ABSTRACTS
OF LECTURES AND POSTERS
THE 6TH
BENEFICIAL
MICROBES
CONFERENCE
PRE- and PROBIOTICS
for LIFELONG Human
and Animal Health
9–11 October 2017
AMSTERDAM
THE NETHERLANDS
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### Key to the abstracts of lectures and posters:
- abstracts of lectures and posters are grouped separately
- lectures are grouped according to the daily programme
- posters are grouped in an alphabetical order according to the corresponding author

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PREBIOTIC FIBRES
FOR HUMAN & ANIMAL NUTRITION

Tereos’ short chain fructo-oligosaccharides (FOS) is a science-based health ingredient with consistent characteristics and reliable health benefits. It has been used in animal and human nutrition, including infant formulae, for more than twenty years.

HUMAN NUTRITION
ABOUT 200 STUDIES CONDUCTED WORLDWIDE

Ongoing research programme:
- Gut health: digestive comfort, transit
- Obesity and diabetes: glycemia, glucose tolerance, fat mass reduction
- Early life nutrition
- Immunity modulation
The EU health claim on glycemia reduction by non-digestible carbohydrates is applicable to FOS.

ANIMAL NUTRITION
ABOUT 200 STUDIES CONDUCTED WORLDWIDE

Many studies report important benefits of FOS in various species: pets, horses, rabbits, pigs, poultry, calves, aquaculture and mink. It can be used to increase the performance of livestock as well as to promote health and well-being in companion animals. The benefits of FOS come from its effects on the balance of the microbiota - which, in turn, modifies gut structure, modulates immune response, glucose homeostasis and improves performance.
WELCOME

Welcome to the 6th Beneficial Microbes Conference, 9-11 October 2017, Amsterdam, the Netherlands!

The impact of pre- and probiotics seems too diverse to be beneficial to the average man/woman as different life stages have different needs: pregnancy period; infancy and childhood; adulthood; and older adults. This applies to animals, such as pigs, poultry, cattle, horses, dogs and cats, as well. The 6th Beneficial Microbes Conference will provide an overview of the latest scientific results and future developments related to pre- and probiotics and their importance to human and animal health across the lifespan. Topics include pre- and probiotics and the gut microbiome, the gut-brain axis, the microbiome beyond the gut, and more.

High quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are conference values we want to uphold creating a platform for new initiatives for the application of beneficial microbes in food, feed, and healthcare. You are invited to take part in the discussions with participants from different disciplines and meet business relations in your area. The members of the Advisory Committee wish you an active and fruitful meeting!

On behalf of the Advisory Committee,

Dr. Koen Venema
Conference chair

ADVISORY COMMITTEE

Prof. Dr. Koen Venema, conference chair Beneficial Microbes Consultancy and University of Maastricht, the Netherlands
Dr. Frédérique Chaucheyras-Durand Lallemand, France
Prof. Dr. Richard Ducatelle Ghent University, Belgium
Dr. Emily B. Hollister Baylor College of Medicine, USA
Dr. Marjorie Koenen consultant, the Netherlands
Dr. Thomas D. Leser Chr. Hansen, Denmark
Dr. Annick Mercenier Nestlé, Switzerland
Dr. Jiro Nakayama Kyushu University, Japan
Dr. Gregor Reid University of Western Ontario, Canada
Dr. Guus Roeselers Nutricia Research, the Netherlands
Prof. Dr. Henk Schols Wageningen University & Research, the Netherlands
Dr. Elaine Vaughan Sensus, the Netherlands
## PROGRAMME AT A GLANCE

### MONDAY 9 OCTOBER 2017

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<tr>
<td>13:00</td>
<td>Opening of the <strong>6th Beneficial Microbes Conference</strong></td>
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| 13:15 – 14:15 | Plenary meeting
**Pre-/probiotics – keynote topics**                                  |
| 14:15 – 15:35 | Plenary meeting
**Pre-/probiotics and the gut**                                        |
| 15:35 – 16:00 | Networking break & poster viewing                                     |
| 16:00 – 17:45 | Plenary meeting (continued)                                           |
| 17:45 – 18:20 | Plenary meeting
**Speed presentations**                                               |
| 18:20 – 19:30 | Poster viewing & drinks                                               |

### TUESDAY 10 OCTOBER 2017

<table>
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| 08:30 – 10:15 | Parallel session 1
**Pre-/probiotics and the gut-brain axis**                             |
| 10:15 – 10:45 | Networking break & poster viewing                                     |
| 10:45 – 12:50 | Parallel session 1 (continued)                                        |
| 12:50 – 14:00 | Lunchbreak & poster viewing                                           |
| 14:00 – 15:45 | Parallel session 3
**Pre-/probiotics beyond the gut**                                        |
| 15:45 – 16:15 | Networking break & poster viewing                                     |
| 16:15 – 17:10 | Parallel session 3 (continued)                                        |
| 17:10 – 17:40 | Plenary meeting
**Speed presentations**                                               |
| 17:40 – 19:00 | Poster viewing & drinks                                               |

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| 10:45 – 12:50 | Parallel session 2
**Pre-/probiotics in productive animals**                               |
| 12:50 – 14:00 | Lunchbreak & poster viewing                                           |
| 14:00 – 15:45 | Parallel session 4
**Pre-/probiotics in animals**                                           |
| 15:45 – 16:15 | Networking break & poster viewing                                     |
| 16:15 – 17:10 | Parallel session 4 (continued)                                        |

### WEDNESDAY 11 OCTOBER 2017

<table>
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| 08:30 – 10:45 | Plenary meeting
**Pre-probiotics – health, disease and longevity**                   |
| 10:45 – 11:15 | Networking break & poster viewing                                     |
| 11:15 – 13:00 | Final plenary meeting
**Facing the future – challenges ahead**                               |
| 13:00         | Closing of the **6th Beneficial Microbes Conference**                 |

* Short presentation by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters.
CONFERENCE PROGRAMME

MONDAY 9 OCTOBER 2017

13:00 Opening of the 6th Beneficial Microbes Conference
Prof. Dr. Koen Venema – conference chair

PLENARY MEETING: PRE-/PROBIOTICS – KEYNOTE TOPICS

Chair: Prof. Dr. Koen Venema, Beneficial Microbes Consultancy and Maastricht University, the Netherlands

13:15 Microbes matter more than ever – innovating in probiotics
Dr. Johan van Hylckama Vlieg, Chr. Hansen, Denmark

13:45 Prebiotics in metabolic disorders associated with cancer and obesity
Dr. Laure Bindels, Louvain Drug Research Institute, UCL, Belgium

PLENARY MEETING: PRE-/PROBIOTICS AND THE GUT

Chair: Dr. Annick Mercenier, Nestlé, Switzerland

14:15 Chair’s introduction

14:20 Influence of pre- and probiotics on early life dynamics of infants’ large intestinal microbiota composition and activity
Prof. Dr. Hauke Smidt, Laboratory of Microbiology, Wageningen University & Research, the Netherlands

14:45 Human milk oligosaccharide consumption patterns in baby’s large intestine
Prof. Dr. Henk Schols, Laboratory of Food Chemistry, Wageningen University & Research, the Netherlands

15:10 Early benefits of a starter formula enriched in pre- and/or probiotics on the gut microbiota of healthy infants born to HIV+ mothers
Dr. Philippe Steenhout, Département femme-mère-enfant, Centre hospitalier universitaire vaudois, Switzerland

15:35 Networking break & poster viewing

16:00 Gut microbiota, probiotics, and vaccine responses in children
Dr. Katri Korpela, Department of Bacteriology and Immunology, University of Helsinki, Finland

16:25 Ecology of gut microbiota in Japanese children: possible link with pre- and probiotics research
Dr. Jiro Nakayama, Faculty of Agriculture, Kyushu University, Japan

16:50 Looking for alleviation of IBS symptoms: what about beneficial yeast?
Dr. Bertrand Rodriguez, Lesaffre Human Care, France

17:15 The human gut mycobiome – obesity and cardiovascular risk
Dr. Matilde Rodríguez Chacón, Pere Virgili Health Research Institute (IISPV), Spain

17:40 Chair’s summary
MONDAY 9 OCTOBER 2017

PLENARY MEETING: SPEED PRESENTATIONS

Chair: Prof. Dr. Koen Venema

17:45 – 18:20
Short presentations (7-minutes) by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters.

P4: Emerging sampling and microbiota analysis tools that simplify and facilitate experimental setup of clinical trials
Dr. Tom van den Bogert, BaseClear, the Netherlands

P18: The efficacy of a novel probiotic on glucose metabolism: a randomised controlled trial
Talia Palacios, The Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, The University of Sydney, Australia

P17: Efficacy of pre-selected bacilli as probiotic feed additives in F4+ Escherichia coli challenged post-weaning piglets
Dr. Bea Nielsen, Chr. Hansen A/S, Denmark

P15: The effect of additional galacto-oligosaccharide prebiotic on iron absorption with micronutrient powders in Kenyan infants
Nadja Mikulic, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland

P24: Can a commercial probiotic or a faecal transplant delay Campylobacter jejuni transmission in broiler chickens?
Prof. Dr. Paul Wigley, Institute for Infection and Global Health, University of Liverpool, UK

18:20 – 19:30
Poster viewing & drinks
TUESDAY 10 OCTOBER 2017

PARALLEL SESSION 1: PRE-/PROBIOTICS AND THE GUT-BRAIN AXIS

Chair: Dr. Emily B. Hollister, Baylor College of Medicine, USA

08:30 Chair’s introduction

08:35 Probiotics in pregnancy for anxiety and depression: a pilot randomised controlled trial
Pamela Browne, Behavioural Science Institute, Radboud University and Athena Institute, VU University, the Netherlands

09:00 Gut-brain axis in early life: a prime time for probiotics?
Dr. Marko Kalliomäki, Department of Paediatrics, University of Turku, Finland

09:25 Long-term effect of early life antibiotic and probiotic exposure on brain and behaviour
Dr. Sophie Leclercq, Institute of Neuroscience and Louvain Drug Research Institute, UCL, Belgium

09:50 Examining the role of the microbiota-gut-brain axis in the development of hypertension
Dr. Dave J. Durgan, Department of Anesthesiology, Baylor College of Medicine, USA

10:15 Networking break & poster viewing

10:45 Dietary prebiotic supplements prevent stress-evoked sleep disruptions, anxiety and gut microbial dysbiosis
Dr. Monika Fleshner, Department of Integrative Physiology, University of Colorado Boulder, USA

11:10 Microbiome-derived therapeutics, the gut-brain axis and central nervous system disorders
Dr. Helene M. Savignac, 4D Pharma Research Ltd., UK

11:35 Probiotics – a novel antidepressant? Lessons from preclinical studies
Dr. Anders Abildgaard, Translational Neuropsychiatry Unit, Aarhus University and Department of Clinical Biochemistry, Aarhus University Hospital, Denmark

12:00 Lactobacillus plantarum PS128 rescues motor deficits in MPTP mouse models of Parkinson’s disease
Prof. Dr. Ying-Chieh Tsai, Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taiwan

12:25 The gut-brain axis – knowledge and knowledge gaps
Prof. Dr. Robert Jan Brummer, School of Medical Sciences, Örebro University, Sweden

12:50 Lunch break & poster viewing
TUESDAY 10 OCTOBER 2017

PARALLEL SESSION 2: PRE-/PROBIOTICS IN PRODUCTIVE ANIMALS

Chair: Prof. Dr. Richard Ducatelle, Ghent University, Belgium

08:30  Chair’s introduction

08:35  Potential of using pre- and probiotics in early life of ruminants to program the rumen microbial ecosystem
  Dr. David R. Yáñez-Ruiz, Estación Experimental del Zaidín, CSIC, Spain

09:00  Fibrolytic potential of rumen microbiota in young lambs and impact of probiotics
  Dr. Frédérique Chaucheyras-Durand, Lallemand Animal Nutrition, France

09:25  CALIMERO, an in vitro dynamic, computer-controlled Chicken ALIMEntary tRact mOdel
  Prof. Dr. Koen Venema, Beneficial Microbes Consultancy and Maastricht University, the Netherlands

09:50  Antimicrobial activity of Bacillus – from genomic potential to effective probiotic activity in the gastrointestinal tract of broilers
  Dr. Marion Bernardeau, Danisco Animal Nutrition, DuPont Industrial Biosciences, France

10:15  Networking break & poster viewing

10:45  Bayer’s gut health solution for poultry through a novel, patented strain of Bacillus subtilis
  Dr. V.K. Shankar, Bayer Animal Health GmbH, Germany

11:10  Lactobacillus reuteri suppresses enterohaemorrhagic Escherichia coli O157:H7 in bovine ruminal fluid
  Dr. Lysiane Dunière, UMR MEDIS 454 INRA-UCA and Lallemand Animal Nutrition, France

11:35  Prevention of metritis in dairy cows by lactic acid bacteria
  Dr. Anna Aris, Department of Ruminant Production, IRTA, Spain

12:00  Effects of the probiotic Saccharomyces cerevisiae var. boulardii on behaviour, metabolism and faecal microbiota of finishing pigs submitted to heat stress
  Dr. Caroline Achard, Lallemand Animal Nutrition, France

12:25  The usefulness of the ‘omics’ tools to deeply characterise the effect of probiotic administration on the pig
  Dr. Paolo Trevisi, Department of Agricultural and Food Sciences, University of Bologna, Italy

12:50  Lunch break & poster viewing
TUESDAY 10 OCTOBER 2017

PARALLEL SESSION 3: PRE-/PROBIOTICS BEYOND THE GUT

Chair: Dr. Thomas D. Leser, *Chr. Hansen, Denmark*

14:00 Chair's introduction

14:05 The use of probiotics to modify the gut-skin axis in health and disease
Dr. Catherine O’Neill, *Centre for Dermatology Research, The University of Manchester, UK*

14:30 *Staphylococcus aureus* and the ecology of the nasal microbiota
Dr. Paal Skytt Andersen, *Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark*

14:55 How do probiotics benefit athletes?
Dr. Danica Michaličková, *Department of Pharmacology, Charles University, Czech Republic*

15:20 Effects of probiotics on sperm quality
Dr. Vanesa Robles, *Plantas de El Bocal, IEO, Spain*

15:45 **Networking break & poster viewing**

16:15 Vaginal probiotics to prevent recurrence of bacterial vaginosis
Prof. Dr. Janneke van de Wijgert, *Institute of Infection and Global Health, University of Liverpool, UK*

16:40 New generation of vaginal probiotics
Dr. Magdalena Strus, *Department of Microbiology, Jagiellonian University Medical College, Poland*

17:05 Chair's summary

PLENARY MEETING: SPEED PRESENTATIONS

Chair: Prof. Dr. Koen Venema

17:10 – 17:40
Short presentations (7-minutes) by selected poster presenters to provide an overview of their research and to inspire the audience to visit their posters.

P1: A link between human milk oligosaccharides, infant faecal community types, and risk of infection
Dr. Bernard Berger, *Nestlé Research Center, Switzerland*

P5: Monitoring intestinal immune and microbiome responses at the gut level in ruminants
Dr. Christopher Chase, *Department Veterinary & Medical Science and Department of Animal Science, South Dakota State University, USA*

P6: Adipose tissue microbiota: the new target of prebiotics in diabetic mice model
Dr. Bérengère Coupé, *Vaiomer, France*

P23: Molecular effects of GOS delivered in ovo in chickens challenged with acute heat stress
Dr. Anna Sławinska, *University of Molise, Italy and UTP University of Science and Technology, Poland*

17:40 – 19:00
**Poster viewing & drinks**
TUESDAY 10 OCTOBER 2017

PARALLEL SESSION 4: PRE-/PROBIOTICS IN ANIMALS

Chair: Dr. Frédérique Chaucheyras-Durand, Lallemand, France

14:00 Chair’s introduction

14:05 Faecal microbiota transplant in veterinary medicine: the egg of Columbus for autologous probiotics alimentary integration
Prof. Dr. Giacomo Rossi, Department of Veterinary Science, University of Camerino, Italy

14:30 Applications for pre- and probiotics to modify the canine intestinal microbiota – what is the evidence?
Dr. Silke Salavati, Hospital for Small Animals and The Roslin Institute, University of Edinburgh, UK

14:55 Relationships between gut microbiota and glucose homeostasis in obese dogs fed with scFOS or oligofructose-enriched diets
Dr. Emmanuelle Apper, Tereos, France

15:20 The use of prebiotics in a strict carnivore, the cat
Prof. Dr. Myriam Hesta, Laboratory of Animal Nutrition, Ghent University, Belgium

15:45 Networking break & poster viewing

16:15 Modulation of the equine hindgut microbiota using pre/probiotics: current knowledge, applications and perspectives
Dr. Pauline Grimm, Lab To Field, France

16:40 Probiotics in turtles – their effects on growth performance, shell mineralisation and microbiota
Mateusz Rawski, M.Sc., Department of Animal Nutrition and Feed Management, and Division of Inland Fisheries and Aquaculture, Poznań University of Life Sciences, Poland

17:05 Chair’s summary

PLENARY MEETING: SPEED PRESENTATIONS

For details, see page 8.

17:40 – 19:00
Poster viewing & drinks
WEDNESDAY 11 OCTOBER 2017

PLENARY MEETING: PRE-/PROBIOTICS – HEALTH, DISEASE AND LONGEVITY

Chair: Dr. Jiro Nakayama, Kyushu University, Japan

08:30 Chair’s introduction

08:35 Where probiotic therapy saves lives
Dr. Johanna Maukonen, DuPont Nutrition & Health, Finland

09:00 Translating microbiota knowledge to improved outcomes in the intensive care unit: where do we stand?
Dr. Joost Wiersinga, Center for Experimental and Molecular Medicine, Academic Medical Center, the Netherlands

09:25 Probiotic Saccharomyces cerevisiae and enterohaemorrhagic Escherichia coli: an effective strategy against a deadly enemy?
Dr. Stéphanie Blanquet-Diot, UMR 454 Microbiote Environnement Digestif et Santé, Université Clermont Auvergne, France

09:50 Dietary fibre, the gut microbiota and the colonic mucus barrier: implications for health and disease
Dr. Mahesh S. Desai, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg

10:15 Dietary fibres as oral adjuvant
Dr. Coen Govers, Food & Biobased Research, Wageningen University & Research, the Netherlands

10:40 Chair’s summary

10:45 Networking break & poster viewing

FINAL PLENARY MEETING: FACING THE FUTURE – CHALLENGES AHEAD

Chair: Prof. Dr. Koen Venema – conference chair

11:15 Chair’s introduction

11:20 Lactic acid bacteria convert human fibroblasts into multipotent cells
Dr. Kunimasa Ohta, Department of Developmental Neurobiology, Kumamoto University, Japan

11:45 Host-niche specialisation in the gut – clues from bacterial genomes and transcriptomes
Dr. Lisa Crossman, University of East Anglia and SequenceAnalysis.co.uk, UK

12:10 Harnessing magneto-aerotactic bacteria to deliver therapeutics in regions of active cancer cells
Prof. Dr. Sylvain Martel, Department of Computer and Software Engineering, Polytechnique Montréal, Canada

12:35 Top five lessons learned at the 6th Beneficial Microbes Conference
Prof. Dr. Koen Venema

13:00 Closing of the 6th Beneficial Microbes Conference
Take your packed lunch to eat along the way!
LECTURES

MONDAY 9 OCTOBER 2017

PLENARY MEETING
PRE-/PROBIOTICS – KEYNOTE TOPICS

MICROBES MATTER MORE THAN EVER – INNOVATING IN PROBIOTICS

Johan van Hylckama Vlieg

Chr. Hansen, Denmark
dkjohv@chr-hansen.com

This presentation will highlight some recent advances in probiotic research and development. In particular, it will highlight how:

- the elucidation of mechanisms of microbe-host interaction provides novel targets for probiotic intervention;
- the microbiome acts as a source probiotics and live bacterial therapeutics and how this may impact the probiotic industry in the future; and
- scientific and technical challenges in developing next generation probiotics will drive innovation.
PREBIOTICS IN METABOLIC DISORDERS ASSOCIATED WITH CANCER AND OBESITY

Laure B. Bindels

Louvain Drug Research Institute, Université catholique de Louvain (UCL), Belgium
laure.bindels@uclouvain.be

During this presentation, I will discuss our recent studies aiming to evaluate the role of the gut microbiota and the interest of prebiotics in malnutrition, going from overnutrition to undernutrition.

The first part of the talk will be dedicated to the systematic evaluation of the role of the gut microbiota in the health benefits conferred by a candidate prebiotic, resistant starches, in metabolic syndrome. Resistant starches (RS) improve insulin resistance in human nutritional trials and animal experiments, and these benefits are often hypothesised to be mediated through modulation of the gut microbiota. Using comparative studies performed in conventionalised and germ-free mice fed a Western diet, we demonstrate that some metabolic benefits exerted by dietary RS, especially improvements in insulin levels, can occur independently of the microbiota. This work also sets a precedent for future mechanistic studies regarding the role of the gut microbiota in mediating the benefits of other functional foods.

