbitol agar (SMAC) at 37 °C. The analysis time, including a 6 h enrichment and plate incubation was 48 h. Sorbitol negative colonies were agglutinated with *E. coli* O157:H7 antiserum. Between 1999 and 2003 in the Zagreb area, 500 food samples were investigated for the presence of *Escherichia coli* O157:H7. The foodstuffs included 300 uncooked foods (100 raw vegetables, 100 raw cow's milk and 300 raw meats and meat-products. *Escherichia coli* O157:H7 was present in five samples of raw meat. The frequency of *Escherichia coli* O157 in the Zagreb area is very low.

Dynamics and spreading of antibiotic resistant enterobacteria in ovine gastrointestinal tract. R. Malik, J. Ivan, P. Javorsky, P. Pristas (Institute of Animal Physiology, Slovak Academy of Sciences, Soltesovej 4-6, 04001 Košice, Slovak Republic).

Antibiotic-resistant strains or resistance element occurrence are found in animal husbandry, and the route of transmission from animals to humans by contaminated meat products has been established. Dissemination of resistance is mediated by clonal spread of a particular resistant strain and/or by the spread of resistance genes. There is some evidence that certain colonisers of the gastrointestinal tract, e.g. *E. coli*, possess a pronounced capacity for the acquisition and dissemination of resistance genes. The occurrence and spread of ampicillin, kanamycin and tetracycline resistance in natural populations of *Enterobacteriaceae* obtained from the ovine gastrointestinal tract (rumen and colon) during a six month period were analysed. The significant seasonal dynamics were observed with culmination of antibiotic-resistant enterobacteria during the summer months. The presence of selected antibiotic resistance genes was subsequently analysed by a PCR method. The $tem1_{bla}$, *aphA1* and *tetB* genes were found to be predominant in the tested isolates. Conjugative transfer of *tetB* and *aphA1* genes was observed

in a laboratory *Escherichia coli* strain under in vitro conditions. ERIC (Enterobacterial Repetitive Intergenic Consensus) PCR as the molecular typing method was used to elucidate the spread of antibiotic resistance among *Enterobacteriaceae*. The comparison of ERIC-PCR profiles of ruminal and faecal isolates suggested that horizontal gene transfer was a main source of antibiotic-resistant bacteria in the rumen during the whole period tested. However, the clonal spread was found to be responsible for the dissemination of antibiotic resistance into the lower part of the GI tract.

Resistance of *Campylobacter jejuni* and *Campylobacter coli* strains to antibiotics. M. Mikulicova, I. Steinhauserova (University of Veterinary and Pharmaceutical Sciences Brno, Department of Meat Technology and Food Hygiene, Palackeho 1-3, 61242 Brno, Czech Republic).

Campylobacteriosis is the second most important alimentary illness nowadays. The main reason of resistant bacterial strains development in *Campylobacter* spp. is feeding medicated food mixtures to food animals in the past which challenged the selection of resistant strains therefore causing therapy complication in human illness. In total, 88 strains from slaughtered pigs, 49 strains from slaughtered poultry and 53 strains from human patients suffering from colitis were examined in 2003. In 88 pig strains, 69 *Campylobacter* spp. strains were isolated and detected as *C. coli,* 19 strains as *C. jejuni.* From the poultry samples 40 strains presented *C. jejuni* and 9 strains presented *C. coli.* In the 53 human strains, 35 strains were detected as *C. coli* and 18 strains as *C. jejuni.* The results were confirmed by molecular biology methods (PCR, PCR-RFLP). The sensitivity of certain microbiological strains was tested by an agar dilution method. In order to set the sensitivity to antibiotics, five of them were selected: tetracycline, nalidixic acid, ciprofloxacin, erythromycin, and chloramphenicol. After incubation, the isolates were classified in compliance with NCCLS M31-A2. The higher resistance was observed in *C. coli* compared with *C. jejuni*, particularly in the case of macrolide resistance in pigs. In poultry products, our results were consistent with the case of nalidixic acid and ciprofloxacin, where the strains were mostly sensitive to these antibiotics. For human strains the situation was similar. Poultry strains may cause campylobacteriosis in man and their high sensitivity to antibiotics facilitates the therapy.

***Laribacter hongkongensis* in fish is associated with gastroenteritis and traveller's diarrhea.** P.C.Y. Woo[a], S.K.P. Lau[a], J.L.L. Teng[a], T.L. Que[b], R.W.H. Yung[c], W.K. Luk[d], R.W.M. Lai[e], W.T. Hui[a], S.S.Y. Wong[a], H.H. Yau[f], K.Y. Yuen[a] ([a] Department of Microbiology, The University of Hong Kong; [b] Tuen Mun Hospital, Hong Kong; [c] Pamela Youde Nethersole Eastern Hospital, Hong Kong; [d] Tseung Kwan O Hospital, Hong Kong; [e] United Christian Hospital, Hong Kong; [f] Department of Accident and Emergency, Tuen Mun Hospital, Hong Kong).

