INTERNATIONAL™
MILK GENOMICS
CONSORTIUM

12th International Symposium on

Milk Genomics and Human Health

"Translation of Omics into Production and Health"

October 26-28, 2015

Amora Hotel Jamison, Sydney, Australia

Dear Participant,

Welcome to Sydney for the 12th International Symposium on Milk Genomics and Human Health. This is the second time the symposium has been held in Sydney, and the third time in Australia. This time we will explore the diverse sciences where milk intersects with genomics, nutrition and metabolism.

The Symposium is always a highlight of the year when old and new colleagues bring together the advances that have been happening in their own specialist fields for a truly multi-disciplinary perspective in milk science. It provides a unique snapshot of the most recent advances and current activities, with plenty of opportunity for cross-fostering ideas and developing networks between researchers and industry.

View of the Sydney Opera House with Harbour Bridge.

The organizers are also delighted to welcome you to the city of Sydney – the largest city in Australia. Sydney is a metropolis of over 4.5 million people. The venue for our symposium sits above the birthplace of European settlement, and is adjacent to the harbor and downtown area.

We have planned various social events as part of the symposium. On Monday afternoon from 6:00 pm to 8:00 pm, we invite you to the poster reception, and on Tuesday evening from 7:00 pm onwards, we invite you to participate in an evening cruise on the harbor. These events are limited to paid and registered guests only.

For those who wish to extend their stay, I would be pleased to offer you a visit to my home campus, The University of Sydney, which is only 10-15 minutes from our venue, or for those interested in what's happening on the farm, to visit our experimental dairy farms, about one hour's drive away.

We look forward to an intensive three days of interactive science and fun that these symposiums have come to represent. Welcome to IMGC 2015.

The University of Sydney.

Kind regards,

Peter Williamson.
Local Convener on behalf of the Organizing Committee

Program Schedule

Monday - October 26, 2015

9:00 am **Welcome:** *Peter Williamson, Faculty of Veterinary Science, University of Sydney, Australia*

9:15 am **Opening: Milk Genomics: How Lactation Can Teach Us about Lifelong Health**
Bruce German, Foods for Health Institute, University of California, Davis, USA

9:45 am **Emerging Hot Topics in Milk Science**
Danielle Lemay, University of California Davis Genome Center, Davis, USA

10:30 am **Coffee Break**

11:00 am **Cow Milk-Derived Extracellular Vesicles Possess Immunoregulatory - and Anti-Inflammatory Activities and Oral Administration Ameliorates Experimental Arthritis**
Fons van de Loo, Experimental Rheumatology, Radboud University Medical Center, Nijmegen, The Netherlands

11:40 am **Supplementation with *Bifidobacterium Infantis* in Combination with Bioactive Milk Components to Alter Microbial Composition in Children with Autism**
Megan Sanctuary, Department of Nutrition, University of California, Davis, USA
Student Travel Award Recipient

12:00 pm **Metabolic Programming in Utero - How a Mother's Diet Shapes Her Child's Future**
Jennie Brand-Miller, School of Molecular Biosciences and Boden Institute of Obesity, Nutrition and Exercise, University of Sydney, Australia

12:40 pm **Lunch**

2:00 pm **Sensor Derived Health Phenotypes for Improved Dairy Cattle Management and Genetic Selection**
Cameron Clark, Dairy Science Group, The University of Sydney, Camden, Australia

2:40 pm **Harnessing the Benefit of Functional Genomics Information When Combining Two Unrelated Populations for Genomic Selection**
Antonio Reverter, CSIRO Agriculture Flagship, Queensland Bioscience Precinct, St. Lucia, Australia

3:20 pm **Collection of Data for the Genetic Improvement of Health Traits in Australian Dairy Cattle**
Mary Abdelsayed, Faculty of Veterinary Science, The University of Sydney, Camden, Australia
Student Travel Award Recipient

3:40 pm **Coffee Break**

4:00 pm **Human Breast Milk miRNA, Maternal Probiotic Supplementation and Atopic Dermatitis in Offspring**
Melanie Rae Simpson, Department of Public Health and General Practice, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
Student Travel Award Recipient

4:20 pm **Freed Milk Glycan Monomers Create a Niche for Gut Pathogens in Vivo**
Steven Frese, Department of Food Science and Technology, University of California, Davis, USA

5:15 pm – 6:15 pm Joint Scientific Advisory Council/Steering Committee Meeting (closed session)
6:00 pm – 8:00 pm Poster Reception

Tuesday - October 27, 2015

8:30 am **Novel Genetic Makers Affect Rennet-Induced Milk Coagulation**
Vivi Gregersen, Department of Molecular Biology and Genetics, Aarhus University, Tjele, Denmark

9:10 am **Not Sex but Birth Weight of the Calf Affects Its Mother's Milk Production**
Yu Wang, Animal Breeding and Genomics Centre, Wageningen University, The Netherlands

9:50 am **An Evaluation of Extended Lactation in the Australian Dairy Industry**
Peter Thomson, Faculty of Veterinary Science, The University of Sydney, Camden, Australia

10:30 am **Coffee Break**

10:50 am **Characterization of Regulatory Elements Associated With Mammary Gland Function in Mouse and Cow**
Monique Rijnkels, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, USA

11:30 am **Maternal Mechanisms of Energy Mobilisation during Lactation in Mouse Models**
Peter Williamson, Faculty of Veterinary Science, The University of Sydney, Australia

12:10 pm **A Role for Sim2s in Elongation Regulation of Milk Protein Gene Transcription in the Mammary Gland**
Jessica Elswood, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, USA
Student Travel Award Recipient

12:30 pm **Lunch**

2:00 pm **Peptidomics of Human and Bovine Milk Revealing Novel Antimicrobial Peptides**
David Dallas, Department of Food Science and Technology, University of California, Davis USA

2:40 pm **Survival of New Probiotic Strains with Anti-Inflammatory & Anti-Obesity Effects Used in Non-Fat Yogurt and Low-Fat Cheddar Cheese Making**
Véronique Demers-Mathieu, Dairy Science and Technology (STELA), Institute of Nutrition and Functional Foods (INAF), Department of Food Sciences and Nutrition, Laval University, Quebec City, Canada
Student Travel Award Recipient

3:00 pm **Molecular Determinants of Milk Lactoferrin as A Bioactive Compound in Early Neurodevelopment and Cognition**
Bing Wang, School of Medicine, Xiamen University, China, and School of Animal and Veterinary Sciences, Charles Sturt University, Sydney, Australia

3:40 pm **Coffee Break**

4:00 pm **Packaging of Endogenous and Exogenous Milk miRNAs in the Mammary Gland: New Opportunities for Milk as a Therapeutic**
Meagan Gillespie, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia

4:40 pm **Comparative Analysis of Milk miRNA**
Christophe Lefevre, The Walter and Eliza Hall Institute of Medical Research, and Melbourne University, Australia

7:00 pm **Group Dinner: Sydney Harbor Dinner Cruise**

Wednesday – October 28, 2015

9:00 am **Effects of Maternal Periconception Overnutrition on the Adipose Tissue Epigenome of Offspring**
Ross Tellam, CSIRO Agriculture, Brisbane, Australia

9:40 am **The Nature of Nutrition: An Integrative Framework from Animal Adaptation to Human Obesity**
Stephen J. Simpson, Charles Perkins Centre, The University of Sydney, Australia

10:20 am **Modulation and Programming of Immunity and Intestinal Microbiota through Early Life Supplementation with Human Milk Oligosaccharides in an Autoimmune Mice Model**
Belinda van't Land/Dr. Bernd Stahl, Nutricia Research, Utrecht, The Netherlands

11:00 am **Coffee Break**

11:20 am **Homeostasis and the Composition of Mammalian Milk**
David Raubenheimer, Charles Perkins Centre, The University of Sydney, Australia

12:00 pm **Human Complex Milk Lipids: Concentrations, Benefits and Implications for Human Infant Formula**
Alastair MacGibbon, Fonterra Co-operative Group, Palmerston North, New Zealand

12:40 pm **Metabolomic and Proteomic Insights into Milk Biochemistry: Genetic, Seasonal and Feed Induced Changes in Milk**
Simone Rochfort, AgriBio, Centre for AgriBioscience, Biosciences Research, DEDJTR, and School of Applied Systems Biology, La Trobe University, Bundoora, Australia

1:20 pm **Closing Remarks**

1:30 pm **Lunch**

List of Speakers

Mary Abdelsayed, Ph.D.
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Mary Abdelsayed is originally from Sydney and did her undergraduate and postgraduate studies at the University of Sydney. She has recently submitted her Ph.D. which looked at the genetics of extended lactation in Australian dairy cattle on pasture based systems. For the past 10 months she has been living in Melbourne after being offered the position with Holstein Australia as the project manager and researcher working on a Gardiner funded project 'Health data for healthy cows', where she is exploring the ability to estimate genomic breeding values for common health disorders using farm collected data.

Jennie Brand-Miller, Ph.D., AM, FAIFST, FNSA
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B.Sc. (Food Science and Technology), Ph.D., A.M., Fellow of Nutrition Society of Australia, Fellow of Australian Institute of Food Science and Technology, Personal Chair in Human Nutrition in the School of Molecular Bioscience and Charles Perkins Centre at the University of Sydney. Brand-Miller is an academic and researcher with 30 years experience in a variety of research techniques including dietary intervention studies in pregnancy and animal models, observational cohorts, randomized controlled trials, systematic reviews and meta-analysis. Her research has focused on all aspects of carbohydrates—diet and diabetes, diet and pregnancy, insulin resistance, lactose intolerance and oligosaccharides in infant nutrition, with more than 250 scientific publications. She currently leads the Australian arm of the world's largest diabetes prevention study (the PREVIEW study) with responsibility for its dissemination and exploitation. She plays a major role health promotion in Australia as President of the Glycemic Index Symbol Program (www.gisymbol.com). Her popular books have translated research to practice and made the GI a household word, with over 3.5 million copies sold worldwide in 12 languages. She Chairs the University of Sydney's Disability Action Plan committee, the Shepherd Centre's Research Advisory Committee and is a past-President of the Nutrition Society of Australia and immediate-past Chair of the National Committee for Nutrition of the Australian Academy of Science.

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As a senior research fellow in the University of Sydney's Dairy Science Group, Cameron is heavily involved in research associated with the integration of the farming system and technology such as automatic milking systems.

In particular, Cameron's experience as a farm systems scientist in New Zealand is helping to pave the way to turn the increasingly large amounts of data generated by dairy systems into meaningful information to improve farm productivity, profitability and animal health.

Cameron works closely with farmers and domestic and international research institutes to develop novel systems and solutions for current and emerging industry needs and issues.

David Dallas, Ph.D.
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After receiving graduating from Rice University in 2008, David Dallas earned his Ph.D. in Nutritional Biology at the University of California, Davis under Dr. Bruce German. Since July of 2012, David has been a post-doctoral fellow at UC Davis. In January, 2016, David will be an Assistant Professor of Nutrition at Oregon State University. His research employs state-of-the-art mass spectrometry, bioinformatics and functional assays to understand milk protein digestion in infants and identify functional peptides.

Veronique Demers-Mathieu, Ph.D.
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After a B. Sc. in Food Science (2009) and a M. Sc. in Biochemistry (2011) at Laval University (QC, Canada), Dr. Demers-Mathieu did a Ph.D. in Food Microbiology and Molecular Medicine (2015) at Agriculture and Agri-Food Canada and Quebec Heart and Lung Institute (QC, Canada). Her doctoral thesis work was focused on viability, technological, anti-inflammatory and anti-obesity properties of new probiotic strains in dairy products. She will work as Post-doctoral researcher at Oregon University (OR, US) in the lab of Dr. Dave Dallas from January 2016.

Jessica Elswood
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Jessica Elswood is a member of the Porter Lab in the Veterinary Integrative Biosciences Department at Texas A&M University in College Station, Texas. Her work focuses on mammary gland development and the regulatory networks thereof. Stemming from her background in production livestock, Jessica has a passion for contributing to the dairy science industry and the advancement of our understanding of lactation.

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Steven Frese is a Postdoctoral Fellow at the University of California Davis in the Department of Food Science and Technology, supported by a Fellowship from the National Institutes of Health. Dr. Frese studies how the gut microbiome of nursing mammals is shaped by milk, how the gut community contributes to digestion, and how problematic populations in the gut can be reduced by understanding this ecosystem in light of evolutionary and ecological principles.

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Bruce German received his Ph.D. from Cornell University, joined the faculty at the University of California, Davis in 1988, in 1997 was named the first John E. Kinsella Endowed Chair in Food, Nutrition and Health is currently Director of the Foods for Health Institute and professor, at University of California, Davis. His research interests include the structure and function of dietary lipids, the role of milk components in food and health and the application of metabolic assessment to personalizing diet and health.

Bruce and colleagues founded the milk genomics consortium in 2001 which he now co-directs with Johan von Arendonk and Peter Williamson (http://www.imgconsortium.org). Bruce and colleagues have published more than 350 papers on lipids and food, metabolism and metabolite measurements and food functions and patented various technologies and applications of bioactive agents. The research articles from the lab rank in the top 10 most cited in Agriculture at www.ISIhighlycited.com.

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Meagan Gillespie is a post-doctoral fellow at CSIRO Australian Animal Health Laboratory. She completed her Ph.D. through Deakin University and CSIRO in 2012 on the transcriptomic characterization of pigeon 'milk' production and its effect on young. Currently she is investigating the bio transfer of milk microRNAs from mother to baby and the potential of microRNAs as a new functional component of milk.