In the second part of the talk, I will present our recent findings on the role of the gut microbiota and the gut dysfunction in the context of cancer cachexia. Cancer cachexia is a complex multi-organ syndrome characterised by weight loss, muscle atrophy and fat browning. With a prevalence of 1 million people in Europe and only limited therapeutic options, there is a high medical need for new approaches to treat cachexia. In this context, we started a few years ago studying the therapeutic interest that prebiotics may represent. Our experimental results suggest that microbial metabolites can influence cancer progression and that nutritional modulation of the gut microbiota could constitute an interesting adjuvant therapeutic tool for cancer and associated cachexia.
Soon after birth, the gastrointestinal (GI) tract of humans and animals undergoes a rapid microbial colonisation. Diet is one of the key factors influencing the development of the GI tract microbial ecosystem in early life. High inter-individual variation in faecal microbiota composition between human infants exists, and cluster analyses revealed three different cluster types, two of which are characterised by high relative abundance of either *Bifidobacterium* or *Bacteroides*, whereas a third community type is characterised by a mixed microbial community with relatively low abundance of the aforementioned groups.

Human milk oligosaccharides (HMOs) found in breastmilk stimulate microbial communities that are dominated by *Bifidobacterium* and *Bacteroides*. Infant formulas lack HMOs, but are currently supplemented with other prebiotics such galacto- and fructo-oligosaccharides. We found that modern, prebiotic-fortified infant formulas result in higher relative abundance of faecal bifidobacteria and overall faecal bacterial community patterns that are more similar to those found in the breastfed (BF) infants, as compared to formulas without prebiotics. Although a strong direct link between specific HMOs and microbial community composition in one-month old infants could not be observed, we found that intestinal degradation of specific HMOs can be correlated with the significant increase in relative abundance of various phylotypes within the genus *Bifidobacterium*, and to a lesser extent within *Bacteroides* and *Lactobacillus*. In addition, BF infants belonging to different microbial cluster types differed in their ability to degrade various HMOs.
HUMAN MILK OLIGOSACCHARIDE CONSUMPTION PATTERNS IN BABY’S LARGE INTESTINE

Henk Schols

Laboratory of Food Chemistry, Wageningen University & Research, the Netherlands
henk.schols@wur.nl

Human milk oligosaccharides (HMOs), as the third most abundant component in human breastmilk, are health-beneficial for newborns. There are more than a hundred different structures of HMOs identified, all composed of the five monosaccharides: glucose, galactose, N-acetylglucosamine, fucose and sialic acid. The presence and abundancy of HMOs depends on the genetic profile of the mother. HMOs are indigestible by infants, but instead will enter the gut and be fermented. HMOs are selectively utilised by a number of health beneficial gut bacteria, amongst them various Bifidobacterium species. Depending on the development status of the gut, specific HMOs and HMO’s glycosidic metabolisation products might end up in infant’s faeces. However, the utilisation patterns of HMOs by infants are not fully understood yet. Therefore, the fermentation behaviour of HMOs in infant gut was investigated. The structures and quantities of HMOs and metabolites present in human milk and in faeces of the related infant were determined and correlated. There are around 250 mother-baby dyads involved in the present research, and milk/faeces samples were monitored at three time points within the period of 2-12 weeks after birth. Inter-individual differences were found in the HMO composition among different mother milk samples, as well as in the HMO profiles remaining in corresponding infant's faecal samples. By comparing the HMOs profiles in mother milk and corresponding infant faeces, the utilisation ability by the infant colon microbiota can be speculated. The utilisation ability of infants varied along the time points, and also varied among different infants at the same time point. In the near future, the difference in utilisation ability will be correlated to infant colon microbiota composition, which on their turn, will be influenced by other factors, such as, e.g., delivery mode and feeding mode.
EARLY BENEFITS OF A STARTER FORMULA ENRICHED IN PRE- AND/OR PROBIOTICS ON THE GUT MICROBIOTA OF HEALTHY INFANTS BORN TO HIV+ MOTHERS

Philippe Steenhout

Département femme-mère-enfant, Centre hospitalier universitaire vaudois, Switzerland philippe.steenhout@chuv.ch

Infants born to HIV+ mothers are at high risk to acquire HIV through mother-to-child transmission and for this reason at the onset of this infectious disease, bottle feeding was recommended in place of breast feeding. The risk of formula feeding in region with poor hygiene conditions and non-availability of safe water to prepare the bottle is well known and WHO recommend also breastfeeding in those circumstances [1]. With the development of antiretroviral therapy prescribed before birth and during breast-feeding, the risk of vertical transmission is significantly reduced. Consequently, breastfeeding should be recommended also for those infants [2]. Recent research also shows that maternal HIV infection influences the microbiome of HIV-uninfected infants [3]. Breast milk of HIV-positive mothers has potent and species-specific in vivo HIV-inhibitory activity [4]. The human milk (HM) contains specific complex oligosaccharides (HMO) and also probiotics bacteria [5]. HM is known to promote the development of a microbiome with high level of bifidobacteria. Several pathways have been tested to mimic the effects of those components of HM

**Addition of probiotics**

A meta-analysis of 5 studies with formula supplemented with *Bifidobacterium lactis* (BL) [6], among infants from HIV-positive mothers, has shown a positive effect on the weight gain. A study using BL + *Streptococcus thermophilus* showed that probiotics have immune stimulatory properties and might be helpful in the treatment of HIV-infected children [7].

**Addition of prebiotics**

Infants born from HIV mothers fed with a formula supplemented with fructo- (FOS) and galacto- (GOS) oligosaccharides were showing a normal growth as control born from non-infected mothers (breastfeeding or mix-feeding) [8].

**Addition of symbiotic**

A formula [9] containing BL and a mixture of prebiotics extracted from bovine source (BMOS) showed at 10 days a significantly higher faecal bifidobacteria counts in the test formula than in the control among infants with caesarean birth and a lower faecal pH. At 3 months, the pH of the caesarean control group was still higher than in the tests groups.

In conclusion, supplementation of infant formula with pre-and/or probiotics fed to infant born from HIV-mothers is safe, promotes a healthy grow and the development of a microbiome with high level of bifidobacteria. Future research should evaluate the effect of newly available oligosaccharides similar to HMO.

**References**

GUT MICROBIOTA, PROBIOTICS, AND VACCINE RESPONSES IN CHILDREN

Katri Korpela

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The early development of the gut microbiota is emerging as a key factor involved in the immunological and metabolic programming of the host. The microbiota development in early life can be characterised as primary ecological succession, where specific successional stages are evident. This process may be compromised when the child is born by caesarean section, given antibiotics, or fed formula instead of breast milk. These early-life microbiota-disrupting factors have been found to be associated with later immunological diseases.

A potential way to restore the natural colonisation process is supplementation with probiotic bacteria, such as lactobacilli and bifidobacteria. Using data from two double-blind placebo-controlled studies in high allergy-risk infants and in healthy day care-attending children, we showed that long-term probiotic supplementation altered the microbiota composition and function in the children. In infants, a probiotic mixture reduced C-section- and antibiotic-associated changes in the microbiota, and in the day care children, a single probiotic strain prevented some of the antibiotic-associated changes in the microbiota. These results indicate that probiotics have the potential to alleviate microbiota disturbances in children, and that it may be beneficial to select specific strains depending on the age of the child or the aim of the treatment.

As the gut microbiota are intimately involved in stimulating and controlling the host’s immune responses, we have studied the association between vaccine responses and gut microbiota composition in two cohorts of infants in Asia and Africa. In both cohorts, we found that the intestinal microbiota composition correlated significantly with rotavirus vaccine immunogenicity. This suggests that modulation of the gut microbiota may be a novel avenue for improving rotavirus vaccine responses in developing countries.
ECOLOGY OF GUT MICROBIOTA IN JAPANESE CHILDREN: POSSIBLE LINK WITH PRE- AND PROBIOTICS RESEARCH

Jiro Nakayama

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Mutualism between gut microbiota and host fully begins after birth. Thereafter, colonisation of microbes and metabolism of their community greatly influence the development of host physiology, including metabolic, nervous, and immune systems. To understand the impact of developmental microbiota in the beginning of life on later life, birth cohort studies are being performed extensively all over the world. We have also demonstrated the link between microbiota in infancy and host health in later life, notably allergy development.

We compared faecal microbial composition between one-month-old Japanese infants who later did and did not develop allergic diseases (n=11 vs. 11). Allergic infants were more commonly colonised by bacteria of the genus Bacteroides and less often colonised by bacteria of the genera Acinetobacter and Clostridium [1]. Interestingly, it has been reported that Acinetobacter isolated from a farming environment showed a potent allergy-protection effect, suggesting hygiene hypothesis [2]. Also, a comparative cohort study in Northern Europe reported that Bacteroides species were not abundant in Russian infants, but dominate in Finnish and Estonian infants who had developed allergy more frequently than Russian children [3]. Bacteroides, known as an immune-silencing microorganism may, have precluded aspects of immune education in allergic children in Japan as well as in Finland and Estonia.

We have also found an association of gut microbiota in early life with development of food allergy [4]. In the lactation period, organic acid producers, such as Leuconostoc, Weissella, and Veillonella tended to be underrepresented in subjects who developed food allergies (FA, n=14) within the first two years. Also in the weaning period, children in the FA group were highly colonised by unclassified Enterobacteriaceae and two Clostridium species closely related to Clostridium paraputrificum and C. tertium, and the whole tree phylogenetic diversity (PD) index was significantly lower in the FA group. A higher abundance of unclassified Enterobacteriaceae was also found in the other allergic group (n=15), whereas the two Clostridium species were highly specific to the FA group.

In conclusion, the timing and maybe also the dose of colonisation of each species are critical for normal development of the immune system in early life. Further studies warrant the need to develop preventive programmes for allergy diseases, such as by using pre- or probiotics.

References
LOOKING FOR ALLEVIATION OF IBS SYMPTOMS: WHAT ABOUT BENEFICIAL YEAST?

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Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder with high prevalence affecting up to 20% of the population in some geographical areas. Diverse GI symptoms occur in IBS, mainly abdominal pain, bloating and altered bowel habits. Affected people also report some extra-GI symptoms such as anxiety and depression. Facing a lack of satisfactory pharmacological treatments suitable for a long-term administration, both patients and caregivers turn to alternative methods and probiotics proved to be a promising option. Although there are some discrepancies, the role of the gut microbiota is suggested in the pathogenesis of IBS, and recently the mycobiome has been proposed to be involved as well.

Saccharomyces cerevisiae CNCM I-3856, a proprietary yeast strain from Lesaffre, showed analgesic effects in a preclinical model. Therefore, it has been studied for its beneficial properties in alleviating GI symptoms in two randomised placebo controlled clinical trials (RCT) involving together a total of 579 unselected IBS subjects. In both trials, the primary outcome was the decrease of the abdominal pain/discomfort score expressed on a Likert scale and secondary endpoints were other IBS symptoms scores, e.g., bloating, bowel movement difficulty, and stool consistency. In the first trial, the percentage of subjects who experienced a significant decrease of their abdominal pain/discomfort score for at least 2 out of the 4 last weeks of the study was significantly higher in the probiotic group compared to the placebo group. In the second RCT, a significant improvement of GI symptoms (pain and bloating) was reported in the probiotic group compared to the placebo group in the constipated (IBS-C) subpopulation, suggesting that S. cerevisiae CNCM I-3856 may be more effective in alleviating IBS pain in this specific patient category. A recent individual patient data meta-analysis has confirmed that S. cerevisiae CNCM I-3856 significantly improve abdominal pain/discomfort in both overall IBS population and IBS-C subpopulation after 2 months of treatment. Moreover, bloating and stool consistency were significantly improved in IBS-C volunteers consuming the probiotic as compared to placebo.

All together, these data support a promising role for S. cerevisiae CNCM I-3856 as an alternative for clinicians looking for new opportunities of natural treatments on a long-term perspective. Beyond these promising clinical results, the meta-analysis highlighted the fact that one specific strain may be more active on a specific sub-population, in particular when addressing disorders, such as IBS, with strong intra- and inter-individual variability. In addition, such analysis is of great support for the design of further clinical studies.
THE HUMAN GUT MYCOBIOME – OBESITY AND CARDIOVASCULAR RISK

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The human intestine is home to a diverse range of bacterial and fungal species, forming an ecological community that contributes to normal physiology and disease susceptibility. Recent findings disclosed a specific fungal microbiota profile (mycobiome) of obese subjects. A specific fungal composition could also distinguish metabolically ‘healthy’ from ‘unhealthy’ obesity. The relative abundance of the phylum Zygomycota, comprising the family Mucoraceae and genus Mucor, was negatively associated with carotid atherosclerosis (cIMT) even after controlling for false discovery rate. Clusters according to genus abundance co-segregated with body fatness, fasting triglycerides and HDL-cholesterol. Obese subjects with detectable Mucor spp. had a similar cardiovascular risk profile as non-obese subjects. Interestingly, the relative abundance of Mucor racemosus was negatively associated both with the Framingham Risk Score and cIMT. Partial least square discriminant analyses modelling, evaluating the potential relevance of gut mycobiota in patients stratified by mean values of cIMT, showed that even a 1 component model had a high accuracy (0.789), with a high $R^2$ value (0.51). Variable importance in projection scores showed that M. racemosus abundance had the same impact in the model as waist-to-hip ratio, HDL-cholesterol, fasting triglycerides or fasting glucose, suggesting that M. racemosus relative abundance in the gut may be a relevant biomarker for cardiovascular risk. M. racemosus and M. fuscus were the species more represented in non-obese subjects compared to obese counterparts. The decreased relative abundance of the Mucor genus in obese subjects was reversible upon weight loss. Some metabolites, such as hexadecanedioic acid, caproic acid and N-acetyl-L-glutamic acid, were linked to the mycobiome. In summary, the mycobiota composition is associated with obesity and cardiovascular disease.
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Plenary Meeting
Speed Presentations

Short presentations by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters.

P4
Emerging sampling and microbiota analysis tools that simplify and facilitate experimental setup of clinical trials
Tom van den Bogert
BaseClear, the Netherlands

P18
The efficacy of a novel probiotic on glucose metabolism: a randomised controlled trial
Talia Palacios
The Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, The University of Sydney, Australia

P17
Efficacy of pre-selected bacilli as probiotic feed additives in F4+ Escherichia coli challenged post-weaning piglets
Bea Nielsen
Chr. Hansen A/S, Denmark

P15
The effect of additional galacto-oligosaccharide prebiotic on iron absorption with micronutrient powders in Kenyan infants
Nadja Mikulic
Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland

P24
Can a commercial probiotic or a faecal transplant delay Campylobacter jejuni transmission in broiler chickens?
Paul Wigley
Institute for Infection and Global Health, University of Liverpool, UK
PROBIOTICS IN PREGNANCY FOR ANXIETY AND DEPRESSION: A PILOT RANDOMISED CONTROLLED TRIAL

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Maternal prenatal depression or anxiety are risk factors for adverse health and behaviour outcomes in offspring. With prevalence rates of prenatal depression or anxiety ranging between 10-20%, attempts to identify feasible and effective interventions to reduce symptoms are priority in the prenatal care and clinical setting. This ongoing pilot study (n=40) evaluates the feasibility of probiotic food supplement intervention in pregnant women, as an adjuvant therapy, to reduce prenatal depression and anxiety. Our study is based on the fact that there are indications that probiotics, as a food supplement, by improving the intestinal microbiota, can improve mental well-being. Indeed, several human clinical studies in non-pregnant populations demonstrated that ingestion of a probiotic mixture can decrease levels of anxiety and depression in humans [1-3]. Additionally, the probiotic mixture used in this study has been shown to significantly reduce negative thoughts associated with sad mood in healthy adults [4].

Three general pathways have been described on how the gut microbiota influences anxiety and depression via the microbiome-brain axis: through low-grade inflammation, neurotransmitter signalling, and the HPA-axis [5-7]. Probiotics, with their anti-inflammatory and neuroregulatory properties, may improve intestinal microbiota in pregnant mothers and consequently their mood. Additionally, prenatal ingestion of probiotics by mothers may improve their vaginal microbiota, which may in turn positively influence their offspring’s developing microbiota [8,9].

References
THE GUT-BRAIN AXIS IN EARLY LIFE – A PRIME TIME FOR PROBIOTICS?

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Enteral and central nervous systems develop postnatally in parallel with the evolution of gut microbiota. Experimental studies demonstrate that gut microbiota impact on the development and function of the nervous systems in many ways, thus suggesting microbial modulation as a potential tool for intervention in human diseases related to the gut-brain axis. Indeed, infant colic is such a disorder in which duration of crying has significantly been diminished by probiotics. Moreover, probiotic intervention has been successful in the prevention of social behavioural deficits in a murine model of autism spectrum disorder. The potential role of dysbiosis in autism spectrum disorder is further supported by alterations of gut microbiota found in children with the disorder. In addition, our preliminary finding demonstrates that early probiotic supplementation may reduce the risk of later development of neurodevelopmental disorders including autism spectrum disorder and attention deficit hyperactivity disorder. Thus, early probiotic intervention may offer substantial health benefits for the gut-brain axis disorders.
LONG-TERM EFFECT OF EARLY LIFE ANTIBIOTIC AND PROBIOTIC EXPOSURE ON BRAIN AND BEHAVIOUR

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Antibiotics (AB) are the most frequently dispensed drugs in paediatric patients and there is currently increasing concern that AB exposure early in life may have long-term consequences for health. Epidemiological studies revealed that early life AB exposure increases the risk of developing allergies, inflammatory bowel diseases and obesity. The effects of AB on brain and behaviour have been demonstrated by using very high doses of a cocktail of antibiotics administered to adolescent or adult rodents.

In this study, we investigated the long-term effects of clinically relevant dose of penicillin administered early in life on gut, brain and behaviour, in both male and female mice. Pregnant dams received penicillin V in drinking water 1 week before pups’ delivery and until weaning. Penicillin is absorbed by the gastrointestinal tract, crosses the placenta and is found in the breast milk. The pups therefore received penicillin in utero and during the first 3 weeks of life while nursing. At weaning, pups were separated from the mothers and received regular drinking water. At 6-weeks old, the offspring was subjected to a battery of behavioural tests and gut (ileum, colon) and brain (hippocampus, frontal cortex) tissues were collected after the last test. We found that early life AB exposure had lasting effects on gut microbiota composition, modified the tight junctions of blood-brain barrier, induced inflammation in the frontal cortex and was associated with changes in brain neurochemistry. Also, AB-treated mice exhibited decreased anxiety-like behaviour, reduced social behaviour, reduced preference for social novelty as well as an unexpected aggressive behaviour. Supplementation with \textit{Lactobacillus rhamnosus} JB-1 during AB treatment restored certain biological and behavioural parameters. These results warrant further studies on the potential role of early-life antibiotic use in the development of neuropsychiatric disorders, and the possible attenuation of these by beneficial bacteria.
EXAMINING THE ROLE OF THE MICROBIOTA-GUT-BRAIN AXIS IN THE DEVELOPMENT OF HYPERTENSION

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Obstructive sleep apnoea (OSA) is an independent risk factor for systemic hypertension, and the most common underlying cause of resistant hypertension. The importance of a healthy gut microbiota on host physiology is becoming increasingly evident.

We have shown that gut dysbiosis plays a causal role in the development of OSA-induced hypertension. The mechanisms linking gut dysbiosis to hypertension are unknown. We tested the hypothesis that OSA-induced dysbiosis leads to gut barrier dysfunction, systemic inflammation, and neuroinflammation, which is linked to hypertension. We exposed rats to 2 weeks of sham or OSA (60 apnoeas/h). OSA led to a >100-fold increase in TNF-α expression in the cecum wall (n=5, \( P<0.001 \)), and decreased goblet cells/crypt (8.4 vs. 11.4; \( n=6, P<0.05 \)). Consistent with gut barrier dysfunction and bacterial translocation, we found bacterial 16S rRNA in adipose tissue, as well as a 4-fold increase in adipose IL-6 mRNA expression following OSA (n=4-7, \( P<0.05 \)). Flow cytometric analysis revealed a decrease in the percentage of Treg cells in the brain of OSA vs. sham rats (0.08% vs. 0.25%; \( n=4-6, P<0.05 \)). In addition, the percentage of activated microglia was increased following OSA (20% vs. 10%; \( n=4-6, P<0.05 \)). Next, we treated sham and OSA rats with a prebiotic (20% resistant starch diet) or probiotic (C. butyricum; \( 10^9 \) cfu gavage every three days) to increase short chain fatty acids, important in maintaining gut barrier integrity and regulating immune responses. Pre- and probiotic prevented OSA-induced loss of goblet cells and TNF-α expression in the cecum. Compared to control rats, pre- and probiotic increased the percentage of Treg cells in the brain of OSA rats by 10- and 5-fold, respectively (n=4-6, \( P<0.05 \) for each). Additionally, pre- and probiotic prevented OSA-induced activation of microglia (n=4-6). Importantly, pre- and probiotic prevented OSA-induced hypertension (prebiotic sham=158.5 mmHg vs. OSA=160.3 mmHg, probiotic sham=147.4 mmHg vs. OSA=145.6 mmHg; \( n=6-7, NS \)). These data demonstrate a causal role for gut dysbiosis in the development of hypertension that involves gut barrier disruption, bacterial translocation, and neuroinflammation. Manipulation of the gut microbiota may serve as a novel therapy in the prevention of hypertension.
DIETARY PREBIOTIC SUPPLEMENTS PREVENT STRESS-EVOKED SLEEP DISRUPTIONS, ANXIETY, AND GUT MICROBIAL DYSBIOSIS

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Repeated, chronic or extreme stressor exposure can negatively impact health; and individuals differ in their vulnerability to stress. Although some factors that produce differences in stress vulnerability cannot be changed (e.g., age and genetics), others can. There is, for example, evidence that physical activity, nutrition, and probiotic gut microbes can reduce stress vulnerability and promote a stress robust phenotype. I will present published and preliminary results from a series of recent pre-clinical studies that support the hypothesis that gut microbial modulating nutrients reduce the negative impacts of stressor exposure on mental and physical health and promote a stress robust phenotype.

