Pet nutrition – Art or Science?

Abstracts

Cambridge, UK

September 16-18, 2010
Veterinary medicine at the University of Cambridge

The University of Cambridge has just celebrated its 800th Anniversary, however, the Veterinary School has only been in existence for just over 70 years. It was founded in 1949, but its origins go back to 1909 when the Department of Pathology set up an outstation to study diseases of large animals.

In 1935 the University entered into an arrangement with the Royal College of Veterinary Surgeons whereby it ran a pre-clinical course and a postgraduate diploma, with the final two years spent at one of the existing veterinary schools. The recommendation of the Loveday Report that students completing the Natural Sciences Tripos could go on to take a course leading to the VetMB degree was put into effect in 1949 with the arrival of the first eight students. The Veterinary School was officially opened by HM Queen Elizabeth II on the 20th October 1955.

The Cambridge Veterinary School is now at the forefront of veterinary science and education and is a centre of excellence for teaching and research. Its mission is to improve the prevention and treatment of diseases of animals by defining and applying best clinical practice, by understanding and developing the science underpinning best practice, and by embedding an education programme in the veterinary sciences that delivers the best veterinary practitioners, academics and research scientists.

Talented individuals are educated in the veterinary sciences so that they develop into leading clinicians and researchers. The Veterinary School maintains and develops research excellence in basic and applied biomedical and veterinary sciences and embeds its clinical veterinary training in this strong scientific foundation. We aim to produce practitioners, academic clinicians and researchers of the very highest calibre. Many prestigious posts in the various branches of the veterinary profession are occupied by Cambridge graduates.

The Queen's Veterinary School Hospital is an integral part of the Veterinary School, offering the best professional care as a teaching and a referral hospital. Each year the Hospital sees more than 4,000 new patients referred from veterinary surgeons throughout the UK.
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*All abstracts are in chronological order*

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**The WALTHAM International Nutritional Sciences Symposium**

**Co-hosted by the University of Cambridge and The Nutrition Society**

**September 16-18, 2010, Cambridge, UK**

**PET NUTRITION – ART OR SCIENCE?**

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**WEDNESDAY, 15 September**

**18:30 – 20:30** Welcome Reception at the Crowne Plaza Hotel, Cambridge
  Registration, cocktails and canapés

**THURSDAY, 16th September**

**07:30 – 08:00** Coffee and registration at The Guildhall, Cambridge

**08:00 – 08:30** Welcome – Karyl Hurley, Global Scientific Affairs, Mars Petcare

  Welcome to Cambridge – Dean Mike Herrtage, Cambridge Veterinary School

**SESSION I: WEIGHT MANAGEMENT**

**08:30 – 08:40** The Nutrition Society
  Ian McDonald

**08:40 – 09:30** Advances in comparative genetics – influence of genetics on obesity
  Daniel Pomp

**09:30 – 09:50** Effects of weight loss on adipokines and markers of inflammation in dogs
  Wakshlag, J., Struble, A., Levine, C., Bushey, J., LaFlamme, D., Long, G.

**09:50 – 10:10** Chronic obesity in cats does not lead to a systemic low grade inflammation
  Van de Velde, H., Janssens, G.F.J., Cox, E., Buyse, J., Hesta, M.

**10:10 – 10:30** Comparison of energy expenditure of pet cats estimated using the double-labeled water method with metabolizable energy intake
  Chen, C-A., Hill, R.C., Scott, K.C., Tutela, S.M., O’Donnell, K., Morris, P.J.

**10:30 – 11:00** COFFEE BREAK

**11:00 – 11:20** Omega-3 fatty acid supplementation improves insulin sensitivity and increases EPA and DHA tissue content in obese insulin resistant dogs
  Le Bloc’h, J., Leray, V., Ouguerram, K., Nguyen, P.

**11:20 – 11:40** Plasma estrogen level after estradiol dosage to normalize food intake in neutered cats supports hormone replacement use in obesity treatment
  Backus, R.

**11:40 – 12:00** Portion control after neutering for a period of 18 weeks may help prevent post-spaying weight gain in growing female kittens
  Alexander, L., Salt, C., Thomas, G., Butterwick, R.

**12:00 – 13:00** LUNCH
SESSION II: CHALLENGES IN DEVELOPING NUTRIENT GUIDELINES
13:00 – 15:00 Objectives – to understand issues in development of nutrient guidelines for both humans and animals. How can we apply the learnings from development of human guidelines to animals and vice versa, and ultimately stimulate discussion on how to achieve more frequent and timely updates on nutrient guidelines in dogs and cats

Dietary Reference Intakes (DRIs) for Humans: What are the implications for animal nutrient guidelines?
Dr. John Erdman

National Research Council nutrient recommendations for dogs and cats
Dr. Richard Hill

Developing Nutrient Guidelines – a view from farmed livestock
Prof. Colin Whittemore

The challenges of putting together an NRC report on the nutrient requirements of animals
Dr. Austin Lewis

15:00 – 15:30 BREAK

15:30 – 15:50 Birth weight and postnatal growth of purebred kittens
Moik, K., Kienzle, E.

15:50 – 16:10 The effect of feeding Vitamin A to puppies up to 52 weeks of age
Morris, P., Salt, C., Raila, J., Brenten, T., Kohn, B., Schweigert, F., Zentek, J.

16:10 – 16:30 Effects of selenium sources on semen characteristics and semen antioxidant status in dogs
Putaros, T., Sartori, J., Vasconcellos, R., Barducci, R., Guimarães, A., Carciofi, A.

16:30 – 17:30 BREAK

17:30 – 19:00 POSTER SESSION: Authors of the even numbered posters will be standing by their work for the first hour, and authors of the odd-numbered posters for the second hour.

Drinks and hors d'oeuvres

Dinner at leisure in Cambridge – lists of local eateries provided

Friday, 17 September

08:00 – 08:30 Coffee at The Guildhall, Cambridge

Session III: Advances in Applied Nutrition

08:30 – 09:15 PLENARY: Feline paleolithic nutrition: A consideration of its nature and its implications for nutrition of domesticated cats
Dr. Wouter Hendriks

09:15 – 09:35 The effects of dry and wet diets on faecal bacterial populations in the domestic cat
Bermingham, E., Kittelmann, S., Basset, S., Weidgraaf, K., Hekman, M., Roy, N., Thomas, D.

09:35 – 09:55 Frequency and extent of nutritional imbalances in ‘bone and raw food’ (BARF) rations
Dillitzer, N., Becker, N., Kienzle, E.

09:55 – 10:15 Effects of feeding polydextrose on fecal characteristics, microflora, and fermentative end products in healthy adult dogs
Beloshapka, A., Wolff, A., Swanson, K.

10:15 – 10:45 COFFEE BREAK

10:45 – 11:05 Correlation of a feline muscle mass score with body composition determined by DEXA
Michel, K., Anderson, W., Cupp, C., Laffamme, D.

11:05 – 11:25 In vitro evaluation of fiber and protein fermentation substrates in cats
Rochus, K., Janssens, G., Bosch, G., Hendriks, W., Vanaenecke, L., Hesta, M.

11:25 – 11:45 The potential for enhancement of immunity in cats by dietary supplementation
Rutherfurd-Markwick, K., Hendriks, W., McGrath, M., Weidgraaf, K., Thomas, D.

Queau, Y., Larsen, J., Kass, P., Glucksman, G., Fascetti, A.

12:05 – 13:00 LUNCH

13:00 – 13:20 Association between serum 25-hydroxyvitamin D (25-OH-D3) level and mast cell tumors in Labrador retrievers
Malone, E., Wakshlag, J., Rassnick, K., Struble, A., Vachhani, P.

13:20 – 13:40 The effect of diet composition on glucose, insulin and leptin concentrations, weight gain and food efficiency in healthy cats
Coradini, M., Rand, J.S., Morton, J.M., Arai, T., Ishioka, K., Mori, A., Rawlings, J.M.

13:40 – 14:15 COFFEE BREAK

SESSION IV: PET FOOD SAFETY – A SHARED CONCERN

14:15 – 14:45 Food safety challenges facing the pet food industry
Dr. Robert Buchanan

14:45 – 15:05 Microbiological challenges facing the pet food industry
Robert C. Baker

15:05 – 15:25 Non-targeted analysis of foods and feed
Adrian Charlton

15:25 – 15:45 Novel ingredients: assuring safety and sustainability
Dr. Jim E. Riviere

15:45 – 16:05 Pet food safety: the role of new technologies
Dr. Robert Standaert

16:05 – 16:30 BREAK

16:30 – 17:15 OPEN DISCUSSION

SATURDAY, 18 September

08:30 – 08:50 Coffee at The Guildhall, Cambridge

SESSION II: CHALLENGES IN DEVELOPING NUTRIENT GUIDELINES

08:30 – 12:30 Interactive sessions – be prepared to vote, discuss, share and learn from other participants!

12:30 – 13:30 CLOSE OF WINSS 2010 and LUNCH
The WALTHAM International Nutritional Sciences Symposium

Pet Nutrition –
Art or Science?

KEY SPEAKERS

Daniel Pomp  PhD, MS, BS
Professor, Departments of Genetics and Cell and Molecular Physiology (School of Medicine) and Nutrition (School of Public Health) at the University of North Carolina – Chapel Hill

Daniel Pomp is a Professor in the Departments of Genetics and Cell and Molecular Physiology (School of Medicine) and Nutrition (School of Public Health) at the University of North Carolina – Chapel Hill. He is a member of UNC’s Carolina Center for Genome Science, Nutrition Obesity Research Center, Lineberger Comprehensive Cancer Center, and Center for Environmental Health and Susceptibility. He holds a BS in Agricultural Sciences from the Hebrew University of Jerusalem, a MS (1986) in Quantitative Genetics from the University of Wisconsin – Madison, a PhD (1989) in Animal Genetics and Biotechnology from North Carolina State University, and received Postdoctoral research and teaching experience at the University of California-Davis. Dr. Pomp specializes in the genetic and genomic analyses of complex traits such as obesity using polygenic animal models. He also focuses on how genetics and environmental factors, such as nutrition, interact with each other to control energy balance and disease. His research program has attracted funding from the NIH, USDA, NSF and private industry. Dr. Pomp has published more than 125 journal papers and many review articles and book chapters on genetics with applications to both the biomedical and agricultural sciences. He has served as Chair of the US National Animal Genome Research Program, and is currently Chair of the Committee on the Scientific Evaluation of Dietary Reference Intakes (DRIs) and is currently Chair of the Committee on Military Nutrition Research. For his extensive contributions to the NAS, he was named as Lifetime National Associate of the NAS in 2001 and was elected as a Member of the Institute of Medicine, NAS in 2003. Other honors include receipt of the Samuel Cate Prescott Award for Research and the William Cruess Award for Teaching from IFT: the Borden Award from ASN; being named as an Original Member in Agricultural Science by ISI as an Highly Cited Researcher (top 0.05%); and several University of Illinois Excellent and Outstanding Teaching awards. Dr Erdman received his BS, MS, MPh, and PhD in Food Science from Rutgers University.

Erdman’s training and expertise encompass the nutritional and physiological biochemistry of man and animals. He has written more than 160 original research articles on these subjects and has more than 100 other articles and chapters to his credit. He is a member of a variety of professional organizations including the American Society for Nutrition (ASN), the Institute of Food Technologists (IFT), and the American Heart Association (AHA). He is past President of the American Society for Nutritional Sciences (now ASN), has been elected Fellow for both AHA and IFT. He has been extensively involved with the Food and Nutrition Board (FNB) of the Institute of Medicine, National Academy of Sciences (NAS), where he served on the FNB for nine years, six as Vice Chair. Among other committees of the FNB, Dr. Erdman recently served as Chair of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (DRIs) and is currently Chair of the Committee on Military Nutrition Research. For his extensive contributions to the NAS, he was named as Lifetime National Associate of the NAS in 2001 and was elected as a Member of the Institute of Medicine, NAS in 2003. Other honors include receipt of the Samuel Cate Prescott Award for Research and the William Cruess Award for Teaching from IFT: the Borden Award from ASN; being named as an Original Member in Agricultural Science by ISI as an Highly Cited Researcher (top 0.05%); and several University of Illinois Excellent and Outstanding Teaching awards. Dr Erdman received his BS, MS, MPh, and PhD in Food Science from Rutgers University.

Richard Hill  MA, VetMB, PhD, DACVIM, DACVN, MRCVS
WALTHAM Associate Professor in Small Animal Internal Medicine and Clinical Nutrition, University of Florida, Member National Research Council

Richard Hill qualified as a veterinarian at the University of Cambridge in 1980 and spent five years as an assistant veterinarian at the small animal hospital of a large mixed practice in Aylesbury, Bucks., north of London. He then completed a residency in small
animal internal medicine at the University of Pennsylvania and a PhD at the University of Florida (UF). He is currently the WALTHAM Associate Professor of Small Animal Internal Medicine and Clinical Nutrition at the UF College of Veterinary Medicine and Small Animal Medicine Service Chief. He is a diplomate of the American Colleges of Veterinary Internal Medicine and Veterinary Nutrition. His clinical responsibilities include teaching clinical small animal internal medicine and nutrition and running the small animal nutrition service at UF. He conducts research into the gastrointestinal physiology and nutrition of companion animals. His nutrition research has involved establishing the nutritional requirements of racing greyhounds on a racing track at UF and he is currently assessing the energy requirements of pet dogs and cats while also supervising an antioxidant laboratory. As a member of the Subcommittee on Dog and Cat Nutrition of the National Research Council Committee on Animal Nutrition, he was a co-author of the Nutrient Requirements of Dogs and Cats published in 2006 by the National Academy of Sciences and was primary author of the chapter that discusses the effects of natural foods on the health and performance of domestic animals. His nutrition research has resulted in the development of novel preservation techniques for the food industry and the safety of meat and poultry products. A native of the United Kingdom, he received BS and PhD degrees from the Universities of Reading and Nottingham, respectively. Before joining the National Academies, Austin was a professor of animal science at the University of Nebraska. He has been active in the American Society of Animal Science, serving on several committees and spending a three-year term as Editor-in-Chief of the Journal of Animal Science.

Austin Lewis is a Senior Program Officer with the National Research Council of the National Academies. Austin Lewis is a Senior Program Officer with the National Research Council of the National Academies. As a member of the Board on Agriculture and Natural Resources, one of his primary responsibilities is to manage the Animal Nutrition Program. This program publishes reports on the nutrient requirements of animals. The publications are used worldwide to ensure the proper feeding of animals. Other recent projects are the status of the US sheep industry and the safety of meat and poultry products. A native of the United Kingdom, he received BS and PhD degrees from the Universities of Reading and Nottingham, respectively. Before joining the National Academies, Austin was a professor of animal science at the University of Nebraska. He has been active in the American Society of Animal Science, serving on several committees and spending a three-year term as Editor-in-Chief of the Journal of Animal Science.

Wouter H. Hendriks, PhD Professor in Animal Nutrition at the Department of Animal Science of Wageningen University and the Faculty of Veterinary Medicine of the University of Utrecht in the Netherlands

Wouter Hendriks, PhD, is full Professor in Animal Nutrition at the Department of Animal Science of Wageningen University as well as the Faculty of Veterinary Medicine of the University of Utrecht in the Netherlands. He obtained his Ingenieur degree in Animal Nutrition from Wageningen University in 1992 investigating lysine bioavailability and the effect of heat processing. In 1992 he immigrated to New Zealand to start a PhD in Monogastric Nutritional Physiology at Massey University investigating “Protein Metabolism in the Adult Domestic Cat”. In 1996 he was appointed as Director of Massey University’s Feline Unit and in 2000 he became the head of the Department of Animal Nutrition & Physiology of the Institute of Food, Nutrition and Human Health at that same University. In 2005 he immigrated back to the Netherlands to take up his current position. His research interests include companion animal nutrition, pet food processing and nutrient bioavailability, nutritional requirements, feline metabolism, digestive physiology, and metabolism in the cat.

Robert Buchanan, PhD, MPhil, MS, BS Professor and Director, Center for Food Safety and Security Systems, University of Maryland

Dr. Buchanan received his BS, MS, MPhil, and PhD degrees in Food Science from Rutgers University, and post-doctoral training in mycotoxicology at the University of Georgia. Since then, he has 30 years’ experience teaching and conducting research in food safety, first in academia, then with the USDA Agricultural Research Service and the Food and Drug Administration. He recently joined the faculty of the University of Maryland as professor and director of the new Center for Food Safety and Security Systems. His scientific interests are diverse, and include extensive experience in predictive microbiology, quantitative microbial risk assessment, microbial physiology, mycotoxicology, and food safety systems. He has published more than 400 manuscripts, book chapters and abstracts on a wide range of subjects related to food safety, and has given hundreds of invited lectures on five continents. Additionally, he is one of the co-developers of the widely used USDA Pathogen Modelling Program, and served on the boards of editors of several journals. Dr. Buchanan has an ongoing interest in the development of science-based public health policy. He served as the FDA Center for Food Safety and Applied Nutrition’s Senior Science Advisor, as the Director of the CFSAN Office of Science, the FDA Lead Scientist for the U.S. Food Safety Initiative, and as Deputy Administrator for Science with the USDA Food Safety and Inspection Service. Dr. Buchanan has served on numerous national and international advisory bodies, including as the U.S. Delegate to the Codex Alimentarius Commission Committee on Food Hygiene and a permanent member of the International Commission on Microbiological Specification for Foods. He has also served as a member of the National Academy of Science’s Institute of Medicine Committee on Emerging Microbial Threats, the National Advisory Committee on Microbiological Criteria for Foods, and numerous international expert consultations for the FAO and WHO. Dr. Buchanan has received numerous national and international honors and awards, and is a Fellow of the American Academy for Microbiology and the Institute of Food Technologists.

Robert C. Baker, MS, BS Head of Food Safety for Mars Incorporated

Mr. Robert C. Baker is Head of Food Safety for Mars Incorporated, where he is responsible for leading the development of Food Safety programs for Mars Globally. He started his career in the pharmaceutical industry in 1984 as a microbiology technician, moving to the food industry as a microbiologist in 1987. Mr. Baker joined Mars in 1987 as a microbiology technologist, responsible for quality control testing of snack food materials and products. In 1998, Mr. Baker was asked to support the development of innovative petcare products for Mars’ Germany business through the development of novel preservation techniques and the construction of an on-site microbiology laboratory. Mr. Baker has held multiple positions of increasing scope and responsibility across his 20 plus years in the area of quality and food safety management, and is the co-developer of a patent for sterilizing low acid foods using ultra-high pressure. Before his most recent position, Mr. Baker was asked to oversee Mars’ quality programs across the Asia Region. He received his BS degree in microbiology from Fairleigh Dickinson University and MS degree in Food Science from Rutgers University. Mr. Baker is a registered microbiologist and a member of several professional organizations, including the Institute of Food Technology, the International Society for Microbiology, and the American Society for Microbiology.

Adrian Charlton BSc, PhD Head of Chemical and Biochemical Profiling at the UK Food and Environment Research Agency

Adrian is Head of Chemical and Biochemical Profiling at the UK Food and Environmental Research Agency. Adrian graduated from the University of Sheffield with a first degree in biochemistry and a food industry sponsored PhD studying the interaction between salivary proteins and polyphenols. He joined the Food and Environment Research Agency (Fera, formerly the Central Science Laboratory) as lead NMR spectroscopist in 1999. Various roles at CSL/Fera followed and he is currently Principal Scientist heading the Biochemical and Chemical Profiling team. The team undertake research in a range of sectors using advanced analytical approaches such as NMR
Dr. Jim E. Riviere

Dr. Jim E. Riviere is the Burroughs Wellcome Fund Distinguished Professor of Pharmacology, an Alumni Distinguished Graduate Professor, and Director of the Center for Chemical Toxicology Research and Pharmacokinetics, College of Veterinary Medicine at North Carolina State University in Raleigh. Dr. Riviere received his BS (summa cum laude) and MS degrees from Boston College, his DVM and PhD in pharmacology as well as a DSc (hon) from Purdue University. He is an elected member of the National Academies’ Institute of Medicine, serves on its Food and Nutrition Board, and was chair of the 2008 NRC University. He is an elected member of the National and Chemicals across Skin; and the Food Safety and Biomathematics to Problems in Toxicology, including Development of Animal Models; Applying His Current Research Interests Relate to the Chapters, Holds 6 U.S. Patents, Has Authored/Edited 10 Veterinary Pharmacology and Toxicology. Dr. Riviere from the Consolidated University of North Carolina, His Honors Include the 1999 O. Max Gardner Award Residue Avoidance and Depletion (FARAD) Program. Though, I will introduce a new paradigm for the study of obesity, namely analysis of host genes that one day, I will talk about the wide variety of mouse models that currently exist (and new, more powerful emerging models) and how they are used to understand the genetic predisposition of traits like exercise, appetite, dietary response, and other components of obesity. And second, I will introduce a new paradigm for the study of obesity, namely analysis of host genes that influence composition of the gut microbiome, a climax population of thousands of microbial species that enter into intimate metabolic and immune interactions with host GI tissues and potentially affect many nutritionally relevant traits and diseases.

Part 1: Mouse Models of Complex Traits Related to Obesity

Most phenotypes displaying continuous variation, including nearly all traits related to energy balance and obesity, are exceptionally complex, with varying contributions of genetic susceptibility and interacting environmental factors. The use of mouse models has been a powerful driving force in understanding the genetic architecture of polygenic traits such as obesity. In addition to the many mouse models of obesity caused by spontaneous mutations and targeted gene knockouts and insertions, the commonly used inbred laboratory strains of mice constitute the primary mammalian model system and are an integral component of obesity research. Within these lines and their derivatives there exists a vast array of obesity-relevant genetic and phenotypic variation. The study of such variation has shed significant light on the genetic and genomic architecture of nearly all aspects of energy balance regulation, how body weight and body fat are controlled, and the impact of dietary influences.

 Appropriately designed animal models can uncover networks of functionally important relationships within and among diverse sets of biological and physiological phenotypes that can be altered by relevant external factors (for example, diet and exercise), and thus incorporate multiple genetic, environmental and developmental variables into comprehensive models describing susceptibility to obesity and its progression. Such a model is represented by a new paradigm for complex-trait analysis, the ‘collaborative cross’ (CC). The CC is a large panel of recombinant inbred mouse lines derived from a genetically diverse set of eight founder strains. Existing data on the founder strains and on many of the early generations in development of the CC demonstrate broad variability in many obesity-related phenotypes, indicating that the CC will represent an excellent resource for identifying genes controlling predisposition to many traits relevant to obesity. For example, we have identified strains of CC mice that voluntarily run in wheels nearly 20 km per night, while other strains hardly run at all! Identifying the factors that contribute to this remarkable variation will shed valuable light on why some people (and animals) are “couch potatoes” while others are “born to run”.

ADVANCES IN COMPARATIVE GENETICS – INFLUENCE OF GENETICS ON OBESITY

Pomp, D., MS, PhD

Professor, Department of Genetics (School of Medicine) and Nutrition (School of Public Health). Member, Nutrition and Obesity Research Center, Carolina Center for Genome Sciences, Lineberger Comprehensive Cancer Center, Center for Environmental Health and Susceptibility. The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7264.

Few research topics capture the public’s imagination like the search for genes that predispose to obesity. Ever since the discovery that the obese mouse mutation was caused by a deficiency in the protein leptin, each new finding is hailed in the headlines with promises of pharmaceutical or nutritional intervention to prevent weight gain. However, it is clear that complex diseases such as obesity are not caused by genes alone, but involve interplay between genetics, diet, infectious agents, environment, behavior and social structures. This interactive tapestry, combined with the fact that complex traits are controlled by many genes, most with small effect, has rendered the search for obesity genes exceedingly difficult.

In this talk, I will focus on how mouse models play valuable roles in understanding the genetics of traits related to energy balance and obesity in mammalian species including companion animals and humans. First, I will discuss the wide variety of mouse models that currently exist (and new, more powerful emerging models) and how they are used to understand the genetic predisposition of traits like exercise, appetite, dietary response, and other components of obesity. And second, I will introduce a new paradigm for the study of obesity, namely analysis of host genes that influence composition of the gut microbiome, a climax population of thousands of microbial species that enter into intimate metabolic and immune interactions with host GI tissues and potentially affect many nutritionally relevant traits and diseases.

Part 1: Mouse Models of Complex Traits Related to Obesity

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Dr. Robert Standaert

Dr. Robert Standaert is a staff scientist in the Biosciences Division at Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee, USA. A chemist by training, he received a Bachelor’s degree from Cornell University (1985), a Master’s degree from Yale University (1998) and a PhD from Harvard University (1992), where he also was a post-doctoral fellow (1992–1995). Prior to joining the staff at ORNL in 2005, he served on the research faculty in the departments of chemistry at Texas A&M University (1995–2001) and the University of Illinois at Chicago (2001–2005). His research has emphasized the application of chemistry to problems of biological interest, ranging from the interaction of drugs and other bioactive molecules with their protein targets to manipulation of biological systems with light. Since joining the staff at ORNL, he has become increasingly involved in nanoscience and the development of new technology for basic research, national security and food safety.

Dr. Robert Standaert PhD, MS, BS

Staff scientist, Biosciences Division, Oak Ridge National Laboratory (ORNL)

Oak Ridge, Tennessee, USA

The WALTHAM International Nutritional Sciences Symposium 2010
Part 2: Host genetic Control Over Composition of the Gut Microbiome
Mammals are born with a sterile GI tract which is rapidly colonized by successive waves of microorganisms until a dense microbial population stabilizes at about the time of weaning. This population is dominated by thousands of bacterial species that belong to a small number of phyla. The composition of the adult gut microbiota varies dramatically from individual to individual, including differences in the relative ratios of dominant phyla and variation in genera and species found in an individual host. Once established, these compositional features are highly resilient to perturbation. A mechanistic insight into the assembly of the gut microbiota is immediately relevant to our understanding of complex traits and diseases human diseases: obesity, coronary heart disease, diabetes and digestive maladies have all been associated with composition of gut microbiota.

