THE FIFTH BENEFICIAL MICROBES CONFERENCE

PRE- and PROBIOTICS for LIFELONG HUMAN and ANIMAL HEALTH

10-12 October 2016
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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WELCOME

The 5th Beneficial Microbes Conference will focus on pre- and probiotics for lifelong human and animal health.

Pre- and probiotics have been studied for both human and animal applications, and worldwide research on this topic has accelerated in recent years. Reviews on the impact of pre- and probiotics on human and animal health and disease are numerous and have emphasised different components of the field, such as oral and gut health, immunomodulation, gut-brain communication, obesity, diabetes, cancer and drug efficiency.

The impact of pre- and probiotics seems too diverse to be beneficial to the average man/woman as different life stages have different needs: newborns; infants; children; adults; women (pregnant, breastfeeding, menopausal); and seniors. In addition, age-related health issues may develop in these stages. Stages of exercise and physical activities, blood groups, ethnicity, etc., are of influence, too.

The above considerations do not only apply to humans but also to animals, such as pigs, poultry, cattle, horses, dogs and cats, as well as to aquaculture. Species differences also play a role here.

The 5th Beneficial Microbes Conference will inform you on the latest scientific results and future developments from a new perspective, that is, the impact of pre- and probiotics related to the specific needs of human and animal growth and development.

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 Board wish you an active and fruitful meeting!

ADVISORY BOARD

Prof. dr. Koen Venema, conference chair
Beneficial Microbes Consultancy and University of Maastricht, the Netherlands

Dr. Frédérique Chaucheyras-Durand
Ghent University, Belgium

Prof. dr. Richard Ducatelle
Lallemand, France

Dr. Léon Knippels
Danone Nutricia Research, the Netherlands

Dr. Marjorie Koenen
consultant, the Netherlands

Dr. Thomas D. Leser
Chr. Hansen, Denmark

Dr. Jiro Nakayama
Kyushu University, Japan

Dr. Arthur Ouwehand
DuPont Nutrition & Health, Finland

Dr. Gregor Reid
Lawson Health Research Institute and University of Western Ontario, Canada

Prof. dr. Henk Schols
Wageningen University & Research, the Netherlands

Dr. Elaine Vaughan
Sensus, the Netherlands

Dr. Jessica Younes
Winclowe Probiotics, the Netherlands
## PROGRAMME AT A GLANCE

### MONDAY 10 OCTOBER 2016

<table>
<thead>
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<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>12:30 – 12:45</td>
<td>Opening of the 5th Beneficial Microbes Conference</td>
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<tr>
<td>12:45 – 14:00</td>
<td>Plenary meeting&lt;br&gt;Introduction to the conference theme</td>
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<tr>
<td>14:00 – 18:00</td>
<td>Parallel session 1&lt;br&gt;<strong>Pregnancy &amp; early life – humans</strong></td>
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<td>Parallel session 2&lt;br&gt;<strong>Pregnancy &amp; early life – animals</strong></td>
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<tr>
<td>18:00 – 19:00</td>
<td>Poster viewing &amp; drinks</td>
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### TUESDAY 11 OCTOBER 2016

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<tr>
<th>Time</th>
<th>Event</th>
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<tr>
<td>08:30 – 12:35</td>
<td>Parallel session 3&lt;br&gt;<strong>Infants &amp; children – humans</strong></td>
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<td>Parallel session 4&lt;br&gt;<strong>(Young) animals</strong></td>
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<tr>
<td>12:35 – 14:00</td>
<td>Lunchbreak &amp; poster viewing</td>
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<tr>
<td>14:00 – 17:35</td>
<td>Parallel session 5&lt;br&gt;<strong>Adolescents &amp; adults</strong></td>
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<td>Parallel session 6&lt;br&gt;Targeting specific groups</td>
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<tr>
<td>17:35 – 18:15</td>
<td>Parallel session 7&lt;br&gt;<em>Speed presentations: topics in human research</em></td>
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<td>Parallel session 8&lt;br&gt;<em>Speed presentations: topics in animal research</em></td>
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<tr>
<td>18:30 – 21:45</td>
<td>Canal tour &amp; conference dinner (reservations only)</td>
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* Short presentation by selected poster presenters to provide an overview of their research and inspire the audience to visit their posters.

### WEDNESDAY 12 OCTOBER 2016

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<th>Time</th>
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<tr>
<td>08:30 – 10:45</td>
<td>Parallel session 9&lt;br&gt;<strong>Seniors &amp; age-related health issues</strong></td>
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<tr>
<td></td>
<td>Parallel session 10&lt;br&gt;<strong>Pets &amp; farm animals</strong></td>
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<tr>
<td>11:15 – 13:00</td>
<td>Final plenary meeting&lt;br&gt;<strong>Facing the future – challenges ahead</strong></td>
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<tr>
<td>13:00</td>
<td>Closing of the 5th Beneficial Microbes Conference</td>
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CONFERENCE PROGRAMME

MONDAY 10 OCTOBER 2016

12:30 Opening of the 5th Beneficial Microbes Conference
Prof.dr. Koen Venema – conference chair
Beneficial Microbes Consultancy and Maastricht University, the Netherlands

PLENARY MEETING: INTRODUCTION TO THE CONFERENCE THEME

Chair: Prof.dr. Gregor Reid, Lawson Health Research Institute and University of Western Ontario, Canada

12:45 Pre- and probiotics for lifelong human and animal health
Prof.dr. Koen Venema, Beneficial Microbes Consultancy and University of Maastricht, the Netherlands

13:10 Can microbes change our destiny?
Prof.dr. Gregor Reid, Lawson Health Research Institute and University of Western Ontario, Canada

13:35 Longevity lessons from freshwater crustaceans
Prof.dr. Hajime Watanabe, Department of Biotechnology, Osaka University, Japan
MONDAY 10 OCTOBER 2016

SESSION 1: PREGNANCY & EARLY LIFE – HUMANS

Chair: Dr. Léon Knippels, Danone Nutricia Research, the Netherlands

14:00 Chair’s introduction

14:05 Origin of the species – the infant microbiome and lifelong health implications
Lei Lu, Department of Pediatrics, University of Chicago, USA

14:30 Targets for microbiota modulation during pregnancy and lactation
Dr. Esther Jiménez, Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Spain

14:55 Potential mechanisms for the preventive effect of probiotics on atopic dermatitis
Dr. Anne Dorthea Bjerkenes Rø, Department of Public Health and General Practice, Norwegian University of Science and Technology, Norway

15:20 Effect of early nutrition on microbial composition and metabolism in allergy risk reduction
Dr. Gabriele Gross, Mead Johnson Nutrition, the Netherlands

15:45 Networking break & poster viewing

16:15 Allergo-protection through microbial contact
Prof.dr. Harald Renz, Institute for Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps-University Marburg, Germany

16:40 A synbiotic mixture supports the healthy development of C-section delivered infants
Charmaine Chew, Danone Nutricia Research, Singapore

17:05 The gut-lung axis: role of gut microbiota in asthma
Prof.dr. B. Brett Finlay, Michael Smith Laboratories, University of British Columbia, Canada

17:30 Role of pre- and probiotics on microbiome and brain development in newborns
Prof.dr. Ruurd van Elburg, Emma Children’s Hospital AMC, University of Amsterdam and Nutricia Research, the Netherlands

17:55 Chair’s summary

18:00 – 19:00
Poster viewing & drinks
MONDAY 10 OCTOBER 2016

SESSION 2: PREGNANCY & EARLY LIFE – ANIMALS

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

14:00 Chair’s introduction

14:05 In ovo injection of prebiotics and synbiotics as a novel and alternative strategy to feed supplementation in broiler chicken production
Prof.dr. Giuseppe Maiorano, Department of Agricultural, Environmental and Food Sciences, University of Molise, Italy

14:30 Long-term effects of in ovo microbiome stimulation in chickens
Prof.dr. Marek Bednarczyk, Department of Animal Biochemistry and Biotechnology, UTP University of Science and Technology, Poland

14:55 Early colonisers in the intestinal tract of baby chicks
Dr. Ivan Rychlik, Department of Bacteriology, Veterinary Research Institute, Czech Republic

15:20 Gut colonisation after birth in preterm pigs – do probiotics help?
Dr. Thomas Thymann, Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark

14:45 Networking break & poster viewing

16:15 Imprinting power: early-life supplementation with prebiotic impacts immune system and energy metabolism in pigs subjected to challenge
Dr. Emmanuelle Apper, Tereos, France

16:40 Probiotics: a second mother for newborn lambs in ruminal microbial establishment?
Dr. Frédérique Chaucheyras-Durand, Lallemand, France

17:05 Inclusion of a species-specific probiotic in calf milk replacer: effect on health status and performance
Dr. Alessandro Agazzi, Department of Health, Animal Science and Food Safety, University of Milan, Italy

17:30 Mammary probiotics: a new jedi against bovine mastitis
Dr. Sergine Even, Science and Technology of Milk and Eggs (STLO), INRA, France

17:55 Chair’s summary

18:00 – 19:00
Poster viewing & dirnks
TUESDAY 11 OCTOBER 2016

SESSION 3: INFANTS & CHILDREN

Chair: Dr. Marjorie Koenen, consultant, the Netherlands

08:30 Chair’s introduction

08:35 Establishment of microbiota in infants and young children: is there a need for next-generation probiotics?
Dr. Reetta Satokari, Department of Bacteriology and Immunology, University of Helsinki, Finland

09:00 Room to grow: characterising the structure and function of the healthy child’s gut microbiome
Dr. Emily B. Hollister, Department of Pathology and Immunology, Baylor College of Medicine, USA

09:25 Pre- and probiotics for the management of allergy in infants
Dr. Lucien Harthoorn, Nutricia Research, the Netherlands

09:50 Do probiotics have a role in the prevention of type 1 diabetes?
Prof.dr. Suvi Virtanen, Department of Health, National Institute for Health and Welfare, Finland

10:15 Investigation of microbial biomarkers in the gut microbiota of children at risk for type 1 diabetes
Alessia Martina, Department of Biotechnology, University of Verona, Italy

10:45 Networking break & poster viewing

11:15 Do pre- and or probiotics have a role in common infections in children?
Prof.dr. Yvan Vandenplas, Department of Paediatrics, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Belgium

11:40 Non-alcohol fatty liver disease and probiotics in obese children
Dr. Valerio Nobili, Hepato-Metabolic Disease Unit and Liver Research Unit, Bambino Gesù Children’s Hospital, IRCCS, Italy

12:05 Establishing a causal link between gut microbes and body weight – towards the identification of key players
Dr. John Penders, Department of Medical Microbiology, Maastricht University, the Netherlands

12:30 Chair’s summary

12:35 Lunch break & poster viewing
TUESDAY 11 OCTOBER 2016

SESSION 4: (YOUNG) ANIMALS

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

08:30 Chair's introduction

08:35 Microbial and nutritional factors influencing gastrointestinal tract development in calves
Dr. Michael Steele, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Canada*

09:00 Lifestart sets life performance: microbiota development in young animals
Dr. Petra Roubos-van den Hil, *Trouw Nutrition R&D, the Netherlands*

09:25 Should we consider developing more tailored approaches to provide probiotics in animal production?
Prof.dr. Paolo Bosi, *Department of Agricultural and Food Sciences, University of Bologna, Italy*

09:50 Fibre supplements affect colonic fermentation characteristics in piglets
Prof.dr. Henk Schols, *Agrotechnology and Food Sciences, Wageningen University, the Netherlands*

10:15 Pros and cons of pre- and probiotics in horses
Dr. Kathleen Crandell, *Kentucky Equine Research, USA*

10:45 **Networking break & poster viewing**

11:15 Development of a host specific, multi-species probiotic for poultry meeting regulatory requirements in the EU
Dr. Verity Ann Sattler, *BIOMIN Research Center, Austria*

11:40 Identifying alternatives to antimicrobial growth promoters in chicken feed through metatranscriptomics
Dr. John Parkinson, *Department of Molecular Genetics, University of Toronto, Canada*

12:05 Microbiomes in farm animals - combining omics to explore the active communities
Prof.dr. Jana Seifert, *Institute of Animal Science, University of Hohenheim, Germany*

12:30 Chair's summary

12:35 **Lunch break & poster viewing**
TUESDAY 11 OCTOBER 2016

SESSION 5: ADOLESCENTS & ADULTS

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

14:00 Chair's introduction

14:05 Reducing vulnerability to depression in healthy adults: multispecies probiotics reduce cognitive reactivity to sad mood
Dr. Laura Steenbergen, Institute of Psychology, Leiden University, the Netherlands

14:30 Enteric-colonising Lactobacillus gasseri CP2305 improves stress-related adverse behaviours
Prof. Dr. Kazuhito Rokutan, Institute of Biomedical Sciences, University of Tokushima Graduate School, Japan

14:55 Cultivated faecal microbiota transplant to RCDI (recurrent Clostridium difficile infection) patients
Dr. Elisabeth Norin, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Sweden

15:20 Probiotics can improve compliance and eradication rate for Helicobacter pylori therapy
Dr. Goran Hauser, Department of Gastroenterology, Clinical Hospital Centre Rijeka, Croatia

15:45 Networking break & poster viewing

16:15 Prebiotics, short chain fatty acids and appetite regulation
Prof. Dr. Gary A. Frost, Department of Medicine, Imperial College London, UK

16:40 Small player, large role: microbial metabolic pathways associated with metabolic risk of cardiovascular disease
Dr. Jingyuan Fu, Department of Genetics, University Medical Center Groningen, the Netherlands

17:05 Short chain fatty acids: the link between our gut microbiota and metabolic health
Prof. Dr. Ellen E. Blaak, Department of Human Biology, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, the Netherlands

17:30 Chair's summary

SESSION 7: SPEED PRESENTATIONS – TOPICS IN HUMAN RESEARCH

Chair: Dr. Elaine Vaughan, Sensus, the Netherlands

17:35 – 18:15
Short presentations by selected poster presenters to provide an overview of their research and to inspire the audience to visit their posters.

18:30 – 21:45
Canal tour & conference dinner (reservations only)
For details, see page 15
TUESDAY 11 OCTOBER 2016

SESSION 6: TARGETING SPECIFIC GROUPS

Chair: Dr. Jiro Nakayama, Kyushu University, Japan

14:00 Chair's introduction

14:05 The human uterine microbiome: the microbial core of human reproduction?
Prof.dr. Hans Verstraelen, Department of Obstetrics and Gynaecology, Ghent University, Belgium

14:30 Preventive effects of probiotics on mastitis incidence in lactating women
Dr. Mónica Olivares, Biosearch Life, Spain

14:55 Form, function and diversity within the vaginal environment; the implications for dysbiosis and health
Dr. Jessica Younes, Winclove, the Netherlands

15:20 Gut microbiota and metabolic markers in postmenopausal women with obesity
Dr. Lesli H. Larsen, Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark

15:45 Networking break & poster viewing

16:15 Effects of probiotics on oral immune function in athletes
Yukichi Hanaoka, Sports Research and Development Core, University of Tsukuba, Japan

16:40 Changing dietary habit is changing Asian gut microbiota
Dr. Jiro Nakayama, Department of Bioscience and Biotechnology, Kyushu University, Japan

17:05 The intestinal microbiota of the Hadza hunter gatherers: new perspectives on gut microbiota-human host co-evolution
Dr. Simone Rampelli, Department of Pharmacy and Biotechnology, University of Bologna, Italy

17:30 Chair's summary

SESSION 8: SPEED PRESENTATIONS –TOPICS IN ANIMAL RESEARCH

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

17:35 – 18:15
Short presentations by selected poster presenters to provide an overview of their research and to inspire the audience to visit their posters.

18:30 -21:45
Canal tour & conference dinner (reservations only)
For details, see page 15
WEDNESDAY 12 OCTOBER 2016

SESSION 9: SENIORS & AGE-RELATED HEALTH ISSUES

Chair: Prof.dr. Bobbi Langkamp-Henken, University of Florida, USA

08:30 Chair’s introduction

08:35 Identifying targets for microbiota modulation in the elderly; now, tomorrow or never? The challenges ahead
Dr. Miguel Gueimonde, Dairy Research Institute of Asturias, Spanish National Research Council, Spain

09:00 Probiotic lactobacilli can reduce oral Candida in elderly
Dr. Mette Rose Jørgensen, Department of Odontology, University of Copenhagen, Denmark

09:25 Pre- and probiotics in the modulation of gut microbiota in older adults and immune and health outcomes
Prof.dr. Bobbi Langkamp-Henken, Department of Food Science and Human Nutrition, University of Florida, USA

09:50 Cognitive impairment in the elderly: gut microbiota and perspective of probiotics
Dr. Gaspar Pérez Martinez, Instituto de Agroquímica y Tecnología de Alimento, Spanish National Research Council, Spain

10:15 Probiotics to improve cardiovascular risk factors in older people
Prof.dr. Luzia Valentini, Department of Agriculture and Food Sciences, Neubrandenbrug University of Applied Sciences, Germany

10:40 Chair’s summary

10:45 Networking break & poster viewing

FINAL PLENARY MEETING: FACING THE FUTURE – CHALLENGES AHEAD

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

For details, see page 14
WEDNESDAY 12 OCTOBER 2016

SESSION 10: PETS & FARM ANIMALS

Chair: Prof.dr. Henk Schols, Wageningen University, the Netherlands

08:30 Chair’s introduction

08:35 Modulating the canine and feline microbiota: dietary intervention, prebiotic, and probiotic supplementation
Dr. Maria Cattai de Godoy, Companion Animal Nutrition, Department of Animal Sciences, University of Illinois at Urbana-Champaign, USA

09:00 Effects of yeast on feed intake and milk yield of dairy cows
Dr. Renata Breitsma, Micron Bio-Systems, UK

09:25 Butyrate-producing bacteria are key players in gut health maintenance in broiler chickens
Prof. Filip Van Immerseel, Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Belgium

09:50 Resolution of a Clostridium difficile outbreak at a large foaling operation using a rationally designed probiotic
Dr. Steven A. Frese, Evolve Biosystems, USA

10:15 Probiotic applications to improve fish health and disease resistance
Dr. Ana Rodiles, School of Biological Sciences, Plymouth University, UK

10:40 Chair’s summary

10:45 Networking break & poster viewing

FINAL PLENARY MEETING: FACING THE FUTURE – CHALLENGES AHEAD

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

For details, see page 14
WEDNESDAY 12 OCTOBER 2016

FINAL PLENARY MEETING: FACING THE FUTURE – CHALLENGES AHEAD

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

11:15 Chair’s introduction

11:20 Human gas capsules sniffing out a better future for gut health
Prof. dr. Kourosh Kalantar-zadeh, School of Engineering, RMIT University, Australia

11:45 Next generation diagnostic biomarker detection for gut microbiota: breathomics
Prof. dr. Frederik-Jan van Schooten, Department of Pharmacology and Toxicology, Maastricht University, the Netherlands

12:10 Designing next generation prebiotics for lifelong health
Prof. dr. Robert Rastall, University of Reading, UK and ILSI Europe Expert Group

12:35 Top five answers learned at the 5th Beneficial Microbes Conference
Prof. dr. Koen Venema

13:00 Closing of the 5th Beneficial Microbes Conference
‘HET GRACHTENHUIS – THE MUSEUM OF THE AMSTERDAM CANALS’

TUESDAY 11 OCTOBER 2016

18:30 – 21:45
Canal tour & conference dinner (reservations only)

As a special end to the 2nd day of the 5th Beneficial Microbes Conference, there will be a unique dinner in ‘Het Grachtenhuis – The Museum of the Amsterdam Canals’.

The Amsterdam canals make the city. For centuries here, money has been earned, art created, feasts celebrated and life enjoyed. This is the story that the Museum of the Canals brings to life. The museum is situated in a monumental building on the Herengracht, where you are taken on a whirlwind journey through four hundred years of history. The Museum of the Canals shows you not only why the creation of the Amsterdam canals was so unusual, but also why they are still special today. This museum is for everyone who loves or is just about to fall in love with the city of Amsterdam. A journey through the Amsterdam canals begins here in the Museum of the Canals.

IMPORTANT NOTES

- THE CONFERENCE DINNER IS ONLY OPEN TO PARTICIPANTS WHO REGISTERED IN ADVANCE. YOU WILL FIND YOUR TICKET FOR THE CONFERENCE DINNER AT THE BACK OF YOUR NAME BADGE.

- PARTICIPANTS WHO HAVE REGISTERED FOR THE CONFERENCE DINNER MUST WEAR AND SHOW THEIR NAME BADGE WITH THE TICKET FOR ENTRANCE TO THE MUSEUM AND THE CONFERENCE DINNER.

- PARTICIPANTS WHO HAVE REGISTERED FOR THE CONFERENCE DINNER SHALL GATHER IN THE LOBBY OF HOTEL CASA 400 AT 18:30 SHARP.

LECTURES

MONDAY, 10 OCTOBER 2016

PLENARY MEETING
INTRODUCTION TO THE CONFERENCE THEME

PRE- AND PROBIOTICS FOR LIFELONG HUMAN AND ANIMAL HEALTH

Koen Venema
Beneficial Microbes Consultancy and Maastricht University, the Netherlands
koen.venema@outlook.com

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 (not only in the gut, but also in and on other bodily sites) is important for health and disease, and is probably involved in practically every disease and disorder that can affect the host, from gastrointestinal diseases to disorders of the brain. It has also become evident that changes in the composition and activity early in life may predispose to disorders later in life, with allergy and obesity as examples that are heavily investigated in humans. On the other hand, although unwanted in man, increased weight gain is desirable in farm animals, although not necessarily restricted to increased fat.

Pre- and probiotics are important throughout the lifespan of the host. In early life they may prevent allergy or infections or may affect brain development and cognition, in adulthood they could influence body weight, while in old hosts they are thought to contribute to Alzheimer’s or Parkinson’s disease. Although their role in some of these examples is not yet fully recognised, and certainly the mechanisms are unclear, the fact that they influence lifelong health is stunning when you think of it. Concepts that our gut microbiota drives us to buy certain products in the supermarket seem unrealistic, but may contain some truth. However, effects on the host are clearly strain-dependent for probiotics, and also different prebiotics have different effects. Although there are large ongoing studies trying to determine the structure-function relationships of both, research in this area is slowly progressing. Partly, in humans this may be because the host is genetically diverse, and factors such as ethnicity, blood group, etc., may define gut microbiota composition and/or the efficacy of pre- and probiotics. On top of that, we all have a different diet. Perhaps this is why in animal trials (both in laboratory animals as models for man, and in farm animals) results are usually much more consistent, as there is less genetic variation, and diets are much more strictly controlled.

This contribution will give an overview of some of the recent aspect in the benefits of pre- and probiotics in lifelong health of the host.

CAN MICROBES CHANGE OUR DESTINY?

Gregor Reid
Lawson Health Research Institute and Western University, Canada
gregor@uwo.ca

The more we discover about the human microbiome, the more we realise the importance of the microbes we acquire in early life, and the disruptions that occur with exposure to chemical agents throughout life. The trend of increased C-section delivery and formula feeding in ‘developed’ countries has further increased the incidence of chronic diseases. The globalisation of the food chain and energy production has resulted in higher exposure to pesticides and heavy metals also affecting health. The impact on the healthcare system is crippling many countries trying to cope with managing chronic diseases. The question is can we reduce the burden of these diseases and thereby change the destiny of life? I would suggest the answer is yes through primarily lactic acid bacteria.

The foetus when developed to term is exposed to lactobacilli and its metabolites at the placental-amnion interface and upon vaginal delivery and breast feeding. The application of probiotic lactobacilli clearly
influences breast milk microbiota composition, just as use of antimicrobials disrupts it. It also reduces adsorption of some toxic chemicals. The success of probiotics to prevent necrotizing enterocolitis in premature babies is clearly an example of a radical change in destiny attributed to beneficial microbes. While it has been reported that the infant gut microbiota reaches an adult composition irrespective of early life events, this is difficult to believe. More likely, early dysbiosis becomes ingrained in the host.

Long term consumption of fermented food appears to delay the onset of type 2 diabetes, one of the major chronic diseases that shortens lifespan, as well as reduce the incidence of some cancers. Once chronic diseases have developed, their course is more difficult to alter and some attempts using probiotics have failed. But there remains sufficient evidence that certain probiotics and faecal transplants can improve disease management and quality of life.

While it is difficult for individuals to alter how industries and societies pollute the planet, we can influence what we eat, how we nurture the foetus and infants, and encourage the medical profession to better manage our microbiome. In that way, we can indeed change our destiny for the better.

LONGEVITY LESSONS FROM FRESHWATER CRUSTACEANS

Hajime Watanabe
Department of Biotechnology, Osaka University, Japan
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How symbioses between bacteria and aquatic animals influence food webs in freshwater ecosystems is a fundamental question in ecology. We investigated symbiotic bacteria on ecologically important life-history traits, such as population dynamics and longevity, in *Daphnia*. *Daphnia* is a filter-feeding small crustacean which is one key organisms in freshwater ecosystems.

By disinfection of the daphnid embryos with glutaraldehyde, aposymbiotic daphnids were prepared and cultured under bacteria-free conditions. Removal of bacteria from the daphnids was monitored by quantitative polymerase chain reaction (qPCR) for bacterial 16S rRNA gene. The population of aposymbiotic daphnids was reduced 10-fold compared to that of the control symbiotic daphnids. Importantly, reinfection with bacteria, either by co-culturing with control symbiotic daphnids or by dipping in homogenates of control symbiotic daphnids caused daphnids to regain bacteria and increase their fecundity to the level of the control daphnids, suggesting that symbiotic bacteria regulate *Daphnia* fecundity. To identify the species of symbiotic bacteria, 16S rRNA genes of bacteria in daphnids were sequenced, revealing that 50% of sequences belonged to *Limnohabitans* sp., one of the Betaproteobacteria. *Limnohabitans* is an abundant and globally distributed freshwater Betaproteobacteria. Aposymbiotic juvenile *Daphnia* were prepared and exposed to any of four *Limnohabitans* sp., *Limnohabitans* strains DM1, 2KL-3, 2KL-7, and *Limnohabitans* planktonicus strain II-D5, all previously found in *D. magna* digestive tract or culture. Reinfected *Daphnia* were cultured until they produced the first clutch of juveniles. *Limnohabitans* strain DM1 and *L. planktonicus* strain II-D5 successfully reinfected *Daphnia* through single exposure at the first instar juvenile stage. In contrast to aposymbiotic *Daphnia* that produced non-viable juveniles, reinfected *Daphnia* produced viable juveniles and increased fecundity to levels of that of symbiotic *Daphnia*. Reinfected *Daphnia* did not increase their number of eggs nor growth rates. *Limnohabitans* strains 2KL-7 and 2KL-3 could not recover fecundity even in multiple exposures during culture. This study shows the functional evidence demonstrating that a single bacterium *Limnohabitans* regulates fecundity of the consumer *Daphnia* through symbiosis.

Our results indicated that symbiotic relationship between major bacterioplankton and zooplankton is important for maintaining the population of zooplankton in freshwater ecosystems.
MONDAY 10 OCTOBER 2016

SESSION 1
PREGNANCY & EARLY LIFE – HUMANS

ORIGIN OF THE SPECIES – THE INFANT MICROBIOME AND LIFELONG HEALTH IMPLICATIONS

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The functional microbiota begins its dynamic progression around birth and stabilises in early childhood. Despite later environmental alterations and resulting microbiota changes, the earliest microbes to encounter the naïve intestine shortly after birth potentially has the greatest opportunities to fundamentally influence intestinal development and immune function. Studies of premature infants represent a unique opportunity to observe and gain insight into development of the microbiota. Our previously published data demonstrate distinct microbial community patterns with Gammaproteobacteria dominance in infants with the preterm infant inflammatory bowel disease NEC and temporal progression of microbiota establishment in preterm newborns, including specific clustering prior to 2 weeks of age. Since the NICU is the closest approximation to a controlled laboratory setting for the examination of the influence of environment on human phenotype, we therefore use intestinal microbiota (faecal) samples from premature infants in NICU for our studies. By transplantation of the faecal samples from prior to 2 weeks of life of two preterm infants with differing growth rates by weight to pregnant germ free mice, we allowed pups spontaneously and naturally acquired the microbiota of interest during delivery and lactation. Our data demonstrate that different microbiota from early (<2 weeks) life produce distinct phenotypic changes. Using weight gain as a surrogate marker for health, we found that early microbiota from a preterm infant with normal weight gain (MPI-H) induced increased villus height and crypt depth, increased cell proliferation, increased numbers of goblet cells and Paneth cells, enhanced TJs and decreased inflammatory signalling, whereas, in contrast, the early microbiota from a poor weight gain preterm infant (MPI-L) induced a lower rate of weight gain, poor intestinal growth and differentiation, and increased inflammatory signalling. Our data demonstrate that microbial communities have differential effects on intestinal development. Future studies to identify pioneer settlers in neonatal microbial communities necessary to induce maturation may provide new insights for preterm infant microbial ecosystem therapeutics.

TARGETS FOR MICROBIOTA MODULATION DURING PREGNANCY AND LACTATION

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In the last years, it is becoming evident that microbes play a relevant role in the human reproduction and this is challenging traditional scientific dogmas. In fact, the composition of the genitourinary tract of women and their partners can be crucial for fertility. Although there may be multiple sources of microorganisms from conception to the childhood, mother-to-infant vertical transmission during pregnancy, delivery and after birth seem to be critical in the physiological acquisition and evolution of human microbiota. As pregnancy progresses, woman’s body undergoes many adaptations affecting, virtually, to all tissues, organs and systems; some of such adaptations favour a physiological translocation of selected bacteria from the oral and gastrointestinal tract, which may be transferred to the foetus and to the mammary glands. Unfortunately, some women desiring having a baby, during pregnancy and/or during lactation suffer dysbiosis processes affecting their digestive and/or genitourinary tracts, including the mammary glands. Dysbiosis may lead to disease, frequently because of the overgrowth of opportunistic pathogens, and may significantly alter mother-to-infant transmission of microorganisms and, as a result, the initial colonisation process, with consequences that may be life lasting. Therefore, pregnant and lactating women constitute a population in which the use of probiotics seems particularly interesting since the modulation of the microbiota inhabiting their intestinal, mammary and/or genitourinary tracts may have a strong effect on the health of the mother-infant dyad.
applications of probiotics in such populations include the prevention and/or treatment of vaginal and urinary tract infections (which may affect fertility and are frequent during pregnancy), the reduction of foetal exposure to potentially harmful abiotic (e.g., heavy metals) and biotic (e.g., STD-related pathogens, *Listeria monocytogenes*) agents during pregnancy, and the prevention and/or treatment of mastitis.