Juvenile or adult, male rats were fed diets that contained in combination or isolation the following nutrients: lactoferrin, galacto-oligosaccharide, polydextrose, and/or bioactive milk fractions. These nutrients were chosen because they promote the growth and metabolic function of probiotic gut microbes, such as *Lactobacillus* spp., that are linked to positive health. Rats were exposed to either an acute extreme stressor (100, 1.5mA, 5 s, tail shocks) or a repeated stressor (chronic circadian disruption-weekly light/dark reversal). These established animal stress models produce several negative health consequences including anxiety-like behaviour (exaggerated fear), depressive-like behaviour (shuttle box escape failure), sleep disruptions (*in vivo* biotelemetry EEG), gut microbial dysbiosis (reduced alpha diversity), and inflammatory proteins elevations (sterile inflammation). Faecal gut microbial ecology was assessed using selective bacterial culture and/or 16S rRNA sequencing; and faecal metabolites were measured using untargeted mass spectrophotometry. We found that compared caloric/macronutrient matched control diet, rats fed gut microbial modulatory diets for 4 weeks prior to acute extreme acute stressor exposure displayed reduced or no anxiety- and depressive-like behaviours, gut microbial dysbiosis, and sterile inflammation. Both acute extreme stress and chronic circadian disruption produced changes in sleep. Acute extreme stress produces an initial reduction in paradoxical (REM) sleep, followed by increase in REM or ‘REM rebound’. Compared to caloric/macronutrient control diet, rats fed gut microbial modulatory diets had increased REM rebound after acute extreme stress, a highly adaptive response. Chronic circadian disruption increased wakefulness and fragmented REM sleep bouts during the sleep cycle. Gut microbial modulatory diet prevented this effect. Gut microbial modulatory diet had limited impact on the microbial ecology of abundant gut bacterial phyla, but did impact less abundant bacteria and increased *Lactobacillus* spp., and *Lactobacillus rhamnosus*. Gut microbial modulatory diet significantly impacted the faecal metabolome, and several identified metabolites (using GNPS), were associated with sleep changes. Finally, gut microbial modulatory diets impacted brain systems responsible for stress-induced anxiety and depression. Using *in situ* hybridisation, gut microbial modulatory diets reduced acute extreme stress-evoked activation of the dorsal raphe nucleus, and protected down-regulation of DRN 5HT1Ar. Interestingly, constraint of DRN activation was associated with diet-induced increases in faecal *Lactobacillus* spp. Thus, lactoferrin, prebiotic fibre, and bioactive milk fractions are dietary nutrients that support the growth and metabolic function of health promoting gut bacteria, and reduce the negative impacts of stressor exposure on mood, sleep, gut dysbiosis, and sterile inflammation.

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References
MICROBIOME-DERIVED THERAPEUTICS, THE GUT-BRAIN AXIS AND CENTRAL NERVOUS SYSTEM DISORDERS

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There is a high unmet need for novel therapeutic intervention strategies for brain-related disorders. Recent research points towards targeting bidirectional communication in the microbiome-gut-brain axis to help alleviate, and even treat, a variety of central nervous system (CNS) disorders. A promising strategy is to use microbiome-derived live biotherapeutic products (LBPs) to manipulate the gut-brain axis. LBPs are a revolutionary new class of medicines, consisting of single strains of gut commensal bacteria isolated from healthy human donors. We have undertaken a vast programme of research to explore the therapeutic effects of LBPs on neuroinflammatory, neurodevelopmental and neurodegenerative disorders. LBPs were selected from our proprietary MicroRx platform. Both host cellular responses and microbiological properties were investigated to match key physiological readouts of disease. Candidate strains were tested in animal models of multiple sclerosis (MS), autism spectrum disorder (ASD) and Parkinson's disease (PD). Animals were dosed daily with LBPs throughout the course of the studies. Clinical signs, disease severity and body weights were monitored throughout. Behavioural testing was conducted to assess disease-specific impairments (e.g., motor or cognitive impairments following PD induction).

The transgenic BTBR model was used to test the effects of MicroRx strains on autism development. Mice underwent behavioural testing relevant to ASD-like symptoms, specifically targeting social, anxiety and stereotype-related behaviours. The candidate strain MRx1234 induced improvements on anxiety and motor-related behaviours. In the Experimental Autoimmune Encephalomyelitis (EAE) model of MS, the strain MRx0002 reduced disease severity (clinical scores and disease-associated weight loss). This was coupled with a reduction in inflammatory loci and immune markers in the periphery. Finally, ongoing preclinical studies have highlighted several LBP candidates that affect PD signalling pathways and produce metabolites with relevance for gut-brain axis communication.

In summary, we have identified a panel of live biotherapeutic strains that can specifically target clinically-relevant and translational markers of disease in neuroinflammatory, neurodevelopmental and neurodegenerative disorders. Our data suggests that LBPs have a positive impact on CNS diseases, in a strain-dependent manner, and that these products may offer new treatment perspectives.
PROBIOTICS – A NOVEL ANTIDEPRESSANT? LESSONS FROM PRECLINICAL STUDIES

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The gut microbiota has recently emerged as an important regulator of brain physiology and behaviour in animals, and ingestion of certain bacteria (probiotics) therefore appear to be a potential treatment for major depressive disorder (MDD). Also, we have previously shown that depressive-like behaviour in rats may hold a dysmetabolic component. The gut bacteria are believed to affect the brain through interactions with the immune system and hypothalamic-pituitary-adrenal (HPA) axis and through their release of metabolites into the host circulation. In a series of pre-clinical studies, we therefore aimed at evaluating the potential of probiotic treatment as a novel anti-depressant and further studied how probiotics could interfere with the plausibly involved mechanisms.

Outbred Sprague-Dawley (SD) rats were used in addition to selectively bred Flinders Sensitive Line (FSL) rats that inherently display an increased level of depressive-like behaviour. A mix of eight bacterial species (Lactobacillus, Lactococcus and Bifidobacterium strains) was chosen as probiotic intervention, and a high-fat diet (HFD), consisting of 60 kJ-% animal-derived fat, was used as a metabolic stressor. Depression-related behaviour was evaluated, and HPA axis regulation and immunological markers were examined. Finally, the plasma metabolome was analysed to identify metabolites associated with probiotics. Probiotic treatment reduced depressive-like behaviour in SD rats independently of diet, while leaving FSL rats on control diet completely unaffected. In addition, probiotic treatment protected against a pro-depressant like effect of HFD in FSL rats. These behavioural findings were highly equivalent to changes in T lymphocyte CD4/8 ratios in the brains only. The metabolomics revealed an increased level of a potential neuroprotective microbial metabolite indole-3-propionic acid (IPA) associated with probiotic ingestion. Finally, probiotics and HFD had a major impact on hippocampal HPA axis regulation.

These findings clearly support the novel concept of ‘psychobiotics’ and lend inspiration to further studies into probiotics as a potential antidepressant treatment. Furthermore, our results suggest that MDD may indeed hold a dysmetabolic component that potentially involves the gut microbiota. The underlying mechanisms may involve an altered regulation of the immune system and HPA axis as well as the plasma metabolome.
**LACTOBACILLUS PLANTARUM PS128 RESCUES MOTOR DEFICITS IN MPTP MOUSE MODELS OF PARKINSON’S DISEASE**

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Parkinson’s disease (PD) is a common neurodegeneration disease caused by dopaminergic neuron degeneration in brain. The dopamine signalling collapsed and resulted in motor deficits like shaking, difficulty with walking and gait in PD patients. Recent studies have revealed that gut microbiota influence neurodevelopment, modulate behaviour, and contribute to neurological disorders through the microbiome-gut-brain axis (MGBA). Certain probiotics strain, or ‘psychobiotics’, even showed unique psychotropic effects in many animal studies and clinical trials. Lately, we found a special psychobiotic *Lactobacillus plantarum* PS128 which improved dopamine transmission in brain specific regions and modulated behaviours in different mice models, raising the possibility that PS128 might show beneficial effect on host’s CNS dopamine system through MGBA.

In this study, we used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to establish a PD-like mice model to investigate whether PS128 also show neuroprotective effect on host’s CNS dopamine system. PS128 was oral administered to mice for 4 weeks before 5-day MPTP injection. We found PS128 significantly improved the pole test, narrow beam test, and rotarod test performance, indicating that it rescued MPTP-induced motor deficits. Further brain tissue analyses showed that PS128 prevented MPTP-induced dopaminergic neuron death in substantia nigra and rescued dopamine and noradrenaline total level in striatum. In conclusion, PS128 could prevent MPTP-induced motor deficits, dopaminergic neuron death, and neurotransmitter signalling collapse. PS128 might show neuroprotective potential on host’s CNS dopamine system leading to clinical application for treating and preventing PD or other dopamine-related neuropsychiatric disorders.
THE GUT-BRAIN AXIS – KNOWLEDGE AND KNOWLEDGE GAPS

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In addition to the clear role of nutrition and microbiota in primary intestinal disorders, as well as the role of the brain in affecting gut function, there is increasing evidence that certain aspects of brain function are affected by gut function, which in turn is affected by its intraluminal nutritional and microbial composition. This bidirectional relationship between gut and brain function is strengthened by findings of aberrant gut microbial composition in various gastrointestinal as well as behavioural and psychiatric disorders.

This lecture will focus on how basic knowledge on the nutrition/microbe-gut-brain axis can be transformed to health-improving applications. The prerequisite for this innovation process is knowledge about the ‘Mode-of-Action’ (MoA) of nutritional and/or microbial interventions in the human setting. Generally, there is a substantial lack of these methods and development is mainly based on in vitro and animal studies. One specific MoA, i.e. modulation of intestinal barrier function, will be taken as an example and various human approaches to elucidate this mechanism will be discussed. Novel developments within ‘-omics methodology’ will be highlighted.

Traditional pharmaceutic paradigms are traditionally applied in trying to prove the efficacy of nutritional and/or microbial interventions. The lecture will discuss the obvious differences between pharmaceutical compounds and nutritional/microbial bioactives, as well as its consequences. Finally, future research and development challenges filling the knowledge gaps and applying the results will be presented.
The gastrointestinal tract of ruminants is host to a diverse microbial ecosystem that can vary depending on both host genetic and environmental factors. Studies have shown that even minor shifts in these populations can have significant impacts on livestock nutrition and productivity. Recent work has reported that the microbiome structures naturally occurring in the rumen are highly correlated with and predictive of the feed efficiency phenotype of an animal [1].

The rumen is quickly colonised by all type of microorganisms straight after birth and the colonisation pattern may be influenced by several factors, such as presence/absence of adult animals, the first solid diet provided, and the inclusion of compounds that prevent/facilitate the establishment of some microorganisms or the direct inoculation of specific strains. Recent studies suggested that it is possible to promote different microbial populations establishing in the rumen of the young animal by manipulating the feeding management early in life that persisted in later life [2]. This would create differences in adaptive capacity due to different early life experiences, leading to the idea of ‘microbial programming’. The work conducted in our team and collaborations with other research groups shows substantial effects of altering the weaning management of young animals, using probiotics (bacteria, yeast or rumen fluid) and prebiotics (synthetic compounds and essential oils). The main effects relate to speeding rumen development, absorption capacity, enhancing the establishment of desirable microbial groups and improving the long-term efficiency of the animal. However, despite this significant research effort, there is still a lack of understanding of the mechanisms governing microbial/host cell interactions, the immune factors involved, the development of the rumen and its microbial community, and the implications for the host when microbial colonisation patterns are altered, especially the long-term effects. The latest results obtained in this research area and the main limitations to overcome in future will be presented and discussed.

References
FIBROLYTIC POTENTIAL OF RUMEN MICROBIOTA IN YOUNG LAMBS AND IMPACT OF PROBIOTICS

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The rumen harbours a great diversity of microorganisms, comprising prokaryotes (bacteria, archaea) and eukaryotes (protozoa, fungi), which cover essential functions for their host. Part of these microorganisms is specialised in the degradation of plant polysaccharides and thereby constitutes a keystone community for the energy supply to the host. Overall, the genomes of fibrolytic microorganisms harbour hundreds of genes encoding carbohydrate active enzymes (CAZymes), mainly glycoside hydrolases (GH) and carbohydrate esterases (CE) that act synergistically to deconstruct dietary cellulose and hemicelluloses.

In young animals, the rumen microbiota establishes from birth. The factors influencing microbial colonisation of the rumen are not well known, but it is believed that repeated contacts with the dam or older animals ensure optimal inoculation of functional microbes. Separation of newborns from the dam and distribution of milk replacer could impair rumen microbial colonisation in early life, which would affect the rumen function and notably the fibre degradation process, which would ultimately impact animal health and performance. In this context, feed additives, such as probiotics, could be used as a strategy to optimise rumen microbial establishment. To this aim, a study was carried out in 16 newborn lambs separated from the mothers before 24 h of life and allocated to two groups, control and probiotic. The probiotic supplement was a specific combination of a live yeast additive (S. cerevisiae CNCM I-1077) and yeast metabolites, and was distributed through the milk replacer and the starter feed, and lambs were weaned at 42 days of age. Rumen samples were collected from birth to 56 days of age to analyse microbiota establishment by qPCR and DNA sequencing technology, and data presented at the 5th Beneficial Microbes Conference in 2016 have shown that probiotic treatment was beneficial for promoting establishment of fibrolytic bacteria, fungi and protozoa. Samples collected at 56 days were also analysed using an in house-developed DNA microarray targeting CAZyme families known to contain very efficient cellulases and hemicellulases, and present in the major rumen fibrolytic microorganisms. The current version of the DNA microarray allows the detection of 394 genes and it targets the coding sequence of catalytic domains from 8 CAZyme families involved in cellulose and hemicellulose degradation (i.e., glycoside hydrolases GH5, GH9, GH10, GH11, GH43 and GH48, and carbohydrate esterases CE1 and CE6). Here, we present the DNA hybridisation data obtained from 4 lambs of each group, which indicate different GH and CE gene profiles between the two groups, suggesting that lambs supplemented with probiotics harbour a greater fibrolytic potential that is key to the expression of efficient fibre digesting capacities.
Over the past few decades, research on the beneficial effects of prebiotics and probiotics (and their combination) has continued to increase, despite disappointing results regarding claim approval by regulatory bodies. It has become clear that the host microbiota is important for health and disease, and is probably involved in practically every disease and disorder that can affect the host, from gastrointestinal infections to weight gain to disorders of the brain.

Pre- and probiotics are important throughout the lifespan of the host, including farm animals. In early life they may prevent infections, in adulthood they could influence body weight. Their role in some of these diseases and disorders is not yet fully recognised, and certainly the mechanisms are unclear. Validated in vitro models to study these mechanisms are greatly needed to progress the research field. We have modified the TNO in vitro models of the gastrointestinal tract with parameters from the literature such that it mimics chickens and have named this system CALIMERO, for Chicken ALIMEntary tRact mOdel. Initial experiments in the upper GI tract model pertained to starch and fat digestion. Next experiments are planned to study survival of probiotics. The colon model is used for microbiota research.

This contribution will give an overview of the recent development of CALIMERO, which can be used to study the benefits of pre- and probiotics for lifelong health of the host.
ANTIMICROBIAL ACTIVITY OF *Bacillus* – FROM GENOMIC POTENTIAL TO EFFECTIVE PROBIOTIC ACTIVITY IN THE GASTROINTESTINAL TRACT OF BROILERS

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No abstract available.
**BAYER’S GUT HEALTH SOLUTION FOR POULTRY THROUGH A NOVEL, PATENTED STRAIN OF *BACILLUS SUBTILIS***

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The concept of probiotics usage in poultry attracted limited attention for long time due to the inherent limitations of probiotics, such as stability in feed pelleting temperatures, stability inside the gut, ability to exert antibacterial effects on pathogens, and overall consistent performance in different kinds of farm settings. Of late, the raising pressure on cost of production coupled with increase in gut health issues in livestock warrants the need to look at reliable, proven and cost-effective solutions to maintain gut health to enhance productivity.

BAYER has been very successful in crop protection business for a long time with a range of biopesticides developed from a unique strain of *Bacillus subtilis* viz., QST 713, which is screened and selected from a huge library of isolates. QST 713 strain has a patent for enhancing productivity in poultry and it overcomes all the limitations of conventional probiotics. Unlike most probiotic products, QST 713 strain is produced at a biopharmaceutical plant that has the highest quality and manufacturing standards, assuring consistency. QST 713 strain of *B. subtilis* is known for its stability in feed pelleting temperatures, stability in the gut and importantly proven data in the laboratory in valid challenge models as well as in field conditions against necrotic enteritis in broilers. QST 713 strain has 50% more antibacterial genes as compared to other *Bacillus* and the specific category of antibacterial metabolites from this strain have been identified. In the last 6 years, QST 713 strain is available as feed premix under the brand name Grobig BS delivering value to poultry producers across the globe (USA, Asia, Latin America and Middle East) and is expected to serve the swine industry as well in the future.
**LACTOBACILLUS REUTERI SUPPRESSES ENTEROHAEMORRHAGIC ESCHERICHIA COLI O157:H7 IN BOVINE RUMINAL FLUID**

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The bovine gastrointestinal tract (GIT) is the main reservoir for enterohaemorrhagic *Escherichia coli* (EHEC) responsible for foodborne infections. Therefore, it is crucial to develop strategies, such as EHEC suppression by antagonistic microorganisms, to reduce EHEC survival in the GIT of cattle and to limit shedding and food contamination.

Most human-derived *Lactobacillus reuteri* strains produce hydroxypropionaldehyde (HPA), an antimicrobial compound, during anaerobic reduction of glycerol. The capacity of *L. reuteri* LB1-7 to produce HPA and its antimicrobial activity against EHEC FCH6 were evaluated in bovine rumen fluid (RF) under strict anaerobiosis. EHEC was totally suppressed when incubated in RF inoculated with *L. reuteri* LB1-7 and supplemented with 80 mM glycerol. The addition of LB1-7 or glycerol alone did not modify EHEC survival in RF. Glycerol was converted to HPA (up to 14 mM) and lactate (30 mM) by LB1-7, but only HPA production seemed to be responsible for EHEC suppression. Furthermore, *L. reuteri* LB1-7 did not have any major adverse effects on ruminal digestion of common feedstuffs. The bactericidal activity of *L. reuteri* LB1-7, the concentration of glycerol required, and the level of HPA secreted depended on physiological and ecological environments. *In vitro* experiments also showed that EHEC inoculated in rumen fluid and exposed to *L. reuteri* and glycerol had a very limited growth in rectal contents.
PREVENTION OF METRITIS IN DAIRY COWS BY LACTIC ACID BACTERIA

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About 40% of dairy cattle develop uterine disease following parturition and in turn hampering fertility in most instances. Some studies indicate that uterine infection, predominantly caused by Escherichia coli in the first week postpartum, is associated with metritis, a uterus inflammation in which the cow fails to completely clear bacterial contaminants. The aim of this study was to evaluate the potential of four lactic acid bacteria (LAB) (Lactobacillus rhamnosus, Pediococcus acidilactici, L. reuteri, and L. sakei) to modulate E. coli infection and inflammation in primary endometrial cells. A combination of LAB was selected and tested in vitro, ex vivo and in vivo with 135 cows to evaluate their potential impact on prevalence of metritis and endometrium inflammation. The treatment groups of in vivo experiment were: (1) two intravaginal doses of LAB per week during 3 weeks pre-calving (VAG); (2) one intra-uterine dose 1 day after calving (END); and (3) no intervention (CON). Metritis was diagnosed 6 days after calving considering cows as affected when body temperature >39.5°C and purulent vaginal discharge (>50% pus or worse) was observed. Endometrial biopsies were taken from 30 cows, 15 from CON and 15 from the END group to assess the expression of pro-inflammatory markers at days 1, 3, and 6 after calving.