Using sophisticated mouse models as described above, we have now, for the first time, identified host genetic loci that control variability in the abundances of different taxa in the mouse gut microbiota. We found that gut microbiota composition as a whole can be understood as a complex, polygenic trait influenced by combinations of host genomic loci and environmental factors. These findings clearly establish host genetics as a factor in determining composition of the gut microbiome, a climax population of thousands of microbial species that enter into intimate metabolic and immune interactions with host GI tissues. This genetic control appears to encompass, for example, host genetic factors such as those influencing mucosal immunity. Consequently, host genetic loci that affect composition of the gut microbiome are likely to partially contribute to an individual’s overall predisposition to obesity and other nutritionally relevant traits and diseases. How changes in nutrition may influence this host-microbiome relationship, and thus impact weight regulation, remains an interesting yet untested question.

DIETARY REFERENCE INTAKES (DRIs) FOR HUMANS: WHAT ARE THE IMPLICATIONS FOR ANIMAL NUTRIENT GUIDELINES?
Erdman, J. W. Jr., PhD
Professor of Food Science and Human Nutrition, Professor of Internal Medicine, University of Illinois at Urbana

Evolution of Dietary Guidance
Dietary guidance for humans can be traced back to the British Merchant Seaman’s Act in 1835 which suggested lime or lemon juice for sailors to prevent what we know today as scurvy. The UK, The Netherlands, France, Germany and the USA developed dietary recommendations and standards to prevent starvation or to provide the basic needs for military between 1860–1900. Generally, these guidelines focused on energy, protein and “protective foods”. Between 1900 and 1940, there were extensive advancements in discovery of essential nutrients, particularly vitamins and minerals, as well as a more detailed establishment of dietary requirements and recommendations by the United States and the League of Nations1–2. In 1940, the Committee on Nutrition was appointed by the US Department of Defense to assist in nutrition planning with the anticipated entry into WWII. This committee evolved into the Food and Nutrition Board (FNB), which resides in the Institute of Medicine (IOM), National Academy of Sciences.

Recommended Dietary Allowances (RDAs)
The initial RDAs were published in 1941 and made recommendations for energy, protein, two minerals and six vitamins. By the 10th edition in 1989, there were recommendations for 18 vitamins and minerals and “safe and adequate daily dietary intake” recommendations for seven others. RDAs were defined as “levels of intake of essential nutrients considered, in the judgment of the FNB on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons”. RDA committees met over five-year periods, most often behind closed doors, and published updated dietary recommendations considering new research advances over that period of time.

Dietary Reference Intakes (DRIs)
In the early 1990s, the FNB began to consider a new conceptual approach for establishment of dietary guidance. One driving force for this was the consideration of nutrient requirements for optimal health or reduction of chronic diseases, not just for prevention of nutrient deficiency diseases. The new concept was reflected in the landmark FNB document, “Diet and Health: Implications for Reducing Chronic Disease Risk” in 1989 which stimulated consideration of nutrients and disease prevention3. In 1994, the IOM, with guidance from FNB, undertook activities that resulted in a new framework for development of reference values, the DRIs3. It was recognized that a single RDA value alone was not sufficient to meet the breadth of the intended reference value needs. In addition to the RDAs, values for the Estimated Average Requirement (EAR), Tolerable Upper Intake Level (UL) and Adequate Intake (AI) were defined and introduced. In addition, the Acceptable Macronutrient Distribution Range (AMDR) was developed for macronutrient recommendations. From 1995 to 2004, a large number of nutrient-based reports were published in addition to reports focused on applications of DRIs for dietary planning and dietary assessment. In addition, a summary guide “DRIs: The Essential Guide to Nutrient Requirements” for students and end users was published in 20065. There has not been any intention of a complete revision of the DRIs. Instead, new committees were only to be convened to consider revisions when new research suggested such a need. The first such panel on Vitamin D and Calcium is expected to publish revised DRIs for these two specific nutrients during the summer of 2010.

Challenges for setting Human DRIs
There are a large number of limitations in setting the DRIs. The primary challenge in setting DRIs for males and females of different age ranges, for lactating and pregnant women, and for different ethnicities, is a lack of available human data. Many DRIs are established based upon studies of a few individuals and the true biological variance around the mean is usually unknown.
Extrapolation of small amounts of data from (usually) white, young males to other age, ethnic and gender groups is problematic. There is a lack of specific, sensitive, functional biomarkers. Use of stable isotopes enhances our ability to make assumptions about metabolism and storage of nutrients, but we are far more restricted than what can be done in animal studies. Compliance of humans during clinical trials or reliability of dietary recalls is often poor. Many ULs are set based upon acute toxicity or adverse events and not upon chronic excess dietary exposure. The cost for carrying out a comprehensive dietary requirement study is in the millions of dollars and no federal funding programs are in place to fund these types of studies. Many federal and state feeding programs are legally bound to comply with RDAs. Since the RDA for a number of nutrient is close to the UL, it is difficult to design dietary programs that achieve RDA intake levels for all without having some individuals exceed the UL for these nutrients. With the new DRI framework that considers nutrient requirements for chronic disease outcomes, the establishment of specific DRI numbers becomes more difficult.

Learnings and Implications for Animal Requirements

During the period of 1996–2004, there was a funded Standing Committee on the Scientific Evaluation of DRIs. This committee oversaw all of the nutrient panel4, the application committee and the upper levels committee to assure that all reports and recommendations were co-ordinated. The standing committee assured logical and timely movement from report to report. Since then, there has not been continuous federal funding for additional DRI activities. Funding for standing committees for both DRIs and the National Research Council (NRC) activities and updates of requirements for animals is highly recommended. These committees can help set the priority of species or nutrients to evaluate. The DRI and NRC processes to establish requirements is costly despite the volunteer effort of hundreds of scientists. Thus, stable funding is sorely needed.

One clear difference between the FNB and NRC work is that humans have varies diets and it is particularly difficult to control dietary intakes. NRC: requirements of dogs, cats, rodents and some other species assumes that a single feed will provide 100% of the animal’s needs. Compliance, or measurement of feed intake, is much easier with these species. In addition, study of males and females of different ages and during reproduction is more easily accomplished with animals. Setting ULs for humans is also more difficult than with animals. Both the FNB and NRC share the issue of lack of acceptable biomarkers. More dialogue between groups may speed the development of better biomarkers of nutrient status and overall health. Enhanced research funding is of critical need to more clearly establish DRIs and nutrient requirements of animals and humans.

References:

At high levels of nutrient inclusion, nutrients become toxic and health and performance deteriorate. The 2006 NRC recommendations give a safe upper limit (SUL) for some nutrients whose an SUL is defined as the maximal concentration or amount of a nutrient that has not been associated with adverse effects. These give some indication of how much of a nutrient may be included in the diet safely.

Critical Analysis: strengths and weaknesses
1) The 2006 NRC document represents a substantial improvement from the previous version but has also stimulated some controversy and provides some lessons for the future. It provides a good review of the literature generated since the last edition, and provides updated recommendations for growth, maintenance and reproduction. There are also several important new chapters. Nevertheless, the publication has become too expensive and too opaque with the result that the information included has not been widely disseminated.

2) The new NRC guidelines clarify the quality of information on which recommendations are based by distinguishing MRs from As. They also allow for differences in availability of nutrients by distinguishing a requirement from an AA and provide an indication of safe maximum rates of inclusion as a SUL for some nutrients. Unfortunately, there remain many gaps in the tables listing MRs because there has been little research performed over the last 20 years directed at better determining the essential nutrient requirements of healthy dogs and cats. Most requirements have been established using growth rate as a criterion for adequacy and there remains little information on the MRs for maintenance and reproduction or any other physiological state. There is also no distinction between SULs that are known quite precisely from toxicological studies, and SULs that are known less precisely. In the latter situation, higher amounts might be safe but there are no published reports of feeding higher concentrations with impunity. This lack of clarity has resulted in some controversy because some manufacturers maintain that they have fed concentrations of nutrient above the SUL to cats and dogs without causing illness. Nevertheless, the more recent NRC report published in 2008 that discusses the safety of dietary supplements of dogs, cats and horses, provides a more precise framework that could be used in the future in that it distinguishes a no observed adverse effect level (NOAEL), a presumed safe intake (PSI) and a historical safe intake (HSI), as well as a safe upper limit (SUL).

3) The guidelines provide some suggestions as to how to accommodate for some of the factors that affect nutrient requirements other than bioavailability and life-stage. Such factors include the energy density of the diet, the energy requirements of an individual under different amounts of activity or under different physiological conditions, the animal’s breed, sexual status, body size and condition, and the measure by which performance or health is assessed. These accommodations include reporting nutrient requirements per kg, per 1000 kcal and per metabolic body weight, but the result is an increase in complexity and there are many caveats in the text and footnotes to the tables. This makes make understanding the recommendations very difficult for both lay people and professional nutritionists.

Determining how such factors affect requirements is particularly difficult because most studies have not been reported in enough detail. Many studies, for example, do not report the energy density of the diet, the size of animal or the amount consumed. To interpret the results of such studies, some assumptions have been made. The 2006 NRC guidelines assume an energy density of 4 kcal/g of diet and a fixed size of animal requiring an average amount of kcal equivalent to that expected in an average laboratory dog. Furthermore, the recommendations assume that the amounts per 1000 kcal do not change in dogs and cats of different sizes but recognize that nutrient density may have to increase in sedentary animals requiring less energy and may not need to be so nutrient dense in a working dog with increased energy needs. The validity of all these assumptions is very uncertain and different assumptions can give different minimum and maximum recommendations.

4) One great improvement is that the guidelines recognize that modified Atwater factors underestimate the ME density of pet foods and provide an alternative method of estimating ME that adjusts the energy digestibility based on the fiber content of the diet. This should result in higher estimates of the energy density in foods that have not undergone feeding trials and reduce the likelihood that the amount of food to feed to dogs will be overestimated as is the case with the current AAFCO methodology.

Recommendations for the future:
- The chairperson should be a dog and cat nutritionist with an overall understanding dog and cat nutrition and of controversial issues.
- Employees of pet food companies should not be excluded where possible within the rules of the NRC and NAS. Pet food employees bring a wealth of expertise and practical experience concerning pet food and there are many pet food employees who are capable of maintaining an objective view. This is particularly important when considering the feasibility of formulating diets within narrow ranges of nutrient intake.
- Instead of waiting another 20 years to accumulate the enthusiasm and funds to update the whole document, the nutrient requirements of dogs and cats should become a ‘living document’ that responds to scientific developments as they occur. The requirements of only a few nutrients or one major nutrient group should be attempted at a time. Committees would then be smaller and reviews less expensive to perform, which would facilitate more frequent reviews. Evaluating fewer nutrients would also allow review committees to contain more than one expert for each nutrient and result in more informed discussion of controversial decisions. Within the 2006 committee, there was often only one expert with a detailed knowledge of a particular topic. It was, therefore, difficult for other committee members with less intimate knowledge of the topic to challenge a controversial opinion offered by that expert.

- The scope and cost of the publication should be limited. Otherwise, there is a need for two documents: a detailed one for academic nutritionists, pet food manufacturers and regulators that explains the complexities of dog and cats nutrition; and, a simpler one to inform the general public but is easier to understand. If the scope is limited, it may be easier to explain recommendations to all audiences.

- Some chapters such as that on the physiology of digestion should be excluded, whereas additional topics should be addressed such as the composition of milk and milk replacers, the effects of age and neutering, the prevention of obesity, diabetes, urinary calculi, dental disease and cognitive dysfunction, the effect of cooking on nutrient availability and the merits and risks of feeding raw food, novel ingredients. The temptation to include nutrient recommendations for disease should be avoided because there are too many diseases and too little scientific information about requirements for each disease.

- The basis for critical and controversial decisions such as the size of safety factors and listing EPA and DHA as essential nutrients in the tables, whether based on a review of the literature or expert opinion, should be better explained. With this in mind, authors should be given sufficient time to write the report and meetings scheduled after the initial draft to discuss controversial decisions.

- There should be an opportunity also to amend the report after outside review by industry and other interested groups.

- The recommendations for daily energy requirements should be reduced where appropriate based on new information.

- There should be flexibility to change the format for recommendations among tables such as using amounts per metabolic weight for maintenance and amounts per weight in growing animals.

- Encourage studies to resolve some of the issues discussed above, such as the requirements for maintenance, the allometry of nutrient requirements in dogs and cats of different sizes and breeds, how requirements for lactation differ from those for pregnancy, how nutrient requirements in sedentary animals eating little food and working dogs differ from average laboratory dogs and cats, the minimum amount of indigestible protein and carbohydrate for colonic health, the nutrient content and digestibility of common pet food ingredients, the minimum requirements for trace minerals and more accurate documentation of the SUL for nutrients such as fat.

- With this in mind, it is important to establish minimum standards for reporting of information in studies of companion animals.
DEVELOPING NUTRIENT GUIDELINES – A VIEW FROM FARMED LIVESTOCK
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Introduction
Developing nutrient guidelines: three words, three issues. The first supposes (correctly) that the determination of the nutrients that animals need is an on-going developmental issue that is forever in flux, with no definitive ‘absolute answer’ end point. The second supposes that we share with the animals an understanding of what a nutrient actually is. We – necessarily – define nutrients in terms of what is measurable in the laboratory, not in terms of functionality in the animal. The body of a dairy cow is not a bomb calorimeter. Neither are cows properly schooled in concepts of differences in polysaccharide types nor even in the Blaxterian understanding of what is ‘metabolisable’ and what is not. Cattle have not been made adequately conversant with the proposition that protein is N x 6.25, nor are the bugs in their rumens educated in the math determining that which is degradable, and that which is not. A ‘guideline’ is neither a requirement, nor a standard. If it is guidelines we are seeking, then we have progressed far from the earlier unashamedly deterministic and didactic published values for ‘Nutrient Requirements’ of both US NAS-NRC and UK ARC. With a ‘guideline’ we can move away from a pedagogical approach to one that is more reasoning. Nonetheless, it remains easier to define explicit solutions to feeding malpractices and overt nutrient deficiencies than to define optima for ‘normal’ life. From the livestock production perspective, if there is malnutrition or overt deficiency, then all is already lost. Normal life feeding for farmed animals is efficiently to provide meat, milk, eggs.

A UK perspective for farmed livestock
The UK Agricultural Research Council that was created to improve agricultural output and efficiency is no more. Its replacement, the Biotechnology and Biological Sciences Research Council, has wider responsibilities, including to medicine. Similar position-shifting has occurred with the relevant Government departments; the (down-sized) Department for Environment, Food and Rural Affairs now replacing the previous Ministry of Agriculture Fisheries and Food. The two earlier champions of the search for Nutrient Requirement Standards are thus gone. The British Society of Animal Science managed the publication of an updated Standard for Pigs (BSAS, 2003), but nothing has followed for other species. The ‘Feed into Milk’ program (for dairy cows) of the early 2000s has helped forward thinking and practice, but aspires more to understanding through modelling than the setting of requirements. Presently in UK there seems a lack of will for updating nutrient requirement standards. EU initiatives might do better, but it has to be said that with swine (the easiest one), there has been failure even to agree on a common unit for defining energy!

We have lost our appetite for seeking the ultimate goal of correctly defined nutrient requirements; likely because all our efforts through the second half of the last century to achieve the same are perceived to have failed. The whole concept has taken something of a knock. Deficiency disease is no longer a significant livestock farming problem. Requirements for minerals and vitamins are the on the one hand seen as out of date with the substantially increased rate of animal productivity since their original determination, while on the other hand safety issues have ramped up some allowances to now verge upon the profligate. Massive shifts in the genetic composition of farm livestock have had unexpected consequences. Some genotypes need greater rates of energy and protein supply to allow potential to be expressed, while others appear to require a different ratio of nutrients to match a re-balancing of their partition rules. Targets have also shifted, becoming at the same time more diverse. Thus longevity and reproductive success have gained in importance over frank daily growth and lactation performance.

So how are animals to be seen to be properly fed? For many practitioners there has been something of a return to empirical methodologies. For the scientists there is a contrary move to the deductive modelling of processes. Both approaches have in common the concept that nutrient requirement is a variable quantity, flexing with purpose (target output), genotype, environment, economic climate and nutrient source (feedstuff). Practicing animal feed formulators do not need to be instructed by scientists as to absolute values for nutrient supply needs. Rather they need to know the ways and means (equations, algorithms, conceptual frameworks such as supplied in rudimentary form by BSAS, 2003) to calculate for themselves nutrient needs in given, specific and often unique circumstances. The best determination of an animal’s nutrient needs will be highly specific (not in the least general). In brief, nutrient requirements will indeed be ever-changing with no determinable end point of definition, and the guidelines required are not didactic statements, but methodologies for deductive (and variable) resolution.

The calculation of an adequate feed allowance requires; first the definition of the nutrient in ways that properly reflect what the animal does with it, second the knowledge (or control) of feed intake, and third a statement (qualitative and quantitative) of the purpose (or purposes) for which the nutrient is to be supplied. We have imperfect science for each of these. This can only mean that a specific dietary nutrient concentration determined in absolute terms (however dedicated the scientific committees who decide upon such things) has to be also imperfect. A potentially useful way forward is the use of response prediction models to calculate nutrient requirement streams that respond robotically to the automatic measurement of animal performance in relation to chosen targets. There is additionally a social dimension to the feeding of farm animals; often related to the public’s conception of ‘welfare’ and ‘carbon footprint’. Social dimensions can be at odds with reality, at odds with economic (and carbon-efficient) production, and at odds with each other.

This paper forwards a challenge to the idea that published values for nutrient requirement may properly be used as a base-line standard for the adequate nutrition of farmed animals; such standards may, for specific animal groups in specific circumstances be either too high, too low, or in the wrong balance. Presently in UK, there is a real possibility that recommendations for fundamentals such as phosphorus, protein and essential amino acid concentrations for swine may be set too high. The crucial questions in the debate must be not “What is the requirement?” but “What and who is the requirement for?”

BSAS (2003). Nutrient Requirement Standards for Pigs. (Authors: C.T. Whittemore, M.J. Hazzledine and W.H.Close) BSAS, Penicuik. bsas@sac.ac.uk
THE CHALLENGES OF PUTTING TOGETHER AN NRC REPORT ON THE NUTRIENT REQUIREMENTS OF ANIMALS

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Introduction
Since the 1940s the National Research Council-National Academy of Sciences (NRC-NAS) has released reports on the nutrient requirements of numerous species of animals. The reports are updated when new information is available. Although the emphasis has been on the primary agricultural species (poultry, swine, dairy cattle, and beef cattle) other species, including companion animals (e.g., Dogs and Cats in 2006 and Horses in 2007), are also addressed. A committee of experts is appointed to develop each of the publications. This process ensures that the information published in NRC reports is unbiased and of the highest technical quality.

Institutional (NRC) Challenges
The NRC receives no direct financial support for the nutrient requirements series and therefore is dependant on sponsorship for each report. There are also restrictions on the proportions of funding that can be accepted from sponsors who could be perceived as having a financial interest in the findings of a report. These financial challenges are the largest impediment to more frequent updates. Fortunately, a portion of the profits from the sale of previous nutrient requirement reports is available as seed money to leverage contributions from sponsors.

With so many species to cover, decisions have to be made about which species take priority for revision. In general, reports that cover species with the greatest economic significance receive more frequent updates than do others, but issues such national and international priorities and the extent of new information are considered.

The NRC is subject to U.S. government regulations (e.g., the Federal Advisory Committee Act and The Freedom of Information Act) that affect the work of committee members and staff. In general, these regulations have little impact on the work of committees dealing with nutrient requirements, but they sometimes inhibit flow of information from the public to committee members.

To protect its reputation as an institution that produces reports based on high-quality science and debate and that are, as far as possible, free from external influence and bias, the NRC has well-defined practices that all committees must follow. These practices include reviews of bias and conflict of interest among committee members, public access to information about committee composition and meetings, and protocols for external review of a draft of the report before release. These important measures add to the time required to complete a report (and also to the cost).

Members of all NRC committees serve without compensation (except for reimbursement for the expenses associated with attending meetings). Sometimes the work involved in preparing and writing the draft report and responding to reviewer comments exceeds that which was anticipated. This can lead to delays that can be frustrating for everyone including staff and sponsors.

Committee Challenges

Although all of the nutrient requirement reports have the same general format and committees are assigned a similar task, each species presents unique challenges. Nevertheless, many challenges are common to all committees. Some of the most significant are discussed below.

While the primary focus of these reports is the establishment of nutrient requirements for specific stages of life and functions, most reports contain additional background material. Examples are the anatomy and physiology of digestive tracts, methodology, and nonnutrient feed additives. Each committee has to wrestle with how much background material to include. Recent reports for several species have included far more material than previous editions.

Committees also have to decide on the most appropriate modes of expressing nutrient requirement values. For example, requirements can be expressed as a percentage of the diet (on an as is or DM basis) or as a function of energy (GE, DE, ME, or NE) and be on a total, digestible, or bioavailable basis. Modes of expression most appropriate for one species and stage of life may not be suitable for other situations. In addition to minimum requirements, allowances, daily recommended intakes, and safe upper limits are sometime provided.

Mathematical models are valuable tools in the estimation of requirements and committees have to decide whether a model is appropriate and if so what type (static vs. dynamic; deterministic vs. probabilistic/stochastic). Computer programs can be very time consuming to develop and test and so whether to include a model is a key decision that each committee must make early its deliberations. The user interface is also an important component.

Feed composition tables are included in the nutrient requirement publications and often take up a significant portion of the committee’s time. A national or international database that could be used in all reports would be a great asset.

Publication challenges
All of the publications in the Nutrient Requirements of Animals series have been published as books, initially as paperback and now as hardback. There is increasing interest in publication in an electronic format (e.g., PDF). Suggestions have also been made that the reports should be much more “dynamic” with frequent minor updates in addition to periodic full revisions. Electronic publication would enable more frequent updates and numerous enhancements such as hyperlinks to references and other sources of information. On the other hand, frequent updates create problems for regulatory agencies that use the report to determine diet adequacy. Frequent updates also present challenges to the NRC in ensuring that there is adequate discussion and review of changes.

Conclusions
Despite several challenges and limitations, the NRC reports on the Nutrient Requirements of Animals have a long history and are used throughout the world as a key source of information on the nutritional needs of numerous species of animals.
FELINE PALEOLITHIC NUTRITION: A CONSIDERATION OF ITS NATURE AND ITS IMPLICATIONS FOR NUTRITION OF DOMESTICATED CATS

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Evolution and metabolism of cats

The domestic cat (Felis silvestris catus) is a much loved pet in millions of homes and can be considered as one of the most popular companion animals world-wide. The more than 250 million pet cats were domesticated relatively recently on multiple occasions in separate locations from the wild cats residing in the Near East and Central Asia. The domestic cat’s wild ancestors, consisting of five subspecies of Felis silvestris (E.S.lybica, F.s.silvestris, F.s.cafra, F.s.bielli and F.s.ornata), are known to be obligatory carnivores, consuming prey which are high in protein, moderate in fat, and contain a minimal amount of carbohydrates. Evolutionary events adapted the core metabolism and physiology to this diet strictly composed of animal tissues over a period of millions of years. This has led to multiple unique digestive and metabolic adaptations/idosyncrasies, especially in the protein and carbohydrate metabolism but also fatty acid and vitamin metabolisms. Cats require a high dietary crude protein level and are unable to synthesise taurine and arginine. With regard to carbohydrates, hepatic gluconeogenesis activity is low, hepatic fructokinase activity is lacking, salivary amylase is absent and pancreatic amylase activity is reduced while the non-functional Tas1R2 receptor does not allow cats to taste sugar. The modern domestic cat still closely resembles its wild ancestor, genemically, morphologically, and behaviourally. The carnivore connection of domestic cats is well recognised, there is a paucity of information on the precise nutrient profile to which the digestive physiology and metabolism of the cat has adapted throughout evolution.

Study design and results

A literature study was performed to assess the nutrient profile of the wild feeding strategy of the cat. Data from dietary habits of feral cats, as free-living representatives of the domestic cat, were combined with compositional data of the consumed prey species. An electronic literature search was conducted in Scopus and Web of Science to identify potentially eligible articles reporting dietary items consumed by free-ranging feral cats and whole body nutrient composition of wild prey items. To be able to ascertain the “wild” and “human-independent” lifestyle of the feral cats, studies were only included if the percentage of human linked foods was below 5%. Dietary items were expressed as percentage of weight (PW) of complete diet consumed. No whole body composition data for wild cats were found in the literature and therefore body composition data of captive rats were used. The metabolisable energy content was calculated using the standard Atwater factors for cats. Fifty-seven studies were found through database searches of which 27 studies were selected to be suitable for inclusion in this study. The most frequently consumed dietary items of feral cats reported in these studies were mammals (mainly rodents and lagomorpha), followed by birds, reptiles, and invertebrates. The amount of plant material consumed was negligible in virtually all studies. The results of the calculated nutrient profiles are shown in Table 1. The typical diet consists of 29.7% dry matter with a crude protein, crude fat and NFE content in the dry matter of 63.1, 20.1 and 8.9%, respectively. The range with which mean nutrient intake varied was low.

Optimal nutrient intake

In human nutrition, breast milk is regarded as the ideal food for infants and the composition of breast milk is used to define the optimal nutrient requirements of human infants. The assessed nutrient intake (Table 1) here reflects the profile which the cats’ metabolic system can be expected to have adapted to and could therefore be seen as the “optimal diet” for feral cats. It goes without saying that a distinct difference must be made between the “optimal diet composition” and the “physiological nutrient requirements”. The latter is defined as the dietary nutrient level above the minimum and below the maximum nutrient requirement of the animal and represent the maximum level of adaptation of the animal. Supplying nutrients above the maximum level will result in toxicity and deficiency symptoms, respectively. The nutrient intake levels as assessed in the study here reflect the intake by an adult cat living in a stable, healthy, and reproducing population of cats. As such the intake levels do not discriminate between reproducing animals or animals at maintenance. There are situations imaginable, like old age or disease, in which the adaptational ability of the cats’ metabolism declines resulting in changes in the nutrient requirement levels. The question to what extent this decline would affect the cats’ ability to adjust to a suboptimal nutrient profile is difficult to answer, and remains open for much debate.