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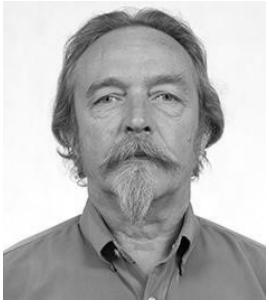
Vivi R. Gregersen is a Postdoc at Department for Molecular Biology and Genetics at Aarhus University. Her main interests are within Milk Genomics where her focus is on genome-wide association studies and method development for identification of causal mutations affecting milk traits utilizing NGS sequencing techniques. She has a B.Sc. in Biology, a M.Sc.IT. in Bioinformatics and a Ph.D. in Genetics from Aarhus University.



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Christophe obtained a Doctorate degree in Biochemistry from the University of Montpellier in 1984. He went on to conduct post-doctoral research in Southern California (USC, UCSD, UCLA) and Japan (Tokai University). After leading bioinformatics software developments at Gentech in southern France, he joined Organon AkzoNobel, a pharmaceutical company in the Netherlands. In 2002 he moved to Australia, working at the Victorian Bioinformatics Consortium at Monash University and the CRC for innovative dairy products at the University of Melbourne. In 2008 he became Associate Professor of Bioinformatics at Deakin University and in 2015 he has joined the Division of Bioinformatics of the Walter+Eliza Hall Institute of Medical Research and the Peter Mac Callum Cancer Research Institute.

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Danielle Lemay is research faculty at the Genome Center of the University of California, Davis where her research program encompasses the "omics" of milk, mammary biology, and milk-oriented microbes. She is also the Executive Editor of the IMGC's monthly e-newsletter, "*SPLASH!* milk science update". She has a Ph.D. and M.S. in Nutritional Biology from UC Davis and a B.S. in Electrical Engineering & Computer Science from MIT.

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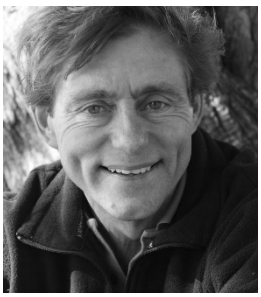
Alastair MacGibbon is a Principal Research Scientist at the Fonterra Research and Development Centre in Palmerston North, New Zealand. His research in milk lipids is wide ranging stretching from animal genetics, to production and through to the health effects with particular interest in structure-function relationships. Recently he has been focusing on the milk fat globule membrane components and their potential implications for health.



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David Raubenheimer is a nutritional ecologist, whose work includes both laboratory and field studies, on animals from insects and spiders to reptiles, fish, birds, and mammals including dogs, cats, giant pandas, several species of non-human primates and humans. In 1992 he graduated with a Ph.D. from Oxford University, and is currently the Leonard P. Ullman Chair of Nutritional Ecology and Nutrition Theme Leader at the Charles Perkins Centre, University of Sydney.

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Shortly after graduating from a B.Sc. in Veterinary Sciences in Barcelona, Spain, Antonio Reverter (Toni) was awarded an INIA/USDA scholarship and in 1990 moved to Colorado State University to pursue a double degree of M.S. in Statistics and a Ph.D. in Animal Sciences. In 1995, Toni joined the Animal Genetics and Breeding Unit (AGBU) at the University of New England in Armidale, NSW, Australia. At the AGBU, Toni performed computer programming and quantitative analyses related to BREEDPLAN, the Australian national genetic evaluation system for beef cattle. Since 2002, Toni has been based in Brisbane with CSIRO where he provides skills in computational and systems biology, quantitative and molecular genetics. In 2005, Toni was the recipient of the Eureka Prize for Bioinformatics Research. With a total of 173 publications (as of September 2015), Toni has an h-index of 30 and averaged 11 papers per year over the past 5 years. In 2010-11, Toni was awarded the Helen Newton Turner CSIRO Award. In 2013, Toni was appointed Research Team Leader of Computational and Systems Biology.

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Her research interests are milk protein gene regulation, genetic and epigenetic regulation in mammary gland development and disease. She trained at Leiden University, the Netherlands, with Pharming Group NV, and Baylor College of Medicine. She is currently at the department of Veterinary Integrative Biosciences, College of Veterinary Medicine & Biological Sciences, Texas A&M University. As part of the bovine genome consortium she led the lactation group for the annotation and analysis of the bovine genome.

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Dr. Simone Rochfort has a Ph.D. in chemistry and over 20 years' experience in both the pharmaceutical and agricultural research sectors. Her current focus is on metabolomics, metagenomics and proteomics for agricultural and human health outcomes. Dr. Rochfort leads the Molecular Phenomics team, a dedicated group of research scientists and students at AgriBio. The AgriBio institute is a joint venture between the Victorian State Government and LaTrobe University for agricultural outcomes.

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Megan received her M.S. in Physiology from the University of Illinois, USA, and is currently pursuing her Ph.D. in Nutrition from the University of California at Davis, USA. Her work in progress is focused on gaining a greater understanding of the gut-brain-immune axis in children with autism and how perturbations in gastrointestinal immune and microbial activity influence behavior. Future work will be aimed at designing personalized diets for optimization of health during critical periods of development.

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Melanie Simpson is a Ph.D. candidate at the Norwegian University of Science and Technology, Trondheim, Norway. Her doctoral work investigates miRNAs, bacteria and cytokines in human breast milk. More specifically, these breast milk components are being assessed as potential mediators of the observed preventative effect of probiotics, given to pregnant and lactating women, on the development of atopic dermatitis (eczema) in their children.

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Professor Stephen Simpson is Academic Director of the Charles Perkins Centre, a University of Sydney cross-faculty initiative aimed at researching and implementing cross-disciplinary approaches to alleviating the individual and societal burden of obesity, diabetes and cardiovascular disease, and is the Executive Director of Obesity Australia.

Stephen was born in Melbourne and grew up in Australia. After completing his undergraduate degree at the University of Queensland, he undertook his Ph.D. at the University of London. Simpson spent 22 years at the University of Oxford, first in Experimental Psychology, then in the Department of Zoology and the Oxford University Museum of Natural History, before returning to Australia in 2005 as an ARC Federation Fellow.

Stephen developed an integrative modelling framework for nutrition (the Geometric Framework), which was devised and tested using insects. This has since been applied to a wide range of organisms, from slime moulds to humans, and problems, from aquaculture and conservation biology to the dietary causes of human obesity and ageing. He has also revolutionised understanding of swarming in locusts, with research spanning neurochemical events within the brains of individual locusts to continental-scale mass migration.

In 2007 Stephen was elected a Fellow of the Australian Academy of Science, in 2013 was elected a Fellow of the Royal Society of London as "one of the world's foremost entomologists and nutritional biologists", and in 2015 was made a Companion of the Order of Australia "for eminent service to biological and biomedical science."

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Bernd Stahl, is R&D Director of Human Milk Research at Danone Nutricia Research: Early Life Nutrition in Utrecht, The Netherlands. He received his education and his Ph.D. as biologist at the Westfaelische Wilhelms-University Muenster, Germany. His research was focused on characterization of biologically relevant molecules obtained from human milk and other natural sources by mass spectrometry in connection with other analytical methods. He has a strong background for more than 20 years in human milk research with a significant contribution to research on specific prebiotic principle for early life nutrition. He was confered the Mattauch-Herzog Award of the German Society for Mass Spectrometry. Since 2008 he is chair of ILSI Europe Task Force Prebiotics. He is author and co-author of more than 100 scientific publications, reviews and book chapters. He is member of the American Society for Nutrition (ASN), American Society for Mass Spectrometry (ASMS), American Chemical Society (ACS), German Biochemical Society (GBM), German Society for Mass Spectrometry (DGMS), European Milk Bank Association (EMBA), International Milk Genomics Consortium (IMGC) and the International Society for Research in Human Milk and Lactation (ISRHML).

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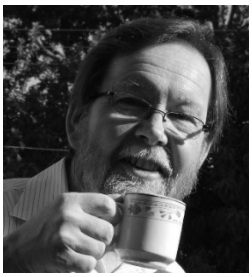
Dr. Ross Tellam is a Chief Research Scientist with CSIRO Agriculture and based in Brisbane, Australia. He gained his Ph.D. from the University of Queensland and has held positions at the Washington University School of Medicine in St. Louis and the John Curtin School of Medical Research at the Australian National University in Canberra.

His current research interests are focused on the identification of epigenetic modifications associated with development and maternal nutrition.



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Peter Thomson is an Associate Professor in Biometry in the Faculty of Veterinary Science, The University of Sydney. He has worked as an academic statistician, primarily in the agricultural and veterinary science sector. His research has covered a wide range including ecology, epidemiology, but the main focus been in statistical genetics. As well as this research role, a large component of his time involves training and consulting, across a range of levels.

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Fons van de Loo is a principle investigator at the department of Rheumatology at the Radboud university medical center in Nijmegen, the Netherlands. His group is dedicated to develop advanced biological therapies for arthritis by targeting or modifying the intercellular communication. Current research focus is to unravel the pathological role of extracellular vesicles in rheumatic diseases. As environmental factors are known risk factors of disease, the effect of food-derived vesicles (cow's milk) is also studied.

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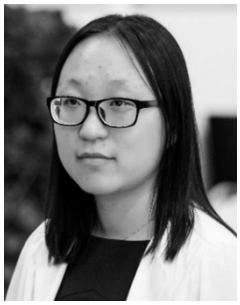
Professor Bing Wang received her Medical degree from Tianjin Medical University, China and her Ph.D. in Nutritional Science from the University of Sydney. She led *Nutritional Neurodevelopment Research* programs in academia and industry before joining Charles Sturt University in Dec 2012. Her research is focused on elucidating the molecular basis of how nutrients alter metabolic responses important in neurodevelopment from fetus to late life, and in translating basic science discoveries to application.



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Yu Wang, a Ph.D. candidate of Farm Animal Genetics and Breeding Group, University of Hohenheim. She just successfully finished her Master study in European Master in Animal Breeding and Genetics program in both Wageningen University and University of Natural Resources and Life Sciences, Vienna. Her research interest is quantitative genetics and future work will be mainly focused on genome-wide selection method in local cattle populations. This presentation is her thesis work during first year master study.

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Presentation Abstracts

Milk Genomics: How Lactation Can Teach Us about Lifelong Health

Bruce German, Foods for Health Institute, University of California, Davis, USA

J. Bruce German, Samara Freeman and Bethany Henrick
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Chronic and degenerative diseases from obesity to autoimmunity are epidemic around the world and will afflict more than half the world's population. Thus, the opportunities facing food development by improving human health are epic. Precision medicine is the emerging field that takes a more personalized approach to therapeutic intervention to address these diseases. However, these conditions arise gradually within individuals through imbalances of systemic processes (metabolism, physiology, immunity) for which traditional pharmacologic interventions using targeted small molecules are ineffectual. Rebalancing whole body processes through dietary mechanisms is ostensibly exactly what milk does for infants. Research over the past decade of the IMGC has documented that milk guides the appropriate development and function of whole body fuel metabolism, both innate and acquired immunity, physiological processes even tissue remodeling and neurological development. Yet, these potentially transformative discoveries have yet to be brought to commercial practice in the food marketplace. What are we doing wrong? The diabolical complexity of improving health as prevention as commercial opportunity is that efficacy must be demonstrated by mechanism of action within individuals. Thus, the path to commercial success will likely need to include mechanism based precision diagnostics paired to precision diets. The information age has brought the knowledge management tools to practice and it is now only necessary for scientific research and its translation to bring accurate measures of human health to practice in parallel with milk---based interventions. The development of the infant microbiome and its functions provides a tantalizing example of what such a future will look like.

Emerging Hot Topics in Milk Science

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In April 2012, the IMGC began publishing an e-newsletter, "*SPLASH!* milk science update," which features four articles on emerging topics in milk science each month—that's 48 new articles on milk science each year. By the time of the IMGC conference in 2015, we will have published 168 articles! This talk will reveal the most exciting milk science topics of the previous 12 months. It will also include a behind-the-scenes tour of "*SPLASH!* milk science update,": who are the current writers and editors, how topics are selected, and the basics of our publication cycle. The *SPLASH!* newsletter has helped to grow the IMGC: we have ~3,000 subscribers with ~500 people reading each newsletter. Nearly all traffic to the IMGC website is the result of *SPLASH!* content. The talk will also reveal milk science topics expected to emerge in the coming year and beyond. A vision for the future will be presented with suggestions solicited from the audience to further increase the impact of IMGC publications.