**POTENTIAL MECHANISMS FOR THE PREVENTIVE EFFECT OF PROBIOTICS ON ATOPIC DERMATITIS**

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Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disease affecting 20% of children in Western countries. AD typically starts in infancy and may have a detrimental impact on normal development and daily life due to the associated itch and sleep deprivation. The pathophysiology involves barrier defects, environmental factors and immune dysfunction. Currently, there is no cure, thus there have been an increasing interest in primary prevention. Perinatal supplementation of probiotics has shown a preventive effect on AD, with the combination of pre- and postnatal supplementation being most effective.

In the randomised, controlled study 'Probiotics in the Prevention of Allergy among Children in Trondheim (ProPACT)', maternal probiotics supplementation 1 month before and 3 months after birth reduced the incidence of AD in the offspring by 40%. The mechanism for this preventive effect is unclear. We are now exploring potential mechanisms of this effect. In ProPACT, 415 pregnant women were randomised to ingest a combination of *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium animalis* subsp. *lactis* Bb-12 (Bb-12), and *Lactobacillus acidophilus* La-5 (La-5) or placebo. Their offspring were assessed for AD during the first 2 years of life. To explore potential mechanisms, we have studied the effect of probiotic supplementation on gut microbiota in the mothers and their children, cytokines and microRNA molecules in breast milk, and T cell subset balance in the children. Normal maturation of T-cells in a child is dependent on colonisation with a minimum of microbial diversity in the neonatal period. An important source for colonisation is the mother. We have previously reported that probiotic supplementation modified the gut microbiota in the mothers, and increased LGG levels in their children, and are now investigating whether maternal probiotic supplementation may affect the immune system in the children. The objective of this study was to examine whether Th cells are affected by maternal probiotic supplementation and whether the preventive effect of probiotics on AD is mediated through Th cells. The proportions of Th subsets were analysed in peripheral blood collected at 3 months of age. Regulatory T cells were analysed in 140 samples, and Th1, Th2, Th8, Th17, Th22 in 77 samples. The proportion of Th22 cells was reduced in children from the probiotic group compared to the placebo group (median 0.038 vs. 0.064, *P*=0.009), whereas there were no differences in the proportions of other Th subsets or the Th1/Th2 ratio. Mediation analysis indicated that the preventive effect of the probiotics was partially mediated through the reduction of Th22 cells.

In conclusion, perinatal maternal probiotics supplementation with a combination of LGG, Bb-12 and La-5 reduced the proportion of Th22 cells in 3 months old children. This effect may be a mechanism for the preventive effect of probiotics on AD.
EFFECT OF EARLY NUTRITION ON MICROBIAL COMPOSITION AND METABOLISM IN ALLERGY RISK REDUCTION

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The human gut microbiome comprises a complex ecosystem of microorganisms. Typically, microbial diversity after birth is low and the bacterial community structure changes towards that of an adult in the first years of life. During early development of this ecosystem, factors such as mode of birth, environment and type of feeding have a crucial impact on intestinal bacterial colonisation. Such differences in intestinal microbiota composition can persist into childhood.

In recent years, the intestinal microbiota has been recognised to play an important role for human health; increasing evidence demonstrates that dysbiosis contributes to disease development. In the postnatal period, microbial colonisation can influence important functions, such as intestinal and immune maturation, mucosal barrier integrity and nutrient absorption. Such host-microbe interactions can be mediated by gut microbial metabolites. However, programming of the infant gut microbiota is not only relevant for the health status of the newborn, but can also affect disease outcomes later in life. For instance, gut microbiota perturbations during infancy, such as low abundance of bifidobacteria, have been linked with allergy susceptibility in childhood. Therefore, early life represents an important window of opportunity to impact disease development later in life.

Consequently, there is increasing interest in possibilities to influence health outcomes via nutritional interventions targeting beneficial changes in the composition of the gut microbiota. Various nutritional factors have been suggested to exert an effect on human gut microbiota composition and metabolic activity. Supplementation with pre- and probiotics has been widely studied for potential health benefits through their effects on the intestinal microbiota, but also other interventions, such as extensively hydrolysed casein formulas can be of particular interest for paediatric nutrition. This presentation will highlight current examples for early nutritional interventions on gut microbiota community in the context of allergy risk reduction.

ALLERGO-PROTECTION THROUGH MICROBIAL CONTACT

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The hygiene hypothesis is currently the leading concept to explain the dramatic increase in chronic inflammatory disease, including autoimmunities and allergies. Conversely, several Gram-positive and Gram-negative bacteria have been isolated from rural environmental communities that provide protection of allergic asthma. Overwhelming evidence indicates a strong impact of environmental microbes on the programming and the development of (early) immune responses. Based on clinical and epidemiological data, a certain exposure of environmental microbes – particularly of bacteria – seems to be an important prerequisite in order to programme immune responses towards the tolerance default programme. This programme on the level of the adaptive immune responses is necessary and required in order to prevent unwanted (chronic) inflammatory diseases that may develop early in life, such as allergies and asthma, or which may occur even later in life, such as many autoimmune diseases at the gut, the brain, or other organs.

The great challenge is to define the appropriate microbial environment on the cellular and molecular level in order to delineate the underlying mechanism of microbe-host interaction. An important concept in this context is the microbial diversity. Conversely, reduced diversity is closely linked to several clinical phenotypes, particularly in early life, such as allergies and asthma. Even more compelling, reduced diversity precedes the clinical onset of the disease, suggesting a cause-effect relationship. This concept implies the loss of (ancient) evolutionary co-evolved microbial strains and is the result of changes in lifestyle condition, particularly under westernised and industrialised environmental conditions. Therefore, microbial exposure seems to be a surrogate marker for biodiversity or the loss of biodiversity,
as observed in the above mentioned living conditions. The great challenge in this research field is to delineate the molecular pathomechanism of gene-environment interactions and the impact of microbial communities on this complex and intimate relationship. Only through a better understanding of these mechanisms, we will be able to define novel and attractive strategies for the prevention of chronic inflammatory diseases. Therefore, it is urgently needed to move this research field towards translational activities. The next few years will provide a compelling amount of novel data, which will hopefully improve the understanding of mechanisms of this important communication between the host and microbial communities.

A SYNBOTIC MIXTURE SUPPORTS THE HEALTHY DEVELOPMENT OF C-SECTION DELIVERED INFANTS

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Infants born by C-section miss the exposure to the maternal vaginal microbiota and this absence of microbial inoculation has been associated with a delayed colonisation of commensal bacterial members, such as *Bifidobacterium*. Epidemiological data from cohort studies indicate associations between C-section, immune and metabolic disorders, such as asthma and obesity. The objective of this study was to determine the effect of a specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS, ratio 9:1) and *Bifidobacterium breve* M-16V in restoring the delayed colonisation of *Bifidobacterium* observed in term C-section delivered infants.

In a multi-country, double-blind, randomised controlled study, 153 infants born by C-section were randomised to receive either (i) basic cow-milk based infant formula (control), (ii) supplemented with scGOS/lcFOS (prebiotic) or (iii) scGOS/lcFOS with *B. breve* M-16V (synbiotic) from birth until 4 months of age. 30 vaginally delivered, breast-fed infants were studied in parallel as reference. Stool samples were collected at day 3 and/or day 5, week 2, week 4, week 8, week 12, week 16, and 6 weeks post-intervention. *Bifidobacteria, B. breve* M-16V, pH and SCFA were measured. Safety and tolerance were recorded in the diary weekly. The data confirmed the delayed colonisation of *Bifidobacterium* in C-section delivered infants. The synbiotic supplementation resulted in a significant higher estimated mean of total bifidobacteria gene count from day 3/5 (GLMM, *P*<0.0001) and this bifidogenic effect remained significant until week 12 (*P*<0.0032) compared to the control group. In the prebiotic group, the estimated mean of total *Bifidobacterium* gene count was comparable to the control group. In the synbiotic group, *B. breve* M-16V was still detected in 38.7% of the infants at week 22, indicating a persistence of the probiotic strain. A significant lower estimated mean faecal pH was observed in the synbiotic group at day 3/5 (*P*<0.0001) and this remained significant until week 4 (*P*<0.001) compared to the control group. The estimated mean amount of acetate observed was significantly higher in the synbiotic group at day 3/5 (*P*<0.0001) compared to the control group. A lower number of subjects with adverse events of eczema/atopic dermatitis was reported in the synbiotic group (n=3) compared to the control (n=10) and prebiotic group (n=9) after correction for family allergy history (*P*<0.05).

In conclusion, supplementation with a specific synbiotic mixture of scGOS/lcFOS and *B. breve* M-16V in C-section delivered infants is able to restore the delayed colonisation early in life and improves gut eco-physiology milieu. These effects may be important to support healthy development of C-section delivered infants.
THE GUT-LUNG AXIS: ROLE OF THE GUT MICROBIOTA IN ASTHMA

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Asthma is an inflammatory disease of the lungs whose incidence is increasing rapidly, making it a major problem worldwide. Although the exact cause is not known, environmental conditions, such as the use of antibiotics, mode of delivery, etc., impact on asthma. Using an experimental murine asthma system, we demonstrated that shifts in microbiota triggered by antibiotics affected asthma outcome. We were able to show that this shift needs to occur very early in life, and that certain microbes are associated with it. We also found that intestinal Treg cells were affected, but not lung Tregs. Using a clinical cohort of children (CHILD), we analysed faeces from 3-month old and one-year old children. Remarkably, we found that certain microbiota species from the 3-month old population were associated with protection from asthma. Additionally, there were significant metabolic changes mediated by microbiota in those at risk for asthma. By transplanting these particular microbiota, along the faeces from an asthmatic child, we found that these microbiota decreased lung inflammation in the murine asthma model. We have also studied a cohort of children from rural Ecuador. Similar to what we had previously found in Canadian babies, at risk Ecuadorian babies also exhibit gut microbial dysbiosis very early in life. However, the microbial alterations between healthy and at risk children were different and more pronounced in Ecuadorian babies. Predicted metagenomic analysis showed significant differences in genes involved in carbohydrate and taurine metabolism.

Collectively, we have found that microbiota play a profound impact on the host very early in life, which has later effects in asthma susceptibility.

ROLE OF PRE- AND PROBIOTICS ON MICROBIOME AND BRAIN DEVELOPMENT IN NEWBORNS

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Brain growth and development is one of the key factors for quality of life in newborn infants. Growing evidence supports the existence of the microbiome-gut-brain axis. The microbiome interacts with the brain through immunological, endocrine, and neural pathways. After birth, enteral nutrition plays a key role in the growth and development not only by delivering the building blocks for the brain but also by supporting the microbiome and immune development of the newborn infant. Preterm born infants are especially at risk for brain injury with both short term and long term effects. White matter injury is the most common pattern of brain injury in preterm infants. Inflammation and perinatal infection play a major role in white matter injury. In addition, preterm infants are also more often born by caesarean section and often and repeatedly exposed to broad-spectrum antibiotics which both have major impact on microbiota and immune development.

For optimal brain development and prevention of brain injury in preterm infants, early postnatal enteral nutrition, preferentially with expressed human milk, is important. Because of the delayed microbiota development, supplement with specific prebiotics, e.g., galacto-oligosaccharides and fructo-oligosaccharides, or specific probiotics or their combination (synbiotics) may play a role in preventing and restoring the normal development of the microbiota, and thereby may have a neuroprotective role against white matter injury through reduction of inflammation and infections. Although meta-analyses on probiotics show a reduction in the incidence of necrotising enterocolitis and in the largest meta-analyses to date also a reduction of late onset sepsis, no benefit on neurodevelopment has been shown so far.

Evaluation of brain development and maturation by ultrasound and MR imaging of the brain in the first weeks after birth may predict long term outcome of neurodevelopment of nutritional interventions in these preterm infants. Future studies on nutritional support through prebiotics and probiotics may further unravel the role of the microbiome-gut-brain axis in brain development and maturation especially in preterm infants.
The efficiency of poultry in converting feed into meat plays a key role in the economics of the broiler industry. Therefore, improvements in the feed conversion ratio (FCR) will considerably increase the margin of profit. A large amount of antibiotics has long been used to control pathogenic diseases and as growth promoters to improve performance in livestock. However, this approach had significant and unwanted side-effects, such as the development of antimicrobial resistance and carry-over of the antibiotic residues in poultry products. In light of this, the use of antibiotics as growth promoters (AGPs) has been banned by the European Union since 2006, based on their possible negative consequences for animal health and food safety. This ban has led to animal performance problems and a rise in the incidence of enteric diseases in farms, with serious economic consequences. Proposed alternatives to AGPs include probiotics, prebiotics and synbiotics.

A variety of probiotics and prebiotics have been used extensively in several feeding trials in broiler chickens. A major problem to overcome is efficient administration under fully controlled conditions. To ensure effectiveness, these compounds have to be administered as early as possible. In ovo technology enables delivery of sustainable bioactives, such as pre-/probiotics and their combination, directly into the egg air chamber at day 12 of embryonic incubation; it allows for a precise delivery of the bioactive substance to all embryos, which equalises the effects across the flock and assures proper development of the gut microflora in all chicks. Previously, different types of prebiotics and their synergistic combinations with probiotics were tested in field and laboratory studies, also by our research groups. Evaluated prebiotics included commercial types (‘DN’ (DiNovo®, BioAtlantis Ltd., Ireland) extract from Laminaria spp. containing laminarin and fucoidan, and ‘BI’ (Bi2tos, Clasado Ltd., Malta), a non-digestive trans-galacto-oligosaccharides), and in-house developed extract from lupin seeds (RFO, raffinose family oligosaccharides). Also, different synbiotic preparations (SYN1: RFO + Lactococcus lactis subsp. lactis; SYN2: RFO + Lactococcus lactis subsp. cremoris; SYN3: commercially available symbiotic Duolac; SYN4: BI + Lactobacillus salivarius; and SYN5: RFO + Lactobacillus plantarum) were tested in different trials. Some of the obtained results are briefly summarised below.

Regarding the performance in vivo, diverse results were obtained depending on the kind of pre-/synbiotics and the mode of their administration. In a trial conducted under experimental conditions, 3 prebiotics (DN, BI and RFO) were administered by in ovo injection, combined with in-water delivery. The prebiotics increased body weight (BW), especially during the first 21 days of life. The feed intake (FI) and FCR increased upon prebiotics delivery with each method used. Considering only the in ovo delivery, the birds from the DN and RFO groups had high pectoral muscle (PM) weight and a greater fiber diameter. Furthermore, delivery in ovo combined with in-water supplementation of prebiotics did not show synergistic effects on broilers performance compared to in ovo injection only. These results confirm that a single injection of prebiotics into the chicken embryo can successfully replace prolonged in-water supplementation post hatch. However, other research with prebiotics conducted under commercial conditions did not prove any effect of prebiotic treatment on mortality, body weight, FI, FCR and European Broiler Index of broiler chickens. In ovo injection of different synbiotics (SYN1, SYN2, SYN3) showed different results based on the kind of synbiotic preparations used: SYN2 and SYN3 increased FCR, SYN3 reduced carcass yield. On the contrary, another trial showed that SYN4 and SYN5 injected in ovo had no negative effects on growth and slaughter traits. Meat quality traits (pH, colour, WHC, cholesterol and collagen content) were not affected by the kind of pre-/synbiotics injected in ovo, but the fatty acid (FA) profile may be leveraged. In fact, prebiotics significantly improved the FA profile and nutritional ratios of meat (lower amount of monounsaturated fatty acids, higher polyunsaturated fatty acids, lower n-6/n-3 ratio). In ovo administration of synbiotics had a marked effect on total lipid and FA composition: SYN4 and SYN5 decreased the meat lipid content; SYN4 negatively influenced meat FA profile; meat from control and SYN5 birds showed a better FA profile, with a positive
effect on nutritional properties of chicken meat. The undesirable effect on the FA composition exerted by the injection in ovo of SYN4 could be related to a marked influence of this prebiotic on lipid metabolism. Prebiotic treatments increase lipid oxidation in meat, even if the detected TBA reactive substances are below the critical value recognised for meat acceptability.

In conclusion, pre-/synbiotics injected in ovo affect in vivo performance and nutritional properties of chicken meat depending on their compounds. Prebiotics may increase FI and FCR which is a typical trade-off of the energy and nutrients use by intestinal microflora. On this basis, we propose that a single in ovo injection can replace prolonged and costly in-water and in-feed supplementation of the broiler chickens with bioactive compounds. As such, the in ovo method should be further recommended to the poultry industry.

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LONG-TERM EFFECTS OF IN OVO MICROBIOME STIMULATION IN CHICKENS

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There are different ways to deliver bioactive substances, such as prebiotics, probiotics or synbiotics into the avian gastrointestinal tract. To be effective, these compounds have to be administered in fully controlled conditions, as early as possible. In birds, bioactive compounds can be introduced as a solution to a particular compartment of the egg. This allows for a precise delivery of the bioactive substance to all embryos at an early stage of development, which unifies the effects of prebiotics across the flock and assures proper development of the gut microflora in all chicks.

In our studies, we have determined the 12th day of incubation as the optimal time for prebiotic or symbiotic injection into the air cell of the incubated egg. The procedure of in ovo injection was performed with the use of a dedicated automatic system. Studies in which a model of the chicken embryo is used to compare the biological activity of various bioactives comprise of few steps. First of all, the substance has to prove complete solubility in physiological salt. Second, the bioactive has to be delivered in ovo in a specific dose that assures high hatchability of the eggs and microflora development already at hatching. Third, prebiotics, probiotics or synbiotics should confer beneficial properties to the host in performance and fitness traits. Synergistic effects of different combinations of prebiotics and probiotics were estimated in vitro, using an automated analyser, on the basis of growth curves of the bacteria in the presence of different prebiotics. To assess the specific properties of probiotic bacteria to stimulate immune responses an in vitro test had been also carried out using a chicken macrophage cell line or using a DT40 cell line. Our results show that the single in ovo administration of prebiotic or symbiotic has long term effects on the chicken organism, in terms of gastrointestinal tract microbiota modulation, broiler chicken performance, meat quality, histological composition of intestinal tissue, immune system development and morphology, hormonal regulations in the pancreas and immune-related gene expression.

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EARLY COLONISERS IN THE INTESTINAL TRACT OF BABY CHICKS

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The development of caecal microbiota in egg laying hens in commercial production, from the day of hatching until 60 weeks of age can be separated into 4 different stages of caecal microbiota development. The first stage lasts for the first week of life and is characterised by a high prevalence of Enterobacteriaceae (phylum Proteobacteria). The second stage lasts from week 2 to week 4 of life and is characterised by nearly an absolute dominance of Lachnospiraceae and Ruminococcaceae (both phylum Firmicutes). The third stage lasts from month 2 to month 6 and is characterised by the succession of Firmicutes at the expense of Bacteroidetes. The fourth stage is typical for adult hens in full egg production and is characterised by a constant ratio of Bacteroidetes and Firmicutes formed by equal numbers of the representatives of both phyla. When microbiota from donor hens of different age representing the 4 developmental stages were used as competitive exclusion products for the protection of newly hatched chickens against Salmonella Enteritidis infection, microbiota from 3-week-old or older chickens protected them against S. Enteritidis challenge. On the other hand, microbiota from 1-week-old donors failed to protect newly hatched chickens against S. Enteritidis challenge. Gut microbiota, or ideally its individual microbiota members, can be used as a preventive measure against S. Enteritidis infection but its composition, or selection of individual microbiota members, is critical for its efficacy.

GUT COLONISATION AFTER BIRTH IN PRETERM PIGS – DO PROBIOTICS HELP?

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At birth, the newborn must meet the acute challenges of circulatory changes, respiration, thermoregulation, microbial colonisation, and enteral nutrition. When the foetal membranes rupture during the birth process, the piglet becomes exposed to microorganisms in the birth canal and in the extra-uterine environment and the gut become quickly colonised. Under normal circumstances, gut colonisation is well tolerated by the host, but in situations such as preterm birth, gut colonisation is a risk factor for complications like necrotising enterocolitis (NEC). In human preterm neonates, the gut microbiome has been studied extensively using faecal samples, yet no single organism has been identified as causal for NEC. Antibiotic therapy is extensively used for preterm neonates, which makes it difficult to determine any causal relationship to specific microorganisms.

The use of probiotics has been studied to some extent in human preterm neonates and has shown preventive effects toward NEC and all-cause mortality [1]. At the other hand, a recent large scale phase 3 study [2] showed no effect of probiotic supplementation on any of the measured clinical outcomes. The discrepancy in clinical outcome among the human intervention trials, indicate that fundamental questions regarding timing, dosing and choice of strain remain to be answered before a reproducible preventive effect can be achieved.

Appropriate animal models may offer one way to study some of the fundamental questions regarding early life dysbiosis and strategies to intervene with probiotics and other compounds of interest. We have for over 15 years used preterm piglets delivered by caesarean section as a model of preterm infants. The piglet model displays many of the same clinical complications as seen in preterm infants (e.g., reduced pulmonary and circulatory function, increased susceptibility of NEC and sepsis [3]). We have characterised the spontaneous development of gut dysbiosis in preterm pigs relative to term pigs, and studied multiple prophylactic and/or intervention strategies. Under these experimental condition we have shown that a marked reduction in the general microbial abundance using either antimicrobials [4] or entirely germ free conditions [3], is preventive against NEC. We have also shown that carbohydrate maldigestion in the small intestine may lead to more available substrate in the colon which appears to be a predilection site for pathological lesions [5]. Collectively, the studies indirectly support the notion that probiotic supplementation can offer some protection if the most optimal strains, dosages, and timing can be identified. We have found both positive and negative effects of inoculation with specific strains very early after birth. In a current experiment under the research programme NEOMUNE.
Although more research is needed to determine if the same positive effects can be achieved with a more defined flora, there appears to be a window for probiotic interventions in preterm neonates.

References

IMPRINTING POWER: EARLY-LIFE SUPPLEMENTATION WITH PREBIOTIC IMPACTS IMMUNE SYSTEM AND ENERGY METABOLISM IN PIGS SUBJECTED TO CHALLENGE

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Early microbiota colonisation is crucial for development of immune and metabolic functions in a sustainable way, with consequences on the susceptibility to develop diseases. Both early colonisation and diversity of the microbiota represent key factors of such nutritional programming. Our study aimed at evaluating effects of perinatal short-chain fructo-oligosaccharides (scFOS) consumption on intestinal functionality. Furthermore, we investigated whether perinatal scFOS supplementation impacts adult metabolic responses to a high-fat (HF) diet.

In the two studies, sows received a diet supplemented with scFOS (Profeed®) or maltodextrins (CTRL) for the last third of gestation and the entire lactation. In the first study, at postnatal day (PND) 28, pigs of each litter were weaned and divided into two groups receiving CTRL or scFOS until PND56. Short-chain fatty acids (SCFA) production was assessed at PND21 and PND56. Intestinal functionality was assessed at PND56. An oral vaccine challenge against Lawsonia intracellularis was performed and serum and ileal specific IgA measured at PND56. In the second study, once weaned, pigs received scFOS or maltodextrin according to maternal diet for one month. Then, pigs were reared until 6 months of age under standard diet and received a HF diet providing 22.6% energy from lipids for 12 weeks. Faecal SCFA production was determined 3 and 9 weeks after the beginning of the HF diet. The enteropancreatic, endocrine pancreas and glucose metabolism were measured at the end of the experiment. In the first study, faecal contents of suckling pigs from scFOS mothers displayed a higher level of total SCFA (P<0.001), indicating a maternal diet-induced change in offspring gut microbiota. The number of goblet cells per crypt was increased in pigs from scFOS mothers (P=0.06) together with ileal concentrations of IFN-γ (P=0.05) and IL-4 (P=0.07) known to play a role in the regulation of mucin synthesis. Furthermore, ileal and serum specific IgA concentration was increased in pigs from scFOS mothers (P=0.08 and 0.004, respectively). In the second study, early scFOS supplementation increased faecal SCFA production after 3 weeks of HF diet (P=0.02), revealing a transient modulation of fermentative activity. Ongoing taxonomic analysis will give more insights on microbiota changes. The number of caecal GLP-1-secreting L-cells (P=0.03) and the basal plasma concentration of GLP-1 (P=0.10) increased in scFOS group and were positively correlated (P=0.003; R=0.59), indicating an increased capacity to secrete GLP-1. scFOS animals tended to secrete more insulin (P=0.09) in response to a glucose stimulus without glucose profile modification.

Our results highlight the key role of perinatal nutrition on later adaptations to diverse challenges, involving the microbiota which, in turn, modify immune system and energy metabolism.
PROBIOTICS: A SECOND MOTHER FOR NEWBORN LAMBS IN RUMINAL MICROBIAL ESTABLISHMENT?

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In ruminants, rumen microbiota is of paramount importance for digestion efficiency as the microbial fermentation supplies the host animal with essential sources of energy and nitrogen. Indeed, a great diversity of microorganisms (bacteria, protozoa, fungi and archaea) combined with a huge abundance of these communities play a key role in plant biomass degradation. In young animals, the gastrointestinal tract (GIT) 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 artificial milk could impair rumen microbial colonisation in early life, which would not only affect rumen function but also have possible negative effects on hindgut homeostasis, and ultimately impact animal health and performance.

In this context, feed additives like probiotics could be used as a nutritional tool to optimise rumen microbial establishment. To this aim, we monitored microbial communities in the GIT of 16 lambs from birth to 60 days, in the absence or presence of a live yeast probiotic. Lambs were fed with milk replacer (MR) from 24 h of life and allocated to 2 groups: control (no probiotic), and treatment consisting in supplementation of MR and starter feed with a specific combination of the live yeast Saccharomyces cerevisiae CNCM I-1077 and yeast metabolites (SCYM). MR was fed until weaning (at day 42), starter feed was distributed from day 8 until the end of experiment. Rumen contents were collected at 2, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, and 56 days of age by stomach tubing, faecal samples were obtained at 14, 35 and 56 days. Microbial groups and targeted bacterial species were quantified by qPCR and microbial diversity and composition shifted as the lambs aged. SCYM induced significant changes in rumen colonisation by functionally important microbial populations, which was corroborated by amplicon sequencing analysis. NGS data also revealed differences between faecal and ruminal microbiota diversity, and composition shifted as the lambs aged. SCYM induced significant changes in relative abundance of 8-23 bacterial OTUs across time in the rumen samples (P<0.05), among which some known to be greatly involved in rumen function (e.g., Fibrobacter), and favoured establishment of Trichostomata and Neocallimastigaceae eukaryotic families. Sequences belonging to Saccharomyces genus were more abundantly retrieved (P<0.05) in rumen contents of SCYM lambs than in control animals at J7 and J14. Our data suggest that this additive combination improves microbial colonisation in the maturing rumen, which could have an important impact on lamb gut development and digestive efficiency.

INCLUSION OF A SPECIES-SPECIFIC PROBIOTIC IN CALF MILK REPLACER: EFFECT ON HEALTH STATUS AND PERFORMANCE

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Different stress factors, such as weaning, dietary changes and transportation, can cause high incidence of intestinal and respiratory diseases in veal calves reducing growth rates and performance. Probiotic bacteria have been proven to be a valid alternative to the use of EU-banned auxinic antibiotics in animal nutrition because of their efficacy in improving animals’ health and performance. This happens in particular following a ‘species-specificity’ approach, thus when the source of isolated bacteria and the target animal to which they are administered are the same. Two different projects were financed by Regione Lombardia Agricultural Department according to the plan of research and Development d.g.r. no. 7/20734, namely ‘Identification of biotype of intestinal lactic acid bacteria with probiotic purpose in veal calves and check in vivo of their efficacy-PROVIT’ and ‘Study and evaluation of a species-specific
lactobacilli (LAB) are good candidates due to their generally recognised as safe (GRAS) status and their recognised technological and inhibitory properties. LAB have been investigated for many years for their beneficial effects on human health. Introducing selected bacterial strains, such as LAB, has been demonstrated to be efficient in human health to restore the microbiota homeostasis and has led to the development of vaginal and intestinal probiotics. Similarly, the barrier effect of LAB isolated from bovine mammary gland or from exogenous ecosystems against mastitis pathogens is being explored. LAB are able to interfere with the infectious cycle of mastitis pathogens by several means, including a direct inhibition of the pathogen growth, titration of the pathogen by co-aggregation, competition with pathogens for tissue aggregation, competition with pathogens for tissue colonisation or modulation of the innate immune system. These mechanisms will be reviewed and illustrated by our own results as well as other studies. Among steps of the infectious cycle, tissue colonisation deserves special attention. It is suspected to be used by some pathogens, such as Staphylococcus aureus, to evade host defences and persist in the mammary gland. We have notably shown that Lactobacillus casei was able to reduce S. aureus internalisation into bovine mammary epithelial cells. This interesting property could help to counteract persistence of S. aureus infections.

The beneficial properties of LAB in mastitis context have been mainly explored in vitro, whereas only few studies have evaluated LAB potential in vivo. Nevertheless, these in vivo studies revealed the lack of adverse effects of LAB on mammary tissues and promising results compared to conventional antibiotic treatment against mastitis. Altogether, these studies pointed out interesting properties of LAB that opens avenues to the development of alternative strategies to prevent or treat intramammary infections.
The human body is colonised by a myriad of microbes, collectively called the microbiota and the densest microbial population is found in the gastrointestinal tract, where anaerobic bacteria prevail. Genomic technologies have enabled in-depth characterisation of the gut microbiota and have revealed complex and individual-specific microbial communities. While the microbiota of healthy adults is typically highly diverse and temporally relatively stable, the microbiota of children is less diverse and more prone to fluctuations due to external disturbances, e.g., by antibiotics. Previously, it has been considered that the intestinal microbiota reaches adult-like configuration by the age of three years, but our recent data suggest that the microbiota maturation in Western children may take much longer [1,2]. Today, it is acknowledged that microbiota affects the human health and well-being in many ways. A number of human diseases have been linked with microbiota dysbiosis, which is characterised by altered composition/diversity of the microbial community. Atopic/allergic children have been shown to have dysbiotic microbiota [3] and means to modulate their intestinal microbiota for health benefits are under investigation. Traditional probiotics, such as lactobacilli and bifidobacteria, have been shown to alleviate atopic/allergic symptoms, but recent research results on microbiota characterisation suggest that so-called next-generation probiotics, i.e., species of intestinal origin that have not yet been used as probiotics, could play a role in paediatric health-care. In the future, bacterial therapies may provide new options for the prevention and treatment of both GI tract and systemic diseases.