Table 1. Calculated nutrient composition of the natural diet (n=30) of free-ranging feral cats

<table>
<thead>
<tr>
<th>Item</th>
<th>5%</th>
<th>Median</th>
<th>95%</th>
<th>Mean</th>
<th>SE</th>
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<tr>
<td>Dry matter (g/100g)</td>
<td>25.4</td>
<td>29.1</td>
<td>34.1</td>
<td>29.7</td>
<td>0.5</td>
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<tr>
<td>Macro nutrients (g/100g DM)</td>
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<td></td>
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<tr>
<td>Crude protein</td>
<td>7.6</td>
<td>9.3</td>
<td>9.7</td>
<td>8.9</td>
<td>0.1</td>
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<td>Crude fat</td>
<td>15.8</td>
<td>18.5</td>
<td>27.1</td>
<td>20.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Crude ash</td>
<td>4.7</td>
<td>8.3</td>
<td>12.0</td>
<td>8.6</td>
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<tr>
<td>Macro minerals (g/100g DM)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>2.56</td>
<td>2.61</td>
<td>3.02</td>
<td>2.66</td>
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<td>Phosphorus</td>
<td>1.53</td>
<td>1.74</td>
<td>2.04</td>
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<td>0.03</td>
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<td>0.39</td>
<td>0.49</td>
<td>0.59</td>
<td>0.50</td>
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<td>Potassium</td>
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<td>0.94</td>
<td>1.04</td>
<td>0.93</td>
<td>0.01</td>
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<td>Trace elements (mg/100g DM)</td>
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<tr>
<td>Iron</td>
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<td>29.3</td>
<td>45.1</td>
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<td>1.1</td>
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<td>Copper</td>
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<td>3.8</td>
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<td>10.0</td>
<td>12.0</td>
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<td>Magnesium</td>
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<td>156</td>
<td>152</td>
<td>128</td>
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<tr>
<td>Energy Real/100g DM</td>
<td>440</td>
<td>490</td>
<td>510</td>
<td>484</td>
<td>4</td>
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</table>

References

FOOD SAFETY CHALLENGES FACING THE PET FOOD INDUSTRY
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Companion animals are an integral part of the family unit in many societies. As the size of families has decreased in most developed countries, the role of pets as “family members” has increased dramatically. This is, in part, reflected in most pets living in the family domicile. The health and well-being of pets is a responsibility that most owners take very seriously. As a result, owners increasingly rely on commercially prepared pet foods to provide sound nutrition through the different phases of an animal’s life phases. However, as consumers increasing rely on commercially produced pet foods, recognition of potential hazards associated with the manufacture, distribution, and use of pet foods are amplified as a result of common sourcing and lot size.

Pet food safety represents a substantial challenge over traditional food safety concerns because the hazards can potentially impact both the animal and the humans that share its environment. The direct impact of chemical contaminates in pet foods on the animal health has been dramatically demonstrated in 2007 when cases of kidney damage and failure in cats and dogs were reported in the United States. Ultimately the source of disease was traced back to the adulteration of protein sources with melamine and cyanuric acid. However, this is not the only example of chemical hazards being associated with pet food ingredients. For example, the year before there was more than 100 deaths of dogs in the United States linked to the consumption of pet foods containing high levels of aflatoxins, again emphasizing the impact that improper ingredient sourcing can have on the safety of the product.

The ability of companion animals to serve as reservoir for disease agents that can impact their owners and other humans has long been recognized. For example, the common canine parasite Toxocara canis can also infect humans. The transmission of this nematode can be associated with either direct contact with the animal or through the contamination of the environment by fecal material. Another example is the warning given to pregnant women to avoid handling of feline fecal material to reduce the risk of fetal Toxoplasma gondii infections.

It has only been widely appreciated recently that pet foods can be a source of pathogenic microorganisms that impact pet owners. Pet foods can serve as a vehicle for foodborne pathogens, resulting in direct or indirect transmission. In the former, the handling of contaminated pet foods by the pet owner is the route of transmission. In the latter case, the pet becomes asymptomatically infected, and, in turn, serves of reservoir for the pathogenic microorganism either through direct contact between the pet and the pet owner, or through fecal contamination of the environment.

The safety of pet foods has traditionally been held to a higher standard than animal feed for domestic animals. However, the recent outbreaks associated with chemical and microbiological contamination of pet foods has resulted in several countries revisiting the requirements associated with manufacture and sale of these products. For example, the U.S. Food and Drug Administration clearly articulated its expectations that pet food be manufactured under conditions similar to those that it requires for human food. A number of large recalls of dry pet foods that have been found to be contaminated with Salmonella enterica or manufactured with ingredients that were subsequently found to be contaminated with the pathogen. For example, a substantial portion of the products recalled as a result of the widespread contamination of peanuts and peanut flours with S. enterica were pet foods. Other countries have similar concerns and changes in regulatory approaches are being implemented in Europe, Japan and other countries.

These changes in consumer expectations and accompanying regulatory interest are likely to have major impacts on the pet food industry, particularly for dry pet food products. The most obvious will be a wide scale need to upgrade facilities so that they meet the requirements for the production of human foods. However, it will also impact all operations as the need for more care in the acquisition of ingredients, the maintenance of records, and the level of quality assurance will be expected by both retail markets and consumers.
MICROBIOLOGICAL CHALLENGES FACING THE PET FOOD INDUSTRY
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Food safety goes beyond traditional factory quality management processes and must cover the entire production pipeline (“farm to fork approach”). Failure to do so can place your consumers, products and business at risk of a food safety incident. Historical information demonstrates that food safety incidents can be traced back to issues involving raw materials, production, distribution and mishandling prior to consumption. Looking further into these food safety incidents, it is possible to categorize the root cause(s) as chemical (involving contamination with toxic chemicals), physical (involving contamination with foreign materials) and microbiological (involving contamination with hazardous microorganisms and/or their toxic metabolites). Of these categories, food safety issues of microbiological origin have been the most challenging for the Food Industry and have received increasing focus over the past five years. This has been escalated with recent advances in forensic epidemiology and trending capabilities, able to detect outbreaks on regional and national levels.

During the past five years there have been numerous food recalls due to microbiological issues related to mycotoxins (primarily aflatoxin contamination) and Salmonella contamination. The mycotoxin issues are typically traced back to the usage of contaminated raw materials (typically grains) which are not detected prior to the food manufacturing process. The Salmonella issues are not that straightforward and in several recent incidents have involved food types that have been traditionally viewed as low risk (i.e. high acid or low water activity foods). To highlight this, during the past two years there have been Salmonella incidents involving tomato-based salsa (high acid food), peanuts (low water activity food), chicken pot pies (frozen food) and dry pet food (low water activity food). Past beliefs were that Salmonella would not survive in or on these foods, therefore being low risk of a food safety incident. Recent understanding into Salmonella biology has demonstrated that it can survive in or on these products and for extended periods (up to a year or longer). In addition, it is still capable of causing illness upon ingestion with an infectious dose as low as 1 cell ingested.

Focusing on dry pet food, recent evidence has shown that not only can Salmonella survive on the product, but it can be a vehicle of contamination and infection to the pet and pet owner. This is primarily driven by the fact that, in many instances, pets and their food are in the home. If the food is contaminated, this is a route of bringing Salmonella into the home, presenting a risk of illness to the pet owner via the food or via the pet if contaminated food is consumed (fecal oral route). Based on this, it is critical that pet foods are produced in a manner to prevent Salmonella contamination.

Managing Salmonella contamination in the production of dry pet foods can be very challenging as many of the raw materials are naturally contaminated (i.e. grains, meats and meat meals, poultry, etc.). Therefore, it is crucial to perform a risk assessment to understand which materials and processes pose the most risk and develop a control plan which addresses and minimizes the potential product safety concerns. This must be comprehensive and take into account key risk areas and risk management efforts involving the “people, plant and process”. In terms of “people”, it should look at the competency and awareness of the factory teams related to Salmonella risks and management requirements. For “plant”, it should look at the process and equipment design, segregation of microbiological hot and cold zones, airflow and condensation management and overall maintenance to keep the facility in a fit for purpose state. For “process”, it should look at the critical control point identification and validation, material and personnel flows, sanitation programs, water control efforts and the overall quality and food safety management process. Basically, the 3 “Ps” form a triangle and are fundamental in managing Salmonella (and other microbiological risks). Failure to have all in place would increase the risk of a potential Salmonella contamination issue.
NON-TARGETED ANALYSIS OF FOODS AND FEED
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Current laboratory methods for the identification of chemical contaminants in food use the so-called ‘target list’ approach. A predefined list of compounds is detected by the employment of customised extraction and analysis methods. This approach is appropriate for the quantification of compounds at trace levels for routine monitoring purposes where the target compound is known, but is limited when an unanticipated contamination threat arises. In these circumstances the exhaustive deployment of targeted analysis methods is time-consuming, expensive and often unsuccessful. Much research is now focussed on the development of non-targeted detection methods that are able to rapidly determine the presence of unknown contaminants. By obtaining a detailed understanding of what is naturally and normally present in foods it becomes possible to monitor the food chain for abnormalities. A profiling or screening approach thus facilitates the determination of a wide range of issues in the areas of food fraud and food safety. Whilst holistic monitoring of food has been the “holy grail” for a number of years, it is only in recent times that instrumentation technology has advanced to the point where this has become feasible.

A number of high profile issues have highlighted the need for broader ranging determination of the composition of foodstuffs. Recently, milk products were adulterated with melamine and the products mislabelled in relation to their protein content, a practice that was clearly financially motivated. The fraud resulted in several fatalities in children who were exposed to adulterated infant formula. Similarly, melamine addition to wheat and corn gluten and rice protein used for pet food manufacture led to more than 5,000 pet food products being recalled following international reports of renal failure in cats and dogs. Reported levels in some products were as high as 6.6%, but as melamine was not routinely measured in foods and ingredients, there was wide penetration of the supply chain. Other widely reported adulteration issues include the use of carcinogenic dyes (e.g. Sudan I) to enhance the colour of products such as spices, an adulteration motivated. The fraud resulted in several fatalities in children who were exposed to adulterated infant formula. Similarly, melamine addition to wheat and corn gluten and rice protein used for pet food manufacture led to more than 5,000 pet food products being recalled following international reports of renal failure in cats and dogs. Reported levels in some products were as high as 6.6%, but as melamine was not routinely measured in foods and ingredients, there was wide penetration of the supply chain. Other widely reported adulteration issues include the use of carcinogenic dyes (e.g. Sudan I) to enhance the colour of products such as spices, an adulteration designed to improve the saleability of goods by enhancing their appearance. Other issues such as the addition of protein from cows and pigs to chicken products pose ethical and religious questions to humans and present a safety risk in the animal feed sector due to the potential for the perpetuation of transmissible spongiform encephalopathies. All of these issues would have benefited from recent advances in non-targeted detection methodology which may have helped to avoid costly product recalls. Fera is at the forefront of the development of non-targeted detection technologies. Measurement techniques include analytical methodologies such as nuclear magnetic resonance (NMR) spectroscopy and high-resolution liquid chromatography mass spectrometry (HR-LC-MS). Fera has also developed software that aids the process of identifying unknown chemicals in complex mixtures.

NMR spectra usually possess a unique combination of chemical properties such as J-couplings, chemical shifts, NOEs, and diffusion rates potentially facilitating automated molecular characterisation. In recent years, rapid developments in instrument design have resulted in significant improvements to both resolution and sensitivity. The correlation between sensitivity and measurement time leads to a compromise between the desired detection limit and the time taken to acquire the NMR spectrum. Recent improvements in NMR sensitivity can therefore be used to obtain more rapid measurements and thus improve sample throughput.

In order to extract key information from the NMR spectra of complex mixtures, a range of chemometric techniques have been used in combination with NMR spectroscopic data to, for example, detect characteristic differences in the chemical composition of foodstuffs. This approach identifies deviations from normality in relation to product and ingredient composition. Once detected an abnormal sample can be scrutinised using a range of NMR techniques to determine the nature of the problem.

The complementarity of NMR spectroscopy and HR-LC-MS will be demonstrated using a number of case studies. The main benefits of using this combined approach are the relatively easy sample preparation strategy and short analysis times. Very high mass accuracy from the mass spectrometer complements the highly specific data generated by NMR spectroscopy. Analysis of a variety of contaminants in relatively dirty extracts at very low concentrations can be achieved. The structure elucidation power of the NMR is then used to categorically determine the nature of an unexpected contamination incident. Using this approach the detection of small quantities of unknown compounds in complex mixtures has been demonstrated without a priori knowledge of potential contaminants. Examples will be taken from a range of studies.

This presentation will discuss non-targeted analysis for the detection of toxins in food and feed focusing on recent developments in the fields of NMR spectroscopy and HR-LC-MS. Other uses of non-targeted methodologies within a metabolomics context and for identifying high value compounds will also briefly be discussed.

References
The identification and determination of safety of any chemical additive or contaminant in pet food is always a daunting challenge. This is particularly an issue when the chemicals of concern are novel ingredients that have not been previously used in food or were produced using novel technologies. The focus of this presentation is to highlight the aspects of comparative toxicology pertinent to this discussion, overview why novel ingredients present different concerns, and review recent events in regulations that impact the issue.

Comparative Toxicology

There are two aspects of general toxicology that are particularly pertinent to this topic: species differences in sensitivity to chemicals and the use of in vitro technologies to screen novel compounds to predict the in vivo effects. It is well known and documented in the veterinary pharmacology literature and embedded in animal health regulations, that species differences exist relative to all aspects of chemical and drug processing by animals, from absorption to metabolism to elimination and inherent biological activity. The National Research Council (NRC, 2009) study on safety of dietary supplements in animals clearly states that safety in humans does not predict safety in pets (e.g. garlic, aspirin); suggesting that historic use in human food does not guarantee safety in pets. Similarly, safety in laboratory rodents does not equate to safety in pets, nor would widespread use in dog food assure safety in cats. There are many other factors unique to individual species nutrition and feeding practices that confound this issue.

Based on a similar logic, the human toxicology and risk assessment community is realizing that reliance on laboratory animal screening tests alone does not predict human effects. This has led to a paradigm shift, best embodied in the NRC (2007) concept of toxicity testing in the 21st century, which would first identify active principles and define mechanism of action to help select appropriate animal models for testing. Importantly, an animal model or experimental system useful for assessing absorption may not be relevant for assessing toxicity. A bank of model systems of different complexities linked by quantitative models is needed. A similar scheme could be developed for pets, however relevant in vitro screens are not available and endpoints in target species (e.g. dogs or cats) have not been well defined as they have in humans.

“Novel” Ingredients:

What are “novel” ingredients? The comparative toxicology discussion above could define a novel ingredient as one which has not been used in the species of concern since use in another species does not assure safety. However, the more interesting case is a truly novel ingredient with unique chemistry which has never been used as a feed additive or supplement. This could be a chemical found in a new raw material or natural food source, or one synthetically produced using novel chemistry or biotechnology approaches. What is the best method to screen these compounds? A variation on this theme is a “normal” chemical produced using “novel” techniques including nanotechnology or genetically modified organisms. What about nanoformulations of existing substances? There are situations where new research identifies “novel” clinical syndromes, an excellent example being the association of Balkan Endemic Nephropathy with aristolochic acid from plant contaminants, rather than as historically believed from ochratoxin exposure due to mold. Finally, there are the recent cases of economic adulterants being introduced in the food supply (e.g. melamine / cyanuric acid). How can these compounds be detected using screening methods before a toxicologic incident defines the endpoint? In recent years, events such as globalization of trade and global climate shifts exacerbate many of these issues and greatly increase the number of “novel” ingredients of all definitions. This issue is multifaceted since techniques to detect and assess safety must be developed and then once an ingredient is determined to be of concern, screening methods instituted to insure production of a safe food is sustainable in the face of changing raw material sourcing and production methods.

Regulatory issues:

Despite the wide scientific uncertainty associated with novel ingredients, the international regulatory environment is often incoherent and definitely not harmonized for pet food additives! Many issues exist in the classification of supplements versus additives versus functional foods versus contaminants and adulterants. The US FDA has recently issued guidelines to clarify GRAS (Generally Regarded As Safe) status. Of great importance to the toxicology community, there are differences in regulatory philosophies concerning specific additives (e.g. hormones and GMO) and the use of animals to detect adverse effects. In Europe, movement continues to ban all animal testing and replace with in silico quantitative structure activity relationship (QSAR) risk Modelling (e.g. REACH). However, accurate QSAR models cannot be developed in the absence of sound biological data to define relevant endpoints. The European Parliament in July proposed suspension of sale of all food containing ingredients derived from nanotechnology.

Conclusion:

This brief review suggests that determination of safety of novel ingredients in pet food is a complex issue, much as it is for human food. The difference is that we know much more about human sensitivity to chemical toxicity than we do for pets and we have much greater resources in human food safety to define syndromes and even screen feed sources. In the pet food industry, manufacturers are under economic pressure, quality of raw materials are less than that used for human food, and in many cases science just has not identified if a specific chemical is toxic under conditions of use for a specific species. Uniform definitions of dose must be established and active ingredients identified. Studies conducted in target species for palatability or nutritional effect should be properly characterized and data collected so that results can be used in hazard identification analyses should adverse effects be detected. However, the greatest challenge is to identify sensitive in vitro and in silico computational techniques that can screen out potential toxicants with a reasonable degree of accuracy. Only in this fashion can this field move forward on the foundation of strong science.

Relevant readings

PET FOOD SAFETY: THE ROLE OF NEW TECHNOLOGIES

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An increasingly global and complex supply chain enhances the already substantial challenge of assuring food safety. At the same time, new technologies continually emerge that can have an impact on every link of the supply chain, from farm to factory to food bowl. In this regard, several areas stand out as being ones to watch.

Supply chain monitoring, decreasing costs and increased sophistication for bar-code, radio frequency identification (RFID), wireless communication and global positioning satellite (GPS) technologies make it feasible to trace the path of food ingredients and products throughout their life. On top of the basic when-and-where chronology, it is possible to gather additional data through sensor networks. For example, temperature, light, humidity, oxygen concentration or output from specialized analytical instruments can be monitored at relevant points. A simple implementation involves the use of smart packaging that can provide an instant visual check for mishandling or adverse conditions. Information of this nature can be of great value for both real-time decisions and retrospective analysis of problems.

Geographic Information Systems (GIS) and Data Analytics: As noted, the value of when-and-where data can often be boosted by metadata. For example, if we know the origin of a given ingredient, what else do we know about the local conditions at the time it originated? One thing we can easily find out about is weather. Was it unusually hot, cool, wet or dry, and are the weather conditions associated with threats to food such as fungi or pests? Another thing we might want to know about is regionally specific problems and threats. Had there been any recent outbreaks of disease in people, pets or livestock? Are their known, localized biological threats, such as the toxic plant Aristolochia clematitis in the Balkans? Are conditions especially ripe for economic adulterers, criminals, or terrorists? Taken together, these types of metadata constitute intelligence. When the food supply was local, this intelligence was relatively easy to gather and share in a community. With a global food supply, it is much more difficult for food manufacturers to keep tabs on what is happening in far-flung regions of the world. GIS technologies can help manage the relevant data and, with the assistance of analytics, build local intelligence on a global scale.

Laboratory Technology: Foods and food ingredients are notoriously difficult to analyze. They are complex, inconsistent, and unstable. Further, they are physically heterogeneous, with multiple phases (such as fat, water and solids) that can sequester organic compounds, microbes and foreign material. To recover analytes of interest from this complex matrix, tedious sample preparation, extraction and purification procedures have traditionally been required. New technologies that allow for direct analysis of food materials with little or any sample preparation are greatly needed, and real progress is being made on this front. One example is new surface-sampling mass spectrometry techniques that bypass the traditional extraction methods yet still provide high sensitivity and specificity. Some useful analyses can now be performed in stand-off mode, where no direct contact with food materials is required. Another trend is the steady miniaturization and automation of analyses through microfluidic or lab-on-a-chip technologies. These trends work synergistically, for as samples get smaller, and their preparation gets simpler, automation becomes more feasible. The ultimate outcome will be faster routine analyses that require less skilled labor and deliver greater return on capital investment.

Field-Deployable Technologies: Taking technology out of the laboratory and pushing it deep into the supply chain promises better quality and earlier warning of safety problems. Instrumentation and tests that can be used by farmers, truck drivers, factory workers, and government inspectors to make quick decisions are of particular value. Likewise, equipping the consumer with simple quality indicators can prevent ingestion of unsafe or nutritionally compromised food. In terms of analytical instrumentation, two examples of versatile technologies that have been successfully miniaturized are mass spectrometry and Raman spectroscopy. Both of these are powerful methods for analyzing mixtures and have great potential for the food industry. Genomic technologies are also advancing at a rapid pace and will certainly lead to new field tests, as well as field-sampling procedures to be accompanied by laboratory analysis. In many cases, development of fieldable devices and tests has been driven by law enforcement, defense, and security needs. Some of the products on the market can be used directly or adapted for food safety, but purpose-specific products will also be needed. Cooperation between technology developers, device manufacturers, food manufacturers and government will be required to accelerate the deployment of new tools for food safety.

All told, widely dispersed, inexpensive and easy-to-use technologies can be powerful weapons in the battle for food safety. Their proliferation can thus be of great benefit, but it also presents big challenges for the food industry: how to stay current on a large, rapidly growing set of technologies; how to perform cost-benefit analyses quickly and effectively; how to manage a swelling body of data and extract useful information from it; and how to allocate scarce resources in selecting the technologies to adopt.
EFFECTS OF WEIGHT LOSS ON ADIPOKINES AND MARKERS OF INFLAMMATION IN DOGS

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Obesity has been associated with many chronic diseases in humans, horses, dogs and cats, including Type II diabetes. Adipokines may play a part in the exacerbation of these conditions. However, Type II diabetes is rare in dogs and previous findings suggest that serum adipokine changes in dogs are mild, and in many cases, yet to be quantified. Our study was designed to examine a series of adipokines and markers of inflammation in dogs before and after successful weight loss.

Initial power of the study was set at $\beta = 0.2$ and an $\alpha = 0.05$ for all analyses. The study included fasting serum samples from 25 dogs before (mean BCS = 8 of 9) and after (mean BCS = 5 of 9) a successful weight loss program (average 27% weight loss). Serum C-reactive protein (CRP), monocyte chemotactic factor-1 (MCP-1) were measured as indicators of chronic inflammation, while serum adiponectin, high molecular weight (HMW) adiponectin, resistin and leptin were also examined. Statistical significance was determined using paired Wilcoxon Rank sum testing.

Median CRP (pre = 9.7 ug/ml; post = 4.9 ug/ml) and MCP-1 (pre = 212 ng/ml; post = 185 ng/ml) decreased ($p < 0.05$) after weight loss. Resistin showed a mild yet significant reduction (pre = 67.1 pg/ml; post 60.5 pg/ml), while leptin showed a dramatic decrease after weight loss (pre = 18.9 ng/ml; post =6.6 ng/ml). Serum adiponectin and HMW adiponectin were unchanged.

Markers of inflammation were modestly decreased, and leptin, which functions in food intake control, was dramatically reduced, which may influence increased food seeking during weight loss. Importantly there was no increase in serum adiponectin after weight loss, confirming recent canine studies. The lack of change in adiponectin and its more active counterpart, HMW adiponectin, may result in better insulin sensitivity explaining why insulin resistance and Type II diabetes are less prevalent in obese dogs versus other species.
CHRONIC OBESITY IN CATS DOES NOT LEAD TO A SYSTEMIC LOW GRADE INFLAMMATION
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In humans and rodents, chronic obesity causes a systemic low grade inflammatory reaction, with an increase of pro-inflammatory and a decrease of anti-inflammatory cytokines. This has not been investigated in cats before. The aim of this study was to examine whether pro-or anti-inflammatory (adipo) cytokines differ between obese and lean cats, and change after weight loss.

Fifteen adult cats were included in this trial. Eight cats were in a chronic obese body condition state and seven cats had an ideal body weight. All cats were fed a low energy diet during 16 weeks. The control group (CG) was fed at maintenance energy requirement (MER) to maintain constant body weight. The obese group (OG) followed a standard weight loss program. Blood samples were taken at week 0, 6, 11 and 16 for leptin and cytokine analyses.

A mean weight loss of 13.7% was reached in the OG after 16 weeks. Leptin concentration was significantly higher in the OG, compared to the CG. This concentration decreased significantly in the OG after 6 weeks weight loss. Cytokines TNF-α, IFN-γ, IL-6 and IL-10 were significantly lower in the OG before and during weight loss. During weight loss, IL-10 increased significantly in the OG, however, no alteration was seen in this group for TNF-α, IFN-γ and IL-6.

As in humans and rodents, a chronic low grade inflammation was expected. However, chronic obesity in cats does not lead to an increased serum concentration of both pro-and anti-inflammatory cytokines. These cytokines might be released in a different way in cats, and remain at the site of adipose tissue. Further investigation of mRNA expression in adipose tissue is necessary to clarify this.

COMPARISON OF ENERGY EXPENDITURE OF PET CATS ESTIMATED USING THE DOUBLE-LABELED WATER METHOD WITH METABOLIZABLE ENERGY INTAKE
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The double-labeled water (DLW) method measures energy expenditure (EE) in free-living animals. It has been validated in dogs but not cats. The purpose of this study was to compare EE estimated using the DLW method with metabolizable energy (ME) intake in pet cats maintaining stable body weight while being fed individually. Food intake was measured daily for 2 weeks in 10 neutered pet cats of both sexes, 2-10 years of age, kept strictly indoors. The ME density of food was calculated (NRC 2006) from the proximate analysis of representative samples. Body weight and condition were measured at the start and after 1 and 2 weeks. Enrichment of the stable isotopes, deuterium (2H) and oxygen (18O), was measured in blood samples obtained immediately before, and then 2 h, 1 and 2 weeks after the subcutaneous injection of saline enriched with these two isotopes. The EE was estimated from the decline in enrichment.