Cow Milk-Derived Extracellular Vesicles Possess Immunoregulatory – and Anti-Inflammatory Activities and Oral Administration Ameliorates Experimental Arthritis

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Recently extracellular vesicles that contain proteins and microRNAs (miRNA), are detected in human and cow milk but their biological function has not yet been described. We isolated extracellular vesicles (EVs) from commercially available semi-skimmed bovine milk by ultracentrifugation. The isolated EVs showed an average size of about 100nm using different techniques (DLS, NTA, AF4), and confirmed by electron microscopy. Proteomic analysis identified 64 proteins and some are characteristic for EVs (CD63, CD81, Rab-1b, actin, myosin-XIX), while others are precursors of secreted milk proteins (casein, Albumin, osteopontin). PCR analysis identified the presence of different miRNAs (e.g. mir-30a,-223,-92a) and also messenger RNA of milk

specific proteins (α-casein, β-lactoglobulin). The milk particles we isolated fulfilled the international criteria of EVs and in particular that of exosomes. We found that the cow milk EVs were extremely pH and heat stable, and probably can survive the harsh condition in the gastrointestinal tract. Confocal microscopy showed that these EVs can be taken up by numerous cell types (macrophages, splenocytes and intestinal cells) and this process requires an active cell metabolism. Interestingly, the milk EVs can inhibit LPS-induced cytokine and chemokine expression in cultures of murine splenocytes. On naïve murine CD4+ T-cells the milk EVs can either support the differentiation towards the pathogenic IL-17 producing T-helper (Th17) subset or the immunosuppressive regulatory T-cells (Tregs) dependent on the culture condition chosen. This effect is at least in part mediated by TGFβ as the differentiation into both T-cell lineage is blocked by the addition of anti-TGFβ1,2,3 antibodies. Using a (CAGA)12-luc reporter assay we confirmed that cow milk EVs possess bioactive TGFβ. Acidification, up to gastric acid level not only kept the exosomes intact but also did not reduce the inhibitory effect they had on NFκB-activation in a NFκB specific reporter assay. This data prompted us the test the effect of cow milk EVs on mouse models of rheumatoid arthritis, the spontaneous arthritis model in the IL-1 receptor antagonist (IL-1Ra) gene deficient BALB/c mice and the collagen-induced arthritis (CIA) model in DBA/1J mice. IL-1Ra$^{-/-}$ mice received cow milk EVs by daily oral gavage starting at week 5 till 15 after birth and CIA mice via their drinking water starting 1 week before immunization till day 40. Macroscopically, EV treatment delayed the onset of arthritis and histology showed diminished cartilage pathology and bone-marrow inflammation in both models. EV treatment also reduced the circulation levels of MCP-1 and IL-6 and their production by splenic cells. EV treatment diminished the anti-collagen IgG2a levels which was accompanied by reduced splenic Th1 (Tbet) and Th17 (RORγT) mRNA. We showed that EVs obtained from pasteurized cow milk possess immune regulating and anti-inflammatory properties This is the first report that oral delivery of bovine milk derived EVs ameliorates experimental arthritis and this warrants further research to determine whether this beneficial effect of milk EVs can also be seen in rheumatoid arthritis patients.

Supplementation with *Bifidobacterium Infantis* in Combination with Bioactive Milk Components to Alter Microbial Composition in Children with Autism

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Student Travel Award Recipient

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A feature of the evolution of lactation is the explicit development of a symbiotic relationship between microorganisms and the mammalian host that is absolutely essential for proper physiological function. Human milk guides the colonization and composition of the infant gut microbiota more than any other environmental factor and compelling evidence is emerging that a milk-oriented microbiota (MOM) guides the development of various systemic processes (immunity, metabolism, neurological networks) and sets the infant up for a lifetime of health, including optimal neurodevelopment. The extent of the effect of microbial composition and activity on human physiology has only begun to be explored with neurodevelopment positioned at the very edge of this investigation. Examination of neurodevelopmental disorders such as autism spectrum disorders (ASD) and their connection with gut microbial dysbiosis will provide insight into the explicit but often elusive gut microbiota-brain axis in human development. Children with ASD, characterized by a range of cognitive and social deficits, are also commonly plagued by gastrointestinal (GI) co-morbidities including chronic constipation and diarrhea. The severity of autism symptoms is often correlated with the extent of GI symptomology as well as degree of microbial dysbiosis in the gut. This study was designed to explore characteristics of microbial dysbiosis that may contribute to the severity of GI and behavioral symptoms in children with ASD and whether supplementation with the probiotic *Bifidobacterium infantis* with bioactive

milk components (bovine colostrum product – BCP) can ameliorate this microbial dysbiosis. This pilot study is a randomized, double blind, controlled trial of a synbiotic (BCP + *B. infantis*) vs. BCP alone. Each participant was randomized to receive either the synbiotic first or the BMO first in a cross-over study design. The study lasted 12 weeks, including 5 weeks of BCP use, 5 weeks of synbiotic use, and a two-week washout period in between. Stool, urine, and blood samples were collected throughout the study and analyzed for microbial composition. Results of this study will provide insights into the dependence of human neurodevelopment on the natural progression of the gut microbiota.

Metabolic Programming in Utero - How a Mother's Diet Shapes Her Child's Future

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In fetal life, tissues and organs undergo critical periods of rapid development and cell division. According to the Barker hypothesis, the future physiological attributes of an organism are being defined from the moment of conception. Metabolic programming is orchestrated through epigenetic events, including histone modification and methylation of DNA. In this way, genes become up or down-regulated for specific biological functions. The mother's diet plays an important role in the metabolic programming of her fetus, preparing it for the environment after birth in which it has the best chance of survival. In particular, the link between maternal glucose levels and offspring birth weight makes the carbohydrate content and glycemic index (GI) highly relevant to maternal nutrition. Both GI and glycemic load (GL = GI x carbohydrate) have been controversially associated with the rise in obesity and type 2 diabetes. The GI is a measure of the relative glycemic potency of the carbohydrates in different foods and most modern starchy foods, such as white bread, potatoes and breakfast cereals, have a high GI. In controlled, well-designed clinical trials, we showed that low GI dietary advice during the second and third trimester of normal pregnancy reduced the risk of macrosomia and infant ponderal index compared with advice to consume a high fibre, low sugar diet. In gestational diabetes, we showed that low GI diets halved the need for prescription of insulin without adverse effects on birth outcomes. In large, prospective observational cohort studies of normal women, low GI/GL diets have been associated with reduced risk of gestational, type 2 diabetes and birth defects. Ongoing mechanistic studies are exploring the role of GI in changing the expression of the FTO gene, the gene most commonly associated with obesity in European populations. These findings suggest that dietary GI has important implications for women before, during and after pregnancy.

Sensor Derived Health Phenotypes for Improved Dairy Cattle Management and Genetic Selection

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Before the advent of machine milking, small numbers of dairy cows per farm enabled farmers to 'know' each animal, often on an individual name basis. Cows were milked by hand and any deviation in the quality/appearance of milk, and general animal health, was detected early. Over the past 20 years, the Australian dairy industry average herd size has more than doubled with large herds (>600 cows) becoming the norm as farmers strive for increased efficiency. In this regard, 19% of Australia's dairy farms now produce over half of the national milk supply. Such large herds have been enabled by incorporating various forms of technology and the ability to manage large amounts of animals per labour unit.
Dairy farmers already work 63 hours per week, almost double the average Australian 35 hour working week. As such there is very little room to increase the working hours associated with increasing cow numbers on farm. This scenario results in one of two outcomes. Either lower-skill levels of employed labour will be

inevitable and/or the amount of time spent visually monitoring each animal will be further reduced. Also, subjective, limited animal health data are currently available across the dairy industry, this is particularly so for lameness. Many animal health issues are either not observed, not recorded, or currently cannot be detected by humans. Together these factors combined result in the reduced profitability and health of Australian dairy cows. As such, farmers are seeking new systems to aid in monitoring individual animals to improve their decision making and associated animal health and profit. Early disease detection is ambitious but very beneficial from all angles of the production system including welfare benefits, improved treatment response and cure-rates, reduced production losses and reduced antibiotic usage. Current on-animal sensor based systems now enable farmers to monitor almost all aspects of behavior from the time spent eating, lying, walking, standing, ruminating and even sleeping. Current research in the field now has a strong focus on combining these and other novel behaviours into predictive models of use by farmers. Further, technology now has the capability to 'learn' from humans to 'observe' discrete behaviours (machine learning). New phenotypes are emerging from these sensor systems to benefit not only to farm management such as the timing of artificial insemination and health treatment but also for genetic selection. Genomic selection is very well suited to traits that are difficult or expensive to measure on a large scale, as genomic predictions can be developed in a reference dataset that comprises animals with phenotypes and genotypes that can then be applied to bulls or cows that are genotyped but do not have phenotype information. Therefore, there is a distinct opportunity to start building phenotype datasets in herds where cows are genotyped focusing in on current areas of reduced health observed in the Australian dairy industry. New valuable traits that cannot be (accurately) measured can then be added to the breeding goals with widespread benefits at both the herd and industry levels.

Harnessing the Benefit of Functional Genomics Information When Combining Two Unrelated Populations for Genomic Selection

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Background: The success of genomic prediction strategies in animal breeding hinges on the availability of a large reference population upon which to build the genomic-based predictions of additive genetic or breeding value. Here we explore the benefit of using functional genomics information when combining two unrelated populations into a single reference population.
Methods: The datasets we use consist of 1,829 Brahman and 1,973 Tropical Composite cattle with measurements on five phenotypes of relevance to tropical adaptation and genotypes for 71,726 genome-wide single nucleotide polymorphisms (SNP) markers. The underlying genomic correlation for the same phenotype across the two breeds is explored on the basis of consistent linkage disequilibrium (LD) phase and marker effect in both breeds.
Results: The proportion of genetic variance explained by the entire set of 71,726 SNP ranged from 37.5% to 57.6%. The estimates of genomic correlation were drastically affected by the SNP selection process and changed from near zero for most traits to > 0.80 when using the set of SNP with significant effect and same phase in both breeds. We find that the use of functional genomics information when selecting SNP to build the genomic relationship matrix allows for the recovery of the missing heritability, which in turns improves the accuracy of genomic predictions.
Conclusions: Our results offer a hope for the effective implementation of genomic selection schemes in situations with many breeds, small sample sizes within any single breed and many phenotypes in the breeding objective.

Collection of Data for the Genetic Improvement of Health Traits in Australian Dairy Cattle

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A growing concern for dairy farmers is the improvement of dairy cow health through genetic selection. Healthy cows are more productive, easier to manage, require less intervention, have improved animal welfare and contribute to profitability by reducing production costs. However, in many countries, including Australia, industry collection of data on common health events has been sub-optimal or absent, which means there is no ability to provide breeding values and apply genetic selection for common health disorders. Also, such traits are low in heritability, meaning that although genetic progress is feasible, it will be slower. While many farmers may collect some of this information on farm, there is likely to be variation in the completeness of these data sets. In Australia, there is little storage or export of such information from some on-farm software packages into industry databases for research or reporting purposes.

Before any work can begin on providing Australian farmers with breeding values for common health disorders, it is important to quantify what data is already being collected on farm and in veterinary practices. As a result of this challenge, the health data for healthy cows (HDHC) project has commenced to help improve our understanding of the extent of health data recording in the Australian dairy industry. The HDHC project will use infrastructure through the Dairy CRC in the form of the 100 'Ginfo' (Genomic information) herds to collect all health data that is currently being amassed on farm. The Ginfo data is being used as a genomic reference population for genomic breeding values. One of the advantages of having a genotyped population is that it opens up new opportunities for new breeding values, such as dairy health traits. A survey was given out to the 100 GINFO farmers to get an understanding of the health data collection and storage methods that occur on farm. Health data files were collected from the respective herd test centres of these herds. Health data obtained from herd test centres totalled 275,729 records from just 46 out of the 100 herds (To date). The four most recorded health diseases identified were mastitis, reproductive problems, lameness and metabolic disorders. This is fairly consistent with the survey conclusions on what farmers indicated they record and what they think is most important. Mastitis having the highest incidence with 20% of cows affected, followed by reproductive problems (12%), lameness and metabolic disorders (5% and 3% respectively). This project has provided an insight into what health information is actually being collected on farm and that there is a source of health data available which can be accessed and potentially used for the genetic improvement of health traits in Australian herds and potentially providing genomic breeding values for new health traits such as lameness which is a trait in demand requested by Australian dairy farmers.

Human Breast Milk miRNA, Maternal Probiotic Supplementation and Atopic Dermatitis in Offspring

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Background: Perinatal probiotic ingestion has been shown to prevent atopic dermatitis (AD) in infancy. This has been observed in a number of randomised clinical trials using a variety probiotics strains and supplementation regimes, and the strongest evidence comes from studies which include both pre- and post-natal supplementation. Results from our own study, Probiotics in Prevention of Allergy among Children in Trondheim (ProPACT), suggest that maternal supplementation alone is sufficient to prevent AD in offspring. The mechanisms behind this preventative effect are incompletely understood and may be partially mediated

by changes in breast milk composition. Micro-RNAs (miRNA) are abundant in mammalian milk and may influence the developing gastrointestinal and immune systems of newborn infants. In this study, we investigated breast milk miRNAs as possible mediators of the observed preventative effect of probiotics using samples collected during the ProPACT trial.
Objective: To describe the miRNA profile of human breast milk, and to investigate the association between miRNAs, maternal probiotic supplementation and the development of atopic dermatitis in offspring.
Methods: Fifty-four breast milk samples were obtained 3 months postpartum from women participating in the ProPACT trial, a double blinded, placebo-controlled randomised trial of perinatal probiotic supplementation. The probiotic milk consumed by the women randomised to the probiotic arm of the trial contained 5 x 1010 CFU of *Lactobacillus rhamnosus GG* and *Bifidobacterium animalis* subsp. *lactis* Bb-12 and 5 x 109 CFU of *L. acidophilus* La-5. Breast milk samples were enriched for extracellular vesicles using ExoQuick™, total RNA was isolated and small RNA sequencing was conducted on the Illumina HiSeq 2000 platform. Differential expression of miRNAs was assessed for the probiotic vs placebo and the AD vs non-AD groups. Target gene and functional predictions were conducted using TargetScan and DAVID bioinformatics resources, respectively. The ProPACT trial is registered at ClinicalTrials.gov (identifier NCT00159523).
Results: The RNA isolates obtained from the human milk samples contained between 12.4 and 247.5ng/µL of RNA, and were found to contain RNA fragments up to 1000nt in length which aligned primarily to rRNA, tRNA and miRNA. The miRNA profile revealed a relatively stable core group of highly expressed miRNAs, including miR-148a-3p, miR-22-3p, miR-30d-5p, let-7b-5p and miR-200a-3p. Functional analysis of the target genes for the 20 most highly expressed miRNAs revealed enrichment of a broad range of biological processes and molecular functions and significant overlap with genes that are upregulated in brain and epithelial tissues. Although several miRNAs were found to be differentially expressed on comparison of the probiotic vs placebo and AD vs non-AD groups, none had an acceptable false discovery rate and their biological significance in the development of AD is not immediately apparent from their predicted functional consequences.
Conclusion: Whilst breast milk miRNAs have the potential to be active in a diverse range of tissues and biological process, individual miRNAs in breast milk 3 months postpartum are unlikely to play a major role in the prevention of atopic dermatitis in infancy by probiotics ingestion in the perinatal period.