After its discovery in Hong Kong in 2001, *Laribacter hongkongensis* was recovered from six patients with gastroenteritis from Hong Kong and Switzerland. However, the association of *L. hongkongensis* with gastroenteritis is still unproven and the source of it is unknown. We conducted a prospective study to investigate the association of *L. hongkongensis* with gastroenteritis. Faecal samples from patients with community-acquired gastroenteritis and controls were cultured for *L. hongkongensis*. A case-control study and targeted food surveillance were performed to identify the potential source of *L. hongkongensis*. All *L. hongkongensis* isolates were characterized by pulsed-field gel electrophoresis (PFGE) and ribotyping. During a four-month period, *L. hongkongensis* was recovered from 17 out of 3 788 patients with community-acquired gastroenteritis, but none of the 1894 controls ($P < 0.005$). *Laribacter* gastroenteritis was associated with recent histories of travel (59% vs. 6% in the controls, $P < 0.001$), fish consumption (94% vs. 56% in controls, $P < 0.01$), and minced freshwater fish meat consumption (29% vs. 3% in controls, $P < 0.05$). Twenty-seven additional *L. hongkongensis* isolates were recovered from intestinal samples in 25% of freshwater fish (29% of mud carp, 59% of grass carp, 53% of bighead carp, and 6% of large-mouth bass) and 15% of minced freshwater fish meat from retail markets in Hong Kong. *L. hongkongensis* of the same PFGE pattern and ribotype was recovered from a patient and minced freshwater fish meat from the retail market where he had recently purchased minced freshwater fish

meat for cooking. In conclusion, *L. hongkongensis* is associated with community-acquired gastroenteritis and traveller's diarrhea. Freshwater fish is the source of *Laribacter* gastroenteritis.

Genetic loci involved in bile tolerance and pathogenesis of *Listeria monocytogenes*. M. Begley, C. Hill, C.G.M. Gahan (Department of Microbiology, University College Cork, Cork, Ireland).

Listeria monocytogenes must resist the deleterious actions of bile in order to infect and subsequently colonise the gastrointestinal tract. We have previously shown that the bacterium can tolerate concentrations of bile greater than those encountered in vivo and is capable of adapting and cross-adapting to bile (Begley, Gahan and Hill, Applied and Environmental Microbiology 2002, 68(12):6005-6012). Present studies are focusing on the molecular mechanisms underlying tolerance. One approach involves screening a transposon bank for bile sensitive mutants. To date, a number of bile tolerance loci have been identified and characterized including *btlA*, *gadA*, *zurR* and *lytB*. Interestingly all play putative roles in the maintenance of membrane integrity or in stress responses. A second approach involves the targeting of specific genes following analysis of the published *L. monocytogenes* EGDe genome. The initial three genes targeted were *bsh*, *pva* and *btlB*. Analysis of deletion mutants revealed a role for all three in bile tolerance. *Bsh* was shown to encode a functional enzyme that hydrolyses bile salts and animal (murine) studies revealed that BSH contributes to persistence in the intestinal tract. Transcriptional analyses and activity assays revealed that while regulated by both the principle virulence regulator PrfA and the alternative stress sigma factor SigmaB the latter appears to play a greater role in modulating *bsh* expression. A mutant lacking a functional SigmaB was shown to be exquisitely sensitive to bile salts further demonstrating a central role for SigmaB in modulating listerial resistance to bile.

Identification and disruption of *opuB*, a novel bile tolerance locus linked to the virulence potential of *Listeria monocytogenes*. R.D. Sleator, C.G.M. Gahan, C. Hill (Department of Microbiology and Alimentary Pharmabiotic Centre, University College, Cork, Ireland).

Listeria monocytogenes is the causative agent of listeriosis, which accounts for ~35% of all deaths caused by known bacterial foodborne pathogens in the US yearly. Following consumption, the pathogen is exposed to the low pH of the stomach and the volatile fatty acids, bile salts, high osmolarity and low oxygen content of the small intestine. The ability of *Listeria* to proliferate under such adverse conditions is attributed, at least in part, to the extrusion of harmful compounds produced by the host. *In silico* analysis of the *L. monocytogenes* genome revealed a novel two-gene operon, designated *opuB*, which exhibits significant sequence similarity to known ABC transporters. Computer aided structural analysis suggests that OpuB is an efflux pump, extruding harmful bile salts from inside the cell. Preceded by the alternative transcription factor sig^B- we demonstrate that *opuB* is transcriptionally up regulated at elevated osmolarities and reduced temperatures (stresses known to induce sig^B). Furthermore, a significant reduction in the level of *opuB* transcription was observed in a sig^B mutant. In addition, we identified an important role for PrfA, the master regulator of virulence potential, in coordinating *opuB* expression in *L. monocytogenes*. Inactivating OpuB by chromosomal exchange mutagenesis resulted in a ~4 log decrease in the ability of the bacterium to tolerate bile, a phenotype which translates to a significant reduction in gut colonisation and subsequent systemic infection following oral administration to a murine model. OpuB thus represents a novel virulence factor in *L. monocytogenes*, and a potential target for novel antibiotic therapies.

Downregulation of L-arginine uptake by spermine inhibits *H. pylori*-induced macrophage iNOS translation and enhances bacteria survival. F.I. Bussière[a], R. Chaturvedi[a], A.P. Gobert[a], Y. Cheng[a], D.R. Blumberg[a], P. Kim[a], H. Xu[a], R.A. Casero Jr.[b], K.T. Wilson[a] ([a] Department of Medicine, Division of Gastroenterology, University of Maryland, Baltimore, MD, USA; [b] Johns Hopkins University School of Medicine, Baltimore, MD, USA).

We showed that stimulation of macrophages with *H. pylori* (Hp) results in upregulation of inducible nitric oxide synthase (iNOS), producing NO; arginase II, converting L-arginine to

ornithine; and ornithine decarboxylase (ODC), synthesizing polyamines from ornithine. The polyamine spermine has been reported to inhibit iNOS in LPS-stimulated macrophages, but the mechanism is unknown. Since L-arginine availability can regulate iNOS translation, we speculated that Hp-stimulated spermine synthesis could inhibit iNOS protein translation via inhibition of L-arginine uptake. Moreover, since we reported that NO kills Hp, we speculated that spermine contributes to Hp survival. Addition of spermine to a coculture of Hp SS1 and murine macrophages inhibited NO production in a concentration-dependent manner. Spermine did not alter Hp-induced iNOS mRNA expression, but significantly decreased iNOS protein levels and translation, while pulse-chase experiments showed no iNOS degradation. Spermine prevented killing of Hp by > 3.5 log. We detected a 4-fold increase in L-arginine uptake and a concomitant increase in mRNA levels of the *y*+ cationic amino acid transporter (CAT2B), the macrophage transporter of L-arginine. Spermine inhibited L-arginine uptake by > 80%, but did not affect CAT2B mRNA levels. Intriguingly, there was an inverse relationship between L-arginine uptake and spermine levels. Addition of L-arginine to Hp-stimulated cells increased iNOS protein levels in a concentration-dependent manner. Increased expression of ODC in Hp-stimulated macrophages downregulates L-arginine uptake and thus inhibition of iNOS protein translation. This loss of NO antimicrobial defense contributes to the dysregulated host response to Hp and the persistence of the organism.