There was no evidence of a change in body weight over two weeks. Cats consumed a mean of 182 kcal ME (range 72-331) daily, whereas daily EE was estimated by DLW to be a mean of 192 kcal ME (range 157-252). The difference between ME intake and EE by DLW was small in eight cats but large in two cats and the correlation was modest (R²: 62%). After removing the two outliers, R² was only 69%. This data suggests that the DLW method may be insufficiently accurate to measure EE in cats.
OMEGA 3 FATTY ACIDS SUPPLEMENTATION IMPROVES INSULIN SENSITIVITY AND INCREASES EPA AND DHA TISSUE CONTENT IN OBESE INSULIN RESISTANT DOGS

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Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are well-known for their hypotriglyceridemic effects. A potential improvement of insulin sensitivity (IS) has also been suggested in cell culture, whereas nutritional studies have brought conflicting results. The effects of EPA and DHA have never been demonstrated in obese insulin resistant dogs.

Eight obese insulin resistant dogs (19.2±1.1 kg), were given for six weeks, a supplementation of EPA and DHA (920 mg/d and 760 mg/d respectively). Prior and at the end of the supplementation period, IS was assessed by the gold standard method, the euglycemic hyperinsulinemic clamp. Body composition was assessed by isotopic dilution, and plasma glucose, insulin and non esterified fatty acid (NEFA) concentration was measured. Fatty acid composition of liver, muscle, visceral and subcutaneous adipose tissues was determined.

No variation in body weight or in body composition was observed. Insulin sensitivity was improved by EPA and DHA (IS index: 0.085±0.009 vs 0.103±0.015, p<0.05). Basal glycemia, insulinemia or NEFA concentration were unchanged. We observed an accumulation of EPA and DHA in liver (EPA + DHA increased from 4.51±0.29 to 7.20±0.63% of total fatty acid, p<0.0001) at the expense of arachidonic acid (p<0.01). In skeletal muscle, only EPA was increased (p<0.05). Subcutaneous adipose tissue was also enriched in EPA and DHA (p<0.05), whereas visceral adipose tissue was not affected by the supplementation.

We showed that EPA and DHA improve insulin sensitivity in obese insulin resistant dogs without changes in body composition or NEFA concentration, that are described as two major determinants of insulin sensitivity. The insulin sensitivity could be at least in part explained by a modification of membrane fluidity, as a consequence of an accumulation in EPA and DHA in liver and muscle.

PLASMA ESTROGEN LEVEL AFTER ESTRADIOL DOSAGE TO NORMALIZE FOOD INTAKE IN NEUTERED CATS SUPPORTS HORMONE REPLACEMENT USE IN OBESITY TREATMENT

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The increase in food intake causing post-neutering weight gain in cats is prevented by low doses of 17β-estradiol (E2). The physiological relevance of the E2 administration on post-neutering food intake was presently evaluated in 6 males (1.3-1.6 years, 4.7-7.0 kg, 20-25% body fat). Jugular venous plasma E2 and estrone (E1) were determined weekly after orchiectomy (OX), when daily subcutaneous injections of E2 (0.5 µg) or vehicle (sesame oil, 0.1 mL/kg) were given in a crossover trial of two, 21 d periods. Preceding OX, means of plasma E1 and E2 were 32±8.3 and 4.3±1.0 pg/mL, respectively. After OX, plasma was sampled 4 hr after E2 injections while food presentation was limited to 110% of pre-OX intake to prevent excessive weight gain. When E2 compared to vehicle was given after OX, weekly-sampled mean plasma of E2 was greater (3.8±0.5 vs. 2.1±0.3 pg/mL, P<0.05) and E1 was not significantly different (29±8.1 vs. 28±8.0 pg/mL). Compared to pre-OX, plasma E2 after vehicle injections were lower (P<0.05) while those after E2 injections were not significantly different. Plasma E2 among the cats after a single E2 injection was 190, 149, 124, 133, and 160 % of baseline at 2, 4, 8, 16 and 24 hr. Plasma E1 and E2 were determined weekly during the last three weeks of two 56 day periods of a crossover trial in which food was continuously presented or restricted to pre-OX amounts.

Continuous compared to restricted food presentation resulted in increased (P<0.03) body weight (6.4±0.4 vs. 5.6±0.4 kg) and fat (23±3 vs. 31±2%). Plasma E2 and E1 while cats were overweight were not significantly different from those when they were at ideal weight. Hence, circulating E2 appears reduced by OX but unchanged by body fat gain, and exogenous E2 in the dosage used appears to be a physiological replacement of E2.
PORTION CONTROL AFTER NEUTERING FOR A PERIOD OF 18 WEEKS MAY HELP PREVENT POST-SPAYING WEIGHT GAIN IN GROWING FEMALE KITTENS

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The objective of this study was to investigate the influence of spaying on food intake and body composition in female kittens.

Twelve pairs of 11-week-old female kittens (from 12 litters) were randomly assigned to two groups. All kittens were offered ad libitum access to a dry diet (protein energy 29%, fat energy 44%, carbohydrate energy 27%). One group was spayed at 19 weeks. Food intake was recorded daily, and body weight (BW) recorded weekly. Body condition score (BCS) using a 9-point scale and body composition (determined using dual-energy X ray absorptiometry) were recorded at 11, 18, and 52 wks.

At 52 wks of age entire kittens had a BCS of (mean [95% confidence intervals]) 5.2 [4.8-5.6] versus 6.1 [5.6-6.5] in neutered kittens and a percentage of fat of 23.5 [19.8-27.3] versus 30.2 [25.8-35.4]. In order to maintain BCS entire kittens consumed 95% of the NRC (2006) recommended allowance at 26 wks of age. By 52 wks this had further reduced to 79%.

Increased intake (Kcal/kgBW) in spayed kittens occurred immediately post-spaying, becoming significant from 4 wks post-spaying. This difference in intake peaked 10 wks after spaying, 17% [8-27] and subsequently decreased, with no significant difference between groups by wk 18 post-spaying. Spayed kittens became increasingly heavy compared to their entire littermates, significantly so from 11 wks post-spaying. By 52 wks of age, the spayed kittens were 24% [11-39] heavier than entire littermates.

Entire female kittens appear to be more able to regulate dietary intake when consuming a dry diet during growth, compared to spayed female kittens. The intake peak following spaying suggests that portion control for 18 weeks post-spay may prevent excess weight-gain. Further studies are required to understand the role of dietary regime and activity in the regulation of BW in growing animals.

<table>
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<th>Breed</th>
<th>Birth weight (gm)</th>
<th>Body weight at 28 days (gms) males</th>
<th>Body weight at 28 days (gms) females</th>
<th>Body weight at 84 days (gms) males</th>
<th>Body weight at 84 days (gms) females</th>
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</tr>
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</table>

* significant difference between males and females

BIRTH WEIGHT AND POSTNATAL GROWTH OF PUREBRED KITTENS

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Data on birth weight and growth of kittens are important for factorial calculation of requirements. In addition they are very helpful for health monitoring of kittens. In the present study data on body weight of 263 pure bred kittens (Maine Coon, Norwegian Forest Cat, Birman, Persian, Siamese/Oriental Shorthair Cat) from birth to 12 weeks of age were obtained from breeders. Mean and standard deviation was calculated. Two means were compared by student’s t-test, more than two means by ANOVA and Holm-Sidak-test (p<0.05). Relative birth weight and relative litter weight was expressed as percentage of normal body weight of female cats of the same breed as determined in another study (Kienzle and Moik, submitted to Waltham Symposium).

Absolute birth weight in g was higher in larger breeds than in smaller breeds, whereas relative birth weight tended to be higher in smaller breeds (Siam 3.2 %, Maine Coons 2.3 %). In all breeds it was below literature data on cats without pedigree. Relative litter weight was highest in the Norwegian Forest cats (14.6 %) and lowest in the Birman cats (8.8 %), the other breeds were in between (11.9 %). Absolute postnatal growth was slower in smaller breeds. In relation to birth weight, however, there was no systematic effect of breed size during the first 84 days.
EFFECTS OF SELENIUM SOURCES ON SEMEN CHARACTERISTICS AND SEMEN ANTIOXIDANT STATUS IN DOGS

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The aim of this study was to evaluate the effects of two sources of selenium on dogs’ semen production and quality.

Twenty-four male beagle dogs were distributed in three groups of 8 dogs each, and fed with three diets: control (7.3 µg of Se/MJ), inorganic (20 µg of Se/MJ of sodium selenite) and organic (20 µg of Se/MJ of selenium yeast). The experiment followed a randomized blocks design; age and semen concentration (spermatozoa per mL) were the blocking factors. Diets were fed for 80 days and the semen collected every 20 days and analysed for volume, motility, and total sperm count per ejaculate (TSC). Glutathione peroxidase activity (GSH-Px), thiobarbituric acid reactive substances (TBARS), and total antioxidant capacity (TAC) of the seminal plasma, together with sperm morphology and membrane integrity were measured on days 0, 40 and 80. Selenium concentration in seminal plasma was determined in days 0 and 80 of food consumption.

On day 60, animals fed organic Se showed higher semen concentration than the control group (p<0.05), and on day 80 higher semen concentration than the others two groups (p<0.05). On days 40 and 80 organic selenium fed dogs showed higher TSC than the control group (p<0.05). The consumption of organic Se also resulted in reduced percentage of minor defects and total defects in the semen than the control group (p<0.05). Selenium concentration, GSH-Px, and TBARS on seminal plasma did not differ (p>0.05), but TAC was higher for organic Se than for control group (p<0.05).

At least 20 µg of Se/MJ is important for semen production in dogs. Yeast selenium is more effective than sodium selenite in supporting dog spermatogenesis.
FREQUENCY AND EXTENT OF NUTRITIONAL IMBALANCES IN “BONE AND RAW FOOD” (BARF) RATIONS

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Interest of pet owners in bone and raw feeding rations (BARF) increased in the last years in Germany. We offered (on the internet) to check BARF rations for a special price in order to prevent malnutrition by barfing. A standardized questionnaire for ration calculation was used. 77 rations were calculated with the software Diet Check Munich©, which is based on recommended allowance (RA; NRC 2006). The actual weight of the dogs was compared with the body weight at about 1.5 years which was considered to be close to the normal weight. Most of the barfers fed many different meats (e.g. horse, lamb, chicken, and beef), different vegetables and fruits, nuts, linseed, egg yolk, dairy products and plant oils. Some of the rations were supplemented with mineral mixtures, others with liver, bones or eggshell. There were also rations with cooked carbohydrates (rice, potatoes, pasta) and raw meat. Sometimes dry kibbles were used as treats. The mean actual body weight was 24.9 kg (range 5.4 to 72 kg), the mean age 4.5 years. Incidence of overweight was very low, only 11.5 % of the dogs had more than 10 % overweight. 76% of the raw feeding rations showed at least one nutritional imbalance (table. 1). 24 % of the rations had neither a deficiency nor an oversupply in nutrients.

### Table 1: Incidence of nutritional imbalances in BARF rations for adult dogs (n =77)

<table>
<thead>
<tr>
<th>Imbalance</th>
<th>% of rations</th>
<th>Mean supply [% of recommended allowance (RA)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>calcium excess &gt; 200% RA</td>
<td>33</td>
<td>440 ± 220</td>
</tr>
<tr>
<td>phosphor excess &gt; 200% RA</td>
<td>33</td>
<td>326 ± 141</td>
</tr>
<tr>
<td>calcium deficiency &lt; 85% RA</td>
<td>18</td>
<td>41 ± 24</td>
</tr>
<tr>
<td>phosphor deficiency &lt; 85% RA</td>
<td>12</td>
<td>65 ± 14</td>
</tr>
<tr>
<td>magnesium deficiency &lt; 85% RA</td>
<td>16</td>
<td>60 ± 11</td>
</tr>
<tr>
<td>potassium deficiency &lt; 85% RA</td>
<td>4</td>
<td>60 ± 22</td>
</tr>
<tr>
<td>iodine deficiency &lt; 85% RA</td>
<td>57</td>
<td>21 ± 19</td>
</tr>
<tr>
<td>zinc deficiency &lt; 85% RA</td>
<td>53</td>
<td>57 ± 17</td>
</tr>
<tr>
<td>copper deficiency &lt; 85% RA</td>
<td>40</td>
<td>47 ± 16</td>
</tr>
<tr>
<td>manganese deficiency &lt; 85% RA</td>
<td>54</td>
<td>57 ± 22</td>
</tr>
<tr>
<td>iron deficiency &lt; 85% RA</td>
<td>4</td>
<td>60 ± 19</td>
</tr>
<tr>
<td>vitamin A deficiency &lt; 85% RA</td>
<td>27</td>
<td>52 ± 24</td>
</tr>
<tr>
<td>vitamin D deficiency &lt; 85% RA</td>
<td>60</td>
<td>24 ± 26</td>
</tr>
</tbody>
</table>

THE EFFECTS OF DRY AND WET DIETS ON FAECAL BACTERIAL POPULATIONS IN THE DOMESTIC CAT

Birmingham, E.1, Kittelmann, S.1, Basset, S.1, Weidgraef, K.2, Hekman, M.2, Roy, N.1, Thomas, D.2

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Obesity levels in domestic cats are increasing1. This may be due to their consumption of highly palatable, carbohydrate (CHO) rich diets (30-60%).2 Obesity has been linked to changes in intestinal microbiota3. We hypothesise that increased weight gain in cats fed high CHO diets may be due to changes in their intestinal microbiota.

Eighty colony-housed domestic short-haired cats (4 male and 4 female; c. 1-10 years old; c. 3.4 kg) were fed a commercially available, complete and balanced, wet diet (crude protein 51.7%; fat 28.9%; carbohydrate 8.9%; ash 10.6% DM; AAFCO, 2009), for 4 weeks. On the 5th week, individual feed intakes and faecal outputs were determined. Faecal samples were collected twice daily, and stored at -85°C until analysis. The cats were then switched to a commercially available, complete and balanced, dry diet (crude protein 33.0%; fat 11.0%; carbohydrate 49.4%; ash 6.6% DM; AAFCO, 2009) and sampled as above.

Faecal bacteria populations were assessed by extracting genomic DNA from faeces. The V2-V3 region of the 16S rDNA gene of bacteria in each sample was amplified using primers HDAl-GC and HDa-24. PCRs products were digested with Mung Bean nuclease and DGGE was performed with 6% polyacrylamide gels containing a 35 to 70% (w/v) gradient5. DGGE profiles were compared by determining the Pearson similarity coefficient using Bionumerics software (Applied Maths, TX, USA).

UPGMA cluster analysis of bacterial community profiles using Pearson correlation revealed diet-specific clustering when the same cats were fed on either a dry or a wet diet (dissimilarity between groups: 88.6%; P<0.001). We aim to identify the major bacterial species that are influenced by the dietary shift by means of cloning and sequencing of their 16S rRNA genes.

CORRELATION OF A FELINE MUSCLE MASS SCORE WITH BODY COMPOSITION DETERMINED BY DEXA
Michel, K.1, Anderson, W.2, Cupp, C.2, Laflamme, D.2

Body condition scoring (BCS) systems primarily assess body fat. Both overweight and under-weight animals may have loss of lean tissue that may not be noted using standard BCS systems. Catabolism of lean tissue can occur rapidly, may account for a disproportionate amount of body mass loss in sick cats and can have deleterious consequences for outcome. Therefore, in addition to evaluation of body fat, patients should undergo evaluation of muscle mass to estimate lean tissue status. We previously reported that a subjective 4-point muscle mass scoring (MMS) system for cats showed moderate inter-rater agreement in cats scored normal or severely wasted ($\kappa$=0.48-0.53). Intra-rater agreement was substantial ($\kappa$=0.71-0.73). In this study, we investigated the correlation between MMS and lean body mass (LBM) as determined by dual energy x-ray absorptiometry (DEXA).

The MMS was as follows: 3: normal muscle mass, 2: slight wasting, 1: moderate wasting and 0: severe wasting. The BCS was based on a 9 point scale. Cats were purposely selected for evaluation based on age, BCS and MMS to generate a study population that represented the scope of each of the 3 parameters. Body composition was determined using DEXA. Correlation between MMS and BCS, age, %LBM and LBM (g) was determined using Spearman rank-order correlation.

Thirty-three cats ranging in age from 3-17 years (median=11 years), MMS from 0-4 and BCS from 3-7 (median=6) underwent DEXA. The MMS was significantly correlated with BCS ($r=0.76$, $P<0.0001$), age ($r=-0.75$, $P<0.0001$), LBM (g) ($r=0.62$, $P<0.0001$), and %LBM ($r=0.49$, $P<0.005$). The negative correlation between MMS and %LBM may be the consequence of including overweight cats (BCS>5).

In conclusion, there was fair correlation between a subjective feline MMS and LBM as determined by dual energy x-ray absorptiometry (DEXA).

Additional investigation is desirable to determine whether the MMS can be further refined and to assess its clinical applicability.
THE POTENTIAL FOR ENHANCEMENT OF IMMUNITY IN CATS BY DIETARY SUPPLEMENTATION

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Increasingly, functional foods which non-invasively modulate and optimise the human immune system are being used for companion animals, as owners try to optimise their pet’s health. To date, dietary supplementation trials primarily carried out in humans and mice have demonstrated that a number of bioactive ingredients modulate immune function. Despite little published data existing for cats, pet food companies are already manufacturing immune modulating diets by adding a plethora of substances. In this study we assessed the potential immune enhancing benefits of dietary supplementation with arginine, salmon oil and yeast-derived nucleotides to adult cats.

A study using 40 adult domestic cats (2-11 yrs; 2.2-5.9kg) was conducted at the Centre for Feline Nutrition, Massey University. Eight animals per group were fed either a low protein diet (LPD) with or without supplementation, or a commercial moist diet (MD). LPD basal diet was formulated using a commercial moist diet (42.1% DM basis), starch (37.5% DM basis), lard (17.4% DM basis) and vitamin/mineral mix (3% DM basis). Dietary supplements added to the LPD were yeast-derived nucleotides (9.25%), salmon oil (9%) and free L-arginine (1.9%). Feed and water were available ad libitum. The cats were fed the diets for 35 days, with immune assessment at days 0, 14 and 35.

Supplementation had no effect on levels of expression of CD4+ , CD8+ , B+ or CD11b+ cells. Nucleotide supplementation enhanced lymphocyte proliferative responses to ConA (P<0.01). Salmon oil supplementation enhanced lymphocyte proliferative responses to PHA (P<0.05) with trends (P<0.1) observed in the nucleotide and arginine supplemented groups. Increased phagocytic activity (P<0.05) was observed following supplementation with arginine, salmon oil and nucleotides compared to the LPD and MD fed animals. These results indicate that cats receiving dietary supplementation with arginine; salmon oil or yeast derived nucleotides may have a greater ability to fight infection and disease than control-fed animals.

IN VITRO EVALUATION OF FIBER AND PROTEIN FERMENTATION SUBSTRATES IN CATS

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The purpose of this study was to investigate the fermentation kinetics and end products of different substrates in vitro. The emphasis was on propionate and putrefactive substance production and kinetics. Twenty-three cats were fed two diets in two consecutive 14 day periods. The first diet contained highly digestible protein and several sources of fermentable fiber, the second had a high protein content, consisting of less digestible protein. They intended to increase carbohydrate and protein fermentation respectively. After each period fresh fecal samples were collected and processed into an inoculum to ferment fructans of different degree of polymerization, citrus pectin, guar gum, cellulose and an amino acid mixture. For 72 hours gas production was measured and samples were taken at different time points for analysis of short- and branched-chain fatty acids, ammonia, protein fermentation products and to characterize the microbial flora.

Some preliminary results are already available. Cellulose and the amino acid mixture were poorly fermentable with gas production curves similar to the curves of the blanks. Citrus pectin and the three fructans (short-chain fructooligosaccharides (scFOS), oligofructose and inulin) had higher gas productions than the guar gum at all time points except for the last one.

Cellulose was confirmed to be poorly fermentable. Citrus pectin was used as a positive control substrate and indeed showed to be highly fermentable. The higher gas production of guar gum compared to citrus pectin at the latest time point was a confirmation of the more moderate rate of fermentation of this substrate. The results of gas production appear to be similar in the two diet periods for the different substrates.
ASSOCIATION BETWEEN SERUM 25-HYDROXYVITAMIN D (25-OH-D3) LEVEL AND MAST CELL TUMORS IN LABRADOR RETRIEVERS

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Studies have demonstrated an association between vitamin-D deficiency (low serum 25-OH-D3) and development of various cancers in people. In dogs, mast cell tumors (MCT) are common with a potentially heritable predisposition in Labrador retrievers. Our data demonstrate that the vitamin-D receptor is expressed in canine MCTs and calcitriol (1,25 OH-D3) diminishes proliferation in canine mastocytoma cells. Hence, we hypothesized that low 25-OH-D3 serum levels are a risk factor for MCTs in Labradors.

Ninety-four dogs were included (25 Labradors with MCT, 42 healthy Labradors, and 27 dogs of other breeds). Dogs were fasted for 12h and serum 25-OH-D3 was measured by radioimmunoassay. Owners completed a questionnaire about recent food and supplement intake. Manufacturers supplied information about diet vitamin-D3 content and kilocalories per cup or can. Vitamin-D intake per kilogram body weight was calculated and compared to serum 25-OH-D3 concentrations.

There was no difference in serum 25-OH-D3 concentrations or kilocalories consumed between Labradors with or without MCT. Compared to other breeds, however, Labradors had a significantly lower vitamin-D intake (P=0.015) and kilocalories consumed per kg body weight (P=0.011). Despite this finding, serum concentrations of 25-OH-D3 were not different between Labradors and other breeds. Among all dogs, there was no association between calculated vitamin-D intake and measured serum concentrations of 25-OH-D3.

In conclusion, low serum 25-OH-D3 is not a risk factor for MCTs in Labradors. Lower vitamin-D intake in Labradors is not reflected in serum concentrations, and is likely due to a decrease in kilocalories consumed per kg body weight. Feeding non-commercial diets appears adequate to maintain serum 25-OH-D3 levels and there is a risk of hypovitaminosis-D when feeding non-commercial diets.
The WALTHAM International Nutritional Sciences Symposium

Pet Nutrition – Art or Science?

The EFFECT OF DIET COMPOSITION ON GLUCOSE, INSULIN AND LEPTIN CONCENTRATIONS, WEIGHT GAIN AND FOOD EFFICIENCY IN HEALTHY CATS
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1Centre for Companion Animal Health, University of Queensland, Australia; 2School of Veterinary Medicine, Nippon Veterinary and Life Science University, Tokyo, Japan; 3WALTHAM Centre for Pet Nutrition, Leicestershire.

A low carbohydrate (CHO), high protein diet is currently recommended for management of diabetic cats because it is associated with increased remission rates. However, the effect of dietary macronutrient composition on insulin sensitivity, adipocytokines and food efficiency is not known. The aims of this study were to compare the effect of feeding a low CHO-high protein diet (19 & 51% ME) containing moderate fat (30% ME) with a high CHO-low protein diet (52 & 24% ME), also containing moderate fat (24% ME). Variables compared between diets were fasting insulin sensitivity, fasting and postprandial (PP) blood glucose, insulin and leptin concentrations after 3 weeks of maintenance feeding and 8 weeks of ad libitum feeding, and weight gain and energetic efficiency of each diet after 8 weeks of ad libitum feeding. Thirty-two neutered, lean, mixed breed cats (16 males, 16 females) were studied. Cats were fed a baseline diet of moderate protein (34%ME), CHO (37%) and fat (29%) for 3 weeks, and were then tested after being fed the test diets at maintenance energy levels for 3 weeks, and again after being fed the test diets ad libitum for 8 weeks. At each testing period, cats underwent a DEXA scan, an intravenous glucose tolerance test and a 24-hour meal feeding test.

Results showed that feeding a low CHO diet at maintenance energy requirements results in lower PP glucose and insulin concentrations compared to a high CHO diet, and is indicated for management of diabetic and pre-diabetic cats. However, ad libitum feeding of a low CHO diet results in greater weight gain (P < 0.01) than a high CHO diet, and is associated with greater food efficiency (5.825 kcal/kg weight gain), and therefore ad libitum feeding is not recommended for cats predisposed to diabetes, or ideal or overweight diabetic cats.
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P1) USE OF PEDOMETERS TO MEASURE THE RELATIONSHIP OF DOG WALKING TO BODY CONDITION SCORE IN OBESE AND NON-OBESE DOGS

Wakshlag, J., Warren, B., Maley, M., Struble, A., Panasevich, M., Farrell, T., Wells, M.
Cornell University, Ithaca, US

The objective was to develop an accurate pedometer methodology for dogs and use it to assess the relationship between dog walking and body condition. Initially, 20 large, medium and small dogs were assessed to determine the accuracy of pedometer methodology using video-taping. In 15 of 15 dogs above 10kg weight, there was no significant difference between steps counted from video tapes and pedometers (mean ±SEM = -3.3 %±1.2). Pedometer assessed steps among smaller dogs under 10kg was significantly less than steps from videotaping (-21%±3.0).

During an observational study 77 obese and non-obese dogs over 14 inches shoulder height were recruited from a dog obesity clinic and a community sample for evaluation of body condition score and pedometer assessed walking over 10 weeks. Observational assessment of body condition score (9-point system) and count of dog walking steps for 3 one week periods within a 10 week time period were collected. The range of daily step counts was 5,555- 39,970 steps per day among 77 medium and large dogs (>10 kg). Dogs’ body condition scores were inversely correlated with their average daily steps (Spearman’s rho = -0.515, p-value <0.0001); higher steps were associated with better body condition. The mean difference in walking steps between obese (BCS = 8 or 9) and non-obese dogs (BCS = 4 or 5) was 7,098 steps a day which equates to about 2.5 miles or 45 to 50 minutes of walking per day.

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P57) EFFECTS OF WEIGHT LOSS ON THE CARDIAC STRUCTURE AND FUNCTION OF OBESE DOGS
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PROJECT ANNOUNCEMENT:
CANINE LIFETIME HEALTH PROJECT
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P3) LIFESTYLE CHARACTERISTICS OF PET OWNERS VERSUS NON-OWNERS
Heuberger, R.1, Wakshlag, J.2
1Central Michigan University, Mount Pleasant, MI, US; 2Cornell University, Ithaca, NY, US

The purpose of this study was to ascertain differences in diet, exercise and lifestyle between owners and non-owners of companion animals (dog or cat). Ongoing recruitment in the rural U.S. yielded 105 owners matched to non-owners on demographics (n=210). The design was cross-sectional, convenience sampled, and participants were uncompensated. Pet owners were primarily dog owners (n=72; 69%) and cat owners (n=33; 31%).