Freed Milk Glycan Monomers Create a Niche for Gut Pathogens in Vivo

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Milk profoundly shapes the gut microbiome of the neonate, and while nursing is generally associated with protection from enteric infections relative to formula feeding, it is not an impervious protection for the neonate. Despite their role as a highly relevant and tractable model of human neonates, little is known how milk shapes the gut microbiome of nursing pigs. To address this, we profiled the fecal microbiome of pigs from birth through weaning using 16S rRNA marker gene sequencing and shotgun metagenomic sequencing. Metagenomic sequencing identified functional differences between key bacterial taxa in nursing pigs, and found that primary degraders of porcine milk glycans (*Bacteroides*) did not consume all of the freed monomers, in a distinct example of diet-specific crossfeeding to other taxa (*γ-Proteobacteria)*. We confirmed that these freed monomers, which compose a major proportion of sow milk glycan structures, could be measured in neonate feces and were dietary in origin. Bacterial isolates from these different taxonomic groups reprised these roles in consuming and releasing milk glycan monomers in *in vitro* experiments and we tested how these freed milk glycan monomers shaped microbial populations *in vivo*. This work demonstrates

that dysbiotic communities in the distal gut, enabled by *Bacteroides* or taxa with similar glycolytic capacities, can promote the growth of populations of pathogenic taxa associated with enteric infections (eg. γ-*Proteobacteria* and *Clostridia*) and drive diseases in nursing mammals by freeing otherwise inaccessible milk glycans. However, we found that by limiting the availability of these free monomers, we could reduce dysbiosis *in vivo,* suggesting a new, but critical, mechanism by which infant-associated gut microbes such as *Bifidobacterium* can protect infant health.

Novel Genetic Makers Affect Rennet-Induced Milk Coagulation

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The importance of κ-casein in cheese renneting is well established. We conducted a GWAS utilizing 655,378 SNP markers in 377 Danish Holstein cows that confirmed, by the presence of a major quantitative trait locus, the relation between *CSN3* and the milk coagulation trait; curd firming rate (CFR). The three most significant SNPs were used, together with a SNP known to differentiate the genetic B-variant, to search for haplotypes. All animals were genotyped for the genetic variants of *CSN3* and this knowledge was utilized for classification of the haplotypes. The B-variant SNP made it possible to predict the genetic variants of the remaining haplotypes as the genetic B-variant was completely linked to a single haplotype. Hence, we predicted the other haplotypes based on homozygotes as well as the heterozygotes carrying a single copy of the B-variant. Interestingly, the haplotype analysis divided the data into groups not only based on the *CSN3* allelic variants. The most frequent haplotype (57%) had the poorest mean CFR (7.3 Pa/min) representing animals carrying the *CSN3* E-variant, but also animals carrying the *CSN3* A-variants (AE haplotype). The second most frequent haplotype (25%) was related to animals carrying the *CSN3* B-variant, but this group was not the one with the highest mean CFR (10.8 Pa/min). Instead, the least frequent haplotype group (8%) had the highest mean CFR (11.8 Pa/min) and these were all A-variant animals. The last haplotype group (10%) were also represented by animals carrying the A-variant and this group had a relatively high mean CFR (9.5 Pa/min). To corroborate the results we analysed the Swedish Red breed and found exactly the same inheritance pattern and effect of the haplotypes on CFR, however, with slightly different frequency distributions. To further investigate the effects of the haplotypes we sampled milk from Holstein cows that were homozygous for the AE haplotype (low mean CFR, n=33), the B haplotype (n=26) or the A haplotypes that were combined due to low frequency (n=12). High quality milk coagulation properties have previously been identified to relate to higher protein content, high relative concentration of κ-CN and glycosylation degree of κ-CN. Further, it has been speculated to affect casein micelle size and stability and thereby influence the primary and secondary phases of milk coagulation. Hence, the collected milk samples were analyzed for rennet-induced milk coagulation by free oscillation (ReoRox4), protein profile were analysed by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and micelle size were determined by dynamic light scattering (Nanosizer).
The results of the analysis are depicted in Table 1.

Table 1. Descriptive statistics of protein percentage, curd firming rate (CFR), rennet coagulation time (RCT) and micelle size in relation to haplotype.

	AE haplotype (n=33)	A haplotype (n=12)	B haplotype (n=26)
Protein%	3.59[a]	3.57[a]	3.45[a]
CFR (Pa/min)	6.24[a]	13.17[b]	11.82[b]
RCT (sec)	1546.75[a]	1085.96[b]	1058.36[b]
Micelle size (nm)	165.98[a]	150.41[b]	146.86[b]

Different letter in the same row shows significant differences for $p<0.05$

The AE haplotype had significantly longer RCT and lower CFR compared with the A and B haplotypes (Table. 1). This was shown to be related to the casein micelle size and relative concentrations of κ-CN and partly α_{S1}-CN, whereas the protein contents did not differ significantly among haplotypes. Furthermore, three AE haplotype milk samples were non-coagulating. The results presented here clearly document that genetic influence of the casein gene cluster is complex and not only related to the well-defined known α_{S1}-, κ-, β-CN variants. Furthermore, it is evident that the former association between the haplotypes and milk coagulation properties are due to a higher genetic influence on relative concentration of κ-CN and micelle size rather than protein content.

Not Sex but Birth Weight of the Calf Affects Its Mother's Milk Production

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Giving birth to either a male or a female young can affect the amount and/or quality of the milk that is subsequently produced by the mother. Usually, if sex-bias is present, giving birth to a male young results in a higher milk volume and/or milk with a higher energy content. In contrast to this, higher milk production across lactation after giving birth to female calves was shown in a study on US dairy cows. The aim of our study was to investigate the effect of the sex of the calf on the subsequent milk production of the mother across lactation in Dutch dairy cows. Milk production records of 1,615,765 lactations of 861,273 Holstein Friesian cows from 7,303 herds throughout the Netherlands were used for analysis. This data comprised 815,432 lactations after giving birth to male calves (50.5%) and 800,333 lactations after giving birth to female calves (49.5%). Plain averages suggest that giving birth to male calves results in higher 305-day milk production of the mother: differences up to 90 kg are found in lactation 2 and 4. Differences in milk production are considerably different when performing a statistical analysis including gestation length, lactation length, sex of the calf, calving ease and birth weight of the calf as fixed effects, and sire of cow and herd-year-season as random effects. The effect of the sex of the calf on the subsequent 305-day milk production of the mother is significant only for lactation 1 and 3, with a small positive effect (25 kg) after giving birth to a female calf in the first lactation and a small negative effect (21 kg) in the third lactation. Interestingly, the effect of the birth weight of the calf is significant in all lactations and seems more substantial: an increase in birth weight of the calf from 30 kg to 50 kg is associated with an increase in subsequent 305-day milk production of the mother of about 450 kg in the first lactation up to about 750 kg in the second lactation. What at first sight appears to be an effect of the sex of the calf on the milk production of the mother is actually a confounding effect of the birth weight of the calf, because male calves tend to be heavier than female calves. It is not clear whether previous reports of sex-biased milk production can also be attributed to differences in birth weight of the offspring of different sexes.

An Evaluation of Extended Lactation in the Australian Dairy Industry

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In the Australian dairy industry, and in particular Victoria, there has been a shift away from seasonally concentrated calving patterns. Reasons for such a shift stem largely from improved feeding of cows with year-round feed supplies, and the introduction of new germplasm from North American Holstein Friesians into some Australian dairy herds. Such impacts have resulted in an increase in the phenotypic and genetic potential of cows to produce more milk and be milked for longer than the standard 305 days under seasonal

calving systems. The increase in high-output cows has come at an associated cost with a national decrease in reproductive performance and many highly productive cows being prematurely culled for failing to get in calf. One strategy of interest to the dairy industry is for high-producing cows being mated out of season and then carried over and milked for an extra 6 months (18 month calving interval). In Australia this is regarded as the most optimal and economical method on pasture-based systems. Limited information was available about the potential for genetic improvement of extended lactation under the Australian dairy system. To address this shortcoming, a very large data set with over 158 million test-day records was assessed to examine the scope for genetic improvement in extended lactation in the Australian dairy industry. A data handling pipeline was developed to assist in analysing and editing large scale industry test day records. Variation in Australian dairy cattle in respect to their lactation curves over an extended lactation was explored and derived traits obtained that were then used in the genetic analysis using mixed model methodology. Based on previous work, the Wood model was chosen as the basis for lactation curve modelling. These models were fitted to milk yield records divided into 8 random subsets of 1 million pure Holstein cows with 20 million test-day records. Two traits of interest, namely persistency and extended lactation, were quantified, and these were derived for three yield traits, namely milk, protein and fat yield. Estimates of heritability for milk, fat and protein yield under extended lactation (0.06 to 0.09) and lactation persistency (0.06 to 0.1) were low, while the cows' repeatability for extended lactation and lactation persistency milk traits (0.20) was higher by comparison. Such findings indicate the potential for early cow performance to be used as an indicator for a cow's successive lactation performance. Extended lactation and lactation persistency were found to have low phenotypic and genetic correlations with other economically important traits of milk, fat and protein yield (-0.05 to 0.1). This suggests that selection for increased extended lactation will not adversely impact economically important and highly selected traits, namely cumulative 305 day yields and the Australian Selection Index (ASI). Despite the low heritabilties, estimated breeding values (EBVs) were generated for extended lactation and lactation persistency for milk, fat and protein yields, to assist with the selection of cows and bulls. Genetic trends showed a steady increase (1 genetic SD) in EBVs for extended lactation characteristics in the last 20 years. This increase is potentially a reflection of the increased selection for milk production and introduction of new germplasm from North American Holstein Friesian cattle into Australian herds. This research will provide the Australian dairy industry with the breeding tools to enable selection of bulls to produce cows that are best suited to extended lactation in pasture-based systems. It has also opened avenues for genomic predictions for these traits.

Characterization of Regulatory Elements Associated With Mammary Gland Function in Mouse and Cow

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Milk is the primary source of nutrition for the newborn, providing all the essential components for healthy growth and development in all mammalian species. While we are learning more about the components of milk and how they contribute to milk's unique role in mammalian life, our knowledge of the factors involved in establishing and maintaining lactation is far from complete. To fully understand the biological processes involved in establishing and maintaining lactation, we are using the mouse\ and\ cow\ as model\ systems. We are performing genomic, genetic, epigenomic, and transcriptomic analyses. Results from these studies indicate that, during development, changes in the epigenome enable differentiation by coordinating the expression of groups of genes and the functioning of regulatory elements. DNA binding---motif analysis and transcription factor binding data for regulatory elements give further insight into the signaling pathways

necessary to achieve a fully functional mammary gland. The integration of mammary gland development associated genetic variants with the epigenetic data indicates how genetic variation can contribute to lactation and shows how this approach can help identify mammary gland related traits. We conclude that in the mammary gland an epigenetic state is established during early development that is poised to respond to the systemic hormonal signals of pregnancy and lactation to achieve the full functional capacity of the mammary gland. Genetic variation and environmental exposures that change the epigenetic state will likely affect the establishment and maintenance of lactation.

Maternal Mechanisms of Energy Mobilisation during Lactation in Mouse Models

Peter Williamson, Faculty of Veterinary Science, The University of Sydney, Australia

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Lactation results in one of the greatest energy demand during the female life-cycle. This requires a sustained increase in maternal metabolism, often resulting in a change in body weight and body composition. Impaired regulatory responses to such a metabolic challenge may lead to metabolic disturbances in the mother and/or lactation failure, leading to serious consequences for neonatal growth and development. We used a complex phenotyping strategy to measure variation in maternal physiological parameters during lactation across 32 inbred strains of mice. An *in silico* genome-wide association analysis was performed to identify regions that contribute to energy expenditure and body composition during lactation. Dams and their litters were studied for 8 days postpartum during second lactation. On day 1 postpartum (L1), 10 one-day old CD1 pups were cross-fostered to each lactating dam to standardise variation in pup suckling drivers during the study. Food intake and litter weight gain were recorded daily. Data for food intake and milk energy output (litter weight gain) between day 7 (L7) and day 8 postpartum (L8), were used to calculate energy balance. Body composition was measured using quantitative magnetic resonance on L1 and again at L8 to assess changes during the study period. Genotyping was based on a132k SNP panel, and derived haplotag SNPs were then utilized for genome-wide association analysis. Significnt strain differences ($P < 0.001$) were observed in food intake, milk energy output, maternal body weight variation, and body composition during lactation. GWAS revealed multiple regions associated with these traits, but only one predominant association when evaluating energy utilization. The implications of the candidates for these traits will be discussed.