References

Advances in sequencing technology and an increasing recognition of the importance of our microbial world have led to unprecedented discoveries with respect to the human microbiome. This growing body of evidence suggests that the gut microbiome may play important roles with respect to development, immunity, and health outcomes. Although it is recognised that the human gut microbiome changes as we age, information regarding the structure and function of the gut microbiome during childhood tends to rather limited. To address this need, we characterised the structure, function, and variation of the paediatric gut microbiome in healthy, school-aged children (ages 7-12 years) and compared them with that of healthy adults previously recruited from the same region. Although the healthy paediatric gut microbiome harbours several adult-like features, it also retains many of its own distinct compositional and functional qualities. Such characteristics could contribute to age-adjusted definitions of the healthy gut microbiome, serve as diagnostic biomarkers to delineate life stage and direct appropriate medical treatment, particularly in the case of treatments geared toward microbiome restoration. This lecture will address these findings, as well as additional research aimed at addressing our understanding of the paediatric gut microbiome and its development over time.
PRE- AND PROBIOTICS FOR THE MANAGEMENT OF ALLERGY IN INFANTS

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Food allergies are a growing health problem affecting up to 2-5% of infants and young children in the Western world. Cow’s milk protein allergy (CMPA) is the most common form of food allergy in early life with the majority of children outgrowing this disease before the age of 5 years. There are studies, however, that show that CMPA and other allergies persist into later life. CMPA can generally present as an IgE- and a non-IgE-mediated disease and often affects multiple organ systems, with the most common being gastrointestinal, cutaneous and respiratory systems. Among these systems the gastrointestinal tract is suggested as the key mediator in immune development and responses. Dietary management of CMPA requires avoidance of the allergenic cow’s milk proteins until immunological tolerance is acquired. Guidelines advise the use of extensively hydrolysed formulas (eHF) for mild to moderate CMPA whereas an amino acid-based formula (AAF) is preferred for the management of more complex cases of CMPA, multiple food allergies (MFA), including their non-IgE-mediated form, or when an eHF is not tolerated.

The gastrointestinal tract and its microbial population play a pivotal role in immune responses. The developing gut microbiota in infants and children with CMPA has been shown to be different compared to that of healthy infants. This aberrant gut microbiota or ‘dysbiosis’ has been suggested to play a role in the infants’ CMPA pathogenesis and development of other allergies in the future. This dysbiosis has now become a major target for allergy research and studies have demonstrated possible benefits of modulating the gut microbiota in allergic infants and children using pre- and probiotics. Patients with moderate to severe CMPA or MFA require an AAF, but none of the currently available formulas are supplemented with pre- or probiotics. This means a potential lack for allergic infants who are fully reliant on an AAF to develop an appropriate gut microbiota and immune responses. Hence there is a strong rationale to introduce a suitable AAF with specific pre- and probiotics for use in infants with CMPA or MFA.

The short term aim, when managing infants with CMPA and MFA by using AAF, remains achieving fast and effective resolution of allergic symptoms, however, new clinical studies are being conducted to demonstrate whether utilising an AAF supplemented with specific pre- and probiotics could rebalance the gut microbiota of CMPA infants and bring it close to that of healthy infants to potentially have beneficial effects on the immune system development.

DO PROBIOTICS HAVE A ROLE IN THE PREVENTION OF TYPE 1 DIABETES?

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TEDDY (The Environmental Determinants of Diabetes in the Young) is an ongoing prospective cohort study that started in 2004. Children from six clinical centres, three in the U.S. (Colorado, Georgia/Florida, Washington), and three in Europe (Finland, Germany, and Sweden) were followed up for type 1 diabetes (T1D) related autoantibodies. The aim of the study results of which will be presented in the meeting was to examine the association between supplemental probiotic use during the first year of life and islet autoimmunity among children at increased risk of T1D. Altogether, 8,676 infants with eligible genotype were enrolled in the follow-up study before the age of 4 months. The final sample consisted of 7,473 children with the age range of 4-10 years (as of October 2014). Blood samples were collected every 3 months between 3 and 48 months of age and every 6 months thereafter to determine persistent islet autoimmunity. Details of infant feeding including probiotic supplementation and infant formula use were monitored from birth using questionnaires and diaries. We applied time-to-event analysis adjusting for country, family history of T1D, HLA-DR-DQ genotype, sex, birth order, mode of delivery, exclusive breastfeeding, birth year, child’s antibiotic use, and diarrheal history, as well as maternal age, probiotic use and smoking.
Early probiotic supplementation (at the age of 0-27 days) was associated with a decreased risk of islet autoimmunity, when compared to probiotic supplementation after 27 days or no probiotic supplementation (HR, 0.66; 95% CI, 0.46-0.94). The association was accounted for by children with DR3/4-genotype (HR, 0.40; 95% CI, 0.21-0.74) and was not significant among other genotypes (HR, 0.97; 95% CI, 0.62-1.54). Early probiotic supplementation may reduce the risk of islet autoimmunity in children at the highest genetic risk of type 1 diabetes. The result needs to be confirmed in further studies before any recommendation of probiotics use can be made.

**INVESTIGATION OF MICROBIAL BIOMARKERS IN THE GUT MICROBIOTA OF CHILDREN AT RISK FOR TYPE 1 DIABETES**

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During the last decade, several investigations have highlighted an increasingly more important involvement of gut microbiota in metabolic diseases, such as type 1 diabetes (T1D). Evidences that alterations of gut microbiota composition can occur both in the pre-pathologic and in the pathologic conditions of T1D have been reported in studies on children from different countries. These findings open the possibility to the search for biomarkers that could be correlated to the development of the pathology, and/or that can be useful for monitoring intervention studies aimed at the manipulation of the dysbiosis.

In a previous study, we evaluated the intestinal permeability – a key factor related to the onset of T1D – and gut microbiota composition of 10 Italian children with beta cell autoimmunity at risk for T1D (pre-diabetic), in comparison with 10 healthy subjects. PCR-DGGE analyses based on the 16S rRNA gene had revealed that pre-diabetic children were characterised by significantly higher intestinal permeability, and by three potential biomarkers of the pre-pathological conditions, namely Dialister invisus, Gemella sanguinis and Bifidobacterium longum. Following those observations, we designed a study using an in vitro model of the proximal colon (TIM-2) to compare the effects of different nutritional treatments on the gut microbiota. In this second work, the goal was the characterisation of the composition of three different microbiota, at the baseline of the intervention experiments.

In detail, we individuated three groups (control, pre-diabetic and diabetic group) from the first case-control study. Indeed, two of the former pre-diabetic children developed T1D some months after the first study. Faecal samples were collected and pooled in order to obtain the three microbiota, and 16S rRNA gene-based microbial profiling analyses were performed after an adaptation period in the in vitro model. Results obtained showed that the three microbiota differed in the composition and abundances of different species: Gemmiger formicilis and Faecalibacterium prausnitzii were found at high levels in the control group (23.7% and 13.2% of the total microbial composition, respectively), Bacteroides vulgatus resulted at a high level in the diabetic group (18.5% of the total composition), and Bifidobacterium adolescentis was characteristic of the pre-diabetic and diabetic groups (26.8% and 23.3% of the total composition, respectively), while the biomarkers highlighted by PCR-DGGE analyses were not detected or they were not associated with a specific microbiota in this second experiment.

In conclusion, specific microbial species characterised the healthy, pre-pathologic or pathologic microbiota. These newly detected microbial biomarkers could be helpful in evaluating the outcomes of the in vitro intervention study, where a reduction of a negative biomarker could be considered a beneficial effect at the gut level of a specific nutritional treatment.
DO PRE- AND OR PROBIOTICS HAVE A ROLE IN COMMON INFECTIONS IN CHILDREN?

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The optimal healthy microbiota during early life still needs further evaluation. Different types of prebiotic oligosaccharides (OS) are used in infant formula, including galacto-oligosaccharide, fructo-oligosaccharide, polydextrose and mixtures of these OS, but none adds human milk OS. Prebiotics change gut metabolic activity (by decreasing stool pH and increasing SCFA), have a bifidogenic effect and bring stool consistency and defecation frequency closer to those of breast-fed infants. Although there is only limited evidence that these changes in GI microbiota induce a significant clinical benefit for the immune system, interesting positive trends have been observed for some markers. Additionally, adverse effects are extremely seldom. Prebiotics are added to infant formula because breast milk contains human milk OS. Because most studies suggest a trend of beneficial effects and because these ingredients are very safe, prebiotics bring infant formula one step closer to the golden standard of breast milk.

Although many studies show promising beneficial effects, the long-term health benefits and eventual risks of probiotic supplementation during early life are not clear. In general, results with probiotics in therapeutic indications are disappointing. Probiotic supplementation may be beneficial in prevention and management of disease, such as e.g., reducing the risk of necrotising enterocolitis in preterm infants, prevention and treatment of acute gastroenteritis in infants, etc. The use of the following probiotics (in alphabetical order) may be considered in the management of children with acute gastroenteritis in addition to rehydration therapy: Lactobacillus (L.) rhamnosus GG (low quality of evidence, strong recommendation) and Saccharomyces (S.) boulardii (low quality of evidence, strong recommendation). Less compelling evidence is available for L. reuteri DSM 17938 (very low quality of evidence, weak recommendation) and heat-inactivated L. acidophilus LB (very low quality of evidence, weak recommendation). The latter, although traditionally discussed with other probiotics, does not fit with the definition of probiotics. Other strains or combinations of strains have been tested, but evidence of their efficacy is weak or preliminary. Symbiotics are equally effective as probiotics alone, but prebiotics are not effective. Both pro- and prebiotics have limited to no efficacy in the prevention of acute gastroenteritis. If the use of probiotics for preventing antibiotic associated diarrhoea (AAD) is considered because of the existence of risk factors, such as class of antibiotic(s), duration of antibiotic treatment, age, need for hospitalisation, comorbidities, or previous episodes of AAD diarrhoea, L. rhamnosus GG (moderate QoE, strong recommendation) or S. boulardii (moderate QoE, strong recommendation) are recommended. If the use of probiotics for preventing Clostridium difficile-associated diarrhoea is considered, S. boulardii (low QoE, conditional recommendation) can be considered. Other strains or combinations of strains have been tested, but sufficient evidence is still lacking. Based on currently available evidence suggests L. rhamnosus GG if the use of probiotics for preventing nosocomial diarrhoea in children is considered. For other strains or their combinations, the evidence is either lacking or it is insufficient to formulate a recommendation.

It is likely that ongoing research will result in the use of specific probiotic organisms and/or prebiotic oligosaccharides during the first 1,000 days of life, with the goal to develop a healthy microbiota from conception over birth into the first two years of life with a lowered risk of infections and inflammatory events.

NON-ALCOHOL FATTY LIVER DISEASE AND PROBIOTICS IN OBESE CHILDREN

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The term ‘gut-liver axis’ is used to describe the close relationship that is established between the gut and liver beginning in the very early stages of foetal life. The liver receives most of its blood supply from the intestine through the portal vein and is therefore one of the organs that is most exposed to potentially toxic factors originating in the gut, including all or part of the gut microbiota, and bioactive components
of food that have been processed by the gut microbiota. Thus, quantitative and qualitative variations in the bacteria that compose the gut microbiota may actively contribute to the pathogenesis of several liver diseases, including non-alcoholic fatty liver disease (NAFLD), alcoholic steatohepatitis, and cirrhosis.

Considering that obesity is a major risk factor for NAFLD in humans, there is evidence that the gut microbiota can directly influence body weight in several ways: (i) it affects the proportion of calories obtained from the intestinal contents; (ii) bile acids have bacteriostatic effects, thereby affecting the absorption and emulsification of fats and lipid-soluble vitamins in the small intestine; (iii) it contributes to increased intestinal permeability through loss of epithelial barrier integrity; (iv) bacterial translocation into the systemic circulation is increased, allowing more hepatic access for ethanol and bacterial endotoxins such as lipopolysaccharide (LPS); (v) microbial LPS is recognised by Toll-like receptor 4, which triggers nuclear factor B-mediated proinflammatory cytokine production; (vi) dietary choline modulation; and (vii) development of insulin resistance in the host. Recently, we were able to show that in the complex scenario of NAFLD, the interrelated enterotype-metabotype framework appears to contribute to creating a signature that changes during disease progression. In particular, the combination of a low abundance of *Oscillospira* with high levels of 2-butanone may be a specific intestinal MG and MB profile for liver steatosis in children. The high relative abundance of *Lachnospiraceae, Ruminococcus*, and *Dorea* observed in paediatric patients with NASH suggests that changes in the gut microbiota are associated with disease severity. These findings might provide development of a specific metabolic diagnostic profile of steatosis and suggest a first-line probiotic candidate for treating NAFLD.

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**ESTABLISHING A CAUSAL LINK BETWEEN GUT MICROBES AND BODY WEIGHT – TOWARDS THE IDENTIFICATION OF KEY PLAYERS**

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Over the past years, evidence from both experimental animal and human studies on a link between the gut microbiota and (over)weight has been accumulating. Yet, the cross-sectional designs of the majority of human studies, in which the microbiome is studied once overweight or obesity has already manifested, limit the possibility to identify microbial taxa that might play a causal role in the onset of overweight and obesity. It is likely that obesity-related gut dysbiosis has its origins during early infancy, at a time when first colonisers of gut microbiota lay the foundation for subsequent colonisation by obligate anaerobes. Studies on the establishment of the infant and childhood microbiome and its subsequent link to weight development are therefore crucial to unravel the temporal relationships between microbial perturbations and weight development.

Within the Dutch KOALA Birth Cohort Study, the microbiota was characterised both in early infancy (n=1,032) as well as at school-age (n=476) and related to subsequent height and weight development during childhood. Moreover, antibiotic use (as collected from GP registries) throughout childhood was additionally linked to anthropometric data. These latter data confirmed that early infancy is the most critical period during which exposure to antibiotics has the most pronounced effect on subsequent weight development, likely mediated through perturbations in the establishing microbiome. Comprehensive analysis of the microbiota composition at school-age amongst others confirmed the negative association between *Akkermansia* and (over)weight as well as the positive association between methanogenic archea, in particular *Methanobrevibacter smithii*, and weight development. Metagenomic sequencing also revealed notable differences on a functional level, including enrichment of amino acid biosynthesis and transport and bacterial growth-related pathways in the group of children with the highest BMI.

Altogether these data illustrate the link between the gut microbiota composition and function and childhood weight development, even before the manifestation of overweight and obesity.
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The gastrointestinal tract (GIT) of the young calf plays a critical role in the protection of the host from pathogens, while supporting the absorption and metabolism of nutrients for efficient growth. In dairy production, GIT diseases and disorders are the number one cause of mortality and morbidity, which has reached alarming levels (8% mortality and 50% morbidity) in the first month of life. There is a need to better understand how factors such as GIT microbiota and function can influence calf GIT health. Yet, our knowledge of GIT development, especially from a microbiological standpoint, is quite limited. Recent studies using molecular based techniques have shed new light on the role microbes play at different stages of calf GIT development. Colonisation of GIT microbiota starts immediately after birth, with clear differences between the GIT microbiome of the calf and both the maternal and environmental microbiome. The GIT microbiota and host gene expression related to GIT function have been shown to be dynamic during the first week of life, and the colostrum and milk nutrition that the calf receives can impact GIT microbial colonisation during the same period. For example, the heat treatment (pasteurisation) of colostrum prior to feeding calves decreased *E. coli* and increased *Bifidobacterium* colonisation in the GIT during the first day of life. There also is strong correlation between bacterial populations and gene expression of the host GIT, which further suggests that the first week postpartum – when it is responding to rapid changes in microbial composition – is a critical time in calf GIT development.

Along with microbial colonisation during the first day and week of life, there is also a need to understand more about how the calf transforms into a fully functional ruminant at weaning. Weaning is conducted early and abruptly in dairy production systems, and such, is associated with depressed growth and disease. To date, most weaning studies have focused on methods to improve ruminal development during the pre-weaning period to improve growth and health status. We recently showed that delaying the age of weaning and providing a step-down weaning protocol increases post-weaning growth and coincides with a more gradual shift in ruminal microbiota to a post-weaned state. Nutrient flow to the lower gut also changes dramatically during weaning, coinciding with a wide array of microbiological and structural changes. Studies examining structural and gene expression changes suggests that the lower GIT of the dairy calf undergoes alterations that may reduce barrier function when solid feeds are consumed.

In review, our knowledge of calf GIT microbiology has improved in recent years and there appears to be opportunities to alter management and use probiotic technology to effectively improve ruminal and lower GIT health. An improved understanding of how microbiota and diet interact and impact GIT functional development at key stages of life would benefit the dairy calf industry significantly.

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During the neonatal period, the development of the microbiota in the gastrointestinal tract is characterised by rapid and large changes in abundance, composition and diversity. From the first day of life, the gastrointestinal tract is growing and undergoes major developments, the microbiota changes go together with maturation of intestinal cells and development of the immune system. These changes are influenced by the mother, environment and feed, but also stressors play an important role. The early life development not only has short term effects on health and growth of animals but also will affect
Introduction of solid feed and the weaning process have shown to be the main determinants of the bacterial succession in early life. Feed composition has a direct effect on the microbiota development and a can play a functional role to achieve a diverse and stable microbiota and in order to reduce the disruption of the microbial ecosystem during stress periods. Organic acids, pre- and probiotics are widely applied nowadays in neonatal and young animal feeds. Study results showed various impacts of those compounds on microbial diversity, but also changed abundance of specific groups of bacteria, which could be related to specific health benefits. Strong relations between growth performance and microbiota composition are found, with specific groups of bacteria being more abundant in high performing animals.

The microbiota development already starts before birth, but is established mostly in the first weeks of life. This neonatal period is critical for gaining a stable and healthy microbiota that can alleviate the effects of stressors later in life. Maternal and neonatal feed plays an important role in modulation of a healthy intestinal system with lifelong effects on health and performance.

**SHOULD WE CONSIDER DEVELOPING MORE TAILORED APPROACHES TO PROVIDE PROBIOTICS IN ANIMAL PRODUCTION?**

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Notwithstanding the abundance of favourable research, the constant use of probiotic additives in feed formulations is hard to diffuse in animal production. A frequent anecdotal explanation is that the ‘in field’ productive response is not constant. This, however, often underlies the absence of clear targeted functions, except for a generic improvement of animal performance. It can be hypothesised that there is still the need to define better the response parameters and characteristics of the responsive subject. A clear definition of intermediate goals would imply a better description of the starting characteristics of the targeted animal.

This presentation will try to assemble some possible reasons to develop more tailored approaches to assign probiotic supplementation in animal production. One of the most frequent intermediate aims when planning a probiotic supplementation are a permanent (or transient) settling to influence the resident gut microbiota activity or composition. This implies that more knowledge of the microbiota profile is required. Modern molecular analytical tools are more and more available at cheaper costs, but practically it is often difficult to disentangle the abundant information provided. A first attempt to categorise the profile of gut microbiota of healthy human individuals led to the hypothesis of three robust clusters, broader gut ‘community types’ called enterotypes. These enterotypes are defined by a predominance of *Bacteroides, Prevotella, or Ruminococcus* genus, respectively. Likely, two enterotype-like clusters were identified in the pig microbiota. This could be a starting point to categorise subjects (or groups) that are respondent or non-respondent to a probiotic treatment. Rapid and selective parameters should be also considered, such as serum or faecal immunoglobulin content, acute phase proteins, etc., and utilising the finding to catering to a niche market defined by production phase, type of production, or disease state.

Among microbiologists, there is still a debate about the relevance of the host genetic background to explain gut microbiota variations. This obviously depends also on the relative importance of environmental factors, such as mother environment, diet, animal mixing, etc. Recent findings in human
and other species support the concept of progressive mutual adaptation of host-microorganism association, related to the recognition at the level of gut mucosal surface and to the foraging opportunities for commensal microorganism or pathogens. Particularly, variation in the composition of the sugar motifs may explain the presence or abundance of certain strains. Examples of association between genetically determined surface variation and some microbes or viruses can be found for blood groups affecting also glycomic motifs in the gut. The genetic variation for AB0 system can affect the sugar variations in the jejunum mucosa in pigs, too. Knowledge of the genetic determinants of bacteria adhesion is more developed for some enteropathogens. In pigs, the determinant gene for the adhesion and pathogenesis of enterotoxigenic E. coli F18 and some genetic markers for F4 are known, but suppression of these unfavourable genetic variants in breeding nucleus cannot be always feasible or convenient. Examples of the relevance of this genetic variation for determining some nutrient requirements are available. This opens the option of targeted supplementation for eventual beneficial microorganism able to prevent or contrast the multiplication of these pathogenic E. coli. However, it can be supposed that commensal variation is also affected by the presence of receptors for intestinal adhesion or for the digestion of superficial branches in the intestinal glycocalyx. The abundances of formerly classified Clostridium bartlettii, now Intestinibacter bartlettii, were lower in healthy pigs susceptible to colonisation by enterotoxigenic E. coli F4 than in not-susceptible pigs.

In conclusion, it could be worthwhile to consider research in the direction of providing knowledge and tools to implement more targeted strategies of probiotic supplementation in animal production.

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FIBRE SUPPLEMENTS AFFECT COLONIC FERMENTATION CHARACTERISTICS IN PIGLETS

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The aim of this study was to investigate the effects of three different soluble pectins on the digestion of other carbohydrates present in the pig’s diet, and their effect on the microbiota composition and SCFA levels in the intestine of pigs. Three percent of soluble pectins were added to a cereal-based pig diet and fed to piglets during 4 weeks. Pectins used were low-methyl esterified pectin (LMP), high-methyl esterified pectin (HMP), and soy pectin, in situ solubilised by a hydrothermal treatment from the soybean component of the basic feed. The LMP diet decreased the ileal digestibility of starch resulting in more starch fermentation in the proximal colon (30% of total carbohydrate fermented). Low-methyl esterified pectin present was already for 40% fermented by the microbiota in the ileum, while high-methyl esterified pectin was mainly fermented by the microbiota in the proximal colon. Treated soybean meal was mainly fermented in the proximal colon and shifted the fermentation of arabino-xylans and glucans being representatives of cereal dietary fibres present in the feed as well to more distal parts. The level, relative composition and the location of production through the digestive tracts was quite different for the different diets. LMP, HMP and soluble soy pectin diets differently affected the digestion processes compared to the control diet and shaped the colonic microbiota from a Lactobacillus-dominating microbiota to a Prevotella-dominating community, with potential health-promoting effects.

References
THE PROS AND CONS OF PRE- AND PROBIOTICS IN HORSES

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There are numerous horse feeds that include yeast, pre- and/or probiotics on the market as well as microbial supplements to complement the horse’s diet. Regardless of the inconsistencies seen in the research as to their efficacy, there have been little to no adverse effects found with their use. For many years, the major focus of study on the equine microbiota was how it benefitted the horse by improving digestion of the feedstuffs in the diet. In more recent years, there has been diverse research on effects of direct-fed microbials on feed utilisation, prevention and treatment of disease, immune function, and improving performance as well as stabilising the gastrointestinal tract (GIT) microbiota.

The major difference in the microbial population found in the equine digestive tract and that of humans is the large number of cellulolytic bacteria that break beta bonds in cellulose and hemicellulose for further digestion to volatile fatty acids (VFA) that the horse uses as a major source of energy. This microbial energy production is so great that it can nearly supply the entire energetic needs of a horse at maintenance. Live yeast and yeast cultures have fairly consistently been shown in vitro and in vivo to improve digestibility of dry matter (DM), organic matter (OM) and fibre fractions (acid detergent fibre (ADF) and neutral detergent fibre (NDF)), which is indicative of better energy production. Improved digestibility of poor quality forage is more consistently seen with yeast and/or mixed microbial supplementation than high quality forage, as are high fibre diets, particularly in horses with compromised gut health. Whether this is through the support yeast gives to proliferation of cellulolytic bacteria in the GIT or the direct effect on breaking down fibre is not totally understood.

The two major causes of death in the equine, colic and laminitis, can both be brought on, in part, by dysbiosis of the GIT. The tradition of feeding high starch diets to increase energy intake of the horse is known to have profound effects on hindgut pH, which in turn can affect the health of the cellulolytic bacterial populations predisposing a horse to colic or a die-off of bacteria releasing endotoxins which provokes laminitis. Further, long term high starch intakes can cause insulin dysregulation which may predispose a horse to laminitis as well. These concerns addressed by forcing change in populations or pH of the digestive tract with yeast/probiotic supplementation has proven challenging in studies on ameliorating the effects of high starch intakes, with inconsistent results so that switching to a high fibre diet is more often recommended as recourse.

Probiotic or yeast supplementation in the treatment of disease, such as gastroenteritis or diarrhea has been moderately successful at reducing severity and duration as well as reducing shedding of pathogenic bacteria for the safety in hospital situations. Combining probiotics with treatments for intestinal disorders like worm infestations or sand build-up has been found to improve effectiveness of dewormer and psyllium treatments. Studies on immune function have demonstrated some effects on systemic immunity with supplementation of a yeast-probiotic combination, while others on immunoglobulins isotypes in mares and foals have not been as effective. Prebiotic research in horses is scarce but preliminary studies indicate possible future applications of short chain fructo-oligosaccharide (scFOS) in amelioration of adverse effects of acidifying microflora and improving insulin sensitivity in obese mares, increased levels of immunoglobulins in mare colostrum with mannan-oligosaccharide (MOS), but no measurable effects on immunoglobulins in healthy horses or semen quality in stallions.

While many more studies in horses have been done on yeasts than probiotics or prebiotics, there are still many questions remaining on what are the most effective types or combinations of microbials to improve digestion, health, and performance in the horse. Perhaps some of the lack of consistent results in the current studies stem from use of an inappropriate or effective microbe for the situation. Microbes that are found effective in human studies may not be the most appropriate for a strict herbivore like the horse. In fact, there is no standard microbiome for the horse but is uniquely adapted to each individual for their circumstances, which further complicates investigation of the appropriate microbe for specific situations. The very limited number of horses used in most of the studies makes finding significant results difficult even though there may be beneficial effects on certain individuals in a study. There can be difficulty comparing results from one study to the next or positive results seen in the general horse public when commercial supplements have been used because of a lack of government oversight as to the quality of microbes used in equine products and control of quantity and specificity of the species indicated. A broader spectrum of research into the connections with GIT microbiota and health and well-
being of the horse are needed, such as the possible link of gut microbiota to the epidemic of obesity in horses.

DEVELOPMENT OF A HOST SPECIFIC, MULTI-SPECIES PROBIOTIC FOR POULTRY MEETING THE REGULATORY REQUIREMENTS IN THE EU

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It has been recognised that a well-balanced gut microbiota is important for animal health and improved performance. Modulating the gut microbiota through feed additives, such as probiotics, are effective means to maintain or improve health especially in young animals. In modern poultry husbandry, newly hatched chicken are particularly susceptible to pathogenic colonisation, since they do not come into contact with the mother hen, delaying the development of a diverse and stable gut microbiota. In this respect, a multi-species probiotic competitive exclusion (CE) product for young chicken was developed during a multinational project funded by the European Union. The bacterial strains were isolated from different parts of the intestinal tract of healthy chickens and were selected for specific beneficial properties such as production of antimicrobial substances, attachment to gut epithelial cells, and good production qualities and stability during storage. Meeting the regulatory requirements of the European Food Safety Authority (EFSA) the host-specific probiotic PoultryStar® was confirmed to be safe (non-toxic, susceptible to therapeutic antibiotics, lack of transferable antibiotic resistance and virulence genes) and efficacious for the use in target species. Although several feeding trials worldwide have shown positive effects of PoultryStar® in alleviating a wide range of pathogenic conditions, such as coccidiosis, necrotic enteritis, and salmonellosis, the mode of action of the probiotic in the gut environment is not fully clarified. Therefore, effort is being put into the development and application of molecular techniques to gain knowledge about the impact of probiotics on the gut microbiome. Furthermore, strain specific real-time PCR assays for accurate identification and quantification of the probiotic bacteria in feed and intestinal samples should improve qualitative assessment of feeding trials.

IDENTIFYING ALTERNATIVES TO ANTIMICROBIAL GROWTH PROMOTERS IN CHICKEN FEED THROUGH METATRANSCRIPTOMICS

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To help maintain efficient production, since the 1950s, the poultry industry has relied on the addition of sub-therapeutic quantities of antibiotics, such as virginiamycin and bacitracin, to feed. Due to their role in promoting growth, these additives have been termed antimicrobial growth promoters (AGPs). AGPs are thought to function through altering the microbial composition (microbiome) of the chicken gastro-intestinal (GI) tract, and provide additional benefits, such as improving resistance to infection (e.g., necrotic enteritis) and lowering the burden of food safety pathogens (e.g., Salmonella and Campylobacter). However, with worldwide pressure to eliminate AGPs as feed additives, cost-effective efficacious alternatives to AGPs are urgently required. Key to the identification of such solutions is a systematic analysis of the microbial composition and function within the chicken GI tract to identify feed additives, such as probiotics, that mimic the action of AGPs, as defined by growth, microbial diversity and reduction in pathogen burden. To address this challenge, we are developing whole microbiome RNA sequencing (metatranscriptomics) as an effective platform for detailed studies that will examine the impact of probiotic feed additives on the composition and function of the chicken microbiome.
The microbiome of the animal’s gastrointestinal tract plays an essential role for the growth and health of the animal. It is well accepted that the structure and function of the microbiome is influenced by external parameters, such as composition of the feed, addition of pro-, pre- and antibiotics, environmental factors as well as the by animal itself. Gathering knowledge about the activity and function of the gut microbiome under different feeding conditions can be important to develop highly adapted animal feed in order to increase the efficiency in livestock production and decrease the emission of undesired substances by ruminants for example. The identification and assessment of the protein inventory of the microbiome is named metaproteomics and displays a valuable interface between gene-based analysis and metabolomics studies. Metaproteomic studies of gut samples or faeces are challenged by a high fraction of eukaryotic proteins, which originate from feed particles and host cells and are co-extracted during the sample preparation procedure. Samples from the gastrointestinal tract of broilers and pigs as well as rumen samples were used to improve sample preparation strategies and to give a first impression of the active microbial fraction in these gut sections. We applied the technique to study the influences of dietary changes in broilers and dairy cows.
TUESDAY 11 OCTOBER 2016

SESSION 5
ADOLESCENTS & ADULTS

REDUCING VULNERABILITY TO DEPRESSION IN HEALTHY ADULTS: MULTISPECIES PROBIOTICS REDUCE COGNITIVE REACTIVITY TO SAD MOOD

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The number of people suffering from mental disorders, depression in particular, is constantly growing and measures to prevent the development of these disorders are therefore rapidly gaining interest. In recent years, it has become clear that the intestinal microbiota play an important role in the bidirectional communication between the intestine and the brain. On a mechanistic level, the evidence is rapidly accumulating. The first studies in humans that are now being reported, have also found a positive effect of probiotics on gut-brain communication. In our study, we have tested the hypothesis that a multispecies probiotic formulation reduces cognitive reactivity to sad mood, which reflects one’s vulnerability to develop a depression. In the current study, we showed that, in healthy students, four weeks of probiotic intake significantly reduced sensitivity to depression in the probiotic group compared to the control. Following these promising results, further studies are being initiated to learn more about the influence of the gut-brain axis in alleviating mental disorders.