Translocation of *Listeria monocytogenes* through M-cells is independent of haemolysin production. 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).

Relatively little is known about the precise route by which the food-borne pathogen *Listeria monocytogenes* gains access to intraepithelial lymphoid cells and mucosal lymphoid tissues. A number of intestinal pathogens (eg. *Vibrio cholerae*) have been shown to utilise M-cells, found in Peyer Patches of the follicle-associated epithelium (F.A.E.) of the small intestine. M-cells transport antigens and bacterial pathogens across the epithelial barrier by a process of vesicular translocation. We utilised an in vitro transwell model that mimics M-cell activity through differentiation of epithelial enterocytes via coculture with lymphocytes. Electron microscopy was used to verify this differentiation to the M-cell phenotype. Upon addition of the bacteria, transcytosis can be observed. As a positive control, we showed that *Vibrio cholerae* ElTor cells are transported across transwell plates ~1 log higher through M-cells compared to undifferentiated enterocytes, indicating a viable M-cell activity. We used this model system to measure the transcytosis of virulent *L. monocytogenes* which we found to be 1.5 log higher through M-cells compared to control enterocytes. This translocation was independent of haemolysin production. This in vitro model can be used to study specific properties of F.A.E and M-cells, the first steps of many infectious diseases and also in screening for vaccines or drug vectors that target M-cells.

Enterohemorrhagic *Escherichia coli* 0157:H7 induce nitric oxide synthesis in human intestinal epithelial cells. A.P. Gobert, A. Durand, C. Martin (Unité de Microbiologie, INRA de Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France).

Infection with Shiga toxin-producing Enterohemorrhagic *Escherichia coli* (EHEC) causes diarrhea and can result in the more severe sequelae, the hemolytic uremic syndrome (HUS). In response to intestinal pathogens, epithelial cells rapidly upregulate the expression of pro-inflammatory genes that contribute to an innate immune response. The products of these newly expressed genes include cytokines (e.g., IL-8, TNF-α) and effector molecules such as iNOS (inducible NO synthase)-derived nitric oxide (NO), a free radical possessing numerous inflammatory and immunological functions. To assay the effect of EHEC infection on iNOS expression and NO production by human intestinal epithelial cells. The human epithelial cell lines T84 and Caco-2 were stimulated for 3 h with different strains of 0157:H7 EHEC. In a first set of experiments, total RNA was purified and iNOS mRNA was analyzed by real-time PCR. After being washed, the cells were also

cultured for 21 h in a fresh medium containing gentamycin; nitrites, the stable product of NO in culture, were then measured in the supernatants. iNOS mRNA expression was increased by ~ 10 fold when the cells were stimulated with EHEC in comparison with the controls. Nitrite concentration increased 2 fold in the supernatants of EHEC-infected cells vs the controls. These results show for the first time the production of NO by human intestinal epithelial cells stimulated with non-invasive bacteria. The elucidations of the mechanism of iNOS induction and of the NO-derived effects on epithelial cells and bacteria are underway in our laboratory.

Cytokine patterns of *L. fermentum* KLD that has an ability to bind to Peyer patches in mice. S.S. Kang, P.L. Conway (School of biotechnology and biomolecular sciences, The University of New South Wales, Sydney, NSW 2052, Australia).

It has been demonstrated that *Lactobacillus fermentum* KLD has an ability to selectively associate with the follicle-associated epithelium of the Peyer patches in mice. In this study, we evaluated whether two probiotic strains, *L. fermentum* KLD and *L. fermentum* LMG, that exhibit different binding capacities onto the Peyer patches, can stimulate cytokine production in the Peyer patches and spleens. *Lactobacillus* strains (10^9 CFU/mouse) were orally administered to healthy Balb/c mice, and expression of gamma interferon (IFN-γ), Interleukin-4 (IL-4), IL-10, and IL-12 in the Peyer patches and spleens were measured by ELISA. The results revealed that the production of IFN-γ and IL-12 by Peyer patch cells in the group fed *L. fermentum* KLD was significantly higher than those by Peyer patch cells in the group fed *L. fermentum* LMG or PBS whereas both cytokines were not strongly induced by spleens from both groups fed *L. fermentum* KLD and *L. fermentum* LMG compared with the control group. In contrast, IL-4 and IL-10 were not markedly induced by Peyer patch and spleen cells in both groups. Therefore, distinct *lactobacillus* strains that displayed different adhesive properties in vitro induced distinct immune response and *L. fermentum* KLD with adhesive ability to Peyer patches can be a potent stimulator of Th1 cytokines.

***H. pylori* colonization, anthropometry and body composition.** A.L. Maldonado[a], E. Marini[b], G. Hidalgo[c], R. Buffa[b], A. Marin[c], W. Racugno[d], L.R. Pericchi[e], G. Floris[b], M.G. Domínguez-Bello[a,f] ([a] CBB, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela; [b] Dept Experimental Biology, University of Cagliari, Italy; [c] Centro Amazónico para la Investigación y Control de Enfermedades Tropicales, CAICET, Venezuela; [d] Dept Mathematics, University of Cagliari, Italy; [e] Dept Mathematics University of Puerto Rico, Rio Piedras Campus, Puerto Rico; [f] Dept. Biology University of Puerto Rico, Rio Piedras Campus, Puerto Rico).