A Role for Sim2s in Elongation Regulation of Milk Protein Gene Transcription in the Mammary Gland

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Identification of the mechanisms underlying the establishment and maintenance of functional differentiation is critical to our understanding of lactation. Genome wide analyses have recently demonstrated that regulation of differentiation occurs in large part by transcription elongation. Elongation proceeds after RNA Polymerase II (RNAPII) is released from its paused state through association with the positive transcriptional elongation factor (P-TEFb) containing catalytic enzyme, Cdk9. However, the mechanism by which regulation of these processes occur has yet to be elucidated in the mammary gland. We have previously shown that Singleminded-2s, (Sim2s) a member of the bHLH/PAS family of transcription factors, is required for establishing and maintaining mammary gland differentiation. Overexpression of Sim2s results in heightened expression of the milk protein genes beta-Casein (Csn2) and Wap. We have further demonstrated that

conditional loss of Sim2s in mouse mammary epithelial cells results in decreased Csn2 gene expression. Building on chromatin immunopreciptitation (ChIP) and re-ChIP analyses documenting the association of Sim2s at the Csn2 promoter and interaction of the RNAPII complex with Sim2s respectively, we further analyzed the role of Sim2s in transcriptional regulation of differentiation-dependent gene expression. ChIP analysis of exon 7 at the Csn2 gene locus uncovered association of Sim2s, RNAPII, and Cdk9. Using mouse mammary epithelial cell lines (HC11 and CIT3) we found that inhibition of Cdk9 caused loss of milk protein gene expression. Additionally, we found an interaction between Cdk9 and Sim2s, which further indicates that Sim2s associates with transcription elongation machinery. This suggests Sim2s acts, not only as a traditional transcription factor, but also plays a role in enhancing transcription elongation of milk protein genes. Together these data demonstrate a novel paradigm of milk protein gene regulation in the mammary gland.

Peptidomics of Human and Bovine Milk Revealing Novel Antimicrobial Peptides

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For over 200 million years, milk has co-evolved with mammalian infants to be nourishing and immunoprotective. Many milk proteins have no known function intact, which would be consistent with the need for rich dietary sources of essential amino acids. However, fragments of milk proteins (peptides) released by enzymatic degradation have actions, including opioid-like activity, immune system modulation and antimicrobial action.

Our work via nano-liquid chromatography tandem mass spectrometry-based peptidomics has identified that milk contains thousands of naturally occurring peptides released within the mammary gland by enzymes specifically secreted into milk. We have shown that many of these peptides are homologous with known antimicrobial and immunomodulatory peptides. We have also shown that milk peptide release is higher in preterm mother's milk than term mother's milk, which may partially compensate for the premature infant's lower digestive capacity. Our work then shows that, despite previous research suggesting that little to no digestion occurs in the newborn infant stomach that thousands of peptides are indeed released and many of these by enzymes produced in mother's milk. Thus, the mother continues to aid the infant in digestion via these secreted enzymes. Beyond our work in human milk, we have identified thousands of naturally occurring peptides in bovine milk and shown that these are highly similar across cows and across time. We have also shown that the bovine milk waste product, whey permeate, serves as a valuable source of endogenous milk peptides for functional applications. Isolation and fractionation of human and bovine milk endogenous peptides reveals fractions that are highly antimicrobial towards a variety of antibiotic resistant bacteria. Recently, we have examined bovine milk-based kefir and shown that the kefir microorganisms degrade milk proteins to a large extent, releasing hundreds of novel peptides. We show that the kefir contains many known functional peptides. Our most recent work follows the degradation of milk proteins in piglets across all parts of the digestive tract.

Survival of New Probiotic Strains with Anti-Inflammatory & Anti-Obesity Effects Used in Non-Fat Yogurt and Low-Fat Cheddar Cheese Making

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Chronic Inflammation can play an important role in the development of the pathogenesis related with obesity. It has been demonstrated that some probiotics (*Lactobacillus*, *Bifidobacterium*) can prevent obesity and inflammation by reducing the metabolic endotoxemia, by inhibiting the production of pro-inflammatory cytokines and by shaping the gut microbiota (commensal bacteria). No dairy product containing anti-inflammatory and anti-obesity probiotics are currently available in North America. The viability of probiotic is important during the manufacture and the storage of dairy product since cheese (50 g) and yogurt (100 g) must contain at least 10^9 colony forming unity (cfu) of the specific probiotic strain with health claims. The objective of this study was to determinate anti-inflammatory (*in vitro*) & anti-obesity (*in vivo*) effects of new probiotics and their viability during production and storage of non-fat yogurt and low-fat Cheddar cheese. Five probiotics isolated from faeces and raw milk, *Bifidobacterium animalis* ssp *lactis* (Bf141, Bf26), *Lactobacillus (Lb.) casei/paracasei* (L79), *Lb. rhamnosus* (Lb102) and *Lb. plantarum* (Lb38), genetically identified, were used. A cell model of inflammation (macrophages) and a mouse model of diet-induced obesity (obesogenic diet) were used. The heat-killed of probiotic strains induced a significant decrease in NO production and were able to increase cytokine IL-10 production (except L79) in macrophages. The body weight gain and epididymal, inguinal and retroperitoneal white adipose tissue masses in mice were more reduced with probiotics milks than control milk without probiotic. At the beginning of the cheese ripening period, all probiotic candidates were at the targeted count (10^9 per 50 g portion, based on health claim regulations for probiotic foods). The counts of both bifidobacteria isolates dropped below 10^9 during cheese ripening, while the lactobacilli counts remained stable or increased. Yogurts were formulated to contain 1.0×10^7 cfu g^{-1} of probiotic candidate for at least 30 days of storage. After 60 days, this count was maintained in the case of lactobacilli, but not in the case of bifidobacteria. Studies are in process to evaluate the effects of these probiotics on the parameters associated with inflammation (cytokines and LPS levels) and on the composition of gut microbiota. Obesity is associated with chronic low-grade inflammation.

Some probiotics (Lactobacillus, Bifidobacterium) have contributed to prevent obesity and inflammation by improving intestinal barrier function with the decrease of lipopolysaccharides (LPS) circulation, by decreasing the liberation of pro-inflammatory metabolites such as cytokine (IL-6, TNF-α) and nitric oxide (NO), and by increasing the production of anti-inflammatory cytokine (IL-10). No dairy products containing anti-inflammatory and anti-obesity probiotics are currently available in North America. The viability of probiotic bacteria is important during the manufacture and storage of dairy products since cheese (30 g) and yogurt (100 g) must contain at least 109 of probiotic strain with health claims, according to the regulation of probiotic foods. The objective of this study was to determinate anti-inflammatory (in vitro) & anti- obesity (in vivo) effects of new probiotics and their viability during production and storage of low-fat yogurt and Cheddar cheese. New probiotic strains were isolated from infants or adults feces and dairy products. The use of 16S genetic identification and specific Q-PCR primers allowed confirming two Bifidobacterium animalis ssp lactis (Bf26, Bf141) and the use of RpoB primers three Lactobacillus strains (Lb38, Lb79, Lb102). Model cell of inflammation and model mice obese with milk as vector were used.

The heat-killed of probiotic strains induced a significant decrease in NO production and were able to increase IL-10 production (except L79) in macrophages. The body weight gain of mice fed with high-fat high-sucrose (HFHS) diet treated with probiotic milks was significantly reduced comparing to mice fed with HFHS diet and milk without probiotic. Probiotic cells were viable (>109 cfu/g) in dairy products after the production and storage of low-fat yogurt and Cheddar cheese as recommended FDA. However, results obtained in vitro study suggests that death probiotic cells could play a role in anti-inflammatory effect.

Molecular Determinants of Milk Lactoferrin as a Bioactive Compound in Early Neurodevelopment and Cognition

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Background/Aims: To test the hypothesis that milk Lf may induce gene expression profiling and function to improve neurodevelopment and cognition in postnatal piglets, an animal model for human infants.
Methods: 3-day-old male piglets were randomly allocated to 2 groups. Group 1 were fed milk replacer supplemented with bovine milk Lf at a dose level of 0.6g/L (n= 17) and Group 2, 0.06 g/L (n= 16; control). Global gene expression profile and the selected target gene expression were determined using a Porcine Affymetrix GeneChips representing 20,201genes from S. scrofa and real-time PCR to validate the microarray findings. The selected protein expression was analysed using western blot and immunohistochemistry techniques.
Results: A total of 1,187 genes were differentially expressed between the control and Lf groups, based on our filter criteria (fold change: 1.15 and $p < 0.05$). A positive global effect of milk Lf on neurodevelopment and cognition was observed, as evidenced by the modulation of a wide range of neuronal processes. Milk LF up-regulated transcriptional and translational levels of the brain-derived neurotrophin factor (BDNF) and increased phosphorylation of the cAMP responsive element–binding protein (CREB), a downstream target of the BDNF signaling pathway, and a protein of crucial importance in neurodevelopment and cognition. Milk Lf enhanced the piglet learning speed and long-term memory.
Conclusions: Milk Lf can improve neural development and cognition in postnatal piglets, an animal model of human infant.
Funding source(s): Medical school of Xiamen University, China, Nestle Research Centre, Beijing.

Packaging of Endogenous and Exogenous Milk miRNAs in the Mammary Gland: New Opportunities for Milk as a Therapeutic

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MicroRNAs (miRNAs) occur in many biological fluids, but are most abundant in milk. These milk miRNAs can be found packaged in nanovesicles called exosomes, or in association with the RNA Induced Silencing Complex (RISC) which both provide protection from miRNA degradation. Here we show that milk could be used as a natural RNAi delivery vehicle for small RNA therapeutics.

As milk contains a large proportion of miRNAs that are predicted to have targets on immune genes and developmental pathways, it is likely that milk miRNAs are delivered to a range of tissues. Milk exosomes have been shown to transfer their miRNA cargo to macrophages *in vitro*. This suggests that milk acts as a natural RNAi delivery system that could be modified to deliver therapeutics. To determine whether an exogenous miRNA can be packaged into milk, we first determined whether milk miRNAs are specifically packaged. Comparison of mammary and milk miRNAs from both transgenic and wild type mice determined that milk miRNAs are a subset of mammary gland miRNAs, indicating that milk miRNAs are not specifically packaged. Indeed, analysis of exogenous miRNA expression in transgenic mouse and cows milk revealed a high level of expression. This study highlights the potential of milk as a stable, abundant delivery vehicle for small RNA therapeutics.

Comparative Analysis of Milk miRNA

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Micro-RNAs (miRNAs) are small RNA molecules known to participate in important regulatory mechanisms through the targeting of mRNAs by sequence specific interactions, leading to targeted inhibition of gene expression. Initial studies have highlighted the importance of miRNA in normal mammary gland development processes associated with the lactation cycle but a role in lactation is not yet completely clear. However, the recent identification of significant quantities of miRNA in the milk of a number of mammals, together with the reporting of functional plant food miRNA in the blood of people, have precipitated speculations about the potential role of miRNAs in milk production as well as immune protection and development of the young. To examine the role of milk miRNAs as informative markers of lactation, maternal physiology or information carrying signals for the timely delivery of development signals to the young, we deploy a comparative framework across the mammalian kingdom. The identification of conserved milk miRNAs allows the characterization of essential miRNA while difference in milk miRNA composition may reveal specific adaptations of a putative secretory miRNA signalling pathway. Towards this, we produce high throughput sequencing data from milk of a number a species, including more distantly related monotremes (platypus and echidna) and marsupials (tamar wallaby) together with additional eutherian species (buffalo and seal). The new results are integrated with publicly available data from other eutherian models (human, cow, pig) to construct a comprehensive catalogue of milk miRNA profiles. This analysis shows a large overlap of miRNA populations present in human and cow milk but a relatively poor correlation in absolute levels between the

species. However, it is possible to identify miRNAs consistently present within high concentration ranges. For example 64 miRNAs have concentrations with less than five-fold difference between cow and human milk, including 5 of the 7 miRNAs previously proposed as milk-enriched quality markers in cow studies (mir-26b, mir-21, mir-30d, mir-26a and mir-148a), and 46 are within a 3-fold difference, including four quality-markers candidates; mir-26b, mir-21, mir-30d, mir-26a. Some of these miRNAs therefore emerge as potential candidates for conserved, perhaps universal, milk markers and we will discuss how these preliminary results extend to a broader number of species. Marsupials, such as tammar wallaby, present one of the most interesting lactation phenotype due to their adoption of a short gestation with a relatively long lactation cycle following birth of an immature neonate with significant development during lactation. Continuous changes of tammar milk composition have been reported and may contribute to development and immune protection of pouch young. Therefore the marsupial model presents a unique opportunity to address the putative contribution of secretory milk miRNA in these processes. Profiling of miRNAs collected from tammar milk at different time points of lactation was conducted by High throughput sequencing. The results show that miRNA are also secreted at relatively high levels in marsupial milk and that milk miRNA composition changes significantly during the course of lactation in this specie. In addition, the difference in miRNA profiles obtained from maternal blood and milk indicates that passive transfer of serum is not a major contributor of miRNA secretion, suggesting that the mammary gland is the more likely major contributor of milk miRNA biogenesis. In contrast, highly expressed milk miRNAs could be detected at significantly higher levels in the blood serum of the neonate in comparison to adult blood, suggesting milk miRNAs may be absorbed through the gut of the young, at least during the early postnatal phase of development. Therefore the results support the notion that milk miRNA may have evolve to contribute protective and developmental signals to the young.