Owners and non-owners ranged in age from 17 -75 years (39.8 ± 13.1). The matched individuals were Caucasian (99%), mostly female (57%) and had some college education (67%). Average height was 67.5 ± 4 inches. Half of all respondents (51%), did no formal exercise. Non-paired T-test was used to detect differences between owners and non-owners on continuous variables and Mann-Whitney U/Wilcoxon Rank Sum was used for non-normally distributed variables. Significant differences were seen with exercise (p< .02) when owner activity plus dog walking was compared to non-owners’ exercise duration. Weight of owners was 164.8 ± 38.7, and non-owners 171.95 ± 41.6. The Body Mass Index (BMI) for owners was 24.6 ± 4 and non-owners was 26.8 ± 6, making both groups overweight. Differences in BMI were statistically significant (p< .03). There were no differences in alcohol, fast food, fish or low fat dairy consumption between groups. Significant differences were observed when servings of fruits, vegetables, whole grains and added fats were compared (p< .01, p< .01, p< .03) respectively. Veterinarian reported pet overweight was 11% in this sample.

Significant differences were seen in dietary patterning, physical activity and lifestyle variables between owners and non-owners. Differences may be useful in targeting educational and marketing strategies to pet owners, and suggests that pet ownership may lead to differences in healthy lifestyle.

P2) EFFICACY OF A DIET DESIGNED WITH A RELATIVE SUPER SATURATION <1 TO DISSOLVE STRUVITE STONES IN THE FELINE BLADDER
Houston, D.1, Weese1, H.1, Evason, M.1, van Hoek, I.2
1Royal Canin, Guelph, Ontario, Canada; 2Royal Canin, Aimargues, France

Previous studies have shown the efficacy of canned and dry magnesium-restricted, urine acidifying diets in dissolving struvite stones in cats; mean times to dissolution in these studies were 26-36 days (canned food) and 34 days (dry food), respectively. Studies have shown that urine supersaturation is primarily responsible for the formation and dissolution of crystals within the urinary tract. A previous study showed the efficacy of a dry diet formulated to generate a urinary struvite relative supersaturation (RSS) < 1, in dissolving feline struvite uroliths in vitro. The present study was undertaken to determine if a diet with efficacy for dissolving struvite stone in vitro also had efficacy of dissolving struvite stones in vivo.

Thirteen privately owned cats suspected of struvite urolithiasis based on radiographs were included. Cats were kept in their home environment and owners were instructed to feed Urinary S/O either wet (6 cases) or dry (7 cases) as the exclusive diet for the duration of the study. Investigational parameters at inclusion included a complete blood count, serum biochemistry, urinalysis, urine culture, and abdominal radiographs. Radiographs and urinalysis were repeated on a weekly basis and evaluated by two board certified internal medicine specialists to mark time of stone dissolution.

Eleven spayed female and 2 neutered male cats with a mean age of 7.50 ± 3.44 years and mean weight of 6.2 ± 2.02 kg were included. Struvite stones dissolved in a median of 19.5 days (range 14-56 days; mean time of 28.33 ± 18. 47 days) and of 18 days (range 10-39 days; mean time of 20.14 ± 10.12 days) in cats fed wet or dry food respectively.

Our preliminary results suggest that a diet designed to create urine undersaturated for struvite (RSS <1) is effective both in vitro and in vivo in dissolution of struvite bladder stones.
P4) LOW MAINTENANCE ENERGY REQUIREMENTS OF OBESE DOGS AFTER WEIGHT LOSS

German, A., Holden, S., Mather, N., Morris, P., Biourge, V.
1University of Liverpool, Wirral, UK; 2WALTHAM Centre for Pet Nutrition, Leicestershire, UK; 3Royal Canin Research Center, Aimargues, France

Weight rebound after successful weight loss, is a well-known phenomenon in humans and companion animals, possibly due to the fact that caloric restriction improves metabolic efficiency, reducing subsequent maintenance energy requirements (MER). Studies have examined post-weight-loss MER in experimental models of canine obesity, but limited information is available from pet dogs. The aim of the current study was to estimate post-weight loss MER in obese pet dogs that had successfully lost weight and did not subsequently rebound.

Twenty-four obese dogs successfully completing a weight management programme at the Royal Canin Weight Management Clinic, University of Liverpool, were included. In all dogs, a period of >14d of stable weight (<1% change) was identified post-weight loss, when food intake was constant, and their activity levels stable (assessed via owners’ diary records). MER was, therefore, indirectly estimated by determining caloric energy consumption during this stable weight period, expressed as energy per kilogram metabolic body weight (MBW; Kcal·kg\(^{-0.75}\)). Multivariable linear regression was used to identify factors that were associated with MER after weight loss.

Median (range) weight loss was 24% (10-40%), rate of weight loss was 0.87%/wk (0.35-1.54%/wk), body fat loss was 32% (14-45%), and food intake during the weight loss phase was 63Kcal·kg\(^{-0.75}\) (44-86Kcal·kg\(^{-0.75}\)). The median length of stable weight was 54d (16-163d). During this time, median (range) MER was 65Kcal·kg\(^{-0.75}\) (52-104Kcal·kg\(^{-0.75}\)). Rate of prior weight loss and food intake during the weight loss phase were positively associated with post-weight-loss MER, whilst the amount of lean tissue lost was negatively associated with post-weight-loss MER.

Maintenance energy requirements are low after weight loss in obese pet dogs (typically only 10% more than required during weight loss MER), which has implications for what constitutes the optimal diet during this period. Preserving lean tissue during weight loss, may maximise post-weight loss MER, and help prevent rebound.

P5) DO FEEDING PRACTICES OF OBESE DOGS, PRIOR TO WEIGHT LOSS, AFFECT THE SUCCESS OF WEIGHT MANAGEMENT?

German, A., Holden, S., Gernon, L., Morris, P., Biourge, V.
1University of Liverpool, Wirral, UK; 2WALTHAM Centre for Pet Nutrition, Leicestershire, UK; 3Royal Canin Research Center, Aimargues, France

Not all obese dogs successfully complete a weight programme, but the reasons for this are unclear. Since dietary factors (e.g. feeding treats and table scraps) can predispose to canine obesity, owners’ feeding habits might also influence the likelihood of dogs successfully losing weight. This study sought to determine whether dietary factors were associated with a variety of outcome measures in a cohort of dogs undergoing conventional weight management.

Information from 95 dogs attending the Royal Canin Weight Management Clinic, University of Liverpool, was reviewed. The effect of different food types (e.g. dry, wet, home-prepared), feeding practices (e.g. method of weighing out food, number of meals per day), and use of treats was assessed on various outcome measures including: outcome of weight loss (reached target versus failed to reach target), rate of weight loss, percentage weight loss, mean energy allocation during weight loss, and the proportion of lean tissue lost.

Most owners (63/95, 66%) fed twice daily, most 72/95 (76%) used complete dry food, and most assigned food either by measuring cup (36/95, 38%) or visual estimation (37/95, 39%). Feeding treats was common and included purchased treats (51/95, 54%), table scraps (71/95, 75%), pet food (83/95, 87%) and human food (80/95, 94%). Types of human food included confectionary, dairy products, fruit, meat products and bread.

The majority of feeding practices had no significant effect on any outcome measure (P>0.05 for all). However, mean energy allocation during weight loss was significantly lower in dogs fed purchased snacks, compared with those who were not fed these snacks (55±5.1% vs. 61±5.5% of MER at target weight; P<0.0044).

The range of feeding habits, prior to weight loss, had limited effects on success of subsequent weight management. This suggests that good education can change owners' feeding habits, minimising the effect of undesirable practices, such as giving treats.
**P6) POST-PRANDIAL GLUCOSE AND INSULIN PROFILES FOLLOWING A GLUCOSE-LOADED MEAL IN CATS AND DOGS**

Hewson-Hughes, A., Gilham, M., Upton, S., Miller, A.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

Data from intravenous (iv) glucose tolerance tests suggest that glucose clearance from the blood is slower in cats than in dogs. Since different physiological pathways are activated following oral administration we investigated the profiles of plasma glucose and insulin in cats and dogs following ingestion of glucose.

Adult male and female cats and dogs (labradors and miniature schnauzers) were assigned to groups and fed either a high protein (HP; protein, fat and carbohydrate to energy ratios 68/26/6) test-meal (15g/kg bodyweight; n=10 cats, 11 dogs) or a HP+glucose test meal (13g/kg bodyweight HP diet + 2g/kg bodyweight D-glucose; n=7 cats, 13 dogs) following a 24h fast. Blood samples were taken before and 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360 and 1,440 minutes after the meal was offered. Data were analysed at the 5% significance level using mixed model analysis and Tukey’s honestly significant difference procedure.

Marked differences in the glucose and insulin profiles were seen in cats and dogs following ingestion of the glucose-loaded meal. In cats, plasma glucose concentration reached a peak at 120 minutes (mean and 95% CI, 10.2mmol/l; 9.7–10.8mmol/l) and returned to baseline by 270 minutes but no statistically significant change in plasma insulin concentration was seen. In dogs, plasma glucose concentration reached a peak at 60 minutes (6.3mmol/l; 5.9–6.7mmol/l) after the glucose-loaded meal and returned to baseline by 90 minutes. The plasma insulin concentration was significantly higher than pre-meal values from 30–120 minutes following the glucose-loaded meal in dogs.

The results of this study indicate that cats are not as efficient as dogs at rapidly decreasing high blood glucose levels. The profiles of glucose and insulin seen in the cat are consistent with a known metabolic adaptation of this species, namely a lack of the enzyme glucokinase which is important for both insulin secretion and glucose uptake from the blood.

**P7) THE EFFECT OF DIETARY CARBOHYDRATE LEVEL ON POST-PRANDIAL GLUCOSE AND INSULIN CONCENTRATION IN CATS AND DOGS**

Hewson-Hughes, A., Gilham, M., Upton, S., Miller, A.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

A charge made against feeding dry foods to cats is that the high carbohydrate content results in high blood glucose levels which over time may have detrimental health effects. This study determined the post-meal concentrations of plasma glucose and insulin in lean cats and dogs fed dry diets with low (LC), medium (MC) or high (HC) carbohydrate levels.

In a crossover design with 7 days between test-diets, plasma glucose and insulin concentrations were measured in male and female adult cats (n=11) and dogs (Labradors, n=9) before and after LC, MC and HC meals (50kcal/kg bodyweight). The protein, fat and carbohydrate to energy ratios of the diets were: 50/41/9 (LC); 36/34/30 (MC); 30/28/42 (HC). Responses were analysed using GLM with 95% LSD intervals used to determine significant differences between diets and time-points.

Only the HC diet resulted in significant post-meal increases in plasma glucose concentration in cats and dogs although the time-course profiles were different between species. In cats, plasma glucose concentration was not significantly increased above the pre-meal concentration (mean±95% CI, 4.4±0.2mmol/l) until 11h after the meal (5.3±0.2mmol/l) and remained elevated until 19h after eating (5.7±0.2mmol/l; the last time-point studied) whilst in dogs a significant increase above baseline (4.9±0.2mmol/l) was seen only at the 7th time-point (5.5±0.2mmol/l). Plasma insulin was significantly elevated in dogs 4-8h following the MC diet and 1-8h after the HC diet and from 3-7h and 11-17h after the HC diet in cats.

It has not been possible to determine the maximal increase or the time for plasma glucose to return to baseline levels in cats following intake of a HC diet. The time-lag (~11h) between eating the HC diet and the subsequent prolonged elevation of plasma glucose concentration seen in cats may reflect metabolic adaptations that result in a slower digestive and absorptive capacity for carbohydrate.
High dietary protein is considered to result in increased urinary oxalate concentrations, which could encourage urolith formation in high-risk groups. However, other studies have shown that higher dietary protein levels seemed to decrease the risk of calcium oxalate formation. The objective of this study was to establish the effects of dietary protein on urine composition in cats.

Eight cats (4 female + 4 male; ø age: 15 months) were included in this study, receiving the same experimental diets in three feeding periods (A-C). In the first feeding period (A), diet contained 32% crude protein (CP) as fed basis, followed by 41% CP (B) and 55% CP (C). After a three-weekly adaptation, cats were housed in metabolic cages to collect urine and feces for seven days. Urinary oxalate, sodium, calcium, phosphate, magnesium and potassium were analyzed using ion-exchange chromatography, and urinary pH was measured with a pH meter. Feed intake was documented per day. For correlation analysis and multifactorial variance analysis, SPSS17 was used.

Protein intake had only minor impact on urine composition, but increased urinary calcium concentrations (Table 1). PH (A: 6.66 ± 0.25a; B: 6.34 ± 0.11b; C: 6.61 ± 0.07a) was different between groups, but no correlation could be demonstrated (correlation coefficient dietary CP: urinary pH = 0.19 (p = 0.345)).

Dietary protein increased urinary calcium indicating that high dietary protein might be a risk factor for calcium oxalate formation within high-risk groups.
A FIELD STUDY ON THE BODY WEIGHT OF CATS IN RELATION TO
BREED, SEX AND BODY CONDITION

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Chair of Animal Nutrition and Diетetics, Munich, Germany

Data on body weight of cats are mostly data on domestic European shorthaired cats without a
pedigree, often lacking information on the body condition of the cats. Feeding instructions on cat
food packages often refer to a normal 4 kg cat. With regard to the incidence of obesity in cats, and
the specific problems of weight reduction in cats, prevention is highly important. One kg of extra
weight in a cat may represent an overweight pet which already has a potential health risk. To alert
veterinarians and owners to the onset and early stages of obesity, we collected data on the rela-
tionship between breed, sex, body condition scores and body weight in cats. 539 pure bred and
75 cats without a pedigree were weighed and scored (BCS by Laflamme 1997, 1 to 9 scale) at cat
shows or in veterinary surgeries. 192 were intact males, 247 intact females, 109 neutered males
and 66 neutered females. Data from cats with a BCS other than 5 (ideal) were not used for statis-
tics on normal body weight. Breeds were grouped by Bartlett’s test. To identify effects of breed
and sex a two-way-ANOVA was carried out.

Five groups of cats (table 1) were identified. Some breeds had a large variation and were repre-
sented in several groups, other breeds had only a rather narrow range. Sexual dimorphism with
larger males was more pronounced in large and very large breeds than in smaller breeds. In Maine
Coons lean intact males were significantly heavier than lean neutered males.

In intact cats the incidence of obesity was below 1%, in neutered cats it was nearly 50%. In pure
bred neutered cats the incidence of obesity was higher in large and very large breeds and lower
in small and very small breeds. Cats with a BCS of 6 had on average 120% of the normal weight
of their breed, cats with a BCS of 7 154% and with a BCS of 8 of 214%.

P10) WHOLE BODY AMINO ACID COMPOSITION OF ADULT FANCY RANCHU
GOLDFISH Carassius auratus

Snellgrove, D., Alexander, L.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

Aqua feeds should be formulated to provide complete and balanced nutrition to achieve optimal
health and growth in fish, including adequate levels of essential amino acids (EAA). There is little
or no data relating to the EAA requirements for ornamental fish species, with the majority of quan-
titative data for these nutrients being available for commercially farmed fish.

The determination of EAA requirements is usually established through dose-response studies, which
can be costly and time consuming especially if calculating the requirement for many amino
acids. An alternative method for predicting the EAA of fish, that is also relatively fast and inexpen-
sive, is the assessment of the whole body amino acid composition (Ogino, 1980).

Eight goldfish with mean wet weight 34.2g ± 1.4g were obtained as a result of a routine cull by
breeders. Fish were freeze-dried and amino acid content analysed by hydrolysis or performic oxi-
dation (Table 1).

These findings are in agreement with those of Gatlin (1987) for juve-
nile common goldfish, suggesting that there are no differences in the
whole body amino acid composition between juvenile and adult, or fancy
and common goldfish. However, these indices do not provide a quanti-
tative total amount of each amino acid required by the fish, but can be
used proportionally to provide guidelines to formulate diets for
ornamental species.

Table 1. Essential amino acid composition of whole body
tissue of adult ranchu goldfish (n=8 ± SEM)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ranchu Goldfish (g/100g amino acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>6.57 ± 0.10</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.53 ± 0.04</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.06 ± 0.10</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.52 ± 0.18</td>
</tr>
<tr>
<td>Lysine</td>
<td>7.90 ± 0.14</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.80 ± 0.06</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.34 ± 0.08</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.51 ± 0.10</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>Valine</td>
<td>4.55 ± 0.08</td>
</tr>
</tbody>
</table>

Table 1. Body weight of cats with a BCS of 5

<table>
<thead>
<tr>
<th>Sex</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>3.0±0.6</td>
</tr>
<tr>
<td>Female</td>
<td>3.2±0.6</td>
</tr>
</tbody>
</table>

P12) AGE RELATED IMMUNE CHANGES IN DOGS UNDER HOME AND COMMERCIAL PET FOOD AND DEVELOPMENT OF JUVENILE ONSET DEMODICOSIS
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Pathogenesis of canine demodicosis is poorly understood. Immunity, nutrition and genetics play roles in the development of the disease. The aim of this study was to evaluate the age related immune changes in young dogs fed with home and commercial foods and to assess the influence of age related immune changes in the development of Juvenile Onset Demodicosis (JOD). This 3 year study comprised of a clinical surveillance in 3970 dogs, among which 1648 cases of JOD were recorded.

Healthy and demodectic dogs were grouped (n=6 in each) based on diet into home based and commercial diet based (Pedigree, Mars India International) and based on age into 1-6 months old, 7-12 months old and 13-18 months old. Total and differential leukocyte counts, lymphocyte subset - CD4 and CD8 counts were assessed. Statistical analysis was done with 3 way ANOVA, using LSMLMW–MIXMDL (1.0).

Incidence of JOD was high (45%) in dogs of age 7-12 months, followed by equal incidence in pups aged up to 6 months (27%) and in dogs of 12-18 months (28%). Among different feeding groups, higher incidence was observed in home fed dogs (67% in pups aged up to 6 months, 59% in 7-12 months age and 62% in 12-18 months age) and lesser incidence in dogs fed commercial food.

Reduction in CD4 count was observed in all three age groups and the reduction was more pronounced in dogs of 7-12 months age. This CD4 reduction could possibly be the cause for the higher incidence (45%) of JOD in 7-12 months age. Least squares means comparison among dogs under home and commercial foods revealed a mildly significant (P< 0.10) increase in CD3 count and a highly significant decrease (P<0.01) in CD8 count in dogs under commercial food, indicating a better immune protection offered by commercial pet food.

Funding: WALTHAM Foundation

P13) INCREASED ANTIOXIDANT CAPACITY IN PLASMA OF DOGS AFTER A SINGLE ORAL DOSAGE OF TOCOTRIENOLS
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1University of Potsdam, Potsdam, Germany; 2University of Hamburg, Hamburg, Germany; 3University of Leipzig, Leipzig, Germany

Tocotrienols (TCTs), a group of vitamin E stereoisomers, represent lipid-soluble antioxidants with a higher efficacy against oxidative damage than that of α-tocopherol (α-TCP). Because the metabolism of TCTs in dogs has not been investigated yet, the objective of this study was to evaluate the intestinal uptake of dietary TCTs and their effect on the plasma antioxidant capacity in dogs.

Eight clinically healthy Beagle dogs (4 male/4 female; 13.5 ± 1.4 kg body weight, BW; mean ± SD) were given a single oral dosage of a Gold Tri vitamin E mixture (Golden Hope, Malaysia; 40 mg/kg BW) containing 32% α-TCT, 29% β-TCT, 14% δ-TCT and 25% α-tocopherol (α-TCP) together with 5 mL cream (30 percent fat). Blood was sampled at time 0 (for baseline measurements), 1, 2, 3, 4, 5, 6, 8, and 12 h after the administration. Chylomicrons were isolated from plasma by preparative ultracentrifugation at density less than 1.006 g/mL. Concentrations of TCTs and α-TCP were determined by HPLC. The antioxidant capacity was measured by Trolox equivalent antioxidant capacity (TEAC) assay. The data were analysed using the general linear model (GLM) procedure for repeated measurement design.

In fasted dogs, levels of TCBs were 0.07 ± 0.03 µmol/L and were detected only in plasma of 3 out of 8 dogs. As a consequence of the administration of the vitamin E mixture, plasma levels of TCBs peaked 2 h after dosing (7.16 ± 3.88 µmol/L; P< 0.01). α-TCT (57 ± 12%) was the predominant form of TCTs followed by β- and γ-TCT (36 ± 13%) and δ-TCT (6.2 ± 3.8%). 12 h after dosing, plasma TCT concentrations were 0.67 ± 0.44 µmol/L and did not return to baseline levels. The chylomicron TCT response parallels the increase of TCTs in plasma with a maximum at 2 h (3.49 ± 2.06 µmol/L; P< 0.01) after dosing. The TEAC values increased from baseline (71 ± 7.79 µmol/L) to a maximum at 12 h (113 ± 7.72 µmol/L; P< 0.01) after TCT supplementation. The results demonstrate for the first time that dogs are able to absorb α, β, γ and δ-TCTs from the gut and incorporate them in the chylomicrons. The increase in plasma antioxidant capacity after TCT intake suggests the beneficial role of TCTs in the prevention or treatment of several diseases in dogs. The discrepancy in the appearance of maximal TCT increment and TEAC plasma concentrations should be elucidated in further studies.
P14) INCREASING BODY WEIGHT DOES NOT AFFECT CELLULAR IMMUNITY BUT ALTERS FAT OXIDATION AND OXIDATIVE STRESS IN DOGS

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Overweight and obesity can cause several obesity-related disorders in dogs but also alter immunity in humans and rodents. The aim of this study was to investigate if increasing body weight alters immunological parameters in adult dogs.

Sixteen adult healthy Beagles were equally divided into a control group (CG) and weight gain group (WGG). All dogs were fed a high energy diet during 47 weeks and received 1.1 and 1.4 x MER respectively for CG and WGG. To evaluate body composition, deuterium was injected and blood samples were taken at week 0, 20, 35 and 47. Blood samples were also taken at week 0, 4, 9, 16, 24, 31, 40 and 47 for measuring proliferation of peripheral blood mononuclear cells (PBMC), subtypes of lymphocytes and phagocytosis. Serum Thiobarbituric Acid Reactive Substances (TBARS), Ferric Reducing Antioxidant Power (FRAP) and ceruloplasmin were measured as markers for respectively fat peroxidation and anti-oxidative status.

Body weight and BCS were significantly elevated in the WGG. Body composition already significantly changed after 20 weeks, as both fat free mass (FFM) and fat mass (FM) increased in the WGG (P<0.05). However, no effect was seen for proliferation of PBMC, subtypes of lymphocytes and phagocytosis (results available until week 31). On the other hand a significant time x treatment interaction was noted for TBARS, FRAP and ceruloplasmin. However, the direction of the alteration depended on the time point.

No alteration in non specific cellular immunity were observed over the first 31 weeks. Over that period some oxidative stress was measured although not repeatedly. Since chronic obesity was not reached and data were not fully available yet, it is possible that alterations in immune parameters may occur later on in the process of the disease.

P15) A FIELD STUDY ON FEEDING OF DOGS AND CATS WITH AND WITHOUT SUSPECTED FOOD ALLERGY IN GERMANY

Kienzle, E., Becker, N., Dillitzer, N., Muller, R., Sauter-Louis, C. Chair of Animal Nutrition, Oberschleißheim, Germany

The aim of two separate surveys was to collect data on feeding of dogs and cats with and without suspected food allergy. Owners of 160 dogs and 18 cats with suspected food allergy (typical dermatologic and/or gastrointestinal symptoms and significant improvement after the elimination diet – may include atopic disease) were interviewed about the feeding including treats and supplements. Furthermore, owners of 865 dogs and 243 cats from all over Germany were interviewed about their feeding practice and the use of treats and supplements. Golden retrievers, West Highland white terriers, German shepherds, boxers and white shepherds were over-represented in the allergy group. In dogs first clinical signs typically occurred during the first year of life (35%) or in young adulthood (1-3 years, 36%). In cats respective peaks were 1-3 years (47%) and 4-6 years of age (24%). Home made elimination diets were more popular in dogs than in cats with suspected food allergy (39% vs. 6%).

However, significantly more owners of afflicted dogs and cats used home made diets compared to those without suspected food allergy (7.5% vs. <1%). Both surveys revealed a high percentage of dog and cat owners using prepared diets exclusively (dogs 36% with and 58% without, cats 50% with and 90% without suspected food allergy). The majority of owners used additional treats, with significantly more frequent use in dogs and cats without suspected food allergy. Furthermore, dog owners used more often supplements than cat owners (results of both studies). There was a discrepancy between answers to questions with different phrasing but same content, indicating effects of wording and interview technique, respectively.
P17) FURTHER DIETARY RESTRICTION IS REQUIRED TO ACHIEVE WEIGHT LOSS IN RESISTANT HORSES

Curtis, G.1, Barfoot, C.2, Dugdale, A.1, Harris, P.3, Grove-White, D.1, Argo, C.1

1University of Liverpool, Cheshire, UK; 2Mars Horsecare U.K. Ltd, Buckinghamshire, UK; 3Equine Studies Group, WALTHAM Centre for Pet Nutrition, Leicestershire, UK

Obesity, a significant risk factor for the development of debilitating disease, is common among domestic ponies. Controlled nutrition to promote weight loss is essential for rehabilitation but evidence-based advice is sparse.