It has been shown that *Hp*+ children have more coinfections with intestinal parasites and *H. pylori* might be considered as a marker for human microbial burden. There are controversial results on the associations between H pylori and child growth and diarrhea. The aim of this study was to investigate the influence of *H. pylori* infection on the nutritional status assessed by anthropometry and body composition analysis. Thirty-four male and 32 female Amerindians of the Jivi tribe in Venezuela were included in the study, after approval by the IVIC bioethical commission, and community and individual consent. *H. pylori* infection was detected by the 14C breath test. Feces from asymptomatic individuals were examined microscopically. Weight, height, BMI, and bioelectrical parameters of resistance (R, Ω) and reactance (Xc, Ω) were measured. Body composition was assessed by means of Bioelectrical Impedance Vector Analysis (BIVA and individual bioelectrical values were adjusted for sex/age utilizing impedance European standards. Regression analysis, including gender and age in the model, was applied to compare BMI in the *Hp*+ and *Hp*- subgroups. (1) *Hp* infection was high (80% of adults) and occurred early (50% children under 10). (2) Regression analysis indicated that *Hp*+ persons had a significantly higher BMI. (3) Impedance vector analysis indicated a better nutritional status (higher soft tissue mass) in *Hp*+ than in *Hp*- subjects. *H. pylori* colonization does not adversely affect body mass and composition of Jivi Amerindians. Indeed, there is a tendency towards a better nutritional status in *H. pylori* positive persons.

Transcriptional response of intestinal epithelial cell/dendritic cell co-cultures to *Lactobacillus plantarum*. S. Pavan[a,b], M. Kleerebezem[a,c], J.J.M. van de Sandt[b] ([a] Wageningen Centre for Food Sciences, 6700 AN Wageningen, The Netherlands; [b] TNO Nutrition and Food Research, 3700 AJ Zeist, The Netherlands; [c] NIZO Food Research, 6710 BA Ede, The Netherlands).

The gut mucosal surface represents a major site of interaction with environmental microorganisms. The intestinal epithelial cell response to non-pathogenic, commensal bacteria is thought to be influenced by the presence of immunocompetent cells. We investigated the epithelial response to *Lactobacillus plantarum* in an in vitro co-culture model. Enterocyte-like CaCO-2 cells were co-cultivated with human blood monocyte-derived dendritic cells in separate compartments of Transwell cultures. Co-cultures were stimulated with *L. plantarum*, and the transcriptional response of epithelial cells was evaluated by using human cDNA microarrays. On the Contrary to the effect of pathogenic microorganisms described in some studies, *L. plantarum* does not induce strong changes of the transcriptional profile of CaCO-2 cells. Nevertheless, this microorganism was able to influence the gene expression of CaCO-2 cells. Interestingly, *L. plantarum*-stimulated CaCO-2 cells co-cultivated with dendritic cells displayed a different transcriptional profile compared to mono-cultivated CaCO-2 cells. This strengthens the observation that the presence of immunocompetent cells greatly influences the reactivity of epithelial cells to bacterial signaling.

Biotic and abiotic factors influence *Escherichia coli* O157:H7 growth in rumen fluid and acid resistance. F. Chaucheyras-Durand[a], J. Madic[a,b], F. Doudin[a,b], C. Martin[b] ([a] Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France; [b] INRA Clermont-Ferrand Theix, Unité de Microbiologie, 63122 St-Genès-Champanelle, France).

The gastrointestinal tract of ruminants is the main reservoir for Enterohemorrhagic *Escherichia coli* (EHEC) strains, which are the most common cause of the hemolytic-uremic syndrome (HUS) in humans due to food poisoning. Our aim was to characterise biotic and abiotic factors influencing EHEC carriage by ruminants in order to develop strategies for reducing EHEC survival in the bovine gastrointestinal tract, therefore limiting food contamination. EHEC (10^6 CFU·mL^{-1}) were grown anaerobically in clarified rumen fluid or faecal fluid at 39 °C for 24 h. The rumen autochtonous flora exerted a high barrier effect against EHEC O157:H7 growth in contrast to the faecal flora. Growth of EHEC was reduced in the rumen fluid collected from animals fed a diet composed of 50% hay/50% wheat compared to 100% hay, due to the inhibitory effect of low pH conjugated to high volatile fatty acid concentration. In addition, EHEC cells incubated in the rumen fluid collected from hay/wheat-fed animals were more resistant to a two-hour acid shock in the acidic abomasum content than cells grown in rumen fluid harvested from hay-fed animals, and thus would be much more likely to colonise the gut and to be a source of faecal contamination of the environment. Preliminary results highlight the use of probiotic yeasts in ruminant feed for limiting the induction of EHEC acid resistance in the rumen.

Effect of salt on growth property of *E. coli* O157:H7 strain. N. Kijima[a], M. Wachi[b], C. Yajima[a], H. Murakami[a] ([a] National Institute of Vegetable and Tea Science, Tsukuba, Japan; [b] Tokyo Institute of Technology, Yokohama, Japan).