Effects of Maternal Periconception Overnutrition on the Adipose Tissue Epigenome of Offspring

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Maternal nutrition during pregnancy is linked with metabolic changes in adult offspring of a number of mammalian species. The metabolic changes can lead to increased adipose tissue deposition and increased risk of metabolic disease in humans and animal models. These effects are encapsulated by the Development Origins of Adult Disease hypothesis, which posits that during critical times in early development maternal nutrition influences developmental programs resulting in permanent changes in offspring metabolism and disease susceptibility in later life. However, the mechanism underpinning these effects is unclear. An established sheep model developed by the Early Origins of Adult Health Research Group (CM, JM, SZ, LN) was used to examine the impact of maternal periconception overnutrition on offspring perirenal adipose tissue deposition and its epigenome in late gestation offspring. Importantly, the animal model used embryo transfer into control recipient ewes for the duration of gestation to separate maternal periconception nutritional influences from maternal metabolic influences during gestation. Genome wide profiling of gene expression (RNASeq) and three chromatin modifications (ChIP-Seq) were performed. A whole genome methylation analysis of a reference sample was also undertaken. Independent of nutritional treatment, there were strong positive and negative relationships between the chromatin marks and gene expression and, DNA hypomethylation features were intimately associated with the chromatin marks. It is suggested that the hypomethylation features define a permissive and lineage specific epigenetic state that is acted upon by chromatin modifications to control gene expression. Periconception overnutrition had little impact on gene expression although there were changes in the histone 3 lysine 27 acetylation chromatin mark suggesting the presence of a latent epigenetic signature of periconception overnutrition in the late gestation fetuses. We speculate that this epigenetic signature directs changes in gene expression that alter adipose tissue metabolism and deposition in subsequent postnatal life.

The Nature of Nutrition: An Integrative Framework from Animal Adaptation to Human Obesity

Stephen J. Simpson, Charles Perkins Centre, The University of Sydney, Australia

Macronutrients (protein, fats and carbohydrates) are fundamental dietary components, yet the question of what represents a macro-nutritionally balanced diet and how this maintains health and longevity remains unanswered. We have developed a set of state-space models called the Geometric Framework (GF) to capture the multidimensional nature of nutritional requirements, the relative values of foods in relation to these requirements, the behavioural and physiological responses when feeding on diets of varying composition, and the growth and performance consequences of being restricted to particular dietary regimes. We have also derived the necessary theory for defining health and performance in relation to nutrient intake, for describing key nutritional traits and assessing trade-offs between different responses. I begin by introducing these models and then show how they have been used to address problems in life-history theory, immunity, gut microbial ecology, ageing, obesity and cardiometabolic health. Along the way I will use examples spanning slime moulds to humans.

Modulation and Programming of Immunity and Intestinal Microbiota through Early Life Supplementation with Human Milk Oligosaccharides in an Autoimmune Mice Model

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Objective: Human milk oligosaccharides (HMOS) are known to have an important impact on microbiome composition and immune development. We investigated the effect of early exposure to HMOS on the development of autoimmune type 1 diabetes in later life.
Methods: From pooled samples donated by healthy donors, the total fraction of lactose reduced HMOS was isolated, and added to semi synthetic AIN-93M diets (1% W/W). Non-Obese Diabetic NOD/ShiLtJ mice received at wk 4-10 of age HMOS supplemented or control diet. During the experiment diabetes incidence was measured by urine glucose test. After two positive tests, or at 30 weeks of age, animals were sacrificed and insulitis was further analyzed histologically. In addition, splenocyte populations were analyzed by flow cytometry, and the microbiome composition as well as the microbial metabolites were studied.
Results: Early life exposure to HMOS significantly ($p<0.05$) reduced the incidence of diabetes up to the age of 30 weeks, as measured by urine glucose test compared to control diet. The programming effects of HMOS were corroborated by reduced insulitis and decreased blood glucose levels. Moreover, decreased T-cell activation was detected in the spleen but were not dependent on the levels of splenic regulatory T cells. In addition, modulation of microbial metabolite ratios was detected.
Conclusion: These results implicate that human milk derived oligosaccharides modulates the immune development and microbiome composition leading to suppression of spontaneous autoimmune development later in life.

Homeostasis and the Composition of Mammalian Milk

David Raubenheimer, Charles Perkins Centre, The University of Sydney, Australia

David Raubenheimer and Stephen J. Simpson

Recent work has shown that homeostasis is a powerful concept that can provide a unifying framework for nutrition science. In this talk I will show how a modified concept of homeostasis which takes into account the interactions among regulatory systems for different nutrients can help to explain and predict animal responses at levels spanning the physiological, behavioural and ecological. I will present examples from animal foraging, and then consider the implications for the special case of suckling mammals which have limited opportunity for independently regulating their nutrient intake.

Human Complex Milk Lipids: Concentrations, Benefits and the Implications for Pediatric Nutrition

Alastair MacGibbon, Fonterra Co-operative Group, Palmerston North, New Zealand

Alastair MacGibbon, Bertram Fong, Angela Rowan and Paul McJarrow
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Complex milk lipids (CML) include phospholipids, gangliosides, cerebrosides and other polar lipids and are found as major components of the milk fat globule membrane (MFGM). Reported levels of these bioactive components in human milk are very variable due to a range of reasons including sample collection, extraction methods and assay methodology.

Gangliosides (GA) are a large family of sialic acid containing glycosphinglipids found in many tissues and fluids such as milk. Diversity within the family is provided through both the polar sugar group and the nonpolar ceramide anchor. Due to difficulties of analysing the whole molecule historically gangliosides have been quantified as lipid bound (or associated) sialic acid (LBSA or LASA) which has left open a large degree of uncertainty on actual levels. Application of more modern LC and LC-MS techniques in recent years has produced more robust quantification. The application of this to human milk from mothers in Malaysia and China has determined the average level along with detailed analysis of longitudinal changes in GA species, concentrations and fatty acid profiles. Phospholipids (PL) in the human MFGM include sphingomyelin (SM), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidyl serine (PS). Application of LC-MS analysis has allowed us to determine the longitudinal changes in PL species and their concentrations through the first 12 months of lactation. CML supplementation of the diet in preclinical studies has shown significant impact *in utero* and in infancy on brain composition, learning and memory, synaptic signalling, immune system and anti-infection. Nutritional infant and early childhood studies indicate benefits seen in preclinical studies can be seen in humans.

This presentation will summarize the up to date science around human milk ganglioside and phospholipid content and suggested benefits and extrapolate the implications to the future formulation of human milk substitutes.

Metabolomic and Proteomic Insights into Milk Biochemistry:
Genetic, Seasonal and Feed Induced Changes in Milk

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It has long been recognized by milk processors that the properties of milk change over the milking season and that the suitability of milk for different products varies accordingly. Modern analytical technologies provide the ability to assess this variation with new depth. Liquid chromatography mass spectrometry (LCMS) methods for the investigation of proteins, oligosaccharides, polar lipids and triacylglycerides have been developed and applied to milk from Holstein cows on a dairy in SE Victoria over a milking season. The same samples have also been analyzed by nuclear magnetic resonance spectroscopy (NMR) and a more traditional fatty acid methyl ester (FAMES) approach by gas chromatography mass spectrometry (GCMS). The techniques allow demonstration of variation in many milk components. For instance NMR studies reveal a decrease in citrate, while lipid analysis shows an increase in certain lipid species, and decreases in others, over the season. These changes affect both major and minor components therefore influencing the value of milk for particular products be they commodity products or higher-value products e.g. with human health benefits.

Perhaps not unexpectedly, diet can also have an effect on milk biochemistry; with changes in metabolites such as urea, choline and certain lipid species observed in milk from animals on different diets. Animal genetics is also critical and significant variation within a herd can be seen (e.g. cholesterol was shown to vary from 50 ug/mL to almost 300 ug/mL across 120 animals at one time point). The potential for these new omic tools to better define milk allows greater opportunities for breeders and processors to understand changes in milk properties and develop strategies to modify milk properties for product optimization.

Poster Abstracts

1.) Collection of Data for the Genetic Improvement of Health Traits in Australian Dairy Cattle

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A growing concern for dairy farmers is the improvement of dairy cow health through genetic selection. Healthy cows are more productive, easier to manage, require less intervention, have improved animal welfare and contribute to profitability by reducing production costs. However, in many countries, including Australia, industry collection of data on common health events has been sub-optimal or absent, which means there is no ability to provide breeding values and apply genetic selection for common health disorders. Also, such traits are low in heritability, meaning that although genetic progress is feasible, it will be slower. While many farmers may collect some of this information on farm, there is likely to be variation in the completeness of these data sets. In Australia, there is little storage or export of such information from some on-farm software packages into industry databases for research or reporting purposes. Before any work can begin on providing Australian farmers with breeding values for common health disorders, it is important to quantify what data is already being collected on farm and in veterinary practices. As a result of this challenge, the health data for healthy cows (HDHC) project has commenced to help improve our understanding of the extent of health data recording in the Australian dairy industry. The HDHC project will use infrastructure through the Dairy CRC in the form of the 100 'Ginfo' (Genomic information) herds to collect all health data that is currently being amassed on farm. The Ginfo data is being used as a genomic reference population for genomic breeding values. One of the advantages of having a genotyped population is that it opens up new opportunities for new breeding values, such as dairy health traits. A survey was given out to the 100 GINFO farmers to get an understanding of the health data collection and storage methods that occur on farm. Health data files were collected from the respective herd test centers of these herds. Health data obtained from herd test centers totaled 275,729 records from just 46 out of the 100 herds (To date). The four most recorded health diseases identified were mastitis, reproductive problems, lameness and metabolic disorders. This is fairly consistent with the survey conclusions on what farmers indicated they record and what they think is most important. Mastitis having the highest incidence with 20% of cows affected, followed by reproductive problems (12%), lameness and metabolic disorders (5% and 3% respectively). This project has provided an insight into what health information is actually being collected on farm and that there is a source of health data available which can be accessed and potentially used for the genetic improvement of health traits in Australian herds and potentially providing genomic breeding values for new health traits such as lameness which is a trait in demand requested by Australian dairy farmers.

2.) **Effect of Probiotic Milk and Probiotic Yogurt to Counteract the Negative Effect of Obesogenic Diet**

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Several studies have demonstrated that the gut microbiota as one of the most origin of metabolic disorders such as obesity and type 2 diabetes. Western obesogenic diet (i.e. rich in saturated/transfat and simple sugars and poor in fibers) can importantly alter gut microbiota (i. e. dysbiosis) leading to the production of pro-inflammatory cytokines and metabolic endotoxemia, therefore promoting metabolic disorders. To counteract these negative effects, probiotics (*Lactobacillus*, *Bifidobacterium*) have been used to shaping the gut microbiota (commensal bacteria) and then prevent inflammation and obesity. No study has compared, until now, the anti-obesity effect of new probiotic strains contained in milk and yogurt. The objective of this study was to compare the anti-obesity (*in vivo*) effect of new probiotics in yogurt and milk and their survival after digestion. Two probiotics isolated from faeces and raw milk, *Bifidobacterium animalis* ssp. *lactis* (**Bf141**) and *Lactobacillus rhamnosus* (**Lb102**), genetically identified, were used. Both freezing-drying strains were added individually in skim milk and non-fat yogurt. The population of each strain was 1 x 10^9 cfu per serving. Male mice fed with High-Fat High-Sucrose (**HFHS**) were treated by daily gavage with yogurt or milk with and without probiotic (**WP**) for 8 weeks. Body weight gain, epididymal white adipose tissue weight (**eWAT**) and blood glucose concentration (glycaemia) were determined as well as the survival of probiotics after digestion. The both probiotic strains survived to the gastrointestinal passage. Bf141 was more resistant than Lb102 to gastrointestinal transit, whether fed in milk or yogurt, but the post-transit counts of both probiotic isolates remained above 1 x 10^8 cfu g^{-1}. The body weight gain, eWAT mass and blood glucose concentration in mice fed with high-fat high-sucrose diet were more reduced with probiotics milks (Bf141 and Lb102) than with control WP milk. The anti-obesity effect was greater when the probiotic was delivered in milk rather than yogurt. These probiotic strains had any anti-obesity effect in yogurt whereas weight gain, fat mass and plasma glucose in mice were reduced similarly with Bf141 and Lb102 in milk. In this study, milk was therefore a better vector than yogurt for probiotic bacteria with anti-obesity effect. Studies are in process to evaluate the effects of these probiotics on inflammation (cytokines, LPS) and on the composition of gut microbiota.

3.) **A Role for Sim2s in Elongation Regulation of Milk Protein Gene Transcription in the Mammary Gland**

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Student Travel Award Recipient

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Identification of the mechanisms underlying the establishment and maintenance of functional differentiation is critical to our understanding of lactation. Genome wide analyses have recently demonstrated that regulation of differentiation occurs in large part by transcription elongation. Elongation proceeds after RNA Polymerase II (RNAPII) is released from its paused state through association with the positive transcriptional elongation factor (P-TEFb) containing catalytic enzyme, Cdk9. However, the mechanism by which regulation of these processes occur has yet to be elucidated in the mammary gland. We have previously shown that Singleminded-2s, (Sim2s) a member of the bHLH/PAS family of transcription factors, is required for establishing and maintaining mammary gland differentiation. Overexpression of Sim2s results in heightened expression of the milk protein genes beta-Casein (Csn2) and Wap. We have further demonstrated that conditional loss of Sim2s in mouse mammary epithelial cells results in decreased Csn2 gene expression. Building on chromatin

immunopreciptitation (ChIP) and re-ChIP analyses documenting the association of Sim2s at the Csn2 promoter and interaction of the RNAPII complex with Sim2s respectively, we further analyzed the role of Sim2s in transcriptional regulation of differentiation-dependent gene expression. ChIP analysis of exon 7 at the Csn2 gene locus uncovered association of Sim2s, RNAPII, and Cdk9. Using mouse mammary epithelial cell lines (HC11 and CIT3) we found that inhibition of Cdk9 caused loss of milk protein gene expression. Additionally, we found an interaction between Cdk9 and Sim2s, which further indicates that Sim2s associates with transcription elongation machinery. This suggests Sim2s acts, not only as a traditional transcription factor, but also plays a role in enhancing transcription elongation of milk protein genes. Together these data demonstrate a novel paradigm of milk protein gene regulation in the mammary gland.