L. sakei and L. reuteri had a positive effect (P<0.001) preventing E. coli infection in primary endometrial cultures (87% and 78%, respectively); however, they were also associated to a dose-variable effect on tissular inflammation that could further exacerbate the pro-inflammatory status of the cows. Infection of E. coli was clearly reduced (P<0.001) up to an 83% with P. acidilactici, whereas, the expression of pro-inflammatory cytokines CXCL8 and IL1β dropped significantly (P<0.001) up to 85.11- and 5.24-fold, respectively, in the presence of L. rhamnosus. The combination of L. rhamnosus, P. acidilactici, and L. reuteri at a ratio of 25:25:2, respectively, reduced E. coli infection in vitro in the presence (89.77%) or absence of basal tissue inflammation (95.10%) compared with single LAB strains. LAB treatment reduced CXCL8 and IL1β expression 4.7- and 2.2-fold, respectively, under acute inflammation. Ex vivo, the tested LAB combination reduced acute inflammation under E. coli infection, decreasing secretion of IL-8, IL-1β, and IL-6 up to 2.2-, 2.5-, and 2.2-fold, respectively. In the in vivo experiment, VAG treatment reduced metritis prevalence up to 62% compared with the CON group. However, prevalence of metritis did not differ between END and CON group. Both, END and VAG treatments reduced neutrophil activity, but the expression of pro-inflammatory markers did not differ between END and CON cows. Metritic cows expressed more CXCL8 and IL1β in the uterus 3 days post-calving than healthy cows, whereas healthy cows expressed more CXCL8 at day after parturition. This study shows a promising potential of LAB probiotics as a preventive treatment against metritis in dairy cows.
EFFECTS OF PROBIOTIC SACCHAROMYCES CEREVISIAE VAR. BOULARDII ON BEHAVIOUR, METABOLISM AND FAECAL MICROBIOTA OF FINISHING PIGS SUBMITTED TO HEAT STRESS

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In pigs subjected to heat stress, decreased feed intake is an essential mechanism to maintain body temperature while metabolic adjustments have negative consequences on growth performance. This study assessed the effects of dietary Saccharomyces cerevisiae var. boulardii (CNCM I-1079) supplementation on performance, energy metabolism and faecal microbiota of finishing pigs housed in thermoneutrality condition or during thermal acclimation at high ambient temperature.

Twelve finishing male pigs were assigned to two dietary treatments: without or with S. cerevisiae var. boulardii (SCB) at a dose of 10⁹ cfu/kg. After 2 weeks of acclimation to the diet, each pig was individually housed in respiratory chamber, exposed to 22°C for 7 days (P1), and thereafter to 28°C for 2 periods of 7 and 6 days (P2 and P3). Feed intake, feeding behaviour parameters, nitrogen and energy balance and components of heat production (HP) were measured for each period. Faecal samples were collected at the end of each period. Microbiota were characterised by sequencing the V3-V4 region of the 16S rRNA gene. The increase in ambient temperature from P1 to P2 was associated with decreased dry matter intake (DMI) in non-supplemented pigs. Growth performance was improved in supplemented pigs (P=0.03). This was associated with an increased DMI (from 2.26 to 2.65 kg/day on average over the 3 periods), a tendency for an increased number of meals (P=0.06) and a lower feeding rate. Metabolisable energy intake and total HP gradually decreased from P1 to P3 (16 and 12%, respectively). Metabolisable energy intake was higher when diet was supplemented with SCB whereas HP was not significantly affected. Energy retention decreased under heat stress condition but was higher in supplemented pigs. Faecal microbiota diversity was neither changed under heat stress challenge nor by SCB supplementation. Heat stress challenge was associated with increased Spirochaetaceae and Christensenellaceae abundances, while Prevotellaceae and Lachnospiraceae abundances were decreased (P<0.05). SCB supplementation had no significant effect on microbiota at the family level. However, both ambient temperature conditions and dietary SCB supplementation influenced microbiota at the OTU (operational taxonomic unit) level (P=0.001 and P=0.07, respectively). Using a discriminant analysis (sPLS-DA), we pointed out 21 OTUs that contribute to the discrimination of non-supplemented vs. SCB supplemented pigs under heat stress condition. The most represented among these belonged to Ruminococcaceae family (6 OTUs). These results suggest that SCB modulates gut microbiota in pigs submitted to heat stress challenge. Causal link with improved growth performance still needs to be elucidated.
THE USEFULNESS OF THE ‘OMICS’ TOOLS TO DEEPLY CHARACTERISE THE EFFECT OF PROBIOTIC ADMINISTRATION ON THE PIG

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The adaptive capacity of the organism in response to specific environmental conditions (e.g., diet, heat stress, pathogens) is mediated by the complex interaction between biological signals that at different levels can modulate the behaviour and wellbeing of the animals. In-depth characterisation of the host by the collection of a large set of phenotypic attributes, such as microbiome, metabolome and transcriptome, offers an unprecedented possibility for deciphering the basic mechanism of these interactions, so leading to the description/prediction of specific traits in terms of sets of biological markers. From one side, the cutting-edge biotechnologies are more and more available at cheaper costs and data generation may no longer be a bottleneck. From the other hand the availability of large set of data requires to take care of the bioinformatics approach adopted, as well as a multidisciplinary team is needed to decipher the obtained data in the biological context. An increasing body of evidence suggests that undesirable modification in the microbiota-gut-brain axis can affect the maturation, morphology, and immunological functions that in turn increases the risks for stress-related disorders. The early imprinting with beneficial microbes seems to be a promising strategy for reducing the psychological distress and probiotic bacteria have been defined as psychobiotic when acting to modulate the microbiota to host immune interactions and synergistically benefit mental health.

Recently, in pigs, the application of ‘omics’ techniques allowed to progress in defragmenting the interplay between gut microbiota, host physiology, behaviour and welfare. The utility of ‘omics’ approaches can be evidenced also for short term association of intestine with beneficial bacteria. Thus, the mediatery effect of cortisol on the relationship between faecal Ruminocci and neurometabolites in pig was evidenced. The pig-specific Lactobacillus amylovorus affected the transcriptome of piglet jejunum by the up-regulation of SLC26A3 gene (congenital chloride diarrhoea), encoding a protein typically expressed in enterocytes to contribute to the chloride reabsorption. The triggering of SLC26A3 by this microbe may compensate the endotoxin-induced activation of chloride channels and reduce the severity of diarrhoeal from enteropathogens. Furthermore L. amylovorus down-regulated the gene of cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a cAMP- and phosphorylation-regulated chloride/bicarbonate channel, regulating the chloride efflux; thus, its activation is involved in enterotoxin-induced secretory diarrhoea. Both these gene regulations could have contributed to the favourable response observed in the oral supplementation with L. amylovorus in E. coli F4-challenged weaning pigs. In conclusion, the application of ‘omics’ techniques to the animal science, can concur to define probiotic strategies targeted for specific needs.
As crucial interface organs gut and skin have much in common. Therefore, it is unsurprising that several gut pathologies have skin co-morbidities. However, the reason for this remains ill explored, and neither mainstream gastroenterology nor dermatology research have systematically investigated the ‘gut-skin axis’.

Here, in reviewing the field, I propose that the gut microbiota may be a potential principle actor along the gut-skin axis. I suggest that probiotics may be a strategy targeting one organ to improve the health of the other.
**STAPHYLOCOCCUS AUREUS AND THE ECOLOGY OF THE NASAL MICROBIOTA**

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Bacteria in humans are often divided into commensals and pathogens. A number of commensals are also known to cause infection given the ‘right’ conditions, such as an immune-compromised host. These commensals often termed ‘opportunistic pathogens’ are source of concern, particularly in hospital environments. Strict measures are undertaken to avoid, e.g., methicillin-resistant *Staphylococcus aureus* (MRSA) to enter hospital wards with immunocompromised patients. Traditionally, we have combatted carriage of MRSA by applying antibiotics prior to surgery and/or hospitalisation. Such preventive action is rarely if at all done for methicillin-sensitive *S. aureus*, although this ‘cousin’ is as virulent as the MRSA counterpart albeit easier to treat.

Generally, 20-30% of all adults carry commensal *S. aureus* in their noses as well as other body sites. All such carriers have increased risk for invasive disease, and they are also sources for transmission when in close contact with other people, such as in hospital environments. A general eradication of *S. aureus* in all individuals that are either hospitalised or may enter nursing homes or the like will impose a major risk for generating new antibiotic-resistant *S. aureus*. Therefore, alternatives are needed.

The nasal microbiome, the very environment where *S. aureus* sometimes reside, is also perhaps the possible solution for avoiding *S. aureus* or other opportunistic pathogens to cause unwanted infection. A number of recent studies have investigated the microbial communities in the nasal cavity. There are several studies that indicate that communities or specific species may act as antagonists of *S. aureus* colonisation and thus serve as potential probiotics or alternative compounds to antibiotics. The present talk will discuss recent advances in the field and pinpoint remaining challenges and future possibilities.
HOW DO PROBIOTICS BENEFIT ATHLETES?

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Moderate physical activity (PA) exerts positive health effects, such as prevention and risk reduction of chronic diseases (obesity, diabetes, and cardiovascular diseases). Intensive PA, however, causes transient and long-term perturbations of the immune system, resulting in increased incidence of upper respiratory tract illness (URTI) and gastro-intestinal (GI) infections. Strenuous training also causes increased production of free radicals, causing the destruction of important biomolecules and muscle damage. In addition, reduced splanchnic perfusion and elevated body core temperature during PA could result in compromised gut permeability and consequent (GI) discomfort in athletes. This can lead to performance impairment.

The most commonly studied probiotic effect in athletes and active individuals is their influence on URTI. Emerging number of clinical studies have showed that probiotic supplementation reduces the incidence, duration and severity of URTI symptoms in elite athletes. In addition, another potential benefit of probiotics was reported – a reduced risk of GI infections, which are a particular concern when travelling abroad. These effects were ascribed to probiotic interaction with the gut-associated lymphoid tissue, leading to positive effects on the immunity: increase of salivary IgA (sIgA) antibodies, elevation of pro-inflammatory cytokines, preservation of humoral immunity. However, further clinical studies are warranted for the clarification of probiotics interaction with the immune system. In addition, certain probiotic strains have the ability to strengthen the intestinal mucosal barrier by the tight junctions’ enhancement. Probiotics reduce exercise-induced GI permeability and its associated symptoms, such as nausea, stomach and intestinal cramps and diarrhoea.

Probiotics can even exert ergogenic effects, i.e., they can enhance physical performance. There are two studies that provide evidence that probiotic supplementation in combination with protein reduces parameters of muscle damage, improves recovery, and maintains physical performance subsequent to damaging exercise. However, more research in this area is needed to confirm these results. It appears that probiotics can provide clinical benefits in athletes and other highly active individuals. Although this is a promising area of research, more well designed clinical trials are warranted to confirm that taking probiotics can decrease the number of training days lost to illness and to establish the most effective probiotics since their effects are highly strain-specific.
EFFECTS OF PROBIOTICS ON SPERM QUALITY

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Nowadays, an increasing percentage of couples are affected by infertility and it has been suggested that 40% of these events are caused by the male factor [1]. Asthenozoospermia pathology is described by reduced motility or absent sperm motility in the fresh ejaculate and this condition involves a poor fertility prognosis since male gamete motility is critical for spermatozoa migration in the female reproductive tract, for penetration of the oocyte, and for processes involved in fertilisation [2]. Since the introduction of the intracytoplasmatic sperm injection (ICSI), the low fertilisation prognosis of these patients has improved drastically with successful gestations and live births after injection of immotile cells [2]. However, new fields should be explored to try an improvement of sperm motility and facilitate assisted reproductive technologies in clinical centres or natural pregnancies.

Interestingly, our studies in animal model species and human donors demonstrated that probiotics could have a positive effect on sperm quality. Molecular studies carried out in fish showed that after probiotic ingestion molecular markers associated with a good reproductive performance are overexpressed at testicular and germ cell level [3]. This positive effect is particularly relevant in human donors. In this case, the ingestion of two selected antioxidant probiotics strains (Lactobacillus rhamnosus CECT8361 and Bifidobacterium longum CECT7347) significantly improved sperm quality in asthenozoospermic donors [4].

The significant improvement on sperm motility and the decrease in DNA fragmentation reported in this study provides preliminary evidence that probiotics could be administrated to improve motility and decrease DNA fragmentation and reactive oxygen species levels in asthenozoospermic human males.


References
VAGINAL PROBIOTICS TO PREVENT RECURRENCE OF BACTERIAL VAGINOSIS

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Most women have a vaginal microbiota (VMB) that consists predominantly of lactobacilli, and vaginal dysbiosis (a VMB that is not dominated by lactobacilli) can cause symptomatic conditions. The most common condition is bacterial vaginosis, an anaerobic polybacterial dysbiosis. Other dysbiotic states of importance to global health are VMBs with a high abundance of streptococci, staphylococci or Enterobacteriaceae, vaginal candidiasis, and vaginal trichomoniasis. Knowledge about the different types of dysbiosis and their relationship to urogenital and reproductive disease burden has increased in recent years by applying non-culture based techniques but is far from complete. Vaginal dysbiosis has been associated with increased risk of HIV and other sexually transmitted infections, pelvic inflammatory disease, miscarriage, preterm birth, and maternal and neonatal infections. In most settings, vaginal dysbiosis is only treated when it is symptomatic and the recurrence rate after standard treatment (oral metronidazole for 7 days) is high. We recently conducted a randomised controlled trial of antibiotic and probiotic maintenance therapies with as aim to reduce the recurrence rate after standard metronidazole treatment in women at high risk of urogenital infections in Rwanda. After successful treatment (no bacterial vaginosis by Amsel criteria), women (n=68) were randomised to two different vaginal probiotics, oral metronidazole, and behavioural counselling only (control group). Both vaginal probiotics contained lactobacilli but in different combinations and formulations; all interventions were self-administered 2-3 times per week for two months. Endpoints were measured at baseline, week 1, and months 1, 2, and 6.

This presentation will summarise current VMB knowledge, discuss the preliminary results of the above-mentioned trial, as well as other potential interventions to normalise the VMB and/or prevent vaginal dysbiosis.
NEW GENERATION OF VAGINAL PROBIOTICS.

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Vaginal microbiota is dominated by Gram-positive Lactobacillus bacteria, which maintain the acidic pH in the vagina and protect it from pathogen invasion by the production of organic acid, bacteriocins, and hydrogen peroxide. Other bacterial species, such as Gardnerella vaginalis, Peptostreptococcus spp., Prevotella spp., Escherichia coli, Streptococcus agalactiae, and Enterococcus faecalis, are present in limited quantities in a healthy vagina, but in certain conditions, their populations may increase significantly, which can cause diseases such as bacterial vaginosis (BV) or aerobic vaginitis (AV). These diseases are diagnosed using both clinical and microbiological criteria. Clinical criteria include, among other signs and symptoms, elevated vaginal pH, while the microbiological criterion involves the microscopic assessment of vaginal microbiota according to the Nugent score. A score of 4-6 indicates an abnormal condition, also called intermediate vaginal microflora, and a score of 7-10 suggests the presence of BV.

Treating BV and AV with antibiotics and chemotherapeutics is often ineffective and results in relapses; however, administration of viable probiotic strains of Lactobacillus can promote success of these therapies, or they may be used as a prophylactic. The new generation of vaginal probiotics are isolated from the healthy woman vaginal microbiota, because these Lacobacillus strains colonise vaginal environment rapidly and have very strong antagonistic properties toward potential vaginal bacterial pathogens. Moreover, due to their ability to decrease the pH, these bacteria create favourable conditions for colonisation by other Lactobacillus species that are regularly found in a healthy vaginal microbial community. Additionally, the new probiotics should delay the clinical relapse of BV mainly after standard metronidazole treatment together with oral probiotic, but the use of oral probiotic significantly delayed the clinical relapse of clinical symptoms of the inflammatory process by an average of 20-31 days. Each new probiotic product designed to improve vaginal health should be evaluated separately in controlled clinical studies.
TUESDAY 10 OCTOBER 2017

PARALLEL SESSION 4
PRE-/PROBIOTICS IN ANIMALS

FAECAL MICROBIOTA TRANSPLANT IN VETERINARY MEDICINE: THE EGG OF COLUMBUS FOR AUTOLOGOUS PROBIOTICS ALIMENTARY INTEGRATION

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A first mention of faecal material transplantation (FMT) comes from the documents of Chinese medicine. During the fourth-century AD, the Chinese medical doctor Ge Hong mentioned the effects of a special beverage, called 'yellow soup' for gastrointestinal problems. Hong documented a rather unique recipe for treating diarrhoea: yellow soup. It was, as one might expect, not a pleasant dish but rather a broth involving dried or fermented stool from a healthy person. Taken by mouth, the bacteria in the stool would then inhabit the gut of the sick person, and bring about a cure. Medical scholars can debate whether Ge Hong's yellow soup was the first effort at what is now called faecal microbiota transplantation. History is replete with documented examples of the practice, from Renaissance-era veterinarians to a 1958 Colorado case involving four critically ill patients. In veterinary medicine, the practice of stool or GI tract content transplant, has often been practiced. There are dozens of reports that show how the transplant of ruminal content, taken from the rumen of an adult and healthy cattle or sheep, capable to convert very well poorly poor food (i.e., straw, agricultural residues or hay/herb of low-quality) to a young calf or lamb, transfer these capabilities to the recipient young animal. Now, in veterinary medicine, as well as in human gastroenterology, an increase in a hard-to-beat intestinal bacterium has prompted researchers to revisit the effectiveness of FMT: a modern version of Hong's yellow soup. The treatment revolves around intestinal flora. In a healthy donor, the bowels are full of healthy bacteria. When a human or veterinary patient is receiving antibiotics, the balance can be upset; after all, antibiotics kill bacteria indiscriminately, whether harmful or not. The result, all too often, is the development of gastrointestinal disease; clostridia are the most important pathogens involved in these conditions of dysbiosis. In humans, as well as in pet animals, such as dogs and cats, *Clostridium difficile*-related diarrhoea and colitis is the most frequent consequence of a prolonged antibiotic treatment. The problem of clostridial diarrhoea after antibiotic therapy, and diffusion of antibiotic multi-resistant clostridia, is very similar to the dramatic increase of methicillin-resistant *Staphylococcus aureus* (MRSA).

The poultry industry has always been a dynamic and integral part of national economies in many countries, and a good answer to increase demand of high protein and relatively cheap food, produced with low environmental impact. Economic losses incur especially in large-scale rearing facilities, often attributed to the deterioration of environmental conditions, poultry exposure to stressors and development of diseases. While antibiotics have been commonly used for prophylactic purposes and as growth stimulants, extensive documentation of antimicrobial resistance among pathogenic bacteria due to indiscriminate utilisation of antibiotic in the industry has led to public and governmental outcries. Elimination of antibiotics from poultry production has thus encouraged intensive search for alternatives. We propose FMT as an easy, cheaper and most natural way to employ the immense potential of probiotics to fill the gap as alternative growth promoters and evidences of beneficial effects of probiotic application in poultry production. In FMT, we have the possibility to administrate billions of autologous probiotic strains, but also some prebiotic substances as well as immunoglobulins, bacteriocins and other anti-bacterial and anti-parasitic substances.

Chickens acquire naturally bacteria constituting the GI microbiota, and different factors for passive immunisation by eating faeces of hens and brothers, starting immediately after hatching. Starting from particularly screened and treated/reared donors, we have obtained a stool inoculum to use for chickens, starting at aerosolisation in the hatchery chamber and, thereafter, administered daily for the first week of life in three group of animals. 300 one day old, male, Lohmann chickens, were subdivided in 2 groups consisting of 150 chickens each (FMT group, and control group) at week 1. Both groups were only fed with a mix of maize, barley and crushed grain, with vitamin supplementation and water at libitum. The FMT group was treated with faeces diluted in water once a day for the first week, and then repeated weekly for a day until slaughter; the control group received only tap water. For faecal transplantation,
we used as donors chickens breeded naturally (extensive manner) without any treatment in the last three months before the experiment, immunised for Marek’s disease, infectious bronchitis, Newcastle disease and Gumboro disease, and for coccidiosis. Donors were constantly negative for internal parasites, and in excellent BCS. The chickens were weighed daily, at morning, and at the end of the study (4 months) they were slaughtered. Organs were removed, weighed and measured, and formalin-fixed for histological analyses. Samples from litter and from faecal contents were used to perform coccidian oocysts count by the McMaster method (oocysts per gram determination). Histological sections were obtained from duodenum, ileum and caeca and used for calculation: villi height, crypts depth, villi/crypts ratio, thickness of the caecal lamina, diameter of the mucosal lymphoid follicles, lesions score, and degree of oocyst infection. Better growth performance was evidenced in the FMT group than in C group. Significant differences between all the parameters evaluated, with exception of the ileal villi height and V/C ratio, and very important differences in mortality ratio were reported.

In conclusion, FMT may be a good and ‘natural’ way to administer probiotic/prebiotic supplementation in chickens and promote amelioration of all the productive parameters, enhancing local and systemic immune response, protective for bacterial but also viral and parasitic pathogens. FMT could be one of the ‘answers’ to produce broilers without antibiotics as growth promoters (AGPs). With the ban on sub-therapeutic antibiotic usage, FMT may be a potential alternative to AGPs in the poultry industry and other animal production.

References
APPLICATIONS FOR PRE- AND PROBIOTICS TO MODIFY THE CANINE INTESTINAL MICROBIOTA – WHAT IS THE EVIDENCE?