Escherichia coli O157:H7 is a pathogenic bacterium, the infection of which causes severe food poisoning. Clarifying the growth property of *E. coli* O157:H7 in the environment is important for the prevention of food born illness by this organism. As the first step of characterization of this bacterial strain in environment, we investigated the effect of salt on the growth property of *E. coli* O157:H7. Non-pathogenic *E. coli* O157:H7 strain ATCC43888 was incubated in liquid medium with or without sodium chloride and its growth was monitored by measuring OD 660. Sodium chloride (0.5 % to 1.0 %) promoted the growth of *E. coli* O157:H7. On the contrary, with the typical laboratory strain, *E. coli* K12 (W3110 and MG1655), there were little effects on growth, indicating that salt-dependency of growth is a unique characteristic of *E. coli* O157:H7. Sucrose (20%) did not promote the

growth of *E. coli* O157:H7, indicating that sodium chloride does not function as an osmotic stabilizer. Magnesium chloride or ammonium chloride also promoted the growth of *E. coli* O157:H7. These results suggest that the presence of salt in the environment is an important factor for the growth of the *E. coli* O157:H7 strain.

Coarse non-pelleted feed reduces *Salmonella* in the gastrointestinal tract of pigs. L.L. Mikkelsen[a], P.J. Naughton[b], M.S. Hedemann[a], B.B. Jensen[a] ([a] Danish Institute of Agricultural Sciences, Research Centre Foulum, PO Box 50, 8830 Tjele, Denmark; [b] Northern Ireland Centre for Food and Health, School of Biomedical Sciences, University of Ulster (at Coleraine), Cromore Rd, Coleraine, Co. Londonderry BT52 1SA, UK).

A 2 × 2 factorial experiment was conducted to study the effect of feed grinding (fine and coarse) and feed processing (pelleted and non-pelleted) on the physico-chemical properties, microbial populations and survival of *Salmonella enteric* serovar *Typhimurium* DT12 in the gastrointestinal tract of pigs. The results showed that the pigs fed the coarse non-pelleted (C-NP) diet had more solid gastric content, higher dry matter content, significantly increased number of anaerobic bacteria ($P < 0.05$), increased concentrations of organic acids, reduced pH and significantly increased in vitro death rate of *Salmonella* DT12 in the contents from the stomach ($P < 0.001$) as compared with pigs fed the other diets. The concentration of undissociated lactic acid was significantly higher in the gastric content from pigs fed the C-NP diet ($P < 0.001$). A strong correlation between the concentration of undissociated lactic acid and the death rate of *Salmonella* DT12 was found. In the distal small intestine, caecum and mid colon, significantly lower numbers of coliform bacteria were observed in pigs fed the coarse versus the fine diets ($P < 0.01$). The pigs fed the C-NP diet showed the numerically lowest number of coliform bacteria in the gastrointestinal tract. It was concluded that feeding a coarsely ground meal feed to pigs changes the physico-chemical and microbial properties of the contents in the stomach that decreases the survival of *Salmonella* during passage through the stomach. In this way, the stomach acts as a barrier preventing harmful bacteria from entering and proliferating in the lower part of the gastrointestinal tract.

Intestinal bacterial community analysis: *Lawsonia intracellularis* a case study. L. Mølbak, M. Boye (Danish Institute for Food and Veterinary Research, 1790 Copenhagen, Denmark).

The aim was to study the influence of the diet on the distribution, dynamics and stability of the intestinal bacterial communities of pigs, and the relationship between the bacterial community and the presence of *Lawsonia intracellularis*. *L. intracellularis* is an obligate intracellular bacterium causing proliferative enteropathy. The infection causes diarrhea, retarded growth and/or sudden death in pigs and is an economically important disease in the swine industry worldwide. Previous studies indicate that infection with *L. intracellularis* might be influenced by the animals diet and/or the intestinal microbiota. Terminal restriction fragment length polymorphism (T-RFLP) analysis was used for screening the diversity of the microbiota of ileum and colon samples from 52 pigs. The pigs were fed with the same diet either in a meal or in a compressed form (pellets). Faeces and ileum samples were analysed from each pig for *L. intracellularis* by Real-Time quantitative PCR. The T-RFLP results showed a clear difference between the bacterial communities in the ileum and colon. The ileum communities were more heterogeneous than the colon, and there was a significant correlation between the bacterial communities of the colon among pigs from the same pen. A principal component analysis (PCA) showed differences between the bacterial communities of the pigs fed with the different diets. It was possible from the specific band (T-RF) belonging to *L. intracellularis* to see a dependency between the band intensity and the severity of proliferative enteropathy determined by H&E-staining as well as the density of *L. intracellularis* determined by in situ hybridization.

Effect of C_2–C_{18} fatty acids on *Clostridium perfringens* CCM 4435. E. Skřivanová[a], M. Marounek[a,b], V. Skřivanová[a] ([a] Research Institute of Animal Production, Prague 10, 104 01, Czech Republic; [b] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague 4, 14220, Czech Republic).

Antimicrobial activity of $C_2 - C_{18}$ fatty acids was determined in vitro in cultures of *Clostridium perfringens* CCM 4435 grown on glucose. Antimicrobial activity was expressed as IC_{50} (a concentration at which only 50% of the initial glucose was utilized). In cultures of the strain 4435, the IC_{50} values of caprylic, capric, lauric, myristic and oleic acid were 1.92, 0.28, 0.04, 0.08 and 0.49 $mg \cdot mL^{-1}$, respectively. The effects of linoleic acid were very variable. Palmitic, stearic and short-chain fatty acids had no effect on glucose utilization. Antimicrobial activity of myristic, oleic and linoleic acid decreased when bacteria were cultivated in the presence of straw particles. The counts of viable bacteria determined by a plating technique decreased after incubation with caprylic, capric and lauric acid (30 min; 1 $mg \cdot mL^{-1}$) at pH 5.0 from $> 10^8$ to less than $10^2 \cdot mL^{-1}$. Bactericidal effects of myristic and oleic acid were less pronounced. At pH 6.6 the antimicrobial activity of capric and lauric acid still existed, but that of caprylic, myristic and oleic acid decreased. It can be concluded that lauric acid had the highest antimicrobial activity toward *C. perfringens* among the fatty acids tested. Its activity was not influenced by the presence of solid particles in the milieu and did not cease at a pH near 7. (This study was supported by the Czech Science Foundation: project no. 523/02/0460.)