4.) Supplementation with *Bifidobacterium Infantis* in Combination with Bioactive Milk Components to Alter Microbial Composition in Children with Autism

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A feature of the evolution of lactation is the explicit development of a symbiotic relationship between microorganisms and the mammalian host that is absolutely essential for proper physiological function. Human milk guides the colonization and composition of the infant gut microbiota more than any other environmental factor and compelling evidence is emerging that a milk-oriented microbiota (MOM) guides the development of various systemic processes (immunity, metabolism, neurological networks) and sets the infant up for a lifetime of health, including optimal neurodevelopment. The extent of the effect of microbial composition and activity on human physiology has only begun to be explored with neurodevelopment positioned at the very edge of this investigation. Examination of neurodevelopmental disorders such as autism spectrum disorders (ASD) and their connection with gut microbial dysbiosis will provide insight into the explicit but often elusive gut microbiota-brain axis in human development. Children with ASD, characterized by a range of cognitive and social deficits, are also commonly plagued by gastrointestinal (GI) co-morbidities including chronic constipation and diarrhea. The severity of autism symptoms is often correlated with the extent of GI symptomology as well as degree of microbial dysbiosis in the gut. This study was designed to explore characteristics of microbial dysbiosis that may contribute to the severity of GI and behavioral symptoms in children with ASD and whether supplementation with the probiotic *Bifidobacterium infantis* with bioactive milk components (bovine colostrum product – BCP) can ameliorate this microbial dysbiosis. This pilot study is a randomized, double blind, controlled trial of a synbiotic (BCP + *B. infantis*) vs. BCP alone. Each participant was randomized to receive either the synbiotic first or the BMO first in a cross-over study design. The study lasted 12 weeks, including 5 weeks of BCP use, 5 weeks of synbiotic use, and a two-week washout period in between. Stool, urine, and blood samples were collected throughout the study and analyzed for microbial composition. Results of this study will provide insights into the dependence of human neurodevelopment on the natural progression of the gut microbiota.

5.) **Human Breast Milk miRNA, Maternal Probiotic Supplementation and Atopic Dermatitis in Offspring**

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Student Travel Award Recipient

Background: Perinatal probiotic ingestion has been shown to prevent atopic dermatitis (AD) in infancy. This has been observed in a number of randomized clinical trials using a variety probiotics strains and supplementation regimes, and the strongest evidence comes from studies which include both pre- and post-natal supplementation. Results from our own study, Probiotics in Prevention of Allergy among Children in Trondheim (ProPACT), suggest that maternal supplementation alone is sufficient to prevent AD in offspring. The mechanisms behind this preventative effect are incompletely understood and may be partially mediated by changes in breast milk composition. Micro-RNAs (miRNA) are abundant in mammalian milk and may influence the developing gastrointestinal and immune systems of newborn infants. In this study, we investigated breast milk miRNAs as possible mediators of the observed preventative effect of probiotics using samples collected during the ProPACT trial.

Objective: To describe the miRNA profile of human breast milk, and to investigate the association between miRNAs, maternal probiotic supplementation and the development of atopic dermatitis in offspring.

Methods: Fifty-four breast milk samples were obtained 3 months postpartum from women participating in the ProPACT trial, a double blinded, placebo-controlled randomized trial of perinatal probiotic supplementation. The probiotic milk consumed by the women randomized to the probiotic arm of the trial contained 5 x 1010 CFU of *Lactobacillus rhamnosus GG* and *Bifidobacterium animalis* subsp. *lactis* Bb-12 and 5 x 109 CFU of *L. acidophilus* La-5. Breast milk samples were enriched for extracellular vesicles using ExoQuick™, total RNA was isolated and small RNA sequencing was conducted on the Illumina HiSeq 2000 platform. Differential expression of miRNAs was assessed for the probiotic vs placebo and the AD vs non-AD groups. Target gene and functional predictions were conducted using TargetScan and DAVID bioinformatics resources, respectively. The ProPACT trial is registered at ClinicalTrials.gov (identifier NCT00159523).

Results: The RNA isolates obtained from the human milk samples contained between 12.4 and 247.5ng/µL of RNA, and were found to contain RNA fragments up to 1000nt in length which aligned primarily to rRNA, tRNA and miRNA. The miRNA profile revealed a relatively stable core group of highly expressed miRNAs, including miR-148a-3p, miR-22-3p, miR-30d-5p, let-7b-5p and miR-200a-3p. Functional analysis of the target genes for the 20 most highly expressed miRNAs revealed enrichment of a broad range of biological processes and molecular functions and significant overlap with genes that are upregulated in brain and epithelial tissues. Although several miRNAs were found to be differentially expressed on comparison of the probiotic vs placebo and AD vs non-AD groups, none had an acceptable false discovery rate and their biological significance in the development of AD is not immediately apparent from their predicted functional consequences.

Conclusion: Whilst breast milk miRNAs have the potential to be active in a diverse range of tissues and biological process, individual miRNAs in breast milk 3 months postpartum are unlikely to play a major role in the prevention of atopic dermatitis in infancy by probiotics ingestion in the perinatal period.

6.) **Brucellosis as Health Hazards for Smallholder Dairy Farmers in Pakistan**

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Pakistan is the fifth largest milk producer globally, and small-holder dairy farms are the mainstay of most rural communities there. While some large-scale dairy operations are underway and in development in that country, the vast majority of production is via smallholder farms, typically with under ten livestock (cattle and/or buffalo), There are substantial impediments to development of these farms, in terms of animal husbandry, nutrition, and health-related factors. Further, the close contact between farmers and livestock results in many zoonotic transmissions of diseases. Brucellosis is considered by the Food and Agriculture Organization, the World Health Organization and the World Organization for Animal Health (OIE) as one of the most widespread zoonoses in the world, and is still endemic in many developing countries. In Pakistan brucellosis is a neglected disease and is still prevalent at a very high rate due to lack of public awareness and preventive measures.
The aim of this study was to investigate household practices that may act as risk factors for brucellosis transmission from cattle to humans in smallholder dairy farms. A cross-sectional study among smallholder farms (*n*=420) was conducted in the seven districts of Punjab and Sindh provinces. A questionnaire to assess potential risk factors was designed and used during face-to-face interviews. A combination of purposive and random sampling was used to select the participant smallholder farms. Results from the questionnaire indicate that the household practices could be the potential risk factors for human brucellosis. The study found that only 22 % of farmers know that they can get the disease from animals, and only 3 % of farmers have knowledge about the mode of transmission in brucellosis. Further, 65 % of farmer families consume raw milk and its products, and, 48 % farmers live in a shared place with animals. One other major risk factor identified was that 75 % of farmers do not cover their hand cuts before contact with animals. Coupled with this, 17 % have direct contact with foetal membranes during animal parturition, and that is an intense source of bacteria if the animal is infected. In conclusion, results from this study suggest there is a need to estimate the true prevalence of disease in the area and educate the farmer via participatory epidemiology about the risky farm practices and their preventive measures. Through improvements in health, both of people and livestock, and coupled with others gains in livestock management, the economic and social quality of lives of smallholder farms can be improved considerably.

7.) **Variation in Phosphorylation Degree of Bovine As2-Casein**

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Caseins are highly phosphorylated milk proteins interacting with calcium phosphate to form large colloidal structures called casein micelles. Phosphorylation of casein is an important post-translational modification occurring in the mammary gland under the action of kinases. However, the molecular mechanism behind is not fully understood yet. These kinases recognize the consensus motif as a glutamic acid, phosphor-serine residue, or aspartate at the position n+2 relative to the target serine or threonine residue (**Ser**/Thr-x-**Glu**/**SerP**/Asp; Mercier, 1981). This modification is essential for the construction and the stabilization of the micelle structure which is based on interactions between phospho-serine or phospho-threonine residues of casein and calcium phosphate. αs2-casein (αs2-CN) is of particular interest since it is the most phosphorylated casein and exhibits various phosphorylation states. In addition, it has been the least investigated since its phosphorylated

isoforms are difficult to purify and quantify. The objective of this study was to investigate the variation in the phosphorylation degree of αs2-CN among individual cows.
Milk samples from 500 French Montbéliarde cows were collected as part of a R&D project (FROM'MIR)*****, and were analyzed by liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) to determine milk protein composition. Relative protein concentration of each αs2-CN phosphorylated isoform was quantified by mass signal intensity (Miranda *et al.*, 2013). Method reproducibility was assessed by calculating the coefficient of variation (CV) of relative protein concentration for all αs2-CN phosphorylated isoforms from a reference milk sample that was analyzed after every 10 milk samples. In total, 50 reference milk samples were analyzed. αs2-CN was characterized with multiple phosphorylation states containing 10 to 14 phosphate groups (10P-14P). This is the first study that shows the presence of αs2-CN-14P, and the first method to determine the relative concentration for each αs2-CN phosphorylated isoform. CV values for reproducibility of the relative protein concentration were below 6% for all the αs2-CN phosphorylated isoforms. Furthermore, CV values for relative protein concentration regarding all the αs2-CN phosphorylated isoforms (> 10%) were larger than CV values for reproducibility, which implies a significant variation in phosphorylation degree of αs2-CN among individuals. Regarding to the correlation between the relative concentration of the different phosphorylation states of αs1-CN and αs2-CN, αs1-CN-9P had negative correlation with αs2-CN-10P and αs2-CN-11P (-0.74 and -0.72, respectively), but positive correlation with αs2-CN-13P and αs2-CN-14P (0.69 and 0.67, respectively). This observation supports the previous finding on the relationship between αs1-CN-8P and αs1-CN-9P, which suggests that two different sets of genes are involved in regulation of phosphorylation isoforms (Bijl *et al.*, 2014).

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8.) **Using Whole Genome Sequences to Identify Candidate Mutations of Bovine Milk Fatty Acids and Proteins in Dairy Cattle**
Presented by *Zih-Hua Fang, INRA, France*

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In the framework of the PhenoFinlait project, 23 milk fatty acids and 6 major proteins were analyzed by genome-wide association study (GWAS) at the whole genome sequence level in three French dairy cattle breeds. Traits were estimated from Mid Infrared (MIR) spectrometry for about 600,000 test-days of 116,495 cows in first or second parity in Montbeliarde (MO), Normande (NO) and Holstein (HO) cattle breeds. More than 8600 cows (3,032 MO, 2,659 NO, and 2,954 HO) were genotyped with the Illumina 50K Beadchip®. In each breed, genotypes were first imputed to the high density (777k SNP) using HD genotyped bulls as the reference panel (522 MO, 546 NO, and 776 HO). The resulting HD genotypes were subsequently imputed at the sequence level. As a reference panel, we used 27 millions of highly confident sequence variants produced by run 4 of the 1000 bull genomes project, including 1147 sequenced bulls. Imputation was carried out with Fimpute software.
Individual test-day records were first adjusted for environmental effects after a polygenic evaluation and then averaged per cow. Analyses were conducted within breed with GCTA software, accounting for the population structure through a polygenic effect and a HD-based genomic relationship matrix. Among numerous QTL regions identified in each breed, two of them, located on chromosomes 11 (LGB) and 14 (DGAT1), had large effects on both milk fatty acid and protein composition. For fatty acids, other very significant regions were in agreement with already known regions for fat components or fatty acid desaturation, e.g. on chromosomes 19 (FASN), 26 (SCD1), and 27 (AGPAT6). Several strong QTLs were also found on chromosomes 5 (93.9Mb), 17 (53.2Mb) and 20 (32.8). For proteins, 5 other very significant QTL regions were shared by the three breeds. They were located on

chromosomes 1 (144.4Mb), 2 (131.8Mb), 6 (87.4Mb i.e. casein genes region), 20 (58.4 Mb) and 29 (9.5Mb). Other significant effects, partially overlapping across breeds, were found on almost all autosomes. Besides previously described mutations, resolution obtained in the present study was high enough to directly pinpoint a small number of novel candidate mutations for milk fatty acid and protein composition. Several hundred variants were added to the custom part of the EuroG10K chip widely used in genomic selection, for large scale validation and, for some of them, possible use in genomic selection. The authors acknowledge the financial support from ANR, APIS-GENE, Ministry of Agriculture (CASDAR), Cniel, FranceAgriMer and FGE, and the contribution of the 1000 bull genomes consortium.