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Interest in the composition of the canine gastrointestinal (GI) microbiota and possibilities of its therapeutic modifications has soared and more detailed knowledge is available. The healthy canine duodenal microbial community consists of Firmicutes (46.4%), Proteobacteria (26.6%), Bacteroidetes (11.2%), Spirochaetes (10.3%) Fusobacteria (3.6%), and Actinobacteria (1%). Four additional phyla are reported in the jejunum: Tenericutes, Cyanobacteria, Verrucomicrobia, and Chloroflex. The percentage of Fusobacteriales increases in the ileum (30%), and continues in colonic/ faecal samples (30% each Fusobacteria, Bacteroidetes and Firmicutes).

Alterations of the GI microbiota are observed in both acute and chronic GI diseases in dogs. In acute diarrhoea, large-scale changes include increased abundance of Clostridium spp. (C. perfringens), E. coli, Lactobacillus and Enterococcus spp. In inflammatory bowel disease (IBD), microbiome changes are similar to observations in people: Significantly reduced species richness, and increased proportion of Proteobacteria/Enterobacteriaceae with a reduction in other phyla is observed. There is also large intestinal/faecal dysbiosis in canine IBD, with significantly lower bacterial diversity, an increase in Gammaproteobacteria (i.e., E. coli) and decreases in Erysipelotrichia, Clostridia, and Bacteroidia. There is some merit in the use of probiotics in infectious canine GI diseases. Administration of the probiotic mixture VSL#3 to puppies with parvoviral enteritis lead to more rapid clinical improvement, increased leucocyte counts and increased survival. Ancylostoma egg shedding was reduced in dogs treated with a mixture of lactic acid producing bacteria (LABs). However, Giardia cyst shedding was unaltered and local immunity not enhanced using Enterococcus faecium. Non-infectious acute diarrhoea (e.g., kennelling stress) showed some improvement with Bifidobacterium animalis, and LABs, but not E. faecium. Some probiotics (E. faecium, LABs) showed immune-modulatory properties in in vitro or ex vivo studies of canine IBD. However, in vivo results did not mirror those findings, with an E. faecium containing synbiotic having no effect on clinical activity, histology scores or gene expression profiles. More promising results could be achieved with multistrain preparations: In IBD dogs given an LAB cocktail, clinical signs improved, duodenal IL-10 and IFNy expression and the number of faecal Enterobacteriaceae decreased, while numbers of Lactobacillus spp. increased. VSL#3 was as efficient to induce clinical remission in dogs with IBD compared to treatment with metronidazole and prednisolone. FoxP3+ cells and TGFβ increased significantly after treatment with VSL#3 strains.

There is much less evidence for the use of prebiotics/dietary fibre in canine GI conditions, even though some small studies described positive clinical responses (fibre-responsive colitis, IBD, IBS). Psyllium is most commonly used on its own and FOS/MOS and other oligosaccharides are frequently found in synbiotics, but more studies are needed to understand their benefit and mechanisms of action.

In summary, despite widespread use of pro-, pre- and synbiotics, scientific evidence of their beneficial effects in different conditions of the dog is scarce. In some instances, in vitro results have been promising, but could not be transferred consistently into in vivo situations. Depending on the probiotic used, improvements of both acute (infectious) and chronic conditions like IBD can be achieved.
RELATIONSHIPS BETWEEN GUT MICROBIOTA AND GLUCOSE HOMEOSTASIS IN OBESE DOGS FED WITH SCFOS OR OLIGOFRUCTOSE-ENRICHED DIETS

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Weight gain and obesity are closely associated with insulin resistance and are a major issue in companion animals. The dietary amount of lipids and carbohydrates is a causative factor for peripheral insulin resistance. On the contrary, the reduction of insulin resistance is correlated with greater consumption of dietary fibre. Prebiotic fibres are not digestible by the mammalian digestive enzymes, but are selectively fermented by potentially beneficial bacteria. A previous study demonstrated that the supplementation with 1% short-chain fructo-oligosaccharides (scFOS) allowed significant improvement of glucose homeostasis evaluated by the FSIGTT test, while it modified adipocyte gene expression.

The present study aimed to evaluate the relationships between microbiota composition and activity and the metabolic status of obese dogs fed or not with prebiotics in diets differing by the protein content. Six beagle dogs were maintained in obese status and received a diet with 23 or 32% crude protein. These diets were supplemented or not with 1% scFOS or oligofructose in a complete Latin-square design study with 5 week-period. Glucose homeostasis was estimated via fasting collection of insulin and glucose at the end of each period. Faecal and blood samples were also collected and used to make metabolomics and metagenomic by 16S rRNA sequencing. A network of correlations was made to better understand the relationships between faecal microbiota and metabolome, plasma metabolome, and phenotypic variables. For this network, metavariables were built gathering either several genera of bacteria or several molecules (metabolome). No significant effect on insulin resistance index (HOMA IR) was obtained with prebiotics. Both prebiotic tended to decrease faecal branched-chain fatty acids concentrations (P=0.07). A supplementation with scFOS, but not with oligofructose, increased richness of faecal microbiota and significantly modified faecal profile metabolome. Building metavariables allowed putting forwards that certain genera of bacteria, such as Lactobacillus and Dorea, were negatively correlated with Fusobacterium or Campylobacter, known to be potentially pathogenic. Furthermore, preliminary results of the correlations network highlighted that blood cholesterol and body weight were significantly correlated with faecal metabolome and metagenome (P<0.05), while insulinemia tended to be correlated with body weight excess percentage (P<0.07). Further analysis, and notably identification of main molecules of faecal/plasma metabolome is required to better understand the relationships between gut microbiota and glucose homeostasis. This study was a preliminary one showing that in dogs like in other mammals, microbiota seems to play a role on glucose homeostasis.
THE USE OF PREBIOTICS IN A STRICT CARNIVORE, THE CAT

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Domestic cats are strict carnivores and thus have a higher protein requirement than dogs and humans. Consequently, more undigested protein will be fermented in the hind gut of cats. In general, carbohydrate fermentation is regarded as positive compared to protein fermentation, which is associated with the production of putrefactive substances, such as ammonia, indole, skatole, etc. Although these end products of protein fermentation are also found in cats, the cat as a strict carnivore, may have adapted to cope with the negative effects of protein fermentation. Indeed, Lubbs et al. (2009) found Novosfingobium spp. in the faeces of cats fed a high protein diet and these bacteria are able to breakdown aromatic compounds. On the other hand, populations of Bifidobacterium were decreased and those of Clostridium perfringens were increased in adult cats consuming a high protein diet compared to a medium protein diet (Lubbs et al., 2009). Similar results have also been shown in 8-16 week old kittens (Hooda et al., 2013), underlining the potential beneficial effect of supplementing high protein diets with prebiotics.

These results highlight the importance of the composition of the basal diet as this may influence the outcome of prebiotic studies. To my knowledge, feline prebiotic studies have only been performed supplementing traditional commercial diets, although alternative, more natural diets are also popular now. Apart from the higher protein content in these diets, the difference in fibre type may also play a role in the microbial composition of the gut. Cats consuming natural diets do not consume plant fibre; instead they do consume animal fibre. The concept of animal fibre was introduced by De Pauw et al. (2011) and was described as un- or less enzymatically digestible parts of the prey like hair, skin, cartilage etc. that are available for hind gut fermentation. Phylogenetic analysis of faecal samples from captive cheetahs consuming supplemented meat and whole prey showed that Bifidobacteria were underrepresented (Becker et al., 2014). It might thus be interesting to investigate the effect of prebiotics in feline alternative diets.

Most abundant microbiota in the colon and faeces of healthy cats are Firmicutes (especially Enterococcus spp., Streptococcus spp., Lactobacillus spp., Erysipelotrix spp. and Clostridium clusters), Bacteroidetes, Proteobacteria and Actinobacteria (Rochus et al., 2014). Several studies using different types and doses of prebiotics (FOS and/or GOS) showed an increase in Bifidobacterium and/or Lactobacilli populations (Sparkes et al., 1998; Barry et al., 2010; Kanakupt et al., 2011). Another potential target for prebiotic supplementation are elderly cats and IBD inflicted cats as those animals have decrease Bifidobacterium populations (Patil et al., 2000; Inness et al., 2007). However, Barry et al. (2014) found a tendency to a decreased Bifidobacterium population after fructan supplementation in senior cats. To our knowledge, there are no studies investigating the effect of prebiotics in cats with gastrointestinal disease.

Prebiotic supplementation can also influence metabolism in cats. Fructan supplementation to a low protein diet tended to increase faecal N-excretion and decrease urinary N-excretion (Hesta et al., 2005). Similarly, FOS and inulin supplementation decreased urinary N-excretion and was suggested to reduce postprandial amino acid gluconeogenesis in healthy adult cats consuming a high protein diet (Verbrugghe et al., 2010). It might be interesting to apply these findings in cats with renal or hepatic disease.

References
Available upon request.
MODULATION OF THE EQUINE HINDGUT MICROBIOTA USING PRE/PROBIOTICS: CURRENT KNOWLEDGE, APPLICATIONS AND PERSPECTIVES

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Horses are hindgut fermenters and their caecal and colonic microbiota play a crucial role in providing vital nutrients, but also in maintaining gut health and preventing diseases or anxiety-like behaviour. Dietary solutions constitute an easy way for manipulating the microbiota structure and functions. Several studies using probiotics and prebiotics investigated their impact on the ration digestibility, but few evaluated their influence on health – wellbeing – behaviour of the horse, despite their importance considering the specific issues of the equine industry. In this abstract, we will summarise studies that have been conducted in vivo in the hindgut of adult horses.

Among probiotics, yeasts have received more attention than bacteria and were tested with diets considered at risk for colic or laminitis. Horses fed a high-starch diet and supplemented with Saccharomyces cerevisiae (SC) presented a significantly higher concentration of lactate-utilising bacteria in the caecum and right ventral colon compared to non-supplemented horses. This resulted in a decrease in lactate produced by starch fermentation, thus limiting the pH drop and preventing the decline of cellulolytic bacteria (and fibrolytic activity) that are keystone species in herbivores. Hence supplementing horses with SC would prevent acidosis and the resulting microbiota disturbances that could be associated to colic or laminitis. The impact of SC supplementation on the hindgut microbiota was also tested in case of abrupt change of hays, but no significant effect was observed.

The effect of bacterial probiotic on the equine hindgut microbiota is controversial. Whereas an increase of colonic lactobacilli and cellulolytic bacteria concentrations was reported in horses receiving Lactobacillus farciminis and L. rhamnosus, no effect was measured in the caecal bacteria concentration of horses supplemented with L. acidophilus, except for a diminution of E. coli.

Regarding prebiotic supplementation, the impact of short chain fructo-oligosaccharides (scFOS) was evaluated on horses subjected to an abrupt incorporation of starch. Lactobacilli and streptococci concentrations increased in the hindgut of non-supplemented horses after dietary change while they remained stable in horses receiving scFOS. This suggested that scFOS may help stabilising the microbiota under a stressful dietary challenge.

Data on pre/probiotics are sparse in the hindgut. More studies assessed their impact on the faecal microbiota. Indeed, collection of faeces is non-invasive and thus easier and more acceptable ethically than hindgut content collection. However, no study has compared the effect of a probiotic or prebiotic both on the hindgut and faecal microbiota. This raises the question of the representativeness of data obtained with faecal microbiota. Recently, we demonstrated that under a dietary change some bacterial populations in the hindgut and faeces varied concomitantly, providing optimism for studying pre/probiotic effect in the horse using faeces rather than hindgut content. Another challenge in horse supplementation is its particular digestive anatomy, which should be taken into account in the elaboration of pre/probiotics. It appears necessary to systematically verify that probiotics and prebiotics reach the hindgut respectively alive or non-degraded before to study their effect.
The natural history and ecology of turtles has been studied for years, and such information is available for many species. However, the microbiology of the reptilian gastrointestinal tract (GIT), its composition and effects on the host still remain almost unknown, and only a few studies have been published. Most scientific papers are concerned with reptiles only as pathogen carriers and as a zoonotic threat to people. Salmonellosis caused by pet reptiles kept in 1-2% of households are supposed to be responsible for 6-11% of this disease in the USA. Thus, a cycle of long-term 20-52 weeks long experiments was carried out to evaluate the effect of dietary addition of different, single and multiple species of probiotic preparations on growth performance, shell composition, intestinal microbiota, and intestinal histomorphology.

The experiments were conducted on *Trachemys scripta*, *Sternotherus odoratus*, and *Apalone ferox*. In each trail, turtles were randomly distributed to three experimental groups: (1) CON – with no additives; (2) SSPA – with *Bacillus subtilis* PB6; and (3) MSP – with multiple strain probiotic. The growth and development of the experimental turtles were evaluated on the basis of body mass straight carapace length measurements and condition index calculations; additionally, GIT morphology was assessed. Crude ash, Ca and P concentrations were measured in the shell (carapace and plastron). Samples of gastrointestinal content taken during turtle dissection were immediately frozen and stored in -80°C for fluorescent *in situ* hybridisation analysis. The results varied among the species and used probiotics and suggest the high specificity of mutual relations between strains used as probiotics and animal host species. However, the use of probiotics preparations improved the body weight gain, crude ash, Ca and P share in the turtles’ shells. The alteration of duodenal histomorphology was recorded, including increased the villous height and mucosa thickness. In the case of intestinal microbiota, bacteria suppressing effects were observed in each experiment.

In conclusion, the above-mentioned results suggest that probiotics are useful in turtle nutrition due to their positive effects on growth performance, shell mineralisation, duodenal histomorphology, and microbiota.
TUESDAY 10 OCTOBER 2017

PLENARY MEETING
SPEED PRESENTATIONS
Short presentations by selected poster presenters to provide an overview of their research and inspire
the audience to visit their posters.

P1: A link between human milk oligosaccharides, infant faecal community types, and risk of
infection
Bernard Berger
Nestlé Research Center, Switzerland

P5: Monitoring intestinal immune and microbiome responses at the gut level in ruminants
Christopher Chase
Department Veterinary & Medical Science and Department of Animal Science, South Dakota State University, USA

P6: Adipose tissue microbiota: the new target of prebiotics in diabetic mice model
Bérengère Coupé
Vaiomer, France

P23: Molecular effects of GOS delivered in ovo in chickens challenged with acute heat stress
Anna Slawinska
University of Molise, Italy and UTP University of Science and Technology, Poland
WHERE PROBIOTIC THERAPY SAVES LIVES

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Probiotics are “live microorganisms, which when administered in adequate amounts confer a health benefit on the host”, according to the widely accepted definition by FAO/WHO. Most of the currently used probiotics belong to the genera *Bifidobacterium* and *Lactobacillus*. However, probiotic preparations containing species of the genera *Enterococcus*, *Pediococcus*, *Streptococcus*, *Lactococcus*, *Propionibacterium*, *Bacillus*, and *Saccharomyces* are also used. Probiotics do not usually colonise the GI-tract, and therefore the products should be consumed daily for the health benefits.

There are various health benefits that have been described for probiotics. Since the title of this presentation is “Where probiotic therapy saves lives”, we will only concentrate on those health benefits that have been shown to have potential life-saving opportunities as well as clear improvements in the quality of life, such as reducing risk for necrotising enterocolitis (NEC), reducing risk for antibiotic associated diarrhoea (AAD) and *Clostridium difficile*-associated diarrhoea, in addition to reducing risk for respiratory tract infections.

Necrotising enterocolitis (NEC) is a major cause of morbidity and mortality in premature infants. Approximately 7% of infants with a birth weight between 500 and 1,500 g develop NEC. Interestingly, probiotics have been shown to effectively reduce the risk for NEC. Especially bifidobacteria and probiotic mixtures have been shown to be efficacious. Reducing risk for antibiotic associated diarrhoea (AAD) is, on the other hand, one of the best documented health benefits of probiotics, and there are numerous probiotic studies and strains that have been documented to provide benefits against AAD and *Clostridium difficile*-associated diarrhoea. Moreover, it has been shown in meta-analyses that when probiotics are given with antibiotics they reduce the risk of developing *C. difficile*-associated diarrhoea (CDAD) by 64%. Reducing risk for respiratory tract infections (RTI) does not necessarily come to mind when talking about potential life-saving opportunities for probiotics. However, in some rare occasions RTI may lead to more serious secondary infections, which could possibly be prevented without the initial RTI.
TRANSLATING MICROBIOTA KNOWLEDGE TO IMPROVED OUTCOMES IN THE INTENSIVE CARE UNIT: WHERE DO WE STAND?

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The composition and diversity of the microbiota of the human gut, skin, and several other sites is severely deranged in critically ill patients on the intensive care unit (ICU), and it is likely that these disruptions can negatively affect outcome. In this talk, new and ongoing studies that investigate the use of microbiota-targeted therapeutics in the ICU are reviewed ending with some recommendations for future research.

Practically every intervention in the ICU as well as the physiological effects of critical illness itself can have a profound impact on the gut microbiota. Therapeutic modulation of the microbiota, aimed at restoring the balance between ‘pathogenic’ and ‘health-promoting’ microbes is therefore of significant interest. Probiotics have shown to be effective in the treatment of ventilator-associated pneumonia, and the first faecal microbiota transplantations have recently been safely and successfully performed in the ICU. However, all-encompassing data in this vulnerable patient group remain sparse, and only a handful of novel studies that study microbiota-targeted therapies in the ICU are currently ongoing.

Enormous strides have been made in characterising the gut microbiome of critically ill patients in the ICU, and an increasing amount of preclinical data reveals the huge potential of microbiota-targeted therapies. Further understanding of the causes and consequences of dysbiosis on ICU-related outcomes are warranted to push the field forward.

References
PROBIOTIC SACCHAROMYCES CEREVISIAE AND ENTEROHAEMORRHAGIC ESCHERICHIA COLI: AN EFFECTIVE STRATEGY AGAINST A DEADLY ENEMY?

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Enterohemorrhagic \textit{Escherichia coli} (EHEC) are major foodborne pathogens responsible for human diseases ranging from mild diarrhoea to life-threatening complications. The main virulence determinant of EHEC is the production of Shiga toxins and the terminal ileum and large intestine are assumed to be the main sites of bacterial colonisation and pathology in human. As no specific treatment is available and as antibiotic therapy has worsened clinical outcomes, alternative strategies using probiotics are under consideration. Nevertheless, the way how probiotics can affect the survival and virulence of EHEC throughout the human digestive tract remains largely unknown, owing to a lack of relevant model systems.

We aimed to explore the antagonistic effects of the probiotic yeast \textit{Saccharomyces cerevisiae} CNCM I-3856 on the reference strain EHEC O157:H7 EDL933 by using complementary original and relevant \textit{in vitro} and \textit{in vivo} models of the human digestive tract and intestinal mucosa. The effect of the probiotic on EHEC survival and expression of main virulence genes was investigated in multi-compartmental dynamic \textit{in vitro} models of the human upper (TNO Gastrointestinal Model – TIM) and lower (Artificial Colon – ARCOL) gastrointestinal tract. The influence of the yeast on EHEC interactions with the intestinal epithelium was assessed using an \textit{in vitro} model of specialised M cells and mice ileal loops assays. In TIM, the probiotic significantly reduced the growth resumption of the pathogen in the small intestinal compartments. In ARCOL, we observed that both the probiotic and the pathogen had an individual-dependant effect on gut microbiota composition. In this artificial colon, \textit{S. cerevisiae} CNCM I-3856 had no influence on EHEC survival but significantly decreased Shiga toxin-encoding genes expression at 9h and 12h post EHEC infection. In addition, the probiotic favourably influenced gut microbiota activity through beneficial modulation of short chain fatty acid production. Pre-treatment with \textit{S. cerevisiae} CNCM I-3856 also significantly reduced EHEC translocation through M cells, inhibited \textit{in vivo} interactions of the pathogen with Peyer’s patches and significantly reduced the number of haemorrhagic Peyer’s patches in murine ileal loops.

Our results indicate that \textit{S. cerevisiae} CNCM I-3856 may be useful in the fight against EHEC infections via a multi-targeted approach. Additional studies are ongoing to better understand the underlying molecular and cellular mechanisms. The interests and limits of a probiotic-based approach for EHEC infection in humans will be discussed as well as the interest of dynamic artificial digestive systems, such as TIM and ARCOL, for an in-depth understanding of commensal-probiotic-pathogen interactions in the human gut, often considered as a ‘black box’. In particular, these models have been recently adapted to mimic the digestive conditions of specific populations, such as young children and elderly people, who are at-risk populations for EHEC infections.
DIETARY FIBRE, THE GUT MICROBIOTA AND THE COLONIC MUCUS BARRIER: IMPLICATIONS FOR HEALTH AND DISEASE

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Despite the accepted health benefits of consuming dietary fibre, little is known about the mechanisms by which fibre deprivation impacts the gut microbiota and alters disease risk. Using a gnotobiotic mouse model, in which animals were colonised with a synthetic human gut microbiota composed of fully sequenced commensal bacteria, we elucidated the functional interactions between dietary fibre, the gut microbiota and the colonic mucus barrier, which serves as a primary defence against enteric pathogens. We show that during chronic or intermittent dietary fibre deficiency, the gut microbiota resorts to host-secreted mucus glycoproteins as a nutrient source, leading to erosion of the colonic mucus barrier. Dietary fibre deprivation, together with a fibre-deprived, mucus-eroding microbiota, promotes greater epithelial access and lethal colitis by the mucosal pathogen, Citrobacter rodentium.