ENTERIC-COLONISING LACTOBACILLUS GASSERI CP2305 IMPROVES STRESS-RELATED ADVERSE BEHAVIOURS

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Several lines of evidence have suggested that gut microbiota could profoundly modulate the communication between the gut and the brain through neural, endocrine, and immune signalling pathways. This concept is now interpreted broadly as the microbiota-gut-brain axis. Gut microbiota are likely to be important in normal healthy brain function and have been shown to affect stress sensitivity. We originally isolated a unique strain Lactobacillus gasseri CP2305 from stool samples of a healthy volunteer. L. gasseri CP2305 could colonise in the digestive tract of the volunteers at the odds of approximately 40% after 3-time oral administration at the dose of $1.0 \times 10^{11}$ cfu. This unique enteric-colonising strain may enhance to exert the highly influential stimulation activity to the brain-gut axis. Daily administration of L. gasseri CP2305 significantly improved not only the stress-related mental and physical symptoms, but also sleep disturbance in the subjected medical students during the cadaver dissection course. Daily administration of L. gasseri CP2305 also prevented the stress-induced sleep disturbance in medical students preparing for the National Examination for Medical Practitioners (chronic academic stress model), which was confirmed by both the Pittsburgh Sleep Quality Index and the overnight single-channel electroencephalography (EEG) recordings. We also examined the beneficial effects of this unique strain on patients with irritable bowel syndrome (IBS). A randomised, double-blind placebo-controlled study showed that treatment with L. gasseri CP2305 for 4 weeks significantly improved QOL as well as stool properties and the severity of IBS symptoms. Gene expression profiling of peripheral blood leukocytes demonstrated that L. gasseri CP2305 significantly prevented the stress-induced downregulation of a group of genes related to eukaryotic initiation factor 2 (EIF2) signalling in association with the improvement of stress-associated mental and physical symptoms in both healthy medical students and IBS patients. Our results suggest that L. gasseri CP2305 may have potential benefits for prevention of stress-related adverse behaviours.
CULTIVATED FAECAL MICROBIOTA TRANSPLANT TO RCDI (RECURRENT CLOSTRIDIUM DIFFICILE INFECTION) PATIENTS

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Clostridium difficile is a Gram-positive, spore forming, toxin-producing, anaerobic bacterium, which has been associated since the 1970s as a cause of recurrent C. difficile infections. RCDI is now considered a leading nosocomial infection, and the infectious agent has become increasingly virulent in recent years with the outbreak of hypervirulent strains resulting in significant patient morbidity and mortality. We have an anaerobic microecological system comprising anaerobic cultivated human intestinal microbiota (ACHIM) produced by continuous re-culturing of the original faeces homogenate. The faecal culture is originally obtained from faeces collected in 1994 from a healthy Scandinavian donor consuming a customary Western diet. Both the donor and the faecal sample were carefully examined. The faecal sample was thoroughly investigated with respect to absence of hepatitis A, B and C, cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus (HIV), and the intestinal pathogenic bacteria Salmonella, Shigella, Campylobacter, Yersinia and C. difficile, all of which were negative. During these years, ACHIM has been administered to both isolated patients and those taking part in clinical trials where ACHIM and vancomycin treatment were compared and some of the results will be presented and discussed.

PROBIOTICS CAN IMPROVE COMPLIANCE AND ERADICATION RATE FOR HELICOBACTER PYLORI THERAPY

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Helicobacter pylori infection in humans is associated with a spectrum of gastroenterological and haematological diseases. Successful eradication of the bacterium is a major component in the treatment of these conditions. The current eradication rates are low and still decreasing mainly due to increased antibiotic resistance. An additional important contributor of low eradication rates is the high incidence of treatment-related side effects (including diarrhoea, nausea, epigastric discomfort, and dysgeusia with a metallic taste), determining low compliance, and, consequently, incomplete therapy. Many of these gastrointestinal effects result from the modification of the ecologic equilibrium of intestinal microbiota due to the antibiotics. Probiotics represent an alternative/adjuvant treatment to standard eradication protocol. The current report of the European Helicobacter Study Group considers probiotics as an adjuvant treatment in reducing side effects during the standard Helicobacter pylori eradication therapy. The primary objective of the study was the determination of the efficacy of a probiotic preparation as a supportive therapy in eradication of H. pylori.

The study was multicentre, prospective, randomised, placebo-controlled, and double-blind. The enrolment of subjects into the trial was conducted in 121 general practitioners' offices in different regions in Croatia from September 2009 until June 2012. The study was reported according to the CONSORT guidelines and was registered at www.clinicaltrials.gov (NCT01969331). The initial diagnosis of H. pylori infection was established using the rapid urease test, stool antigen, or the urea breath test. The subjects first filled out a specially designed questionnaire in order to assess the severity of the 10 symptoms which can be related to eradication therapy to be monitored during the trial. Each subject then received 28 capsules of probiotic preparation or matching placebo capsules, which they were supposed to take over the following 14 days, twice a day, at least two hours prior to or after the antibiotic therapy administration. A total of 804 patients were enrolled in the trial, of which 650 (80.9%) were included in the analysis. The results show a significantly larger share of cured subjects in the probiotic arm versus the placebo arm (87.38% vs. 72.55%; P<0.001). Additionally, odds ratio, absolute and relative risk reductions as well as number needed to treat all point strongly in favour of the probiotic arm. Overall, at baseline the average value of intensity for all 10 symptoms was 1.17 for subjects on probiotic and 1.07 for subjects on placebo (P<0.001). At follow-up visit 15 days after the start of the trial, the intensity of the same symptoms that were monitored at enrolment was again evaluated. Overall, the average
intensity value for all 10 symptoms was 0.55 for subjects on probiotic and 0.76 for subjects on placebo ($P<0.001$).

In conclusion, adding probiotics to the standard triple therapy for $H. pylori$ eradication significantly contributes to the eradication rates.

PREBIOTICS, SHORT CHAIN FATTY ACIDS AND APPETITE REGULATION

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In recent years, there has been a renewed interest in the role of dietary fibre in obesity management. Much of this interest stems from animal and human studies that suggest increased intake of fermentable fibre can improve body composition. A growing number of reports have demonstrated that the principal products of colonic fermentation of dietary fibre, short chain fatty acids (SCFAs), contribute to energy homeostasis via effects on cellular metabolic pathways and receptor mediated mechanisms. In particular, over the past decade it has been identified that a widespread receptor system exists for SCFAs. These G protein-coupled receptors, free fatty acid receptor 2 (FFAR2) and 3 (FFAR3) are expressed in numerous tissues, including the gut epithelium, adipose tissue and liver. Investigations using FFAR2- or FFAR3-deficient mice suggest that SCFA-mediated stimulation of these receptors at different tissue sites modulates metabolic processes that control energy intake, utilisation and expenditure. The importance of SCFAs to metabolism has been further emphasised in studies where germ-free mice have received gut microbiota transplants. These investigations highlight that the transfer of gut microbiota compositions that produce different levels of SCFAs in the colon influence body weight gain and adiposity. Increasing colonic SCFAs is therefore an attractive target to improve metabolic health in humans. However, translating the positive outcomes observed in animal studies into humans remains a major challenge due to the difficulty in reliably increasing SCFA production in the human colon. Our group in partnership with the University of Glasgow have developed a method to deliver SCFA to the colon orally. We have demonstrated the potential of SCFA to effect body weight over a 6-month period using this technology. However, the mechanism which underlie this observation remains unclear; potential possibilities will be discussed in this lecture.

SMALL PLAYER, LARGE ROLE: MICROBIAL METABOLIC PATHWAYS ASSOCIATED WITH METABOLIC RISK OF CARDIOVASCULAR DISEASE

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Abnormal metabolism is a precursor of cardiovascular disease (CVD). Emerging evidence supports a link between the gut microbiome and the development of metabolic syndrome and CVD. However, we know little about the functional connection between host-microbe metabolism and need interdisciplinary integrative analyses to understand their complex metabolic interaction.

Over the past few years we have built up LifeLines-DEEP, a multi-omics biobank that is part of LifeLines, the large population cohort study in the northern Netherlands. LifeLines-DEEP consists of 1,500 individuals (42% males, age range 18-81 years), from which dietary, genetic, gut flora and metabolic profiles have been generated. To determine an individual’s metabolic risk of developing CVD, we measured 33 serum metabolic biomarkers using a nuclear magnetic resonance (NMR) platform; the biomarkers included various lipoprotein particles, fatty acids, amino acids and glycolysis-related metabolites. We report association to 48 bacterial metabolic pathways, which were derived from metagenomics shotgun sequencing data. In particular, our data showed that microbial energy metabolism and various biosynthesis pathways — leading to the production of pyruvate, amino acids, vitamins, short-chain fatty acids and polyunsaturated fatty acids — might impact CVD risk. Our current study also presents an integrative analysis and provides a deeper insight into the complex diet-microbe-
host dialogue in metabolism and inflammation that are relevant to CVD development. This knowledge can help pave the way towards the development of therapies to modulate the microbial metabolism and help prevent CVD.

**SHORT CHAIN FATTY ACIDS: THE LINK BETWEEN OUR GUT MICROBIOTA AND METABOLIC HEALTH**

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Our gut microbiota is increasingly recognised as an important player in the etiology of chronic metabolic diseases, such as obesity, type 2 diabetes mellitus and cardiovascular disease. Our microbiota may ferment indigestible carbohydrates, thereby producing short-chain fatty acids (SCFA), of which propionate, butyrate and acetate are the most abundant. These SCFA are crucial for intestinal health and may have important metabolic effects on energy homeostasis and substrate metabolism, thereby playing an important role in body weight control, insulin sensitivity and glucose metabolism. They may act as signalling molecules in the gut and may also enter the systemic circulation directly affecting peripheral tissues. In that respect, the site of SCFA may be an important determinant of its metabolic effects. We recently showed that distal colonic administration of physiological concentrations of acetate affected whole-body substrate metabolism, with a pronounced increase in fasting fat oxidation and plasma PYY in overweight males, whilst administration in the proximal colon had no metabolic effects. In this presentation, data will be presented on the impact of gut-derived SCFA and differential SCFA availability on human energy and substrate metabolism. Secondly, implications for dietary interventions targeting SCFA production in the prevention of chronic metabolic diseases will be discussed.
TUESDAY 11 OCTOBER 2016
SESSION 6
TARGETING SPECIFIC GROUPS

THE HUMAN UTERINE MICROBIOME: THE MICROBIAL CORE OF HUMAN REPRODUCTION?

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The epithelial lining of the human uterine cavity elicits a remarkable cyclical transformation in the mid-secretory phase of the uterine cycle, following each ovulation yet regardless of fertilisation, into a receptive state referred to as the decidua. The decidual epithelium is the first point of contact in the uterine environment for the conceptus, and has developed as a specialised tissue that allows apposition, adhesion and implantation of the blastocyst to occur, while also acting as an embryo quality filter. Maternal tolerance of the developmentally competent, yet semi-allogeneic embryo at this stage, has puzzled immunological science for decades. While once attributed to systemic suppression of the maternal immune system, tolerance of the embryo that subsequently develops into a foetus is increasingly recognised to involve specifically trained decidual immune cells, including macrophages and regulatory T lymphocytes. In particular, early pregnancy is paralleled by an expansion of maternal FoxP3+ regulatory T cells (Tregs), which confer immune tolerance in a paternal antigen-specific manner, while blunted maternal Treg expansion is associated with adverse reproductive outcome, including subfertility.

The drivers of Treg expansion remain incompletely defined, but compelling evidence supports the mechanism of repeated exposure to semen from the ultimate father in inducing Treg-mediated maternal tolerance to foetal antigens. Treg priming by semen is however unlikely to be the single driver of maternal-foetal immune tolerance. In the gut, immune homeostasis through induction and maintenance of Treg cell populations has been shown to relate to the gut microbiome, and in particularly to several taxa in the class Clostridia and in the genera Lactobacillus and Bacteroides. In a preliminary study among a selected group of non-pregnant patients, we recently documented that the endometrium harbours a distinct, low-biomass microbiome with a rather high degree of similarity between subjects. In most women, the endometrial microbiome was co-dominated by B. xylanisolvens, B. thetaiotaomicron, and B. fragilis, while a majority of women also harboured typical vaginal Lactobacillus taxa, though at highly variable abundances.

Though currently still under study, Treg cell induction presumably is a general hallmark mechanism for maintaining the homeostatic relationship between the microbiota and the host, while it is further conceivable that in the human uterus host-bacterial homeostasis contributes to maternal tolerance in early pregnancy.

PREVENTIVE EFFECTS OF PROBIOTICS ON MASTITIS INCIDENCE IN LACTATING WOMEN

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During the breastfeeding period, women can experience a range of breast related problems, such as breast and nipple pain, nipple cracks and mastitis. Mastitis is an inflammatory condition of the breast that is usually associated with lactation. The reported incidence is around 15% but can reach values as high as 35% when any clinical mastitis case is considered. Human mastitis is characterised by a mammary bacterial dysbiosis, a process in which the population of mastitis agents, such as Staphylococcus, increases at the expense of the normal mammary microbiota. Previous studies have demonstrated that Lactobacillus fermentum CECT5716, a probiotic strain previously isolated from
breast milk, can be used as an effective treatment of mastitis and painful breastfeeding by reducing pathogen counts in breast milk.

The objective of the present study was to evaluate the preventive effect of the consumption of *L. fermentum* CECT5716 on mastitis incidence in lactating women. A randomised double blind controlled study including 600 women was conducted. This study was carried out according to the Helsinki declaration, and the protocol was approved by the Regional Ethics Committee of the Sistema Andaluz de Salud based in Seville (Spain). Women were recruited 1-6 days after childbirth and randomly assigned to a group. The probiotic group received 1 capsule/day containing *L. fermentum* 3x10⁹ cfu; the control group received 1 placebo capsule/day containing maltodextrin. The intervention period was 16 weeks. The primary outcome of the study was the incidence of clinical mastitis defined as: at least two out of the three breast symptoms (pain, redness, lump) and at least one of fever or flu-like symptoms (shivering, hot sweats or aches). Secondary outcomes were bacterial load in breast milk, growth and health of infants and faecal microbiota of infants. Two hundred and ninety-four women completed 16 weeks of treatment. The incidence rate of mastitis in the probiotic group was significantly lower than in the control group (IR=0.130 in the probiotic group vs. IR=0.263 in the control group; *P*=0.021). Specifically, the consumption of *L. fermentum* CECT5716 during lactation decreased by 51% the incidence rate of clinical mastitis. The probiotic treatment induced a significant decrease in *Staphylococcus* load in breast milk of women (*P*=0.013) demonstrating that breast milk microbiota might be modulated by probiotic treatments.

In conclusion, the consumption of the human milk probiotic strain *L. fermentum* CECT5716 prevents the development of lactational mastitis in lactating women.

**FORM, FUNCTION AND DIVERSITY WITHIN THE VAGINAL ENVIRONMENT; THE IMPLICATIONS FOR DYSBIOSIS AND HEALTH**

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The vaginal microbiome plays a vital role in women’s health and human reproduction. Unique physiological and structural features of the vagina itself are optimally designed to co-exist with and support this synergistic microbial community. Natural biological variation in the vaginal environment (menstruation) as well as additional factors (sexual intercourse, pregnancy, hygiene practices, vaginal infections) directly and indirectly influence the composition and function of the protective microbiome. This presentation will focus on these changes in the vaginal microbiome and environment, especially highlighting their implications for the health of women.

**GUT MICROBIOTA AND METABOLIC MARKERS IN POSTMENOPAUSAL WOMEN WITH OBESITY**

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Experimental studies with gut microbiota transplantations indicate that a specific gut microbiota composition can be the cause and not just the consequence of the obese state and metabolic disease, which suggests a potential for gut microbiota modulation in prevention and treatment of obesity-related metabolic diseases.

To examine the link between dietary habits, the microbiome and metabolic markers, we explored if the associations between metagenomic and metabolic markers persisted after adjustment for body fat, age and habitual dietary intake among postmenopausal women with obesity. Additionally, the effect of a 6-week intervention with a daily intake of either *Lactobacillus paracasei* F19 (9.4x10¹⁰ cfu), flaxseed
mucilage (10 g) or placebo on the gut microbiota and metabolic risk markers were examined. Quantitative metagenomic analysis of faecal DNA was performed to identify the changes in the gut microbiota. Diet-induced changes in metabolic markers were explored using adjusted linear regression models. In total, 114 metagenomic species were associated with metabolic markers (P<0.001), including Akkermansia muciniphila, Bilophila wadsworthia, Bifidobacterium longum, and Faecalibacterium prausnitzii, but also species not previously reported to be associated with metabolic markers, including Bacteroides faecis and Dorea longicatena. The negative associations between insulin sensitivity for B. longum and F. prausnitzii was modified by the intake of dietary fibre and fat. The intervention over 6 weeks with flaxseed mucilage reduced serum C-peptide and insulin release during an oral glucose tolerance test (P<0.05) and improved insulin sensitivity measured by Matsuda index (P<0.05). Comparison of gut microbiota composition at baseline and after 6 weeks of intervention with flaxseed mucilage showed alterations in abundance of thirty-three metagenomic species (P<0.01), including decreased relative abundance of eight Faecalibacterium species. The intake of L. paracasei F19 did not modulate metabolic markers, compared with placebo.

In conclusion, this study shows that several gut bacterial species are linked to metabolic risk markers in obesity, also after adjustment for potential confounders, such as long-term diet composition. The study supports the use of gut metagenomic markers for metabolic disease prediction in a population of postmenopausal women.

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EFFECTS OF PROBIOTICS ON ORAL IMMUNE FUNCTION IN ATHLETES

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Athletes are engaged in heavy training to improve their performance. However, many previous studies reported that repeated intensive exercise might increase the risk of developing upper respiratory tract infections (URTIs) by decreasing in immune function. Therefore, it is one of the most important things for athletes to inhibit the immune depression in the athletic field. Most pathogens irrupt into the mucous membrane causing URTIs. Many athletes have the URTIs symptoms during training camps or competition. For that reason, the mucosal immune system plays a crucial role in the system for protection against infection. Maintaining or improving the mucosal immune function is important for athletes. Some evidence suggests that probiotics may be effective in alleviating URTIs symptom incidence and symptom severity or duration. Lactic acid bacteria are reported to have a wide range of effects on the immune system, including systemic and mucosal immunity. In this presentation, we will discuss the method for probiotics contributing to the health condition of athletes.

CHANGING DIETARY HABIT IS CHANGING ASIAN GUT MICROBIOTA

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The impact of modernisation in life style on the health of human is a global issue at present. Particularly, westernisation of diets links to certain diseases in the developing area. Recently, some studies have revealed that gut microbiota altered by the introduction of Western foods is involved in the increase of risk for certain diseases, e.g., colon cancer, obesity or diabetes. Our international consortium project, Asian Microbiome Project (AMP), has also investigated the impact of globalisation of diets on the gut microbial community in Asian people.
Leyte island in the Philippines is being modernised and children in the rural Baybay city and urban Ormoc city have a different life style, including daily diets. To know the impact of modernisation of dietary habit on gut microbiota in preadolescent children, we compared the faecal microbiota between Baybay and Ormoc children at the age of 7 to 9 years old and examined the correlation of bacterial composition with their dietary data. The dietary survey indicated that the Ormoc children consumed significantly higher levels of fat than the Baybay children by eating modern high-fat foods. Faecal microbiota was analysed by 16S rRNA meta-analysis with the dataset of other five Asian countries. Their microbiota at family level were grouped into two enterotype-like clusters, each defined by the high abundance of Prevotellaceae (P-type) and Bacteroidaceae (BB-type), respectively. Baybay and Ormoc children mainly harboured P-type and BB-type, respectively. Redundancy analysis with their dietary nutrients showed that P-type and BB-type favours carbohydrate and fat, respectively. The fat intake level correlated positively with the Firmicutes-to-Bacteroidetes (F/B) ratio and negatively with relative abundance of the genus Prevotella. Overweight and obese children who were living in Ormoc and took a higher level of fat, harboured the microbiota with higher F/B ratio and lower abundance of Prevotella. The altered gut microbiota may be a sign of modern diet-induced obesity among children in developing areas.

AMP has also observed the impact of Japanese diets on Thai gut microbiota. Among Asian populations, there is a gradient in the abundance of Bifidobacterium. Japanese people tend to harbour a higher amount of Bifidobacterium than Thai people. However, we found that Thai people who consume Japanese foods harboured a gut microbial community shifted to the Japanese type that is highly abundant in Bifidobacterium.

Oriental and Western dietary cultures are now mixing in Asia and remodelling gut microbiota of Asians. To ensure our healthiness in the future, AMP will further address the link between diets, gut microbiota, and health.

THE INTESTINAL MICROBIOTA OF THE HADZA HUNTER GATHERERS: NEW PERSPECTIVES ON GUT MICROBIOTA-HUMAN HOST CO-EVOLUTION

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The gut microbiota (GM) co-evolves with the human host providing the necessary metabolic flexibility to optimise nutrient acquisition from different food sources. Indeed, the GM described an evolutionary trajectory along the course of the human evolutionary and ontogenetic history, changing its phylogenetic and functional configuration in response to the different subsistence strategies. This process involved the continuous tuning of the pattern of metabolic services provided to the host by the GM, depending on diet and lifestyle changes occurring along human evolution. The Hadza hunter-gatherers represent a perfect model of ancestral human population, and the comparison of their GM layout with that of urban Western populations represents a unique opportunity to highlight the process of GM-host co-evolution. Indeed, the Hadza are one of the last remaining modern people that are fully enveloped within their natural environment in a way that is no longer possible for the rapidly westernising world. The Hadza live around the shores of lake Eyasi in north-western Tanzania and maintain a subsistence strategy that relies on wild foods and natural water sources. Compared to the GM of urban industrial populations, the Hadza ecosystem showed a higher microbial diversity, the acquisition of the so-called ‘old-friends’ microorganisms Treponema and Succinivibrio and the absence of Bifidobacterium. The characterisation of the Hadza microbiome and the comparison with that of urban populations defines a pattern of adaptive lifestyle-associated GM differences in populations, which stand at opposite ends of an entire subsistence spectrum, from hunting and gathering to post-industrial urban life.
TUESDAY 11 OCTOBER 2016

SESSION 7
SPEED PRESENTATIONS – TOPICS IN HUMAN RESEARCH

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

P20
Effect of tablets containing probiotic candidate strains on clinical and cytokine markers of gingival inflammation and composition of the salivary microbiome

Mette Kristine Keller
Department of Odontontology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

P21
Oral delivery of pentameric glucagon-like peptide-1 by recombinant Lactobacillus in diabetic rats

Yin Lin
Department of Laboratory Medicine, Division of Clinical Immunology and Transfusion Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Sweden

P25
Gut microbiota composition and dietary intake are related to serum zonulin, a marker of intestinal permeability, of overweight pregnant women

Kati Mokkala1,2,3
1Institute of Biomedicine, University of Turku, Finland; 2Department of Medical Microbiology and Immunology, University of Turku, Finland; 3Functional Foods Forum, University of Turku, Finland

P38
Neuro- and immunological effects induced by non-digestible oligosaccharides during early life

Kirsten Szklany
Division of Pharmacology, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, the Netherlands

P45
Faecal microbiota manipulation prevents dysbiosis and alcohol-induced liver lesions in mice

Laura Wrzosek1,2
1INSERM U996, DHU Hepatinov, Université Paris-Sud, Université Paris-Saclay, France; 2Institut Paris-Sud d’Innovation Thérapeutique, IFR141, Faculté de Pharmacie, Université Paris-Sud, Université Paris-Saclay, France
TUESDAY 11 OCTOBER 2016

SESSION 8
SPEED PRESENTATIONS – TOPICS IN ANIMAL RESEARCH

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

P7
Development of a new in vitro model of the piglet colon: applications to the study of probiotics as an alternative to antibiotics
Stéphanie Blanquet-Diot
EA 4678 CIDAM, Conception Ingénierie et Développement de l'Aliment et du Médicament, Centre de Recherche en Nutrition Humaine Auvergne, Université d'Auvergne, France

P12
Immunological and physiological effects of Saccharomyces cerevisiae RCV016 alone and in combination with deoxynivalenol
Lilia R. Cavaglieri¹,²
¹Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Argentina; ²Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina

P27
Gammaproteobacteria abundance and specific gene functions are highly correlated in the faecal microbiota of the domestic cat fed a canned or kibbled diet
Christina D. Moon
Rumen Microbiology Team, AgResearch Grasslands, New Zealand

P30
Pre- and post-weaning gut microbial profiles of piglets administered Bacillus spp. spores and gentamicin
Ann-Sofie R. Poulsen
Section of Immunology and Microbiology, Department of Animal Science, Aarhus University, Denmark

P41
Prenatal caprine milk oligosaccharide consumption improve development and colonic microbiota in pups at weaning
Caroline Thum¹,²
¹Food Nutrition & Health Team, Food and Bio-based Products Group, AgResearch Grasslands, New Zealand; ²Riddet Institute, Massey University, New Zealand
WEDNESDAY 12 OCTOBER 2016

SESSION 9
SENIORS & AGE-RELATED HEALTH ISSUES

IDENTIFYING TARGETS FOR MICROBIOTA MODULATION IN THE ELDERLY; NOW, TOMORROW OR NEVER? THE CHALLENGES AHEAD

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The ageing process is known to affect the intestinal microbiota composition and the immune status. Therefore, selecting probiotics and prebiotics for microbiota and immune modulation has become a common target in the development of functional foods for the elderly population. Such products promise to expand the period of good health along senescence, but prior to their development the specific targets, for both the intestinal microbiota and immune system, must be defined. The identification of these targets constitutes, however, a challenging task; for example, when compared with younger adults the currently elder persons show an altered microbiota and inflammatory status, but establishing whether these are exclusively due to the ageing process or there is a cohort effect is not an easy task. The high inter-individual variability in the observed alterations, as well as in the response to pro- and prebiotics, further difficult the identification of targets and the development of intervention strategies. In addition, specific diet-microbiota-immune system interactions, different from those found in adults, seem to be present in elderly. Therefore, the design of probiotic and/or prebiotic functional foods for elderly should consider the specific needs of the current elder population, understanding that these may be different for the future elderly. Moreover, the nutritional formulation of the products should also be considered within the framework of the specific nutritional needs and diet-microbiota-immune interactions present in this population. The design of such target-specific products constitutes an interesting opportunity for the development of probiotic and prebiotic products with specific and improved functionalities.

PROBIOTIC LACTOBACILLI CAN REDUCE ORAL CANDIDA IN ELDERLY

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The oral cavity harbours one of the most complex microbiomes in the human body. The composition of the oral microbiota varies between individuals and by location in the oral cavity (smooth mucosal surfaces, deep fissures in teeth, and rough surfaces, e.g., on the tongue, etc.). Candida species – mostly Candida albicans – are commensal microorganisms in the oral cavity in approximately 20-50% of the population, increasing up to 75% in older adults. Hence, instabilities in the oral microbial balance can lead to Candida infection in carriers. Oral candidiasis, or Candida-associated stomatitis, is a common problem in the elderly population and is often associated with recent antibiotic treatment, impaired immune functions and immunosuppressive drugs, polypharmacy, reduced saliva flow, neglected oral hygiene, dentures and smoking. Today, adequate treatment options with antifungals exists, however, more Candida species are getting resistant to the treatments.

The use of probiotic bacteria for oral health maintenance is a fairly new concept and recent insights in the importance of maintaining a natural microbial balance in the oral biofilm have gained our interest. Since oral candidiasis is caused by an ecological imbalance (dysbiosis) in the oral biofilm, a certain interest has been addressed to a bio-ecological approach with probiotic bacteria for prevention and management instead of antifungal treatment. Recent randomised clinical trials have proved that daily intake of probiotic lactobacilli can reduce the counts of Candida in the oral cavity in elderly individuals. Moreover, in vitro studies show that the lactobacilli co-aggregate with Candida species, produce bacteriocins and hydrogen peroxide (H₂O₂), and lower the pH, altogether hampering Candida growth. This presentation will focus on the newest results from our research group, and furthermore, give an overview of our knowledge within probiotic bacteria and the effect on oral Candida.
Ageing is associated with a decline in physical and physiological functions which impact diet and the gastrointestinal tract. Decreased intake of dietary fibre and longer intestinal transit time often result in constipation and gas production in older adults. The immune system also undergoes changes with age leading to a reduction in CD4+ T cells, increased inflammation, and greater susceptibility to infections, cancer, and autoimmune diseases. Altered gastrointestinal function, diet, and immune health lead to changes the composition of the gut microbial community including a reduction in bifidobacteria, bacteria commonly associated with health but found in reduced numbers in aged adults. These changes in gut microbiota may further the age-related deterioration of physiological functions.

Pre- and probiotics are now being administered to older adults to modulate the gut microbiota and immune function. We supplemented the diets of older adults aged 70±1 years with a probiotic mixture containing Bifidobacterium bifidum G9-1, B. longum MM-2, and Lactobacillus gasseri KS-13 for 3 weeks in a randomised, double-blind crossover study with a 5-week washout period between interventions. After 3 weeks on the probiotic versus the placebo, a higher percentage of participants had an increase in faecal bifidobacteria and lactic acid bacteria, as would be expected, and they also had an unexpected decrease in Escherichia coli. Additionally, multiple operational taxonomic units matching closest to Faecalibacterium prausnitzii, a Firmicute shown to be associated with a less inflammatory profile, increased in stool samples provided during the probiotic but not the placebo intervention. Immune changes were also suggestive of a less inflammatory environment in that ex vivo mitogen-stimulated lymphocyte production of interleukin-10 was also higher with the probiotic than with the placebo. The percentage of CD4+ T cells was maintained with the probiotic but reduced with the placebo. Prebiotics have also been used to modulate faecal microbiota composition and immune function in aged adults. The prebiotic, galacto-oligosaccharide (GOS), increases numbers of faecal bifidobacteria. In one of the few studies that examined GOS supplementation and immune function in aged adults, the prebiotic induced a less inflammatory cytokine profile.

Although pre- and probiotics appear to be able to reverse some of the observed age-related changes in the gut microbiota composition and immune function, it is unknown how these changes impact health outcomes.