Rates of body mass (BM) and body condition score (BCS, 1=emaciated, 9=obese) loss were determined for 12 owner-offered overweight/obese horses and ponies (BCS 8.2±0.57), randomly-assigned to one of two diets (hay and a chaff-based complete feed, HC, n=6 or hay with a balancer meal, HB, n=6), offered at 1.25% of BM as daily DM intake (DMI) for 16 weeks. BM, BCS, belly girth (BG) and ultrasound measures of retroperitoneal and subcutaneous fat depths were measured weekly. Despite a common level of restriction, rates of BM (0.16 to 0.79%/week) and BCS (0.01 to 0.16 points/week) loss were normally-distributed between-individuals and independent of diet type, outset values for BCS, BMI, blood glucose/insulin concentrations, breed, gender or body fat content derived following D2O dilution. Animals at the lower-extreme of the weight-loss spectrum were categorised ‘weight loss resistant’ (WLR). Four, WLR animals (HC, n=2; HB, n=2) were monitored for a further 4 weeks during which DMI was reduced to 1.0% of BM as daily DMI. Animals remained healthy. No stereotypic behaviours were seen. Increasing dietary restriction from 1.25 to 1.0% of BM as daily DMI doubled the rate of weight loss from 0.26 ±0.03% to 0.45 ±0.05% weekly, a rate comparable to that of ‘weight loss sensitive’ animals, at the other extreme of the range described for the 12 animal during restriction to 1.25%. Genetic diversity may account for individual differences in sensitivity to weight loss. For practical purposes, initial restriction of food intake to 1.25% can be increased to 1% of BM as DMI after 6 weeks if WLR is identified. Weight loss was most usefully monitored as changes in BG.
P18) HOME-PREPARED DIETS AND THE IMPACT ON ORAL HEALTH IN CATS AND DOGS

Buckley, C.1, Acolyer, C.1, Skrzywanska, M.2, Jodkowski, K.3, Kurski, G.4, Gawor, G.5, Cereczyn, M.2

1WALTHAM Centre for Pet Nutrition, Leicestershire, UK; 2Mars Petcare, Sochaczew, Poland; 3Warsaw Agricultural University, Warsaw, Poland; 4Veterinary Clinic ELWET, Warsaw, Poland; 5Veterinary Clinic ARKA, Krakow, Poland

Many factors influence the oral health status of cats and dogs. This study aimed to elucidate the relationship between feeding HP versus commercial petfood and oral health parameters in these animals. The study, run in conjunction with the Polish Small Animal Veterinary Association (PSAVA), surveyed 17184 dogs and 6371 cats visiting more than 700 veterinary surgeries in Poland in 2006-2007. Examinations of the conscious animals visually assessed dental deposits (0=clean, 1=plaque, 2=tartar on several teeth, 3=extensive tartar), size of mandibular lymph nodes (0=normal, 1=enlarged, 2=markedly enlarged) and gingival health (0=healthy, 2=gingivitis, 3=periodontitis). Information was collected on age and diet (HP=home-prepared solus, HP mix=home-prepared plus wet and dry commercial, dry=dry commercial petfood solus, wet=wet commercial petfood solus, C mix=dry and wet mixture of commercial petfood). An oral health index (OHI) ranging from 0-8 was calculated for each animal by combining examination scores (0=good oral health, 1-2=consultation required, 3-5=problem requiring minor treatment and 6-8=problem requiring intensive treatment).

The mean OHI increased with age in both cats and dogs. Following adjustment for age, ANOVA analysis showed a significant effect of diet on OHI (p<0.001). The mean OHI was significantly reduced in cats fed HP mix (2.97±0.073) and those fed C mix (2.68±0.064) compared to cats fed HP (3.65±0.11). In dogs, C mix feeding resulted in a significantly reduced OHI (2.75±0.044) compared to those fed HP (3.40±0.045) and those fed HP mix (3.00±0.034). There was only a significant effect of feeding commercial petfood solus compared with HP diet when there was an element of dry format present. In the groups of cats and dogs fed commercial petfood solus, introducing an element of dry format significantly improved OHI (p<0.001).

Feeding dry diet solus to cats and dogs results in the greatest benefit to oral health, while feeding home-prepared diet appears to be the least beneficial.

P19) THE EFFECT OF DIETARY WATER ON URINE PARAMETERS IN THE CAT

Buckley, C., Hawthorne, A., Colyer, A., Stevenson, A.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

In humans and dogs, it has been reported that daily fluid intake influences urinary dilution, and consequently the risk of urolithiasis. The current study aimed to investigate the role of dietary moisture on urine parameters and total daily fluid intake in healthy adult cats by comparing nutritionally standardised diets, varying only in moisture content.

Six cats were fed a complete dry food (6.3% moisture) hydrated to 25.4%, 53.2% and 73.3% moisture for 3 weeks in a randomised block crossover design. Cats received 45 kcal kg⁻¹ bodyweight per day in each phase, divided into three meals. Urinary specific gravity (SG), urine volume, water drunk and total fluid intake (water drunk plus water ingested with food) were measured daily; relative supersaturation (RSS) for calcium oxalate (CaOx) and struvite was calculated using the SUPERSAT computer programme.

Cats fed the 73.3% moisture diet produced urine with a significantly lower SG (p<0.001) compared to diets containing 53.2% moisture or lower. Mean RSS for CaOx was approaching undersaturated (1.14 ± 0.21; p=0.001) for cats fed the diet with 73.3% moisture and significantly lower than the 6.3% moisture diet (CaOx RSS = 2.29 ± 0.21). The effect of diet on struvite RSS was less clear, with no significant difference between treatment groups. Total fluid intake was significantly increased (p<0.001) in the 73.3% moisture diet (144.7 ± 5.2 ml, or 30 ml kg⁻¹ day⁻¹) compared with the 6.3% (103.4 ± 5.3 ml), 25.4% (98.6 ± 5.3 ml) and 53.3% (104.7 ± 5.3 ml) moisture diets despite voluntary water intake decreasing as dietary moisture intake increased.

Cats fed the 73.3% moisture diet had a higher total daily fluid intake resulting in a more dilute urine with a lower risk of CaOx when compared with the lower moisture diets.
P21) EFFECT OF DIETARY SUPPLEMENTATION WITH VITAMIN E AND/OR SELENIUM ON THE FELINE IMMUNE SYSTEM
O'Brien, T., Thomas, D., Morel, P., Rutherford-Markwick, K.
Massey University, Palmerston North, New Zealand

Supplementary vitamin E (VitE) and selenium (Se) given individually or in combination, has been reported to enhance immune function in several species. From the limited studies in cats, VitE only has immune enhancing effects in geriatric cats, while cats of all ages have been shown to tolerate higher levels of dietary Se than other species.

This study aimed to determine the effects of supplemental VitE and Se, individually and in combination, on selected parameters of the immune system of the cat. Nine diets were fed in a 3x3 factorial design with no; moderate (MOD); or high (HI) levels of VitE (0, 250 or 500IU/kg DM diet) and/or Se (0, 2 or 10mg/kg DM diet) supplementation. Seventy-two cats (1.5-10 yrs, 2.3-6 kg) were fed 1 of the 9 diets (n=8) ad libitum for a period of 4 weeks, with immune parameters measured at 0, 2 and 4 weeks of feeding.

After four weeks, significant enhancement of lymphocyte proliferative responses (P<0.05) to Concanavalin A and phytohemagglutinin were observed in cats consuming diets containing supplemental VitE (MOD and HI levels), irrespective of Se content. No effect was observed in cats fed diets supplemented with Se alone. Cats in groups supplemented with MODVitE, HIVitE, MODVitE+MODSe, HIVitE+MODSe and HIVitE+HISe showed significant (P<0.001) enhancement of phagocytic activity compared to control animals. These findings differ from those in other species, and may indicate metabolic differences between species.

Se supplementation alone and Se combined with VitE either had no effect on immune function in the cat, or had no additional immune enhancing effects above that seen with VitE alone. Beneficial effects may be seen at a supplemental level of 2500U/kg DM diet VitE. Higher VitE levels appear unlikely to offer additional immune enhancing effects and would add unnecessarily to the manufacturing cost of the diets.

P20) DEPLETION OF PLASMA ARACHIDONATE AND TOTAL PROTEIN OCCURS IN CATS FED A PEROXIDIZED DIET BUT REPRODUCTIVE TISSUES RESPOND MORE SLOWLY
Chamberlin, A., Mitsushashi, Y., Bigley, K., Bauer, J.
Texas A&M University, College Station, TX, US

Because highly unsaturated fats are often included in feline diets, diet peroxidation during storage is of concern. In this study, the effect of an elevated peroxide value (PV) diet on fatty acid (FA) profiles of plasma phospholipid (PL) and reproductive tissues of adult female cats was evaluated. Cats had been maintained on one of three dry extruded diets formulated to be complete and balanced according to AAFCO nutrient profiles. The diets were fed for (n = 9/group) for 300 days prior to spaying (n = 9/group). They were similar; adequate arachidonic acid (AA ~0.35 g/kg); antioxidant content; stored at ambient temperature; but differed in FA composition. Diets were designated; high linoleic acid (diet A), high γ-linolenic acid (diet B), and adequate linoleic acid (diet C).

Diet samples obtained the week before spaying revealed a markedly increased PV of diet A vs. diets B and C (135 vs. 5.80 and 2.12 Meq/kg fat, respectively). Feeding records revealed decreased daily food consumption of cats fed diet A after 240 days but without weight loss; thus an opportunity presented to investigate whether storage of the diets over time might be involved. Total plasma protein and PL-AA concentrations in group A were significantly decreased at 140 and 300 days. Total PL-FA profiles of reproductive tissues collected at surgery revealed only modest decrements of AA in both uterine and ovarian tissues. The AA content in diet A was below minimum standards at 0.015% (minimum for cats: 0.02%) and likely due to oxidation. The precise time at which diet A became unacceptable during feeding is unknown. However, it likely occurred between 60 and 140 days because plasma PL-AA was within our normal colony range (~4-7 relative %) after 56 days of feeding this diet. The possibility exists that high linoleic acid containing diets are more likely to be oxidized requiring more antioxidants. The findings suggest that reduced plasma protein and AA concentrations may serve as biomarkers of diet peroxidation in cats prior to feed refusal, weight loss, or tissue depletion.
P22) MAINTENANCE ENERGY REQUIREMENT DETERMINATION OF CATS AFTER SPAYING
Mitsuhashi, Y., Chamberlin, A., Bigley, K., Bauer, J.
Texas A&M University, College Station, TX, US

Spaying often results in obesity due to decreased energy expenditure. However, the recommendation of a maintenance energy requirement (MER) for these animals has not been clearly defined. This study investigated the MER for spayed cats whose body weights (BW) began to increase shortly after surgery. Twenty-two short-hair adult female cats had been fed complete and balanced diets in amounts to maintain their BW and BCS prior to the study. All cats were then spayed and the diet was fed once daily for 11 weeks using the same MER as previously. During these weeks, all cats gained weight. Beginning on wk 12, a weight loss regimen was initiated until each cat achieved a BCS of 5-9. Cats were fed approximately 65%BW0.67 to achieve a 1-2 % body weight loss per week during this period. After each cat obtained a BCS of 5, an appropriate amount of diet was fed to maintain its BW for at least 4 weeks to determine a new MER. Daily food consumption, weekly BW, and BCS were monitored. Blood was collected before and after weight loss for plasma biochemistry profiles.

BW and BCS increased by 16% and 1 point (p<0.01) respectively during the first 11 weeks after surgery even though food consumption was constant both pre- and post-surgery. During the weight loss regimen, all plasma biochemistry profiles were within normal range. The MER for each cat after obtaining a BCS of 5 was 75.0±5.6 kcal/BW0.67, which is 25% lower than the current National Research Council recommendation and lower than cats before surgery (p<0.05). In conclusion, spaying significantly increased body weight when using MER values for intact cats. Thus, 75×ideal BW0.67 is proposed for the MER of spayed cats.

P23) DEVELOPING METHODOLOGY/APPARATUS TO ASSESS THE EFFECTS OF DIET ON EXERCISE PHYSIOLOGY AND PERFORMANCE IN WORKING DOGS
Rutherfurd, S., O’Flaherty, K.
Massey University, Palmerston, New Zealand

Based on the literature and our previous research, we determined that a sensitive measure for use in canine exercise physiology studies needed to be developed. Such a measure would allow the evaluation of subtle performance differences in response to dietary change. To date many canine exercise studies have been conducted measuring a plethora of physiological and biochemical markers, with limited success. There is a dearth of published information regarding indirect calorimetry use in dogs and no readily available calorimetry equipment for dogs. This study was designed to develop apparatus, indirect calorimetry techniques and protocols to use in these types of studies. It involved the use of four male and six female adult Harrier Hounds (21-29 kg BW).

The dogs were run on treadmills and indirect calorimetry was used to determine which fuels dogs utilised primarily during sub-maximal exercise tests, and whether these changed when dogs were fed diets with different dietary macronutrient proportions. The equipment for this study was designed and engineered at the university. A custom-made mask worn by the dogs was connected to a Douglas bag and samples were collected from this. The respiratory exchange ratio (RER) was then determined indicating the proportion of each fuel utilised during the test. None of the dogs had been taught to exercise on the treadmill while wearing a mask. The dogs were gradually trained to accept the masks, building them up to the speed required during testing. By the first testing day all dogs were accustomed to the masks and could retain their speed for the full sampling period (40 minutes) while serial breath samples were obtained.

The protocol and apparatus designed and utilised during this study proved robust and highly effective, produced a marker that clearly highlighted dietary differences and appeared suitable for use in future studies.
P25) DIETARY FLAXSEED OIL SUPPLEMENTATION IN TWO DOG BREEDS

Purushothaman, D., Brown, W., Wu, S., Vanselow, B.
University of New England, Armidale, NSW, Australia

A nutritional trial aimed at flaxseed oil supplementation was performed to support a genetic project that is analyzing the impact of essential fatty acids on gene expression in leucocytes. This preliminary study analyzed plasma fatty acid composition (%) following oil supplementation over time and measured the effect of breed.

Plasma was extracted at fasting state from five beagles and five greyhounds, which were fed MELROSE® flaxseed oil (52-62% 18:3(n-3) and 17% 18:2(n-6)) at the rate of 2ml/kg BW. Statistical analysis used Fully Nested ANOVA.

Plasma 18:3(n-3), 20:5(n-3) and 18:2(n-6) increased steadily and significantly from Day 0 to Day 22, however no significant breed differences were shown. Plasma 22:6(n-3) level on the other hand showed no significant time difference but a significant breed difference was observed before supplementation, with beagles having a higher plasma level at Day 0. This requires further investigation as both the breeds were fed identical diets for four months prior to the start of the study.

20:4(n-6) in the plasma showed no significant breed/time differences.

These findings suggest that flaxseed oil could be a useful source of essential fatty acids in dogs, especially 18:3(n-3) and 20:5(n-6), and that breed differences may be important.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Day</th>
<th>Ca intake mg/d</th>
<th>ANOVA P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beagles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=5</td>
<td>18(3-5)</td>
<td>0.36±0.07</td>
<td>4.12±1.41</td>
</tr>
<tr>
<td></td>
<td>20(5-3)</td>
<td>0.63±0.16</td>
<td>2.48±0.29</td>
</tr>
<tr>
<td></td>
<td>22(6-5)</td>
<td>3.94±0.51</td>
<td>3.64±0.59</td>
</tr>
<tr>
<td></td>
<td>18(2-6)</td>
<td>24.98±2.10</td>
<td>28.89±4.91</td>
</tr>
<tr>
<td></td>
<td>20(4-6)</td>
<td>19.88±2.25</td>
<td>18.05±1.47</td>
</tr>
<tr>
<td>Greyhounds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=5</td>
<td>18(3-5)</td>
<td>0.33±0.04</td>
<td>5.00±1.00</td>
</tr>
<tr>
<td></td>
<td>20(5-3)</td>
<td>0.67±0.11</td>
<td>3.54±1.11</td>
</tr>
<tr>
<td></td>
<td>22(6-5)</td>
<td>2.97±0.44</td>
<td>2.61±0.42</td>
</tr>
<tr>
<td></td>
<td>18(2-6)</td>
<td>26.24±0.85</td>
<td>28.95±2.72</td>
</tr>
<tr>
<td></td>
<td>20(4-6)</td>
<td>18.33±1.58</td>
<td>15.66±2.41</td>
</tr>
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</table>

The WALTHAM International Nutritional Sciences Symposium 2010
P27) ENDOGENOUS SEASONAL CONSTRAINTS ON APPETITE ARE INSUFFICIENT TO PREVENT THE ATTAINMENT OF OBESITY IN PONIES
Dugdale, A.1, Curtis, G.1, Cripps, P.1, Harris, P.2, Argo, C.1
1University of Liverpool, Chester, Wirral, UK; 2Equine Studies Group, WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK
Seasonally-adapted Native pony breeds commonly become obese when translocated to domestic management with year-round provision of high quality foodstuffs.

Eleven mature Welsh Mountain pony mares were offered ad libitum access to a complete diet of comparable quality to a moderate hay (DE, 8.45MJ/kgDM; DM, 88.06%) for 12 weeks during summer (n=6; BM, 246±20kg) and winter (n=5; BM, 219±21kg) to monitor their appetite, body mass (BM), body condition score (BCS; 1 [emaciated] to 9 [obese]), morphometric measurements and direct (ultrasonographic) and indirect (D₂O dilution technique) measures of body fat. Each group comprised 2 animals of thin (BCS1-3), moderate (BCS 4-6) and overweight (BCS 7-9) condition at outset. Variables recorded for each pony were daily BM (±1kg), daily dry matter intakes (DMI, ±10g) and weekly BCS, morphometric and ultrasonographic measures. Body fat contents, derived from D₂O dilution measures of total body water, were determined at the beginning and end of each study.

Summer ponies of non-obese outset BCS increased their BM most rapidly (non-ObS: average daily gain (ADG), 0.8±0.1kg/day; n=4) compared to winter (non-ObW: ADG; 0.6±0.0kg/day; n=3). Obese animals (Ob; n=4) maintained constant BM regardless of season. Maximal appetites were 4.6 ±0.3% of BM as DMI /day (non-ObS) and 3.5 ±0.1% BM as DMI/day (non-ObW) during the second month of each trial. Appetites for Ob ponies were not different between seasons and even when maximal, were only half those of non-ObS animals (2.3±0.2% of BM/day). Subjective and objective measures of body ‘fatness’ increased for all ponies which were non-obese at outset. An exponential relationship between body fat content and BCS was determined but BCSS >6 were more insensitive determinants of body fat (see figure).

Endogenous seasonal suppression of appetite was insufficient to prevent the development of obesity under domestic conditions. Obese ponies had relatively restricted appetites (~2% BM as DMI).

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P26) THE EFFECT OF B-VITAMIN SUPPLEMENTATION ON FOOD RESPONSES IN HEALTHY DOGS
Sriphavatsarakom, P., Brown, W
University of New England, Armidale, NSW, Australia

B-vitamins are sometimes used in a clinical setting to stimulate appetite in sick animals. The aim of this study was to investigate whether B-vitamin supplementation could influence behavioural and olfactory responses to food in healthy dogs. To test this theory, twelve dogs of mixed breeds received a daily oral supplementation of vitamins B1, B6, and B12 (17mg, 7mg, and 35 mcg per kg BW, respectively) for 6 days in a 2-period crossover design. Food response was tested using a two-bowl preference test comparing an empty food bowl with one containing 1g of tinned food (Trial A) or 1g of tinned food + 200mg activated charcoal (Trial B). All bowls were identical in appearance, and covered with a thin layer of gauze to remove the possibility of visual discrimination, and bowls containing the food target were randomly allocated to the left or right position. Time spent at the food-containing bowl, and a five-point food response score (from 0= no interest to 5= active response) were analysed by Wilcoxon sign-rank test. First approach analysis was by Chi-Square. Feeding rate comparisons were made using repeated measures ANOVA. There was no effect of B-vitamin supplementation on any of the criteria measured in this study (p<0.05).

RESULTS. Values shown are means +/- standard deviation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Supplemented</th>
<th>Non-Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating Rate (g/sec)</td>
<td>3.6 ± 1.8</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>Time at food bowl (sec)</td>
<td>11.9 ± 16</td>
<td>2.7 ± 3.8</td>
</tr>
<tr>
<td>Food Response Score</td>
<td>1.5 ± 1.2</td>
<td>0.7 ± 0.6</td>
</tr>
<tr>
<td>First Approach (%)</td>
<td>55</td>
<td>45</td>
</tr>
</tbody>
</table>

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83
P29) EFFECT OF ZINC, LINOLEIC ACID AND B VITAMIN SUPPLEMENTATION ON COAT QUALITY OF COCKER SPANIELS

Wallis, C., Pelletier, L., Stendhurst, N., Allsopp, J., Barradas, C.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

Previous studies have shown that diets supplemented with zinc (100mg/1000kcal) and linoleic acid (15g/1000kcal) significantly improve coat gloss and reduce dandruff of Labrador retrievers and diets supplemented with B vitamins enhance coat softness. In addition, diets containing increased total dietary fat have been shown to significantly improve coat glossiness and softness of beagles and hound type mixed breeds. The objective of this study was to investigate the effects of supplementing a complete and balanced diet with zinc, linoleic acid and B vitamins on coat gloss and coat silkiness of cocker spaniels.

Adult cocker spaniels were fed Chappie® complete original for eight weeks (phase 1) and then divided into two groups and either maintained on Chappie® complete original or fed Chappie® complete original supplemented with linoleic acid (10g/1000kcal), zinc (62.5mg/1000kcal), vitamin B2 (14.8mg/1000kcal), vitamin B6 (14.5mg/1000kcal), vitamin B5 (38.5mg/1000kcal), niacin (297mg/1000kcal) and biotin (2.5mg/1000kcal) for a further eight weeks (phase 2). Dogs were evaluated in weeks 0, 2, 4, 6 and 8 of phase 2 by a trained sensory panel. Significant improvements in coat gloss (P=0.001) and two of the three aspects of coat silkiness, weighted movement (P=0.012) and coat gloss (P=0.0435), were observed. Silkiness is proposed as a new complex composite attribute that combines gloss, smoothness and weighted movement.

This is the first study to show that dietary supplementation with zinc, linoleic acid and B vitamins significantly increases coat gloss and enhances elements of coat silkiness of cocker spaniels. In addition, it demonstrates that zinc and linoleic acid at levels lower than previously reported are efficacious at improving coat quality.

Table 1: Effects of Ca and P supply and energy requirements in puppies of 2 different breeds

<table>
<thead>
<tr>
<th>Ca excess</th>
<th>Bone growth affected</th>
<th>Foxhound crossbreds</th>
<th>Beagles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Ca digestibility</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Nutrient digestibility</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P deficiency</th>
<th>Clinical symptoms</th>
<th>Foxhound crossbreds</th>
<th>Beagles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy requirements as multiple of maintenance (0.544 MJ ME/kg BW0.75/d)</td>
<td>20-30% adult BW</td>
<td>1.54±0.24</td>
<td>1.69±0.21*</td>
</tr>
</tbody>
</table>

Table 84

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P31) IDENTIFICATION OF THE INTRA- AND INTER-INDIVIDUAL METABOLITE VARIATION IN PLASMA METABOLITE PROFILES OF CAT AND DOG

Colyer, A.1, Gilham, M.1, Kamlage, B.2, Rein, D.3
1WALTHAM Centre for Pet Nutrition, Leicestershire, UK; 2Metanomics, Berlin, Germany; 3Metanomics Health, Berlin, Germany

The purpose of this analysis was to identify drivers of variance in plasma metabolite profiles of cat and dog in an environmentally-controlled study. Fourteen cats and fourteen dogs (neutered female, aged 1-9) housed in environmentally-enriched accommodation were fed a single batch of diet to maintain body weight in two three week blocks three weeks apart (7 cats and 7 dogs per block). On days 14, 16 and 18 fasted blood samples were taken. Metabolite profiling used GC-MS, LC-MS/MS and SPE-LC-MS/MS. Principal component analysis (PCA) indicated 31% and 27% of the variance was explained in PC1 & PC2 for cat and dog respectively, with data for each cat and for most dogs occupying a unique space, with no trend observed for age, sibling group, sampling day, housing lodge, years spent neutered or feeding block.

Having substantiated that the individual was the main driver of variance in the plasma metabolome in this study, the second objective was to identify metabolites that associated with the individual variation observed. After removing metabolites with a high intra-individual variance (SD >0.05) (110 and 115 for cat and dog respectively), the proportion of intra and inter individual variance was calculated for the remaining metabolites (109 and 101 for cat and dog respectively). Fifteen and six metabolites for cat and dog respectively had inter-individual variance accounting for at least 90% of the total variance, of which four metabolites were common to both species (campesterol, docosahexaenoic acid, a cholestenol and a sphingosine moiety). Other metabolites with >75% inter-individual variance were common to both species and to similar areas of metabolism.

In summary, in this study, the individual is an important driver of variance in the plasma metabolome and specific metabolites and areas of metabolism may be differentially regulated by individuals in two companion animal species.

P30) THE PLASMA METABOLOM E RESPONSE IN CAT AND DOG TO A CHANGE IN DIETARY GLUCOSE

Allaway, D.1, Kamlage, B.2, Gilham, M.1, Hewson-Hughes, A.1, Wiemer, J.2, Colyer, A.1, Rein, D.3
1WALTHAM Centre for Pet Nutrition, Leicestershire, UK; 2Metanomics, Berlin, Germany; 3Metanomics Health, Berlin, Germany

The purpose of this study was to evaluate metabolite profiling with respect to supporting companion animal health and nutrition. The response of the fasted plasma metabolome in healthy cats and dogs (neutered females, aged 1-9, n=14 per species) to a change in dietary glucose (3.85% by weight) was investigated. The study consisted of a two-way, crossover design with two diets (base and base + glucose) fed for three weeks each. Blood was sampled from each animal on days 14, 16, and 18 of each feeding block. Plasma was stored at -80°C. General metabolite profiling was undertaken using GC-MS and LC-MS/MS platforms whilst SPE-LC-MS/MS was used to determine catecholamine and steroid levels.