Our work reveals intricate pathways linking diet, the gut microbiome and intestinal barrier dysfunction, which could be exploited to improve health using dietary therapeutics. Our follow-up unpublished work shows that mucus-degrading bacteria, in the context of a fibre-deprived diet, are essential to drive lethal colitis by C. rodentium.
DIETARY FIBRES AS ORAL ADJUVANT

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Adjuvants are immunological agents that can increase the efficacy of other agents like a vaccination and can boost antibody titres or extend the protection of the host. Enhancing vaccination efficacy by supplementation with nutritional compounds in population with malnutrition is an accepted strategy and has led to health claims for some vitamins and minerals.

Several dietary fibres have shown immunomodulating properties in vitro and in vivo and some of these fibres were studied by a follow-up trial based on vaccination response model in animals or human. Human vaccine efficacy studies with dietary fibres as food supplements have been performed with different vaccination protocols, such as influenza, measles, tetanus, pneumococcal and hepatitis B with variable success. The variability is likely caused by variation in immunomodulating potency of the included dietary fibre, the variability in composition of these fibres but also by the vaccination model used and subject’s vaccination history and health status. A review on the current literature on animal and human studies, including vaccination response to dietary, fibres will be given.

Two trials will be discussed in more detail. The first is a piglet trial in which a long chain inulin type fructan (lcITF) was tested alone or combined with a probiotics strain Lactobacillus acidophilus W37 (LaW37). Ingredients were given daily as of day 2 after birth until sacrifice via oral drenches. Piglets were weaned on day 24 and vaccinated with a single dose of Salmoporc STM®. To analyse the effect on protection against Salmonella, animals were challenged with this pathogen before sacrifice. Diarrhoea occurrence and severity was significantly lower in lcITF and lcITF/LaW37 groups compared to the placebo control group. In addition, the combination of lcITF/LaW37 enhanced vaccination efficacy as evidenced by increased anti-body levels. The second trial that will be discussed in more detail is a trial based on healthy postmenopausal woman and men (n=239) aged ≥ 50 years. The dietary fibres tested were: beta-glucan preparation from yeast, a beta-glucan preparation from shiitake, a beta-glucan preparation from oat, an arabinoxylan preparation from wheat, and an exopolysaccharide preparation from L. mucosae as well as a placebo (maltodextrins). The products were consumed for 5 weeks. Two weeks after starting consumption a standard seasonal influenza vaccine was given and antibody titres were assessed before vaccination and 1 and 3 weeks after vaccination. Compared to control the arabinoxylan preparation had higher GMFI for influenza strain H1N1 (P=0.075 in unadjusted Student t test) and the yeast preparation for strain B (P=0.043). The increase in seroprotection rate was higher for H1N1 (P=0.053 in unadjusted Fisher exact test) and B (P=0.078) in the arabinoxylan preparation compared to the placebo control treatment. Based on these results it was concluded that, among these five products tested, the arabinoxylan preparation had the highest potency for adjuvant effects which was also supported by a comparative in vitro study based on human cell lines and primary immune cells, of which the results will be presented as well.
LACTIC ACID BACTERIA CONVERT HUMAN FIBROBLASTS INTO MULTIPOTENT CELLS

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Somatic cells can be reprogrammed into pluripotent stem cells by nuclear transfer into oocytes, ectopic expression of defined transcription factors, and treatment with a particular set of chemical compounds. Previously, we incorporated lactic acid bacteria (LAB) into adult human dermal fibroblasts (HDF) that were treated with trypsin/EDTA. After a few days incubation, cell clusters were generated similar to the embryoid bodies derived from embryonic stem cells. The cell clusters expressed a subset of pluripotent markers and transform into cells derived from three germ layers in vivo and in vitro [1].

In this study, we cultured LAB, homogenised them, and passed them through 3 chromatography columns. The peak fraction for cell cluster formation activity was analysed by MALDI-TOF MS. From the obtained results, we speculate that the ribosome is a candidate for the LAB-derived reprogramming material. Here, we demonstrate the generation of reprogrammed cell clusters by the ribosome isolated from LAB. The ribosome can form cell clusters effectively than the living bacteria. The cell clusters have different properties compared with the original HDF. The cell clusters differentiate into endodermal, mesodermal, and ectodermal cells via appropriate cultivation. The way of reprogramming by the ribosome has potentially wide-ranging implications for elucidating the mechanism of cell generation, reprogramming, and evolutionally tracts.

References
HOST-NICHE SPECIALISATION IN THE GUT – CLUES FROM BACTERIAL GENOMES AND TRANSCRIPTOMES

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The vertebrate gastrointestinal (GI) tract is known to harbour many different genera of symbiotic bacteria. The extent to which these bacteria affect humans in health and disease is only just becoming recognised. Large-scale gut metagenomic studies have revealed that Bacteroidia and Clostridia are amongst the most commonly identified classes of bacteria in the GI tract. It is known that gut symbiont species have diversified into distinct phylogenetic clades. These clades can exhibit a host-specificity signature. Previous studies in Lactobacillus reuteri demonstrated host-specific genetic features in rodents, chickens and in porcine gut by DNA sequence analysis approaches [1,2].

In this study, phylogenetic analyses of two important gut Bacteroides spp. Are presented and their potential for host specific markers are discussed. Whole genomes of Bacteroides thetaiotaomicron and Bacteroides ovatus have been examined for host-specific genetic markers. Bacteroides spp. have previously been surveyed in the context of 16S rRNA signatures in metagenomic studies originating from the GI tract and described in some as displaying host-specificity with local variation, whilst others describe host-specific markers as residing outside of the 16S rRNA gene. New sequencing technologies, such as Oxford Nanopore MinION, together with Illumina and RNA-seq can provide high quality datasets for the re-evaluation of host specific markers. Analysis of these markers from sites, such as contaminated water courses, could reveal the host origin of faecal pollution at an early stage due to the high abundance of these organisms in the gut, and therefore lead to a timely identification of faecal contamination sources.

References
HARNESSING MAGNETO-AEROTACTIC BACTERIA TO DELIVER THERAPEUTICS IN REGIONS OF ACTIVE CANCER CELLS

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Although the large majority of cancers are spatially localised in the form of a tumour or within a known physiological region, modern cancer therapy still relies on systemic delivery to target active cancer cells. The long systemic circulation through the large blood network contributes to not only reduce the amount of therapeutics reaching active cancer cells but contributes also to increase toxicity for the patients. If therapeutics could be navigated, the same blood network would provide access to any parts of the body to increase significantly the amount of effective but also toxic drug molecules reaching the physiological regions that need to be treated while minimising systemic toxicity for the patients. To do just that, microscopic carriers capable of navigating through such complex network to deliver using the shortest physiological route to the active cancer cells would be required. Each of these carriers would need not only a propelling system, but also have a level of autonomy and some level of ‘intelligence’ to be able to navigate towards such active cancer cells being detected by their own sensors. More specifically, each carrier would need to be no larger than the size of a bacterium to be able to reach the active cancer cells through physiological openings leading to the tumour interstitial space, and each carrier would require a compass to allow an external platform to guide them from the injection site to a solid tumour or a region that need to be treated. Since the spatial resolution of modern clinical imaging modalities cannot detect the small blood vessels and other potential microscopic physiological route leading to the active cancer cells, each carrier would have to be sufficiently autonomous to navigate around physiological obstacles. Once in the tumoural volume for instance, each carrier should be able to detect and adjust their trajectory accordingly in order to follow decreasing oxygen gradients and to stop at specific oxygen concentrations corresponding to hypoxic regions where oxygen is being consumed by active cancer cells before releasing the therapeutics. In other words, not simple carriers but cancer fighting nanorobots offering these capabilities would be required. Finally, tens of millions of these nanorobots carrying therapeutics and implemented at low cost would be required to deliver sufficient doses to achieve the required therapeutic effect. Since this is far beyond what can be implemented artificially, the strategy has been to identify a bacterium with the right characteristics and functionalities and to harness it to behave as a natural cancer fighting nanorobot capable of delivering therapeutics directly to regions of active cancer cells where the therapeutic efficacy would be optimal. The MC-1 magneto-aerotactic bacteria have been selected to act as such natural nanorobots when operating under a computer-controlled platform developed by our group and dubbed the magnetotaxis platform. Experiments performed in mice showed that 55% of the drug injected reached the active cancer cells deep in tumours compared to only 0.7% with typical chemotherapy.
POSTERS

P1 A link between human milk oligosaccharides, infant faecal community types, and risk of infection
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P2 Functional and potential probiotic properties of lactobacilli
Š. Horáčková, Kristina Bialasová, M. Kumherová and M. Plocková
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P3 Antimicrobial properties of Saccharomyces cerevisiae CNCM I-3856 against enterotoxigenic Escherichia coli in a simulator of the human upper GI tract
C. Roussel1,2, W. Galia3, A. Collette1, S. Chalancon1, N. Ballet4, O. Le Goff1, S. Denis1, M. Alric1, F. Leriche1, P. Vandekerckove4, T. Van de Wiele2 and Stéphanie Blanquet-Diot1
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P4 Emerging sampling and microbiota analysis tools that simplify and facilitate experimental setup of clinical trials
Bartholomeus van den Boeg1,2 and D. Butler1,2
BaseClear BV, the Netherlands; 2MyMicroZoo BV, the Netherlands

P5 Monitoring intestinal immune and microbiome responses at the gut level in ruminants
Christopher C.L. Chase1,2, A. Young1, C. Rinehart2, B. St. Pierre3 and E. Chevaux4
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P6 Adipose tissue microbiota: the new target of prebiotics in diabetic mice model
Bérengère Coupé, S. Delga, J.L. Insonere, G. Payros, F. Servant and B. Lelouvier
Vaiomer SAS, France

P7 Metabolites from Lactobacillus and bifidobacteria enhance the macrophage response to pathogenic challenge in vitro
Thomas S. Davies1, S.F. Plummer1, J.W.E. Moss2, D.P. Ramji2 and D.R. Michael1
1Cultech Ltd., Unit 2, UK; 2Cardiff School of Biosciences, Cardiff University, UK

P8 Polysaccharides isolated from Lactobacillus casei LOCK0919 probably promote benefits to the host
Petra Hermanova1, M. Schwarzer1, D. Srutkova1, H. Kozakova1, B. Cukrowska2, S. Gorska3 and T. Hudcovic1
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P9  The role of probiotic strain *Escherichia coli* Nissle 1917 in gnotobiotic mice experimental models
Tomás Hudcovic, R. Stepankova, H. Kozakova, D. Srutkova, M. Schwarzer, P. Hermanova, B. Sestakova and H. Tlaskalova-Hogenova
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P10  Beneficial role of selected probiotic *Bifidobacterium longum* ssp. *longum* on the development of DSS-induced colitis in mice
Hana Kozakova, D. Srutkova, T. Hudcovic and M. Schwarzer
Laboratory of Gnotobiology, Institute of Microbiology of the CAS, Czech Republic

P11  Functional properties of vaginal lactobacilli isolated from pregnant women
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P12  Microencapsulation of *Lactobacillus plantarum* LAB12 enhanced pH-resistance, heat-tolerance and targeted release without compromising efficacy
Siong Meng Lim¹,², I.M. Fareez¹,², F.T.Lim² and K. Ramasamy¹,²
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P13  The effect of probiotic supplementation on the efficacy of antidepressant treatment in depression: research proposal
A. Schmidt¹, Laura Mählmann¹, S. Brand²,³, N. Schweinfurt², S. Borgwardt¹, C. Beglinger⁴ and U. Lang¹
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P14  Screening for inhibitory activity of *Lactobacillus salivarius* SGL03 against oral pathogens and its efficacy in improving oral health
Laura Manna¹, F. Federici¹, E. Rizzi¹, E. Galantini¹, U. Marini¹, P. Pidutti² and D. Cecconi²
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P15  The effect of additional galacto-oligosaccharide prebiotic on iron absorption with micronutrient powders in Kenyan infants
Nadja Mikulic¹, D. Paganini¹, M.A. Uyoga², N. Stoffel¹, F. Jeroense¹, C. Zeder¹ and M.B. Zimmermann¹
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P16  Real-time PCR quantification of probiotic strains in clinical faecal samples of healthy adults administered varying doses of a multi-strain probiotic
Varuni Nagulesapillai, A. Piano, T. Fountain, S. Wang and T.A. Tompkins
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P17  Efficacy of pre-selected bacilli as probiotic feed additives in F4+ Escherichia coli challenged post-weaning piglets
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P18  The efficacy of a novel probiotic on glucose metabolism: a randomised controlled trial
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P19  Lactobacilli-supplemented diet improved broiler performance: an interplay between diet, gut microbiota and health
Kalavathy Ramasamy¹,², S.M. Lim¹,², F.T. Lim², C.C. Sieo³ and Y.W. Ho⁴
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P20  Physico-chemical approach for characterising probiotics: example of high concentrated multistrain-based formulation
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P21  Differential development of the ileal microbiome in three broiler breeds
Peter Richards, P. Wigley and J. Fothergill
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P22  Induction of IL-8, IL-12/23 p40, and TNF-α and transcription of TLR2, TLR4, and TLR9 in the ileum of germ-free piglets colonised with E. coli Nissle 1917 or Lactobacillus amylovoorus or infected with Salmonella Typhimurium
Vera Slavikova, A. Splichalova and I. Splichal
Laboratory of Gnotobiology, Institute of Microbiology of the CAS, Czech Republic

P23  Molecular effects of GOS delivered in ovo in chickens challenged with acute heat stress
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P24  Can a commercial probiotic or a faecal transplant delay Campylobacter jejuni transmission in broiler chickens?
R. Gilroy, G. Chaloner, L. Lacharme-Lora, A. Wedley and Paul Wigley
Institute for Infection and Global Health, University of Liverpool, UK

P25  Milk versus milk replacer in preweaning: effects on gut microbiota of dairy calves
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A link between human milk oligosaccharides, infant faecal community types, and risk of infection

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Human milk oligosaccharides (HMOs) may provide health benefits to infants partly by shaping the development of the early life intestinal microbiota. We explored stool microbiota in relation to reported morbidity identified a priori and medication use throughout 12 months in infants fed formula milk supplemented with 2 HMOs. Healthy term infants <14 days old were randomly assigned to infant formula (control, n=62) or the same formula with 1.0 g/l 2'-fucosyllactose and 0.5 g/l lacto-N-neotetraose (test, n=58) from enrolment to 6 months; all infants received the same follow-up formula without HMOs from 6-12 months. Breastfed infants (BF, n=35) served as a reference group. Stool microbiota at 3 and 12 months was analysed by 16S rDNA sequencing. Faecal community types (FCT) were established by Dirichlet Multinomial Mixture model. Their associations with HMOs supplementation of formula, and reported morbidities and medication use through 12 months were established by chi-square tests and Cox-proportional hazard model.

At 3 months, microbiota composition in the test group appeared closer to BF than control by microbiota alpha (within group) and beta (between groups) diversity analyses, and distribution of microbiota community types (A, B, or C). HMOs supplementation decreased the number of infants with formula specific C community and increased those with BF specific B community. Both FCT B and C were dominated by Bifidobacterium spp., but with a higher abundance in B, whereas FCT A was Enterobacteriaceae dominated. In formula fed infants, the likelihood of cumulative reported antibiotic use through 12 months was increased with FCT C compared to FCT B. At 12 months, none of the four FCTs (D, E, F, and G) was associated to any of the formulas or breastfeeding. Amongst these FCTs, three were likely precursors of the enterotypes previously identified in adult faeces. In conclusion, infant formula with HMOs shifted microbiota towards that of breastfed infants. The 3-month FCT promoted by HMOs was associated to lower antibiotic use in the first year of life. Previously reported reduced likelihood of medication use with HMOs may thus be linked to gut microbiota community types. Potential precursor communities for adult enterotypes were identified as early as 12 months.
Functional and potential probiotic properties of lactobacilli

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Recently, great attention has been paid to the application of beneficial microbes not only to dairy products but also to non-dairy foods in combination with different prebiotics. Species Lactobacillus plantarum, naturally occurring on many plant substrates, could be a suitable candidate for this purpose. In this work, the growth characteristics and metabolic activity of several L. plantarum strains on non-traditional fibre source as carbon substrate were tested. Also, their probiotic properties were tested in vitro, such as antimicrobial activity against conditional pathogens and antifungal activity, bile salt hydrolase activity, and stability in conditions simulating those in the gastrointestinal tract. The lactobacilli growth was best supported by the addition of moringa (powder from the leaves of the tree Moringa oleifera) and quinoa (powder from the plant Chenopodium quinoa) to the basic cultivation media. Antibacterial activity caused especially by life cells against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, and Listeria monocytogenes, and antifungal activity against Fusarium culmorum, Candida albicans, and C. parapsilosis were proved. Furthermore, the synergic effect of lactobacilli metabolites (lactic, acetic and phenyllactic acids) against the moulds was confirmed. Low phenyllactic acid concentrations (2-3 mg/100 g agar), corresponding to its real production by lactobacilli, did not inhibit mould growth significantly. The characteristics of two tested lactobacilli strains could succeed in non-traditional synbiotic functional food development. Acknowledgements. The work was supported by grant of Ministry of Agriculture No. QJ1610202 and from specific university research (MSMT No 20-SVV/2017).
Antimicrobial properties of *Saccharomyces cerevisiae* CNCM I-3856 against enterotoxigenic *Escherichia coli* in a simulator of the human upper GI tract

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Enterotoxigenic *Escherichia coli* (ETEC) are major food and water borne pathogens responsible for traveller’s diarrhoea. The main virulence traits of ETEC infections are the production of adhesins such as CFA/I and Tia promoting colonisation of the small intestine, and the secretion of heat-labile (LT) and/or heat-stable (ST) enterotoxins leading to watery diarrhoea. In a context of increase in antibiotic resistance worldwide, probiotics are a promising alternative strategy for the control of ETEC infection. In this study, we investigated the ability of the probiotic yeast *Saccharomyces cerevisiae* CNCM I-3856 to modulate the survival and virulence of the ETEC reference strain H10407 in a dynamic multi-compartmental simulator of the human upper GI tract, the TNO gastrointestinal model (TIM). Two series of *in vitro* experiments (n=4) were performed: ETEC alone at a physiological dose of $10^{10}$ bacteria or ETEC co-administered with the yeast ($10^{10}$). Bacteria and/or yeast were introduced with a glass of water into the TIM system reproducing the main physicochemical digestive parameters of a healthy adult. During *in vitro* digestion, kinetics of ETEC viability were determined by plating, PMA-qPCR and flow cytometry. Production of LT enterotoxins was measured by ELISA and the expression of six major virulence genes was followed by qRT-PCR in the TIM gastric and ileal effluents. Growth resumption of ETEC was noticed at the end of *in vitro* digestions in the jejunal and ileal compartments, which are the main sites of pathogenesis of the bacteria in humans. Co-administration of *S. cerevisiae* CNCM I-3856 led to a decrease in the amount of viable bacteria in the small intestine *in vitro*. When the probiotic yeast was added, we also observed in the gastric and ileal effluents a significant reduction in LT enterotoxin production, as well as a down-regulation of estA, cfa/lb and tia genes encoding for ST enterotoxins, CFA/Ib and Tia adhesins, respectively. Our study allows a better understanding of ETEC pathogenesis in human simulated gastrointestinal conditions. We also showed the potential of the probiotic yeast *S. cerevisiae* CNCM I-3856 in reducing ETEC viability and virulence. The next step will be dedicated to the study of ETEC and probiotic yeast in a simulator of the lower part of the human GI tract integrating gut microbiota, the mucosal simulator of the human intestinal microbial ecosystem (M-SHIME).
Emerging sampling and microbiota analysis tools that simplify and facilitate experimental setup of clinical trials

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During the last decades, considerable research has been performed to elucidate the composition and function of the human microbiota. Most of this research has been focused on the intestinal microbiota for which faecal samples are usually obtained as a representative of the bacteria that inhabit the end of the large intestine. 16S rRNA gene targeted microbial profiling is widely employed to determine the bacterial composition. While this technology is relatively mature, it depends heavily on obtaining adequate concentration and sufficient quality DNA. Therefore, it is no surprise that sample collection and DNA extraction are important factors that influence the outcome of microbial profiling. More importantly, these factors need to be considered when using these approaches for clinical trials. At BaseClear we evaluated and compared faecal collection methodologies and DNA extraction methodologies that led to a simple and established protocol for collection of faecal samples. This method employs DNA stabilising buffers that enable transport of the sample at ambient temperatures instead of using dry ice or liquid nitrogen. Moreover, sampling can be performed at the study participant’s home which facilitates recruitment of participants itself. Microbial communities with a known composition (so-called ‘mock’ communities) are used to evaluate and monitor reproducibility of the pipeline as well as the outcome of composition analysis to reflect that of the original sample. As study participants are more motivated when they are involved in the project, our sister company MyMicroZoo™ can provide the outcome of microbial profiling to the participants in a secure and anonymous manner. In conclusion, the microbiota analysis pipeline of BaseClear and MyMicroZoo can facilitate clinical trial experiment setup using an established and evaluated analysis from sample collection and DNA extraction to 16S rRNA gene sequencing as well as subsequent bioinformatics analysis.
Monitoring intestinal immune and microbiome responses at the gut level in ruminants