Developed societies are facing a remarkable increase of population's longevity and, consequently, of chronic diseases, such as obesity, cardiovascular or neurodegenerative ones. Immunosenescence in older persons is the cause of low immune response against pathogens and a low-grade chronic inflammatory state (inflammaging). This inflammatory state is the origin of many age-related health problems, but also it can sensitise the organism to intestinal microbes and, altogether, inflammatory secretions may cause changes in the gut microbiota composition. Then both intestinal diseases and changes in the gut microbiota have been related to the progression of diseases and frailty in the elderly, establishing a potential inflammatory health deterioration spiral. In addition, there are consistent evidences of the prominent role of gut microbiota in the gut-brain signalling, where it may participate by different mechanisms, such as the processing of hormones and neurotransmitters. Solid evidences related changes in the gut microbiota to stress and mood (depression, anxiety), which may also be associated to increased bacterial translocation in the gut mucosa. On the other hand, a high proportion of older persons suffer cognitive impairment (CI), including Alzheimer disease (AD), which has high levels of inflammatory markers and an altered metabolism of neurotransmitters and hormones. It has
been suggested that there is a favourable chance that CI or AD could be associated to changes in the patterns of microbiota. In a prospective work, we could confirm that the cognitive status is significantly related to specific inflammatory markers, such as serum myeloperoxidase, cortisol and the IgG titre of viral bodies, and also specific oral and gut microbiota were significantly correlated to the cognitive status and to the levels of those markers. Then, a further question is served: could gut microbiota be modulated in order to improve the general inflammatory state, and hence cognitive faculties in the elderly? Intervention studies with probiotics and prebiotics in elderly frequently showed that *Bifidobacterium* populations can be stimulated with a decrease of enterobacteria. Other studies demonstrated that probiotics and prebiotics decreased the synthesis of pro-inflammatory cytokines and increased the levels of activated lymphocytes, suggesting that they may be useful to stabilise inflammation and cognitive deterioration in older persons.

**PROBIOTICS TO IMPROVE CARDIOVASCULAR RISK FACTORS IN OLDER PEOPLE**

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Cardiovascular diseases are a major cost factor in Europe and are associated with high morbidity and mortality. Probiotic modification of the gut microbiota might be able to improve cardiovascular risk in older people, the main risk group for cardiovascular events, such as stroke and myocardial infarct. Cardiovascular risk factors comprise a number of targets such as hypertriglyceridemia, hypercholesterolemia, hypertension, insulin sensitivity and type 2 diabetes mellitus, low grade inflammation and certain vitamin deficiencies. Accumulating evidence shows probiotics to lower low density lipoproteins (LDL)-cholesterol and improve the LDL/high density lipoproteins (HDL) ratio, as well as lower blood pressure, inflammatory mediators and blood glucose levels. Recent meta-analysis showed significant effects of probiotics on reduction of total cholesterol, low-density lipoprotein (LDL), and inflammatory markers. Additionally, several probiotic strains of bacteria are able to produce vitamins, mainly of the vitamin B group. One recent human randomised trial showed that probiotic supplementation increases both serum folate and vitamin B12 concentration and significantly decreases homocysteine concentration in the blood, which is a main cardiovascular risk factor. The effects, however, seem strain specific and dependent on the medium of probiotic supply (fermented milk, yoghurt versus capsules) as well as the duration of consumption. There is also uncertainty regarding the dosages of the probiotics. Also genetics of the individual might affect the efficacy of probiotics.
WEDNESDAY 12 OCTOBER 2016

SESSION 10
PETS & FARM ANIMALS

MODULATING THE CANINE AND FELINE MICROBIOTA: DIETARY INTERVENTION, PREBIOTIC, AND PROBIOTIC SUPPLEMENTATION

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Similar to humans and other mammals, the gastrointestinal microbiome of dog and cats is a complex ecological community comprised of bacteria, fungi, protozoa and viruses. Evaluation of genomic libraries of the gastrointestinal tract of healthy adult dogs and cats revealed that more than 98% of the sequences were from bacteria, with Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria comprising the major phyla in these species. Microbial diversity also increases towards distal portions of the gastrointestinal tract showing predominance of anaerobic bacterial taxa. In contrast to human and rodent literature, limited information is available on effective strategies to modulate the gastrointestinal microbiota of dogs and cats. A few studies investigated the effect of diet format, macronutrient composition, and fibre content and source in altering the canine and feline hindgut microbiota. Diets with moderate concentrations of protein and carbohydrates resulted in greater abundance of Actinobacteria, and lower abundances of Proteobacteria and Fusobacteria in kittens, and adult cats and dogs. Fructo-oligosaccharide supplementation increased Actinobacteria and altered the abundance of genes related to protein and carbohydrate metabolism in cats. Probiotic supplementation has resulted in inconsistent findings. Some studies were able to detect changes in specific bacterial species and strains, without alterations in the overall microbial community.

Significant differences also exist on the gastrointestinal microbiota of healthy vs. diseased pet animals. Gut microbiota of cats with inflammatory bowel disease (IBD) have lower counts of total bacteria, Bifidobacterium spp., and Bacteroides spp. in contrast to healthy cats. Similarly, lower abundance of Bacteroidetes, Clostridia, and increased Proteobacteria have been reported in dogs with IBD. Additionally, antibiotic treatment in adult cats and dogs altered the gastrointestinal microbiota and decreased microbial diversity. The current literature on canine and feline gastrointestinal microbiome serves as a foundation for future studies. A better phylogenetic and functional characterisation of this ecosystem is necessary prior to drawing practical conclusions and interventions to pet animals.

EFFECTS OF YEAST ON FEED INTAKE AND MILK YIELD OF DAIRY COWS

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Humans have made use of the metabolic properties of yeast for thousands of years, in the leavening of bread and in the production of alcohol from grape sugar or cereal starch to produce wines, beers and spirits. Over the last two decades, there has been considerable interest in using yeast and yeast products in livestock production systems in order to improve animal health and performance. The most commonly used live yeast is Saccharomyces cerevisiae. Supplementation with strains of S. cerevisiae in dairy cow nutrition has been shown to improve rumen stability resulting in increased fibre degradation, greater milk yields and increased feed efficiency. The proposed mode of action of yeast in the rumen is both through its oxygen scavenging activity and production of metabolites to stimulate lactate-utilising bacterial populations. Both of these actions create a more favourable environmental for cellulolytic bacteria, fungi and protozoa capable of digesting forage derived fibre. This undoubtedly results in higher rates of total mix ration (TMR) digestion and therefore higher dry matter intakes, which in turn results in either, an increase in the physiological condition of the animal or in increased milk production or both.

The aim of this study was to assess the effect of supplementation with live yeast, S. cerevisiae NCYC R404 (UltraCell®; Micron Bio-Systems, UK) on feed intake, body condition score (BCS) and milk yield of lactating dairy cows. The Holstein Friesians cows (n=62) were allocated into two groups of 31 cows
each at Rodway Farm, Bridgwater, UK. The experimental groups consisted of cows ranging from 14 days post-partum (pp) to 155 days pp. The cows were allocated to two experimental groups in matched pairs according to previous milk yield, parity, BCS and live weight. The cows were all offered ad libitum access to the same TMR either with the addition of *S. cerevisiae* R404 at the rate of $1 \times 10^{10}$ cfu/head/day (treatment group) or with no addition (control group) for a total of 19 weeks. The components of the total mixed ration (TMR) are shown in Table 1. The milk yield was recorded manually on a daily basis and reported as a weekly mean. The BCS was recorded on monthly basis by the same observer in each instance. The BCS was estimated using a five point system where 1=emaciated and 5=obese, with increments of 0.5 according to the method described by Edmondson et al. [1]. The individual cows were palpated over the spinous processes, the pelvic region of the hook and pin bones and the tail head to assess the level of fatty tissue under the cow’s skin in order to determine the body condition score. Feed intake levels of each group were assessed on a daily basis. The TMR offered and refused was recorded on a daily basis and the average intake levels were calculated by deducting the amount of feed refused from the amount of feed offered at the previous days feed allocation.

**Table 1.** Components of the total mixed ration (TMR) as fed per kg/head/day offered to all experimental cows.

<table>
<thead>
<tr>
<th>Component</th>
<th>High production ration</th>
<th>Mid production ration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silage (1st oxylage)</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Maize silage</td>
<td>25.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Grass silage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hay</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Megalac</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Oats</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Caustic wheat</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Urea</td>
<td>0.04</td>
<td>0.125</td>
</tr>
<tr>
<td>Performance energy</td>
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<td>1.0</td>
</tr>
<tr>
<td>Mineral vitamin mix</td>
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<td>0.15</td>
</tr>
<tr>
<td>Concentrate V2SH</td>
<td>4.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**Figure 1.** Average milk yield.

**Figure 2.** Average feed intakes at different stages during the trial (left bar, control group; right bar, treatment group).

**Figure 3.** Average BCS at different stages during the trial (left bar, control group; right bar, treatment group).

The effect of supplementation of *S. cerevisiae* R404 on the milk yield of dairy cows is shown in Figure 1. An average daily increase in milk (3 l/head/day) production was observed in the treatment group throughout the 19 week trial period and this was found to be statistically significant ($P<0.001$). As shown in the Figure 2, the feed intake at the beginning of the trial was slightly numerically higher in the control group compared to the treatment group. The TMR offered and refused was recorded on a daily basis and the average intake levels were calculated by deducting the amount of feed refused from the amount of feed offered at the previous days feed allocation.
the feed intake was almost the same in both groups. Due to oxygen scavenging effect the anaerobic conditions in the rumen are enhanced leading to a stimulation of fibre digesting and lactic acid utilising bacteria. More stable rumen pH and improved fibre digestibility in turn resulted in a higher milk yield.

In conclusion, supplementation with *S. cerevisiae* R404 in TMR of lactating dairy cows at $1 \times 10^{10}$ cfu/ head/day resulted in significantly higher milk yields.

References

**BUTYRATE-PRODUCING BACTERIA ARE KEY PLAYERS IN GUT HEALTH MAINTENANCE IN BROILER CHICKENS**

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Broiler chickens are fast-growing animals that are genetically selected to have a very efficient digestion of high-energy diets. Because of this, these animals are very susceptible to disease conditions caused by small intestinal bacterial overgrowth leading to damage to the gut mucosa. This is generally referred to as ‘dysbiosis’, a condition with decreased performance caused by intestinal inflammation and shortening of villi, due to an unfavourable microbiota shift. The most severe condition is necrotic enteritis, caused by overgrowth of *Clostridium perfringens*, in some cases leading to clinical outbreaks with high mortality. Dysbiosis can also lead to increased intestinal barrier permeability and consequently cause systemic spread of bacteria. *Escherichia coli*, but also *Enterococcus* species, can translocate to the blood stream and to the joints, causing bacterial chondronecrosis with osteomyelitis, leading to lameness and animal welfare issues. Maintaining intestinal homeostasis and preserving the integrity of the intestinal epithelium is thus a key issue in broiler production. In addition to dysbiosis, broilers can also be colonised by zoonotic agents, including the two globally important foodborne pathogens *Campylobacter* and *Salmonella*.

Butyrate has been shown to have beneficial effects on invasiveness of *Salmonella* and *Campylobacter*, and is a key anti-inflammatory molecule that strengthens the epithelial barrier. This has been well documented *in vitro* and *in vivo*. Adding butyrate producing bacteria to the diet of broilers or steering the microbiota towards butyrate production is thus a possible strategy to optimise gut health in broilers. We have tested different prebiotic compounds that stimulate digestibility in dysbiosis models in broilers, showing that these improve villus structure and reduce inflammation, while stimulating colonisation with butyrate producing *Ruminococcaceae* and *Lachnospiraceae*. Administration of specific strains belonging to these families efficiently reduced intestinal necrosis in a *C. perfringens* infection model, and in-feed supplementation of *Butyricicoccus pullicaecorum*, a butyrate producing bacterium from the *Ruminococcaceae* family, significantly lowered the abundance of (opportunistic) pathogens, including *Campylobacter* spp. in the caecum and *Enterococcus* and *Escherichia* spp. in the ileum, while improving broiler performance. These data suggest that colonisation by butyrate producing bacteria is of utmost importance to counteract intestinal dysbiosis and (opportunistic) pathogen colonisation in broilers, and that nutritional interventions (e.g., prebiotics) can support gut health by beneficially affecting metabolic activity of these micro-organisms. Although butyrate-producing bacteria colonise the caeca, butyrate has been shown to stimulate entero-endocrine cells in the distal intestinal tract to produce peptide hormones, such as GLP-2, that enters the bloodstream and affects small intestinal structure.
RESOLUTION OF A CLOSTRIDIUM DIFFICILE OUTBREAK AT A LARGE FOALING OPERATION USING A RATIONALLY DESIGNED PROBIOTIC

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Milk has evolved to not only foster the growth of the neonate, but also to shape the early gut microbiome via complex milk oligosaccharides (MOs). In nursing mammals, these structures play a major role in establishing the initial gut ecosystem and promoting the development of a beneficial microbiome early in life. This role of MOs in the selection of intestinal microbial residents is paramount, as a dysbiotic gut environment can severely affect the health of the neonate. For example, microbial cross-feeding, where MOs are partially degraded by opportunistic microbes, can lead to pathogen blooms in the gut of neonates.

Here we describe the use of this microbe-MO partnership to stem an outbreak of metronidazole-resistant Clostridium difficile at a large foaling operation. The outbreak was clinically confirmed by qPCR and antibody detection in the affected animals, as well as isolation of the causative organism. We collected milk from mares in the facility and characterised the constituent MOs by mass spectrometry. Based on those findings, a probiotic solution was developed and fed to affected foals to stem the outbreak through inhibition of microbial cross-feeding. Faecal samples were collected from foals (n=58) in the facility before, during, and after treatment, and assessed by qPCR and 16S rDNA marker gene sequencing to examine the impact on the faecal microbiome. Introduction of the product was significantly associated with improvements in animal health in the facility. Cases of diarrhoea were ceased in treated, but not untreated animals after introduction of the product. Molecular methods (qPCR and 16S rDNA sequencing) confirmed that the probiotic was able to colonise and dramatically reduce gut dysbiosis and reduce blooms of cross-fed pathogenic taxa in treated animals.

Here, we demonstrate that rational selection of a probiotic organism using ecological criteria can produce beneficial effects in a nursing animal system, with implications across a number of animal production settings.

PROBIOTIC APPLICATIONS TO IMPROVE FISH HEALTH AND DISEASE RESISTANCE

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The gastrointestinal (GI) tract of the fish naturally contains indigenous symbiotic bacteria, such as lactic acid bacteria (LAB) and other beneficial bacteria, however, in the past it has been difficult to assess the non-viable gut bacteria due to the limitations of culture-dependent methods. More recently, the use of molecular methods, including next generation sequencing, have evidenced the presence of LAB and probiont genera, such as Aerococcus, Aeromonas, Alteromonas, Arthrobacter, Bacillus, Carnobacterium, Clostridium, Debyaryomyces, Enterobacter, Enterococcus, Flavobacterium Lactobacillus, Lactococcus, Leuconostoc, Micrococcus, Pediococcus, Phaeobacter, Pseudoalteromonas, Pseudoalteromonas, Roseobacter, Saccharomyces, Shewanella, Streptococcus, Vagococcus, Vibrio, and Weisella. In contrast, the presence of Bifidobacterium is rare in fish.

Probiotics are considered as novel functional ingredients that can be used as a preventive treatment. They can also restore the gut microbiota after a period of antibiotic provision or stress conditions, as well as fight against infectious diseases in aquatic animals. Probiotics were first incorporated into aquaculture feeds over 3 decades ago. Since this time, many studies have demonstrated that probiotic applications in fish can provide both nutritional benefits and improved disease resistance to the host, which can enhance growth performance and fish health. Likewise, probiotics can provide essential nutrients and enzymes to the host that improves survival and reproduction, as well as the metabolism of the fish.
of fat and glucose. The immunological status of the fish can also be improved via increases in cytokine expression, lysozyme and phagocyte activity, complement factor and respiratory bursts. Other benefits are related to the displacement of opportunistic microorganisms by the production of inhibitory compounds (e.g., bacteriocins), competition for adhesion sites and essential nutrients in the GI tract.

Based on these promising findings and the general concern about the use of antibiotics, probiotics are increasingly seen as an environmentally friendly alternative to the use of chemotherapeutic agents. This is especially important in aquaculture due to both the high environmental impact and ease of disease transmission in the aquatic environment.
HUMAN GAS CAPSULES SNiffING OUT A BEtTER FUTURE FOR GUT HEALTH

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We have developed a novel low-cost and non-invasive medical device called ‘human gas sensor capsule’ that has applications in the diagnostics of gastrointestinal disorders and assessing dietary effects on the gut. The product is a capsule size indigestible electronic device that leaves the body after normal bowel transient (Figure 1). The capsule consists of gas sensors, micro-electronic circuits, small-sized harmless batteries and telecommunication components. The capsule allows for the accurate measurement of the concentrations of 4 vital gases of O\textsubscript{2}, H\textsubscript{2}, CO\textsubscript{2} and CH\textsubscript{4}, and also temperature. Intestinal gas profiles are then transmitted to an external small handheld device that communicates with a smart-phone allowing real-time data display and analysis. We have successfully finished the animal trials and the first phase of human trials. The outcomes of these trials will be presented in detail in the talk. The outcomes show some extraordinary phenomena that can potentially revolutionise the fields of gastroenterology and food sciences.

Figure 1. (a) Schematic of a capsule, showing internal components, and also capsule passage through a human gut and communications with a handheld device; (b) illustration without antennae; (c) photo of capsule used in animal trials.

References

NEXT GENERATION DIAGNOSTIC BIOMARKER DETECTION FOR GUT MICROBIOTA: BREATHOMICS

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Since ancient times, physicians valued human breath as a window of diseased and healthy organs. Thousands of volatile organic compounds (VOCs) are produced in different organs which are transported by blood to the lungs where they are released. Inflammatory and deviant metabolic processes change the relative concentrations of these compounds in breath, which can be used for clinical diagnosis and disease monitoring. Comprehensive analysis of gas chromatography-mass spectrometry breath data (breathomics) has revealed profiles of compounds that are strongly associated with active disease states in patients suffering from inflammatory bowel disease (IBD). These exhaled compounds represent a changed microflora and/or bacterial metabolism and/or epithelial disease states. Recent scientific developments clearly show that gut microbiome have a profound influence on gastrointestinal health and disease. Therefore, the identification of the relation between gut microbiome and volatile metabolites is important for interventional studies where the gut microbiome is modified.

The aim of the present study is to study the relation between the microbiome and metabolites in blood and breath in Crohn diseased patients. From 68 Crohn’s disease (CD) patients repeated faecal and breath samples were collected, resulting in a total of 92 active disease and 92 remission samples as defined by a combination of biochemical and clinical parameters. The microbiota composition was assessed by pyrosequencing of the 16S rRNA V1-V3 gene region. The volatile metabolites in breath were detected by gas chromatography-time-of-flight mass spectrometry. Canonical correlation analysis was used to find the relation between volatile metabolites in breath and bacteria species in human gut in active and inactive stage of the disease. The sets of volatiles in breath and faecal bacteria were found to have strong correlation, independently from disease stage. These correlations were remained when single measurements per patients were considered. This result shows that analysis of breath metabolites may offer a potential for rapid non-invasive assessment of therapeutic or dietary interventions for chronic disorders with associated microbial dysbiosis, such as IBD.

DESIGNING NEXT GENERATION PREBIOTICS FOR LIFELONG HEALTH

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There are currently three food ingredient types that are recognised as prebiotics. These are the fructans, inulin and fructo-oligosaccharides (either derived from inulin or from sucrose) and the lactose-derived galacto-oligosaccharides. These carbohydrates have a selective impact on the bacteriology of the colon and on the profile of metabolites produced by these bacteria. There are also accumulating studies showing a positive impact of these prebiotics on health. Given the huge array of carbohydrate structures found in nature or that can be readily synthesised, it is unsurprising that there are many more that are currently being investigated for their prebiotic potential.

Even though we are making extremely rapid progress in understanding the composition and to a lesser degree the functioning of the gastrointestinal ecosystem, we have little understanding of what makes a prebiotic selective in its fermentation. We currently do not have a clear picture of the mechanisms by which carbohydrates of varying structure exert selective influences over the gut microbiome. In order to gain this understanding we need much more information on the linkage specificities of glycosidase enzymes involved in carbohydrate catabolism and on the specificities of carbohydrate transport systems in bacterial cells. Most of the data available on structure function relationships in fermentable carbohydrates comes from in vitro studies in various forms of gut model. Although these are intrinsically limited in their relevance to the human situation, they allow us to make detailed measurements of changes in bacterial population and in metabolite production. We have data from animal and human
feeding studies on various carbohydrates, but these studies have a range of experimental designs, have used varying microbiological methods and have frequently used poorly characterised carbohydrates as substrates. It is not really possible to come to firm conclusions about the influence of structure from the studies currently available in the literature.

ILSI Europe has commissioned an activity to produce an authoritative view of the state of the art in terms of our understanding of structure function relationships in fermented carbohydrates. This will produce a clear picture of the shortfalls in our knowledge at the present time and also provide a route map of where research is needed. As we increase our understanding of the microbial ecosystem that we seek to influence, we will identify more targets for intervention, either specific bacterial groups or specific metabolite profiles. Maybe in time, we will be in a position not only to predict the fermentation profiles of any given carbohydrate structure but also to manufacture carbohydrates targeted and health outcomes.

This presentation will discuss the current state of the art in our understanding of structure-function relationships in fermentable carbohydrates, identify the questions to be answered in this area and introduce the ILSI activity.
P1 A multi-omics approach to characterise the metabolic effects of fermented dairy products on healthy men
G. Pimentel1,2, K.J. Burton2, R. Portmann1, R. Badetscher1, Ueli von Ah1, U. Büttikofer1, M.J. Voir2, F.P. Pralong2, N. Vionnet2 and G. Vergères2
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P2 A dual-environment co-culture system to better evaluate effects of host-microbe-food interactions on intestinal barrier function in physiologically relevant conditions
Rachel Anderson1,2, E. Maier1,2, D. Ulluwishewa1,2 and N. Roy1,2
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P3 Shifts in oral and gut microbiota in older people are associated to age, cognitive impairment and diet
M. Selma Royo1, A.G. Mera Balseca1, Christine Bäuerl1, J.E. de la Rubia2, M.C. Collado1 and G. Pérez Martínez1
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P4 Selective fermentation of okara soybean by-product and amaranth flour by probiotic and starter cultures
Raquel Bedani, M.C.A. Albuquerque, A.D. Vieira and S.M.I. Saad
Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, Brazil

P5 Folate production by Lactobacillus spp. in co-culture with Streptococcus thermophilus in soymilk supplemented with fruit by-product and fructo-oligosaccharides
M.A.C. Albuquerque1, Raquel Bedani1, J.G. LeBlanc2 and S.M.I. Saad1
Department of Biochemical and Pharmaceutical Technology, School of Pharmaceutical Sciences, University of São Paulo, Brazil; 2CERELA-CONICET, Argentina

P6 Lipoproteins attenuate TLR2 and TLR4 activation by bacteria and bacterial ligands with differences in affinity and kinetics
Jeroen van Bergenhengouwen1,2, A.D. Kraneveld2, L. Rutten1, J. Garssen1,2, A.P. Vos1 and A. Hartog1,2
1Nutricia Research, the Netherlands; 2Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands

P7 Development of a new in vitro model of the piglet colon: applications to the study of probiotics as an alternative to antibiotics
O. Le Goff1, M. Fleury1,2, S. Denis1, F. Chaucheyras-Durand1,2, E. Jouy2,3, I. Kempf2,3, M. Alric1 and Stéphanie Blanquet-Diot1
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P8 Extending the potential of in vitro digestion models to the specific elderly population
Stéphanie Blanquet-Diot1, M.-A. Peyron2, S. Denis1, V. Santé-Lhoutellier3, T. Sayd3, F. Olivier1, M. Hennquin1,5 and M. Alric1
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Interest of the dynamic gastrointestinal TIM system to assess the performance of innovative oral dosage forms for probiotics

O. Le Goff¹, S. Denis¹, S. Chalancon¹, S. Kuylle², F. Paul², S. Holowacz³, M. Dubourdeaux⁴, M. Alric¹ and Stéphanie Blanquet-Diot¹
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Electrochemical properties of butyrate producing gut bacteria using different redox mediators

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Lean and obese microbiota: differences in in vitro fermentation of by-products from the Brazilian food industry

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Immunological and physiological effects of Saccharomyces cerevisiae RCV016 alone and in combination with deoxynivalenol

G.R. García¹,³, C.A. Dogi¹,³, D. Payros², C.R. Greco¹, S.N. Chulze¹,³, A. De Moreno³,⁴, I.P. Oswald² and Lilia R. Cavagneri¹,³
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Immunomodulation of Lactobacillus rhamnosus RC007 on the intestinal toxicity of deoxynivalenol

G.R. García¹,³, D. Payros², C.A. Dogi¹,³, C.R. Greco¹, S.N. Chulze¹,³, A. De Moreno³,⁴, Lilia R. Cavagneri¹,³ and I.P. Oswald²
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Sterols influence on colonic microbiota

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From gene to activity – exploit the potential of the Nestlé Culture Collection

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Contribution of gut microbial composition to the development of diet-induced metabolic syndrome in BALB/c mice

Lieke van den Elsen, A. Jones and E. Forbes-Blom
Malaghan Institute of Medical Research, New Zealand

The effects of Lactobacillus johnsonii strain N6.2 and rosmarinic acid on apoptosis biomarkers

L.D. Teixeira, D. Kling, G.L. Lorca and Claudio F. Gonzalez
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P18 A MarR regulator controls the production of flavouring compounds in Lactobacillus brevis
F.A. Pagliai, L. Hjelm, L. Jakobsson, G.L. Lorca and Claudio F. Gonzalez
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P19 Carbs can make the difference: how pectins fuel immunity
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2Division of Medical Biology, University Medical Center Groningen, the Netherlands

P20 Effect of tablets containing probiotic candidate strains on clinical and cytokine markers of gingival inflammation and composition of the salivary microbiome
Mette Kristine Keller, M.R. Jørgensen and S. Twetman
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P21 Oral delivery of pentameric glucagon-like peptide-1 by recombinant Lactobacillus in diabetic rats
Yin Lin1, K. Krogh-Andersen1, J. Pelletier2, H. Marcotte1, C.-G. Östenson2 and L. Hammarström1
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2Department of Molecular Medicine and Surgery, Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska University Hospital, Karolinska Institutet, Sweden

P22 Toward controlled steering of microbiota and immunity in infants by non-digestible carbohydrates
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2Division of Medical Biology, University Medical Center Groningen, the Netherlands

P23 Toll-like receptor activation by live Faecalibacterium prausnitzii using a novel apical anaerobic co-culture model
Eva Maier1,2, R.C. Anderson1,2 and N.C. Roy1,2
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P24 Exopolysaccharides produced by Leuconostoc mesenteroides strain NTM048 as an immunostimulant to enhance the mucosal barrier
Chiaki Matsuzaki, K. Matsumoto, T. Katoh, K. Yamamoto and K. Hisa
Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Japan

P25 Gut microbiota composition and dietary intake are related to serum zonulin, a marker of intestinal permeability, of overweight pregnant women
Kati Mokkala1,2,3, H. Röyttö1,3, E. Munukka1,2,4, S. Pietilä5, U. Ekblad6, T. Rönnema7, E. Eerola2,4, A. Laitio6 and K. Laitinen1,3
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P26 Characterisation of bifidobacteria from dairy calves
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P27

Gammaproteobacteria abundance and specific gene functions are highly correlated in the faecal microbiota of the domestic cat fed a canned or kibbled diet

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Anti-ageing effects of Lactobacillus gasseri SBT2055 and the mechanism of action

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P29

Bifidobacterium spp. in breast milk increases over lactation and might be affected by intrapartum antibiotic therapy

Marina Padilha¹, J. de M. Laucchi¹, N.B. Danneskiold-Samsøe², J.B. Holm², C.R. Taddei¹,²,³, K. Kristiansen² and S.M.I. Saad¹
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Pre- and post-weaning gut microbial profiles of piglets administered Bacillus spp. spores and gentamicin

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P31

Aflatoxin M1 levels reduction in milk after Saccharomyces cerevisiae or mannano-oligosaccharides addition to aflatoxin B1-contaminated diet of dairy cows

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Effectiveness of antimycotoxin additives based on Saccharomyces cerevisiae cell wall in gilts intoxicated with zearalenone

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P33

Development of alginate and alginate-chitosan microparticles containing Saccharomyces cerevisiae produced by external gelation following by a drying process

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Use of probiotic bacteria or yeast in the control of ETEC infections: in vitro investigation of their inhibitory potential

Charliène Roussel¹,², G. Garralt¹, A. Sivignon³, E. Graff¹, F. Deboudard¹,⁴, M. Desvaux⁴, N. Ballet⁴, P. Vandekerckove³, T. Van de Wiele² and S. Blanquet-Diot¹
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P35 Use of the gastrointestinal TIM system to promote the understanding of enteric pathogen behaviour in the infant GIT: application to the evaluation of probiotics as an alternative strategy for infection control
Charlène Roussel1,2, C. Cordonnier1,3, W. Galia1,4,5, O. Le Goff1, J. Thévenot1,3, S. Chalancon1, D. Thevenot-Sergentet4,6, F. Leriche5, M. Alric1 and V. Livrelli3,7, T. Van de Wiele2 and S. Blanquet-Diot1

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P36 The possibility of culturing obligatory anaerobic Faecalibacterium prausnitzii in oxygenated environments
Mehdi Sadaghian Sadabad1, A. Overbeek1, R.E. Steinhart2 and H.J. M. Harmsen1

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P37 Survival of Bifidobacterium longum BB-46 in a fermented soy beverage supplemented with acerola by-product under in vitro gastrointestinal stress
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P38 Neuro- and immunological effects induced by non-digestible oligosaccharides during early life
Kirsten Szklany1, C.G.M. de Theije1, C. de Waard1, N.G. van Staveren1, T.A. van Wagningen1, J. Wu1, M. Verdouw1, K. van Limpt2, H. Wopereis2, L. Groenink1, R. Ooze2, L.M.J. Knippels1,2, J. Garssen1,2 and A.D. Kraneveld1

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P39 Dietary fibres educate macrophages for immune support
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P40 Robustness of Bifidobacterium longum LMG 13197 encapsulated in lyophilised Vegetal BM 297 ATO-inulin lipid based synbiotic microparticles
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P41 Prenatal caprine milk oligosaccharide consumption improve development and colonic microbiota in pups at weaning
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P42 Novel culturing system for isolating commensal gut bacteria
Eleni Sibbald-Tsompanidou1, C. Bus-Spoor4, A.C.M. Veloo1, G. Henderson2, A. Wichmann Gustafsson2, A. Baker2, J. van Hylckama Vlieg2 and H.J.M. Harmsen1

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The impact of polyols on oral microbiome of Estonian schoolchildren
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Prebiotic effect of xylo-oligosaccharides determined by a polyphasic study in the i-screen system containing ex-vivo human gut microbiota
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Faecal microbiota manipulation prevents dysbiosis and alcohol-induced liver lesions in mice
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An anti-oxidant enriched variety of apple alters circulating immune cell gene expression and faecal microbiota composition in healthy adults
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5th Beneficial Microbes Conference
10-12 October 2016, the Netherlands
**P1: A multi-omics approach to characterise the metabolic effects of fermented dairy products on healthy men**

G. Pimentel1,2, K.J. Burton2, R. Portmann1, R. Badetscher1, **Ueli von Ah1**, U. Bütkofer1, M.J. Voïro2, F.P. Pralong2, N. Vionnet2 and G. Vergères2

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Humans have fermented foods for over 7,500 years, first to increase shelf life, then to improve taste. This tradition now translates into dietary patterns in which up to a third of human diets is made of fermented foods, not least because of their impact on health. Dairy products represent a major fraction of fermented foods in western societies. The beneficial properties of fermented dairy products depend on the metabolites released by lactic acid bacteria (LAB) during fermentation as well as on the activity of these strains in the gut. Characterising the interaction of fermented dairy products with humans thus requests a holistic approach that takes these complex interactions into account. To assess the functional properties of fermented dairy products, we have taken a multi-omics approach investigating all three elements of the triad composed of food, bacteria, and the human host. A double-blind, cross-over clinical intervention was conducted on fourteen healthy men fed a yoghurt containing the widely used probiotic *Lactobacillus rhamnosus* GG and a non-fermented milk. Mass spectrometry-based untargeted metabolomics was conducted on the sera of the subjects to assess their postprandial response as well as their fasting status after a two-week intervention. The postprandial metabolome of the subjects after a high-fat metabolic challenge, known to induce a transient inflammatory response, was also evaluated at the end of the intervention phase. Clinical chemistry and quantification of the faecal microbiota completed the analyses of the human samples. Finally, the metabolome of the dairy products as well as the metagenome of the yoghurt were measured. The serum metabolomes of the subjects were able to differentiate milk from yoghurt ingestion. In particular, we have identified products of milk fermentation that were transferred to human blood upon ingestion of the yoghurt. Some of the metabolites differentiating the consumption of the two products under postprandial conditions were also discriminative under fasting conditions after a 2-week intervention. Differentially produced metabolites indicating a specific impact of yoghurt consumption on the endogenous metabolism of the subjects were also identified. Finally, in addition to the metabolic changes induced by the dairy intervention, a significant reduction of the inflammatory response associated with the high fat meal challenge has also been observed. Taken together, this works allows us to put together different parts of a metabolic puzzle composed of the genome of the fermenting LAB, the metabolome of the test products, as well as metabolic and immunomodulatory parameters of the subjects.