Metabolic profiling analysis identified 219 metabolites for cats and 216 metabolites for dogs that were consistently present within quantifiable detection limits. Results showed that both cats and dogs maintained their plasma glucose homeostasis when glucose was added to the base diet. However, statistical analysis (mixed model ANOVA) indicated that 44% and 31% of metabolites changed significantly (p<0.05) with diet in cat and dog respectively. In general the two species responded similarly to additional glucose in the diet. For example, data from both species were consistent with a decrease in fatty acid oxidation, a decrease in catabolism of glucogenic amino acids, sparing of taurine and uptake of branched chain amino acids into muscle. Analysis of the significant metabolite differences supports the view that the majority of metabolic changes are consistent with a normal insulin-regulated response to glucose in both cat and dog. Other responses included an increase in amino acid pools from the urea cycle, especially in dog. In addition, glucose affected markers previously associated with collagen and bone turnover and also increased consumption of meat. In this proof-of-principle study it was concluded that nutritional metabolomics can provide interpretable and novel information with relevance to companion animals.
P33) HAEMATOLOGY AND PLASMA CHEMISTRY OF THE RED TOP ICE BLUE MBUNA CICHLID (Metriaclima greshakei)
Snellgrove, D., Alexander, L.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

Clinical haematology and blood plasma chemistry can be used as a valuable tool to provide substantial diagnostic information for fish. A wide range of parameters can be used to assess nutritional status, digestive function, disease identification, routine metabolic levels, general physiological status and even the assessment and management of wild fish populations. However to accurately evaluate such data, baseline reference intervals for each measurable parameter must be established for the species of fish in question. Baseline data for ornamental fish species is limited, as research is more commonly conducted using commercially cultured fish. This study describes a range of haematology and plasma chemistry parameters measured for the ornamental Lake Malawi cichlid (Metriaclima greshakei).

Although this cichlid is fairly large in comparison with most tropical ornamental fish, two independent small volume blood samples were taken in order to assess a large range of parameters. A total of 16 fish with mean weight of 99.7g were sampled six weeks apart. On each date 8 fish were sampled for blood biochemistry and the remaining 8 for general haematological parameters (in a crossover design).

No statistical differences were noted between sample periods for any parameter except urea, which was significantly lower during the second sampling date; this may be related to the time of last feeding. Values obtained for a large number of parameters were similar to those established for other closely related fish species such as tilapia (Oreochromis spp). In addition to reporting the first set of blood values for Metriaclima greshakei, this study highlights the possibility of utilising previously established data for cultured cichlid species in studies with ornamental cichlid fish. Although consideration would need to be given to the many factors such as; sex, age, water quality, stocking density and diet, as these can all influence blood indices.

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P32) CHARACTERISTICS OF AGING PETS AND THEIR OWNERS: DOGS VERSUS CATS
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1Central Michigan University, Mt. Pleasant, MI, US; 2Cornell University College of Veterinary Medicine, Ithaca, NY, US

The purpose of this study was to ascertain differences in diet and lifestyle between cat (n=155) and dog (n=318) owners and their pets. The design was cross-sectional, convenience sampled, and participants were uncompensated. Trained interviewers administered questionnaires in person to participants who self reported data (mean±SD) on themselves and their pets.

Average cat ownership was 6.1±5 years and average cat age was 6.9±5 years. Cats were reported overweight (14%), fed ad-lib (87%), took medication (11%) and had health conditions (24%). For cats with health conditions, duration was 3.9±3 years. Pearson correlations showed cat age was positively related to cat weight, duration of illness, owner BMI and owner fat, whole grain, fruit and vegetable intake (p< 0.01, 0.05, 0.05, 0.01). Cat age was inversely correlated with activity level, owner’s exercise and owner’s fast food consumption (p< 0.01, 0.05, 0.05). Cat treat feeding was inversely associated with being overweight (p< 0.05). T-tests showed that cat owners were older than dog owners (p< 0.05).

Owners owned their dogs for 5.5±4 years and dog’s age was 5.9±4 years. Dogs were reported overweight (18%), fed ad-lib (49%), took medication (31%) and had health conditions (34%). For dogs with health conditions, duration was 3.6±3 years. Correlations revealed that dog age was positively associated with duration of illness (p< 0.01). Dog age was inversely correlated with amount of food fed, activity and owner’s exercise, fast food consumption and being overweight (p< 0.01, 0.05, 0.05, 0.01).

Similarities in owner diet and lifestyle as pets age, suggest educational and marketing strategies across both species may promote health. However, reporting of weight and overweight in aging dogs versus cats may be different. Pet overweight in this sample is lower than published literature. Therefore, marketing and educational strategies regarding obesity prevention may differ between dog and cat owners and requires exploration.

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P34) ENERGY EXPENDITURE AND WATER TURNOVER IN HUNTING DOGS IN WINTER CONDITIONS

Ahlstrøm, O., Redman, P., Speakman, J.
1Norwegian University of Life Sciences, Department of Animal and Aquacultural Sciences, Ås, Norway; 2University of Aberdeen, Department of Zoology, Aberdeen, UK

In the Nordic countries grouse hunting take place during autumn and winter when the terrain can be covered with snow. Dog owners taking part in this activity have shown interest to know more about the effect of cold climate and snow depth on energy and water requirement in their dogs. Therefore, the present experiment was carried out to determine the energy expenditure and water turnover in eight hunting dogs (Setters and Brittany Spaniels) running for three hours in three following days using doubly labeled water technique. The location was an alpine terrain at 1100 m altitude in Norway. The experiment was carried out in Jan/Feb and ambient temperature was -6 °C and snow depth 20-40 cm. The running distance covered by each dog was recorded by GPS. There was found a significant correlation (0.97) between BW and EE. Covering about the same running distance, the heaviest dog (27.6 kg) spent 16.6 MJ/d while the lightest (14.3 kg) 7.9 MJ/d. The EE based on BW^0.75 was quite equal, but somewhat higher for the heaviest dogs as they tended to run longer than the lightest. Body water turnover were 103 ml/kg BW/d and 0.19 ml/kJ. The EE was approximately 120 % higher than maintenance requirement determined for dogs.

<table>
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<tr>
<th>Average body weights, running distance, energy expenditure and body water turnover in eight dogs during hunting in winter conditions. Standard deviation in parentheses</th>
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<tbody>
<tr>
<td>Body weights (kg)</td>
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<tr>
<td>Running distance (km/d)</td>
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<td>Metabolizable energy expenditure (Mj/d)</td>
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<td>Metabolizable energy expenditure (kJ BW^0.75/kg/d)</td>
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<td>Metabolizable energy expenditure (kJ BW/kg/d)</td>
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<td>Body water turnover (ml/d)</td>
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<td>Body water turnover (ml BW^0.75/kg/d)</td>
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<td>Body water turnover (ml BW/kg/d)</td>
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<td>Body water turnover ml/kJ</td>
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</tbody>
</table>

P35) FOOD INTAKE AND BODYWEIGHT IN CATS FED LOW CARBOHYDRATE WET AND DRY DIETS

Hewson-Hughes, A., Miller, A.
WALTHAM Centre for Pet Nutrition, Leicestershire, UK

It is well recognised that dry food has a higher energy density than wet food but the different macronutrient profiles of wet and dry food could influence food intake and body weight. This study investigated wet and dry diets with similar macronutrient profiles (protein, fat and carbohydrate energy ratios: PER/FER/CER) but different energy densities on energy intake, bodyweight and body composition in the cat.

Twenty-four cats were assigned to two groups (5 males and 7 females per group), evenly matched for bodyweight and % body fat (determined by DEXA). They were offered 150 g of dry food (397.3 kcal/100g; PER/FER/CER 50/43/7) or 400 g of wet food (68.5 kcal/100g; 53/42/5) per day split between 2 x 45 min meals. The amount of food was increased for any cat that ate all that was offered. Cats were removed from trial if they gained 10% from their start bodyweight.

Cats on both diets gained weight over the course of the study. Repeated measures ANOVA revealed no significant difference between diets on bodyweight gain for cats that remained on the study at week 8. However, 9 cats fed the dry diet had been removed for weight gain by the end of the 10 week study while only 4 cats fed the wet diet had been removed. In addition to the higher number of dry-fed cats that had to be removed from the study due to weight gain, % body fat of the cats that remained on the study was significantly higher in cats fed the dry diet for 8 weeks compared to cats fed the wet diet (t-test).

Providing excess calories as either wet or dry food can lead to weight gain in cats and there appears to be greater risk of body fat gain if fed dry food ad libitum.
P36) MUTT CENSUS 2010: OBSERVATIONS ON THE BREED COMPOSITION AND SELECTED LIFESTYLE DEMOGRAPHICS OF AMERICAN MIXED BREED DOGS
Fretwell, N.1, Dibley, M.1, Davison, S.1, Perez, H.2, Sourk, E.2, Hughes, A.2
1Mars Veterinary, Leicestershire, UK; 2Mars Veterinary, Rockville, MD, US

Mixed breed dogs comprise a significant proportion of the dog population in many countries. Genetic tests designed to determine the breeds present in mixed breed dogs are now available commercially. The most comprehensive of the current tests1 covers more than 180 distinct dog breeds and has tested more than 33,000 mixed breed dogs to date. The aim of this study was to use the accumulated results of this testing to inform on the observed profile of breeds detected in mixed breed dogs in the USA. The genotype data from every dog submitted for sampling with the Wisdom Panel test since 2007 was analysed using the latest breed detection algorithm available, and the breeds detected informed the mixed breed population profile by state and across the whole of the USA. The number of breeds detected per dog averaged ~2.5 with German shepherd Dog being the most commonly identified breed detected in the background of mixed breed dogs.

In an adjunct to the main study, a web-based Mutt Census2 was conducted, where selected demographic owner and pet data was collected on veterinary care frequency, weight, feeding and exercise habits from over 15,000 mixed breed dog owners, many of whom had discovered the breed background of their pet using the test3. This survey was intended to inform the pet ownership habits and preferences of dog owners who are interested in discovering their pet’s lineage. The intent is to use this information to increase the future usage of the test through more appropriate communication with owners.

1WISDOM Panel™ Professional
2National Mutt Census™, Mars Veterinary, Rockville, MD http://www.muttcensus.com/

P37) TRYPTOPHAN SUPPLEMENTATION INCREASES VOLUNTARY FOOD INTAKE IN DOGS
Xavier Manteca Vilanova, Maria D. Baucells Sánchez
Universitat Autònoma de Barcelona, Barcelona, Spain

Introduction: The tryptophan is an essential aminoacid for dogs. Dogs fed a tryptophan-free diet exhibit depression in food intake. In pigs, tryptophan supplementation induced a significant increase in food intake. The aim of this study was to verify whether tryptophan diet supplementation increases the voluntary food intake in dogs.

Materials and methods: Eight Beagles were supplied with tryptophan (1 g/day) during 81 days (Tryp) and 8 Beagles were included in the study as a control group (Control). Both groups were fed with an experimental diet (1681 kJ/100g; 18% calories from protein) once a day according to requirements (NRC, 2006). The last 5 days of the supplementation period a voluntary food intake test was performed: 600 grams of the experimental diet were offered individually during 20 minutes twice a day (at 9:00 and 18:30). During the first day an adaptation was performed. The intake of the last four days was considered for statistical analysis using PROC MIXED (SAS, 2002).

Results: The average of total intake during the voluntary food intake test in the Tryp group was 77,49±3,647 g/kg of metabolic weight (MW) while the Control group was 57,99±5,375 g/kg MW. Differences were found between groups (p=0.074) but not among days (p=0.419). Table 1 shows the average of each group in each test day.

| Day 2* | Day 3 | Day 4* | Day $**$
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<tbody>
<tr>
<td>Tryp</td>
<td>79,50±7,037</td>
<td>76,49±6,541</td>
<td>76,58±7,632</td>
</tr>
<tr>
<td>Control</td>
<td>58,08±9,255</td>
<td>69,82±13,099</td>
<td>53,91±10,114</td>
</tr>
</tbody>
</table>

*,** within the same column means statistical differences (*p<0.1; **p<0.05).

Discussion and conclusions: A higher food intake in the supplemented group was observed. This raise could be related with some hormones such as ghrelin, cholecystokinin and leptin, as happens in other species. Further research should be developed in dogs to verify it.

Keywords: Tryptophan, food intake, dogs.
P38) EFFECTS OF FEEDING FREQUENCY ON PHYSICAL ACTIVITY LEVEL IN ADULT CATS
Deng, P.1, Grant, 2., Swanson, K.1,2
1Department of Animal Sciences, University of Illinois, Urbana, IL, US; 2Division of Nutritional Sciences, University of Illinois, Urbana, IL, US

Feline obesity has rapidly increased the past couple decades. Diet composition and feeding patterns may have effects on body weight by influencing physical activity. The objective of this study was to evaluate the effect of feeding frequency on physical activity in adult cats. Twelve healthy adult neutered male domestic shorthair cats (4.74 ± 0.16 kg BW; 4.5–5.5 BCS on 9-point scale) were used in a randomized, crossover study consisting of 48 days (three 16-day periods). In each of the first two periods, six cats were fed either two meals (8am and 8pm) or four meals daily (8am, 12pm, 4pm, and 8pm). A third period, in which all twelve cats were fed once daily (8am), was then performed and used as a reference. Throughout the study, cats were fed the same diet at amounts to maintain BW and BCS. Cats were individually housed from 8–9 am, 12–1 pm, 4–5 pm, and 8–9 pm each day. For the other 20 hours, cats were group housed to allow for voluntary physical activity. Voluntary activity levels were evaluated using Actical activity collars for 7 consecutive days in each period. Activity levels were expressed as ‘activity counts’ per epoch (epoch length = 15 sec). Total average activity level for two-meal-fed cats (20.04 ± 2.19) was not different from four-meal-fed cats (20.14 ± 2.15; P>0.05). Total average activity level for one-meal-fed cats (15.57 ± 2.03) was numerically lower than the multiple-meal treatments. The lower activity level in one-meal-fed cats was mainly due to less activity during the light period. Daily activity levels peaked during meal times, which were all during the light period. In conclusion, multiple feedings/day numerically increased physical activity as compared with once a day feeding. Further study is needed to clarify the effect of feeding frequency on physical activity and potential mechanisms involved.

P39) GENETIC DIFFERENCES OF HORSE, DONKEY AND MULE ARE DETECTABLE BY PROTEIN PROFILING
Henze, A.1, Aumer, F.2, Grabner, A.2, Raila, J.1, Schweigert, F.J.1
1Institute of Nutritional Science, University of Potsdam, Potsdam, Germany; 2Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Introduction: Horse and donkey belong to the genus equus, but nevertheless represent individual species with distinct genetic characteristics. These may result in a specific proteomes and may also influence the posttranslational modification (PTM) of proteins. Since PTMs can alter protein properties, specific PTMs may contribute to species-specific characteristics. Therefore, the aim of this study was to analyse differences in serum protein profiles of horses and donkeys as well as mules, which combine the genetic backgrounds of both. Additionally, changes in PTMs of the protein transthyretin (TTR), an ideal model for studies of PTMs, were analysed.

Materials and Methods: Serum protein profiles of each species were analysed using strong anion exchanger ProteinChips® in combination with SELDI-TOF mass spectrometry. The PTMs of TTR were analysed subsequent to immunoprecipitation with MALDI-TOF mass spectrometry.

Results: Protein profiling revealed 15 peaks, which were present in all species, seven peaks, which were only present in donkey and mule and three peaks, which were only present in horse and mule, seven peaks, which were only present in horse, but no peaks, which were unique for donkey or mule.

TTR of horse and donkey revealed native TTR as well as the modified forms sulfonated, cysteinylated and cysteinylglycinated TTR which all differed by a mass shift of approximately 30 Da between both species. The mass spectra of mule represented a merge from horse and donkey TTR spectra.

Discussion and conclusions: Despite the phylogenetic similarity of horse and donkey, the protein profiling revealed significant differences in the proteomes. Furthermore, although the mule combines the genetic backgrounds of horse and donkey, protein profiling revealed a higher similarity with donkey on protein level. However, with regard to TTR, the mule represents a genetic overlay of horse and donkey and no differences in the kinds of PTM seem to exist between the species.
P41) NITROGEN METABOLISM OF FOUR RAW MEAT DIETS IN DOMESTIC CATS
Kerr, K.1, Beloshapka, A.2, Dikeman, C.3, Burke, S.3, Simmons, L.3, Swanson, K.1,2
1Division of Nutritional Sciences, University of Illinois, Urbana, IL, US; 2Department of Animal Sciences, University of Illinois, Urbana, IL, US; 3Henry Doorly Zoo, Omaha, NE, US

Little nutritional information has been collected from domestic cats fed raw meat diets. Thus, the objective of this study was to evaluate differences in nitrogen (N) metabolism of diets based on meat sources commonly used for domestic or exotic cats: beef (BE: 66% crude protein (CP), 22% fat); bison (BI: 49% CP, 39% fat); elk (E: 79% CP, 6% fat); and horse (H: 60% CP, 28% fat). As is commonly done, diets only included the meat source, solka floc, and a vitamin/mineral premix to meet all nutritional needs. Eight intact adult female cats were fed to maintain BW in a crossover design. Total daily intake and fecal and urinary outputs were measured.

Because of diet differences, N and total amino acid (TAA) intake (g/d) was higher (p<0.05) in cats fed E compared to BE, BI and H, and higher (p<0.05) in cats fed BE compared to BI and H. Apparent total tract N digestibility (96.9 + 0.01%) was not different, but N absorbed (g/d) was higher (p<0.05) in cats fed E compared to BE, BI, and H, and higher in cats fed BE compared to H. Fecal N (g/d) was also higher (p<0.05) in cats fed E compared to H. Urinary N and total N excretion (g/d) was higher (p<0.05) in cats fed E compared to BE, BI, and H, and higher (p<0.05) in cats fed BE compared to H. The urinary N: fecal N ratio (22 + 6.0) did not differ due to diet.

In conclusion, dietary N was highly digestible for all treatments. Given their high digestibility, urinary N accounted for the majority of total N excretion. Differences in N and TAA intake and amount of N absorbed were due to differences in dietary CP. N retention was similar to values reported in the literature for domestic cats.
Diabetes mellitus (DM) treatment is based on insulin therapy and dietary management. Starch is the main nutrient that influences post-prandial glycemic response, but there are no studies of the effect of different starch sources in the glycemic control of diabetic dogs. This study evaluated the effects of diets with two starch sources and two different nutritional managements on the control of DM in dogs.

Two extruded isonutrient diets with 40% of starch and 16% of dietary fiber were manufactured, one with 46% of rice (as-fed) and the other with 42% of sorghum and 10% of lentils as starch sources, remaining the other ingredients unchanged. Ten dogs with naturally acquired DM, clinically stable (without signs of hyperglycemia or hypoglycemia, with fast glycemia between 100 and 350 mg/dL) with insulin therapy were used. Dogs were fed each diet for two months in a crossover design. Five dogs received insulin and food every 12 hours (management 1), and the other five insulin every 12 hours, but were fed three times a day (management 2). Variables assessed were insulin dosage, glycemic post-prandial response during 12 hours (management 1) or 8 hours (management 2), CBC, biochemical profile, and urinalysis. Glycemic curves were analysed by repeated measures ANOVA. Glycemic control parameters and area under the curve (AUC) were analysed by paired T-test (p<0.05).

In management 1 [four castrated females and one intact male, 10.4 (6 to 10) years old and 11.8 (5 to 12) kg], dogs fed the sorghum based diet showed lower mean and minimum blood glucose concentration (p<0.05), and lower maximum blood glucose and glucose AUC (p<0.08) than dogs fed rice based diet. In management 2 [three castrated females, one neutered male and one intact male, 9.6 (6 to 12) years old and 16.8 (7.4 to 29.3) kg], there was no effect of diet on the assessed variables (p>0.05). No difference in insulin dosage (1.1±0.18IU/kg day) were verified (p>0.05). In management 2, the ingestion of food without insulin administration and the lower relationship starch:insulin (g/IU/kg of BW) of the first meal overcame the starch effect.

The management of two meals followed by insulin administration associated with the sorghum-based diet improved glycemic control in diabetic dogs.

**Reference**
P45) EFFECTS OF SUGAR-CANE YEAST CELL WALL (Saccharomyces cerevisiae) AND 1,3/1,6-BETA-GLUCAN ON THE IMMUNE RESPONSE OF ADULT DOGS
Zaine, L.1, Vasconcellos, R.1, Tortola, L.1, Monazzi, L.2, Carlos, I.2, Carciofi, A.1
1Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil; 2Sao Paulo State University (UNESP), Araraquara - SP, Brazil

With the increase in the population of geriatric dogs, the comprehension of how aging can influence immune parameters has become important. Immunoglobulin A (IgA) is the most abundant class of antibody in mucous membranes, where it represents an essential factor in protection against infections, allergens and foreign proteins. Compared to mature dogs, studies shown increased serum and salivary IgA concentration in old animals. Data evaluating the influence of age in the intestinal secretion of IgA were not found. Considering this, we compared fecal IgA concentration in puppies, mature and geriatric dogs.

Twenty four beagle dogs were allotted to three groups of eight dogs each: puppies (5 months old); mature (4.6±0.5 years old) and old (10.6±0.5y old). Fresh fecal samples were collected daily, for 3 consecutive days and frozen (-20°C) immediately. After this, samples were pooled by dog, thawed and submitted to saline extraction. After centrifugation, supernatant was transferred to an Eppendorf containing 20 µL of protease inhibitor cocktail (Sigma-Aldrich). Samples were centrifuged at 15,000 x g for 15 min, and the supernatants were transferred to clean Eppendorf tubes and stored at -20°C. The quantification of IgA was performed by ELISA kit for canine IgA determination (Bethyl laboratories, Montgomery, USA). Optical density was read at 450 nm with a Microplate Reader (MRX TC Plus, Dynex Technology, Chantilly, Virginia, EUA). The results were evaluated using Proc GLM of SAS; means were compared by Tukey test (p<0.05).

An age effect on fecal concentrations of IgA was observed (Figure 1): Puppies shown lower fecal IgA concentration than mature and old dogs (p<0.05). Mature and old dogs did not differ on fecal IgA concentration (p>0.05), but larger mean deviation was observed for old animals.

The results suggest a reduced membrane immunological activity in puppies.

P44) FECAL IgA CONCENTRATION IS INFLUENCED BY AGE IN DOGS
Zaine, L., Ferreira, C., Tortola, L., Vasconcellos, R., Marcia Gomes, M., Carciofi, A.
Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil

With the increase in the population of geriatric dogs, the comprehension of how aging can influence immune parameters has become important. Immunoglobulin A (IgA) is the most abundant class of antibody in mucous membranes, where it represents an essential factor in protection against infections, allergens and foreign proteins. Compared to mature dogs, studies shown increased serum and salivary IgA concentration in old animals. Data evaluating the influence of age in the intestinal secretion of IgA were not found. Considering this, we compared fecal IgA concentration in puppies, mature and geriatric dogs.

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The results suggest a reduced membrane immunological activity in puppies.
P46) OLDER DOGS HAVE REDUCED BACTERIAL FERMENTATION ACTIVITY IN THE COLON AND LOWER PERIPHERAL LYMPHOCYTES CONCENTRATION
Gomes, M., Brunetto, M., Putarow, T., Zaine, L., Filho, F., Carciofi, A.
Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil

The study compared geriatric and mature dogs regarding fecal microbiota composition, gut fermentation end-product formation, and some immunological parameters.

The original experimental design focused on yeast cell wall (YCW) effects, but no interaction between diet and age was verified (p>0.05) allowing the comparison of age independent of diet. The experiment followed a two 4x4 Latin square design, one with four mature beagle dogs (4 years old) and the other with four old beagle dogs (10 years old), resulting in 16 replicates per age. In each period a 15-d of adaptation preceded a 5-d total collection of feces for digestibility trial. On day 21 fresh fecal samples were collected for bacterial enumeration, pH measurement, determination of ten biogenic amines, short chain fatty acids and lactic acid, and blood samples were collected for immunophenotypic quantification of lymphocyte subsets through flow cytometry (CD5+, CD4+, CD5+CD8+, CD45+ and CD45+CD21+). Dogs were fed four isonutrient kibble diets with 0%, 0.15%, 0.30% and 0.45% of YCW (as-fed). Data were evaluated using Proc GLM of SAS software (p<0.05).

Nutrient digestibility and metabolizable energy did not vary between ages (p>0.05). An age effect on fecal bacteria counts was not verified (total anaerobes, *Bifidobacterium*, *Lactobacillus*, *Clostridium* and *E. coli*); only a tendency for total aerobe increases in old dogs (p=0.15) was found. Older dogs presented lower fecal concentrations of butyrate (p=0.01), histamine (p=0.04), agmatine (p<0.01), and spermine (p=0.01), and higher fecal pH (p=0.03) than mature dogs. These findings suggest alteration in bacterial metabolic activity and end product formation, with a decrease in colonic fermentation with aging. Compared to mature, geriatric dogs showed a decrease of T lymphocyte (p=0.01), T-cytotoxic lymphocyte (p<0.01), and B lymphocyte (p<0.01) concentrations.

The study confirmed immunological alterations in geriatric dogs and revealed a reduced fermentation activity in the colon of older dogs.

P47) LOSS OF FAT MASS REDUCES ADIPOKINE CONCENTRATIONS IN OBESE DOGS
Brunetto, M., Sá, F., Nogueira, S., Teshima, E., Jeremias, J., Carciofi, A.
Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil

This study evaluated the serum circulating concentration of TNF-α, IL-2, IL-6 and leptin in obese dogs, before and after 20% of body weight loss.

Ten owned obese dogs, with body condition score (BCS) of 9 and mean fat mass of 45.7±1.51% (determined by deuterium isotope dilution technique) were used. These dogs had their weight reduced by 20%, achieving 33.5±1.92% of fat mass (p<0.001), without change in lean mass (kg; p>0.05)). A control group of 10 beagle dogs were also included (BCS =4.5; 18.3±1.38% of fat mass; p<0.01). TNF-α, IL-2 and IL-6 were determined in cytokines panel MILLIPLEX™ MAP, validated for dogs, on serum sampled after 12h of fasting. Leptin was measure by radioimmunoassay in multispecies kit validated for dogs. Means were analyzed by Wilcoxon no parametric test and the relationship between fat mass and circulating adipokines through Pearson’s correlation (p<0.05).

Obese dogs presented higher serum concentrations of TNF-α, IL-6 and leptin than the same dogs after weight loss and the control dogs (p <0.05), but these last two groups did not differ from each other (p>0.05). A positive correlation between fat mass and TNF-α (r=0.67) and leptin (r=0.67) concentration was found.

Fat loss in obese dogs can reduce the concentration of circulating adipokines and thereby can result in potential benefits to dogs’ health.
P49) THE INTRAVENOUS GLUCOSE TOLERANCE AND POSTPRANDIAL GLUCOSE TESTS MAY PRESENT DIFFERENT SENSIBILITIES IN THE EVALUATION OF OBESE DOGS
Brunetto, M., Nogueira, S., Sá, F., Gomes, M., Pinarel, A., Carciofi, A.
Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil

This study compared intravenous glucose tolerance test (IVGTT) and glucose postprandial response (GPPR) for the evaluation of glucose metabolism of obese dogs before and after weight loss.