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Mucosal immune responses involve the immune modulation of the mucosa epithelial cells. These cells respond to microbial produced ligands and metabolites and modulate the immune response. An experimental approach in cattle to study these interactions was developed. A pilot study was done to determine the effect of feeding a yeast probiotic on intestinal immune function. Stressors (social and physiological) were applied to the animals and cytokines [tumour necrosis factor alpha (TNF-\(\alpha\)), transforming growth factor beta (TGF-\(\beta\)), interleukin 1 beta (IL-1\(\beta\)), interleukin 6 (IL-6), and interleukin 10 (IL-10)] and white blood cell counts were measured in intestinal lymph collected real-time using a lymph node cannulation system. White blood counts, antibody and cortisol were also measured from the peripheral blood. Increases in stress resulted in increases of lymphatic TNF-\(\alpha\), IL-6 and IL-1\(\beta\). The inclusion of a yeast probiotic at times of stress decreased these responses. In addition, one calf had intestinal cannulas placed at the ileum and duodenum and ingesta samples collected for microbiota analysis. Microbial composition was determined using a PCR-next generation sequencing approach, which included taxonomic affiliation and clustering of operational taxonomic units (OTU). This approach to assess immunological function in the gut provides a real-time assessment of tissues directly involved in the immune response.
**Adipose tissue microbiota: the new target of prebiotics in diabetic mice model**

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It is now well established that metabolic diseases are associated with gut microbiota dysbiosis and systemic inflammation. We have shown that gut microbiota dysbiosis is associated with an increase in intestinal permeability leading to bacterial translocation from the gut to tissues (blood, adipose tissue, liver) establishing a tissue microbiota. These bacteria interact with host cells and modify their function leading to metabolic inflammation or other pathologies. It is crucial to assess their genetic signature in tissues and their modulation by prebiotics. In this study, we have shown that a high fat diet for 4 weeks induces glucose intolerance in mice, associated with dysbiosis in gut and mesenteric adipose tissue (MAT) assessed by 16S rDNA targeted metagenomic sequencing. Treatment with a prebiotic reduce glucose intolerance and modify the gut microbiota signature. Moreover, we have shown by 16SqPCR a decrease in bacterial translocation when HFD mice are treated with prebiotics. Treatment of HFD mice with prebiotics also rescue the dysbiosis in adipose tissue. These data suggest that systemic inflammation leading to metabolic complication is regulated by a direct crosstalk between bacteria and host cells and indicate that prebiotics treatment impacts positively gut and adipose tissue microbiota which decrease glucose intolerance.
Metabolites from *Lactobacillus* and bifidobacteria enhance the macrophage response to pathogenic challenge *in vitro*

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The Lab4 and Lab4b consortia of probiotics are composed of *Lactobacillus* spp. and *Bifidobacterium* spp., and supplementation of these consortia has shown a reduction in the incidence of upper respiratory tract infections (URTI) in children and reductions in atopic eczema sensitisation in babies, respectively, in human intervention studies. This implies immune-enhancing/modulating capabilities for both consortia. We have shown that metabolites from Lab4 and Lab4b are able to increase gene expression levels of iNOS and IL-6 while reducing gene expression levels of CD206 and Arg1 in mouse macrophages *in vitro*. This suggests that Lab4 and Lab4b are able to polarise such cells to the classical 'M1' pro-inflammatory phenotype. In human macrophages, gene expression and protein levels of the pro-inflammatory cytokine IL-1β were increased by both consortia, thus, highlighting the involvement of the inflammasome – a key intracellular regulator of innate immune response. Gene expression analysis of key components of the inflammasome; a key intracellular regulator of IL-1β secretion and the innate immune response, revealed differential expression patterns with Lab4 able to upregulate NLRP1, NLRP3 and AIM2 and Lab4b able to upregulate NLRP3 and NLRC4. Both probiotic consortia also enhanced macrophage IL-1β secretion in response to challenge with pathogen bacteria (modelled with LPS and ATP). In addition, Lab4 and Lab4b upregulated gene expression of IL-12p35 and IL-12p40 and secretion of IL-12 by human macrophages, suggesting anti-viral activity by these cells. Macrophage secretion of IL-12 in response to poly I:C, a synthetic viral mimic, was also enhanced in the presence of metabolites from Lab4 and Lab4b. Taken together, these data suggest that metabolites generated by the Lab4 and Lab4b probiotic consortia promote an anti-bacterial and anti-viral response by macrophages *in vitro* and enhance the response of macrophages to bacterial and viral challenge. These data provide some mechanistic insight into the *in vivo* Lab4 and Lab4b outcomes.
Polysaccharides isolated from Lactobacillus casei LOCK0919 probably promote benefits to the host

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Lactobacilli are currently the most frequently used bacteria in probiotic products. We recently demonstrated that mixtures of Lactobacillus rhamnosus LOCK0900, L. rhamnosus LOCK0908, and L. casei LOCK0919 (L919) have synergistic effect in induction of anti-allergic and regulatory cytokines in human blood cell culture. Germ-free mice were colonised with the mentioned lactobacilli strains. Bacterial colonisation was evaluated at weekly intervals to day 48. Vortexed faeces were plated onto MRS agar and cultivated. Bacteria were distinguished on the colonies’ morphology basis. To distinguish between L. rhamnosus strains specific qPCRs were used. We investigated that L919 is a dominant coloniser of the mice gut. The signalling pathways were evaluated using HEK 293 cells transfected with TLR2, TLR4, and NOD2, and immunomodulatory properties (cultivation with bone marrow-derived dendritic cells – BM-DC) were assessed with formalised bacteria and isolated and purified polysaccharides from L919. Whole bacterium L919 was recognised by TLR2 and NOD2 receptors and stimulated production of IL-10, TGF-β, IL-12p70 and TNF-α in culture of BM-DC. Two antigens, L919/D and L919/E, were isolated from the molecular mass of L919 according to their sugar composition. The polysaccharides’ structural analysis was determined by classical chemical analysis, nuclear magnetic resonance spectroscopy and mass spectrometry. We showed that the polysaccharides L919/D and L919/E did not signal through the TLR2, TLR4 nor NOD2 receptors and did not induce immune response by themselves in culture of BM-DC. In conclusion, we found that L. casei LOCK0919 is a dominant coloniser of the mice gut. We observed that probably polysaccharides contribute to the host colonisation, which may promote some benefits to the host. L919 produced two different polysaccharides that have distinct immunomodulatory properties compared to the whole bacteria. Acknowledgements. Supported by CSF grant No: 15-07268S.
The role of probiotic strain *Escherichia coli* Nissle 1917 in gnotobiotic mice experimental models

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*Escherichia coli* Nissle 1917 has been recommended as a therapeutic tool for treatment of various diseases and dysfunctions of the intestinal tract. The aim was to study the effect of *E. coli* strains on the development of acute ulcerative colitis (UC) induced by dextran sulfate sodium (DSS) in gnotobiotic mice. Two-month-old germ-free Balb/c mice were used in our experiments. Experimental colitis was evoked by administration of 2.5% dextran sulfate sodium (DSS) in drinking water for 7 days. In the first experimental group, originally germ-free mice were monoassociated with *E. coli* Nissle 1917 and then after weaning (day 21) the mice were reassocciated with uropathogenic *E. coli* O6K13. In the second group, originally germ-free mice were monoassociated with *E. coli* O6K13 first and then reassocciated after weaning by *E. coli* Nissle 1917. DSS was administered to both groups. Colon morphology and mucin production were evaluated. The level of cytokines was determined in the supernatant of cultivated intestinal pieces of colon descendens. The levels oftight junction protein zonulin 1 (ZO-1) and immunoglobulin A (IgA) were detected by immunohistochemistry. Production of myeloperoxidase (MPO) was determined in colon tissue. Mice monoassociated with *E. coli* Nissle 1917 strain and then reassocciated by *E. coli* O6K13 strain developed a lower degree of intestinal inflammation in the colon in the DSS model of ulcerative colitis than the conversely associated group. In the first group, the level of pro-inflammatory cytokine TNF-α and IL-6, ZO-1 and IgA was reduced markedly in the colon compared to the second experimental group. It is concluded that *E. coli* Nissle 1917 colonisation protects mice against intestinal inflammation induced by *E. coli* O6K13 strain in the DSS model of ulcerative colitis. For this reason, we believe that *E. coli* Nissle 1917 is a suitable candidate, which we will further test in other gnotobiotic models. **Acknowledgements.** Supported by grant 15-07268S of the Czech Science Foundation.
Beneficial role of selected probiotic *Bifidobacterium longum* ssp. *longum* on the development of DSS-induced colitis in mice

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Composition and diversity of microbiota and homeostasis are responsible for health or disease. IBD is accompanied with reduced microbiota diversity. In the ulcerative colitis mice model, we studied the effect of two *Bifidobacterium longum* ssp. *longum* strains chosen according to the effects on cytokine production *in vitro*. Nine probiotic candidates – bifidobacterial human isolates – were cultivated with splenocytes and cytokines. Two *B. longum* ssp. *longum* (Bl) strains with different patterns of cytokine induction were further analysed using bone marrow-derived dendritic cells (DC) and human embryonal kidney cells transfected by pattern recognition receptors. During the next step, before treatment with 2.5% dextran sodium sulphate (DSS) solution, mice received *B. longum* strains or saline by intragastric gavage. We found that cytokine induction by bifidobacteria is strain dependent. Strain Bl7952 compared to strain Bl372 stimulated lower levels of IFN-γ, TNF-α and IL-10 in naive splenocytes or DC. Both strains engaged TLR2 receptor but Bl7952 signalisation through NOD2 was stronger compared to Bl367. In the DSS-model, the Bl7952 strain reduced macroscopic and histological signs of intestinal inflammation and downregulated pro-inflammatory cytokines in mesenteric lymph node cells compared to strain Bl372 or the PBS control. The *in vitro* less immunogenic *B. longum* ssp. *longum* strain CCDM 7952 was able to protect the development of DSS-colitis in mice contrary to the strain CCDM372. *B. longum* CCM 7952 promotes epithelial barrier function and prevents acute DSS-induced colitis in a strictly strain-specific manner. We conclude that the probiotic strain inducing less pronounced immune responses might be a good candidate for IBD prevention. Acknowledgements. Grant 15-07268S of the Czech Science Foundation.
P11

Functional properties of vaginal lactobacilli isolated from pregnant women

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The vaginal microbiome of healthy women is a dynamically changing ecosystem. It is affected by a number of factors, such as age, hygiene, hormone levels, menstrual cycle or diet. Bacteria from the genus Lactobacillus are dominated in the vaginal microbiome in the density $10^7$-$10^8$ cfu/g of vaginal fluid in healthy premenopausal women. Vaginal lactobacilli are important because of their protective function (adhesion to the vaginal tissue and production of antimicrobial substances). The most common strains are Lactobacillus crispatus, L. gasseri, L. iners, and L. jensenii but it depends on ethnicity. There is a strain diversity between pregnant and non-pregnant women. The number of lactobacilli decreases during pregnancy and the vaginal microbiome is abnormal. It can cause grow of pathogenic microorganisms (e.g., Escherichia coli, Gardnerella vaginalis, Staphylococcus aureus, Streptococcus agalactiae). The presence of an abnormal microbiome in early pregnancy is recognised as a risk factor for preterm delivery and low birth weight. It follows that the knowledge of the biology and metabolic activity of vaginal lactobacilli is important for the prevention and treatment of vaginal infections of pregnant women. In this study, lactobacilli were isolated from 50 vaginal swabs of pregnant women from the Czech Republic and identified by MALDI-TOF MS. Antibacterial activity of lactobacilli was tested by the agar diffusion method against 7 pathogenic microorganisms. Determination of hydrogen peroxide produced by lactobacilli was tested on MRS agar supplemented with 3,3’, 5, 5’-tetramethylbenzidine and horseradish peroxidase. Antibiotic resistance was tested by the disk diffusion method with 16 antibiotics. Autoaggregation of lactobacilli was observed in PBS buffer by absorbance measurement. It was found that lactobacilli occurred in 56% of the vaginal swabs. The most represented strain was L. crispatus (48%), followed by L. gasseri, L. fermentum, L. jensenii, L. rhamnosus, L. plantarum, and L. iners. The majority of isolates exhibited antibacterial activity against the tested pathogenic microorganisms. The highest antimicrobial activity was measured for L. fermentum and L. rhamnosus. Each of the isolates was resistant to metronidazole and fluconazole. The highest autoaggregation was observed for L. crispatus. Acknowledgements. This work was supported from specific university research (MSMT No 20-SVV/2017).
Microencapsulation of *Lactobacillus plantarum* LAB12 enhanced pH-resistance, heat-tolerance and targeted release without compromising efficacy

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Dysbiosis is associated with increased susceptibility to intestinal and extra-intestinal disorders. It was hypothesised that consumption of probiotics can alter the composition of microflora in the gastrointestinal tract and thereby restore the health of the hosts. Our preliminary studies strongly indicated the potential use of *Lactobacillus plantarum* LAB12 (LAB12), a lactic acid bacteria (LAB) isolated from local fermented food, as cholesterol lowering agent. In spite of their beneficial effects, the vulnerability of LAB12 to low pH (i.e., gastric transit) and intense heat (i.e., industrial processing) remains a major obstacle to their widespread application. This study immobilised LAB12, by means of microencapsulation, within alginate (Alg)-based polymeric matrix incorporated with vegetable-based protein (VP). The survivability of microencapsulated LAB12 exposed to simulated gastrointestinal fluids and high temperatures was assessed. It was found that microencapsulated LAB12 exhibited excellent tolerance against simulated gastric juice (96.4% survivability), intense heat (80.2% survivability at 100°C for 30 min) and targeted release in simulated intestinal fluid (>9 log cfu/g). The fate and release of LAB12 from Alg-based microcapsules in different gut sections were also examined. The microcapsules were intact in the stomach and free LAB12 were found to be present abundantly (>7 log cfu) only in the intestines. The safety of the encapsulated LAB12 was tested via acute and a 90-day subchronic toxicity studies. No treatment-related adverse effects were observed across all vital physiological parameters. The present study then evaluated the cholesterol lowering effect of microencapsulated LAB12 in the context of high cholesterol diet prepared by dissolving cholesterol in diethyl ether to achieve a content of 5% cholesterol-coated feed after ether evaporation. Serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL)/very low-density lipoprotein (VLDL) and liver triglyceride were measured using commercial ELISA kits. Apart from retaining their intrinsic cholesterol lowering effect, it was found that microencapsulated LAB12 conferred additional advantages in terms of lowering body mass index (BMI) and triglyceride as well as increasing HDL. Altogether, this study strongly implied the possibility of having microencapsulated LAB12 safely incorporated into a wide variety of food types to confer beneficial effects to the hosts.
The effect of probiotic supplementation on the efficacy of antidepressant treatment in depression: research proposal

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Recent research demonstrates that the composition of the gut microbiome is a master regulator of key neurophysiological processes that are affected in depression. Contemporary studies showed that faecal microbiota is altered in patients with major depressive disorder (MDD). Furthermore, it has also been shown that supplementation with probiotics ameliorated depressive symptoms in unmedicated patients with mild to moderate depression. However, no study has yet explored the efficacy of a probiotic-based therapy in patients with severe MDD in addition to a standard antidepressant treatment. This project is aimed at investigating for the first time if probiotic supplementation compared to a placebo treatment improves the effect of standard antidepressant medication on depressive symptoms (i.e. better and faster remission) in patients with severe MDD. For the study a double-blind, placebo-controlled, randomised, pre- and post-intervention design will be applied, conducted in a single center. Over a period of 4 weeks, 30 depressive patients will ingest a probiotic food supplementation (Vivomixx®) 4 times a day and will be compared to a placebo (n=30). Once before and after these 4 weeks, the effect of the probiotic supplementation will be examined on the primary and secondary study outcomes compared to a placebo condition. It will be tested if probiotic supplementation modulates immune signalling and inflammatory processes (macrophage migration inhibitory factor and interleukin 1 beta), hypothalamic-pituitary-adrenal (HPA) axis responses (saliva cortisol), neurogenesis (brain-derived neurotrophic factor (BDNF) expression), the release of appetite-regulating hormones (leptin and ghrelin), the composition of gut microbiota (in particular levels of Enterobacteriaceae, Alistipes and Faecalibacterium) and brain perfusion, structure and activation, and if these changes are associated with the probiotic-induced effect on depressive symptoms.
P14

Screening for inhibitory activity of *Lactobacillus salivarius* SGL03 against oral pathogens and its efficacy in improving oral health

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The objective of this study was the characterisation of a new strain *Lactobacillus salivarius* SGL03, isolated in Sintal Dietetics’ cell bank, as a potential probiotic with antimicrobial activity against some human oral pathogenic strains, known to cause various oral diseases such as pharyngitis and tonsillitis, dental caries, bad breath, periodontitis and other oral inflammatory diseases. Various studies have reported probiotics inhibition of oral bacteria, suggesting a promising role in establishing good periodontal health and combating periodontal diseases. For this purpose, after initial biochemical and molecular identification and screening for gastrointestinal survival, *L. salivarius* SGL03, was tested in co-culture experiments, for its inhibitory capacity against some oral commensal bacteria involved in oral diseases. Further study was the identification and characterisation of antimicrobial peptides secreted from *L. salivarius* SGL03 by tricine SDS-PAGE coupled to nano-HPLC-Chip MS/MS technology. The potential antimicrobial activity of the identified peptides was tested by agar well diffusion method against a whole panel of pathogenic bacteria, including the oral pathogens selected. Gastrointestinal tolerance assays showed that *L. salivarius* SGL03 was able to survive passage through the gastrointestinal tract. In co-culture experiments, the strain showed good antimicrobial activity against the tested pathogens *Streptococcus pyogenes*, *S. mutans*, *S. sanguinis*, and *S. uberis*. Proteomic analysis identified eight secreted proteins, two of them with a strong inhibitory activity against *S. pyogenes*. A smaller inhibition was observed against *S. uberis* and *Enterococcus faecium*. The obtained results suggest that *L. salivarius* SGL03 could be considered as promising, safe and natural option, in the prevention and treatment of oral infections. Moreover, the identification of bacteriocins with specific inhibitory activity, represents a crucial step in this field. Future developments will certainly contemplate the development of successful probiotic delivery system in form of chewing gums, tablets, candies containing *L. salivarius* SGL03 and/or its bacteriocins, for prevention and treating of oral infections. Further studies will be carried out to test the antimicrobial activity of the identified proteins of SGL03, against pathogens known cause infections in other body sites (urinary tract, skin, vagina).
The effect of additional galacto-oligosaccharide prebiotic on iron absorption with micronutrient powders in Kenyan infants

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Iron plays an essential role in cognitive performance and growth in infants and children. Iron deficiency anaemia (IDA) is a leading global risk factor for disease, disability and death. In sub-Saharan Africa, infants and young children have high rates of IDA and in Kenya, 73% of <5-year-old children are anaemic. In-home fortification of complementary foods using micronutrient powders (MNPs) has been shown to reduce the risk for IDA. However, iron absorption from these MNPs is low and consumption of iron-containing MNPs may adversely affect the African infant gut by decreasing beneficial ‘barrier’ commensals, while increasing enteropathogens, gut inflammation, diarrhoea and respiratory tract infections. Safer MNP formulations with high bioavailability are thus urgently needed. Addition of prebiotic galacto-oligosaccharides (GOS) to infant formulas selectively enhance growth of beneficial Bifidobacteriaceae and Lactobacillaceae. We investigated the efficacy and safety of an improved MNP formula with 7.5 g GOS combined with a low dose of highly bioavailable iron (5 mg ferrous sulphate (FeSO₄) or a mixture of 2.5 mg ferrous fumarate (FeFum) and 2.5 mg sodium iron ethylene-diaminetetraacetate (NaFeEDTA)), which aims to improve iron status and gut microbiota in infants living in resource-poor areas. Two human absorption studies were conducted in which fractional iron absorption was measured as erythrocyte incorporation of stable isotopes 14 days after consumption of a labelled test meal containing the MNP. In study 1, infants consumed iron-fortified (MNP) meals either with (n=22) or without GOS (n=28) for three weeks daily. GOS consumption significantly increased iron absorption by 62% from the MNP containing the iron mixture (NaFeEDTA+FeFum) (P<0.05), but not from FeSO₄. In study 2, we investigated if the beneficial effect of GOS on iron absorption is an acute effect not requiring pre-feeding. Infants (n=16) were enrolled to consume iron-fortified (MNP) meals either with or without GOS. Although the addition of GOS increased iron absorption by 57% (P=0.12), the increase was not statistically significant. Our findings demonstrate that 3 weeks of feeding a GOS-containing MNP containing a low iron dose of 5 mg sharply increases iron absorption in African infants. This is the first demonstration in humans, which shows that pre-feeding with a prebiotic increases iron absorption measured by stable isotopes. The beneficial effect may be due to increased colonic iron absorption and appears to be iron compound specific.
P16

Real-time PCR quantification of probiotic strains in clinical faecal samples of healthy adults administered varying doses of a multi-strain probiotic