**P2: A dual-environment co-culture system to better evaluate effects of host-microbe-food interactions on intestinal barrier function in physiologically relevant conditions**

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Proper regulation of the human intestinal barrier is vital to prevent antigens and pathogens from entering the body and potentially causing disease. Conventionally, *in vitro* experiments investigating the interactions between host cells, intestinal microbes and foods that regulate intestinal barrier function are carried out in the presence of oxygen to ensure survival of the human cell lines. However, this does not accurately represent conditions in the human intestinal lumen. Oxygen partial pressure in standard cell culture conditions is 151 mm Hg, whereas the human intestinal lumen has a gradient of decreasing oxygen from 33 mm Hg in the small intestine to <1 mm Hg in the rectum. To overcome this limitation, we developed a novel dual-environment co-culture system. The epithelial cell monolayer receives oxygen from aerobic medium in the basal compartment below the cell layer through the semi-permeable membrane, which is sealed off from the apical anaerobic environment of the workstation above the cell layer. The test food ingredients and/or bacteria are added to this anaerobic compartment where they are able to interact with the epithelial cells. Firstly, we applied this model to study the effect of *Faecalibacterium prausnitzii* on the epithelium. Using microarray analysis showed that live *F. prausnitzii* had a greater effect on the regulation of intestinal immune function than dead *F. prausnitzii* particularly in relation to NF-κB regulation. We investigated the effect of *F. prausnitzii* strains on Toll-like receptor (TLR) activation using human TLR reporter cell lines with a NF-κB inducible luciferase plasmid in the dual-environment system. Live *F. prausnitzii* caused higher activation of TLR2 and TLR2/6 than dead
F. prausnitzii, again illustrating the importance of using live obligate anaerobes in co-culture studies. Secondly we applied the dual-environment system to study the effects of dietary proteins on intestinal barrier integrity. We monitored the effect of purified casein, beta-lactoglobulin and lactoferrin on the trans-epithelial electrical resistance (TEER) across epithelial cell layers (a measure of intestinal barrier integrity) in both conventional and apical anaerobic conditions over a 24 h period. None of the three milk proteins altered TEER in conventional aerobic testing conditions, but all increased TEER in apical anaerobic conditions. This indicates that the interactions between the milk proteins tested and host cells are different depending on the environment. The next step in our model development is the incorporation of other intestinal cell types (e.g., enterocytes, goblet cells, immune cells). Long term we aim to develop a robust complex model to study host-microbe-food interactions in more physiologically-relevant conditions, to increase the likelihood of successfully translating in vitro results into in vivo outcomes.

P3: Shifts in oral and gut microbiota in older people are associated to age, cognitive impairment and diet

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Inflammatory processes (inflammaging) are associated to age bound diseases, cognitive impairment and dementia. In this study, we recorded information on the diet, cognitive status and biological samples (blood, saliva and faeces) of 34 volunteers over 60 years, that included individuals with deteriorated cognitive faculties (n=15, average score in the global deterioration scale=4). Adherence to a healthy (mediterranean) diet was difficult to assess due to the special conditions of the group with compromised cognitive faculties (CC). The CC group showed significantly higher plasma concentrations of myeloperoxidase and salivary cortisol and a tendency to have higher IgG titers against Epstein Barr virus. Also, higher proportions of Proteobacteria, family Enterobacteriaceae, and a reduction in the families Prevotellaceae and Veillonellaceae, and the genera Faecalibacterium, Prevotella and Veillonella were found in the gut of the CC group. Oral microbiota had the distinctive feature that Prevotella and Selenomonas were more abundant in CC; however, in the group with normal cognitive faculties (NC), again, Veillonella was predominant, together with Leptotrichia and especially Weekssellaceae. This supports that inflammation linked to neurodegenerative diseases associated to ageing may cause changes in the oral and gut microbiota composition that may create an inflammatory feedback.

P4: Selective fermentation of okara soybean by-product and amaranth flour by probiotic and starter cultures

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Okara, a by-product of the soymilk industry, is rich in fibres, proteins, and lipids. However, this by-product is either used as animal feed or discarded in the environment, causing environmental contamination problems. Although okara has a low market value, it might be employed as a functional ingredient in probiotic-fermented products, improving their functionality and potential health benefits. Another potential substrate that could be used to support bacterial fermentation is amaranth seeds flour. This pseudocereal has attracted much interest in recent years, as it is considered an excellent alternative source of proteins with a balanced composition of essential amino acids, besides having a higher concentration of soluble fibres than many cereals. Therefore, the ability of the by-product okara and amaranth flour to support the growth of the probiotic strains Lactobacillus acidophilus LA-5, L. rhamnosus GR-1, L. rhamnosus LGG, L. paracasei LC-431, L. paracasei F19, L. fermentum PCC, L. reuteri RC-14, Streptococcus thermophilus TH-4, and the starter culture S. thermophilus STM-6 was assessed. The evaluation of growth promotion of the probiotic strains in the presence of okara and amaranth flour was determined on selective agar before (0 h) and after 24 and 48 h of aerobic incubation at 37°C. The basic medium was modified MRS broth plus phenol red (as indicator) supplemented with amaranth flour and okara by-product (1%, w/v). Different growth profiles were verified for each substrate when the probiotic and starter strains were compared. Among the probiotic strains, GR showed the
highest growths in the presence of okara (3.63 log cfu/ml) and amaranth (3.45 log cfu/ml) after 24 h. The starter strain showed higher increase in its population after 24 h in the presence of amaranth (3.42 log cfu/ml) compared to okara (1.48 log cfu/ml). In general, probiotic and starter populations kept stable after 48 h. Different growth profiles were verified for each substrate when the probiotic and starter strains were compared. Although pure culture models do not reflect bacterial interactions in the host, this study reinforces that the ability to metabolise different substrates, such as amaranth flour and okara by-product, is strain-dependent. **Acknowledgements.** Financial support: FAPESP (project 2013/50506-8) and CAPES (project 1575592).

**P5: Folate production by *Lactobacillus* spp. in co-culture with *Streptococcus thermophilus* in soymilk supplemented with fruit by-product and fructo-oligosaccharides**

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Some starter and probiotic cultures may be used for delivering B-group vitamins in fermented foods, such as fermented soy products, enhancing their nutritional and functional values. Therefore, the potential of two *Streptococcus thermophilus* (ST-M6 and TH-4) and two probiotic lactobacilli strains (*Lactobacillus acidophilus* LA-5 and *L. rhamnosus* LGG) to produce natural folates in co-culture during the fermentation of four formulations of soymilk (F1, soymilk (SM); F2, SM with 1% (m/v) of passion fruit by-product (PFBP); F3, SM with 1% of fructo-oligosaccharides (FOS); and F4, SM with 0.5% of PFBP and 0.5% of FOS) was investigated. Before fermentation, each formulation was inoculated with 4-5 log cfu/ml of each strain in co-culture (ST-M6+LA-5, ST-M6+LGG, TH-4+LA-5, TH-4+LGG) and incubated statically at 37°C for 24 h. The folate content of each formulation was determined at 0 and 24 h by microbiological assay. The four co-cultures were able to increase the folate level of each fermented soymilk formulation. Regarding F1 (134±19 ng/ml), the combination TH-4+LA-5 produced the highest amount of the vitamin (1.092±42 ng/ml) and the lowest was produced by TH-4+LGG (683±25 ng/ml); in F2 (177±45 ng/ml), the best producer was TH-4+LGG (1170±93 ng/ml) and the lowest was ST-M6+LA-5 (526±25 ng/ml); in F3 (149±44 ng/ml), the combination TH-4+LGG (1432±24 ng/ml) was the best producer and ST-M6+LGG (1210±23 ng/ml) produced the lowest level. The combination TH-4+LGG produced the highest folate value in F4 (from 156±31 to 1,927±49 ng/ml) and the lowest production was obtained by ST-M6+LA-5 (1,110±50 ng/ml). The use of lactobacilli in co-culture with streptococci increased considerably the initial folate value of each soymilk formulation and the production was co-culture-dependent. Besides, the PFBO and FOS also influenced the folate production by the microorganisms and the highest levels were observed in F3 and F4. Thus, the tested co-cultures may be used to develop new synergetic fermented soy foods bio-enriched with natural folates. **Acknowledgements.** Financial support: FAPESP (project 2013/50506-8) and CAPES (project 1575592).

**P6: Lipoproteins attenuate TLR2 and TLR4 activation by bacteria and bacterial ligands with differences in affinity and kinetics**

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The small intestine is a specialised compartment were close interactions take place between host, microbes, food antigens and dietary fatty acids. Dietary fats get absorbed by epithelial cells and processed into a range of lipoprotein particles after which they are basolaterally secreted and collected in the lymphatics. In contrast to the colon, the small intestine is covered only by a thin mucus coat that allows for intimate interactions between host-cells and microbes. Lipoproteins have long been recognised as protective factors in infectious diseases via the neutralisation of bacterial toxins like lipopolysaccharides. Much less attention has been given to the potential role of lipoproteins as factors contributing to the maintenance of small intestinal immune homeostasis via modulating bacteria-induced immune responses. Lipoproteins VLDL, LDL and HDL were found to neutralise TLR responses towards specific TLR-ligands or a selection of gram-negative and gram-positive bacteria. Attenuation of TLR2 activity was acute and only slightly improved by longer pre-incubation times of ligands and lipoproteins with no differences between bacterial-lipoproteins or bacteria. In contrast, attenuation of TLR4
responses was only observed after extensive pre-incubation of lipoproteins and LPS. Preincubation of bacteria and lipoproteins led only to a modest attenuation of TLR4 activity. Moreover, compared to TLR2, TLR4 activity could only be attenuated by lipoproteins over a small ligand dose range. These results demonstrate the ability of lipoproteins VLDL, LDL and HDL to inhibit TLR responses towards bacterial-ligands and bacteria. The presence of lipoproteins was found to modulate the MAMP-induced cytokine release by primary human monocytes measured as changes in the release of IL-6, TNFα, GM-CSF and IFNγ. Using TLR2 and TLR4-reporter cells, lipoproteins were found to inhibit TLR responses with differences in affinity and kinetics. These data establish a role for lipoproteins as immunoregulatory molecules, attenuating TLR-responses and thereby positively contributing to mucosal homeostasis.

P7: Development of a new *in vitro* model of the piglet colon: applications to the study of probiotics as an alternative to antibiotics

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The use of antibiotics is still widespread in animals, particularly in pigs during livestock critical periods such as post-weaning. This practice can lead both in animal and in human to antimicrobial resistance, which is considered as a major public health problem worldwide. The development of alternative strategies, such as probiotics, is then fully supported by French and European regulation. For ethical, technical, regulatory and cost reasons, *in vitro* methods are increasingly used as an alternative to *in vivo* experimentations. In this context, the objective of the present work was to develop and validate according to *in vivo* data from living animals a new *in vitro* model of the piglet colon under normobiosis and dysbiosis (antibiotic treatment with colistin) states and subsequently used it for the study of probiotic effects on the pig gut microbiota. The parameters of the *in vitro* model (pH, temperature, redox potential and transit time) were first implemented according to *in vivo* data collected from piglets. Then, the model was inoculated with faces from piglets and three series of fermentations were performed: (i) control fermentation, (ii) antibiotic treatment with colistin under field conditions, and (iii) twice daily administration of *Saccharomyces boulardii*. The effect of the antibiotic and probiotic on the piglet gut microbiota composition was followed by qPCR analysis of the main bacterial populations and sequencing. Their influence on microbiota activity was also assessed by measurement of short chain fatty acids (SCFA). *In vitro* data were compared to *in vivo* data in piglets (n=8) under similar experimental conditions (only for control and antibiotic treatment). Under both control and treated (colistin) conditions, qPCR analyses showed that the main bacterial populations of piglet microbiota were similar *in vitro* and *in vivo*, with Pearson correlation coefficient higher than 0.9. The antibiotic led to a significant decrease of the *E. coli* and NGS of gut microbiota evidenced changes in microbial composition of subdominant populations during colistin treatment both in piglets and in the *in vitro* model. SCFA production was not modified by colistin both *in vitro* and *in vivo*. Interestingly, the probiotic yeast strain also led in vitro to a significant decrease of *E. coli* levels. This study shows the relevance of the new piglet colonic *in vitro* model compared to the *in vivo* situation, when both balanced and disturbed conditions are reproduced. It opens up new opportunities for the *in vitro* evaluation of new feed or additives for piglets and will be helpful in the development of non-antibiotic alternative strategies.
P8: Extending the potential of in vitro digestion models to the specific elderly population

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Dramatic changes are taking place in the demographic structure of the developed countries with more than 20 percent of the population being over 65 years of age by 2030. The natural ageing process is associated with various physiological changes affecting multiple organs, including the digestive system. Changes in the ageing gastrointestinal tract (GIT) include the mechanical disintegration of food, gastrointestinal motor function, food transit, chemical food digestion and functionality of the intestinal wall. The aim of this study is to present the extended potential to the specific elderly population of two in vitro models, the mastication simulator AM2 (artificial masticatory advanced machine) and the gastric and small intestinal system TIM (TNO gastrointestinal model). AM2 makes food bolus with properties similar to those produced by human chewing by integrating key variables of human mastication, such as the temperature, number of masticatory cycles, amplitude of the mechanical movements of human lower jaw, masticatory force, and injection of saliva. TIM is a very complete simulator of the human stomach and small intestine reproducing the body temperature, longitudinal and time changes in pH, gastric, pancreatic and biliary secretions, chyme mixing, gastrointestinal transit time, and passive absorption of nutrients and water. An exhaustive review of the literature was made to identify the physicochemical parameters unique to the elderly GIT in vivo and subsequently implement them into the in vitro models. Then, the new protocols were applied for a comparative study of the digestion of pasta (AM2) and meat (TIM) in the adult and elderly populations. By reproducing main facets of the functional decline of the ageing upper GIT, such in vitro models could be advantageously used as an alternative to in vivo studies. They could find many applications in nutritional but also in pharmaceutical, toxicological or microbiological studies.

P9: Interest of the dynamic gastrointestinal TIM system to assess the performance of innovative oral dosage forms for probiotics

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Probiotics are defined as ‘live micro-organisms which, when administered in adequate amounts confer a health benefit on the host’. Viability in the human gut is a key feature in probiotic activity. The survival of probiotics during gastrointestinal (GI) transit could be improved by using specific galenic formulations that protect them against digestive stresses. As in vivo studies in human are hampered by technical, ethical and cost reasons, a relevant alternative approach includes the use of artificial digestive systems, such as the dynamic multi-compartmental TNO gastrointestinal Model (TIM). The aim of this study is to evaluate, using the TIM system, the effects of various oral forms of delivery on the survival of Lactobacillus salivarius LA307 strain throughout the human upper digestive tract. This probiotic strain was selected because of its high sensitivity to GI conditions (acidity and bile salts). The viability of L. salivarius LA307 was investigated by numeration in each digestive compartment (stomach, duodenum, jejunum and ileum) and in the ileal effluents of the TIM model. The strain (1010 UFC) was administered with a glass of water, in three oral forms: powder, capsule and sustained-release tablet. The TIM system was programmed to reproduce the conditions observed during digestion of a liquid meal in healthy adult humans. The probiotic survival rate was widely influenced by its galenic form. In the gastric compartment, no bacteria were released from the capsule, confirming its gastro-resistance. At the end of digestion (4 h), the overall survival percentage of L. salivarius LA307 was 0.003±0.004, 2.8±0.6 and 17.0±1.8 (n=3) for powder, capsule and tablet forms, respectively. This study shows that the highest viability of L. salivarius LA307 was obtained with the sustained-release tablet, which may increase the probiotic activity of sensitive strains of bacteria in the human digestive tract. We also show the benefits of using the TIM system for the evaluation of new oral forms for probiotics. This model may be also relevant to assess the effects of various food matrices and different target populations with
impaired GI conditions (infants, patients, etc.) on probiotic survival and activity throughout the entire length of the human upper digestive tract.

**P10: Electrochemical properties of butyrate producing gut bacteria using different redox mediators**

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*Faecalibacterium prausnitzii* can represent 5 to 15% of the total bacterial account in human gut microbiota [Van Tongeren et al., 2005. Applied and Environmental Microbiology 71: 5438-6442]. As a butyrate-producing bacterium, it is considered beneficial for human health and needed for the normal development of the gastrointestinal tract and immune system [Berni Canani et al., 2011. World Journal of Gastroenterology 17: 1519-1528]. Even though this bacterium is considered a strict anaerobe, it can live in little concentrations of oxygen as in the gut, where oxygen from the blood diffuses to the mucus layer [Tanweer Khan et al., 2012. The ISME Journal 6: 1578-1585]. This survival is possible because of the extracellular electron transfer system (EET) that allows *F. prausnitzii* to reduce the environment, by the use of electrons produced from metabolism (NADH+) in a more effective way through electron bifurcation [Herman et al., 2008. Journal of Bacteriology 190: 784-791]. Requirements for EET in *F. prausnitzii* has been reported previously as: (i) electrons from metabolism in the form of NADH+; (ii) living bacteria; and (iii) an electron transfer mediator (ETM), which it is a molecule able to carry electrons from cell wall to the final acceptor [Tanweer Khan et al., 2012. The ISME Journal 6: 1578-1585]. We reported that *F. prausnitzii* can use riboflavin as ETM, however studies on other molecules that can be used for the bacteria to exploit EET are missing [Tanweer Khan et al., 2012. The ISME Journal 6: 1578-1585]. Therefore, the aim of this experiment is to test different electron mediators in *F. prausnitzii* and other bacteria, in order to know which can be used for EET and propose them as butyrate-producing bacteria boosters. By the use of microbial fuel cell (MFC) technique, we measure the voltage produced by several bacterial strains with different ETM to know if they can exploit EET [Tanweer Khan et al., 2012. The ISME Journal 6: 1578-1585]. We found that mainly substances that have redox functional groups, such as quinones, nitrogen or sulfur double bounds in humic substances, can be used as ETM. Besides that, our research shows that some ETM tested can permeate freely through cell membrane because of their low polarity. The voltage production after the addition of this molecules was dose dependent. Among the tested bacteria only gram-positive ones could use low polarity ETM and each different bacteria produce different voltages at the same dose. This low polarity ETMs can be proposed as growth booster for butyrate-producing bacteria and should be part of our diet.

**P11: Lean and obese microbiota: differences in in vitro fermentation of by-products from the Brazilian food industry**

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By-products generated by the food industry are mostly underused and their disposal can be seen as a waste of valuable material since they still contain a lot of nutrients, such as fibres. At the same time, the consumption of dietary fibres by the general population is lower than the recommended amount, leading to some negative health effects, such as constipation and even obesity. Moreover, gut microbiota is being suggested as an environmental factor that contributes to obesity and interestingly, it can be modulated through diet, especially dietary fibres. This work aimed to test the potential prebiotic effect of diverse by-products from the Brazilian food industry in a validated *in vitro* model of the colon, assessing the differences in fermentation using microbiota originating from lean and obese subjects. The *in vitro* system TIM-2 (TNO intestinal model of the large intestine) that simulates the proximal colon was used as a screening system. Fermentation experiments lasted 72 h using a pooled faecal microbiota derived from lean individuals (BMI 20±1.48 kg/m²) or obese individuals (BMI 32±1.17 kg/m²). In total, 8 by-products were tested for the fermentation experiments, including cassava bagasse, orange bagasse and passion fruit peel, all from different batches and/or brands. Microbiota composition, metabolite (SCFA, BCFA) and energy production were analysed. When analysing microbiota composition using...
PCoA, a clear separation between lean and obese microbiota after 72 h fermentation was noticed. Additionally, test compounds with similar composition were clustered (control and cassavas – starch as main substrate; oranges and passion fruits – pectin as main component). For SCFA, the amount produced by the lean and obese microbiota was dependent of the by-product. Obese microbiota generated more BCFA in almost all test compounds when compared to the lean microbiota. The fermentation of all test compounds by the obese microbiota did not in all cases generate more energy as expected. It can be hypothesised that the obese microbiota is not used to have fibres as substrates and therefore does not have (i) the necessary species to degrade efficiently complex fibres and/or (ii) the machinery to break the substrate and make complete use of that. In conclusion, to give a better destination to these by-products, such as to incorporate back into food products as functional ingredients, could be an important step to tackle obesity and decrease the waste of valuable food material and consequently environmental pollution.

P12: Immunological and physiological effects of *Saccharomyces cerevisiae* RCV016 alone and in combination with deoxynivalenol

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Probiotics have been explored in order to replace antibiotics as growth promoters in animal feed. They represent a potential safe advance to control enteric bacterial diseases and improve gut immunity. *Saccharomyces cerevisiae* RCV016 was previously isolated from gut pig and showed *in vitro* beneficial and mycotoxin adsorbent properties. The aim was to evaluate beneficial properties of *S. cerevisiae* RCV016 in weaned piglets and in *ex vivo* assays. Secretory IgA (s-IgA) levels, intestinal cytokines, goblet cells and production parameters were evaluated in a pig model. For the *in vivo* assays, a total of six pigs were weaned at 21 days of age and assigned to two groups: control group (CG) and yeast group (YG). Animals received yeast strain during three weeks. Feed and water were available *ad libitum*. Animals were weighed every 3 days. The feed given and the remainder were weighed daily. After 22 days, the animals were sacrificed by a lethal injection of sodium pentobarbital. Growth parameters determined were: total weight gain (TWG), feed efficiency (FE), and feed conversion rate (FCR). Small intestine was recovered for determination of goblet cells and s-IgA. For the *ex vivo* assay, jejunal explants were obtained from five-week old crossbred piglets. The treatments were: (i) porcine jejunal explants; (ii) porcine jejunal explants exposed for 3 h to 10 μM deoxynivalenol (DON); (iii) porcine jejunal explants with 10⁷ cfu/ml yeast strain; and (iv) porcine jejunal explants pre-incubated 1 h with 10⁷ cfu/ml yeast strain and then exposed for 3 h to 10 μM DON. The explants were incubated at 39°C. For CCL20, IL-1β, IL-8 and IL-22 gene expression, total RNAs were extracted and then reverse transcription and qPCR steps were performed. Oral administration of *S. cerevisiae* RCV016 increased s-IgA, the number of goblet cells in small intestine and all the growth parameters assayed. In the *ex vivo* model, a toxic effect of DON was observed as an increase of proinflammatory cytokines expression. The presence of the yeast strain showed a strong tendency to counteract this toxic effect. In conclusion, *S. cerevisiae* RCV016 is a promising candidate for feed additives formulation to improve animal growth and gut immune system. This yeast strain could be able to counteract the toxic effect of feed naturally contaminated with DON. The use of additives based on beneficial micro-organisms instead of chemical products is a safer and eco-friendly option to increase animal productivity with a minimum environmental impact.
The legislation of the European Union banned antibiotics as growth promoters in food animals. In addition, the Food and Agricultural Organization of the United Nations estimates that mycotoxins contaminate 25% of the world’s agricultural commodities. Different alternatives, such as the use of probiotics, have been explored to stimulate gut health in weaned pigs. *Lactobacillus rhamnosus* RC007 was previously isolated from maize silage and demonstrated beneficial properties in a mice colitis model. The aim of the present study was to evaluate the immune effect on the intestinal toxicity of deoxynivalenol (DON) in an *ex vivo* porcine model. Jejunal explants were obtained from 5 weeks old crossbred castrated male piglets. The treatments evaluated were: (i) porcine jejunal explants in complete medium; (ii) porcine jejunal explants exposed for 3 h to 10 μM DON in complete medium; (iii) porcine jejunal explants with 10⁸ cfu/ml *L. rhamnosus* RC007 in complete medium; and (iv) porcine jejunal explants pre-incubated 1 h with 10⁹ cfu/ml *L. rhamnosus* RC007 and then exposed for 3 h to 10 μM DON in complete medium. The explants were incubated at 39°C. For the gene expression analysis, total RNAs were extracted and then reverse transcription and qPCR steps were performed to determine IL-1β, TNFα, IL-8 and IL-22. Cellular permeability was measured by a Ussing chamber. Treatment of intestinal explants with DON significantly increased the expression of IL-1β, TNFα, IL-8, IL-22 and increased the paracellular permeability. By contrast, pre-incubation with *L. rhamnosus* RC007 reduced the expression of these pro-inflammatory cytokines and the paracellular permeability. In conclusion, *L. rhamnosus* RC007 is a probiotic candidate to be included as feed additive able to decreases the intestinal toxicity caused by DON.

### P14: Sterols influence on colonic microbiota

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Plant sterols (Ps) are poorly absorbed in the small intestine (1-2%) [Quilez et al., 2003. Clinical Nutrition 22: 343-351] reaching the colon where they are hydrolysed, hydrogenated, dehydrogenated and transformed by colonic bacteria generating degradation products [Keller & Jahreis, 2004. Journal of Chromatography B 813: 1999-207]. The effect of the sterols on colonic microbial populations is not known yet. The aim of this work is to evaluate the influence of Ps in the colonic microbiota after *in vitro* colonic fermentation using a residue of a Ps enriched beverage (BPs) obtained after *in vitro* gastrointestinal digestion [Alvarez-Sala et al., 2016. Journal of Agricultural and Food Chemistry, in press]. In addition, the impact of lyophilisation on the faecal microbiota in three different faeces was evaluated. *In vitro* colonic fermentation assay: a pool of fresh faeces of 5 healthy donors (faeces-pool) was inoculated into culture medium supplemented with BPs (test sample) or not (control) at controlled conditions (37°C, pH 6.7-6.9) and under anaerobiosis for 24 and 48 h. Identification of colonic microbial populations: bacterial DNA extraction was done from the initial faeces-pool lyophilised and from control and BPs samples and from three individual faecal samples before and after lyophilisation using a Stool Total RNA Purification Kit (Norgen Biotek Corp.). The V4 regions and V5 of the 16S DNA gene were amplified. Amplicons were purified and sequenced by MiSeq platform. Data analysis was done using bioinformatics tools, such as Flash, Uchime, Mothur, SILVA, Bayesian RDP Classifier, UPARSE. As anticipated, incubation time influenced bacterial populations, independently of the BPs presence. An increased proportion of taxonomic assignment of the reads (OTUs) of the genera *Bacteroides, Eubacterium, Fecalibacterium and Prevotella* was detected after 24 h fermentation and of *Dorea, Bacteroides* and *Flavonifractor* after 48 h in the presence of BPs. It has been reported [Ayesh et al., 1999. Food and Chemical Toxicology 37: 1127-1138] that after intake a Ps enriched-margarine only *Lactobacillus* content was reduced *in vivo*. Analysis of bacterial DNA showed that the lyophilisation...
process improved the amplification step, finding less chimeric sequences, which may indicate that the microbial DNA has a greater stability and quality. OTUs indicated that the lyophilisation process has a minor impact on faecal bacterial composition. In conclusion, the presence of sterols especially favours the proliferation of *Bacteroides*.

**P15: From gene to activity – exploit the potential of the Nestlé Culture Collection**

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Providing nutritious, healthy and sustainably produced food is one of the main objectives of food companies, such as Nestlé. A culture collection of more than 3,000 food grade strains (Nestlé Culture Collection, NCC), has been integrated into an R&D network covering microbiology, fermentation, food processing, nutrition and health, as well as clinical trials. Thanks to this approach and its research and technological capabilities, Nestlé has been able to develop and produce a large range of functional foods containing beneficial microbes. Today, the genomes of almost all NCC strains have been sequenced, assembled and annotated by using the complementary Illumina or Pacific Biosciences sequencing technologies, followed by assembly and annotation pipelines. Web based bioinformatic software enabling the storage and the analysis of the genomes have been implemented. The resulting genome databank allows direct evaluation and exploration of the NCC microbes for the development of fermented foods with enhanced taste and texture, functional benefits, and/or probiotics. The sequenced NCC can also be used to identify and source specific enzymes. In addition, the gene collection enables an easy screen for the presence/absence of undesired metabolic pathways or antibiotic resistance genes. In this presentation, we will illustrate how the newly developed bacterial genomic platform was used to go from gene to activity, bridging between in silico analysis and preclinical or proof of concept human trials. Examples relate to the use of lactic acid bacteria to deliver functional molecules or enzymes that could alleviate the consequences of specific food adverse reactions. We will also present the identification and application of lactic acid bacteria as delivery systems for micro nutrients like iron and the use of specific lactic acid bacterial enzymes for the conversion of sugars into fibres.