***Enterococcus faecium*-based direct-fed microbials (DFM) on fecal shedding of *Escherichia coli* O157 in cattle**. T.G. Nagaraja[a], K.F. Lechtenberg[b], W.Q. Alali[a], J.M. Sargeant[a], W.P. Kautz[c], J.A.Z. Leedle[c] ([a] Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, USA; [b] Midwest Veterinary Services, Inc., Oakland, NE., USA; [c] Chr. Hansen's Biosystems, Milwaukee, WI, USA).

Two studies were conducted to determine the effect of DFM on fecal shedding of *E. coli* O157 in cattle. Sixteen calves were randomly allotted to one of four treatment groups: control and DFM dosed daily at 2, 20, or 200 g per head. The DFM consisted of two strains of *E. faecium* blended to deliver a 2.5×10^9 CFU/g/hd/day. Calves were orally inoculated with nalidixic acid-resistant (*Nal^r*) *E. coli* O157:H7 (5.2×10^{10} cfu/animal). Feces were collected three times a wk for 4 wk and the presence and concentration of *Nal^r E. coli* were determined. Groups fed DFM at 2 or 20 g shed lower concentrations of *Nal^r E. coli* in the feces compared to the control ($P = 0.06$ and 0.01, respectively). DFM at 200 g had no effect on fecal shedding of *Nal^r E. coli*. In the second study, 240 steers in 30 pens were randomly assigned to: control (no DFM) or 20 g daily dose of *E. faecium*-based DFM. Two weeks later, feces were collected to assess prevalence of *E. coli* O157:H7. The control group had 8/15 pens compared to 3/15 pens in the DFM-fed group with at least one animal positive for *E. coli* O157:H7. Chi square analysis of the probability of at least one animal within a pen testing positive for the control and DFM-fed indicated a trend ($P = 0.06$) for DFM reducing prevalence of *E. coli* O157:H7. Results suggest potential benefit of feeding *E. faecium*-based DFM in reducing prevalence of *E. coli* O157:H7 cattle.

Echinaceae extract in immunocorrection of patients with ulcerous erosion of gastroduodenal zone complicated by gut microflora imbalance. A.I. Sidorov[a], L.E. Smirnova[b], E.M. Sulman[a], V.F. Vinogradov[b] ([a] Tver State Technical University, 22 A. Nikitin str., Tver, 170026, Russia; [b] Tver State Medical Academy, Sovetskaya str. 4, Tver, 170642, Russia).

A group of 95 patients with ulcerous erosion of the gastroduodenal zone complicated by gut microflora imbalance was observed. A control group of 30 patients was treated with a basic therapy (omeprazole, amoxicillin, furazolidone, drotaverine hydrochloride). Sixty-five patients (main group) received the basic therapy and immunopotentiation with Echinacea purpurea extract (30 vol. % ethanol) prepared using ultrasonic treatment (55 w./cm^2, 5 min). The extracts were analyzed by IR- and UV-spectroscopy. Twenty-five drops of the extract were taken 3 times a day. The duration of the medical treatment was 21 days. Clinical, physico-chemical and endoscopic studies were carried out, including the study of biopsy mucous. The patients of the main group were classified according to groups of immunodeficiency (40 patients, 61.5%) and risk group (25 patients, 38.5%). Thirty-seven patients from the main group (57.0%) had a gut microflora imbalance and the expansion of the immunity B-chain. The number

of leucocytes was found to be lower ($P < 0.05$), which can be explained by the emaciation of the immunity cell-chain. After the medical treatment, the indices of the immunity parameters became worse to a lower extent in the main group (7% against 27%, $P < 0.05$). Gut microflora was normalized for 50% of the patients of the main group and 17% of the control group. Such a relationship between the gut microflora characteristics and the immunity status suggests the efficiency and stability of the medicinal effect. The use of Echinaceae extract resulted in immunity status normalization and rapid erosion healing.

Estimation of in vivo probiotic activity of two lactobacilli to uropathogenic *Escherichia coli* in mouse models. D. Teperik, K. Truusalu, T. Karki (Department of Microbiology, Tartu University, Tartu, Estonia).

Historical data indicate that the majority (80%) of UTI is caused by *Escherichia coli*. It is generally accepted that the starting point for uropathogens is the gastrointestinal (GI) microflora. The use of probiotic lactobacilli has received great attention as an alternative remedy to prevent and treat UTI. The mechanism of action may be a direct or indirect antagonistic activity against uropathogens both in the perianal area and GI tract. Using the murine GI system as a model for the reservoir of uropathogenic bacteria, we sought to assess the impact of the administration of two probiotic lactobacilli strains on the colonisation abilities of uropathogenic *E. coli*. Uropathogenic and kanamycin (Km) resistant *E. coli* were isolated from the urine of a patient with pyelonephritis. Lactobacilli used in this study were strains with probiotic properties: *Lactobacillus fermentum* ME-3 and *Lactobacillus plantarum* 299v. To suppress intestinal microflora, all 6-to 8-week-old female BALB/c mice were fed Km in drinking water for 11 days. There were 6 treatment groups. Viable *E. coli* and lactobacilli detected in the faeces of Km treated mice were monitored in freshly void faeces collected on each experimental day. Mice were dissected on day 12 and the bladder, kidneys and liver were investigated for histopathological changes. To compare and administrate changes in microecology, molecular analysis of DNA isolated from the faeces was made (DGGE, PFGE, and PCR).