Ten owned obese dogs (body condition score [BCS] of 9; mean fat mass of 45.7±1.51% determined by deuterium isotope dilution technique) were used. These dogs had their weight reduced in 20% (33.5±1.92% of fat mass [p<0.001], without changes in lean mass in kg [p>0.05]), named weight reduced (WR) group. A control group of 10 beagle dogs were also included (BCS =4.5; 18.3±1.38% of fat mass; p<0.01). Glucose tolerance and insulin sensitive were measured in the three groups by two methods: IVGTT (infusion of 0.5g of glucose/kg BW); and GPPR (consumption of cooked rice to achieve 6g of starch/kg BW).

At IVGTT the area under the curve (AUC) of glucose, maximum and mean glycemia were higher for obese than lean (p<0.05), and intermediate for WR (p>0.05). Basal leptin, basal insulin, insulin response peak, insulinogenic index, AUC of insulin increment between 0-15min and between 60-120min were higher for obese (p<0.05), presenting WR and control dogs similar results (p>0.05). However, glucose disappearance (%) and the time to glucose concentration reduce to half weren’t different among any of the three groups (p>0.05). A t GPPR the A UC of insulin increment between 0-120min was higher for obese than lean (p<0.05), and intermediate for WR (p>0.05), although the later secretion (AUC of insulin increment between 120-360min) was similar for obese and WR (p>0.05), with higher values than for the control group (p<0.05).

The IVGTT and GPPR showed different results, and maybe different sensibilities. While the IVGTT showed that the loss of 20% BW resulted in improvement of glucose control with reduced insulin secretion, these same dogs at GPPR test remain showing higher insulin secretion with values similar to obese.

Urolithiasis is a common clinical problem in dogs. In uroliths formation several factors like breed, sex, age, diet composition, water intake, infection of the urinary tract, environment and drug administration, were recognised. Struvite and calcium oxalate are the predominant mineral types in urolithiasis of the dog, representing overall more than 80% of total urolithiasis case reported.

The aim of this study was to compare the effect of two different commercial dry food formulated for the management of struvite urolithiasis with different acid/base status (-203 and -182 mEq/kg, for diet 1 and 2, respectively) on urinary pH.

Twelve adult dogs (4.3 ± 1.2 years old; live weight 20.2 ± 10 kg) of different breed showing struvite urolithiasis confirmed by urinalyses and uroliths composition, were divided into two group (A and B) fed for 5 month diet 1 or 2, respectively. The urinalyses were repeated 6 times (every 15 days), while blood were analysed only at the beginning and at end of the trial. Data were processed using PROC GLM of SAS.

At the first urinalyses the mean pH values was (8.0 ± 0.5 and 8.0 ± 0.8, respectively for group A and B) and in about 70% of the samples bacteria were present and an antimicrobial (Fluoroquinolones in tablets form) were given for one week. At the second urinalysis no bacteria were found. Both groups showed a progressive decreasing of pH values (Figure).

After two months of therapy in both case the recommended pH value for stone dissolution (5.5-6.1) was achieved. The differences between the groups were significant (P<0.05) from the third analyses. At the end of the trial none of the urine samples showed crystals, but the final pH value of group A was particularly low and could be dangerous.

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The IVGTT and GPPR showed different results, and maybe different sensibilities. While the IVGTT showed that the loss of 20% BW resulted in improvement of glucose control with reduced insulin secretion, these same dogs at GPPR test remain showing higher insulin secretion with values similar to obese.

P48) MANAGEMENT OF STRUVITE UROLITHS IN DOGS
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Urolithiasis is a common clinical problem in dogs. In uroliths formation several factors like breed, sex, age, diet composition, water intake, infection of the urinary tract, environment and drug administration, were recognised. Struvite and calcium oxalate are the predominant mineral types in urolithiasis of the dog, representing overall more than 80% of total urolithiasis case reported.

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After two months of therapy in both case the recommended pH value for stone dissolution (5.5-6.1) was achieved. The differences between the groups were significant (P<0.05) from the third analyses. At the end of the trial none of the urine samples showed crystals, but the final pH value of group A was particularly low and could be dangerous.
P50) EVALUATION OF SELENIUM SOURCES FOR DOGS
Putarow, T. 1, Sartori, J. 2, Vasconcellos, R. 1, Barducci, R. 2, Guimarães, A. 1, Carciofi, A. 1
1Sao Paulo State University (UNESP), Jaboticabal - SP, Brazil; 2Sao Paulo State University (UNESP), Botucatu - SP, Brazil

This study evaluated the bioavailability of two sources of selenium, and their effects on antioxidants status of dogs.

Twenty-four male beagle dogs were distributed in three treatments: control (7.3 µg of Se/MJ), inorganic (20 µg of Se/MJ; sodium selenite) and organic (20 µg of Se/MJ; selenium yeast), with eight dogs per diet. In the first 10 days animals were kept in metabolism cages for total feces and urine collection, and then transferred to kennels. At day 11 a postprandial Se curve was conducted, plasma samples were obtained before feeding and after every 2 hours after feeding during 12 hours. In all samples, Se was analysed by GFAAS and a Shimadzu modelAA-6800 atomic absorption spectrometer was used. Plasma thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC), and glutathione peroxidase activity (GSH-Px) were determined on days 0, 40, and 80, and Se in hair on days 0 and 80. The results were evaluated using Proc GLM of SAS; means were compared by Tukey test (p<0.05).

Control group showed the lowest Se intake, fecal excretion and retention (p<0.05). No differences on Se excretion on urine and Se bioavailability were found (p>0.05). Se concentration in plasma (µg/L) did not change (p>0.05), but Se in hair (pg/g) at day 80 was 2.5 times higher on organic and inorganic groups than in control group (p<0.05). The post-prandial area under Se curve was higher for organic than for inorganic and control groups (p>0.05), showing better absorption. GSH-Px activity decreased from day 0 to 80 in the control group (p<0.05), but remained unchanged in organic and inorganic supplemented groups (p>0.05).

Results suggest that at least 20µg of Se/MJ is required for adult dog maintenance. On the scope of evaluated parameters, no differences between sources were verified.

P51) EVALUATION OF THE ORAL 13C-BICARBONATE TRACER TECHNIQUE FOR THE ESTIMATION OF CO2 PRODUCTION AND ENERGY EXPENDITURE IN DOGS DURING REST AND PHYSICAL ACTIVITY
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For feeding of working dogs during their daily life, illness, routine jobs or sporting activities, an accurate determination of their nutritional requirements is essential to ensure optimal health and performance. In order to predict appropriate guidelines about how to feed dogs, it appears essential to be able to determine the rate of energy expenditure (EE) in a reliable and feasible way. In the present experiment, the oral 13C-bicarbonate tracer technique (o13CBT), i.e. collection of breath samples after oral administration of NaH13CO3, was used for the estimation of CO2 production (RCO2) and EE in dogs.

The RCO2 and EE were estimated from the 13C kinetics of exhaled breath CO2, in six dogs of different breed (2 English Springer Spaniel, 2 Germar Shorthair Pointers and 2 Beagles), age and body weight (BW), collected into breath bags by using a mask with a two-way non-rebreathing valve system at -5, 5, 10, 20, 30, 40, 60, 90, 120, 180, 360, 540 and 720 minutes after tracer administration (5 mg/kg BW). Measurements were conducted during two days of rest, and during three days with three hours of exercise per day.

Average EE was 483 and 876 kJ◊kg-0.75◊d-1 during rest and exercise, respectively. Variation in individual EE between dogs was assumed to be due to individual differences between the dogs (e.g. age, breed, physical conditions and different types of exercise), and also to further need for standardization of the technique.

The results indicate that the o13CBT is appropriate to use as a minimal restrictive and non-invasive method to obtain reliable estimates of EE in dogs at different activity levels under near natural conditions. However, the accuracy of the estimates depends on the values used for the 13C recovery factor and the respiratory quotient in the calculations, and thus the technique needs to be further standardized and validated in large-scale experiments.
P52) A HIGH DIETARY SODIUM CONTENT (1.3% VS. 0.35%) DOES NOT AFFECT RENAL AND CARDIOVASCULAR VARIABLES IN AGED HEALTHY CATS OVER A 12-MONTH PERIOD

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High-salt diets have been proposed to increase water intake and urine volume, as part of the prevention of urinary crystal formation. Potential adverse effects of high sodium intake have been suggested in older cats, especially on kidney and heart functions. This blinded randomized prospective study aimed to compare the effects of 2 dietary salt contents on renal and cardiovascular variables in aging cats.

Twenty healthy neutered cats (10.1±2.4 y.) were included. Following baseline measurements, cats were randomly allocated in 2 groups according to their glomerular filtration rate, gender, age and body weight. They were fed one of two complete balanced extruded diets (4000 kcal ME/kg as fed), only differing in their salt content, normal (sodium 0.35%, chloride 0.70%) or high (sodium 1.30%, chloride 2.27%).

Measures were made before and over 12 months after diet implementation, and concerned (1) body weight, water consumption, urine volume, specific gravity, protein/creatinine ratio, plasma urea, creatinine, total proteins, electrolytes, calcium, phosphate; (2) systolic and diastolic arterial blood pressure, heart rate, myocardial wall thickness, shortening fraction and left atrium/aorta ratio, radial and longitudinal tissue Doppler imaging of the left ventricular free wall; (3) glomerular filtration rate. Statistics were performed using a general linear model. The only significant changes between diets were water intake as well as urine volume and specific gravity (P<0.05). All other variables were unaffected by the diet.

In conclusion, over a 12-month period, a high-sodium intake does promote diuresis but does not affect renal or cardiac function in aged healthy cats.

P53) TRACE AND NUTRITIONAL ELEMENTS IN BRAZILIAN DOG FOODS

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Brazil holds the second largest contingent of domestic dogs in the world, with 33 million dogs, behind the United States. The annual consumption of dog food in the country is 1.7 million tons. Pet food should be able to provide a complete and balanced diet. The concern about food safety has been extended to pets, including not only the assurance of necessary nutrients in correct levels, but also the control of contaminants. This study aimed to evaluate trace and nutritional elements in dog foods of various brands available in Brazil. Thirty-four samples of dog food for puppies, adults and seniors were acquired in the local market of Piracicaba, state of São Paulo.

For analysis, 300 g of each sample were ground in a knife mill and homogenized. Test portions of 400 mg were taken for instrumental neutron activation analysis (INAA). Moisture was determined in separate portions of 1 g, and all pet foods complied with the humidity required by legislation, i.e. lower than 12%. The procedure allowed the simultaneous determination of As, Br, Ca, Co, Cr, Cs, Fe, K, La, Na, Rb, Sb, Sc, Se, U and Zn. The concentration values found for these chemical elements, except for As, Cr, Cs, Sb and U, were higher than the detection limit (DL) in all samples. Amongst the toxic elements, the highest variations were observed for Cr (0.52–6.47 mg kg⁻¹), Sb (0.013–5.42 mg kg⁻¹) and U (0.46–3.99 mg kg⁻¹), while As concentrations lied within a narrow range (0.29–0.49 mg kg⁻¹).

Regarding the nutritional elements, Ca showed concentrations in some samples slightly higher than the maximum limit established by the legislation (2.4% for adults and 1.6% for puppies in dry matter). The results will be further discussed according to the nutritional role and the potential toxicity of each chemical element found in the dog foods.
P54) EFFECT OF DIRLOPATIDE ON PLASMA LIPIDS AND HEPATIC mRNA EXPRESSION IN OBESE DOGS
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Obesity exacerbates or predisposes to serious medical conditions. This can result from excessive dietary fat intake, and leads to dyslipidemia. In enterocytes, microsomal triglyceride transfer protein (MTP) participates in chylomicrons biosynthesis. Dirlotapide (Slentrol®), a MTP inhibitor should be useful to promote weight loss by lowering fat absorption, and improve lipid metabolism disorders. Our aim was to examine the effects of dirlotapide on plasma lipids and expression of hepatic lipid metabolism factors, in obese dogs.

Thirteen obese dogs (initial BW: 12.8±0.6 kg) were randomly allocated to a placebo (n=5) or a dirlotapide (n=8) group. They received amounts of food to maintain their obese BW. Dirlotapide or placebo were administered once daily for 21 weeks according to the Slentrol® dosing regimen. Before and after treatment, body condition score (BCS) was evaluated, and plasma lipid levels were assayed. SREBP1 and PPARα mRNA levels were assessed by real time PCR in liver biopsies.

Neither BW (13.3±1.4 to 13.0 ±1.4 kg) nor BCS (7.0±0.3 to 7.0±0.3) had been changed by placebo treatment, whereas they were lower (12.5± 0.6 to 9.7± 0.3 kg [p<0.0005] and 6.9±0.3 to 4.4±0.4 [p<0.001] in dirlotapide-treated dogs. No difference with pre-treatment values was seen in NEFA levels of either group. In dirlotapide-treated dogs, triglyceride, phospholipid and total cholesterol levels were lower (0.91±0.10 to 0.65±0.04 mmol/L, [p<0.05], 3.62±0.07 to 3.29±0.10 g/L. [p<0.05] and 6.17±0.58 to 4.14±0.32 mmol/L. [p<0.05]). SREBP1 mRNA levels were unchanged in dirlotapide- and placebo-treated dogs. PPARα mRNA levels were higher dirlotapide-group (100±57 to 251±24 %, [p<0.05] and lower in placebo group (100±19 to 39±13 %, [p<0.0001].

The dirlotapide treatment resulted in a BW and BCS decrease, accompanied by a lower plasma lipid level, and a higher expression of transcription factors involved in lipid metabolism. Dirlotapide could be useful to improve lipid metabolism in obese dogs.

P55) EFFECT OF DIRLOPATIDE ON ACUTE PHASE PROTEIN EXPRESSION IN OBESE DOGS
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Obesity can exacerbate medical conditions. Especially inflammation and insulin resistance have been described. Dirlotapide (SlentrolTM), a microsomal triglyceride transfer protein (MTP) inhibitor could be useful to promote weight loss. Its effect on insulin sensitivity (IS) and inflammation is not well established. Our aim was to examine the effects of dirlotapide on insulin sensitivity and expression of inflammation factors in the liver of obese dogs.

Thirteen obese adult beagle dogs (2.8y, initial BW: 12.8±0.6 kg) were randomly allocated to a placebo (n=5) or a dirlotapide (n=8) group. They received amounts of food to maintain their obese BW. Dirlotapide or placebo were administered once daily for 21 weeks according to the Slentrol® dosing regimen, to maintain a BW loss of >0.7%/wk. BW was recorded weekly. Before and after treatment, body condition score (BCS) was evaluated, and IS assessed (euglycemic/hyperinsulinemic clamp). Haptoglobin, ceruloplasmin and serum amyloid A (SAA) protein mRNA levels were assessed by real time PCR from liver biopsies.

Prolonged dirlotapide treatment resulted in lower BW, improved IS, and lower acute phase protein expression, directly or indirectly. Dirlotapide could thus be also useful to improve the inflammatory state associated with obesity in dogs.
**P56) EFFECT OF CITRUS AND CURCUMA SUPPLEMENTED DIET ON BODY WEIGHT AND INFLAMMATION STATE IN OBESE CATS**

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Obesity can exacerbate medical conditions. Especially low-grade inflammation state has been described. The putative therapeutic properties of citrus and curcuma could be associated with anti-inflammatory effects. Our aim was therefore to examine the effects of citrus or curcuma extract supplementation on body condition and inflammatory state in obese cats.

Two diets supplemented with either curcuma or citrus extract were fed to eight obese cats for two 8-week period, in a cross-over study design. BW was measured weekly. Plasma acute phase proteins (α1-glycoprotein, serum amyloid A [SAA] and haptoglobin) were assessed prior and at the end of each test period. mRNA levels of markers of inflammation (TNFα, IL1B, IL2, IL4, IL5, IL10, IL12, IL18, TGFβ), IFNγ) were determined in leucocytes by real-time PCR.

BW was unaffected by diet. Compared to pre-period values, plasma α1-glycoprotein level was lower for both diets, plasma SAA level was unchanged, and plasma haptoglobin level was lower after the citrus-supplemented diet. TNFα, IL1B, IL4, IL5, IL10, IL12, IL18, TGFβ mRNA level was unchanged by any diets. IL2 mRNA level was lower after curcuma-supplemented diet, whereas IFNγ mRNA was lower after citrus-supplemented diet.

Our results show no effect of citrus or curcuma supplementation on BW. They also show a poor effect on inflammatory markers expressed by leucocytes, whereas a lower expression of acute phase proteins expressed by liver after citrus-supplemented diet. This could suggest a beneficial effect of citrus, targeted in liver, without effect on immune system, and citrus supplementation could to improve the inflammatory state associated with obesity.

**P57) EFFECTS OF WEIGHT LOSS ON THE CARDIAC STRUCTURE AND FUNCTION OF OBESE DOGS**

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This study evaluated the cardiovascular alterations of obese dogs and the effects of weight loss. Eleven mature healthy obese dogs and eleven mature ideal weight dogs were evaluated. Animals were assigned into three groups: GI (obese dogs – 12.33 ± 2.94 kg), GII (obese dogs after weight loss – 9.77 ± 2.44 kg) and GIII (ideal weight dogs – 14.14 ± 1.87 kg). The obese dogs were submitted to a weight reduction with commercial hypocaloric diet in which they were fed 60% of their maintenance energy requirements calculated by their estimated target weight (TW) considered as actual weight less 20%. The cardiac structure and function were assessed by two-dimensional, M-mode and doppler echocardiography and arterial blood pressure measurement.

Obese dogs demonstrated 20.56% mean weight loss (p<0.0001) in sixteen weeks. It was observed mild left atrial dilation in GI that reduced 12.66% after weight loss (p=0.0340). The remaining echocardiographic variables analyzed (left ventricle diameter, left wall and interventricular septum thickness in systole and diastole; E-point to septal separation, left atrium-to-aorta ratio, fractional shortening, ejection fraction, mean velocity of circumferential fiber shortening, velocities flows across mitral, tricuspid, pulmonic and aortic valves, pre-ejection time, ejection time and isovolumetric relaxation time) did not show significant variations. A decrease in arterial pressure was seen in all obese dogs after weight loss, with 13% for systolic (p=0.0059), 11% for mean (p=0.0296) and 8.5% for diastolic (p=0.0451). Systemic blood pressure did not differ between groups I and III.

The study conclude that obese dogs can develop mild left atrial dilation that is correct with weight loss and do not show significant structural and functional cardiac alteration that can lead to a sub-clinical obesity cardiomyopathy. Moreover, obese dogs do not show systemic arterial hypertension and systolic, mean and diastolic arterial pressures can reduce after weight loss.
P59) LIPID PROFILE AND INSULIN SENSITIVITY IN RATS FED HIGH-FAT OR HIGH-FRUCTOSE DIETS

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Obesity is a major factor leading to many clinical disorders including insulin resistance (IR) and dyslipidemia. To study obesity-related dyslipidemia and IR, an appropriate animal model is needed. Use of rat, as animal model of obesity and IR is under study, but the occurrence and severity of obesity-induced metabolic disorders could vary according to diet. The aim of this study was to examine the effects of high-fat or high-fructose diets on body condition, insulin sensitivity (IS) and lipid profile in rats.

Twenty-one Wistar male rats (12 week-old, initial BW: 394.1±12.4 g) were fed control, high-fat diet (65% lipid calories) or high-fructose diet (65% calories from fructose) for 10 weeks. BW was recorded weekly. Before and at the end of 10-week diet, body composition (isotope dilution method) and IS (euglycemic-hyperinsulinemic clamp technique) were assessed. Plasma basal glucose, non-esterified fatty acids (NEFA), triglyceride (TG) and total cholesterol were assayed. TG and cholesterol were measured in plasma lipoproteins separated by FPLC.

BW was higher (p<0.0001) in all groups at the end of 10-week diet, whereas fat mass percentage was higher (p<0.05) in high-fat group and high-fructose group. Fasting glycemia was unchanged in all groups. IS was lower (p<0.05) in high-fat group, but unchanged in control group and high-fructose group. Fasting plasma NEFA and TG were unchanged in all groups. In control group, VLDL-cholesterol was lower, while LDL-cholesterol was higher (p<0.05). In high-fat group, we observed higher total cholesterolemia, VLDL-cholesterol, LDL-cholesterol and HDL-cholesterol (p<0.05). In high-fructose group, LDL-cholesterol was higher (p<0.05).

Our results show that both high-fat and high-fructose diets lead to obesity, but high-fructose diet has no obvious diabetogenic properties. It seems that dietary fat could induce IR independent of visceral obesity. Further experiments will be required to understand limited fat accretion (possibly reduced hepatic lipogenesis).
P60) INFLUENCE OF DIFFERENT LEVELS OF RESISTANT STARCH ON FECAL QUALITY OF DOGS VARYING IN BODY SIZE
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Undigested starch can influence fecal quality. This has not been well documented in canine breeds other than beagles. The aim of our study was to assess the effects of different amounts of resistant starch on fecal score in dogs, differing in breed and size.

Twenty-one healthy female dogs (5-8 years old) of four breeds were used: 6 miniature poodles (5.0±1.0 kgBW), 6 miniature schnauzers (6.9±0.5 kg), 3 medium schnauzers (16.0±0.4 kg) and 6 German shepherds (27.3±3.3 kg). Each dog was fed to maintenance (144±6 kcal/kgBW0.75/day) the same commercial diet (Royal Canin, Medium Adult, 100g starch/1000 kcalEM) supplemented with 0, 5, 10 or 20% of starch flour (Hi-Maize 260, National Starch; 42.4% RS/DM). The supplemented diets were tested successively from the lowest to the highest concentration of starch (7 test days followed by 7 washout days). Fecal scores were evaluated by a single person using a scoring scale ranging from 1 for hard and dry feces to 5 for liquid stools. Scores were considered optimum when ranging from 2.5 to 3.0, acceptable from 3.0 to 3.75 and unacceptable when higher than 3.75.

In medium schnauzers and especially German shepherds, frequency of unacceptable scores was higher (p<0.05, MacNemar’s test) at a dose as low as 5%. They were doubled with the 10% supplementation. On the contrary, in miniature poodles and miniature schnauzers, feces quality was unchanged and minimal alteration were observed at the highest supplementation level (20%).

Those results show a lower digestive tolerance (i.e. higher fecal score) of larger dogs to undigested starch whereas small dogs appear less sensitive even to high levels of resistant starch. This observation might be linked to a higher colonic fermentative activity as reported in large breed dogs. Cooking of starch appears to be a critical step for petfood manufacturers, especially regarding to large breeds.

P61) EVALUATION OF THE USE OF ANIMAL COMPOUNDS AS SUBSTRATES BY FELID FECAL MICROBIOTA: A CHEETAH MODEL
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Although strict carnivores like felids do not consume plant fibre, considerable intestinal fermentation has been measured. This indicates that certain animal-derived compounds can serve as substrates for fermentation and microbial proliferation in the feline hindgut. It is often claimed that enzymatically indigested animal material exerts the production of toxic compounds in the hindgut, but to date, only little peer-reviewed documentation is available, whereas in contrast, recent findings point to specific microorganisms in the feline hindgut that use proteolysis products as a substrate.

The present study investigated to which extent faecal microbiota of the cheetah (as model for strict carnivores) ferment animal tissues and which would be the concomitant end product profile. Fresh faecal samples of captive cheetahs were collected and processed into an inoculum, combined with following substrates: casein (pure protein), bone, skin, hair, cartilage, collagen, chondroitin-glucosamine mixture, glucosamine, fructo-oligosaccharides (FOS, positive control), cellulose (negative control). During 72 hours, cumulative gas production was continuously registered. Thereafter, incubates were sampled for analysis of volatile fatty acids (VFA).

Gas production was highest for FOS, followed by glucosamine, chondroitin-glucosamine mixture and cartilage. Casein gave intermediate gas production, but still more than collagen. Bone, skin, hair and cellulose were poor fermentation substrates. Interestingly, maximum gas production rate of FOS occurred much later compared to all animal substrates.

VFA production was highest with FOS. Glucosamine and glucosamine-chondroitin showed high VFA production, whereas cartilage and casein produced moderate VFA. Despite the low gas production, collagen had high short-chain fatty acids production and showed, in contrast to all other substrates, a very high ratio of acetic to propionic acid.

The present data indicate that cartilage and cartilage related compounds are well-fermentable animal tissue for faecal microbiota of the cheetah. Moreover, collagen can also contribute to microbial fermentation, but behaves differently and needs to be look at thoroughly.
Small dog breeds are at a higher risk of urolithiasis than larger breeds. Factors known to influence this risk include dietary water intake. The current study aimed to assess the effect of different regimes of system feeding with wet and dry diets on urinary parameters in small dogs.

Eight healthy small breed adult dogs were included in the study. The trial was conducted as a four phase crossover in which four different dietary systems were offered (where the proportions of each complete and balanced diet were based on energy): diet 1: 100% dry diet; diet 2: 75% dry, 25% wet; diet 3: 50% dry, 50% wet and diet 4: 100% wet. Each phase lasted for 10 days, during which urine was collected daily (using a non-invasive urine collection system) and analysed for volume, pH and specific gravity (SG). During the last 2 days of the trial, urine was collected for calcium oxalate (CaOx) and struvite relative supersaturation (RSS) analysis. Full data sets were collected from five dogs.

CaOx RSS was significantly reduced in dogs receiving 25% of their calories from wet diet (9.33 ± 3.74) compared to feeding 100% dry diet (19.92 ± 7.36). There was no significant effect of diet on struvite RSS or SG. When nutritional composition of the four feeding systems was analysed by Principal Component Analysis (PCA), a negative correlation was found between increasing moisture and sodium intakes and CaOx RSS. A number of other nutrients showed some influence on CaOx RSS, these effects being less clear.

Feeding at least 25% of the daily calorie allowance in a wet food format significantly reduced the risk of CaOx stone formation in small dogs, primarily driven by increased dietary moisture and increased sodium intake although the relative influence of these two nutrients cannot be ascertained from this study.
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