**P16: Contribution of gut microbial composition to the development of diet-induced metabolic syndrome in BALB/c mice**

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The gut microbiota contributes to the metabolic phenotype. Germ-free mice are protected against diet-induced obesity and transfer of gut microbiota from obese to germ-free mice results in fat mass gain. Furthermore, the development of obesity correlates with low *Bacteroidetes* and high *Firmicutes* in the gut. We investigated the susceptibility of BALB/c mice to develop diet-induced metabolic syndrome and the role of the gut microbiota in this process by using 2 lines of BALB/c mice with divergent gut microbial compositions (BALB/c A versus B). Mice were fed a control or high fat/high sugar (HFHS) diet for 15 weeks. BALB/c A had a higher *Firmicutes/Bacteroidetes* ratio at baseline than BALB/c B. The HFHS diet increased the ratio for both BALB/c lines, however, their microbial signature remained very different with specific bacterial species present. BALB/c A fed the HFHS diet gained body weight compared to the control group, whereas BALB/c B did not. Fat pad mass was larger in BALB/c A vs. B and increased significantly when fed the HFHS diet in BALB/c A only. This is in line with increased serum leptin concentrations. HFHS diet-fed BALB/c A also showed increased expression of proinflammatory cytokines including IL-6 and TNF-α mRNA in adipose tissue. However, BALB/c B but not A developed impaired glucose tolerance and insulin sensitivity when fed HFHS diet. This was associated with more lipid accumulation in liver tissue. In conclusion, the HFHS diet induced different hallmarks of the metabolic syndrome in these two lines of BALB/c mice with divergent gut microbiota, which is associated with the abundance of specific bacterial species present in the gut.
P17: The effects of Lactobacillus johnsonii strain N6.2 and rosmarinic acid on apoptosis biomarkers

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Bio-breeding diabetes prone rats (BBDP rats) orally fed with Lactobacillus johnsonii strain N6.2 displayed lower rate of type 1 diabetes development (T1D). Probiotic fed animals showed variations in the gut microbiota composition associated to local changes in the ileal mucosa. The changes observed included lower expression of inflammatory and oxidative stress markers in the ileum, a Th17 bias in the mesenteric lymph nodes, the strengthening of the tight junctions and an increased amount of Paneth cells in the Lieberkühn crypts. The healthier status of the gastrointestinal system (GI) was positively correlated with important systemic consequences. L. johnsonii feeding resulted in a 17% reduction in serum kynurenine compared with that in vehicle-fed controls, correlating with a 1.4-fold elevation in 5-HT levels. This is a consequence of the direct modulation of the indoleamine 2,3-dioxygenase activity at GI level. Lowering the levels of serum kynurenine may also affect the regulation of downstream pathways like those controlled by the regulators AhR and mTOR. A subset of assays was performed to evaluate the effects of L. johnsonii and bioactive phenolic on downstream pathways (i.e., apoptosis). To this end, BBDP animals were orally fed with the probiotic or the probiotic in combination with the natural T cell apoptotic inducer, rosmarinic acid. Herein, we present the preliminary data obtained regarding the synergistic effects of the L. johnsonii and rosmarinic acid on biomarkers of the apoptotic pathways. We observed that the administration of L. johnsonii increased the mRNA levels of CASP1 on ileum while these effects were reverted by co-feeding the animals with rosmarinic acid. The administration of both treatments repressed the mRNA level of CASP1, PYCARD and TP73. The mRNA levels of CASP1 from liver samples showed the same pattern of expression.

P18: A MarR regulator controls the production of flavouring compounds in Lactobacillus brevis

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 Biosynthesis of flavouring compounds is one of the most relevant characteristics observed during selection of food quality strains. In Lactobacillus, the butanoate pathway is used to produce a variety of volatile flavouring compounds (i.e., acetoin, diacetyl). However, the genetic regulation of several enzymes involved in the biosynthetic pathway has been minimally studied. LVIS_0490 is a member of the MarR-family of transcriptional regulators in Lactobacillus brevis. This uncharacterised transcription factor is encoded in the same genomic cluster of LVIS_0491 and LVIS_0492. These genes encode for an acetolactate synthase and acetolactate decarboxylase respectively, which may be involved in the production of acetoin. We used a high-throughput biochemical approach to identify ligands that modulate the activity of LVIS_0490 in L. brevis ATCC 367. A subset of metabolic intermediates of the butanoate pathway were identified as potential ligands. The objectives of this work were to determine the regulatory mechanisms of LVIS_0490, to establish its biological role, and to identify which metabolites modulate its regulatory function. It was found, that LVIS_0490 acts as a repressor of its own transcription, as well as of LVIS_0491 and LVIS_0492. DNase I foot-printing and in vitro DNA binding assays confirmed that LVIS_0490 binds within its own promoter in a region of 18 nucleotides located in close proximity of the start codon. Using LacZ fusions to LVIS_0490, we found that one or more intermediates of the pathway catalysed by LVIS_0491 and LVIS_0492 modulated the regulatory activity of LVIS_0490 in vivo. The effect of these compounds as modulators of LVIS_0490 were evaluated on DNA binding experiments in vitro. To gain insight into the regulatory mechanism at molecular level, the ligand binding pocket in LVIS_0490 was identified. Using structural modelling, a ligand binding pocket formed by residues Y6, Q9, R16, C37, and E67 was predicted in silico. Site-directed mutagenesis followed by DNA binding assays confirmed the involvement of these residues in ligand binding. Our results indicate that acetoin is the molecule that modulate the expression of the acetolactate synthase and acetolactate decarboxylase genes in L. brevis.
P19: Carbs can make the difference: how pectins fuel immunity

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A imbalance in microbiota communities in the intestine is implicated in a large number of Western diseases. This correlates with low intake of dietary fibre in Western diets. Pectin is a dietary fibre that might be essential for prevention of Western diseases. Recently, evidence has been found that beneficial effects of pectin are highly dependent on its chemical structure and the effects go beyond prebiotic effects. Earlier research has demonstrated that low methyl esterified pectins have direct, microbiota-independent effects due to their direct interaction with TLRs in the small intestine [Tian et al., 2016. Molecular Nutrition & Food Research, in press; Vogt et al., 2016. Journal of Functional Foods 22: 398-407]. High methyl esterified pectins mainly impact the microbiota in the colon to enhance formation of pectic oligosaccharides and SCFA [Sahasrabudhe, 2016. PhD thesis, University Medical Center Groningen, the Netherlands]. The aim of our research is to fully characterise bioactive pectins and their intermediate degradation products upon fermentation by enzymatic fingerprinting techniques. Pectin molecules from various sources will be applied and transformed in molecules with a combined beneficial effect. The already available data on pectin health effects will serve to produce a mixture of pectins with a desired molecular weight and methyl esterification pattern. Results obtained will be used to further tailor pectin structure to yield optimal bioactivity. This study will contribute to the understanding of the structure-function relationship of oligosaccharides and ultimately lead to a tailored design of non-digestible carbohydrates with desired health effects.

P20: Effect of tablets containing probiotic candidate strains on clinical and cytokine markers of gingival inflammation and composition of the salivary microbiome

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The oral microbiota does not play a passive role in the oral cavity but contributes actively to the maintenance of oral health. The use of probiotic bacteria may help to influence the composition of the oral microbiota. The potential avenues are: (i) co-aggregation with pathogens and growth inhibition, (ii) bacteriocin and hydrogen peroxide production, (iii) competitive exclusion through antagonistic activities on adhesion sites and nutrition, and (iv) systemic immunomodulation. The aim of the study was to investigate the effect of tablets containing probiotic candidate strains on gingival inflammation and the levels of selected pro- and anti-inflammatory cytokines in gingival crevicular fluid (GCF). A secondary aim was to describe the effect of the tablets on the salivary microbiome. The study was a double-blind placebo controlled randomised trial with two parallel arms. The intervention period was 4 weeks with a two-week follow-up after the intervention. After informed consent, baseline examination and sampling, 50 participants were randomly assigned to one of the study groups and given supply of either probiotic tablets or placebo tablets. The probiotic tablets contained Lactobacillus rhamnosus PB01 and L. curvatus P2-2 at a dose of not less than 1x10⁸ cfu/tablet. At each visit, saliva and GCF was collected and then plaque index (PI) and bleeding on probing (BOP) was registered. Forty-eight participants completed the study. A statistic significant difference in the GCF volume between the two groups was registered. Both groups displayed a statistical significant decrease in BOP but no differences between the two groups were seen. In conclusion, the volume of gingival crevicular fluid was statistic significantly decreased in the probiotic group compared to the placebo group.
P21: Oral delivery of pentameric glucagon-like peptide-1 by recombinant Lactobacillus in diabetic rats

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone produced by intestinal cells and stimulates insulin secretion from the pancreas in a glucose-dependent manner. Exogenously supplied GLP-1 analogues are used in the treatment of type 2 diabetes. An anti-diabetic effect of Lactobacillus in lowering plasma glucose levels and its use as a vehicle for delivery of protein and antibody fragments has been shown previously. The aim of this study was to employ lactobacilli as a vehicle for in situ production and delivery of GLP-1 analogue to normalise blood glucose level in diabetic GK (Goto-Kakizaki) rats. In this study, we designed pentameric GLP-1 (5×GLP-1) analogues, which were both expressed in a secreted form and anchored to the surface of lactobacilli. Intestinal trypsin sites were introduced within 5×GLP-1, leading to digestion of the pentamer into an active monomeric form. The E. coli-produced 5×GLP-1 peptides delivered by intestinal intubation to GK rats resulted in a significant improvement of glycemic control demonstrated by an intraperitoneal glucose tolerance test. Meanwhile, the purified 5×GLP-1 (trypsin-digested) from the Lactobacillus cultures stimulated insulin secretion from HIT-T15 cells, similar to the E. coli-produced 5×GLP-1 peptides. When delivered by gavage to GK rats, non-expressor Lactobacillus paracasei significantly lowered the blood glucose level but 5×GLP-1 expression did not provide an additional anti-diabetic effect, possibly due to the low levels produced. Our results indicate that lactobacilli themselves might be used as an alternative treatment method for type 2 diabetes, but further work is needed to increase the expression level of GLP-1 by lactobacilli in order to obtain a significant insulinotropic effect in vivo.

P22: Toward controlled steering of microbiota and immunity in infants by non-digestible carbohydrates

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Bacteria colonising the infant mucosa guide the development of a balanced immune system and support maturation of the gut-barrier. Breast milk has been considered the golden standard for guiding this colonisation. Human milk oligosaccharides (HMOs) serve as an energy source for the microbiota and also supports immune function directly. For those infants where mother-milk is not a feasible option, cow-milk formulas supplemented with non-digestible carbohydrates (NDC) are used. An important function of these NDCs is a preferred support of Th1-responses responsible for fighting infections. However, we recently found that not all NDCs currently applied support Th1 responses and that induced responses are dependent on the composition of NDCs. From this information it could furthermore be concluded that the optimal NDC composition of cow-milk formula may be different for healthy and for disease-prone infants. To unravel the relation between specific NDCs and the immunity of infants a system will be developed that mimics the gastrointestinal tract. Herein, various NDCs will be co-cultured with infant-microbiota. By the addition of appropriate cytokines, the system could also represent an allergic or premature microenvironment. Methods will be developed to enable the characterisation and quantification of the specific NDCs. These will be used to determine the remaining NDCs and their glycocid-degradation products, the kinetics of degradation and the effect on the microbiota composition after certain incubation in the system. Furthermore, the effect of the specific NDCs on the barrier-function and immunity will be determined at the Division of Medical Biology of University Medical Center Groningen. This will result in the capability of designing tailored NDC-supplements for healthy and disease-prone infants to support the immune response development.
Various intestinal diseases, for example inflammatory bowel disease, are linked to an inappropriate activation of mucosal immune responses causing chronic inflammation. The activation of innate immune receptors, including Toll-like receptors (TLRs), is important in triggering inflammation in the defence against pathogens. However, uncontrolled immune responses can lead to intestinal diseases. Even though excessive TLR activation causes detrimental effects, it is becoming increasingly evident that a ‘tonic’ level of TLR activation by commensal bacteria is required for intestinal homeostasis. In this research the activation of TLRs by strains of *Faecalibacterium prausnitzii*, an abundant Gram-negative obligate anaerobe of the human intestinal microbiota associated with anti-inflammatory properties, was determined. To test the effect of live *F. prausnitzii* on TLR activation, a novel apical anaerobic co-culture model was used that allowed the co-culture and direct interaction between live obligate anaerobes and oxygen-requiring human cells by separation of anaerobic and aerobic compartments. TLR activation was tested using nuclear factor-κB reporter cell lines (HEK293) transfected with either one or two human TLRs (TLR2, TLR2/6 and TLR4). The TLR activation assay was adapted to the apical anaerobic co-culture model. The assay was successfully conducted in the new model using cells seeded on collagen-coated Transwell inserts and known TLR ligands as positive controls. The TLR cell lines remained viable throughout the 6 hours of incubation. No oxygen was detected in the apical anaerobic compartment at any time point ensuring the viability of obligate anaerobic *F. prausnitzii*. In the basal aerobic compartment 60% of the starting dissolved oxygen concentration remained after 6 hours of incubation providing sufficient oxygen for the TLR cell lines. The TLR activation assays showed that live *F. prausnitzii* induced higher activation of TLR2 and TLR2/6 than dead bacteria. Neither live nor dead *F. prausnitzii* activated TLR4. The higher TLR2 and TLR2/6 activation by live *F. prausnitzii* may contribute to its immune-regulatory effects and to the maintenance of immune homeostasis in the gastrointestinal tract. The different effects on TLR activation between live and dead bacteria highlight the importance of understanding physiologically relevant co-culture systems, such as the apical anaerobic co-culture model, to decipher the mechanisms of action of live obligate anaerobes in the gastrointestinal tract.

**P24: Exopolysaccharides produced by *Leuconostoc mesenteroides* strain NTM048 as an immunostimulant to enhance the mucosal barrier**

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In recent years, the use of pre- and probiotics as supplements in food has rapidly increased. Lactic acid bacteria (LAB) have attracted significant attention for their probiotic properties, such as their beneficial effects on the host gastrointestinal conditions and immune systems, including their ability to promote host mucosal immunoglobulin A (IgA) secretion. The generation of massive amounts of IgA is indispensable for mucosal protection because IgA prevents antigens and pathogens from binding to cell-surface receptors. To explore novel probiotics, we isolated 173 LAB strains from various sources and evaluated their ability to induce IgA production using murine Peyer’s patch cells. Among these bacteria, strain NTM048, isolated from green peas, showed the highest activity and was identified as *Leuconostoc mesenteroides* subsp. *mesenteroides*. This strain was found to produce large amounts of exopolysaccharides (EPS) possessing the IgA-inducing ability [Matsuzaki et al., 2014. Journal of Applied Microbiology 116: 980-988]. The induction of IgA through a T cell-dependent pathway leads to the production of high-affinity IgA because of the occurrence of somatic hypermutation, which is responsible for affinity maturation. We performed animal experiments to clarify the pathway which EPS induces IgA. NTM048-EPS-supplemented drinking water was given to BALB/c mice for 42 days, resulting in the stimulation of intestinal IgA secretion. Gene expression analysis of their Peyer’s patch cells revealed that the gene expression of TGF-β receptor 2, RALDH and BCL6, which are involved in T cell-dependent IgA induction, was upregulated by the administration of NTM048-EPS. This result indicates that NTM048 EPS stimulates intestinal IgA secretion through a T cell-dependent pathway and implies its potential utility in promoting the production of not only total IgA but also high-affinity IgA [Matsuzaki et al., 2015. Journal of Agricultural and Food Chemistry 63: 7009-7015]. Mucosal adjuvants that enhance the production of high-affinity IgA are strongly desired for effective mucosal vaccination.
Based on the results of our gene expression analysis, we next investigated the adjuvant activity of NTM048 EPS using influenza A (H1N1) virus antigen. Stimulation with H1N1 antigen and EPS induced a significant (1.5-fold) increase in the secretion of H1N1-specific IgA by Peyer’s patch cells compared with that secreted by cells stimulated with H1N1 antigen alone. In conclusion, we demonstrate NTM048 as a probiotic strain and NTM048 EPS as an immunostimulant to enhance mucosal barrier against pathogen and antigens. We are currently investigating the enzymes involved in EPS biosynthesis [Ishida et al., 2016. Biotechnology Letters 38: 681-687] and EPS structure with the aim to determine its immunoneactive structure.

P25: Gut microbiota composition and dietary intake are related to serum zonulin, a marker of intestinal permeability, of overweight pregnant women

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Alterations in intestinal permeability, as a result of obesity, dietary factors and gut microbiota dysbiosis, may precede adverse metabolic conditions including diabetes and obesity. Whether pregnancy is associated with alterations in intestinal permeability and the extent to which diet and gut microbiota composition contribute are poorly understood. Our aim was to investigate whether the gut microbiota, abundance of certain bacteria and richness, and diet differs according to serum zonulin concentration, a marker of intestinal permeability, in overweight pregnant women. Serum zonulin, determined by ELISA, were analysed from 100 overweight women (mean age 29 years; median body mass index 30 kg/m²) in early pregnancy (gestational weeks <17, median 13). Gut microbiota were assessed by 16S rRNA sequencing and dietary intake of macro- and micronutrients calculated from three-day food diaries. Women were divided into Low (<46.4 ng/ml, median 38 IQR 35-43ng/ml) and High zonulin groups (≥ 46.4 ng/ml, median 53 IQR 49-60) based on the median value of zonulin (46.4 ng/ml). Richer gut microbiota was related to lower intestinal permeability, established as higher Chao 1, observed species and phylogenetic diversity in the Low zonulin group (P=0.01). Further differences between the Low and the High zonulin group were detected in the abundance of following bacteria: Bacteroidaceae and Veillonellaceae. Bacteroides and Blautia were lower and Faecalibacterium and Faecalibacterium prausnitzii higher (P<0.05) in the Low compared with the High zonulin group. In addition to the gut microbiota composition, dietary quantitative intake of n-3 polyunsaturated fatty acids, fibre, and a range of vitamins and minerals, were higher (P<0.05) in women in the Low compared with the High zonulin group. The present finding shows that serum zonulin concentration in overweight pregnant women is related to gut microbiota composition and diet. Lower serum zonulin concentration, i.e., lower intestinal permeability is associated with higher gut microbiota richness and abundance of anti-inflammatory species (Faecalibacterium prausnitzii) and lower abundance of pro-inflammatory genus (Bacteroides). Modification of the gut microbiota and diet may beneficially affect intestinal permeability leading to improved metabolic health of both the mother and foetus.

P26: Characterisation of bifidobacteria from dairy calves

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Ruminant animals rely on a complex specialised rumen microbial community for feed fermentation. However, newborn ruminants have a physically and metabolically underdeveloped rumen which means they naturally rely on milk during their early stages of life, and gradually transition and adapt to solid feed during which time the rumen develops. Current rearing strategies for dairy and dairy-beef calves separate the newborns from their dams, and restrict offerings of milk, or milk replacer, to encourage solid feed intake and accelerate rumen development for early weaning. Consequently, the limited exposure to maternal gut microbes and the nutritional benefits that milk provides may negatively impact calf development, health and productivity. Bifidobacteria are a group of microbes considered highly beneficial to young infants, and the use of *Bifidobacterium* strains as direct-fed microbials may provide
beneficial effects for young livestock in early life when the risks of morbidity and mortality are high. We have isolated a collection of bifidobacterial cultures from fresh calf faecal material. Phylogenetic analyses of 16S rRNA gene sequences revealed that the isolates represent five species of *Bifidobacterium*: *B. bifidum*, *B. breve*, *B. choerinum*, *B. longum* subsp. *suis* and *B. pseudolongum* subsp. *globosum*. The genomes of representative isolates from each species were sequenced through the Hungate1000 project, and range in size between 1.9 and 2.3 Mb. Functional assignment of the ORFeomes based on the Clusters of Orthologous Groups (COG) database indicates a functional designation for 66% of the predicted protein-coding genes for each genome on average. A comparative analysis of the ORFeomes shows a conserved set of 1,019 gene families shared between the five genomes examined. Genes unique to each of the genomes have been identified and these highlight potential strategies each of the calf bifidobacterial species employ to successfully adapt, co-exist, and survive within the pre-ruminant gastrointestinal tract. Our current research aims to understand the impact of these strains on gut colonisation, gut health and immune response in calves. Furthermore, we are exploring the abundance and diversity of bifidobacteria populations in calf gastrointestinal microbial communities using culture-independent approaches. These investigations will be used to help identify strains with potential in enhancing calf health and performance.

**P27: Gammaproteobacteria abundance and specific gene functions are highly correlated in the faecal microbiota of the domestic cat fed a canned or kibbled diet**

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Interest in the effects of diet on the intestinal microbiota composition and the clear links to health and wellbeing have extended from humans to that of domestic companion animals. Complementary to the changes in microbial composition associated with diet, are the effects of the diet on the function of the intestinal microbiome. Investigating these two factors in parallel gives insight into not only the effects of dietary change on microbiome composition, but the impact that this has on the function of the microbiome. Pet cats in the home environment are typically fed specific formats of pet food such as kibbled or canned diets. These diets differ in the levels of macro- and micro-nutrients, and previous studies have shown dramatic differences in faecal microbial populations between these diets. The aim of this study was therefore to investigate the effects post-weaning (kibbled or canned) diet on the composition and function of faecal microbiota in the domestic cat using a shotgun metagenomic sequencing approach. Kittens were weaned at 8 weeks of age and randomly assigned to a moderate protein:fat:carbohydrate kibbled (35:20:28% dry matter) or high-protein:high-fat:low-carbohydrate canned (45:37:2% dry matter) diet for 17 weeks (n=10). Faecal metagenomic DNA was shotgun sequenced and sequences were analysed using the metagenomics Rapid Annotation using Subsystem Technology server to determine taxonomic identity and predicted gene function. Wide ranging differences in faecal microbiota were observed between kittens fed the two diets, with significant increases (false discovery rate, FDR<0.05) in the relative abundance of *Bifidobacterium*, *Ruminococcus*, *Eubacterium* and *Lactobacillus* genera in kittens fed the kibbled diet. In contrast, kittens fed the canned diet showed significant (FDR<0.05) increases in taxa, such as *Bacteroides*, *Prevotella*, *Fusobacterium*, *Clostridium*, and a wide range of genera within the class *Gammaproteobacteria*, including *Escherichia*, *Enterobacter*, *Shigella*, *Citrobacter* and *Salmonella*. Although the *Gammaproteobacteria* were not highly abundant overall (kibbled 0.3% vs. canned 4.2%), across all cats, particular species within this group were highly correlated (R>0.9) with the abundance of specific genes involved in amino acid, sugar, and sulphate transport systems, secretion systems, and lipopolysaccharide biosynthesis. This strikingly high correlation suggests that this group of bacteria possess specific functions that may not be replicated in other members of the domestic cat lower gastrointestinal microbiome. The *Gammaproteobacteria* are known for including members which are opportunistic pathogens, however, our results suggest these commensal bacteria may fill unique ecological niches in the healthy cat gastrointestinal ecosystem.
P28: Anti-ageing effects of Lactobacillus gasseri SBT2055 and the mechanism of action

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Lactobacillus gasseri SBT2055 (LG2055) has been shown to exert beneficial effects in mice and humans, such as improvement of the intestinal environment, prevention of infection by influenza A virus, augmentation of IgA production, and lowering of the serum cholesterol concentration. It has been suggested that LG2055 could exert beneficial effects on longevity and ageing. Therefore, we investigated whether feeding with LG2055 extended the life span of and delayed ageing in Caenorhabditis elegans and then analysed the underlying mechanisms. We analysed the life span after administration of LG2055 or control E. coli OP50 in wild type N2 and several mutants of C. elegans. We observed that LG2055-fed worms showed approximately a 37% increase in median survival, with a statistically significant right-shifted survival curve. LG2055 feeding up-regulated the gene expression of skn-1 gene and its target genes. Therefore, we hypothesise that LG2055 inhibits the accumulation of oxidative damage associated with ageing by stimulating the immune system, including p38 MAPK signalling and other pathways. Feeding with LG2055 marginally increased the life span of the tir-1 mutant of C. elegans but did not increase the mean lifespan of the nsy-1, sek-1, or pmk-1 mutants. We found that feeding with LG2055 effectively stimulated NSY-1-SEK-1-PMK-1-SKN-1 signalling pathway. We showed that feeding with LG2055 effectively extended the life span of C. elegans by increasing stress resistance and stimulating the immune response. Furthermore, we examined the influence of LG2055 treatment on the mitochondria membrane potential, total ATP levels, mitochondria mass, and anti-oxidative defence in mammalian cell damaged by CCCP (carbonyl cyanide m-chlorophenyl hydrazone). Interestingly, mitochondrial mass and ATP level were significantly increased by LG2055 feeding in comparison with the control group. In mammalian cell, CCCP decreased the number of mitochondria, but LG2055 treatment inhibited the decrease of mitochondrial mass. LG2055 also enhanced the function of mitochondria, increased ATP levels and decreased ROS levels via Nrf2-ARE signalling. Therefore, it was suggested that LG2055 activated the Nrf2-ARE signalling pathway, and it strengthened the anti-oxidative defence system against mitochondrial damage in mammalian cells.

P29: Bifidobacterium spp. in breast milk increases over lactation and might be affected by intrapartum antibiotic therapy

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Bifidobacterium spp. is one of the most important genera related to health beneficial effects, especially for infants. Particularly regarding breast milk, it is a source of Bifidobacterium for the infant gut colonisation. Identifying the factors which affect Bifidobacterium counts in breast milk and consequently the infant gut colonisation, could be a key to reduce the risk of diseases and promote health. The aim of this work was to evaluate the effect of the intrapartum antibiotic therapy on Bifidobacterium counts in human milk, in short (1 week) and medium (1 month) term. A total of 10 healthy lactating women, with healthy babies who were delivered vaginally and were exclusively breastfed, living in São Paulo city (Brazil) were recruited: 5 who had received intrapartum antibiotic therapy (IAT group), and 5 who had not received antibiotic therapy (NAT group). The samples of human milk were taken at the first week (7±3 days) and around one month (30±4 days) after delivery. The presence of DNA from Bifidobacterium spp. was assessed by quantitative polymerase chain reaction (qPCR) using genus-specific primers for the bacterial 16S rRNA gene. Bifidobacterium spp. DNA was identified in all samples. No differences were found in Bifidobacterium spp. counts between IAT and NATI groups for the first week after delivery (2.2±0.2 vs. 2.1±0.1 log copy numbers/ml for IAT and NAT, respectively; P=0.834). However, Bifidobacterium spp. counts were higher on 30 ± 4 days after delivery, for both groups (2.6±0.2 and 2.2±0.2 log copy numbers/ml for IAT and NAT, respectively; P<0.05). Furthermore, we observed that IAT group showed Bifidobacterium spp. counts higher than NAT group in the first month (P=0.0367). Our results suggest that Bifidobacterium spp. counts change over lactation and the intrapartum antibiotic therapy seems to affect it. We believe that changes on the breast milk microbiota may occur even in
short term, influencing the *Bilidobacterium* community. However, more studies are being conducted in order to evaluate if the intrapartum antibiotic therapy could affect the breast milk microbiota diversity.

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**P30: Pre- and post-weaning gut microbial profiles of piglets administered *Bacillus* spp. spores and gentamicin**

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Diarrhoea is highly prevalent in neonatal piglets, and is often treated with antibiotics like gentamycin. Administrating antibiotics to newborn piglets may have short- and long-term consequences on gut microbiota and immune system development. We hypothesise that the consequences may be alleviated by concurrent probiotic administration. The objective was therefore to investigate the effect of administrating gentamicin and a mixture of *Bacillus licheniformis*, *B. subtilis* and *B. amyloliquefaceans* spores on the gut microbiota of piglets pre-and post-weaning. Twenty-four sows and their litters were randomly allocated to four treatment groups and administered: (i) *Bacillus* spore mixture to sows and piglets (PRO); (ii) gentamicin to piglets day 4, 5 and 6 of age (AB); (iii) spore mixture to sows and piglets, and gentamicin to piglets (PRO+AB); or (iv) no probiotics or antibiotics (CTRL). The study included 12 piglets from each litter. Faecal samples were collected day 7, 14, 21, 28, 35 and 42. Piglets were sacrificed for intestinal digesta and tissue day 3, 28 and 42. Selected samples were subjected to amplicon sequencing of the 16S rRNA gene, culture counts, and organic acid, biogenic amine and tissue gene expression analysis (TNF-α, IL-10, COX-2, ZO-1, OCLN, CLDN-4 and CLDN-2). Treatment had a significant effect on composition of the faecal microbial community on day 28 \((P_{\text{adonis}}=0.003)\) and 42 \((P_{\text{adonis}}=0.008)\), and the colonic community on day 28 \((P_{\text{adonis}}=0.017)\). Faecal \((P=0.001)\) and colonic \((P<0.047)\) species richness and diversity were higher for AB- than PRO-piglets on day 28, and were not significantly different from day 42. Species richness and diversity were numerically lower for CTRL-piglets compared to AB-piglets, and numerically higher compared to PRO-piglets. Significant differences in low abundant OTUs were observed between treatment groups. PRO piglets had the highest faecal concentration of iso-butyric acid on day 7 \((P=0.04)\) and a higher butyric acid concentration compared to CTRL piglets \((P=0.02)\); otherwise were no significant effects observed on organic acid and biogenic amine concentrations, gene expression or bacterial counts. The results show that administrating oral gentamicin to piglets shortly after birth may affect gut microbial composition and is counteracted by concurrent administration of *Bacillus* spores, suggestively due to spores competitively excluding early colonisers. We conclude that both gentamicin and *Bacillus* spores influence the microbial gut diversity of young piglets, although administration of the tested antibiotic did not result in severe dysbiosis. The importance of the microbiological findings in relation to gut and animal health needs further investigation.