Optical interactions between bacteria: the response of native *Escherichia coli* culture to rifampicin-treated culture of the same species. M.V. Trushin (Kazan Institute of Biochemistry and Biophysics, PO Box 30, 420111, Kazan, Russia).

The problems associated with resistance of microorganisms to antimicrobial drugs are becoming more real today. It has been shown that communication between microorganisms may play a key role in the formation of the microbe community that is beyond the reach of treatment. In this way, the many-sided study of microbial communication has an important significance. Experiments were done with *E. coli* 1061 cells that were grown in LB medium in a "flask in a flask" device. Rifampicin was added to the "sender" culture during different phases of its growth at a final concentration of 30 μg per mL medium. Growth was monitored spectrophotometrically at 590 nm. The experiments were done in 10 replicates. The results were treated with the Student test and Kolmogorov-Smirnov test. It was found that the response of native *E. coli* culture to a rifampicin-treated culture depended on the time of antibiotic addition. Namely, the addition of rifampicin to the "sender" culture resulted in an increment of the lag phase duration of a native (non-treated) culture. A significant increase of harvest of the non-treated culture was observed when the antibiotic was added during the active growth of the "sender" culture. Finally, there were no alterations in growth of the native culture when rifampicin was added to the stationary phase "sender" culture. Since the native and treated antibiotic cultures were separated chemically and mechanically, it is reasonable to suggest that the effect observed was mediated by light signals. Such communication has been detected previously in many microorganisms.

Assessment of the effects of a defined probiotic preparation and cultured caecal contents on a *Salmonella typhimurium* 29E challenge in vivo. S.M. Waters[a], K.A. Horgan[a], R.A. Murphy[a], R.F.G. Power[b] ([a] Alltech Biosciences Centre, Dunboyne, Co. Meath, Ireland; [b] Alltech Inc., North American Bioscience Center, 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA).

Salmonella spp. are not native members of the microbiota in poultry, but readily colonise intestines of young chicks and persist during rearing. Cross-infection in the flock and transfer of salmonella to carcasses by faecal contamination during processing are common. These non-host adapted species do not cause any obvious ill effects in birds and rarely cause economically disruptive losses to the poultry industry. However, poultry products contaminated with salmonella are a major source of foodborne disease in most developed countries. Nurmi-type cultures have been used successfully for decades to prevent salmonella colonisation in poultry. Such cultures are derived from the caecal contents of specifically pathogen-free (SPF) birds and are administered via drinking water or spray application onto eggs in the hatchery. In this study, the efficacy of cultured caecal contents and a defined dual-strain probiotic, containing *Enterococcus faecalis* and *Pediococcus pentosaceus*, was tested in vivo by performing *Salmonella typhimurium* 29E challenge trials in a chick model. These trials involved dosing SPF chicks with each treatment on day 1 and subsequently challenging the birds with salmonella two days later in chick isolator chambers. The efficacy of each preparation was determined by measuring the level of salmonella colonisation in each bird seven days post-salmonella challenge. The defined probiotic did not offer the same degree of protection as cultured caecal contents, indicating that defined cultures are less effective salmonella control agents than preparations generated from the complete caecal microflora.

New B-type microcin and non-specific resistance to it. A.A. Yakovleva[a], B.V. Tarakanov[b], V.V. Aleshin[a] ([a] A. N. Belozersky Inst. Physico-Chemical Biology, Moscow M.V. Lomonosov State University, Russia; [b] All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Farm Animals, Russia).

Microcins are a family of low weight peptides produced by Enterobacteriaceae strains and active against related bacteria. Microcin B17 is a ribosomally synthesized peptide antibiotic which inhibits DNA gyrase. In this work the cloning and sequence of the genetic determinants of new Microcin type B from a natural isolate of *Escherichia coli* S 5/98 obtained from healthy swine intestine is described. The new microcin producer is characterized by a wide spectrum of antibacterial activity against the natural isolates of enterobacteria *Escherichia* and *Salmonella*, a great variety of colicinogenic *E. coli* and different species of museum salmonellas. It is noteworthy that the cells of the microcin producer under study contained no plasmids; the genes of microcin synthesis and immunity to it had a chromosomal localization. At the same time microcin type B determinants studied earlier are located in plasmids. Apparently antibiotic production or multiple antibiotic resistance are different survival strategies in microbial communities. The natural microcin producer *E. coli* S 5/98 was marked by colicin genes from the plasmid ColEI. The dynamics of its mixed population in conditions of tube fermentation in full and deficient culture media as well as in the natural intestine community of experimental animals was studied.

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Host-defending effects of *Lactobacillus plantarum* 299v. Siv Ahrné (Department of Food Technology, PO Box 124, SE-22100 Lund, Sweden).

Following a survey of *Lactobacillus* species on human intestinal mucosa where seventy-five persons were sampled by biopsies, an administration study was undertaken in order to find strains with outstanding capability to thrive in the intestine. This ability was scored as positive if a strain was detected as one of the dominating ones in the mucosa 11 days after the administration was stopped. The strains administered were chosen to represent the different lactobacilli found in the mucosa. The detection of a specific strain in its natural habitat (a probiotic strain in the GI-tract) demands means of identification that are very hard to obtain. In the administration study, restriction endonuclease analysis (REA) of total chromosomal DNA was successfully used for this purpose and *Lactobacillus plantarum* 299v was found to be the best. *L. plantarum* 299v belongs to the gastrointestinal subcluster of *L. plantarum* as defined by REA. Members of this subcluster possess a mannose-specific adherence mechanism conferring binding to human colonic cells. The effect of *L. plantarum* 299v on the intestinal mucosa status and barrier function has been extensively studied in rat models. Treatment with this strain before insults showed improvements of parameters such as protein, RNA, DNA contents, secretory IgA concentration, myeloperoxidase activity, permeability and translocation. In the liver injury model the liver status was improved and the translocating bacteria were different than those in untreated animals. In humans, intake of a fruit based product containing *L. plantarum* 299v protect from bloating and pain in Irritable Bowel Syndrome patients. Moreover, in smokers, several risk factors in coronary artery disease (fibrinogen, F_2-isoprostanes, IL-6, systolic blood pressure, insulin, leptin) decrease.