9.) **Precision Diets: Bringing Milk Research to Practice**

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Chronic and degenerative diseases from obesity to autoimmunity are epidemic around the world and will afflict more than half the world's population. Thus, the opportunities facing food development by improving human health are epic. Precision medicine is the emerging field that takes a more personalized approach to therapeutic intervention to address these diseases. However, these conditions arise gradually within individuals through imbalances of systemic processes (metabolism, physiology, immunity) for which traditional pharmacologic interventions using targeted small molecules are ineffectual. Rebalancing whole body processes through dietary mechanisms is ostensibly exactly what milk does for infants. Research over the past decade of the IMGC has documented that milk guides the appropriate development and function of whole body fuel metabolism, both innate and acquired immunity, physiological processes even tissue remodeling and neurological development. Yet, these potentially transformative discoveries have yet to be brought to commercial practice in the food marketplace. What are we doing wrong? The diabolical complexity of improving health as prevention as commercial opportunity is that efficacy must be demonstrated by mechanism of action within individuals. Thus, the path to commercial success will likely need to include mechanism based precision diagnostics paired to precision diets. The information age has brought the knowledge management tools to practice and it is now only necessary for scientific research and its translation to bring accurate measures of human health to practice in parallel with milk---based interventions. The development of the infant microbiome and its functions provides a tantalizing example of what such a future will look like.

10.) Concentration and Distribution of Sialic Acid in Sow Milk during Lactation

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Sialic acids (Sia), a family of 9-carbon acidic sugar molecules, are key monosaccharide units of brain gangliosides and glycoproteins and a major component of sialylated glycotope in human milk. Human milk Sia has been proposed as a bioactive compound promoting immune function, gut maturation and neurodevelopment of the newborn. Porcine milk however, has received little attention. The aims of the present study were to quantify and compare the levels of *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc) and ketodeoxynonulpsonic acid (KDN) in oligosaccharide, glycoprotein and glycolipid in sow milk during course of lactation. Milk samples from 22 sows were collected by manual expression on 3 occasions, day 1 (colostrum), day 3 (transition milk) and day 15-21 (mature milk) respectively. Theconcentration of Neu5Ac, Neu5Gc and KDN were analyzed using

UHPLC. The results showed that sow milk contained significant amounts of Sia with the highest concentration found in colostrum (1238.50 mg/L) followed by transition milk (778.32 mg/L) and then mature milk (347.21 mg/L). Most of the Sia in sow milk was conjugated to glycoproteins (41-46%), free oligosaccharides (31-42%) and then glycolipid (12-28%) throughout the course of lactation. Neu5Ac was the major form of Sia (93-96%) and then Neu5Gc (3-6%), KDN however contained as little as 1-2%. This distribution was common to each milk fraction and to each time point in lactation. In conclusion porcine milk contains a rich source of sialylated glycan in the forms of glycoproteins, free oligosaccharides and glycolipids. The high concentrations of Sia in porcine milk suggest that Sia is an important nutrient that may contribute to the optimization of immune function, neurodevelopment and growth and development of piglets.

11.) Identification and quantification of triacylglycerols containing omega-3 long-chain polyunsaturated fatty acids in bovine milk

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Omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA) are low-abundance components in milk fat, but have great potential in promoting human health and development, such as prevention of cardiovascular disease, enhancing brain development in infants and anti-inflammatory activity. A comprehensive survey on triacylglycerol (TAG) molecular species that contain at least one type of ω3 LC-PUFA, namely EPA (C20:5), DHA (C22:6) and DPA (C22:5), has been conducted in this work using LC-LTQ-Orbitrap and LC-Triple Quadrupole mass spectrometry (MS) techniques. A combination of neutral loss scan, accurate mass data and MS2 profile allowed us to identify over 60 TAG species in milk that contain ω3 LC-PUFA. Among them, 51 species were present at a level that allowed reliable quantification. The fatty acid composition of these 51 TAG species was established using MS/MS fragmentation pattern. The majority of the species were found to be composed of even numbered fatty acids, but one odd numbered saturated fatty acid (C15) was detected in three species. All ω3 LC-PUFA were esterified together with either two medium-chain fatty acids (C10-C18), or one short-chain (C4-C8) and one medium-chain fatty acids. A LC-Triple Quadruple MS-based method was developed in order to quantify all the identified TAG species. TAG containing DHA were found in much smaller number and at much lower abundance compared with those containing EPA or DPA, and in all cases the distribution of ω3 LC-PUFA in TAG was not random. Our study confirmed that when the cow's diet is not fortified with DHA, milk is not an important source for DHA intake, but the significant amount of EPA and DPA found in milk is expected to contribute substantially to human intake of ω3 fatty acids. When this method was applied to the analysis of milk samples collected from cows after different dietary treatments, the abundance of all EPA-containing TAG species was found to be significantly reduced after intake of grape marc diet. This is the first systematic characterisation of low-abundance TAG molecular species that contain ω3 LC-PUFA residues in bovine milk to our knowledge. Our study has revealed that the conversion of DPA to DHA is very limited in cows, and the feed has a huge impact on the fatty acid composition of milk fat.

12.) **Isolation of Putative Mammary Stem/Progenitor Cells from Cultured Primary Bovine Mammary Epithelial Cells using Fluorescence Activated Cell Sorting.**

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Introduction- The proliferation of mammary stem cells in the bovine mammary gland may influence milk production (Capuco et al., 2009). While populations of mammary stem cells have been identified and characterized in various species, little is known about this cell population in the bovine mammary gland (Tiede and Kang, 2011). It has been proposed through investigations in mice that putative mammary stem and progenitor cells exhibit different immunophenotypes (Visvader and Stingl, 2014) with the potential populations of putative mammary progenitor cells (*Lin-/CD24+/CD29+/CD61+*) and putative mammary stem cells (*Lin-/CD24+/CD29+/CD49f+*). These cell surface marker combinations were used to isolate cells from culture bovine mammary epithelial cells to assess the utility of these markers for enriching populations of putative mammary stem and progenitor cells in this species.
Materials and Methods- Mammary tissue biopsies from three Holstein Friesian cows sampled at 6-20 days prior to parturition were macerated, digested and isolated through a Percoll-Ficoll gradient. The layers (1.03/1.04 density layers) containing bovine mammary epithelial cells (BMEC) were removed and cultured. At passage 4 or 5 the cells were detached, put into solution and depleted of Lin+ (nonepithelial cells positive for antibodies: CD45, CD31, TER119, BP-1) through the use of magnetic bead separation. The remaining cells were treated with a cocktail of antibodies and isolated by FACS. Once isolated the cell populations were tested functionally using proliferation (manual cell count) and mammosphere formation (number and area measurement by image analysis) assays.
Results/Discussion- FACS analysis revealed 12.67 % (± 6.67 %) (n=3) of cells identified as putative stem cells (*Lin-/CD24+/CD29+/CD49f+ phenotype)* and 15.33% (± 4.16 %) (n=3) as putative progenitor cells (*Lin-/CD24+/CD29+/ CD61+ phenotype)*. When cultured *in vitro* the proliferation rate of the sorted putative stem/progenitor populations showed a significantly higher mean proliferation rate at 133% (± 18 %) and 130 % (± 8 %) respectively compared to unsorted control cells. When the same populations were isolated and investigated with Matrigel culture there were fewer observed mammospheres formed by both sorted populations, with 75.9 % (± 1.02 %) and 80.6 % (± 9.57 %) for putative stem and progenitor cells respectively compared to unsorted control cells. There was very little difference in the size of the mammospheres seen in both the putative stem and progenitor cell population compared to the unsorted control cells.
Conclusions- These observations suggest that the immunophenotypes observed in mice for enrichment of mammary stem and progenitor populations may have similar characteristics and putative function in bovine mammary cell populations. Isolation of these cell populations may be useful in future experiments investigating mammary cytodynamics in the lactation cycles of dairy cows.
References- Capuco, A., Evock-Clover, C., Minuti, A., and Wood, D., In Vivo Expansion of the Mammary Stem/Progenitor Cell Population by Xanthosine Indusion. J. Experimental Biology and Medicine, 2009. 243:4, 475-482.
Tiede, B., and Kang, Y., From Milk to Malignancy: The Role of Mammary Stem Cells in Development, pregnancy and Breast Cancer. Cell Research, 2011. 21, 245-257.
Visvader, J., and Stingl, J., Mammary Stem Cells and the Differentiation Hierarchy: Current Status and Perspectives. Genes and Development, 2014. 28, 1143-1158.

13.) **Bottom-up and top-down analyses of cow's milk proteins using LC-MS**

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Cow's milk is a complex fluid whose proteome displays a diverse set of proteins of high abundance such as caseins (CN) and medium to low abundance whey proteins such as beta-lactoglobulin (b-LG), alpha-lactabumin (a-LA), lactoferrin, immunoglobulins, glycoproteins, peptide hormones and enzymes. CNs represent 80% of milk proteins and possess important nutritional and functional properties, whilst b-LG (largely unknown function) and a-LA (involved in lactose biosynthesis) represents approximately 10 and 4%, respectively. As genetic variants of CNs and whey proteins and post-translational modifications (PTMs) influence many properties of milk that are essential to the dairy industry, the development of robust analytical methods for the separation and quantification of variants of milk proteins within single protein fractions is of interest. A sample preparation method that enables high reproducibility and throughput is key in reliably identifying proteins present or proteins responding to conditions such as a diet, health or genetics.
This work had two purposes:
1) Optimizing a bottom-up proteomics procedure to identify as many proteins as possible from skim milk samples in a high-throughput fashion. Three extraction procedures that have not previously been applied to milk were compared using protein assays, SDS-PAGE profiling, and trypsin digestion followed by nLC-MS/MS (LTQ-Orbitrap) analyses. Replicates were used at each analytical step (extraction, digestion, injection) to assess reproducibility.
2) Establishing a top-down proteomics method to study intact major proteins from skim milk. The effects of HPLC column temperature, mobile phase composition, and gradient conditions were evaluated to improve protein separation prior to analysis using ESI-Qq-TOF MS. High resolution spectra subsequently allowed accurate mass determination and sequencing of major proteins, along with the identification of PTMs.

Applying these bottom-up and top-down methods on bulk milk samples from Jersey and Holstein cows, we demonstrate the complementarity of both approaches.

14.) Microbiota Identification and Diversity of Australia Kefir

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Kefir is a traditional cultured dairy product that originated from the Caucasus mountain regions. Kefir is produced by the microbial activity of kefir grains which contain a unique symbiotic complex mixture of bacteria, yeast and polysaccharides. The health benefits of kefir and its constituents have been claimed to include lowering cholesterol, anti-microbial and anti-mutagenic activity, immunomodulatory activity and improved lactose digestion. However, the microbial profiles of kefir grains are considered likely to be variable given the diverse geographical origin of the grains, the climatic conditions, the methods, culture temperature and source of the milk.In this study, we firstly investigated the profile of microbiota in milk kefir grains collected from the Victoria region of Australia and its fermented milk using culture-independent high-throughput DNA sequencing technology. The results show that the bacterial population in the kefir grains is much less diverse in comparison with

raw milk, and instead rich in probiotic related bacteria, which are microorganisms thought to have beneficial effects on human health. The composition of the kefir grains is dominated by 1 phlya, the *Firmicutes* (97.00-98.59%), followed by the *Proteobacteria* (0.61-1.62%) and *Actinobacteria* (0.56-1.22%). The most abundant genus identified in the kefir grains is *Lactobacillus* with an abundance of 84.5 to 88.4%. The sub-dominant genus includes *Pediococcus*, *Lactococcus* and *Streptococcus*. In the *Lactobacillus* genus, a *Lactobacillus kefiranofaciens* species predominated with an abundance of 74.64% to 80.99%, which is consistent with other studies. However, the less abundant species present are different from what has been reported in other countries. The species we identified in our study are related to *Pediococcus argentinicus*, *Lactobacillus gigeriorum*, *Lactobacillus senmaizukei*, and *Lactobacillus letivazi.* Moreover, yeasts have also been identified in the kefir grains and are related to *Kazachstania exigua* and *Kazachstania unispora.*

Gut microbiota features have are linked to human physiology. Aberrations of microbiota composition have been associated with many conditions and diseases such as allergies, intestinal inflammatory diseases, cancer, diabetes, cardiovascular disease, dyslipidaemia and brain diseases. Our study indicates that the sub-dominant microflora are diverse in composition, and consistent with the literature, there is a predominance of *Lactobacillus kefiranofaciens*. Kefir in Australia is a source of interesting probiotics which can be readily monitored using advanced culture-independent DNA technologies.

15.) Variation of the Natural Milk Microbiome of Victorian Dairy Cows

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The microbiological profile of raw bovine milk is of major importance at both the on-farm level, for animal health and milk quality as well as at the factory level, for process suitability and product performance. There can be several contributors to the milk microbiome, firstly, the natural milk microbiome within the udder of the healthy cow and subsequent to milking, the environment, hygiene practices and storage/transport conditions encountered on route to the factory. In this study, we attempt to characterize the natural milk microbiome of Victorian dairy cows and aim to provide information about how on-farm variation can contribute to the milk microbiome profile. Factors such as breed, animal health status, animal to animal variation, different feeding regimes and seasonal effects were all examined. Raw bulk milk and raw individual animal milk was collected from two farms on many occasions. Microbes were purified from milk samples, gDNA extracted, 16S rRNA PCR undertaken, DNA libraries prepared and sequencing performed using the Illumina MiSeq platform. The sequence data obtained was quality filtered and analyzed using an automated bioinformatics pipeline, beginning from the sequence file to a table of taxonomy assignments. This approach typically resulted in the identification of over 1000 bacterial OTUs of which only a minor component was culturable under standard conditions. Microbial diversity was reasonably consistent when stored at four degrees for several days, typical of ideal milk transport conditions but as expected, diversity decreased with increased storage temperature and time. Most notably, variation of the microbial profile was evident between animals within the herd at a given time and at different time points across the lactation cycle and season. These variations in the natural microbiome will be demonstrated, correlations investigated and possible implications for animal health, milk production and product quality discussed.

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