**P31: Afatoxin M1 levels reduction in milk after *Saccharomyces cerevisiae* or mannan-oligosaccharides addition to aflatoxin B1-contaminated diet of dairy cows**

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The purpose of this study was to evaluate the ability of *Saccharomyces cerevisiae* (SC47) and a mannanoligosaccharide (MOS) to bind aflatoxin B1 (AFB1) in the diet of dairy cows fed with 200 μg/kg an AFB1-contaminated diet daily to reduce the aflatoxin M1 (AFM1) levels in milk. Toxigenic fungi that grow on crops can produce highly carcinogenic metabolites called aflatoxins (B1, B2, G1, G2). AFM1 is a hydroxylated AFB1 metabolite secreted (0.3-6.2%) in milk of mammary glands of lactating animals. The study was conducted in a commercial farm in Rio de Janeiro State and supervised by technicians of PESAGRO and UFRJ. Thirty-six early to mid-lactation dairy cows averaging 90 days were used in a 4x4 latin square design with 3 replicates. Cows were blocked by parity, body weight and milk production. *Ad libitum* access to feed and water was provided. Within each replicate, cows were randomly assigned to six dietary treatments for 2 consecutive 7-day periods. Dietary treatments included: T1, basic diet (BD); T2, BD + AFB1 (200 μg/kg dry matter (DM)); T3, BD + 10 g MOS/cow/day;
T4, BD + AFB1 (200 µg/kg DM) + 10 g MOS/cow/day; and T5, BD + 10 g SC47/cow/day and T6- BD + AFB1 + 10 g SC47/cow/day. Milk production and composition, AFM1 determination and quantification, fat and protein concentrations on milk, animal productivity were evaluated daily. Milk samples were collected from the 1st to 4th day, 7th and 10th to 14th day of the experimental period. The cows of treatments T2, T4 and T6 were fed the AFB1 contaminated diet until the 10th day of the experiment. High-performance liquid chromatography (HPLC) was used for AFM1 detection on no-fat milk (50 ml) using an immunoaffinity column. The samples were injected in triplicate. Adding SC47 or MOS to basal or AFB1-contaminated diets at 10 g/day/animal had no effect on lactation performance. The maximum levels of AFM1 averaged at the 1st day were 2.04±0.18 µg/l, 0.7±0.12 µg/l and 0.14±0.03 µg/l, respectively, for cows fed T2, T4 and T6. Transfer rates of AFB1 from feed to milk (AFM1) averaged 1.02, 0.35, and 0.07% for cows fed T2, T4 and T6, respectively.

P32: Effectiveness of antymycotoxin additives based on *Saccharomyces cerevisiae* cell wall in gilts intoxicated with zearalenone

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Mycotoxins are present in grains and cereals, and represent a global health problem of pigs and other farm animals, causing deleterious effects to the health of consumers. They are secondary metabolites produced by certain fungi that grow naturally in grains and cereals. Zearalenone (ZEA) is a mycotoxin produced mainly by *Fusarium graminearum*; swine is a highly sensitive species. ZEA causes a lot of problems in the reproductive tract, being related to hyperestrogenism in gilts. Preventive measures to prevent fungal growth are not always effective; the use of antymycotoxin additives (AMA) added to food products, capable of adsorbing, neutralising or biotransforming mycotoxins, preventing the development of mycotoxicosis are required. The cell wall of *Saccharomyces cerevisiae* has become the focus of numerous studies and is used as AMA, because it has high binding capacity and adsorption of mycotoxins. The aim of this study was to evaluate the effectiveness of AMA based on yeast cell wall (YCW) in 36 prepubertal Topigs gilts experimentally intoxicated with ZEA. Rice contaminated with ZEA produced by *F. graminearum* was added to achieve a concentration 0.25 mg/kg ZEA in feed. A randomised blocks' design was used and treatments consisting of: T01, basal diet (BD); T02, BD + 0.2% AMA; T03, BD + 0.25 mg/kg ZEA; and T04, BD + 0.25 mg/kg ZEA + 0.2% AMA. Body weight, feed conversion, weight gain, feed intake, vulvar volume, relative weights of liver, total reproductive tract (uterus, ovarian, vagina set) and reproductive tract length were calculated. No statistically significant differences in performance parameters body weight, feed consumption and feed conversion were observed (P≤0.0001). There was difference (P≤0.0001) in reproductive parameters, where gilts from T02 had the highest average in vulvar volume and weight of the reproductive tract. There was no statistically significant difference (P≤0.0001) between the treatments for relative liver weight. ZEA showed a great oestrogenic action at these concentrations. AMA was effective at a concentration of 0.2% (2 kg/ton) to prevent the oestrogenic effects of 0.25 mg/kg ZEA in gilts.

P33: Development of alginate and alginate-chitosan microparticles containing *Saccharomyces cerevisiae* produced by external gelation followed by a drying process

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Encapsulation of probiotic microorganisms has been used to increase yeast survival and viability, promoting the protection against external factors during storage, passage and controlled release in the animal digestive tract. The technique mobilises the microorganisms in an encapsulating material, generating particles of smaller diameter that are able to maintain their structure even under adverse environmental conditions. Among materials studied, hydrogel formed from sodium alginate is considered an excellent microencapsulation system because of its characteristics, such as being non-toxic, mechanically strong and stable in acidic media. Drying of the microparticles could increase their application and stability. However, few studies reported on drying process and particles characterisation.

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Therefore, the objective of this work was to develop alginate and alginate-chitosan microparticles as protectors of *Saccharomyces cerevisiae*. The yeast cells were grown in YPD at 30°C. The obtained suspension was centrifuged and the precipitate was added to the sodium alginate solution (2%). The microparticles were formed by external gelation by dripping alginate in a solution of calcium chloride (2%), inducing polymer chains crosslinking. For increasing the strength and stability of the formed microparticles, a coating of their pores surface was performed with chitosan (1%). Then, the formed microparticles were dried in a chamber with air circulation (30°C). Characterisation of wet and dried microparticles, with and without chitosan, was carried out in terms of moisture content, morphology and microencapsulation efficiency. The wet particles had a moisture content of about 97% and 95% for alginate and alginate-chitosan systems, respectively. After drying, microparticles showed a moisture content of around 13% for both systems. Microparticles showed an irregular and a wrinkled surface. There was no log reduction in the cell concentration in the free-form and microencapsulated yeast (in wet form), whereas the drying showed one log cycle reduction in alginate microparticles and two log cycles in those coated with chitosan. The results are promising and increase the possibility of the use of sodium alginate as a protective material for probiotic cultures.

**P34: Use of probiotic bacteria or yeast in the control of ETEC infections: in vitro investigation of their inhibitory potential**

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Enterotoxigenic *Escherichia coli* (ETEC) are a leading cause of traveller's diarrhoea and infant diarrhoea mostly in developing countries. The main virulence traits of ETEC are the production of adhesins that promote the attachment of bacteria to host enterocytes, allowing them to colonise the small intestine, and the secretion of enterotoxins that disrupt fluid and electrolytes homeostasis leading to watery diarrhoea. Treatments are mainly symptomatic and can involve antibiotherapy. Given the rise of antibiotic resistance worldwide, there is an urgent need for the development of new preventive strategies for the control of ETEC infections. Among them, a promising approach is the use of probiotic strains. The aim of this study was to investigate the inhibitory properties of various bacteria and yeast probiotics against ETEC strains isolated from humans. Complementary *in vitro* assays were used to assess the ability of these probiotics to inhibit ETEC growth in LB culture media and prevent pathogen adhesion to mucus layer and intestinal Caco-2 cells. Our results indicate that probiotic strains can show beneficial properties against ETEC through competitive exclusion and inhibition of adhesion and that these effects are highly strain-dependent. The next step of this study will aim to assess the antagonistic properties of the best probiotic strains against ETEC survival and virulence in human simulated digestive conditions by using relevant in vitro models, such as TIM (TNO gastrointestinal model) or the SHIME (simulator of the human intestinal microbial ecosystem). Complementary assays will be also performed in Caco-2 cells to investigate the immunomodulatory properties of the probiotics.

**P35: Use of the gastrointestinal TIM system to promote understanding of enteric pathogen behaviour in the infant GIT: application to the evaluation of probiotics as an alternative strategy for infection control**

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Enterohemorrhagic *Escherichia coli* (EHEC) are major foodborne pathogens that constitute a serious public health threat, mainly in young infants where they cause the life-threatening haemolytic uraemic syndrome. The main virulence determinant of EHEC is the production of Shiga toxins (Stx). As no
specific treatment is available and as antibiotic therapy has worsened clinical outcomes, alternative strategies using probiotics are under consideration. Survival and virulence of EHEC strains in the human gastrointestinal tract (GIT) are key factors in bacterial pathogenesis but remain poorly understood, particularly in infant, owing to a lack of relevant model. The aim of the present study was to use a new infant protocol (6 months – 2 years) in TIM (TNO gastrointestinal model), which is the most complete simulator of the human upper digestive tract, for a comparative study of EHEC O157:H7 survival and virulence under adult and infant digestive conditions. Survival kinetics in the in vitro digestive tract were determined by plating, while bacterial viability was assessed by flow cytometry. Expression of stx genes was followed by RT-qPCR and the production of Shiga toxins was measured by ELISA. A significant higher number of cells in a better physiological state was found in the ileal effluents of infants compared to adults. The stx genes were over-expressed under infant conditions compared to the adult ones, in accordance with a significantly higher toxin production. Our results show for the first time that differences in digestive physicochemical parameters of the upper GIT may partially explain why infants are more susceptible to EHEC infections than adults. Such data are essential for a full understanding of EHEC pathogenesis and would help in designing novel therapeutic approaches, particularly in the infant high-risk population. The next step of this work will be to use the TIM system to study the ability of probiotic strains to inhibit EHEC growth and down-regulate the expression of virulence genes in the infant GIT. The effect of the food matrix or galenic form on the antagonistic properties of probiotics could also be assessed in the in vitro model.

P36: The possibility of culturing obligatory anaerobic Faecalibacterium prausnitzii in oxygenated environments

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Faecalibacterium prausnitzii is a dominant beneficial bacterium in the human gut, which is strictly anaerobic and accounts for 5-15% of the total number of bacteria in the gut. F. prausnitzii is one of the main butyrate producers in the gut, has proven anti-inflammatory properties and its numbers are reduced in patients with inflammatory bowel disease, especially Crohn’s disease. Previously it has been shown that F. prausnitzii has the unique ability to use riboflavin (vitamin B2) as an extracellular electron transporter, which will make it possible for it to tolerate limited amounts of oxygen [Khan et al., 2012. Antioxidants & Redox Signaling 17: 1433-1440]. Even though this oxygen tolerance provided by riboflavin is limited and will not facilitate the aerobic growth of this obligatory anaerobic bacteria, it can help F. prausnitzii to produce current in the microbial fuel cell (MFC). The most difficulty in anaerobic culture is the sensitivity of the beneficial bacteria to the oxygen. Previous research has shown the possibility of the aerobic growth of some strains of anaerobic bacteria with the help of ascorbic acid (vitamin C) in solid media with agar [La Scola et al., 2014. European Journal of Clinical Microbiology & Infectious Disease 33: 1781-1783]. However, the possibility of growing F. prausnitzii outside of the anaerobic chamber especially in broth media stays elusive yet. Our results showed a strong effect of ascorbic acid on the growth of F. prausnitzii in oxygenated environments, such as open air. This indicated the possibility of culturing this bacterium outside of the anaerobic chamber with different amounts of ascorbic acid and making it possible to perform the complete cycle of inoculation and growth in the aerobic environment. However, unlike riboflavin, the current production in MFC with ascorbic acid was independent of F. prausnitzii, due to the similar redox potentials of ascorbic acid and oxygen. This indicates the differences in the ways that riboflavin and ascorbic acid could help F. prausnitzii tolerate oxidative stress. Since our culturing was in broth medium and not in the agar tubes, which are limited in their applications, it now provides the opportunity for developing new techniques for host-microbiota interaction as well as the possibility for large-scale production of F. prausnitzii biomass. In conclusion, our results shed a new light on the role of ascorbic acid as an antioxidant, which seems to have a superior effect compared to riboflavin in protecting the strictly anaerobic F. prausnitzii from oxidation in the open air environment. Acknowledgements. Mehdi Sadaghian Sadabad was supported by DSM Nutritional Products Ltd., Basel, Switzerland.
P37: Survival of *Bifidobacterium longum* BB-46 in a fermented soy beverage supplemented with acerola by-product under *in vitro* gastrointestinal stress

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The fruit processing industry generates a huge amount of waste (seeds, peels, and other parts) that is disposed in the environment, becoming a source of pollution. However, fruit by-products from acerola present significant levels of dietary fibres that may confer functional properties to foods, leading to potential increase of intestinal beneficial bacteria, including *Bifidobacterium* spp. Therefore, bearing in mind that acerola by-product may support the growth of probiotic bacteria, the aim of this study was to evaluate the effect of acerola by-product supplementation on the probiotic survival in a fermented soy beverage under simulated gastrointestinal stress. Two formulations of soy beverage fermented by probiotic (*Bifidobacterium longum* BB-46) and starter (*Streptococcus thermophilus* TH-4) strains were evaluated: SF1 (control) and SF2 soy beverage with acerola by-product powder (2 g/100 ml). The beverages were stored at 4°C and the probiotic survival was performed at the beginning (day 1) and at the end (day 28) of storage, using genera-specific quantitative real-time PCR combined with propidium monoazide (PMA-qPCR). Initial BB-46 populations were 7.7 (day 1) and 8.0 (day 28) log cfu equivalents/ml for SF1, and 7.5 (day 1) and 8.3 (day 28) log cfu equivalents/ml for SF2. After the gastric phase (2 h, pH−2), BB-46 presented a higher survival in SF2 than in SF1 (P<0.05). The mean survival rates varied from 76.7% (day 1) to 73.1% (day 28) in SF1, and from 88.0% (day 1) to 82.6% (day 28) in SF2. After the enteric phases (6 h, pH−7), BB-46 mean survival rates were 95.4% (day 1) to 79.7% (day 28) for SF1, and 91.5% (day 1) to 76.4% (day 28) for SF2. Therefore, although the presence of acerola by-product in soy beverage has conferred a protective effect on BB-46 during the gastric phase of the assay, it did not prevent the decreased survival after the enteric phase. Our findings suggest that the protective effect of acerola by-product was not significant for the probiotic strain tested.

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P38: Neuro- and immunological effects induced by non-digestible oligosaccharides during early life

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Prebiotic fibres have immunomodulatory properties by changing the intestinal microbiome composition. Recent studies have shown that changes in microbiota composition also affect brain development and function. In a dietary intervention study with short chain galacto- and long chain fructo-oligosaccharides (scGOS:lcFOS), we investigated the effects of changes in microbiome metabolism on immuno- and neuromodulation in the brain of healthy BALB/c mice. Dietary supplementation with or without 3% scGOS:lcFOS (9:1) was tested in male BALB/c mice starting from day of birth (n=10 per group). Social, anxiety-like, and stereotypic behaviours were longitudinally studied by conducting social interaction, marble burying and self-grooming tests, respectively. In addition, colonic histone deacetylase (HDAC) activity, caecal fatty acids, mRNA expression of several brain markers and monoamine levels in the brain have been assessed. Male offspring receiving scGOS:lcFOS from day of birth and onwards showed changes in the serotonergic system. These neurological modulations were associated with behavioural changes; scGOS:lcFOS fed mice showed less anxious and repetitive behaviour during development and increased social interest in adulthood compared to mice fed control diet. Altered mRNA expression of astrocytic glial fibrillary acidic protein (GFAP) was detected in the brain of the scGOS:lcFOS group. Furthermore, in scGOS:lcFOS fed mice an enhanced mRNA expression of brain-derived neurotrophic factor (BDNF) was observed in the PFC. Moreover, relatively increased levels of butyric acid and decreased levels of valeric, isobutyric and isovaleric acid were observed in caecal content of the scGOS:lcFOS fed mice. No significant differences in colonic HDAC activity between control and scGOS:lcFOS fed mice were observed. However, a significant correlation between SCFA and HDAC activity was observed in control diet fed mice. In conclusion, dietary supplementation with...
scGOS:lcFOS changes the serotonergic system and behaviour in healthy mice. These neurological changes were accompanied by a suppression of astrocyte activation in the brain. In addition to this immune modulation in the brain, levels of the neuroprotective BDNF were enhanced. These neurological changes may be induced by the altered microbiota metabolism that was observed in these mice.

**P39: Dietary fibres educate macrophages for immune support**

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Non-digestible polysaccharide (NDP) which includes glucans, pectins, and arabinoxylan have showed immunomodulatory properties and support of intestinal homeostasis. The intestinal immune system is formed by epithelial cells and antigen processing cells (APCs). Macrophages, as one of the important APCs, have been proven to reside at the lamina propria as M1 subset and at the muscularis as M2 subset responsible for sampling bacteria or tissue protection, respectively. Here, we have setup an M1 and M2 subtype specific culturing in vitro and revealed specific gene expression markers for M1 (IDO1, LAMP3, CXCL11, and Dectin-2) and M2 (IL17RB, CD209, MRC1, and MGL) to characterise the differentiation towards these subtypes. Following NDP exposures, macrophages (M0) differentiated to a more NDP specific subtype compared to M1 and M2. In addition, microarray analysis revealed that NDPs exposures to M0 macrophages induced the expression of an impressive list of chemokine genes but not those encoding cytokines. Changes in gene expression could be confirmed by protein product detection based on Bio-plex multiplex analysis. To our surprise, CCL1, CCL5, and CCL20 were all more than 100 times higher expressed than medium control. Ingenuity Pathway Analysis revealed that, as a result of NDP exposures, phagosome formation and the antigen presentation pathway were inhibited. Indeed, based on functional assays it could be proven that these macrophage functions were reduced. Especially for Naxus (an arabinoxylan product), the phagocytosis function was lowered to 67% compared to medium and antigen processing was lowered to 36%. Research in which M0 were first differentiated into M1 or M2 and then exposed to NDP indicated that these macrophages are still have their plasticity to change towards other subset, including the NDP specific subset. This effect was associated with a change in phagocytosis efficiency and antigen processing function. Our data revealed that NDPs, which also might have a prebiotic activity, can have a direct immunomodulating activity by modulating macrophage plasticity (inflammatory macrophages and tolerogenic macrophages) and by that macrophage function, which is likely linked to the attraction of other immune cells to enhance priming of the defence. This indicates the specific immunomodulatory function of NDP and suggests implication for disease prevention and other clinical application. Acknowledgements. This research is part of the FibeBiotics EU project supported by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 289517.

**P40: Robustness of Bifidobacterium longum LMG 13197 encapsulated in lyophilised Vegetal BM 297 ATO-inulin lipid based synbiotic microparticles**

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Sensitivity of probiotics to technological and gastrointestinal stress factors, which compromises their much desired viability for them to confer beneficial effects in hosts, remains a huge challenge to the probiotic industry. Microencapsulation of probiotics for improvement of their robustness both in products during storage as well as after their ingestion is one of the strategies that have been explored to curtail this problem. Although various types of encapsulating materials have been extensively tested for their suitability for such applications, the same cannot be claimed for lipid-based food grade encapsulating materials. We have explored in this study the potential of a lipid-based excipient, Vegetal BM 297 ATO, alone or in combination with a prebiotic, inulin, for encapsulation of probiotics, using Bifidobacterium longum LMG 13197 as a test probiotic strain. Firstly, morphological properties, particle size distribution and encapsulation efficiency of the resulting microparticles were evaluated. Secondly, we evaluated survival of encapsulated B. longum during storage as well as in simulated gastrointestinal fluids and in yoghurt. Lastly, the effect of incorporation of the microparticles on physico-chemical properties of

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yoghurt was examined. The formulation containing Vegetal plus 2% (w/v) inulin resulted in better protection of *B. longum*. The microparticles produced were irregular, porous with concavities and contained high number of bacterial cells. Microparticles with or without inulin had average particle sizes of 33.4 μm and 81 μm with encapsulation efficiencies of 82% and 88%, respectively. Vegetal-inulin matrix protected *B. longum* during exposure to simulated gastric fluid (SGF) and subsequently released the viable cells into the simulated intestinal fluid (SIF). By the end of sequential exposure to these fluids encapsulated cells were >5 log units higher than their unencapsulated counterparts. Furthermore, Vegetal-inulin increased the shelf life of *B. longum* in powder form by 3 and 5 weeks at 25°C and 4°C, respectively. However, the use of Vegetal alone did not offer improved survival to *B. longum* cells. Vegetal-inulin matrix improved viability of *B. longum* in yoghurt by a week. There were no significant differences in pH values of yoghurts containing encapsulated cells throughout storage (P>0.05). However, significant differences in the lightness and yellowness of these yoghurts were recorded (P<0.05), although the total colour change was negligible. Vegetal-inulin encapsulation protected *B. longum* in gastrointestinal fluids and yoghurt with negligible effects to its appearance, thus can be used for fortification of yoghurt with probiotics. Thus Vegetal BM 297 ATO is a suitable vegetable lipid based food grade material for encapsulation of probiotics.

P41: Prenatal caprine milk oligosaccharide consumption improve development and colonic microbiota in pups at weaning

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The composition of the gastrointestinal (GIT) microbiota (commensal vs. detrimental), particularly in early life, influences the development of metabolic diseases later in life. The maternal microbiota is the main source of bacteria colonising the infant GIT and can be modified by dietary prebiotics, such as milk oligosaccharides. Caprine milk contains oligosaccharides structurally similar to human milk oligosaccharides, known to stimulate the development and maturation of the neonate’s GIT and bacterial colonisation. However, important differences in the profile of goat and human milk oligosaccharides have also been described. The impact of these different milk oligosaccharide profiles on the GIT microbiota and host physiology have been poorly explored. Our objective was to determine the effects of prenatal consumption of prebiotic caprine milk oligosaccharides (CMO) on the large intestine of female mice, milk composition and offspring development. C57BL/6 mice were fed either a control diet, CMO diet, or galacto-oligosaccharide (GOS) diet from mating to weaning. From weaning, half of the pups nursed by CMO, GOS or control-dams were fed the control diet for 30 days. CMO or GOS-fed dams had increased colon length and milk protein concentration compared to control-fed dams. At weaning, pups from CMO-fed dams had increased body weight and colon length, increased proportions of colonic *Bifidobacterium* spp. and increased concentration of caecal butyric acid compared to the pups from control-led dams. Thirty days after weaning, pups from CMO-fed dams had increased visceral fat weight compared to pups from control-fed dams. Metabolite profile of the blood plasma showed increased (2 fold) lysophosphatidylcholine (LPC) (20:4) in dams and pups 30 days post weaning and decreased (4.5 fold) LPC 16:0 in pups at weaning. In conclusion, the consumption of CMO by the dams during gestation and lactation improved the development of the pups, and the relative abundance of bifidobacteria in the colon, at weaning. Future work would determine/examine the effects of maternal CMO consumption on lipid metabolism.

P42: Novel culturing system for isolating commensal gut bacteria

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The human body contains a large and highly diverse community of microorganisms in its intestines. This gut microbiota benefits the host by providing nutrients, by competing with pathogenic bacteria, and by stimulating the host’s immune system. Aberrant compositions of the gut microbiota have been linked to several diseases. Our aim is to identify beneficial gut microbiota that could restore microbial
imbalances relevant for prevention or treatment of disease. Unfortunately, culturing and identifying beneficial anaerobic bacteria can be troublesome and elaborate. We established a broad-range culturing method using a variety of carbohydrate substrates, which allowed us to culture isolates that belong to the most abundant bacterial species in the human gut. In total, we isolated 800 bacteria from eleven healthy volunteers. We used matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) to identify the bacteria to genus and/or species level. Since MALDI-TOF/MS can only identify species of which there is a reference spectrum present in the database, we used 16S rRNA sequencing to identify isolates that could not be identified by MALDI-TOF/MS. As expected, we observed large differences between the gut microbiota of the eleven individuals. The human gut is dominated up to 90% by two phyla, namely Bacteroidetes and Firmicutes. We managed to identify many representatives of these two phyla, as well as bacteria belonging to Actinobacteria, Proteobacteria, and Fusobacteria. By fermenting carbohydrates gut microbes are able to produce short chain fatty acids (SCFAs) that are beneficial for the host. Especially butyrate is an important energy source for the colonocytes. Thus butyrate-producing bacteria are of great interest. Many butyrate-producing bacteria belong to Clostridium clusters IV and XIVa+b. We managed to culture a large number of bacteria belonging to these two clusters including, Faecalibacterium prausnitzii. Interestingly, we identified bacteria that could grow on all tested carbohydrates. Most butyrate-producing bacteria were isolated either from glucose- or pectin-supplemented media. Fewer were isolated from mucin-supplemented media, with the most frequent species being Dorea longicatena. In conclusion, we established a novel culturing method, which enables the isolation of potentially beneficial gut microbes, such as butyrate-producing bacteria, with great efficiency.

**P43: The impact of polyols on oral microbiome of Estonian schoolchildren**


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The oral microbiome is comprised of about 1000 microbial species and has significant impact on both oral and general health. Polyols have been promoted as potential sugar substitutes in the prevention of oral diseases. A three-year intervention study including 485 Estonian first and second grade primary school children was performed to study the effect of daily consumption of erythritol, xylitol, and sorbitol candies (200 days/year; 7.5 g polyols/day) on caries development in mixed dentition. At the end of the intervention period, time to enamel/dentine caries development, dentine caries development, increase in caries score, and dentist intervention was significantly longer in the erythritol group as compared to the sorbitol treatment. The aim of this study was to reveal in a subcohort of this group the effect of the carbohydrates on the salivary microbiome and some indicator bacteria of caries activity or early onset periodontitis. The study group included 90 children (age at the end of trial 11.3±0.8 years). All children were randomly allocated into 3 polyol groups (n=30). Microbial communities in the saliva of the children were profiled using Illumina HiSeq 2000 sequencing and quantitative PCR. The dominant phyla in saliva, independently of the type of polyol consumed, were Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria, and the most prevalent families were Neisseriaceae, Streptococcaceae, Prevotellaceae and Veillonellaceae. However, 3-year consumption of erythritol, xylitol or sorbitol did induce clear differences in the salivary microbiome. According to PERMANOVA analysis and principal component analysis the microbiome of erythritol group significantly differed from that of the other groups. The relative abundance of Veillonella and Streptococcus increased while that of Gemella and Neisseria decreased after erythritol treatment. The prevalence of caries-related mutants streptococci and the clinical caries markers (DMFT, DMFS, FT) were also the lowest in the erythritol group. In conclusion, the impact of daily consumption of sorbitol or xylitol on the salivary microbiome was found to be rather similar while different from that of erythritol, the latter being associated with the lowest prevalence of caries-related mutants streptococci and the lowest levels of clinical caries markers. This is the first study revealing the effect of polyols on the salivary microbiome and their association with oral health applying Illumina sequencing.
P44: Prebiotic effect of xylo-oligosaccharides determined by a polyphasic study in the i-screen system containing ex-vivo human gut microbiota

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In the 7th Framework EU-project Lignofood, the prebiotic effect of different hemp-derived xylo-oligosaccharides (XOS) with different degree of polymerisation and ratio of xylobiose in comparison to other existing prebiotics inulin and/or GOS has been studied. Different XOS types show bifidogenic effects in the i-screen platform, an intestinal screening multi-well platform simulating the human colonic microbiota conditions. Different types of XOS yield an increase in beneficial microbes, particularly Bifidobacterium spp. The effects are oligo-sugar type dependent as well as concentration dependent. At appropriate concentrations beneficial microbial metabolites, mainly butyrate, are produced. Moreover, a decrease in opportunistic pathogens such as Clostridium spp. and Enterobacteriaceae (E. coli/Shigella) was observed. By applying metatranscriptome analysis of the XOS exposed colonic microbiota, different XOS types were shown to affect gene expression of the colonic microbiota. The metatranscriptome was determined and compared to the metatranscriptome of the non-exposed negative control exposure without these oligosaccharides, thereby showing a significant difference. Differential expression of genes involved in xylose metabolism in bifidobacteria was observed in the presence versus absence of various types of XOS. This polyphasic study in the i-screen platform shows the effects of XOS on the human colonic microbiota and supports the great potential of XOS as a prebiotic.

P45: Faecal microbiota manipulation prevents dysbiosis and alcohol-induced liver lesions in mice

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Alcoholic liver disease (ALD) is a leading cause of liver failure and mortality. In humans, severe alcoholic hepatitis is associated with key changes of intestinal microbiota (IM), which influences individual sensitivity to develop advanced ALD. We used the different susceptibility to ALD observed in two distinct animal facilities to test the efficiency of two complementary strategies (faecal microbiota transplantation and prebiotic treatment) to reverse dysbiosis and prevent ALD. Mice were fed alcohol in two distinct animal facilities within a Lieber DeCarli diet. Faecal microbiota transplantation was performed with fresh faeces from alcohol-resistant donor mice to alcohol-sensitive receiver mice three times a week. Another group of mice received pectin during the entire alcohol consumption period. Ethanol induced steatosis and liver inflammation, which were associated with disruption of gut homeostasis, in alcohol-sensitive but not alcohol-resistant mice. IM analysis showed that the proportion of Bacteroides was specifically lower in alcohol-sensitive mice. Principal coordinate analysis showed that the IM of sensitive and resistant mice clustered differently. We targeted IM using two different strategies to prevent alcohol-induced liver lesions: (i) pectin treatment, which induced major modifications of the IM; and (ii) faecal microbiota transplantation, which resulted in an IM very close to that of resistant donor mice in the sensitive recipient mice. Both methods prevented steatosis, liver inflammation, and restored gut homeostasis. In conclusion, manipulation of IM can prevent alcohol-induced liver injury. The IM should be considered as a new therapeutic target in ALD.
Polyphenols, such as anthocyanins, are a type of phytochemical which add colour to fruit and vegetables and are known to have anti-oxidant and anti-inflammatory properties. Researchers have identified the genes that regulate the anthocyanin-specific part of a polyphenol pathway and have selectively bred this into several lines of Royal Gala apple through New Zealand plant breeding programmes. These apples have naturally high anthocyanin and other polyphenol content in the skin and flesh. When tested in a mouse model for anti-inflammatory and anti-oxidant properties, these apples showed promise not only in reducing inflammation but also in influencing the large bowel intestinal microbiota. The effects of these apples on human immune function and faecal microbiota composition was subsequently studied using a randomised, placebo controlled, cross-over trial. Twenty-six healthy adult volunteers were asked to consume daily portions of the anti-oxidant enriched (test) apple or a placebo control apple for two weeks followed by a one-week washout and further two-week crossover period. During the study the volunteers provided faecal samples for microbiota analysis and blood samples for peripheral blood mononuclear cell (PBMC) gene expression analysis. Analysis of the faecal microbiota showed subtle differences in the microbiota of subjects that were fed the different apples, with significant reductions (P<0.05) in relative abundances of Streptococcus, Ruminococcus, Butyricicoccus, and Roseburia in those fed the test apple. Interestingly, the archaeon Methanobrevibacter was increased in subjects fed the test apple. However, the overall prevalence of this eukaryote was low; 0.022% in the test apple group vs. 0.009% in the placebo group. In contrast, changes in PBMC gene expression were more prominent with 529 mRNA transcripts differentially expressed between test and placebo apple groups (FDR<0.05, fold change >1.5). Pathway analysis showed these genes were primarily involved in cell motility, cell cycle, and immune regulation pathways, such as ‘regulation of chemotaxis’, ‘cellular response to cytokine stimulus’, and ‘response to bacterium’. To date, results from this study show that an anti-oxidant enriched apple can potentially influence immune function compared to non-enriched apples, and these changes may be associated with differences in the faecal microbiota. However, the mechanistic links between changes in the gastrointestinal microbiota and PBMC gene expression in this study remain to be determined.
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