Balancing ruminal microbial activities with live yeasts used as feed additives. F. Chaucheyras-Durand[a,b], G. Fonty[b,c] ([a] Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Toulouse Cedex, France; [b] Unité de Microbiologie, INRA CR Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France; [c] Laboratoire de Biologie des Protistes, Université Blaise Pascal, 63177 Aubière Cedex, France).

Live yeasts (*Saccharomyces cerevisiae*) are more and more widely used as feed additives for ruminants. They are considered as allochtonous microorganisms in the rumen environment, however, distributed daily to dairy cows or beef cattle they can survive in the digestive tract and interact with autochtonous microbial populations. The effects and modes of action of *S.cerevisiae* I-1077 in the rumen were studied extensively. The positive effects of the yeast on bacterial and fungal species implicated in key functions in the rumen ecosystem are demonstrated, and several modes of action of live SC I-1077 were identified. SC was particularly efficient for limiting acidosis, by stabilising rumen pH and avoiding lactate accumulation. Using conventional and gnotobiotically-reared lambs, we clarify the role of SC I-1077 on ruminal microbial colonisation and the yeast impact on the set up of fermentative processes during the pre-weaning period. A more recent work was focused on the interactions between SC and rumen proteolytic bacteria. The results indicate that in co-incubations associating bacteria and live SC I-1077, proteinase activities were reduced compared to those observed in bacterial monocultures. Measurement of peptidase activities and microbial enumerations in the co-incubations suggest that live yeasts and proteolytic bacteria could partly interact in a competitive way, inducing a decrease in peptidase activities. The precise mechanisms responsible for such an effect are currently under investigation. Moreover, we observed that ammonia concentration was consistently lower in the rumen of lambs and sheep receiving SC daily, compared to control animals. Therefore, SC I-1077 may be a useful tool to control nitrogen metabolism in the rumen and prevent nitrogen loss.

How probiotics may favourably modify intestinal structural aspects in piglets at weaning. A. Di Giancamillo[a], C. Domeneghini[a], V. Bontempo[a], V. Dell'Orto[a], E. Chevaux[b], G. Savoini[a] ([a] Department of Veterinary Sciences and Technologies for Food Safety, University of Milan Via Celoria 10, 20133 Milan, Italy; [b] Lallemand SAS, 19 rue des Briquetiers, BP 59, 31702, Blagnac, France).

The main function of diet administered probiotics is to reinforce the intestinal microbial ecosystem in cases of gut stress. In farm animal species, probiotics are currently being used to improve digestive functions and health, thus allowing optimal performance. Microbial metabolism also serves as a source of energy for the intestinal mucosa, providing up to 50% of the daily energy requirements. The effects on selected histometrical aspects of the piglet ileum after weaning as consequences of: i) live yeast (Levucell® SB, CNCM I-1079, Lallemand, France: Y group), and ii) Pediococcus (Bactocell®, CNCM MA18/5M, Lallemand, France: P group) administrations with the diet were compared with control animals fed with a starter diet. Histological examination showed that the ileum of the treated piglets maintained its normal structural aspect after both types of supplementation. Histometrical analysis of the ileum of the Y and P animals resulted in an increase in villi height (V) and crypt depth (C) ($P < 0.01$), as well as in a decrease in the V:C ratio ($P < 0.01$) compared with the controls. A thicker mucous gel layer was observed in the controls than in the Y and P piglets ($P < 0.01$). These findings may signify that both live yeast and Pediococcus have potentially positive effects on the piglet intestinal mucosa. This is important in the view of food safety and consumer health. This work may contribute to focus on how probiotics locally act in a certain species, taking into account that the full efficacy of the diet treatment is strictly dependent upon the knowledge of the mechanism of action.

Interactions between probiotic bacteria and the intestinal epithelium. K.L. Madsen (University of Alberta, 536 Newton Building, AL T6G 2C2, Edmonton, Canada).

Currently, the beneficial effects exerted by probiotic bacteria may be broadly classified as those effects which arise due to activity in the large intestine and are related to either colonization or inhibition of pathogen growth; and those effects which arise in both the small and large intestine, and are related to enhancement of the host immune response and intestinal barrier function. With the demonstration that immune and epithelial cells can discriminate and respond in a differential fashion to different bacterial strains, it has become clear that the functioning of the immune system, at both a systemic and a mucosal level, can be modulated by bacterial strains in the intestine. Probiotic bacterial strains have been shown to have a protective role in both humans and animal models through varying mechanisms, including a probiotic-induced enhancement of barrier function involving alterations of tight junctional proteins and a stimulation of mucus and sIgA production. In addition, probiotic bacteria and isolated bacterial DNA from certain strains can modulate epithelial immune responses through alterations in the NF- B pathway. This effect is mediated through at least two potentially related mechanisms: first, a reduction in the translation of IL-8 mRNA through a reduction in p38 MAPK activity, and second, through an inhibition of proteasome activity. Systemic and mucosal immune function can also be modulated through consumption of either live probiotic bacteria or isolated probiotic bacterial DNA. However, it is also becoming quite clear that not all probiotic bacteria have similar therapeutic effects. Understanding of the mechanisms of action of these probiotic bacteria will permit the development of definitive criteria for the selection of probiotic strains useful for clinical application. It will also allow for the determination of optimal doses, timing of administration and synergistic combinations of bacterial species.