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A randomised, double-blind, dose-response clinical study (NCT02693314) investigated the effects of two doses (5 billion cfu or 25 billion cfu/capsule) of a multi-strain commercial probiotic on faecal microbiota, gastrointestinal function, and general wellness in healthy adults. The probiotic formulation contains *Lactobacillus rhamnosus* R0011 and *L. helveticus* R0052 among other strains. The objective of this sub-study was to enumerate the probiotic strains in the faecal samples by real-time PCR (qPCR), while comparing two faecal DNA extraction kits. Participants of the clinical study were randomised to one of three groups: placebo (n=21), multi-strain probiotic (5 billion cfu/capsule) (n=22), or multi-strain probiotic ‘Higher Potency’ (25 billion cfu/capsule) (n=22). After the baseline period, participants received a placebo or probiotic capsule daily for 28 days, followed by a seven-day wash-out period. Faecal samples were collected at the end of the baseline, intervention, and washout periods for a total of three stool samples per participant. DNA was isolated from all faecal samples by both the Qiagen QIAamp DNA Stool Mini Kit (n=195), and MP Biomedicals FastDNA SPIN kit for faeces (n=195), and then analysed by SYBR-green based qPCR assays with strain-specific primers. The *L. rhamnosus* R0011 strain was detected in 80% of the participants with the QIAamp kit, and in 84% of the participants with the FastDNA kit during probiotic consumption. *L. helveticus* R0052 was detected in fewer participants: 68% (QIAamp kit), and 55% (FastDNA kit). Both DNA isolation kits detected R0011 or R0052 at similar levels during probiotic intervention. The mean R0011 levels detected in the 5 billion and 25 billion groups by both kits was 5.78±0.64 and 6.39±0.89 log bacteria/g faeces, respectively. Mean R0052 levels observed in the 5 billion and 25 billion groups was 4.63±0.81 and 5.59±0.86 log bacteria/g faeces, respectively. These results demonstrate the use of strain-specific primers for the enumeration of probiotic strains R0011 and R0052 in complex faecal matrices containing numerous gut microbes. The QIAamp and FastDNA faecal DNA isolation kits provided similar detection and quantification results, suggesting that both kits can be used for qPCR analysis of the Lactobacilli strains under investigation.
Efficacy of pre-selected bacilli as probiotic feed additives in F4+ *Escherichia coli* challenged post-weaning piglets

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Weaning is a stressful period for piglets due to changes in diet composition, environmental and bacterial challenges, contributing to reduced feed intake and depressions in growth rate. In-feed antibiotics have been used as growth promoter to counteract these negative effects. Among other feed additives, probiotics have been proposed as alternatives to antibiotics. It has been reported that the dietary inclusion of probiotics can enhance growth performance and modulate intestinal microbiota and immune response in piglets and thus have a positive impact on animal health. Therefore, the objective of the trial was to pre-select candidate *Bacillus* sp. strains by *in vitro* procedures to demonstrate efficacy against pathogenic F4+ *Escherichia coli* by direct antagonism and in different cell line models using IPEC-J2 and Caco-2 cells. Two identified candidate strains (*Bacillus amyloliquefaciens*; DSM 25840, *B. subtilis*, DSM 25841) were tested at the recommended dose level of 1.28 x 10⁹ cfu/kg in maize-wheat-soybean meal based diets for post-weaning piglets from 24 to 45 days of age under experimental F4+ *E. coli* challenge conditions. For comparisons, post-weaning piglets were fed corresponding basal diets without probiotic addition and without (negative control) or with *E. coli* challenge. We could demonstrate that diarrhoea occurred in challenged piglets fed the control diet. The groups fed the diets with added probiotics showed a significantly better outcome in terms of faecal scores and dry matter. This shows that combined *in vitro* and *in vivo* approaches can be useful to select probiotic strains with health-promoting properties.
The efficacy of a novel probiotic on glucose metabolism: a randomised controlled trial

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Type 2 diabetes mellitus (T2DM) is a metabolic condition characterised by a persistent low-grade inflammatory response associated with the development of insulin resistance [Osborn and Olefsky, 2012. Nature Medicine 18: 363-374]. Variations in the type, diversity and metabolic capacity of intestinal microbial communities have shown to alter metabolic and inflammatory pathways within the host by shifting energy balance and storage and promoting metabolic endotoxaemia [Cox and Blaser, 2013. Cell Metabolism 17: 883-894; Le Chatelier et al., 2013. Nature 500: 541-546]. An evidence-based multi-strain probiotic has been developed to restore the intestinal bacterial composition from a disease-prone to a balanced state and to improve metabolic markers associated with T2DM. The aim of this study was to assess the therapeutic effect of this novel probiotic on glucose metabolism in adults diagnosed with prediabetes and early T2DM. Sixty adults with a BMI ≥25 kg/m² and diagnosed with prediabetes or early T2DM (within the previous 12 months) were enrolled in a double-blind controlled clinical trial and randomised to a multi-strain probiotic or placebo for 12 weeks. Outcome measures included anthropometric measurements, metabolic and inflammatory markers and faecal microbial and metabolomic profiles. Sample collection and analysis was performed according the protocol described in Palacios et al. [Palacios et al., 2017. Trials 18: 7]. Preliminary results revealed that probiotic supplementation decreased HbA1c (−0.7±0.1 mg/dl, \( P=0.025 \)) in T2DM participants and insulin resistance (insulin sensitivity index: 1.7±2, \( P=0.045 \)) in those with prediabetes 12 weeks after treatment. Changes in the gut microbial profile such as a decrease in pathogenic bacteria and an increase of butyrate-producing bacteria were found in the probiotic group. In conclusion, intentional manipulation of intestinal microbial profiles may be useful for regulating T2DM-associated metabolic disorders.
Lactobacilli-supplemented diet improved broiler performance: an interplay between diet, gut microbiota and health

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Modern husbandry techniques with genetic selection programmes and intensive farming practices have given rise to dysbiosis, a gut condition often associated with increased incidence of diseases. This has led to attempts to improve the host health status by modulating the intestinal flora via live microbial adjuncts. Capitalising on the interplay between diet, gut microbiota and health, this study assessed the role of 11 \textit{Lactobacillus} spp. (LC) in modulating broiler performance, gene expression, cholesterol metabolism and caecal microflora composition. A total of 180 one-day-old male broiler chicks (Ross 308) were randomly assigned to two groups: basal diet (control), and basal diet + 0.1\% LC for 21 days. LC-fed broilers yielded significantly (\(P<0.05\)) improved feed conversion ratio (-0.053) when compared to the control group. High throughput sequence analysis of bacterial 16S rRNA V3-V4 regions revealed a diverse microbiota in the caecal content. The \textit{Firmicutes}:\textit{Bacteriodetes} ratio in LC-fed broilers was significantly lower (-0.4; \(P<0.05\)) when compared to the control group. A comparative analysis of gene expression between both diet groups was performed using the microarray and selected genes (i.e., actc1, cdkn2b, fgf23-r, gja1, inhba and tf-r) were validated using qRT-PCR. The differentially expressed genes were mainly involved in body development, lipid metabolism and immune response. Interestingly, LC-fed broilers were presented with significantly (\(P<0.05\)) lower serum triglyceride (-31\%), total cholesterol (-31\%) and LDL cholesterol (-22\%) when compared to the control group. Serum HDL cholesterol, on the other hand, was significantly (\(P<0.05\)) higher in LC-fed broilers (+42\%). The liver tissues of LC-fed broilers, which was specifically stained with nile red for lipid (-29\%) and filipin for cholesterol (-11\%), exhibited lower intensity under confocal microscope when compared to the control group. This further verified the serum analyses. Amongst the measured serum immune markers (i.e., interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10) and C-reactive proteins (CRP) and Cluster of differentiation 4 (CD4)), significantly (\(P<0.05\)) lower (-45\%) IL-8 and higher CD4 (+41\%) levels were found in LC-fed broilers when compared to the control group. This went on to show that supplementation of broilers with LC would modulate the gut microbiota in favour of enhanced immune system and lipid metabolism which essentially improved broiler performance.
Physico-chemical approach for characterising probiotics: example of high concentrated multistrain-based formulation

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Probiotics are receiving today unprecedented growing interests in Europe, Asia, and in the rest of the world. Their applications are not only limited to food and health sectors, but cover also agriculture and aquaculture areas. The quality of probiotic products depends on many factors, such as the properties of each individual strain and its proportion in mixed products, the viable probiotic dose, and other selective ingredients like prebiotics and protectant agents incorporated into the formulation [Nguyen et al., 2016. International Journal of Molecular Sciences 17: 867-885]. Several strategies are employed for ensuring high product qualities, which can be controlled by different methods and techniques [Sangami and Radhai Sri, 2017. Current Microbiology & Applied Sciences 6: 194-200]. To date, the physico-chemical approach for characterising and controlling probiotic qualities and performances appears very attractive, but less exploited. It particularly consists in characterising probiotic products in terms of thermal, surface and colloidal properties, which could be correlated to probiotic viability and functionalities. In this communication, we report the efficiency of such an approach when applied to a high concentrated multistrain-based formulation which has been shown a metabolic variability impacting on the inflammatory response, depending on the production site [Fiorucci et al., 2017. Frontiers in Pharmacology 8: 505]. Acknowledgments. We would like to acknowledge the Professor Claudio de Simone for providing samples. Thanks to Mrs Doran for her technical assistance. This work received financial supports from the French Community of Belgium.
Differential development of the ileal microbiome in three broiler breeds

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The intestinal microbiome is a crucial factor in the development of the intestinal immune system. An understanding of how microbial communities develop under normal circumstances can provide a rational basis for probiotic interventions which bolster early immune maturation. The development and succession of the ileal microbiome in three breeds of broiler chickens (Cobb 500, Ross 308 and Hubbard JA87) between 0 and 42 days post hatch (d.p.h) was analysed using an Illumina MiSeq run. DNA extracted from luminal samples at 3, 7, 14, 21, 28 and 42 d.p.h and mucosal samples at 14, 21, 28 and 42 d.p.h was submitted for sequencing of the V4 region of the 16S rRNA gene. In general, the early ileal microbiome is evenly split between Proteobacteria and Firmicutes. There is a transient dominance of the microbiome by Firmicutes between 7 and 28 d.p.h. Candidatus Arthromitus, a segmented filamentous bacterium also known as Candidatus Savagella, is the most abundant genus at 7 and 14 d.p.h after which Lactobacillus becomes the most abundant genus. The dominance by Firmicutes is maintained until 42 d.p.h when the abundance of Proteobacteria increases again. The luminal and mucosal microbiomes are very similar with the exception that Candidatus Athromitus tends to localise to the mucosa. There are differences in the rate of development of the ileal microbiome between breeds. At 3 d.p.h, Firmicutes dominated in Cobb and Hubbard (92% and 99% respectively) while Proteobacteria was the most abundant in Ross (52%). By 7 d.p.h, this pattern had reversed. Ross was now dominated by Firmicutes (93%) with Proteobacteria contributing 48% and 45% to the microbiome in Cobb and Hubbard. The abundance of different genera within Firmicutes was also different between breeds. Candidatus Arthromitus abundance was visibly different between breeds in luminal samples (7 d.p.h) and mucosal samples (14 d.p.h) (Ross = 33% and 44%; Cobb = 6% and 9%; Hubbard = 2% and 55%). While both Hubbard and Ross show a large abundance of Candidatus Arthromitus at different time points, no equivalent abundance was observed in Cobb. The spike in Lactobacillus abundance also occurred later in Cobb chickens (28 d.p.h) compared to Ross (14 d.p.h) and Hubbard (21 d.p.h). Our results suggest that microbial succession in the ileum occurs at different rates between breeds of broiler chickens and gives some insight into potential probiotic targets.
Induction of IL-8, IL-12/23 p40, and TNF-α and transcription of TLR2, TLR4, and TLR9 in the ileum of germ-free piglets colonised with *E. coli* Nissle 1917 or *Lactobacillus amylovorus* or infected with *Salmonella Typhimurium*

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A lower part of the gastrointestinal tract (GIT) harbours a majority of body microbiota. Probiotic and pathogenic bacteria show different actions and Toll-like receptor signalling. The impact of probiotic Gram-negative *E. coli* Nissle (EcN), Gram-positive commensal *Lactobacillus amylovorus* (LA) and pathogenic Gram-negative *Salmonella Typhimurium* (ST) on transcriptions of Toll-like receptors (TLR) 2, TLR 4 and TLR 9 and induction of IL-8, IL-12/23 p40 and TNF alpha in gnotobiotic piglet ileum were evaluated. Hysterectomy-derived colostrum-deprived germ-free piglets (GF) were colonised with EcN or LA 4 h after hysterectomy or bred as GF piglets. One-week-old GF piglets were infected with ST for 24 h. Transcriptions of TLRs (RT-qPCR) and secretion of inflammatory cytokines (xMAP technology) were evaluated and compared. Infection with ST caused bacteraemia, somnolence, anorexia, and diarrhoea in the piglets. The piglets associated with EcN or LA thrived. The transcription of TLR2, TLR4 and TLR9 in EcN and LA piglet groups in ileum were comparable with GF piglets. TLR2 and TLR4 in ST group were highly increased but in contrast the transcription of TLR9 was lower in the ST group. Neither EcN nor LA induced changes in IL-8, IL-12/23 p40 and TNF-α levels in the ileum lavage and their levels were comparable with GF piglets. In contrast, in the piglets infected with *Salmonella* all inflammatory cytokines were highly increased. In conclusion, both EcN and LA colonised the intestine without induction of inflammatory reaction. Enteric pathogen *S. Typhimurium* increased transcriptions of TLR2 and TLR4. Both EcN and ST are Gram-negative bacteria but only ST increased transcriptions of TLR4 that is the receptor for lipopolysaccharide. Transcriptions of TLR4-related molecules as MD-2, CD14, LBP, and adaptor molecules MyD88 and TRIF participate in the TLR4-signaling pathway. We will study transcriptions of these molecules to elucidate differences in activation of the TLR-signalling pathway with *E. coli* Nissle 1917 and *S. Typhimurium*. Acknowledgements. This work was supported by the Charles University, project GA UK No. 1368217, by the Czech Science Foundation, grant No. 13-08803S, and the Institutional Research Concept RVO: 61388971 of the Institute of Microbiology.
Molecular effects of GOS delivered in ovo in chickens challenged with acute heat stress

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Embryonic development inside the egg allows the application of the in ovo technique to deliver prebiotics, such as galacto-oligosaccharides (GOS). This is a potent method for early stimulation of intestinal microbiota, which protects the animal from heat-induced gut dysbiosis. Goal of this study was to determine the molecular effects of GOS delivered in ovo in chickens challenged with acute heat stress. Fertilised eggs of broiler chickens were incubated. On day 12 of egg incubation, a single dose of 3.5 mg GOS/egg (GOS) or physiological saline (0.9% NaCl) (C) was injected in ovo into the air chamber (1000 eggs/group). After hatching, 600 chicks (150 birds/groups) were reared in floor pens: 2 groups (GOS and C, 6 pens/group, 25 birds/pen) were reared in thermoneutral conditions (25°C) (TN) and 2 groups (GOS and C, 6 pens/group, 25 birds/pen) were reared under acute heat stress conditions (HS) induced on day 32 (35°C for 8 h). Animals (n=6), randomly chosen, were sacrificed and tissues (spleen and liver) were sampled for RNA isolation. Three panels of genes were analysed: (1) immune-related genes (IL-1β, IL-6, IL-2, IL-10, IL-12A, IL-17A and IFNβ) in spleen; (2) metabolic genes (APOA1, PPARGC1A, ANGPTL4, ABCG8, ITIH5, GTPase, GIMAp5, CCDC79, ACOX2 and BRSK2) in liver; and (3) stress-related genes (HSP90AA1, HSP70, HSP25, SOD, CAT and BAG3) in both spleen and liver. Two reference genes (UB and ACTB) for relative gene expression analysis were used. There was significant increase in expression of IL-2 and IL-4 (P<0.05) and suggestive of IL-6 and IL-10, IL-12 and IL-17 (P>0.05) in C-HS group in comparison to GOS-HS. It indicated that heat led to endotoxin release but GOS mitigated stress-induced cytokine production. Stress-related genes, including oxidative stress (SOD and CAT), were up-regulated in spleen of C-HS (P<0.05) compared to the other groups, which reflected thermal damage in those animals. Metabolic genes in liver were not affected as much by acute heat stress. In conclusion, GOS delivered in ovo mitigated heat-induced immune system activation in chickens and decreased stress-response. Acknowledgements. The research was supported by a project OVOBIOTIC (RBSI14WZCL, MIUR, Rome, Italy).
Can a commercial probiotic or a faecal transplant delay *Campylobacter jejuni* transmission in broiler chickens?

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*Campylobacter jejuni* is the most commonly reported cause of human bacterial foodborne gastrointestinal illness worldwide. With over 9 million estimated cases of infection each year within the EU alone, it results in extensive medical and productivity burden within countries at every level of development. With current strategies aimed at reducing *C. jejuni* burden within the broiler chicken showing limited success, a pragmatic means of large scale on-farm control is necessary. We examined the effect of commercial probiotic microflora preparation Aviguard® or faecal transplantation (FT) on transmission of *C. jejuni* in broiler chickens in small experimental flocks. In experiment 1, one group (n=10) was given the Aviguard preparation in drinking water at 1 day of age with a second group (n=11) acting as control. In experiment 2, one group (n=20) was hatched within our experimental unit and orally inoculated with a non-cultured caecal content preparation taken from 8-week old chickens within 4 h post hatch as a faecal transplant (FT), whilst the second group (n=19) was obtained from the same commercial hatchery as the eggs. At three weeks of age two birds in each group (seeder birds) were orally infected with $10^6$ cells of *C. jejuni*-M1 whilst the remaining birds were unchallenged. Cloacal swabs were taken on several days post-infection (dpi) to follow transmission and at 14 days following challenge all birds were killed and *C. jejuni* load quantified within the ileal and caecal content and liver tissue. In experiment 1, *C. jejuni* was detected within the control group from four days post challenge whereas the group treated with Aviguard remained negative until 8 days post challenge. At 14 days post challenge, both groups were *C. jejuni* positive with no significant difference in load within caecal content between groups at post mortem analysis. In experiment 2, we found that administration of an ‘at hatch FT’ greatly reduced *Campylobacter* transmission. *C. jejuni* was detected from 2 days post challenge in the control group compared with 12 days post challenge in birds given FT. In contrast to Aviguard, both ileal and caecal *Campylobacter* load was significantly ($P<0.05$) lower in FT birds than hatchery birds at 14 days post challenge with median levels around 5 log10 cfu/g lower in birds receiving a FT. Results have since been replicated in subsequent trials. Our findings indicate that at-hatch transplantation of an adult microbiome both significantly delays *C. jejuni* infection and transmission at a flock level and significantly reduces the intestinal load up to slaughter age and could offer an effective, low-cost control strategy for *Campylobacter* in broiler chickens.
Milk versus milk replacer in preweaning: effects on gut microbiota of dairy calves

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Early gut microbiota plays a key role in the development of the gastrointestinal tract and in gut health. To enhance digestion efficiency and animal health, the improvement of knowledge about these microorganisms is required. However, few studies exploring the establishment of gut microbiota in dairy calves are available. The present study aimed at describing the effect of whole milk (WM) versus milk replacer (MR) on ruminal and faecal microbiota of dairy calves, from birth to weaning. Two groups of five female Holstein calves each were immediately separated from their mother at birth (day 0) and placed in individual pens without any contact with adult animals. Until three days (day 0 – day 2), they were offered colostrum. From day 3 to day 66 (weaning), calves received individually twice a day, WM or MR. From day 10 to weaning, calves received a starter concentrate and hay. Samples of rumen fluid and faeces were collected on each calf at day 3, 10, 35, and 66. High-throughput sequencing was performed on Illumina MiSeq platform. Moreover, diarrhoea was monitored by the assessment of faeces dry matter (DM) and the most important enteropathogens (\textit{Escherichia coli}, rotavirus, coronavirus, \textit{Cryptosporidium} and coccidia) were counted. Our study revealed a difference in bacterial composition between ruminal and faecal contents ($P<0.001$) and a change in bacterial community from day 3 to weaning ($P<0.001$). \textit{Bacteroidetes} was the most abundant ruminal phyla whereas in faeces \textit{Firmicutes} was more abundant before day 66. \textit{Proteobacteria} was present at high abundance at day 3 in ruminal and faecal contents and decreased drastically in faeces but not in rumen fluid. Inverse Simpson index increased with age ($P<0.001$) showing less richness at birth. However, no difference in bacterial composition was noted between calves offered WM or MR ($P=0.11$). Faeces dry matter of calves receiving WM was numerically higher compared to calves offered MR before day 35, but no significant difference was noted. Enteropathogens counting was very different between calves (high individual effect). Our results revealed variations in microbial communities depending on the age of calves. A difference in microbiota composition was noted between ruminal content and faeces but there was no effect of the nature of liquid diet suggesting a stronger effect of the environment than the effect of diet in the establishment of gut bacteria. These results need to be confirmed with a larger number of animals (expected results).
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